Projecting the effects of climate change on prey selection and dietary competition in communities of European reedbed warblers

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Doctor of Philosophy

By

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Summary

Climate change remains one of the greatest threats to natural systems worldwide. Songbirds are sensitive to changes in invertebrate prey resources and may respond to climate-mediated shifts by either broadening or narrowing the range of prey types consumed. This PhD used a latitudinal gradient as an analogue for future climate change, to explore how warming might alter diet, prey selectivity and dietary overlap in reedbed warblers breeding in Europe. The diets of six reedbed warbler species along the gradient were elucidated via metabarcoding of prey remains in faecal samples. Local weather, prey availability and warbler diets in the reedbeds of Catalonia (southern Europe) were monitored and compared with northern and southern reedbed sites in England and Wales (UK), to infer future patterns of prey availability and changes to potential competitive responses by warblers to future warming across the gradient.

Each warbler species showed subtle differentiation in diet; as did different age classes of the reed warbler (*Acrocephalus scirpaceus*); including the size of prey captured, taxonomic composition, and the proportion of aquatic and terrestrial arthropods consumed (Chapter 2, 3 and 4). Partitioning was often masked by high dietary overlap when local prey abundance was high, but lower overlap was observed during scenarios of lowered prey abundance. Changes in dietary overlap were also associated with maximum temperature and rainfall, with contrasting responses in the two countries, likely mediated through impacts upon local warbler prey (Chapter 5). The extent of overlap along the gradient could be predicted using a continuum of prey abundance and richness.

Our findings have implications for sympatric birds in both the UK and Spain, potentially affecting future competitive interactions and coexistence. These consequences may be alleviated by carefully controlled habitat restoration and management, with the aim of promoting aquatic biodiversity, and enhancing cross-system subsidies across wider landscapes.

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For my grandmother Mary

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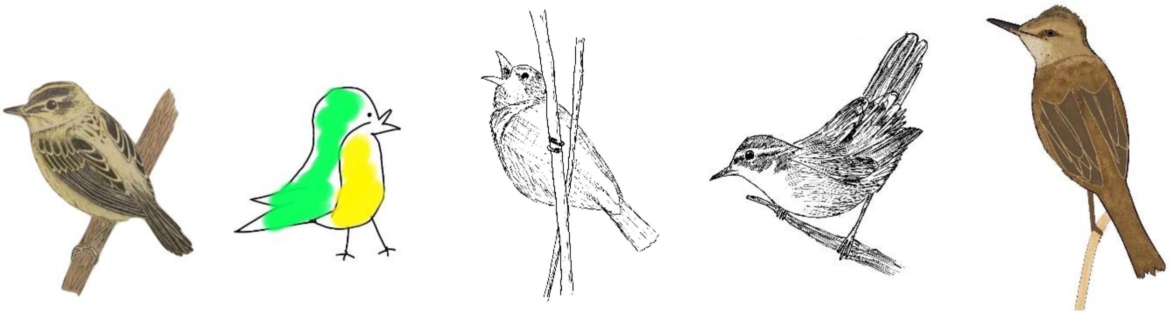
Chapter One - General introduction



Warbler illustrations by Rebecca Young, Ryan Smith, Nia Thomas, Josie D'Urban Jackson and Jordan Cuff.

1.1. The effects of climate change on migratory birds

Understanding how future climate change will affect organisms and their interactions with one another presents a monumental challenge to ecologists and conservation scientists (Harmon et al. 2009). It is unclear how competitive interactions between species may change under warming, as organisms respond to future changes in resources (Traill et al. 2010) and select prey to best maximise fitness, using optimal foraging strategies (Krebs and Davies 1991, see section 1.2.).

Responses to elevated mean or seasonal temperatures have already been documented in a wide range of taxa and one of the first groups to receive scientific attention were migratory songbirds (Berthold 1990, McCarty 2001*b*, Walther et al. 2002, 2010). Due to their complex ecology and distribution over multiple spatial scales and ecosystems, populations of different species may respond to climate change in various, unexpected ways (Rosenzweig et al. 2007, Traill et al. 2010). For example, some species have responded via changes in the timing of migration and breeding (Lehikoinen et al. 2004, Schaefer et al. 2006, Covino et al. 2020). However, long-term data on the potential fitness outcomes of responses to climate change have so far only been collected for a restricted set of bird species (e.g., Winkel and Hudde 1997, Sillet et al. 2000, Stevenson and Bryant 2000, Eglington et al. 2015), most of which are non-migratory. Migratory birds are more constrained by events in their life-cycle compared to resident birds and therefore might be expected to show fewer adaptations to future environmental change, which would then have severe impacts on their population dynamics (Berthold 1990, Lemoine and Boenig-Gaese 2003). Measured impacts to date, have varied greatly both within and between migrant species (Sanz 2003, Visser et al. 2003).

An important factor to consider, is the effect of warming on seasonal prey availability on the breeding grounds and stopover sites to which the birds return in spring (Winkler et al. 2002). Here, prey availability is interpreted as the relative abundance or richness (at the species, genus or family level) of invertebrate taxa recorded in the foraging habitats of the consumer. Thus, it is also assumed that all prey items occurring in a given study area are “available” to birds as a food resource.

The distribution and relative abundance of many invertebrate populations that form the prey of insectivorous birds, is likely to be highly sensitive to warming in future years.(Bale et al. 2002, Walther et al. 2002). For many invertebrate species, an increase in environmental air temperature can lead to more rapid development (Barlow 1962, Bryant et al. 2002) and emergence (Sardiña et al. 2017) or may increase the number of generations in a year (Eggermont and Heiri 2012) leading to higher recruitment. Contrastingly, if temperatures increase above an organism’s optimal threshold, recruitment decreases, and species may shift the southern edges of their geographic range northwards (Parmesan 2006). Invertebrates with northerly distributions, low phenotypic plasticity and low optimum development thresholds are expected to be most at risk from future warming (Bale et al. 2002, Pearce-Higgins 2009). The resulting changes to a songbird species' prey base is likely to result in dietary changes and could change interspecific competition within communities of songbirds for access to prey.

The migration strategies of passerine birds are variable, and subject to selective pressure such that the timing, route, length of flight stages, and habitat and diets at stopover sites should be under selective pressure to enhance the likelihood of survival (Bibby and Green 1981). Importantly, the spatio-temporal availability of food resources has a strong influence on migration patterns. For example, birds optimise their reproductive success by timing their arrival and subsequent laying dates on the breeding grounds with local peaks of invertebrate prey so that chicks hatch during periods of plentiful food resources (Both et al. 2009).

Phenological mismatches occur when spring temperatures advance and cause earlier blooms of host plants, thereby accelerating the development of invertebrate prey (Visser et al. 1998). If migrants cannot adjust their arrival times to allow for earlier spring emergence, the timing of chick hatching with the narrow window of peak food availability becomes asynchronous and chick survival, breeding success and recruitment decreases. It has been suggested that specialists living in highly seasonal habitats such as temperate woodland (e.g., the pied flycatcher *Ficedula hypoleuca* (Both et al. 2006, 2009) and blue tit *Cyanistes caeruleus*) are likely to be worst affected because newly-hatched young are dependent on caterpillars which emerge with the oak bud burst (Buse et al. 1996, 1999). The indirect effects of warming on migrants with more generalist diets, such as old-world warblers, inhabiting less strongly seasonal habitats such as reedbeds, are less well known.

1.2. Warblers in the UK

Old world warblers (Sylvioidea) are common and widespread throughout Europe, occupying a variety of natural habitats such as scrub, woodland, wetland and riverine systems, and man-made environments such as reservoirs, ditches, parks and gardens (Cramp and Brooks 1992). Most species are migratory and breed in the Palearctic during the summer months, departing in the late summer/autumn to overwinter in tropical or subtropical climates, often sub-Saharan Africa (Moreau 1972). Migrating to warmer climates is an evolved strategy to avoid the shorter daylength, lower temperatures and reduced food availability of the northern winter (Bibby and Green 1981).

In the UK, two acrocephalid species, the Eurasian reed warbler (*Acrocephalus scirpaceus* [Herman, 1804] hereafter termed "reed warbler"), and the sedge warbler (*Acrocephalus* *schoenobaenus* [Linnaeus, 1758]) are typical of wetland habitats, especially reedbeds and marshes. In some southern parts of the UK, they have in recent decades been joined by the Cetti's warbler (*Cettia cetti* [Temminck 1820]), a bush warbler associated with low vegetation close to aquatic environments. The Cetti's warbler is a recent arrival to the UK, originally from southern Europe (Bonham and Robertson 1975, Harvey 1977). Since the 1970s a population has become established in the southern part of the country, and populations are slowly expanding their range northwards. Cetti's warblers are now resident year-round, but dependent on mild winter temperatures for overwinter survival, such that populations are not currently present in much of northern UK (Robinson et al 2010). Willow warblers (*Phylloscopus trochilus* [Linnaeus, 1758]) and common chiffchaffs (*P. collybita* [Vieillot, 1817]) are small leaf warblers that use a range of habitats, mostly open woodland and scrub, but they can inhabit wetland environments as well (Simms 1985). The chiffchaff is a partial migrant with only a small proportion of the population breeding in the UK overwintering in temperate north-western Europe but most of the population breeding in Southern Europe remain as year-round residents. Reed, sedge and willow warblers overwinter in sub-Saharan Africa and return to the UK breeding grounds in late spring.

Long distance migrants often demonstrate a high degree of dietary flexibility and opportunism, a response to often highly variable prey that change in availability within and among the breeding grounds, overwintering grounds and migratory stopover sites, which themselves may be formed of different habitats (Poulin et al. 1994, Gerwing et al. 2016). In many habitats, the most available or abundant prey groups fluctuate spatially and temporally. This generates a selective pressure for migratory warblers to become plastic in their prey choice, so that they are able to modify their diet over time to track changes in prey availability (Laursen 1978, Poulin et al. 1994, Marchetti et al. 1996, Mallord et al. 2016). Optimal foraging is achieved by a consumer when the costs and benefits of capturing and consuming a prey item are balanced, to maximise energy gained over energy expended, which should serve to enhance fitness (Krebs and Davies 1991). Therefore, species should differ in their preferences for habitat and food resources according to their efficiency at using a particular foraging habitat and/or feeding technique or behaviour (Partridge 1978, Bibby and Green 1981). During times of environmental change, selection should favour dietary generalists if they are more resilient to changes in food resources and can adjust to taking a wider range of prey types beyond those that they have evolved to consume most efficiently (Colles et al. 2009, Mallord et al. 2016). Therefore, it might be expected that warbler species should be relatively robust to the effects of climate change and might be able to absorb any impacts on prey availability by altering their diets accordingly. However, changing competitive pressure between species is also possible under future climate change, and this is yet to be explored in warblers.

1.3. Climate change and prey availability for reedbed warblers

Reedbeds comprise very nutrient rich, productive habitats for both aquatic and terrestrial organisms (Rodwell 1995, Greenberg and Marra 2005). Invertebrate food sources are renewed regularly as potential prey develop in the water, soil and vegetation, and emerge in succession (Leisler and Drycz 1988). The high diversity and abundance of prey in reedbeds provides continuous availability of emergent prey throughout the summer (Swift and Racey 1983, Sabo and Power 2002, Paerzold et al. 2011, Dunn et al. 2011, Hambäck et al. 2016) so it is not surprising that they comprise suitable foraging and/or breeding habitat for generalist, foraging, migratory warblers. Unlike seasonal habitats with lower productivity, such as deciduous woodlands, reedbeds are thought to respond readily to environmental change, a trait which renders them model systems for climate change studies (Engloner 2009).

Studies suggest that currently, primary productivity in reedbeds in northern Europe is thermally limited, without an obvious seasonal peak, meaning that under current temperatures food availability may be limiting for reedbed birds (McKee and Richards 1996, Halupka et al. 2008). The timing of invertebrate emergence is expected to be earlier in the south of a range if spring temperatures are warmer than in the north, such that the number of growing degree days increases earlier in the year (Vannote and Sweeney 1980, Lee et al. 2008). If warming increases the abundance of many invertebrate groups by increasing development rates and the number of invertebrate generations, then generalists may be able to exploit higher levels of potential prey, resulting in higher fecundity and reproductive success.

Using supplementary feeding trials, Vafidis experimentally demonstrated that under future climate change, reed warblers should benefit from the higher abundances of prey produced by warming in reedbeds (Vafidis 2014, Vafidis et al. 2016). Recent increases in the breeding productivity of reed warblers in both the Netherlands (Kampichler and van der Jeugd 2011) and the UK (Baillie et al. 2012) are thought to be a result of increases in resource availability from increases in spring temperatures. A study by Eglington et al. (2015) showed that reed warblers are showing increased productivity (in the number of juveniles produced per breeding season, per site) at northern European sites as the summer temperatures are rising, and as a result this species is expanding its breeding range within the UK (Balmer et al. 2013).

In addition, long running studies show that reed warblers on average arrive at their breeding grounds up to two weeks earlier than 30 years ago and have advanced their onset of breeding by the same length of time, resulting in a lengthened breeding season and a greater likelihood of re-nesting, both of which are likely to increase reproductive success and fitness (Schaefer et al. 2006, Halupka et al. 2008, Kovács et al. 2012). The height of growing reed stems is a known constraint in determining when reed warblers can initiate nesting (Vafidis 2014, Vafidis et al. 2016), as this species relies on nest concealment to reduce predation risk. Vafidis et al. (2016) showed this experimentally, revealing that reed warblers responded to supplementary feeding in a warm year by shifting their laying date to an earlier point in time, because the reeds had already grown sufficiently tall for nest building, but not during cool years when the reeds were still too short. The growing season and development of the *Phragmites* reed itself is dependent on ambient temperature and day length (Dykyjova et al. 1970), and thus reed growth has also accelerated with increasing spring temperatures in recent years, such that stems reach a sufficient height for reed warbler nesting earlier in the year (Schaefer et al. 2006). The great reed warbler (*Acrocephalus arundinaceus*, [Linnaeus, 1758]), a congener of the reed warbler, has responded similarly to the reed warbler under recent climate change in Poland, by breeding earlier, but net breeding productivity has been neutral, suggesting warming is neither advantageous or disadvantageous for this species (Dyrcz and Halupka 2009). However, a study by Schaefer et al. (2006) that combined breeding data from reed and great reed warblers from 30 years in a marshland site in Bavaria, Germany, found that while great reed warblers only changed their breeding window slightly compared to reed warblers, they did experience higher reproductive success. This suggests that the outcomes of warming for reedbed warblers might vary in time and space, depending on a variety of interacting environmental and biotic factors.

Although reed and great reed warblers are showing adaptation to current climate change, the same may not be true for other warbler species present in reedbeds. The remaining warblers in the Eglington et al. (2015) study; sedge warblers, chiffchaffs, willow warblers, whitethroats (*Sylvia communis*), blackcaps (*S. atricapilla*) and garden warblers (*S. borin*) all have their highest breeding productivity in the mid-latitudes of Europe, but range changes have not yet occurred northwards as expected. These six species all showed evidence of adaptation to local conditions, with their productivity declining as temperatures deviate from local averages. For sedge warblers, reed warblers and blackcaps the optimum temperature was greater than the local average at the majority of study sites. It has been suggested that there is an indirect effect of temperature on both habitat quality and prey availability which likely explains variation in productivity over space (Smith et al. 2011, Ferger et al. 2014), even though short-term variation over time is more likely to be directly related to temperature (Julliard et al. 2004).

The reasons for these patterns are unclear, but other warbler species may show less dietary plasticity than the reed warbler, or may be dependent upon specific invertebrate groups, or feeding habitats with different invertebrate groups, at different stages of the breeding season, as has been shown in other passerines (e.g., Kent and Sherry 2020). Many warbler species feed in scrub and field vegetation as well as reedbeds (Simms 1985, Cramp and Brooks 1992). In these more seasonal habitats, it follows that prey emergence and prey availability follow a seasonal pattern, and such habitats are more likely to experience a shift in the peak of prey abundance such that it misaligns with the time when demand for adult and nestling food is highest (Visser et al. 1998, Both et al. 2009). Contrastingly, prey emerging from the aquatic environment may cross over into these terrestrial habitats (Nakano and Murakami 2001, Sardiña et al. 2017, Lewis-Phillips et al. 2020), providing a dependable source of food even to the warblers more strongly associated with scrub habitats (López-Iborra et al. 2005, Trevelline et al. 2018).

Moreover, future altered rainfall patterns could affect conditions on the breeding grounds, causing usually semi-submerged reedbed habitats to dry out, prey communities to shift and overall prey availability to decline. In a study by Poulin et al. (2002) in the south of France, a food availability index for *Acrocephalus* warblers was negatively correlated with length of time between June and December where no surface water was present in the reedbed. In Mediterranean reedbeds, increases in maximum temperature coupled with reduced rainfall could lead to reductions in water levels and ultimately result in food shortages for reedbed warblers. Reedbeds are commonly managed to prevent drying by manipulation of water levels, usually involving seasonal or permanent flooding, but this is in itself is highly disruptive to invertebrate communities and may have knock-on effects on their avian predators (Poulin et al. 2002, Bedford and Powell 2005).

1.4. Diet optimisation in birds

It is now widely understood that prey quality is just as critical to fitness, survival and reproductive success as prey abundance (Piersma 2012, van Gils et al. 2005, Oudman et al. 2014, Twining et al. 2014). Birds are under selective pressure to choose the most profitable prey possible under the physical and environmental constraints they experience. The optimal diet of birds, as described by optimal foraging theory, is achieved by the ability of a consumer to balance a number of cost-benefit functions that together will influence fitness in a net positive way (Krebs and Davies 1991). Individuals must weigh up the costs and benefits of capturing a given prey resource (for either themselves or in the case of parent birds, their offspring), using information such as foraging costs, predation risk, handling and ingestion time, thermoregulatory costs, reduced time for other activities such as territory defence, nesting and mate searching, and potential consumption of toxins or inhibitory substances (reviewed in Brodmann and Reyer 1999, Orłowski et al. 2017). Aquatic prey in particular are valuable to birds, both in terms of their high availability during mass emergence events (Lewis-Phillips et al. 2020) and their high content, as compared to terrestrial invertebrates, of highly-unsaturated omega-3 fatty acids (HUFA) which are important for growth, development and immune function (Hixson et al. 2015, Twining et al. 2018). The difference in HUFA availability between terrestrial and aquatic habitats means that there is a possibility of nutritional mismatches in terrestrial animals such as birds that consume aquatic prey, if aquatic resource phenology and/or availability is altered due to habitat degradation or climate change (Twining et al. 2018).

1.5. Resource partitioning in birds

Resource partitioning is a central issue in ecology explaining patterns of coexistence among morphologically similar species in sympatry (MacArthur 1958, Hutchinson 1959, Schoener 1974*b*, Pigot et al. 2018). The impact of competition can be effectively reduced by the diversification of traits in congeners or similar, related species, that allow them each to use i) different resources or ii) the same resources at a different time, or in a different area in space (Chesson 2000).

Examples of resource partitioning have been described across the animal kingdom; in songbirds (e.g. Lack 1976, Ferguson 1988, Poling and Hayslette 2006, De Leon et al. 2014, Trevelline et al. 2018, Kent and Sherry 2020), seabirds (Barger et al. 2016), reptiles (Pianka 1974, Durso et al. 2015), amphibians (Steele and Brammer 2006), bats (Razgour et al. 2011, Emrich et al. 2014, Salinas-Ramos et al. 2015, Arrizabalaga-Escudero 2018, Vesterinen et al. 2018, Gordon et al. 2019), mammalian carnivores (Vieira and Port 2006, Fortin et al. 2007, Hayward and Kerley 2008, Cupples et al. 2011, Wang et al. 2014, Smith et al. 2018), small mammals (Kronfeld-Schor and Dayan 1999), ungulates (Heldová 1996, Mysterud 2000, Kartzinel et al. 2015), cetaceans (Spitz et al. 2006), fish (Dineen et al. 2007, Davis et al. 2015, Waraniak et al. 2019) and arthropods (Friberg et al. 2008, Albrecht and Gotelli 2001).

One of the main mechanisms in which insectivorous birds coexist in spite of limiting resources and assumed presence of competition, is via the partitioning of food resources, with different species of birds foraging on a narrower set of prey (Pyke 1984, Perry and Pianka 1997). This reduces the number of prey types shared between potential avian competitors and alleviates extreme competition, which could be costly for fitness (Schoener 1974*b*, Dhondt 1977, Dhondt 1989). Birds may also forage in different habitats to their competitor, where different prey types or compositions of prey can be found. This could result in separation of habitats horizontally, (i.e., in space), or vertically (i.e., feeding at different heights in vegetation) (Catchpole 1972, Dyrcz 1981) or the air (Orłowski and Karg 2013). Alternatively, birds may use diverging feeding methods to obtain prey items, such as picking, gleaning, hawking or sallying, which each serve to capture different prey (Bibby and Green 1983). Taking prey of different size classes has also been documented as a mechanism of resource partitioning in passerines, especially if sympatric passerine species differ in body size themselves (Bibby and Green 1983, Marchetti et al. 1996, Orłowski and Karg 2013). Segregation of breeding times, (i.e., breeding either earlier or later than your competitor) is another common strategy to reduce competition for prey during chick rearing stages (Catchpole 1972, Ceresa et al. 2016).

Since most bird species differ somewhat on some anatomical, behavioural and physiological level; for example, body size, wing length or bill morphology, their ability to capture different prey types and their nutritional requirements should also differ, meaning that the optimal prey for one species may not be the optimal prey of another (MacArthur 1958, Pigot et al. 2018). Thus, even in scenarios of resource partitioning, foraging birds are able to make species-specific decisions based on optimal foraging to select the prey groups that will offer the best balance of calories per unit of time and energy expended (Turner 1982, Robinson and Wilson 1998). Some high-quality prey items may be universally preferred, and these might be in higher demand in foraging habitats, (e.g., protein-rich caterpillars (Greenberg 1995) and spiders (Ramsay and Houston 2003)). Others are able to cross over habitat boundaries and be consumed by birds using different habitats (Poulin and Lefebvre 1996, Wilson 2010, Kent and Sherry 2020). Therefore, while resource partitioning may not result in a separation of diet between neighbours that is complete, it should prevent overexploitation of multiple important prey groups at the same time.

When food supply is very limited, sympatric species can be driven to consume a broader range of prey, since feeding on preferred, optimal prey that are declining is restrictive (MacArthur and Pianka 1966, Pulliam 1985, Martínez-Curci et al. 2015). This often results in a greater extent of dietary overlap between sympatric species. Increased dietary overlap might have implications for future coexistence, as prey groups could become overexploited via added pressure of being exploited by multiple consumers, potentially driving up competition. Eventually this could lead to the loss of one consumer species from the area (Chesson 2000). Conversely, when prey become highly abundant over long periods, or if several groups of prey emerge *en mass* at the same time and are locally superabundant (e.g., MacDade et al. 2011, Lewis-Phillips et al. 2020), generalist songbirds should opportunistically feed on these groups. This may cause dietary overlap between species to increase, potentially masking the existing species-specific dietary associations (e.g., Dhondt 2011, Trevelline et al. 2018).

1.6. Altered patterns of dietary competition as a result of a changing climate

Ecological processes occurring between organisms, such as inter- and intra-specific interactions with predators, parasites and competitors have so far not received wide attention in climate change studies on songbirds (Møller et al. 2004). Similarly, little attention has been given to the impacts of climate change on the habitat selection of sympatric species, which could potentially reveal new shifts in the use of foraging and/or breeding habitats (Martin et al. 2005). Comparative studies that investigate these influences should contribute to a more holistic understanding of the likely mechanisms of responses to future warming and associated cascading effects across trophic levels.

Competition between species could be impacted by climate change if warming drives either an increase or decrease in multiple abundant prey groups simultaneously or if a particularly nutritionally significant prey taxon is affected (Pearce-Higgins 2009, Marshall et al. 2016). Changes in prey availability could lead to dietary partitioning or dietary overlap between similar consumer species, which has the potential to alter competitive pressure (Chesson 2000). In addition, coexistence of species in a given location may be destabilised if a competitive advantage is gained by one or a few species, with interactions between species influenced by climate driven changes in phenology or niche occupancy (Traill et al. 2010).

To predict future changes relating to interspecific competition it is necessary to generate detailed dietary information for insectivorous birds likely to be affected by climate change. One approach is to monitor the prey choice of sympatric species (Razgour et al. 2011, O'Shea et al. 2013, King et al. 2015). In addition, if changes in prey resources occur, it is expected that the diet composition of songbirds should change in response, and this may lead to stronger overlap or partitioning, both of which have consequences for competition and coexistence. However, this has never been examined using an approach that compares both diet composition and dietary overlap scenarios under different climatic conditions, alongside monitoring of available prey, to determine i) whether diets and dietary overlap change with warming and ii) if this is reflected by a similar change in the abundance, availability and/or diversity of prey resources in the field.

1.7. Metabarcoding as a platform for revealing ecological interactions

Where direct observation was not possible, past research on reed warbler diet has been based on invasive methods such as the use of emetics or neck ligatures to sample the prey delivered by breeding adults to their nestlings. Although these methods allow prey items to be quantified, they raise serious ethical questions since there is a risk of causing physical harm or physiological stress to the birds.

Collection of bird faeces is a far less invasive method of investigating diet, but traditional morphological methods of identifying prey from faecal or stomach contents is extremely time consuming and requires specialised training and expertise in systematic taxonomy (Barrett et al. 2007, Deagle et al. 2007, Pompanon et al. 2012). Studies are largely restricted to very broad taxonomic resolution (e.g. Bibby and Thomas 1985, Rosenberg and Cooper 1990, Grim 1999, Kerbiriou 2011) and cannot always detect soft-bodied or easily digested invertebrates (Pompanon et al. 2012). This introduces an element of bias in favour of larger, hard-bodied organisms that survive digestion, skewing results in favour of certain taxa (Clare et al. 2009, Clare 2014).

Recent technical developments in high-throughput DNA sequencing have dramatically altered the research landscape for ecologists. The emergence of molecular techniques for dietary analyses have enabled scientists to disentangle ecological interactions, enhancing our understanding of network dynamics and wider ecological processes (Symondson 2002, Pompanon et al. 2012, Clare 2014, Evans et al. 2016). In recent years, high throughput sequencing (HTS) technology has become more accessible and affordable, becoming a powerful tool for ecological studies (Valentini et al. 2009*b*). HTS costs per sample are decreasing rapidly while throughput rates are increasing (Glenn 2011, Shokralla et al. 2012). These developments have been coupled with a substantial expansion of DNA sequence databases as a result of increased efforts to barcode plants and animals (Herbert et al. 2003, Chase et al. 2007, Taberlet et al. 2007). The extension of HTS methods to characterise many species from an environmental sample simultaneously, using DNA barcode sequencing became known as DNA metabarcoding (Pompanon et al. 2012). DNA metabarcoding is able to identify prey/host plant DNA in a large number of samples via simultaneous sequencing of short fragments of highly variable sections of DNA.

Metabarcoding is now used frequently for trophic studies and has been successfully applied across a wide range of organisms (reviewed in: Symondson 2002; Deagle et al. 2005, Pompanon et al. 2012, Clare 2014, Symondson and Harwood 2014). Collection of samples is relatively non-invasive to the study organism, analyses offer a high-resolution snapshot of diet even when dietary content has undergone degradation from digestion (Deagle et al. 2006, Pompanon. et al. 2012), and the amount of biological data produced from samples is far greater in HTS approaches than is obtained from morphological identification or Sanger sequencing (Soininen et al. 2009, Alonso et al. 2014, Gibson et al. 2014).

Dietary analyses using species-specific primers are well documented but require existing knowledge of prey species (Kuusk and Agusti 2008). More recently, the sequencing of dietary contents without detailed prior knowledge of prey groups has become more commonplace (Clare 2014). While species-specific markers detect the presence of a particular species, group-specific markers detect all species within a taxonomic group (Jarman et al. 2004, Zeale et al. 2011). Newer developments such as the design of “universal” markers to identify all potential food taxa, in theory means that the whole breadth of an organism’s diet can more easily be ascertained (Meusnier et al. 2008, O’Rorke et al. 2012, Jarman et al. 2013). Although these markers are rarely truly “universal” in practise (e.g., Elbrecht and Leese 2017), they still have very wide taxonomic coverage and can be utilised in dietary studies where the consumer’s diet is wide enough to span several orders, (e.g., piscivores, insectivores or generalist herbivores). In some cases, (e.g., omnivores or generalists consuming a very diverse range of taxa) resolution or coverage may be lacking by a single barcode, and the use of several barcodes simultaneously may also be appropriate (Deagle et al. 2009, Rayé et al. 2011).

Metabarcoding can be adapted to characterise the diet of most organisms and has so far been used to in the study of both herbivorous and carnivorous animals from a diverse spread of taxa to include mammals, reptiles, birds, fish, insects and spiders (Deagle et al. 2009, 2010, Valentini et al. 2009*a*, Bohmann et al. 2011, Brown 2011, Eitzinger and Traugott 2011, Wang et al. 2014, Kartzinel et al. 2015, Leray et al. 2015, Waraniak et al. 2019, Shutt et al. 2020). Metabarcoding can also be used for a wider variety of applications within diet studies, for example to identify predators of crop pests (Jedlicka et al. 2017) and vectors of human disease (Puig-Montserrat et al. 2020) or inform invasive species management (Egeter et al. 2019). It is also possible to examine the use of analogue species for ecological replacement (Moorhouse-Gann 2017), compare diet across seasons (Clare et al. 2014*b*, Moorhouse-Gann 2017, Buglione et al. 2018, Waraniak et al. 2019) and across geographic locations (Clare et al. 2014*a*, Pearson et al. 2018, Eitzinger et al. 2019), assess the impact of environmental change on diet (Bhattacharyya et al. 2019), and reveal dietary partitioning, overlap and potential competition in sympatric species (e.g. Razgour et al. 2011, Brown et al. 2013, Crisol-Martínez et al. 2016).

Metabarcoding studies on songbirds are still relatively uncommon (but for recent examples see: Crisol-Martínez et al. 2016, Gerwing et al. 2016, Stockdale et al. 2016, Trevelline et al. 2016, 2018, Jedlicka et al. 2017, Rytkönen et al. 2019, Moran et al. 2019, Shutt et al. 2020) but numerous studies on resource partitioning in insectivorous bats have emerged in recent years (Razgour et al. 2011, Emrich et al. 2014, Salinas-Ramos et al. 2015, Arrizabalaga-Escudero et al. 2018, Vesterinen et al. 2018, Gordon et al. 2019), providing a basis on which to make predictions about prey availability and the impacts on dietary competition in other ecologically similar, generalist terrestrial insectivores.

The main advantage of metabarcoding is that it allows the detection and species-level identification of multiple prey groups, but it can be limited by reliance on well-populated reference libraries of taxon-specific sequences for the target gene (Pompanon et al. 2012, Clare 2014). GenBank and the Barcode of Life Data Systems BOLD (www.barcodinglife.org; Ratnasingham and Hebert 2007) are relatively comprehensive for invertebrates, thanks to successful barcoding campaigns focusing on a wide range of taxonomic orders (reviewed in Raupach and Radulovici 2015, Raupach et al. 2016). This represents a fantastic opportunity for applying molecular tools to study the diet of European insectivores.

Limitations to metabarcoding using HTS include potential difficulties in determining the exact origin of the consumed prey, failure to detect secondary predation and the potential for biases in DNA amplification which could confound downstream quantitative analyses (King et al. 2008, Pompanon et al. 2012, Clare 2014, Deagle et al. 2013, Brooks et al. 2015). Solutions to the majority of these issues are rapidly becoming available however, and biases from HTS are greatly reduced when compared to alternatives such as traditional morphological analyses (Pompanon et al. 2012). Despite their shortcomings, HTS based approaches have tremendous power and show applicability to a wide range of ecological questions.

1.8. Project aims, objectives and methodological approach

Closely related sympatric species may be affected by climate change differently and may each adapt to future environmental changes using very different strategies. Although studies predict that generalist migrants such as the reed warbler will be able to thrive under future warming, few consider the potential effects of dietary competition with other sympatric warbler species. It is expected that reedbed warbler species that currently breed in more northerly areas in Europe will experience a change towards the climatic conditions currently present further south, which has the potential to cause dietary shifts and altered competition pressures.

Under future warming, multiple abiotic and biotic parameters within a location will shift simultaneously (IPCC 2013), and predictive studies need to consider encompassing these complexities, and examine these characteristics in concert. Environmental gradients are beneficial for climate change studies since they cover a wide range and variety of abiotic conditions across an organism’s distribution. Specific locations along a gradient can be studied and compared to improve understanding of ecological trends across space and time (e.g., Blois et al. 2013, Sundqvist et al. 2013, Eitzinger et al. 2019). Gradients can be used to form “space for time” studies, where observed changes in environmental measurements across a range are used as surrogates for future conditions (Körner 2007).

The main aim of this study was to assess the current and future impacts of warming on sympatric reedbed warblers, using a “space for time”, climate gradient approach, using latitudinal differences in climate as a surrogate/proxy for future climate change. The diets of the most common warbler species within reedbeds will be studied along this gradient (Table 1.1).

Research questions

Current and future UK climates will be represented by a network of reedbed sites in the UK and southern Europe. Field sites were chosen where at least two of the study species live in sympatry and where climate matches up with future climate projections for the UK. A combination of techniques from field and molecular ecology will be applied to address the following research questions:

- Which prey taxa (family, genera, species) comprise the diets of different species of reedbed warblers and how does this change across a European climate gradient?
- Does nestling diet and juvenile diet differ significantly from adult diet in reed warblers?

- How does warbler prey availability (abundance and diversity) differ across reedbed sites along the climatic gradient?
- How does prey consumed (identified in the diet by HTS) compare with the prey available (monitored invertebrates) in each region?
- What evidence is there to support the theory that warblers use an optimal foraging approach when selecting prey?
- To what degree do warbler diets overlap within each region and how does this change among the sites studied and over the breeding season?
- How is dietary overlap and resource partitioning between sympatric species affected by climate and prey availability?
- What are the implications for UK warbler species with respect to interspecific competition under future climate change?

To answer the above research questions, the objectives of the study are to i) examine current warbler diets within and across sites and breeding seasons, ii) measure the extent of dietary overlap between warbler species at different sites, iii) compare prey availability with prey consumed by warblers to identify specific preferences, relating these to optimal foraging predictions, iv) identify mechanisms which promote resource partitioning and coexistence among species, and v) use these findings to project the impacts of climate change on future prey selectivity and dietary competition in European warblers.

Table 1.1. Information about the distribution, habitat, migratory strategy and conservation status in the UK, for the six European warbler species under study.

Species	Latin Name	First Described	UK status	Spain status	Wintering grounds	Primary Habitat	UK Conservation Status
Eurasian Reed Warbler	*Acrocephalus scirpaceus*	Hermann 1804	Migrant breeder	Migrant breeder	Sub-Saharan Africa	Reedbed/ marsh	Stable/increasing
Sedge Warbler	*Acrocephalus schoenobaenus*	Linnaeus 1758	Migrant breeder	Not present/ vagrant	Tropical and south Africa	Reedbed/ marsh	Stable/declining
Great Reed Warbler	*Acrocephalus arundinaceus*	Linnaeus 1758	Not present/ vagrant	Migrant breeder	Sub-Saharan Africa	Reedbed/ marsh	Rare visitor to the UK, stable elsewhere in Europe
Cetti's Warbler	*Cettia cetti*	Temminck 1820	Resident breeder	Resident breeder	Europe	Reedbed/ marsh	Increasing in England and Wales
Chiffchaff	*Phylloscopus collybita*	Vieillot 1817	Migrant breeder, winter visitor	Migrant breeder, winter visitor	Western Europe, Mediterranean, North Africa east to India	Open woodland/ scrub, may be present in reedbeds	Stable/increasing

Species	Latin Name	First Described	UK status	Spain status	Wintering grounds	Primary Habitat	UK Conservation Status
Willow Warbler	*Phylloscopus trochilus*	Linnaeus 1758	Migrant breeder	Migrant breeder	Tropical and south Africa	Open woodland/ scrub, may be present in reedbeds	Declining -especially in southern UK

Chapter outline

In Chapter 2, the diets of warblers cohabiting reedbed habitats in several sites in the UK will be examined, to characterise the diet and prey preferences of each species and measure current levels of dietary overlap. Whether, and how, diets may change as a result of climate will also be explored by i) comparing sites in southwestern UK with a site in northern England and ii) by comparing a single site over two years in different weather conditions. Chapter 3 focusses on the diet of reed warblers at different stages of their life cycle and seasonal changes in diet over the course of the breeding season will be assessed. In Chapter 4, the same methodology as Chapter 2 will be applied to sites selected in Catalonia, Spain, where the warbler assemblage differs by the presence of a larger competitor, the great reed warbler. How birds at these sites might avoid competitive exclusion, via resource partitioning on a number of temporal, spatial and taxonomic axes will be investigated. Chapter 5 will combine datasets from both the UK and Catalonia, to evaluate how dietary selectivity and dietary overlap changes across Europe with changes in climate and predict how warblers in Europe will respond to future warming. An evaluation of the accomplishment of project aims and the implications of the findings for conservation, habitat management and future responses to climate change will be presented in Chapter 6.

Chapter Two - Spatial and temporal dietary patterns in reedbed warblers of the UK; the influence of local climate, prey availability and potential competition.

Members of the Gower Ringing Group at Oxwich Marsh, South Wales during a bird ringing session in summer 2017. All birds were captured, handled and ringed by licensed ringers endorsed by the British Trust for Ornithology (BTO). Photo credit: Owain Gabb; Gower Ringing Group.

2.1. Abstract

Songbirds are expected to match their diets to changes in the abundance of their invertebrate prey. A deeper understanding of how diet composition and overlap in birds changes over multiple timescales and across space, in response to changing food availability, could shed light on how birds might respond to environmental change in future years. Here, DNA metabarcoding of invertebrate remains in warbler faeces was used to compare the diets of six sympatric warblers in the UK. Sample collection and invertebrate monitoring incorporated a multi-site approach over time (both within and between breeding seasons) and space (high *versus* low UK latitude). Although warbler diets generally showed greater than expected pairwise overlap, each warbler species consumed a subtly different suite of prey. Seasonal patterns in diet and dietary overlap were uncovered, with greater overlap coinciding with an overall increase in prey abundance as the summer progressed, likely as a result of rising temperatures in the middle and late summer (with respect to early summer) promoting greater prey emergence. Diet composition and richness, dietary breadth and overlap varied by site and with changes in growing degree days, suggesting a role for climate and prey abundance. Evidence was found for increased dietary partitioning with lowered food availability, as a result of elevated summer temperatures and lowered rainfall at one site in 2018, compared to 2017. Chironomids and other insects derived from aquatic habitats were a consistent part of the diet of warblers, but these groups declined in abundance in the field in the drier year and this was mirrored in their lowered frequency in the diets. The resulting implications for songbird coexistence and survival under future climate change alludes to the need for conservation approaches that promote aquatic habitats, notably wetter zones within reedbeds, and their cross-system subsidies to terrestrial insectivores. Future work on this topic would benefit from the use of long-standing datasets that comprise large sample sizes across several sites, seasons and years.

2.2. Introduction

2.2.1. Background to study

Access to food is one of the greatest sources of competition in birds, determining individual survival, reproductive success and lifetime fitness (Nour et al. 1998). The widespread declines in invertebrate prey communities currently occurring across multiple systems, have negatively impacted songbirds at the community, species and population level (Hallmann et al. 2017). The consequences of reductions in the abundance of songbird prey are multi-faceted, but one outcome that has so far received little attention is a possible increase in interspecific competition between consumers.

Landscapes in the UK are often highly fragmented, composed of a variety of habitat patches. In these heterogeneous landscapes, the presence of nearby, productive, wetland habitats may supplement neighbouring terrestrial habitats through the exchange of cross system subsidies that may comprise organisms, nutrients and detritus (Nakano and Murakami 2001, Sanzone et al. 2003, Lewis-Phillips et al. 2019, 2020). This in turn, draws in a variety of consumers from the wider landscape to wetland zones.

2.2.2. Habitat overlap in old-world warblers

Warbler communities often comprise multiple closely-related species co-occurring within a locality, many of which obtain a generalist diet of potentially similar arthropods, despite showing some ecological differences in habitat use (Catchpole 1972, Cramp and Brooks 1992, Surmacki 2005, Leisler and Shulze-Hagen 2011). They thus provide a good model system for examining dietary overlap under changing conditions.

During the breeding season, aggressive territoriality and dominance hierarchies are documented between warbler species, but most known competition between sympatric warblers is tied to access to high-quality breeding habitat (Brown and Davies 1949, Catchpole 1972, Garcia 1983, Cody 1978, Saether 1983a, Saether 1983b, Cramp and Brooks 1992). Sylviine warbler species show overlap in the foraging habitats used on the breeding grounds, which may comprise habitat edges and/or adjacent habitats away from their immediate breeding and/or nesting habitat (Catchpole 1972, Bonte et al. 2001, Surmacki 2005). For example, although *Phylloscopus* species are primarily warblers of woodlands, they might utilise adjacent reedbed habitats when foraging (Laursen 1978, Bibby and Green 1983, López-Iborra et al. 2005). Conversely, the archetypal reedbed warbler, the Eurasian reed warbler (*Acrocephalus scirpaceus*), often forages outside of the reedbed (Catchpole 1972, Thomas 1984, Simms 1985). A study by Ezaki in 1992 showed that the closely related great reed warbler (*Acrocephalus arundinaceus*) was prepared to make longer foraging trips away from its breeding grounds to exploit larger food items from nearby paddy fields.

2.2.3. Dietary overlap in warblers

Competition for resources is costly for fitness if the prey resources utilised by one species are depleted by another (Dhondt 1977,1989, Massa et al. 2004). When competitive pressure for food increases, resource partitioning between species can reduce the likelihood of competitive exclusion (Chesson 2000, Navalpotro et al. 2016, Sherry and Kent 2020). If prey are limiting, different warbler species should consume different prey taxa, or different relative proportions of prey families within shared orders (Marchetti et al. 1998).

In cases where dietary overlap is high, coexistence can still be maintained via niche partitioning on other ecological dimensions (Dyrcz and Flinks 2003, Vieria and Port 2007, Whitfield et al. 2003). For example, different species can exhibit distinct feeding strategies within the same habitat, which may result in different prey being consumed among warblers (Catchpole 1972, Bibby and Green 1983, Martin and Thibault 1996). Within the warblers of reedbeds, Cetti's warblers (*Cettia cetti*) feed in low vegetation or directly from the ground, sedge warblers (*Acrocephalus schoenobaenus*) rely on a "picking" or "gleaning" prey capture method and spend more time in the lower strata of reedbeds than reed warblers, whose active hunting, often climbing up the *Phragmites* stems or sallying from a perch, targets mobile prey (Catchpole 1972). Reed warblers also frequently take prey in the highest vegetation zone or catch it out of the air (hawking) (Bibby and Green 1983). The consumption of different prey size classes compared to other species is another way to limit the potential for interspecific competition and has been observed in several studies on sympatric passerines (Bibby and Green 1983, Poulin and Lefebvre 1996, Marchetti et al. 1998, Orłowski and Karg 2013).

If food supplies are plentiful, warblers may simply consume the most abundant or available prey (Cramp and Brooks 1992, Trevelline et al. 2018). However, food quality may be more important to a bird's fitness than availability of prey alone (van Gils et al. 2005, Piersma 2012, Oudman et al. 2014, Twining et al. 2018). Thus, warblers should also exhibit individual preferences for the most profitable prey resources available to them (Pulliam et al. 1985, Orłowski and Karg 2013, Kent and Sherry 2020). Profitability is often a balance between prey quality, abundance and the energy expended to capture a particular item (Krebs and Davies 1991, Brodmann and Reyer 1999). However, optimal foraging theory and overlap theory are not incompatible. For example, if warblers have species-specific nutritional requirements and prey preferences, they may disproportionately search for those prey, while also supplementing their diet with the most readily abundant, or easily captured prey. If the latter prey types are abundant enough in the environment, they may be shared among warblers without causing intense competition. High dietary overlap can therefore mask a degree of resource partitioning, mediated by the optimal foraging method of each warbler species (Nagelkerken et al. 2009, Dhondt 2011, Trevelline et al. 2018). In scenarios of extreme food limitation however, high dietary overlap between consumers may be an indication that consumers have widened the breadth of prey taken to capture whatever food remains, regardless of preferences and feeding strategy (Tebbich et al. 2004, Clare et al. 2014*b*). This causes diets to converge on a small suite of prey items, which could increase competitive pressure between consumers.

Without identification of prey to genus or species level, dietary overlap between bird consumers can appear very high. For example, a high ecological niche overlap of 0.93 was demonstrated between reed and sedge warblers (Henry 1979). However, more nuanced differences in diet are likely to be revealed with genus or species-level identification of prey items, which can be achieved with high-throughput sequencing of diet samples (Symondson 2002, Deagle et al. 2005, Moszczynska et al. 2009, Pompanon et al. 2012, Clare 2014).

2.2.4. Warbler diet: what is currently known?

Songbirds consume a very wide array of invertebrate groups; largely arthropods (Grundel 1990, Gilroy et al. 2009, Maziarz and Wesołowski 2010, Jedlicka et al. 2017, Trevelline et al. 2018, Shutt et al. 2020), and similarly, breeding warblers are described as generalist insectivores (Cramp and Brooks 1992). A common strategy for generalists is to focus upon particular high-value prey groups when they become abundant, to maximise profitability (Quevedo et al. 2009, Pagani-Núñez et al. 2015). For example, reed warblers consume a wide range of larger-bodied, active Diptera, notably hoverflies (Syrphidae) (Bairlein 2006, Grim 2006, Kerbiriou et al. 2011) alongside an array of smaller arthropods. Leaf warblers including the Chiffchaff (*Phylloscopus collybita*) and willow warbler (*P. trochilus*) might consume different prey items to the reedbed warblers, simply by foraging in different habitat types (Sherry and Kent 2020), however some prey can disperse across a wide range of habitats and be consumed by different species of birds with very different foraging habitats (Wilson 2010).

Some warbler species may have more specialised diets than others. King et al. (2015) studied the diets of reed, sedge and Cetti's warblers in Cardiff Bay, South Wales and ascertained that while reed and Cetti's warblers had highly variable diets, the majority of the sedge warbler diet was composed of chironomids. Chernetsov and Manukyan (2000) suggest that sedge warblers are not dietary specialists but probably focus on prey aggregations that are patchily distributed and thus are more energy efficient to consume in one bout once discovered (e.g., Ornes et al. 2013, Sherry 2016). This aligns with the foraging strategy of the sedge warbler, which is focused on gleaning sessile, abundant, small prey taxa such as small beetles, aphids and less mobile Diptera (Fry et al. 1970, Kennerly and Pearson 2010).

Seasonal dietary switching: the reed plum aphid

In north western Europe, the migratory strategy of the sedge warbler is thought to involve shifting the diet to slow-moving or inactive prey, particularly aggregations of the reed plum aphid (*Hyalopterus pruni*) and invertebrates associated with them (Chernetsov and Manukyan 2000). *H. pruni* is a common aphid species associated with the common reed (*Phragmites australis*) in Europe, but its distribution is often patchy, and abundance varies greatly spatially and temporally. The development and emergence of aphids is dependent on external temperatures, and they are often used as study species in studies investigating the effects of temperature on insect development (e.g., Asin and Pons 2001, Diaz and Fereres 2005, Latham and Mills 2011).

Foraging on sugar-rich aphids allows sedge warblers to build up fat reserves facilitating a non-stop flight to their wintering grounds (Green and Davies 1972, Bibby and Green 1981, Chernetsov and Manukyan 2000). Within Western Europe, a north-south cline occurs where the timing of peak plum aphid super-abundances mirrors the climatic gradient and resulting growth of its host plant, the common reed (Bibby and Green 1981, Bayly 2007) as mean summer temperatures increase. Bayly (2007) argued that sedge warbler fuelling behaviour has evolved to respond to the temporal and geographic distribution of aphid abundance, with early migrating birds (early August) fuelling at southern sites, whereas later migrating birds (mid-late August) fuel at northern sites. Climate change could disrupt this pattern leading to earlier peaks in aphid prey but leaving an "aphid gap" prior to autumn migration.

Chernetsov and Manukyan (2000) suggested that during migration, sedge warblers simply consume the most abundant and aggregated prey they can find. Regardless, chironomids and other Diptera remain prevalent in the diet throughout the late summer, so the dietary flexibility exhibited by sedge warblers may be advantageous in years and sites with low aphid numbers, when birds must still gain enough fat reserves to migrate (Ormerod et al. 1991, Chernetsov and Manukyan 2000). Obtaining dietary information for comparing fattening sedge warblers feeding prior to migration, to non-fattening or breeding sedge warblers would be beneficial in assessing the importance of the reed plum aphid to this warbler species at different times of the breeding season.

Temporal changes in arthropod prey availability and dietary overlap

The composition of invertebrate communities in reedbed habitats follows a continuous pattern of emergence strongly influenced by spring temperatures (Schaefer et al. 2006, Halupka et al. 2008). In the northern hemisphere, invertebrate abundance increases from late spring to July and then declines at the end of summer. Within this general broad window of prey availability, multiple peaks are present with the most abundant invertebrate groups fluctuating throughout the summer (Vafidis 2014). Generalist birds such as reed warblers are thought to match their diets opportunistically to the most abundant invertebrates present at a given time (Cramp and Brooks 1992), thus it is reasonable to expect the other warblers in this study to also follow the seasonal patterns of arthropod abundance (Catchpole et al. 1972, Chernetsov and Manukyan 2000). Competition pressure is also likely to change over the course of the summer in relation to changes in prey abundance (Wiens 1977, McMartin et al. 2009, Razgour et al. 2011) and the bird species present. Accordingly, dietary overlap may shift significantly in a short space of time (Laursen 1978).

Insectivores in more northerly latitudes may experience a narrower window of peak prey availability in mid-summer, flanked by lower availability during the cooler periods of early and late summer, compared to more southerly latitudes (Whitaker 2005). Thus, there may be a climate gradient with respect to diet and the extent of overlap and/or dietary competition within temperate locations.

Dietary changes due to climatic variation across years

In temperate wetlands, the abundance of arthropod food for insectivores is often variable in space and time (Whitaker 1994, 2004) as prey development, emergence and activity is directly correlated with summer temperature maxima (Taylor 1974, Bryant et al. 2002, Sardiña et al. 2017). Invertebrate populations in temperate regions, especially those at higher latitudes, are expected to show declines due to climate change, forcing their ranges further north (Bale et al. 2002, Walther et al. 2002, Pearce-Higgins 2009). Lowered rainfall has a detrimental effect on food availability for reedbed warblers, reducing water levels and hydroperiod, potentially leading to reduced recruitment of aquatic and semi-aquatic prey to the surrounding vegetation (Poulin et al. 2002). The diets of migrant warblers at stopover sites are likely to be consistent between years and sites, but a degree of dietary flexibility was observed in migrants at a stopover site in Sardinia between years, as a result of alterations in the distribution, availability and activity of insects, from altered weather patterns (Marchetti et al. 1996). Thus, it might be expected that annual variation in weather could have an impact on dietary overlap between warblers.

2.2.5. Chapter aims and objectives

In this chapter I demonstrate the use of dietary metabarcoding to characterise the diets of five sympatric warbler species. I hypothesise that local climate will i) directly alter prey abundance and richness and ii) indirectly alter the diet of different warbler species that cohabit wetlands. I also hypothesise that the composition of diet and observed dietary overlap in warblers will change over multiple wetland sites from the south of the UK to the northern range margin of the reed warbler in Yorkshire, over two timescales: i) within and ii) between breeding seasons (studied over two years). Dietary information was used to determine the extent of dietary overlap between pairs of bird species as well as the richness of prey consumed and dietary niche breadth of each bird species. Consumed prey was compared with the monitored prey available using sticky traps in the field, to determine species-specific preferences in prey selection. Dietary differences were determined between focal warbler species, with observed differences in diet and selection of specific prey groups assumed to reflect the use of different foraging strategies by different bird species, to best achieve optimal foraging.

In order to incorporate the additional factors influencing warbler diet and interspecific competition considered above, I will address the following predictions:

- Prediction 1: local climate will change prey abundance and richness over the summer across the UK, with Yorkshire showing lower abundance and/or richness in the early summer with respect to the southern sites. The warmer and drier summer of 2018 will lead to a decrease in prey abundance and richness with respect to 2017.

- Prediction 2: dietary overlap will decrease among the warbler species at sites and during periods of the summer/years where prey abundance/richness is increased, as a wider array of prey are available to enable partitioning of diets.

- Prediction 3: warblers will show differences in the consumption of and/or preferences for (i.e., consumed more than expected from the abundance of prey) different prey items according to i) species-specific optimal foraging, and as a result of ii) spatial (site) and iii) seasonal and/or yearly variation in prey availability and climate. Dietary richness, diversity and niche breadth will vary dependent on focal warbler species, sites, seasons and years.

- Prediction 4: characteristics such as body length (i.e., relative size) and habitat associations (i.e., aquatic or terrestrial), will differ in the prey consumed by different warbler species, which will promote dietary partitioning. Patterns of selectivity for these prey characteristics by warblers will be explained by optimal foraging.

2.3. Methods

2.3.1. Site selection

Four study sites were selected, representing differing UK climates across the north and south of the UK ranges of the warbler species. Two wetland sites were chosen in South Wales; Oxwich Marsh a SSSI site in the Gower (Grid ref = SS501872) and Kenfig National Nature Reserve in Bridgend (SS792812), and a further site in Somerset (Chew Valley Lake: ST558596) to represent southerly UK climates. Magor Marsh (ST428866), a SSSI on the Gwent Levels, was selected as a supplementary site (Fig. 2.1). These sites all fall along a latitudinal band of ~51°. To enable a latitudinal comparison, a wetland site in North Yorkshire; Wheldrake Ings Nature Reserve (SE674438), was selected to represent a northern English climate at ~54° (Fig. 2.1). At this latitude reed warblers are approaching their northern limit but are still present in high enough numbers to enable comparison with the southern sites.

The reedbed specialists; reed warbler, sedge warbler and Cetti's warbler were chosen as the target species for diet sampling. At the majority of the sites visited, the reed warbler is the most abundant of the three species, followed by the sedge warbler. The Cetti's warbler has not been recorded at Wheldrake Ings and is largely absent from northern England, so sample collection for this species was only possible in the southern sites. Two leaf warblers, the chiffchaff, and the willow warbler were frequently captured in close vicinity or adjacent to the reedbeds and were sampled in small numbers (n=27-29).

2.3.2. Site characteristics

The four study sites comprised reedbed habitat dominated by *Phragmites australis* and in Wheldrake also by reed-sweet grass (*Glyceria maxima*). In Chew Valley, extensive reedbeds fringed a large reservoir-lake, and in Kenfig and at Wheldrake a series of pools. At Oxwich the reedbeds were more typical of marshland and fen, divided by ditches and streams. At all sites, the reedbed zones were surrounded by other habitats including carr woodland, scrub, field vegetation and grassland/pasture which was grazed by cattle and/or horses in the summer, with the exception of Kenfig which featured coastal dune habitat. The tree flora was dominated by willows (*Salix*) but also oaks (*Quercus*), birches (*Betula*) and at Kenfig and Oxwich, poplar (*Populus*). Scrub comprised bramble (*Rubus*), hawthorn (*Crataegus*) and dogwood (*Cornus*) as well as field vegetation largely in the form of stands of willow herbs (*Epilobium*) and nettles (*Urtica*).

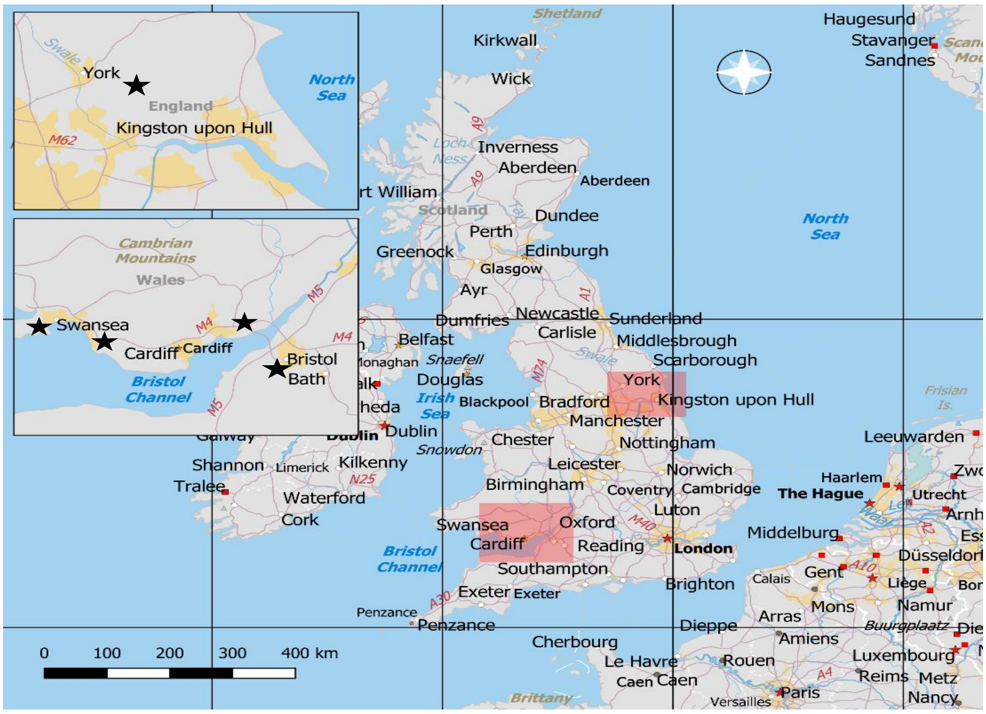


Figure 2.1. Map of the UK with the two regions selected for study highlighted in red. Highlighted areas are expanded on the panel on the left of the map to show the location of each of the four study sites.

2.3.3. Faecal sample collection

Bird ringing groups were fully licensed under the British Trust for Ornithology (BTO) with the appropriate endorsements and permissions for using mist nets and for the capture, handling and ringing of birds. Ringing sessions took place approximately once a week between May and September 2017 at Kenfig, Chew Valley and Oxwich. Sessions were weekly at Wheldrake Ings in early-mid June, increasing to 3-4 times a week between late June and August. Sites were visited on average 12 times over the summer, ranging from 8-21 visits at the main sites in 2017 and Oxwich in 2018. In addition, there was one visit to Magor in 2017 and two visits to Wheldrake in 2018. At each site, mist nets were set through a section of reedbed and surrounding scrub shortly after dawn and taken down at around midday. Nets were checked every 20 minutes for birds, and to maximise the welfare of the birds, ringing was suspended in cases of sudden wet or windy weather.

As the number of visits per site and mist net usage could not be entirely standardised, samples were collected as evenly as possible across the sites and over the summer with an average of 11 samples per session (Chew = 16 (range 5-29), Kenfig = 15.1 (range = 6-30), Magor Marsh = 11, Oxwich 2017 = 7.3 (range = 1-25), Oxwich 2018 = 7.5 (range = 2-13), Wheldrake = 9.8 (range = 2-34), and a maximum of 15 x 40-60m mist nets were used per site at each session (range = 7-13). Sites that were visited more frequently (i.e., Oxwich) were sampled less intensively (as warbler captures were lower in general) and fewer samples per session were collected compared to sites visited on fewer occasions.

Captured birds were placed into a breathable, cotton bag for 10-15 minutes or until the bird defecated. Birds were then ringed and processed before release. During processing, age, sex, moult status, fat score (0-5), presence of breeding characteristics (brood patch score in females, presence of cloacal protuberance in males) were recorded and wing length and body mass measurements were taken. Birds were assigned a fat score using the methodology outlined by the BTO, based on the amount of visible fat viewed in the tracheal pit, abdomen and breast muscles (0 = no visible fat, 1 = trace of fat, 2 = base of tracheal pit obscured by fat to about one third full, 3 = tracheal pit about two thirds full, 4 = tracheal pit completely filled up to end of clavicle but still concave, 5 = convex bulge, perhaps overlapping breast muscles). Birds were aged as juvenile or adult based on plumage (see Chapter 3). Only adult birds could be sexed, provided they showed evidence of breeding with either a brood patch or a cloacal protuberance.

Faecal samples were collected directly from the holding bags using clean, sterile forceps and were placed in a 2ml Eppendorf collection tube containing 100% ethanol for preservation, in a ratio of

3:1 (ethanol to sample). Samples were initially stored in a domestic freezer before transfer to a -80°C laboratory freezer as soon as possible upon return to Cardiff University.

Sample collection took place throughout the breeding and early autumn migration phase of the warbler breeding season between late April and late August 2017 at all study sites, and between May and late August 2018 in Oxwich Marsh. Several reed and sedge warbler samples were also collected in August 2018 at Wheldrake. Several birds were sampled opportunistically at a supplementary site, Magor Marsh in southeast Wales, but these birds are only included in the overall presentation of warbler diet and were not included in site comparison analyses.

2.3.4. Invertebrate monitoring

Double-sided (dry-stick) 10 x 25 cm yellow invertebrate traps, henceforth “sticky traps”, were used to monitor invertebrate abundance at each study site (Oecos UK; http://www.oecos.co.uk/dry%20stick.htm). These traps are effective at monitoring the activity-density of flying insects, especially Diptera (Black and Krafsur 1985, Hogsette et al. 1993, Goulson et al. 2005) that represent a high proportion of known reed-bed warbler diet (Davies and Green 1976; Grim and Honza 1996; Grim 2006; King et al. 2015). Traps were enclosed in a plastic wire gardening mesh with holes 1x1cm to prevent consumption and/or damage of invertebrate specimens by foraging birds and to prevent small birds and mammals from injury.

Each sticky trap site was given a unique waypoint and logged with a handheld GPS (Garmin Etrex) so that the same trap locations could be used consecutively for the three sampling rounds. The time frame of trap exposure for these three periods (across sites) was approximately, late May/early June, late June/early July, and early August 2017, and the same periods in 2018 at Oxwich. Where possible, traps were set up at all three southerly sites within 10 days of each other, with traps set at the northern site no more than 14 days later than at the first southerly site visited. 10 sticky traps per sampling round were set in the scrub vegetation and 10 in the reedbeds at each site.

Reed traps were attached directly onto reeds at varying heights between 0.5 and 1.8m (breakdown of trap heights per sampling round = 0.5m (n =9), 1m (n =15), 1.2m (n=6), 1.5m (n=8), 1.7m (n=2)) to cover the range of warbler feeding heights used between species. For the scrub locations, traps were attached to inner branches of the trees between 1m and 2m (1m (n=5), 1.2m (n =4), 1.5m (n=13), 1.8m (n=8), 2m (n=10). Traps were left for 7 days in the absence of rain or up to 10 days if significant rainfall (more than three consecutive days of rainfall >1mm) occurred to allow for weather related biases. Upon collection, sticky traps were labelled and organized for identification in the laboratory.

Water depth (if water was present) was also estimated at each sticky trap site with the use of a one metre ruler (in cm) and the dominant vegetation type was recorded. For reed traps the dominant vegetation was almost always *Phragmites*, except for a few trap plots at Wheldrake where *Glyceria* dominated. For scrub plots the dominant tree or shrub species in the immediate vicinity was recorded (usually willow, but often oak, ash, birch or hawthorn). A measure of habitat heterogeneity was recorded by estimating the percentage of the vegetation surrounding the trap ($1m^2$) that was made up of vegetation other than the dominant vegetation type (e.g., nettles, willowherb, bramble, flowering plants).

Invertebrates from 25% of each full-size trap (on both sides), were identified to family level (where feasible) from the sticky traps with the aid of a microscope. The total body length of each invertebrate specimen was also measured to give an indication of size. A number of invertebrate groups were identified to order, due to difficulty with accurate identification (e.g., Opiliones, Psocoptera, Thysanoptera, some Lepidoptera) and several families of Diptera were merged together, or identified to superfamily level, where differences between the families could not be discerned (see Appendix 1.8 for a list of affected families). Due to time restraints and the sheer volume of arthropods captured, the contents of only half of the traps were identified, 10 per site (5 x reed and 5 x scrub) per sampling period (120 traps in total). Since most traps were comprised of similar invertebrates, most families present on all the sticky traps were covered with this approach.

Reference invertebrates were collected throughout the study period for use in primer testing and in mock community mixes (see 2.3.6.). A variety of locations accessible to birds were visited, and single invertebrates were captured opportunistically using a hand-held electrical aspirator to avoid cross-contamination. Specimens were stored individually in sterile 1.5ml Eppendorf tubes containing 100% ethanol and stored at -80°C ready for DNA extraction.

2.3.5. Weather data

Weather data for 2017 (and 2018 for Oxwich (Pennard)) was recorded every day from 1st Jan 2017 from local weather station data in close vicinity to each field site (http://www.wunderground.com). Weather stations were selected within 10km of the study site (Chew Valley = Bishop Sutton 2km, Kenfig = Pyle 1.5km , Oxwich = Pennard 7km, Wheldrake Ings = Sutton Upon Derwent 7km). Historical maximum temperature, minimum temperature and rainfall data are available for each day of the year, allowing daily growing degree days to be calculated continuously throughout the year.

Growing degree days (GDD) for two species were calculated; the common reed (*Phragmites australis*), the dominant vegetation in UK reedbeds, and the reed plum aphid (*H.pruni*) an aphid which is dependent on the common reed for part of its life cycle. *H. pruni* is also a significant prey species for sedge warblers (Chernetsov and Manukyan 2000, Bayly et al. 2007) and has been recorded in the diet of the other warblers in the study as well (Bibby and Green 1983). Since this aphid requires external temperatures to be above a given threshold to initiate development and emergence, the accumulation of GDD for *H.pruni* at given temperatures can be used as a proxy of the likely emergence times of other arthropod prey consumed by warblers. Monitoring GDD over the summer may indicate periods of enhanced prey availability at given study sites (Vafidis 2014).

Where the minimum and maximum daily temperature is known, GDD for a given day can be calculated by the following equation:

$$GDD = \frac{Min\,T + Max\,T}{2} - T^{base}$$

where Min T and Max T represent the daily minimum and maximum temperature and T^{base} represents the baseline or minimum temperature required for development of a given species (Nugent 2005). For *P. australis*, $T^{base} = 4$, for *H. pruni*, $T^{base} = 8.65$ (taken from Latham and Mills 2011). Since T^{base} for perennial grasses ranges greatly (from 4-10°C) and no temperature threshold has been identified for reed (Bonhomme 2000, Haslam 2010) a low estimate was used to reduce error. Overestimating T^{base} can have a large effect on developmental date estimations whereas underestimating this value has a more limited effect (Bonhomme 2000, Lovat 2013).

2.3.6. Molecular analysis

The following methodology also applies to the molecular methods referred to in later chapters. I describe the methodology for all faecal samples collected over the entire course of the doctorate but refer to the relevant chapters where applicable.

Extraction

DNA extractions from the faecal samples were performed in batches of 16-23 in extraction rounds using the QIAamp® DNA Stool Mini Kit from Qiagen (Manchester, UK). The kit protocol with modifications from Zeale et al. 2011 were followed, and additional customisation steps specific to avian faeces were included (Nicholls 2017 pers. comm, described in Shutt et al. 2020, see Appendix 1.2 for details). The modifications adjusted for the smaller size of warbler faeces (<200mg) and higher levels of uric acid in avian faeces which can inhibit PCR. The main changes from the original protocol were i) the prior removal of uric acid from the sample using tweezers (sterilised between samples), ii) manual homogenisation of the faecal material with a pestle after adding the first 500µL of InhibitEx Buffer, followed by vortexing after adding the second 500µL, iii) use of a greater volume of sample supernatant, buffer ATL, and ethanol (i.e., 400µL) and proteinase K (20µL), iv) longer incubation times (Appendix 1.2, steps ii and iv) and, v) a smaller volume of buffer AE when eluting samples (50-80µL). For most extracted samples, 80µL of eluate was produced but for very small samples 50-60µL was eluted, to concentrate the DNA and increase the likelihood of amplification success in the polymerase chain reaction (PCR) step. This method was found to improve DNA yields when compared to the standard protocol, evidenced by improved PCR amplification and higher DNA concentration in preliminary tests when the two methods were compared.

Extraction of DNA from the tissue of reference invertebrates were carried out using the DNeasy® Blood & Tissue Kit (Qiagen), following the recommended steps.

During extraction, several steps were taken to reduce the risk of contamination from outside sources. All faecal extractions were carried out under an air flow hood that was cleaned thoroughly between uses with bleach and ethanol. DNA extractions from invertebrate tissue were carried out in a separate location to PCR set up areas to prevent cross contamination. Eppendorf tubes and pestles were autoclaved before use, and these and all pipettes, racks and other tools used in the extraction were cleaned with bleach and placed under ultraviolet light for 20 minutes prior to extraction. Tools for removing uric acid, cutting and transferring samples, were sterilised between samples by washing in bleach, water and ethanol and flaming with a Bunsen burner torch. A new, clean pestle was used for breaking up each sample. Each extraction round contained two extraction negatives to test for the presence of contamination between samples.

PCR

Presence of target prey DNA in the extracts was confirmed using PCR, using a Multiplex PCR Kit (Qiagen). The set-up included a hot start at 95°C for 15 minutes and proceeded with 35 cycles of 94°C for 30 seconds, an annealing temperature of 55°C for 90 seconds, extension at 72°C for 90 seconds followed by 72°C for 10 minutes. After a period of primer testing, the COI primers mlCOlintf, **5'**ACGCTCGACAGGWACWGGWTGAACWGTWTAYCCYCC **3'**(Leray et al. 2013) and Nancy, **5'**ACTAGCAGTACCCGGTAAAATTAAAATATAAACTTC**3'** (Simon et al. 1994) were selected to isolate invertebrate DNA while avoiding the amplification of the host DNA. This primer pair was first used in combination by Stockdale (2018), and they amplify a ~400bp region of DNA. These primers were tested on a range of invertebrate samples of 18 different orders and 50 families, and it was confirmed that they amplified the majority of tested taxa (Appendix 1.1). The chosen primer set is versatile, reliable, and amplifies a wide range of potential prey taxa.

In most PCR reactions, 1ul of DNA was added to each 5µL reaction volume. The remainder of the reaction (4µL) comprised 2.5µl Multiplex PCR Master Mix buffer (containing 3 mM $MgCl_2$, dNTP mix and HotStarTaq DNA Polymerase; Qiagen), 1.3µL nuclease free water, 0.1µL forward primer (concentration = 10µM), 0.1µL reverse primer (10µM) and 0.05µL bovine serum albumin (BSA; New England Biolabs, UK; concentration = 0.05 µg/ml). However, amplification of some individual extracts (i.e., those with lower yields of template DNA) was improved with the addition of 2µL template DNA. In these cases, the reaction volume was kept at 5µL by reducing the volume of nuclease free water to 0.3µL. Extraction negatives (for the same extraction round as the samples to be tested), PCR negatives (nuclease free water) and a positive control (known invertebrate DNA) were included in each reaction.

Results were viewed under ultraviolet (UV) light via a transilluminator, on a 2% agarose gel, with the presence of a band indicating successful amplification. Approximately 70-80% of samples produced a visible band on the agarose gel with a subset of these samples producing a faint band. Many extracts that failed outright were from faecal samples that were very small in size, and the amount of DNA may have been too diluted or too degraded to be detected. Some may have contained PCR inhibitors, however diluting the volume sample in the PCR reaction to 0.5µL did not improve amplification success. Samples that failed to produce a band the first time were re-tested with the same PCR conditions to minimise sample loss and prevent false negative results. Samples that did not produce a band after two PCR tests, even when increasing the amount of DNA in the reaction volume from 1µL to 2µL, were omitted from the next steps.

MID-tag PCR and pooling

All DNA samples that amplified successfully in the initial PCR step were screened again in PCR with MID-tag (Multiplex Identifiers in the form of unique DNA tags) labelled primers. The MID-tags comprised 10 unique base pairs added to the forward and reverse primers using a custom HTS oligos design process (Eurofins Genomics). 24 unique forwards and 15 unique reverses (Appendix 1.3) were used in combination so that each sample could be differentiated after pooling (Brown et al. 2013).

Samples were grouped into PCR plates depending on their initial amplification success (e.g., strong, medium, and faint bands). Ten full (96 well) plates and two half (~48 well) plates were tested each with a column of extraction negatives, at least one PCR negative and 1-2 positive controls (DNA extracts from the tissue of a shrimp (family: Penaeidae) and a mussel species (family: Mytilidae). A mock community (consisting of a mix of DNA from known invertebrates added at different concentrations) was included on eight of the plates and warbler DNA extracted from moulted feathers (to test for host DNA amplification) was included on six plates (Appendix

1.4). In addition, four warbler faecal samples were included more than once as repeats (Appendix 1.5). In each PCR reaction well in the 96 well plate, 2.5µL of forward and reverse MID-tag primer (2µM), 12.5µL Qiagen Multiplex PCR Master Mix, 2.25µL nuclease free water and 0.25µL BSA were combined in a DNA free-zone under an air flow hood. 5µL of DNA from each sample was added to each well to give a total reaction volume of 25µL. For plates with many samples that had initially produced only a faint band, the composition of the reaction volume was adjusted to allow for 7µL of DNA, by reducing the volume of nuclease free water to 0.25µL. The PCR reaction took place for approximately 3 hours on a SimpliAmp thermocycler using the same PCR conditions as above.

As with DNA extractions, all PCRs were carried out under conditions to reduce contamination risk. Work surfaces were thoroughly cleaned, and equipment/materials to be used in the setup were autoclaved and/or cleaned with bleach and placed under ultraviolet light for 20 minutes prior to PCR set up. Separate pipettes were used for adding reagents in PCR setup and for adding DNA, and all non-DNA reagents were removed from the area before DNA aliquots were added. Setup for MID-tag PCR took place under an air flow hood to further reduce the risk of contamination. All plates contained extraction negatives (spread across the plate), and a column of PCR negatives (nuclease free water) to test for the presence of contamination in each row.

A QIAxcel machine (Qiagen) was used to visualise the results of each MID-tag PCR 96 well plate and measure the peak sizes and concentrations (ng/µL) of DNA present in each sample. Plates free of contamination (by checking for DNA in negative wells) were taken forward to pooling. Any rows containing a PCR negative sample that tested positive for DNA were not taken forward and reactions were repeated to ensure that samples were free from contamination. Samples were pooled per plate, and pools were equalised to roughly equimolar concentrations. To retain samples with lower DNA concentration, the DNA samples with the highest concentration (i.e., above 45 ng/µL) were diluted with nuclease free water before pooling. The highest DNA concentration of all the samples per plate (after diluting) was chosen as the base and the correct volume of each of the remaining samples to add to the pool was calculated by dividing the sample DNA concentration by the base sample concentration. This method of pooling is thought to be more accurate than pooling by band strength on an agarose gel.

To avoid over-diluting the pool, samples below ~2ng/µL were excluded from pooling. All other samples were included in the pool provided that their calculated volume to add to the pool did not exceed the threshold of the entire reaction volume (approx. 21µL) once the samples with the highest DNA concentrations had been diluted. PCR and extraction negative controls formed approximately 10% of the samples in each indexing pool, including an extraction negative from every extraction round. The volume of negative control to add to each pool was determined by calculating the mean of all the volumes for the DNA samples to produce an average concentration. This ranged from 4-9µL for each plate.

PCR Clean-Up and DNA size selection

QIAxel electropherograms were checked for primer dimer and unwanted PCR product. PCR clean-up and size selection protocol were carried out to remove products less than 350bp in length using SPRI beads (AMpure) in a ratio of 0.9. The concentration of each of the pools was then measured using a DS DNA qubit (Qiagen) following the high sensitivity protocol. Each PCR plate pool was pooled to create one equimolar indexing pool. Samples from the UK birds formed two indexing pools, the first comprised the reed warbler samples from all UK sites (including nestling samples studied in Chapter 3) and the second comprised the remaining warbler species collected from sites in the UK. A third indexing pool contained faecal samples collected from the warblers in Catalonia (Chapter 4).

Library Prep

The three indexing pools were each normalised to 100ng of DNA by combining with nuclease free water. Sequence libraries were carried out for Illumina MiSeq using the NEXTflex™ Rapid DNA-Seq Kit (1ng – 1µg) (Bioo Scientific Corp 2015) V15.10, compatible with the Illumina sequencing platform. A three-step protocol was followed for preparing the DNA for sequencing; DNA end-repair/adenylation, adapter ligation and PCR amplification. During the adapter ligation step a unique barcode adapter (index) of six base pairs was added to each of the pools to allow greater indexing power. AMPure XP bead clean-up steps throughout the protocol were designed to eliminate unwanted low molecular weight material and purify the pools. Following the final clean, the three indexing pools were combined according to their concentrations to form a final pool, and then diluted to ~ 4ng/µL using nuclease free water. The final pooled and indexed product was added to the flow cell of the sequencing cartridge of an Illumina MiSeq machine and sequencing of the three indexes proceeded until completion.

2.3.7. Bioinformatics and data processing

The following bioinformatics and data processing steps were kept consistent across the three indexing pools described above (which produced three separate sequence data files). Bioinformatic analysis was carried out separately for each of the three sequence data files that resulted from the three indexing pools.

The bioinformatics pipeline was performed using LINUX, comprising several programs and scripts (Appendix 1.6). MID-tag primers were tested for truncation by calculating the percentage of reads with less than 10bp of the MID-tag sequence for the forward and reverse primer. In all cases these did not exceed 15% of the reads. Paired-end Illumina reads were trimmed, aligned and quality checked using FastP v.0.20.0 (Chen et al. 2018). A minimum read length of 200bp and a minimum base quality threshold score of 33 was specified. After filtering, the total number of merged reads for each indexing pool respectively was 1) 9.4 million reads, 2) 5.2 million reads and 3) 2.6 million reads. Mothur (Schloss et al. 2009) was used to assign reads to their respective sample ids, according to each MID-tag sequence combination. Sequences were checked in both directions and a minimum of 1 mismatch was allowed. A demultiplexing step sorted the resulting reads into one file per sample id. Each read was given a header of the respective sample ID before being concatenated into a single file. The Unoise3 command (Edgar and Flyvbjerg 2015, Edgar 2016) in Usearch v.11 (Edgar 2010) removed chimeras and noise and clustered the reads to generate two sets of biological sequences: operational taxonomic units (OTUs) by clustering the reads at 97% sequence similarity and denoised zero-radius OTUs (zOTUs) generated using a clustering threshold of 100%. Read abundance matrices were created for both OTUs and zOTUs (sample ID against numbered unique sequence) but subsequent analyses were carried out and reported using only the zOTUs as these are based on a more conservative clustering algorithm, aiming to identify all correct biological species, reducing identification error and preventing the lumping of several species into one taxonomic unit (Edgar and Flyvbjerg 2015, Edgar 2016).

The Blastn algorithm (Altschul et al. 1990) was used in Blast+ (Camacho et al. 2009) for taxonomic assignment, comparing all sequences to NCBI GenBank. A minimum percentage identity score of 97% was specified for a species level match. The top hit for each zOTU based on BIT score (a combination for percentage identity and e-value) and MEGAN6 (Huson et al. 2016) was used to identify unique dietary items at the species level. Where the requirements for species level identification could not be met, zOTUs were classified to a higher taxonomic level. zOTUs that were not assigned to a rank above order level and/or did not correspond to a blast output sequence by this stage were assumed to be low quality, erroneous, or less than 100bp in length and were discarded.

A combination of scripts and manual processing methods were used to [i] extract the maximum read values found in PCR and DNA extraction negatives and the MID-tag combinations that were not utilised (termed NAs) [ii] remove zOTUs from samples where the read number was lower than the maximum value found in the negatives and NAs; and [iii] collapse and aggregate the matrix so that all prey species detections for each zOTU are represented by a single taxonomic entry. Since multiple zOTUs were often found to corresponded to the same species, it was surmised that aggregating by taxon identification to remove distinctions due to haplotypic variation and cryptic species, was the most practical and ecologically meaningful way to describe the diet.

The resulting dataset was cleaned further by identifying and removing any artefacts and contaminants in samples that originated from positive control and mock community samples. Prey items that occurred in both a diet sample and a positive control/mock community sample, were removed from the sample if its read count (in the sample) was lower than that of the highest count for the same prey item in the control samples or mock community samples. In addition, species that did not occur in the study area and species suspected to be low-level contamination from known invertebrates, fungi and pathogens from the laboratory were also removed (see Appendix 1.7 for full list of species removed). Finally, prey items were removed from a sample if they comprised fewer than 10 reads. This process should have greatly reduced any errors caused by tag-jumping and contamination, producing a conservative dataset from which to commence statistical analyses.

To avoid pseudoreplication, repeated samples were removed from the warbler dataset after checking for successful recovery of prey items. Read abundance data for every prey item in the diet matrix were then converted to presence-absence values. For each warbler species, the frequency of occurrence (FOO) of prey items grouped at the species, family and order level was calculated by counting the number of incidences that a prey item was detected across samples. This was expressed as a percentage (% FOO) by dividing FOO by the number of samples and multiplying by 100. Data were organised by site, year and season (based on the date the bird was captured); which was defined as “early”, “middle” or “late”. Early season was defined as May-early June, mid-season mid-June to mid-July and late season mid-July to August. These three periods broadly corresponded with the timescale of the three invertebrate sampling periods.

2.3.8. Statistical analysis

All statistical analyses were performed in R version 3.5.1. and RStudio version 1.1.463 (RStudio Team 2016, R Core Team 2018). Figures and plots were created using the package *ggplot2* (Wickham 2016). Models were fitted to datasets with an appropriate error family and link function. For each fitted GLM, model fit, and validation was determined by checking residuals plots for normality, residual variances for homoscadiscity, collinearity of variables (using VIF scores) and bias from unduly influential observations where appropriate. Stepwise model refinement was carried out based on AIC using the “drop1” and “step” function (unless otherwise stated). Where applicable, P-values are given with a test statistic from the drop1 results (F for continuous dependent variables, LRT (likelihood ratio test) for non-continuous dependent variables e.g., integer, proportional data). Pairwise differences between factors were measured with Tukey post-hoc tests using the package *emmeans* (Lenth 2020). These reduce the likelihood of type 1 errors in multiple comparisons by adjusting the p-value thresholds. Statistical methodologies are described below for each of the four study predictions.

Prediction 1: local climate will alter the abundance and richness of invertebrate prey families

The invertebrate abundance and richness of invertebrates on sticky traps were compared using two Gamma GLMs with a log-link function. The dependent variables for each were i) the total relative abundance of invertebrates (pooled across all families) and ii) the total number of invertebrate families present (in both cases these values were calculated for each sticky trap). In both models the independent variables were site, season and a three-way interaction between site, season and year, to identify any significant pairwise differences i) between sites, ii) between sites during each of the three sampling periods and iii) among the three periods, including comparisons between Oxwich in 2018 and Oxwich and the remaining sites in 2017.

A third Gamma GLM with an identity link function was used to assess the effect of site, trap habitat (reedbed or scrub), season and estimated water depth on the measured body length of chironomids recorded on sticky traps in 2017. Chironomids were an appropriate choice for this test, since they are an abundant, species-rich family and a known component of warbler diet, with larger species/individuals more prevalent in the diet, and thus selected more than smaller ones (Laursen 1978, López-Iborra et al. 2005).

Prediction 2: dietary overlap between warblers will decrease at sites and/or periods of time when prey abundance increases

Dietary overlap was measured between each pair of warbler species i) for sites and seasons combined, ii) for each season (combined years) and iii) for each site (all sites in 2017 and Oxwich and Wheldrake in 2018) using the R package *EcoSimR* (Gotelli and Ellison 2013). Our input data consisted of the frequency of occurrence of each prey species calculated for each warbler species in the pair. For some combinations of sites and/or seasons, insufficient sample sizes of some warbler species meant they were omitted from certain pairwise tests. Randomization algorithm 3 (RA3) was repeated for 10,000 simulations to generate both i) an expected measure of overlap; Pianka's Index (Pianka 1973) based on the null model and ii) a realised, observed value of Pianka's index based on the input diet data. The observed and expected Pianka values were then compared statistically to determine if overlap was significantly higher or lower than expected. The expected value for overlap usually varies between test scenarios, depending on the input data, but the test is consistent in measuring the difference between the expected and observed value. Dietary overlap between two species is considered high when Pianka's index is above 0.6, moderate between 0.4-0.6 and low when less than 0.4 (Novakowski et al. 2008).

Since much overlap may be due to the presence of common, ubiquitous invertebrates such as *Chaoborus flavicans*, multiple widespread Chironomid species and *Clubiona phragmitis*, a sensitivity analysis was run where overlap was calculated after the removal of the most commonly occurring prey species. This was used only to aid understanding of how overlap functions in the study system, and to determine how much sway these common prey items have in masking subtle resource partitioning. The results displayed are from the full suite of prey species.

Prediction 3: testing for differences in the consumption of prey items among warbler species, across sites and over seasons

The package *mvabund* (Wang et al. 2012) was chosen for assessing the influence of multiple variables on UK warbler diet composition. Invertebrate prey items were grouped at the family level for each individual bird captured. Data from retrapped individuals (comprising 2.3% of total samples) were removed to avoid pseudoreplication. Next, the presence-absence diet matrix was used to create a *manyglm* model which fits an individual general linear model (GLM) to each response variable (in our case, each unique invertebrate family in the diet matrix) with a set of predictor variables; warbler species, age, year, site and the cumulative number of growing degree days for *H. pruni* on the day of capture (to test for the seasonally correlated variables of time and climate). Two-way interaction terms were also included for year and site, and species and age. The model family chosen was binomial with a "clog log" link function, to control for large numbers of zeroes in the dataset. The *anova* function with the Monte Carlo resampling option (resamp = "montecarlo") was applied to test for overall differences in diet composition among the predictor variables, which is the recommended function for hypothesis testing with presence-absence data (Wang et al. 2012). Rather than using stepwise deletion to refine the model, all explanatory variables that were relevant to the hypotheses were retained regardless of significance, and each variable was visualised using NMDS ordination with the *vegan* package in R (Oksanen 2018). The Jaccard distance methodology was applied to plot the sample points in space according to each variable. Stress was high (>0.2), but we did not add a third axis as this would have reduced ease of interpretability.

The dietary richness of each faecal sample in our study was calculated by counting the total number of unique prey items (at species level or at the lowest possible taxonomic rank achieved). To compare dietary diversity and niche breadth across warbler species, site and season; Shannon's Index, Simpson's Index were calculated using *vegan* and Levin's index of niche breadth using *spaa* (Zhang 2016). To estimate the true richness of invertebrates consumed by warblers the *specpool* function in *vegan* corrected for small sample size and calculated Chao's estimator of extrapolated richness (Chao 1987, Palmer 1990, Oksanen 2018). Observed species richness/Chao extrapolated estimate gave the proportion of the total dietary diversity explained by sampling.

The *econullnetr* package (Vaughan et al. 2018) was used for our analysis of dietary selectivity and in determining the strength of dietary interactions. The function *generate_null_net* generated a prey choice simulation from the sticky trap invertebrate abundance data and the molecular diet data. Presence-absence per individual sample for each possible prey family was compared to abundance of that family on the sticky traps and data were organized according to warbler species and visit (corresponding to each site during early, middle and late breeding season captures of birds and invertebrate sampling periods). In some instances, an invertebrate family was detected in the diet of one or more warbler individual but was not recorded on the sticky traps. To allow inclusion of these families in the model the value 0.5 (i.e., half of one individual) was applied as a small constant in the abundance dataset. The simulation was run three times with the consumer data split by i) warbler species, ii) species subset by season and iii) species subset by site. When running the species model, only data from 2017 were included. When species were subset by season, the 2018 data were included with the 2017 data. When subsetted by site the 2018 data for Oxwich were organised separately to the data for 2017, allowing differences to be compared among years. Interactions were assigned as i) stronger than expected from the relative abundance of the prey family (i.e., consumed more frequently than expected), ii) weaker than expected from the relative abundance of the prey family (i.e., consumed less frequently than expected) or iii) consistent with the null model. Stronger than expected interactions can thus be interpreted as preferences and weaker than expected interactions as avoidance. Interactions that are consistent with the null model suggest that the consumer selects the prey item in accordance with its availability. The large number of statistical tests carried out with repeated *econullnetr* analyses run the risk of generating Type 1 errors, and up to 5% of significant interactions can be expected to occur by chance alone (Vaughan et al. 2018). To rectify this problem, preferences and avoidances were scored by standardised effect sizes, rather than focusing on individual *p*-values.

The effect of prey body length and total abundance (for a given prey family) on the dietary preferences of birds were analysed using a Gaussian GLM with an identity link function. The log-transformed standard-effect size from the 2017 *econullnetr* simulation output was used as the response variable (converted to positive values before performing the log-transformation). Standard effect size can be viewed as a proxy for preference with increasing positive SES indicating a stronger preference. An interaction term between consumer (warbler species) and average prey size (for each prey family) was set to determine if prey-size selection occurred in each warbler species. Only prey families which were recorded on sticky traps (i.e., without the 0.5 constant) and were present in the diet of the warblers were included in this model. Residuals *versus* leverage plots indicated one outlier which had strong influence. Removing this outlier did not change the model results or significance of variables but did improve model fit, and so the results of the model with this outlier omitted are reported.

Prediction 4: Characteristics such as body size and habitat associations of prey consumed by warblers will differ depending on the focal consumer and over space and time

Online sources, specialist websites and invertebrate keys were used to find estimates for the body length (in millimetres) of each prey species detected in our study (resources by order: Araneae https://araneae.nmbe.ch/, Lepidoptera: ukmoths.org.uk/, Diptera: http://diptera.info, Coleoptera: www.coleoptera.org.uk, Hemiptera: www.britishbugs.org.uk, others: www.bugguide.net). The average body length from all other species or genera in the family was used when species-specific information could not be obtained. This was commonly used for lesser studied chironomid species and parasitic Hymenoptera not of the more well studied Ichneumonoidea.

As it was not possible to infer the life stage of each prey item consumed, the body lengths for adult stages (rather than nymphs or different instars of larvae) were used for consistency. Since caterpillars often feature in songbird diet, where possible checks were made to ensure that the body length of mid-late instar larvae did not differ greatly from the body length of imagoes. Generally, the body lengths of imagoes and caterpillars were similar enough to choose the adult body length as an estimate of an overall size for a given species, although some age-related size variation is still likely. At times, the latest instar caterpillars were slightly larger than adult imagoes, but the resulting estimates of body length should be sufficient to cover either an adult or a middle/late-stage larva. For each warbler sample an average was taken from the body lengths of all the prey items detected. In addition, invertebrates in the diet were broadly assigned as terrestrial or semi-aquatic/aquatic according to their life cycles. From this information the proportion of prey items that were classed as aquatic/semi-aquatic and terrestrial for each sample were calculated.

Three GLMs were fitted to test for the effects of warbler species, site, year, season/Julian day, climate (e.g., maximum temperature, rainfall, cumulative GDD), age and where necessary, interactions of these effects, on i) sample species richness, ii) average body length of prey detected and iii) the proportion of aquatic prey *versus* terrestrial prey in each sample. For the prey size GLM, a Gamma family and an identity link function was chosen and to compare the proportion of aquatic prey consumed (per sample) a binomial GLM (theta = 1.47) was chosen, weighted by the total number of species per sample (species richness) with a logit link function. The same set of predictors was tested against sample species richness using a Poisson family GLM but overdispersion was above the recommended threshold for this error family (theta = 3.7). The standard errors were therefore corrected by using a quasi-Poisson model with an identity link function, where the variance was theta*mu where mu was the mean of the dependent variable distribution and theta was the dispersion parameter of the quasi-model.

It was not possible to include data from Magor in the dietary overlap tests, *econullnetr*, and GLMs comparing climate data and invertebrate sticky trap data, since climate and invertebrate data were not recorded there. Magor data were also omitted from the dietary diversity index estimations since <5 samples were obtained. However, data from Magor were incorporated into the *manyglm*, NMDS, frequency of occurrence tables and the GLMs describing species richness (per sample) and prey characteristics.

2.4. Results

A total of 501 samples were collected from the focal warbler species across all sites. After extraction, MID-tag PCR, pooling and HTS sequencing, dietary data were successfully obtained from 428 individual faecal samples, 379 in 2017, and 43 and 6 at Oxwich and Wheldrake in 2018, respectively. A total of 895 unique prey items were detected from the diet samples, the majority of which were successfully identified to species or genus level. The final sample sizes for each warbler species were 206 reed warblers, 135 sedge warblers, 31 Cetti’s warblers, 29 willow warblers and 27 chiffchaffs.

Overall, 61.7% of the total dietary diversity was described in the study, where the total extrapolated dietary diversity (Chao Estimate) was estimated at 1424.6 ± 75.06 (see later section Table 2.7). Several prey orders not successfully amplified during primer testing (Appendix 1.1), such as Mecoptera (scorpionflies) and Opiliones (harvestmen), were nonetheless detected at low frequency in the diet of warblers, suggesting the primer set has a wider utility than initial *in vitro* tests suggested. Recovery of known invertebrate sequences in the mock community samples suggested that some biases in amplification success might occur with different MID-tag primer combinations, however most invertebrate groups were successfully recovered in each reaction (Appendix 1.4). All repeat samples contained one or more different invertebrates when used with different MID-tags, which may indicate a low level of tag-jumping or contamination between samples (Esling et al. 2015) or might suggest differential amplification of some invertebrate groups with different MID-tag primer combinations (Deagle et al. 2013), as some combinations were seen to work better than others (see Appendices 1.4 and 1.5 for details). These potential sources of error need to be considered in studies, but broadly should not undermine the main results of the following analyses (see Chapter 6 for in depth discussion).

2.4.1. Differences in climate and growing degree days (Prediction 1)

The four main study sites showed broad similarity in weather patterns over the course of the summer of 2017 with some noticeable between-site variation. Notably, temperatures at Wheldrake

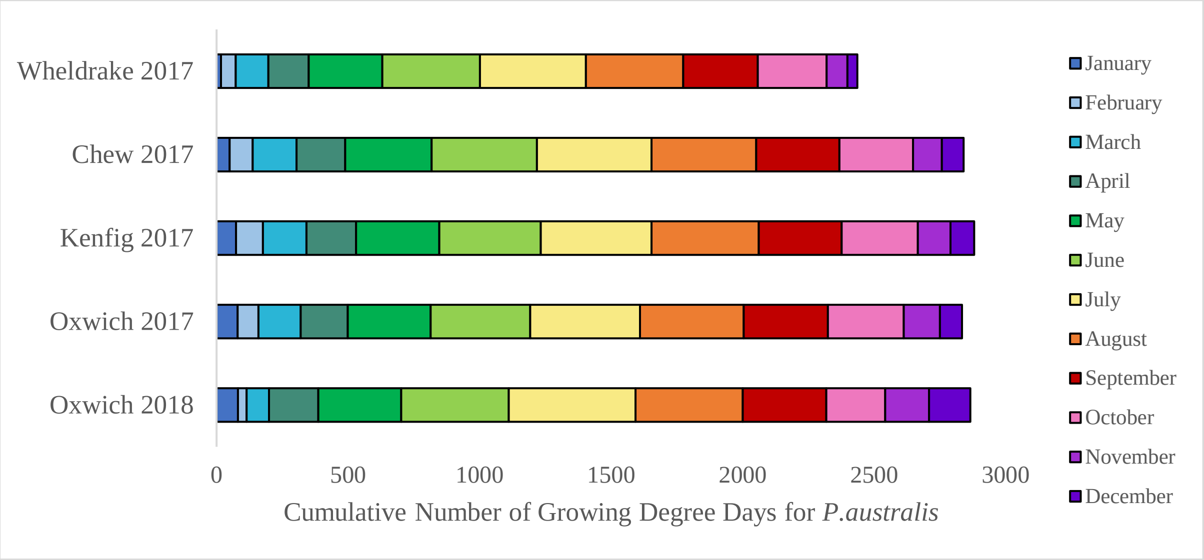
Ings were generally lower than at the remaining sites, showing lower minimum and maximum temperatures each month (Table 2.1) and lower accumulation of GDD for both *P.australis* and *H.pruni* (Fig. 2.2) The summer temperatures recorded in 2018 at Oxwich and Wheldrake were on average several degrees warmer than in 2017, with July maximum temperatures at Oxwich reaching 25.8°C. In 2018, Oxwich Marsh saw elevated summer temperatures with respect to the 2017 sites, alongside lower rainfall.

Table 2.1. Temperature and rainfall data over the study period in the UK. Mean maximum, minimum and average daily temperature ± SD, total precipitation, and number of days with precipitation are given for the four main study sites for the months, May, June, and July, and August and for the entire study period May-August.

Site	**Month**	**Max Temp (°C)**	**Min Temp (°C)**	**Average Temp (°C)**	**Total Precipitation (mm)**	**Days with Precipitation**
Chew Valley (2017)	May	19.72 ± 3.58	9.42 ± 3.19	14.55 ± 2.78	76.6	15
	June	22.05 ± 4.99	12.66 ± 2.17	17.28 ± 3.32	62.6	15
	July	22.51 ± 3.28	13.67 ± 1.62	18.09 ± 2.03	106.7	16
	August	21.30 ± 2.32	12.35 ± 1.96	16.81 ± 1.62	83.5	19
	All	**21.39 ± 3.77**	**12.02 ± 2.78**	**16.68 ± 2.82**	**329.4**	**65**
Kenfig (2017)	May	17.95 ± 3.34	10.52 ± 2.66	14.24 ± 2.71	40.3	11
	June	20.46 ± 3.48	13.27 ± 2.51	16.83 ± 2.64	59.7	14
	July	21.36 ± 2.68	13.82 ± 1.88	17.57 ± 1.68	157	18
	August	20.89 ± 3.48	12.74 ± 2.51	17.14 ± 1.5	69.5	20
	All	**20.16 ± 3.48**	**12.74 ± 2.51**	**16.44 ± 2.64**	**326.5**	**63**
Oxwich Marsh (2017)	May	18.8 ± 2.76	9.52 ± 3.46	14.13 ± 2.67	54.1	16
	June	20.82 ± 4.63	12.43 ± 2.48	16.58 ± 3.06	95.1	16
	July	21.95 ± 2.84	13.01 ± 1.62	17.46 ± 1.51	90.7	18
	August	21.14 ± 2.16	12.27 ± 2.26	16.68 ± 1.59	40.1	23
	All	**20.67 ± 3.38**	**11.78 ± 2.85**	**16.19 ± 2.59**	**280**	**73**
Wheldrake Ings (2017)	May	17.94 ± 4.75	8.09 ± 3.43	13.46 ± 3.38	37.9	10
	June	20.84 ± 4.89	11.91 ± 1.95	16.39 ± 2.97	84.16	11
	July	21.64 ± 3.2	12.36 ± 1.24	16.99 ± 1.67	96.8	15
	August	20.19 ± 2.15	11.65 ± 2.36	15.91 ± 1.69	69.7	16
	All	**20.15 ± 4.1**	**10.99 ± 2.91**	**15.68 ± 2.85**	**288.56**	**52**
Oxwich Marsh (2018)	May	19.90 ± 40.2	8.44 ± 3.95	14.15 ± 3.46	42.7	15
	June	23.18 ± 4.6	12.04 ± 1.99	17.61 ± 2.49	9.8	10
	July	25.77 ± 2.77	14.39 ± 2.13	20.06 ± 1.95	41.7	11
	August	21.45 ± 2.84	12.86 ± 3.54	17.14 ± 2.68	81.1	18
	All	**22.47 ± 4.23**	**11.86 ± 3.77**	**17.15 ± 3.45**	**175.3**	**54**

Site	Month	Max Temp (°C)	Min Temp (°C)	Average Temp (°C)	Total Precipitation (mm)	Days with Precipitation
Wheldrake Ings (2018)	August	23.13 ± 3.66	12.16 ± 3.71	17.69 ± 2.89	48.64	9

A



B

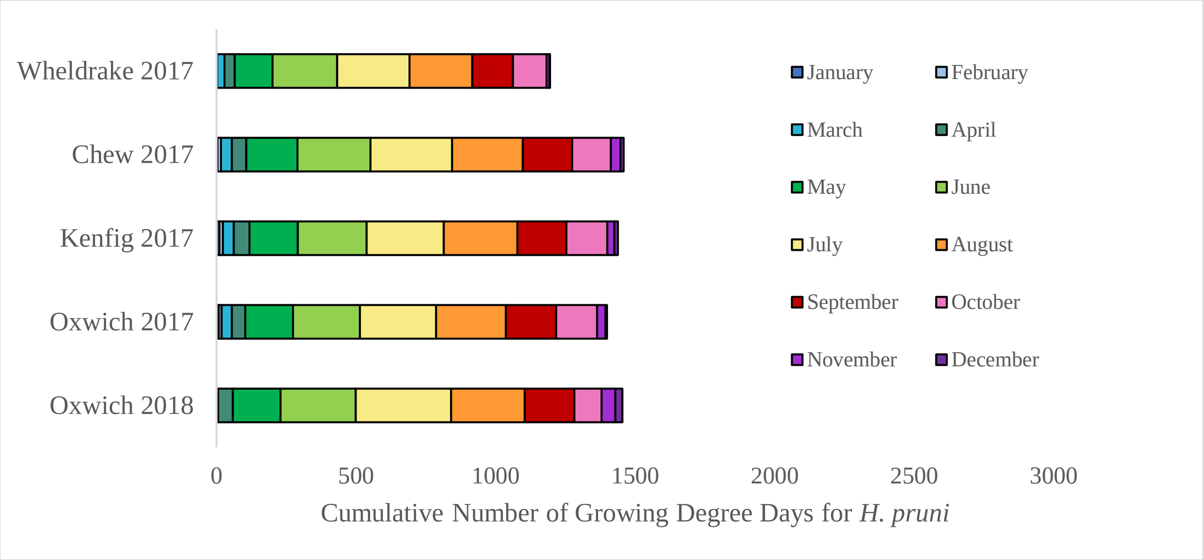


Figure 2.2. The number of growing degree day values accumulated over for each of the four main UK sites in 2017 and Oxwich Marsh in 2018, with the relative contribution of each month indicated by colour, A) values for *P. australis*, and B) values for *H. pruni*.

2.4.2. Measures of arthropod abundance and richness (Prediction 1)

Invertebrate abundance

Invertebrate abundance and species richness varied according to both site and season (Fig. 2.3). Site (LRT = 14.85, p<0.01), season (LRT = 12.06, p<0.01), and the interaction between site, year and season (LRT = 51.2, p<0.001) were significant in explaining sticky trap invertebrate abundance (adjusted R-squared = 0.14, F= 5.79 on 5 and 143 df, p<0.001). Invertebrate abundance was lower at Oxwich in 2018 than at Chew Valley (z = -5.11, p<0.001), Wheldrake Ings (z = -3.16, p<0.05), Kenfig (z = -4.39, p<0.001) and Oxwich in 2017 (z = -3.45, p<0.01). Chew Valley (z =4.56, p<0.001) and Kenfig (z = 3.63, p<0.01) had higher invertebrate abundance than at Oxwich (both years) and Chew had higher invertebrate abundance than Wheldrake (z = 2.58, p<0.05). Sticky traps set in the middle (z = 4.01, p<0.001) and late (z = 2.42, p<0.05) summer sampling periods had higher invertebrate abundance than traps set in the early season. This pattern was mirrored at Oxwich in 2017 where invertebrate abundance in the early season was lower than in the late season (z = -4.19, p<0.01). In Wheldrake however, late season sticky traps were significantly lower in invertebrate abundance than in the mid-season (z = -4.27, p<0.01).

Sites (split by year) did not differ significantly from each other in the early season, however, by the middle of summer, differences could be detected between sites and years. Oxwich in 2018 had lower abundance of prey compared to Chew (z = - 4.74, p<0.001), Kenfig (z = - 4.02, p<0.01), and Wheldrake (z = -4.72, p<0.001). During the late summer, Chew had higher invertebrate abundance than Wheldrake (z= 3.89, p<0.05) and Oxwich in 2018 (z = 3.99, p<0.01). Kenfig had higher invertebrate abundance in the late summer than Wheldrake (z = 3.69, p<0.05). In 2017, Oxwich had higher invertebrate abundance in the late summer compared to Oxwich in 2018 (z = 4.54, p<0.001) and Wheldrake in 2017 (z = 4.45, p<0.001).

Invertebrate richness

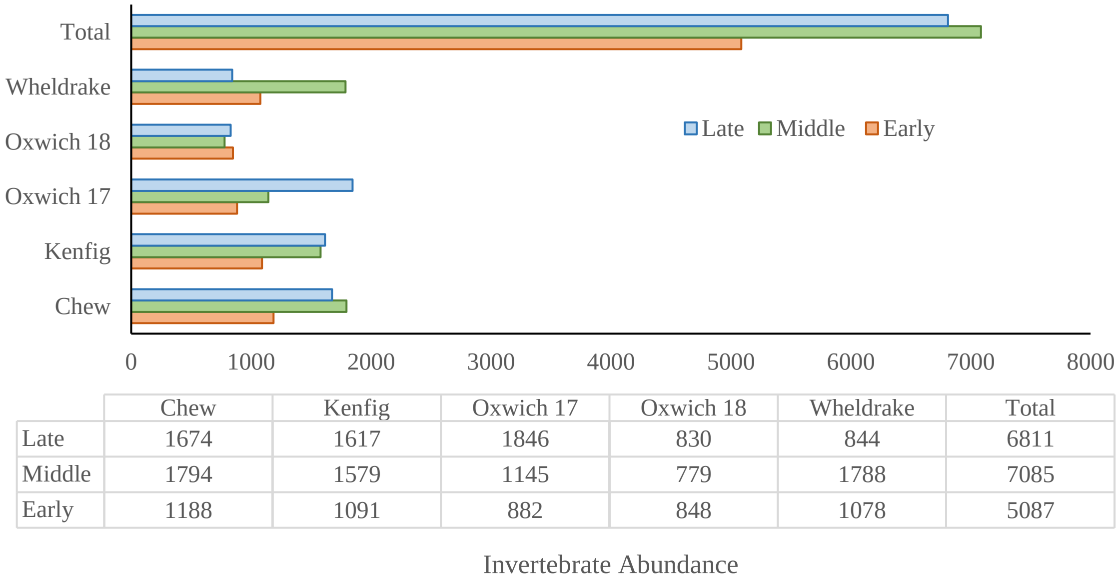
Site (LRT = 76.41, p<0.001), season (LRT = 47.67, p<0.001) and the three-way interaction between site, season and year (LRT = 32.08, p<0.001) were highly significant in explaining invertebrate richness on sticky traps (adjusted R-squared = 0.47, F=27.29 on 5 and 143 df, p<0.001). Oxwich had lower richness than all other sites (p<0.001). Mid-season sticky traps had the highest richness of invertebrate families (p<0.001). Both Wheldrake (z = 6.67, p<0.001) and Kenfig (z = 6.67, p<0.001) saw an increase in their respective invertebrate richness from early to mid-season, after which richness at Wheldrake richness fell significantly (z= -4.72, p<0.001) between mid to late season.

In early summer, Chew had higher invertebrate richness than Oxwich in 2017 (z = 3.85, $p<0.05$) but no other pairs were significantly different during this period. In the late summer, Chew (z = 4.59, $p<0.001$) and Kenfig (z = 5.35, $p<0.001$) had higher diversity than Oxwich in 2018. In the middle of summer, Wheldrake (z = 4.47, $p<0.001$, z = 6.79, $p<0.001$), Kenfig (z =3.83, $p<0.05$, z=6.16, $p<0.001$), Chew (z = 3.84, $p<0.05$, z = 6.16, $p<0.001$) in 2017 had higher invertebrate richness than Oxwich in 2017 and Oxwich in 2018, respectively.

Variation in chironomid body length

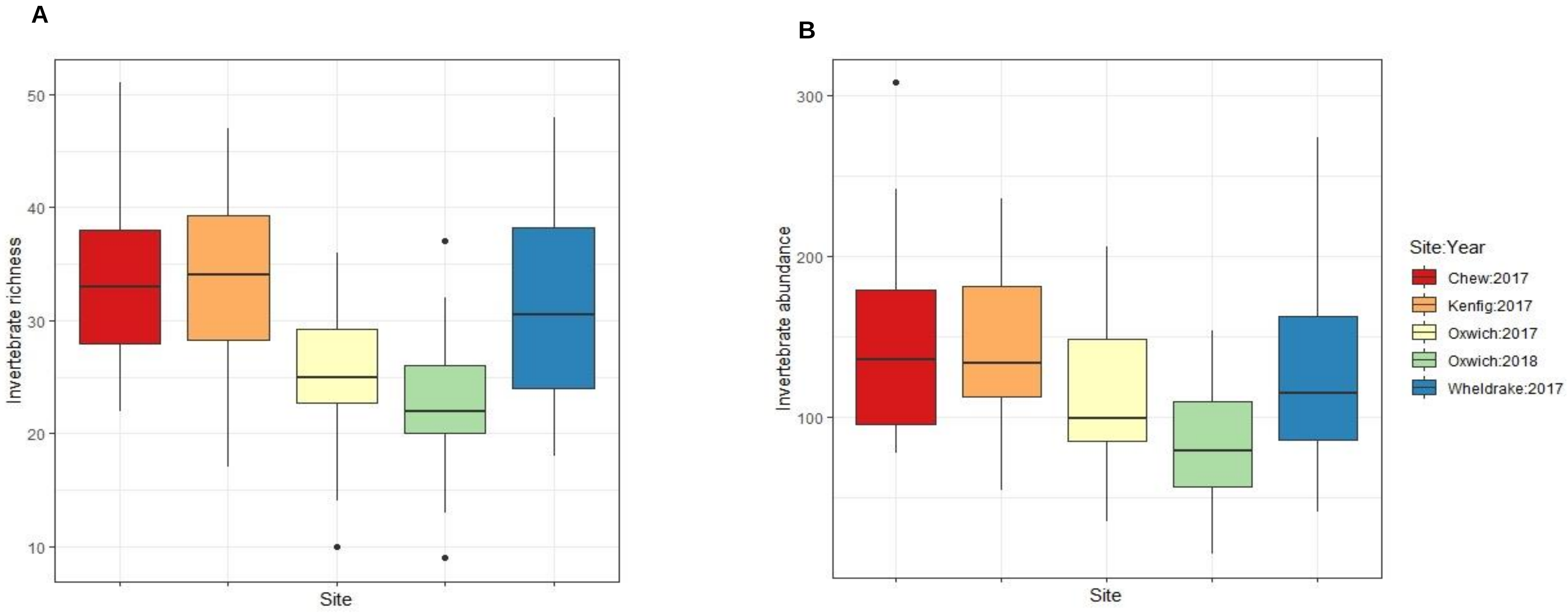
Site, habitat (reed or scrub), season and estimated water depth were all significant predictors of chironomid body length (adjusted R-squared = 0.43, F-statistic =58.44 on 7 and 525 degrees of freedom, $p<0.001$). Sticky trap plots with greater water depth (t=2.11, $p<0.05$) were associated with larger chironomids than drier plots, and scrub habitats harboured larger chironomids than reedbed habitats (z=2.77, $p<0.01$). Traps deployed over the early summer and mid-summer, both had significantly larger chironomids than those deployed in the late summer ($p<0.001$). Most notably, chironomids were significantly larger at Chew Valley and significantly smaller at Oxwich Marsh than at the remaining sites ($p<0.001$).

i)



	Chew	Kenfig	Oxwich 17	Oxwich 18	Wheldrake	Total
Late	1674	1617	1846	830	844	6811
Middle	1794	1579	1145	779	1788	7085
Early	1188	1091	882	848	1078	5087

ii)


A
Invertebrate richness
50
40
30
20
10
Site
B
Invertebrate abundance
300
200
100
Site
Site:Year
Chew:2017
Kenfig:2017
Oxwich:2017
Oxwich:2018
Wheldrake:2017

iii)

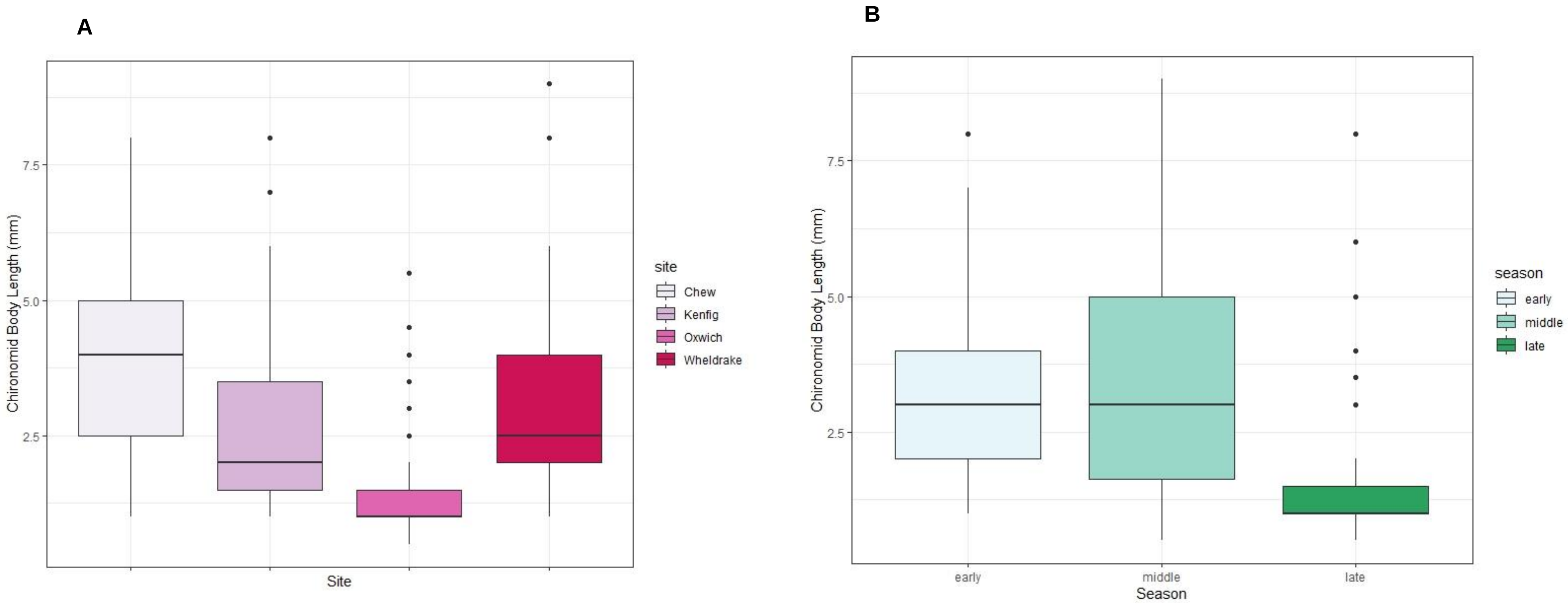


Figure 2.3. Abundance and richness of invertebrates identified from sticky traps in the UK; i) The total number of individual invertebrates collected from sticky traps for each of the four main study sites in 2017 and Oxwich in 2018 split across the three monitoring periods over the summer. ii) Boxplots comparing invertebrate sticky trap data at the four study sites and for 2017 and for Oxwich in 2018. A) invertebrate abundance and B) invertebrate richness. iii) Boxplots comparing chironomid body length (mm) on sticky traps A) at the four main study sites, B) at each of the three sampling rounds corresponding to early, middle (mid) and late summer/breeding season.

2.4.3. Patterns of dietary overlap and resource partitioning (Prediction 2)

The UK warbler assemblage was associated with relatively high dietary overlap when pooled across sites and seasons (Table 2.2i). Every warbler pair showed significant overlap with respect to the null model except for the Cetti's and willow warbler pair which showed significantly less overlap than expected (Pianka index = 0.13, p<0.001). The greatest overlap was between reed and Cetti's warblers (Pianka index = 0.77, p<0.001) and reed and sedge warblers (Pianka index = 0.76, p<0.001). For the remaining species pairs the degree of overlap was "moderate" (Navalpotro et al. 2016) and fell between 0.35 and 0.65.

Generally dietary overlap was significant between species pairs within sites in 2017 (Table 2.2ii). There was a gradient from the high overlap seen at Chew and to a lesser extent Kenfig (especially for reed and sedge warblers) to more moderate overlap at Wheldrake, except the willow warbler – chiffchaff pair. Oxwich was distinctly different, with nine out of ten species pairs showing a Pianka index lower than 0.4 and six with significant dietary partitioning.

There was evidence that between yearly differences in overlap were greater than between sites at Oxwich and Wheldrake (Table 2.2iii). In 2018, sedge and reed warblers showed significant partitioning (Pianka index 0.34, p<0.001) and patterns within 2018 sites showed the same pattern for reed and sedge warblers (both Oxwich and Wheldrake), and reed and Cetti's warblers (at Oxwich). Dietary overlap was not significantly different from the null model between sedge and Cetti's warblers at Oxwich in 2018, which could be a result of the smaller sample size.

Between most species pairs, the degree of overlap increased over the summer, with the greatest partitioning observed during the early summer months (Table 2.2iv). Six out of ten species pairs showed an overlap index of less than 0.4 in the early summer, and two out of the three remaining pairs only showed moderate overlap in diet (<0.6). In the middle of summer, overlap increased but there were still five out of ten pairs that had an overlap <0.4 and one instance of significant partitioning. In addition, none of the pairwise overlap values exceeded 0.6, suggesting that prey sharing was still limited. However, by the late summer, when temperatures were at their peak, elevated dietary overlap occurred in the majority of species pairs (7/10 showing significant overlap) and the highest overlap between reed and sedge warblers was seen (Pianka index = 0.69). In three pairs, overlap was either low (Pianka index = 0.36 between sedge and Cetti's warblers) or significant partitioning occurred (Pianka index = 0.1 between willow warblers and chiffchaffs, and chiffchaffs and Cetti's warblers).

Upon removal of the most common prey species in our sensitivity analysis, dietary overlap decreased in all cases, sometimes enough to produce a result of dietary partitioning. This indicates that at least a proportion of the overlap in our study is due to several bird species sharing widespread, common prey items that may have had high availability in the field.

Table 2.2. Pianka's index of niche overlap (*Ojk*) in observed diet between pairs of warblers in the UK i) for all four of the main sites and seasons in 2017 combined, ii) at each of the four main study sites in 2017, iii) in 2018 at all sites and for Oxwich and Wheldrake, iv) during different stages of the breeding season corresponding to early, middle and late summer. Standard effect sizes (SES) are indicated in brackets. Cells are colour coded according to significance with respect to the null model; green = overlap significantly greater than expected, yellow = overlap not significant, orange = overlap significantly less than expected. Key to species codes: RW = reed warbler, SW = sedge warbler, CW = Cetti's warbler, CC = chiffchaff, WW = willow warbler.

i)

	SW	CW	CC	WW
RW	0.76***(19.9)	0.77***(19.5)	0.62***(13.4)	0.51***(9.3)
SW		0.56***(10.4)	0.75***(10.3)	0.48***(7.5)
CW			0.36***(2.5)	0.13***(-2.6)
CC				0.53***(4.7)

ii)

	SW	CW	CC	SW	CW	CC	WW	SW	CW	CC	WW	SW	CC	WW
	Chew			Kenfig				Oxwich				Wheldrake		
RW	0.79*** (12.2)	0.61*** (8.1)	0.73*** (10.2)	0.76*** (12.4)	0.47*** (5.5)	0.53*** (7.5)	0.52*** (7.2)	0.45** (3.4)	0.27 (0.39)	0.15*** (-3.1)	0.17*** (-3.2)	0.53*** (4.4)	0.42 (1.68)	0.5** (3.2)
SW		0.61*** (5.9)	0.63*** (5.9)		0.43* (2.1)	0.51** (4.6)	0.53** (5.2)		0.32 (1.9)	0.12*** (-2.9)	0.22 (-1.1)		0.40 (1.1)	0.56** (3.7)
CW			0.39 (0.95)			0.28* (-2.1)	0.27 (-1.2)			0.08*** (-3.8)	0.05*** (-4.4)			
CC							0.42* (1.9)				0.16*** (-4.6)			0.52* (1.98)

iii)

	SW	SW	CW	SW
	2018 All sites	Oxwich 2018		Wheldrake 2018
RW	0.34*** (-2.2)	0.32** (-2.7)	0.14*** (-3.4)	0.27* (-1.7)
SW			0.32 (-1.2)	

iv)

	SW	CW	CC	WW	SW	CW	CC	WW	SW	CW	CC	WW
	Early				Middle				Late			
RW	0.65*** (8.1)	0.27 (0.0)	0.43** (3.4)	0.4 (1.67)	0.59*** (9.8)	0.47*** (6.4)	0.47*** (7.1)	0.36*** (3.0)	0.69*** (13.6)	0.36** (3.5)	0.52*** (7.9)	0.45*** (5.4)
SW		0.39 (1.7)	0.48** (4.1)	0.3 (-0.7		0.44*** (3.6)	0.28 (0.78)	0.26 (-0.28)		0.36 (1.9)	0.51*** (6.5)	0.5*** (4.6)
CW			0.32 (-0.14)	0.06*** (-3.6)			0.28 (-1.3)	0.14*** (-3.9)			0.1*** (-5.1)	0.1*** (-5.6)
CC								0.24 (-0.9)				0.54** (2.9)

Notes: Asterisks denote significantly higher or lower overlap than predicted from the null model (* p<0.05, **p<0.01, ***p<0.001). Negative standard effect sizes (SES) and a red shade indicate lower observed overlap than the null model, whereas positive SES and a green shade indicates higher observed overlap than the null model. Yellow shade indicates that observed overlap is not significantly different from the null model.

2.4.4. Multivariate analysis of diet composition (Prediction 3)

Diet composition changed significantly among focal species (LRT = 11.97.4, $p<0.001$), between age groups (LRT – 369.6, $p<0.001$), years (LRT = 359.1, $p<0.001$) and among sites (LRT = 1468.8, $p<0.001$). The interaction between site and year (LRT = 145.9, $p<0.001$) and species and age (LRT = 330.2, $p<0.01$) were also significant. The climatic variables: total rainfall for the week of capture (LRT = 244.7, $p<0.01$) and the cumulative number of growing degree days for *H. pruni* (LRT = 383.1, $p<0.001$) also affected diet composition significantly.

The NMDS plots highlight the patterns of family level consumption detected in the *manyglm* model (Fig. 2.4). The diets of the warblers appeared to be on a continuum with willow warblers and chiffchaffs on one end and Cetti's warblers on the other, with the reed and sedge warblers overlapping in the centre. The univariate tests for the *manyglm* indicated several prey families that significantly differed among warbler species (Table 2.3). Dietary differences between adults and juveniles varied between warbler species, but the family Noctuidae was consumed by a significantly higher percentage of adults than by juveniles (LRT = 14.78, $p<0.01$).

Oxwich appeared distinct from the other sites and the remaining sites showed more subtle differences, each showing limited separation on the NMDS plot. A suite of prey families was consumed differentially by warblers among sites (Table 2.3).

2017 birds showed a different diet to 2018 birds, with the prey families Tomoceridae, Chaoboridae, Ichneumonidae, Coleophoridae and Leptoceridae significantly differing in the diet of birds. Chaoboridae consumption fell from 74% in 2017 to 6.4% in 2018, although this may be due to most of the 2018 coming from Oxwich, which had lower dietary incidences of chaoborids in both 2017 and 2018 compared to the remaining sites. When year was plotted by site it was clear that some of the effects of year were due to differences in diet composition at Oxwich between 2017 and 2018, which is likely a result of the different weather conditions. Diet at Wheldrake also showed little overlap between 2017 and 2018, though this may be partly as result of the limitation that willow warblers and chiffchaffs were only sampled in 2017, and their diet contributions in 2018 are not incorporated.

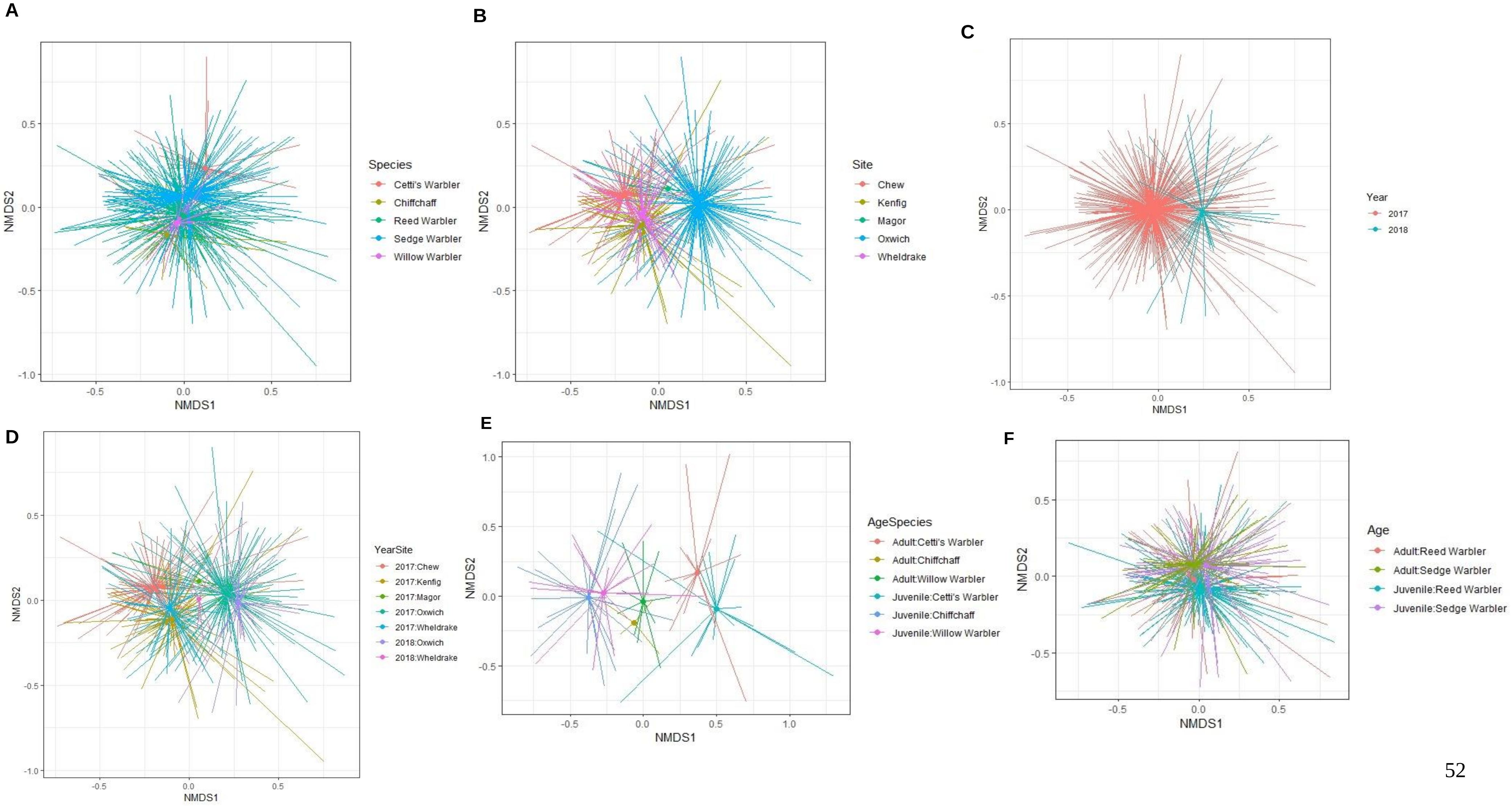
Consumption of reed spiders, Clubionidae, changed in response to changes in cumulative GDD, showing a significant difference in dietary incidence between lower (GDD 0-600 = 18.9%) and higher (GDD 600-1200 = 31.7%) values. This implies they are consumed more frequently both in warmer temperatures and later in the summer (i.e., temporal change). Patterns of rainfall were less clearly shown in the NMDS plot, but samples collected during wetter weeks appeared to cluster together suggesting diet similarity. No individual families were significant in the univariate test, but the majority of families were most prevalent in the diet during weeks where total rainfall was between 2mm and 20mm.

Table 2.3. Results for the univariate *"anova"* test in the *manyglm* model. Prey families showed significant (p<0.05) differences for one or more of the test variables in the final model are shown ordered by their taxonomic orders. Likelihood ratio test values (LRT) and p-values are given for each univariate test. Percent frequency of occurrence values (% FOO) for each prey family across the factor levels and/or with increasing/decreasing values of the numeric predictors are indicated and colour coded, with a darker tone indicating a higher % FOO. Codes: CW = Cetti's warbler, CC = chiffchaff, RW = reed warbler, SW = sedge warbler, WW = willow warbler, C = Chew, K = Kenfig, O = Oxwich, W = Wheldrake.

Predictor Variable	Prey Order	Prey Family	LRT	p-value	% FOO				
					CW	CC	RW	SW	WW
Species	Araneae	Clubionidae	20.32	0.021*	35.5	3.7	26.2	21.5	3.5
Species	Coleoptera	Chrysomelidae	33.07	0.001***	45.2	33.3	39.3	20.0	72.4
Species	Collembola	Tomoceridae	22.22	0.011*	22.6	-	3.4	10.4	-
Species	Diptera	Anthomyiidae	24.27	0.005**	6.5	33.3	22.8	6.7	13.8
Species	Diptera	Hybotidae	38.16	0.001***	3.2	44.4	27.2	8.8	34.5
Species	Diptera	Muscidae	35.82	0.001***	3.2	29.6	29.6	7.4	10.3
Species	Diptera	Ptychopteridae	30.89	0.001***	38.7	-	12.6	7.4	-
Species	Hemiptera	Aphididae	25.41	0.004**	58.1	11.1	21.8	35.5	34.5
Species	Hemiptera	Miridae	22.08	0.011*	9.7	40.7	9.7	7.4	24.1
Species	Hymenoptera	Braconidae	22.89	0.009**	80.7	22.2	46.6	42.9	41.4
Species	Lepidoptera	Gelechiididae	23.26	0.007**	3.2	-	7.7	2.9	6.9
					Adult		Juvenile		
Age	Lepidoptera	Noctuidae	14.78	0.007**	43.68		26.19		
					2017		2018		
Year	Diptera	Chaoboridae	31.22	0.001***	73.5		6.4		
Year	Hymenoptera	Ichneumonidae	17.10	0.003**	22.6		57.4		
Year	Lepidoptera	Coleophoridae	15.98	0.005**	2.1		17		
Year	Trichoptera	Leptoceridae	14.12	0.019*	11.5		-		
					0-600		600-1200		
GDD	Araneae	Clubionidae	18.34	0.017*	18.9		31.7		
					Chew	Kenfig	Oxwich	Wheldrake	
Site	Coleoptera	Scirtidae	37.46	0.001***	0.5	6.5	19.4	3.6	
Site	Collembola	Entomobryidae	39.22	0.001***	2.6	8.6	4.4	29.1	
Site	Diptera	Chaoboridae	184.72	0.001***	78.8	81.7	5.0	43.6	
Site	Diptera	Chironomidae	25.84	0.006**	79.7	73.1	53.8	92.7	
Site	Diptera	Limoniidae	34.76	0.001***	4.4	2.2	4.4	25.5	
Site	Diptera	Micropezidae	24.19	0.012*	3.5	1.1	1.3	16.4	
Site	Diptera	Scathophagidae	24.04	0.012*	20.4	3.2	16.3	14.6	
Site	Hemiptera	Aphididae	28.17	0.005**	23	22.6	38.8	10.9	
Site	Hemiptera	Rhyparochromidae	29.05	0.005**	-	-	10.6	-	
Site	Hymenoptera	Braconidae	25.15	0.007**	33.6	32.3	61.3	34.6	
Site	Hymenoptera	Ichneumonidae	25.17	0.007**	12.4	18.3	43.8	20.0	
Site	Lepidoptera	Geometridae	21.68	0.033*	14.2	38.7	19.4	12.7	
Site	Lepidoptera	Tortricidae	27.94	0.005**	8.9	34.4	18.3	10.9	
Site	Trichoptera	Leptoceridae	47.56	0.001***	19.5	13.9	0.6	14.6	

					CV 2017	K 2017	O 2017	O 2018	W 2017	W 2018
Year: Site	Neuroptera	Chrysopidae	14.88	0.011**	1.8	2.2	4.5	-	4.0	80.0

Notes: Asterisks denote the significance level of the test result (* p<0.05, **p<0.01, ***p<0.001).


A
NMDS1
NMDS2
Species
Cetti's Warbler
Chiffchaff
Reed Warbler
Sedge Warbler
Willow Warbler
B
Site
Chew
Kenfig
Magor
Oxwich
Wheldrake
C
Year
2017
2018
D
YearSite
2017:Chew
2017:Kenfig
2017:Magor
2017:Oxwich
2017:Wheldrake
2018:Oxwich
2018:Wheldrake
E
AgeSpecies
Adult:Cetti's Warbler
Adult:Chiffchaff
Adult:Willow Warbler
Juvenile:Cetti's Warbler
Juvenile:Chiffchaff
Juvenile:Willow Warbler
F
Age
Adult:Reed Warbler
Adult:Sedge Warbler
Juvenile:Reed Warbler
Juvenile:Sedge Warbler
-1.0
-0.5
0.0
0.5
1.0

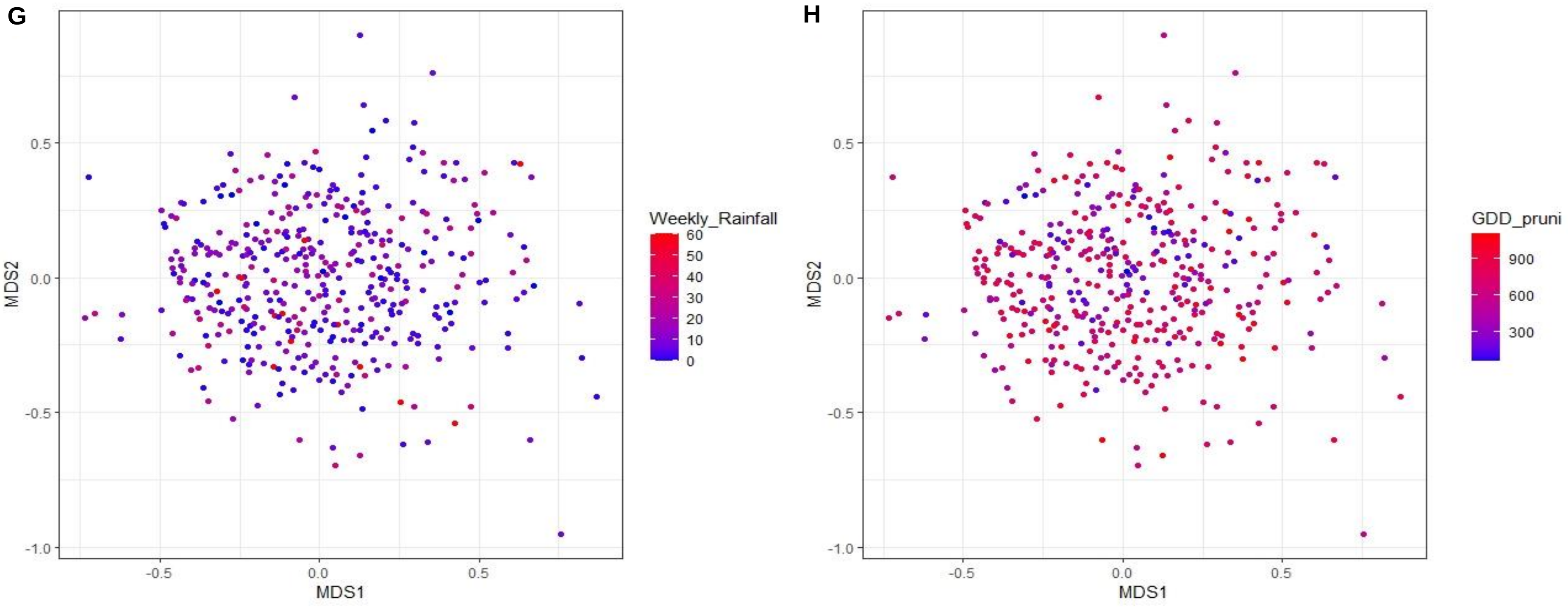


Figure 2.4. NMDS of prey detected in the warbler dietary samples collected in the UK according to the significant predictor variables from the *manyglm* model: A) focal warbler species, B) site, C) year, D) the interaction between year and site E) the interaction between age and species, subset for Cetti's warbler, chiffchaff and willow warbler, F) the interaction between age and species, subset for reed warblers and sedge warblers. G) total rainfall during the week of capture (mm) and: H) cumulative number of growing degree days GDD for *H. pruni.*

2.4.5. Patterns in dietary composition among species, sites and years (Prediction 3)

At the order level, broad differences in diet between the focal species were apparent (Table 2.4). Some aquatic orders such as Ephemeroptera, Odonata, Megaloptera and Plecoptera were not present in the diets of the two *Phylloscopus* leaf warblers, being unique to reed, sedge and Cetti's warblers. Cetti's warblers consumed the lowest frequency of Diptera (87% of samples) but consumption of gastropods, Araneae, Collembola, Neuroptera, Trichoptera and Opiliones was elevated. Consumption of Coleoptera was more variable among warbler species, peaking in willow warblers (79%), but only reaching 44% in the closely related chiffchaffs. Although showing the highest species richness for Hemiptera, reed and sedge warblers consumed this order less frequently than *Phylloscopus* warblers, where % FOO reached 70-80%. Both sedge and reed warblers consumed orders that were absent in the other focal species, but these were all at relatively low frequency.

Table 2.4. The percent frequency of occurrence (% FOO) and species richness (in parentheses) of all invertebrate orders detected in the diet of each focal warbler species. Cells for each warbler are colour coded by increasing % FOO for the respective prey order (increasing % FOO for colours; pink (lowest), orange, yellow, green (highest)).

	% Frequency of Occurrence (Species Richness)				
Order	Willow Warbler (n =29)	Chiffchaff (n = 27)	Cetti's Warbler (n = 31)	Sedge Warbler (n = 135)	Reed Warbler (n = 206)
Acari	0	0	0	0.74 (1)	1.94 (4)
Amphipoda	0	0	3.23 (1)	0.74 (1)	0
Annelida	0	0	0	0.74 (1)	0.48 (1)
Araneae	24.13 (5)	25.92 (5)	58.06 (11)	37.77 (23)	44.66 (16)
Bivalvia	0	0	3.22 (1)	0	0
Cladocera	0	0	0	0	0.48 (1)
Coleoptera	79.31 (16)	44.44 (7)	61.29 (13)	45.18 (41)	53.39 (41)
Collembola	3.44 (1)	0	22.58 (4)	17.03 (5)	18.93 (4)
Diptera	93.1 (63)	96.29 (74)	87.09 (45)	93.33 (178)	96.12 (235)
Ephemeroptera	0	0	3.23 (1)	1.48 (3)	5.82 (4)
Gastropoda	6.89 (1)	7.41 (2)	38.71 (10)	9.62 (6)	10 (7)
Hemiptera	79.31 (26)	70.37 (24)	61.29 (13)	51.85 (48)	54.36 (60)
Hirudinea	0	0	0	0	0.48 (1)
Hymenoptera	58.6 (21)	25.93 (14)	87.09 (22)	57.04 (93)	63.59 (95)
Julida	0	0	0	0.74 (1)	0

Lepidoptera	62.07 (23)	70.37 (16)	83.87 (40)	68.15 (71)	59.71 (66)
Mecoptera	0	0	0	1.48 (1)	0.48 (1)
Megaloptera	0	0	0	0.74 (1)	0
Neuroptera	0	0	35.48 (3)	4.44 (4)	9.22 (6)
Odonata	0	0	6.45 (2)	4.44 (1)	4.85 (1)
Opiliones	3.45 (1)	0	12.9 (2)	3.70 (2)	5.34 (1)
Orthoptera	0	0	0	0.74 (1)	0
Planaria	0	0	12.9(1)	3.70(1)	0.97(1)
Plecoptera	0	0	0	0.74 (1)	0
Psocoptera	10.34 (2)	14.81 (1)	12.9 (1)	14.81 (4)	19.90 (4)
Thysanoptera	6.89 (2)	0	0	2.96 (2)	0.97 (1)
Trichoptera	10.34 (2)	0	22.58 (3)	11.85 (5)	12.14 (6)

Chironomids, especially *Cladotanytarsus atridorsum* and *Endochironomus albipennis* were consistently among the top prey items in the diets of all warbler species (Appendix 1.9, Table A.1.9*a*) but consumed at the highest frequency by *Phylloscopus* species (~ 85%). Cetti's warbler diet was most distinct; their consumption of chironomidae was lower, whereas consumption of terrestrial spider, moth and beetle species were higher.

When prey items were grouped at the family level (Table 2.5), it was clear that Chironomidae, Chaoboridae, Clubionidae, Braconidae, Noctuidae, Chrysomelidae and Aphididae were the most common components of warbler diet . Willow warblers had consumed aggregated prey associated with scrub vegetation, such as chrysomelid beetles (72% of samples) and Cicadellidae (48% of samples), with Miridae in 40% of chiffchaff samples. Moreover, a high frequency of geometrid and tortricid moths were present in the diets of the two *Phylloscopus* warblers. Aphid consumption was particularly elevated in Sedge, Willow and Cetti's warblers (34-58% of samples).

Many of the prey items consumed by birds at Oxwich and Wheldrake in 2017 were also consumed at a similar frequency at these respective sites in 2018 (Table 2.6). Chrysomelidae, Hybotidae and Syrphidae consumption was slightly elevated in reed and sedge warblers in 2018 whereas consumption of Chironomidae, Chaoboridae and Clubionidae fell for all species in 2018, compared to 2017. Scathophagidae and Ptychopteridae consumption by Cetti's warblers also fell by around 50% from 2017 to 2018. Consumption of moth families generally increased from 2017 to 2018 and Hemipterans associated with scrub habitat; Miridae, Cicadellidae and Aphrophoridae doubled in frequency in reed warblers in 2018 compared to 2017.

Aphid consumption in late summer

The reed plum aphid (*Hyalopterus pruni*) was more common in the diet of sedge warblers fattening for migration (fat score 3-5) from middle to late summer (25%, Appendix 1.9, Table A.1.9*b*), compared to sedge warblers in early summer and those in middle and late summer with little or no fat deposit (6.6%). This was also mirrored in higher consumption of Hemiptera by the fattening birds (84.6%; *n* = 13) compared to those without fat deposits (52.75%). However, consumption of Diptera remained above 90% in both groups, suggesting their importance at all life-history stages. In contrast, reed warblers consumed reed plum aphids less often than sedge warblers (14%), with no significant increase prior to migration.

Table 2.5. Percent frequency of occurrence (% FOO) of the top 50 prey items detected in the diet of the five UK warbler species: CW = Cetti's warbler, CC = Chiffchaff, RW = Reed warbler, SW = Sedge warbler and WW = Willow Warbler. Where birds were sampled in both 2017 and 2018 values are given for 2017 samples alone and combined samples from 2017 and 2018. Cells are colour coded based on % FOO with a gradient from low (paler tone) to high (deeper tone).

	Warbler Species and Year								
Family	All (n = 428)	CW 2017 (n = 28)	CW all (n = 31)	CC all (n= 27)	RW 2017 (n = 179)	RW all (n = 206)	SW 2017 (n = 118)	SW all (n = 135)	WW all (n = 29)
Chironomidae	65.55	53.57	51.61	85.19	75.42	71.84	69.49	65.93	86.21
Braconidae	35.51	82.14	80.65	22.22	35.75	41.75	44.92	43.70	48.28
Noctuidae	35.05	53.57	54.84	14.81	26.82	28.16	36.44	38.52	41.38
Chaoboridae	33.38	42.86	38.71	55.56	56.42	50.49	44.07	38.52	51.72
Clubionidae	25.49	35.71	35.48	3.70	27.93	25.73	23.73	22.96	3.45
Chrysomelidae	25.04	42.86	48.39	33.33	37.43	39.32	18.64	20.74	72.41
Aphididae	24.43	53.57	58.06	11.11	21.23	20.87	34.75	34.81	34.48
Ichneumonidae	22.61	14.29	16.13	7.41	27.93	33.50	17.80	20.74	31.03
Geometridae	19.42	17.86	16.13	40.74	17.88	18.93	18.64	17.78	41.38
Muscidae	17.91	3.57	3.23	29.63	30.73	29.61	9.32	8.15	10.34
Culicidae	17.15	7.14	6.45	0	16.76	16.50	11.02	11.85	13.79
Syrphidae	14.57	3.57	3.23	7.41	13.41	15.53	14.41	15.56	10.34
Hybotidae	14.11	3.57	3.23	44.44	26.26	27.18	7.63	8.89	34.48
Tortricidae	13.05	10.71	12.90	33.33	17.32	17.96	11.02	11.85	37.93
Erebidae	12.29	7.14	6.45	14.81	7.82	8.74	9.32	9.63	10.34
Linyphiidae	12.14	35.71	32.26	18.52	16.76	16.02	16.95	16.30	6.90
Scathophagidae	11.38	28.57	29.03	22.22	16.20	17.48	9.32	8.89	6.90
Anthomyiidae	10.93	7.14	6.45	33.33	20.11	21.84	7.63	6.67	13.79
Calliphoridae	10.77	0	0	18.52	10.06	11.65	5.93	5.93	6.90
Crambidae	10.77	53.57	58.06	7.41	7.26	7.77	13.56	14.07	0
Cicadellidae	9.86	3.57	3.23	22.22	7.82	10.19	16.10	15.56	48.28
Miridae	9.26	10.71	9.68	40.74	6.70	9.71	7.63	7.41	24.14
Aphrophoridae	8.50	10.71	9.68	3.70	5.03	8.25	7.63	7.41	6.90

Carabidae	8.35	10.71	12.90	11.11	7.26	9.22	5.08	4.44	0
Gelechiididae	8.19	3.57	3.23	0	13.41	14.56	3.39	2.96	6.90
Dolichopodidae	8.04	3.57	3.23	18.52	16.20	15.53	1.69	3.70	13.79
Scirtidae	7.74	7.14	12.90	3.70	5.59	5.34	12.71	13.33	20.69
Cecidomyiidae	7.44	10.71	9.68	0	6.70	6.31	9.32	9.63	10.34
Ceratopogonidae	7.44	3.57	3.23	7.41	11.17	10.68	9.32	12.59	17.24
Ptychopteridae	7.44	39.29	38.71	0	13.41	12.62	6.78	7.41	0
Leptoceridae	7.28	21.43	19.35	0	11.73	10.19	11.86	10.37	10.34
Pipunculidae	6.53	0	0	7.41	11.73	10.19	12.71	12.59	3.45
Sarcophagidae	6.37	0	0	3.70	9.50	10.68	4.24	5.19	0
Chloropidae	5.46	0	0	0	9.50	11.65	5.08	6.67	0
Entomobryidae	5.31	7.14	9.68	0	11.17	11.17	5.08	5.19	3.45
Philodromidae	5.16	0	0	0	1.68	2.43	0	0	3.45
Psychodidae	5.01	10.71	9.68	0	6.70	6.31	11.02	10.37	0
Limoniidae	4.86	14.29	12.90	0	9.50	8.74	5.93	5.19	3.45
Tetragnathidae	4.55	10.71	12.90	0	6.15	5.34	3.39	2.96	0
Fanniidae	4.55	0	0	3.70	7.26	8.25	0.85	1.48	6.90
Agriolimacidae	4.40	3.57	3.23	3.70	6.70	5.83	1.69	2.22	0
Tomoceridae	4.25	17.86	22.58	0	2.23	3.40	7.63	10.37	0
Tachinidae	4.25	35.71	32.26	3.70	2.23	1.94	2.54	2.22	6.90
Empididae	4.10	3.57	3.23	7.41	10.06	8.74	3.39	2.96	3.45
Coenagrionidae	4.10	7.14	6.45	0	5.59	4.85	4.24	4.44	0
Tipulidae	3.95	3.57	3.23	0	10.61	9.22	3.39	3.70	0
Rhagionidae	3.79	0	0	3.70	7.82	8.25	4.24	3.70	3.45
Tenthredinidae	3.79	3.57	3.23	0	3.35	3.40	7.63	8.15	13.79

Table 2.6. Percent frequency of occurrence (% FOO) of the top 50 prey families detected in dietary samples from the three warbler species collected in both 2017 and 2018 at Oxwich Marsh and Wheldrake Ings: CW = Cetti’s warbler, RW = reed warbler, SW = sedge warbler. Cells are colour coded based on % FOO with a gradient from low (paler tone) to high (deeper tone).

		Warbler species (2017 and 2018 at Oxwich and Wheldrake Ings)					
Order	Family	CW 2017 (n = 9)	CW 2018 (n=3)	RW 2017 (n = 63)	RW 2018 (n = 27)	SW 2017 (n = 67)	SW 2018 (n = 17)
Hymenoptera	Braconidae	77.78	66.67	42.86	81.48	61.19	35.29
Coleoptera	Chrysomelidae	77.78	100	36.51	51.85	14.93	35.29
Diptera: Nematocera	Chironomidae	55.56	33.33	71.43	48.15	59.70	41.18
Hemiptera	Aphididae	88.89	100	15.87	18.52	46.27	35.29
Lepidoptera	Noctuidae	44.44	66.67	33.33	37.04	37.31	52.94
Lepidoptera	Crambidae	100	100	6.35	11.11	14.93	17.65
Hymenoptera	Ichneumonidae	11.11	33.33	46.03	70.37	25.37	41.18
Diptera: Brachycera	Scathophagidae	66.67	33.33	14.29	25.93	7.46	5.88
Araneae	Clubionidae	44.44	33.33	20.63	11.11	25.37	17.65
Diptera: Nematocera	Ptychopteridae	77.78	33.33	12.70	7.41	8.96	11.76

Neuroptera	Hemerobiidae	77.78	66.67	1.59	0	2.99	0
Collembola	Tomoceridae	22.22	66.67	1.59	11.11	10.45	29.41
Coleoptera	Scirtidae	22.22	66.67	9.52	3.70	19.40	17.65
Lepidoptera	Tortricidae	22.22	33.33	17.46	22.22	7.46	17.65
Coleoptera	Carabidae	33.33	33.33	14.29	22.22	7.46	0
Araneae	Linyphiidae	33.33	0	20.63	11.11	25.37	11.76
Diptera: Nematocera	Culicidae	22.22	0	31.75	14.81	13.43	17.65
Diptera: Brachycera	Syrphidae	11.11	0	20.63	29.63	13.43	23.53
Tricladida	Geoplanidae	11.11	66.67	1.59	3.70	5.97	0
Collembola	Entomobryidae	11.11	33.33	19.05	11.11	5.97	5.88
Diptera: Brachycera	Hybotidae	0	0	28.57	33.33	4.48	17.65
Lepidoptera	Geometridae	11.11	0	17.46	25.93	16.42	11.76
Hemiptera	Rhyparochromidae	11.11	33.33	12.70	11.11	4.48	5.88
Diptera: Brachycera	Muscidae	0	0	41.27	22.22	11.94	0
Araneae	Tetragnathidae	22.22	33.33	14.29	0	4.48	0
Diptera: Brachycera	Anthomyiidae	11.11	0	20.63	33.33	8.96	0
Lepidoptera	Erebidae	22.22	0	14.29	14.81	10.45	11.76
Hemiptera	Cicadellidae	11.11	0	4.76	25.93	19.40	11.76
Opiliones	Phalangiidae	11.11	33.33	7.94	11.11	5.97	0
Hemiptera	Miridae	11.11	0	11.11	29.63	10.45	5.88
Hemiptera	Aphrophoridae	11.11	0	9.52	29.63	11.94	5.88
Diptera: Nematocera	Ceratopogonidae	0	0	11.11	7.41	11.94	35.29
Diptera: Brachycera	Chloropidae	0	0	14.29	25.93	7.46	17.65
Diptera: Nematocera	Cecidomyiidae	11.11	0	12.70	3.70	10.45	11.76
Diptera: Brachycera	Pipunculidae	0	0	15.87	0	20.90	11.76
Diptera: Nematocera	Chaoboridae	0	0	20.63	11.11	16.42	0
Lepidoptera	Lasiocampidae	0	33.33	3.17	3.70	1.49	5.88
Diptera: Brachycera	Calliphoridae	0	0	11.11	22.22	7.46	5.88
Diptera: Brachycera	Dolichopodidae	0	0	14.29	11.11	1.49	17.65
Gastropoda	Lymnaeidae	11.11	33.33	0	0	0	0
Lepidoptera	Gelechiididae	0	0	19.05	22.22	2.99	0
Diptera: Nematocera	Limoniidae	11.11	0	22.22	3.70	5.97	0
Diptera: Brachycera	Sarcophagidae	0	0	7.94	18.52	4.48	11.76
Diptera: Nematocera	Psychodidae	11.11	0	12.70	3.70	8.96	5.88
Hymenoptera	Tenthredinidae	11.11	0	3.17	3.70	11.94	11.76
Lepidoptera	Coleophoridae	0	0	6.35	25.93	2.99	5.88
Lepidoptera	Drepanidae	0	33.33	0	0	0	5.88

2.4.6. Indices of dietary diversity (Prediction 3)

Simpson's diversity and Shannon's H were consistently high across the diets of the warblers (Shannon's H range = 4.5 – 5.7) but were elevated in reed and sedge warblers (Table 2.7). Although reed warblers had the highest species richness overall, the Chao estimator of richness indicated that the likely species richness for sedge warblers (1042 species ± 95.23) was greater than for reed warblers (891 species ± 62.98), and this species also had the widest dietary niche. Chiffchaffs showed the lowest scores for all indices, with Cetti's warblers and willow warblers intermediate relative to the other warblers. Reed and sedge warblers both had the greatest niche breadth scores at Oxwich in 2017 followed by 2018 at the same location, but the remaining warblers showed their greatest niche breadth at different sites. Niche breadth was narrowest for each species in the early summer but peaked in either the middle or the late summer.

The GLM investigating species richness in the warbler samples was significant (adjusted R-squared = 0.05, F= 3.24 on 11 and 405 degrees of freedom, $p<0.001$). Site (LRT = 18.41, $p<0.01$), species (LRT = 12.69, $p<0.05$) and age (z = 5.65, $p<0.05$) were significant predictors of species richness in the dietary samples (Fig. 2.5). Year (p = 0.11) and Julian day (p=0.46) were not significant but were retained in the model after using the drop1 function. Adult birds had a higher dietary richness than juvenile birds (z = 2.26, $p<0.05$), and birds captured at Wheldrake (z = 3.86, $p<0.01$) and Oxwich (z = 2.92, $p<0.05$) had significantly higher richness per sample than birds captured at Chew. Most species pairs did not differ significantly from each other in terms of prey richness, but reed warbler samples contained more dietary items on average than sedge warbler samples (z = 3.11, $p<0.05$, Table 2.7iii).

Table 2.7. Indices of dietary richness in the focal warbler species at UK sites, among seasons and years. Species richness (number of unique items detected) and the diversity indices Shannon H, Simpson's Diversity, Levin's Index, Chao Estimate and percentage of species diversity described (Chao estimated diversity/observed species richness) was calculated for each focal warbler species, and all warbler species combined, and split i) across the four main study sites and ii) across seasons. Chao estimator and % diversity described were not calculated for Cetti's warblers at Oxwich in 2018 or birds captured at Magor Marsh, due to insufficient sample size. Table 7 iii) indicates the mean number of unique prey items per sample (± SD/SEM) and the range per warbler.

i)

Warbler Species	Site	Species Richness	Chao Estimator ± S.E.	% Diversity Explained	Shannon H	Simpson's Diversity	Levin's Index
Reed Warbler	All sites	550	891.40 ± 62.98	61.70	5.68	0.99	154.80
	Chew Valley	218	368.36 ± 37.99	59.18	4.81	0.98	60.30
	Kenfig NNR	252	535.25 ± 66.33	47.08	5.04	0.98	79.06
	Oxwich 2017	329	555.83 ± 56.79	59.19	5.43	0.99	176.57
	Oxwich 2018	167	272.07 ± 28.54	61.38	4.89	0.99	100.78
	Wheldrake Ings	145	307.97 ± 51.74	47.08	4.37	0.98	69.88
	All sites	494	1042.02 ± 95.23	47.41	5.66	0.99	157.57

Warbler Species	Site	Species Richness	Chao Estimator ± S.E.	% Diversity Explained	Shannon H	Simpson's Diversity	Levin's Index
Sedge Warbler	Chew Valley	98	234.14 ± 50.12	41.86	4.02	0.97	30.16
	Kenfig NNR	137	380.69 ± 73.26	35.99	4.67	0.98	65.18
	Oxwich 2017	260	538.76 ± 62.83	48.26	5.26	0.99	136.18
	Oxwich 2018	124	388.82 ± 85.16	31.89	4.54	0.99	70.62
	Wheldrake Ings	125	335.25 ± 69.59	37.29	4.54	0.98	69.48
Cetti's Warbler	All sites	172	329.32 ± 43.60	52.23	4.74	0.98	77.32
	Chew Valley	81	327.80 ± 113.76	24.71	4.11	0.98	46.34
	Kenfig NNR	55	107.53 ± 23.49	51.15	3.93	0.97	47.11
	Oxwich 2017	82	259.84 ± 68.75	31.56	4.09	0.98	40.89
	Oxwich 2018	33	NA	NA	3.41	0.96	27.74
Chiffchaff	All sites	143	289.69 ± 44.06	49.36	4.63	0.98	70.94
	Chew Valley	56	116.49 ± 29.75	48.07	3.81	0.97	36.89
	Kenfig NNR	35	125.13 ± 63.62	27.97	2.92	0.94	25.04
	Oxwich 2017	39	201.00 ± 108.74	19.40	3.26	0.96	36.75
	Wheldrake Ings	64	149.49 ± 37.22	38.47	4.01	0.97	45.08
Willow Warbler	All sites	166	362.98 ± 57.32	45.73	4.75	0.98	80.21
	Kenfig NNR	79	192.45 ± 47.68	41.05	3.99	0.98	36.54
	Oxwich 2017	67	174.14 ± 46.62	38.47	4.06	0.97	48.51
	Wheldrake Ings	68	161.09 ± 40.04	42.21	4.03	0.98	44.94
All Species	All sites	895	1424.60 ± 75.06	62.82			
	Chew Valley	320	523.90 ± 43.55	61.08			
	Kenfig NNR	375	621.23 ± 48.89	60.36			
	Oxwich Marsh	571	981.51 ± 70.26	58.18			
	Wheldrake	285	484.11 ± 43.32	58.87			

ii)

Warbler Species	Season	Species Richness	Levin’s Index
Reed Warbler	Early	164	76.98
	Middle	365	134.67
	Late	376	150.26
Sedge Warbler	Early	167	98.26
	Middle	294	161.82
	Late	241	89.64
Cetti’s Warbler	Early	87	46.67
	Middle	112	66.01
	Late	45	39.20
Chiffchaff	Early	32	27.56
	Middle	58	43.76
	Late	98	60.31
Willow Warbler	Early	37	28.25
	Middle	81	49.12
	Late	97	64.51

iii)

Species	Mean number of prey items per sample (± SD (SEM))	Range
Reed Warbler	12.19 ± 7.7 (± 0.55)	1 – 38
Sedge Warbler	10.11 ± 6.6 (± 0.57)	1 – 37
Cetti’s Warbler	12.87 ± 8.1 (± 1.45)	2 – 32
Chiffchaff	10.78 ± 4.5 (± 0.87)	2 – 19
Willow Warbler	12.59 ± 5.5 (± 1.02)	2 – 25

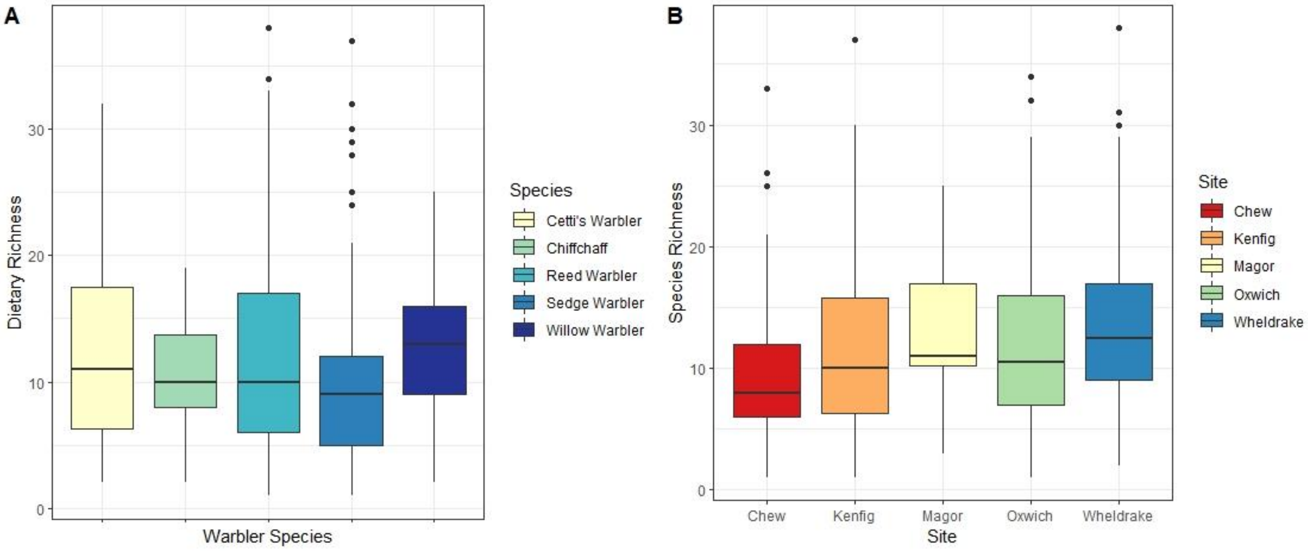


Figure 2.5. Boxplots of dietary richness (the number of unique dietary items per sample) for UK warblers split across A) the five focal warbler species and B) the five study sites (including Magor Marsh).

2.4.7. Prey choice analysis (Prediction 3)

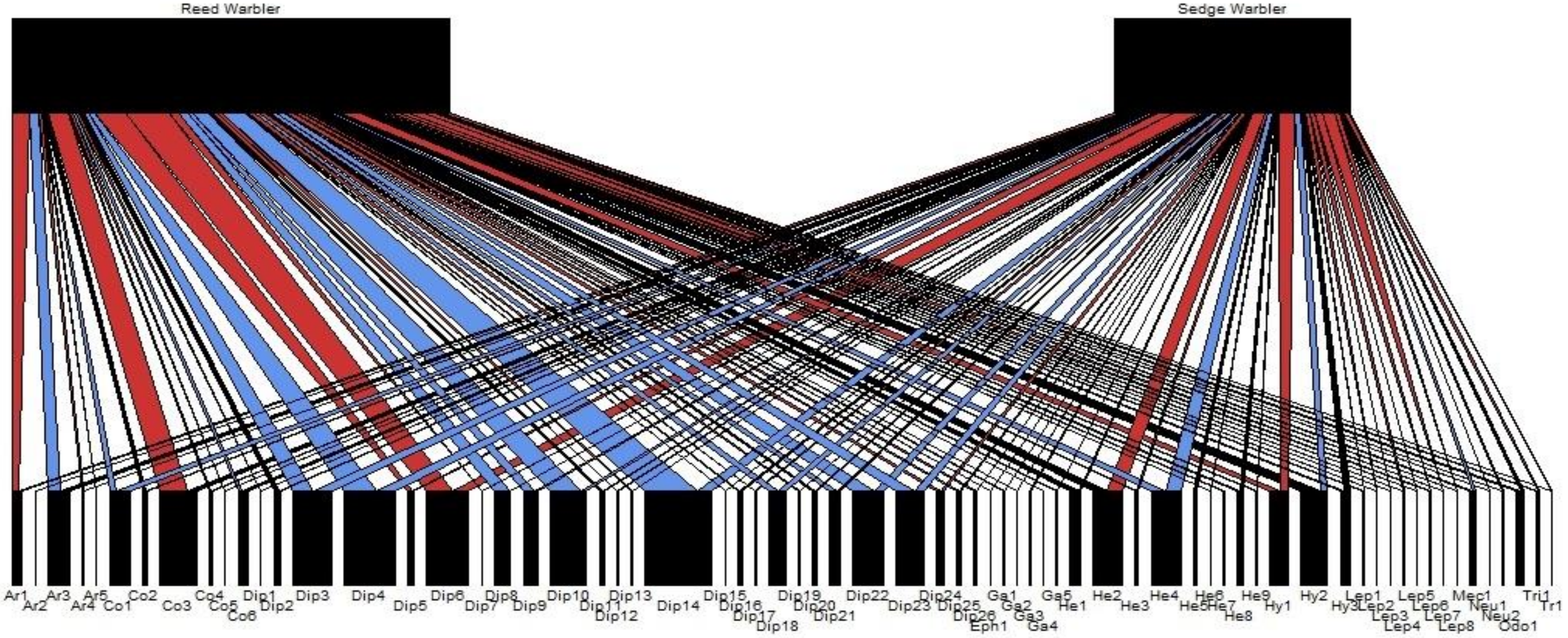
In 2017, 13.12% of trophic interactions between warblers and invertebrate families were significantly higher or lower than expected by the null model. Our GLM explaining prey preferences (SES values from *econullnetr*) in 2017 was significant (adjusted R-squared = 0.46, F = 21.58 on 19 and 443 df, p<0.001). The total abundance of each individual prey family was negatively associated with its SES (t = -9.52, p<0.001). Reed (t=3.1, p<0.01) and sedge (t=2.7, p<0.01) warblers showed stronger preferences for larger prey species. Chiffchaff prey size selection was marginally non-significant (t=1.7, p=0.08) whereas Cetti’s warblers and willow warblers did not show an association with prey size and SES. Lepidopteran prey families were more strongly selected than families of all the remaining main prey orders (p<0.001). In addition, nematocerous flies (Diptera: Nematocera) were more strongly selected as prey than either Coleoptera (z = 3.48, p<0.05), Hymenoptera (z = 3.38, p<0.05) or the brachyceran flies (z = 4.46, p<0.001).

Patterns of prey selection in 2017 (prey taken at a greater rate than expected from their recorded abundance) varied among the warbler species, and many consumer-specific preferences were revealed (Appendix 1.11, Table A.1.11*a*, Fig. 2.6). Nonetheless several prey groups were positively selected by multiple (if not all) warbler species including nematocerous flies: (notably craneflies, mosquitoes and midges) chrysomelids, aphids, braconid wasps and numerous lepidopteran families. In reed warblers, Cetti’s warblers and sedge warblers the families Hemerobiidae

(Neuroptera), Leptoceridae (Trichoptera) and the spider families Clubionidae and Tetragnathidae were jointly selected. Many of the same prey families were avoided by multiple warbler species, especially with increasing effect size. These tended to be small-sized prey items (Chloropidae, Cetatopogonidae, Sciariidae, Pteromalidae), and to a lesser extent, agile, fast-moving prey that may be difficult to capture (muscoid flies).

Patterns of prey group preference and avoidance by warblers varied over the course of the summer when data from 2017 and 2018 were combined (Appendix 1.11, Table A.1.11*a*). Some families were always consumed at a rate disproportionately higher or lower than their abundance no matter when in the summer the interaction occurred (for example chironomids, chaoborids, in all warblers except for Cetti's warbler), whereas others were targeted by different warblers at different periods of the summer, which might indicate dietary switching. For example, in reed, sedge and willow warblers, aphid consumption exceeded that expected from aphid abundance in the late summer, but (with the exception of sedge warblers) they were consumed in proportion to abundance in early and mid-summer.

Site level differences were also apparent from the subset *econullnetr* analysis (Appendix 1.11, Table A.1.11*b*). The most strongly selected and strongly avoided groups (very high or low SES) tended to be consistent both across sites and warbler species but other preferences varied by site and/or species as the availability of the prey item changed. Occasionally a prey item preferred by one species at one site is preferred by a different species at another (e.g., Beraeidae in reed and Cetti's warblers). Between year differences were more subtle. Preferences at Oxwich in 2017 were often consistent and held in 2018 but there were several exceptions. For example, prey associated with aquatic or moist habitats (Beraeidae, Baetidae, Chaoboridae and Chironomidae) were preferred by reed and sedge warblers at Oxwich in 2017 but were consumed at the same frequency as predicted by the null model in 2018 (Fig. 2.7).



prey item codes: Ar1 = Clubionidae, Ar2 = Dictynidae, r3 = Linyphiidae, Ar4 = Tetragnathidae, Ar5 = Theridiidae, Co1 = Cantharidae, Co2 = Carabidae, Co3 = Chrysomelidae, Co4 = Coccinellidae, Co5 = Helophoridae, Co6 = Scirtidae, Dip1 = Anisopodidae, Dip2 = Calliphoridae, Dip3 = Cecidomyiidae, Dip4 = Ceratopogonidae, Dip5 = Chaoboridae, Dip6 = Chironomidae, Dip7 = Culicidae, Dip8 = Dolichopodidae, Dip9 = Empididae, Dip10 = Hybotidae, Dip11 = Lauxaniidae/Drosophilidae, Dip12 = Limoniidae, Dip13 = Micropezidae, Dip14 = Muscidae/Fannidae/Anthomyiidae, Dip15 = Mycetophilidae, Dip16 = Opomyzidae/Tephritidae, Dip17 = Pipunculidae, Dip18 = Psychodidae, Dip19 = Ptychopteridae, Dip20 = Rhagionidae, Dip21 = Sarcophagidae, Dip22 = Scathophagidae, Dip23 = Sciaridae, Dip24 = Syrphidae, Dip25 = Tachinidae, Dip26 = Tipulidae, Eph1 = Baetidae, Ga1 = Agriolimacidae, Ga2 = Arionidae, Ga3 = Helicidae, Ga4 = Lymnaeidae, Ga5 = Succineidae, He1 = Anthocoridae, He2 = Aphididae, He3 = Aphrophoridae, He4 = Cicadellidae, He5 = Delphacidae, He6 = Gerridae, He7 = Lygaeidae, He8 = Miridae, He9 = Psyllidae, Hy1 = Braconidae, Hy2 = Ichneumonidae, Hy3 = Tenthredinidae, Lep1 = Choreutidae, Lep2 = Crambidae, Lep3 = Depressariidae, Lep4 = Erebidae, Lep5 = Gelechiididae, Lep6 = Geometridae, Lep7 = Noctuidae, Lep8 = Tortricidae, Mec1 = Panorpidae, Neu1 = Chrysopidae, Neu2 = Hemerobiidae, Odo1 = Coenagrionidae, Tri1 = Leptoceridae, Tr1 = Geoplanidae

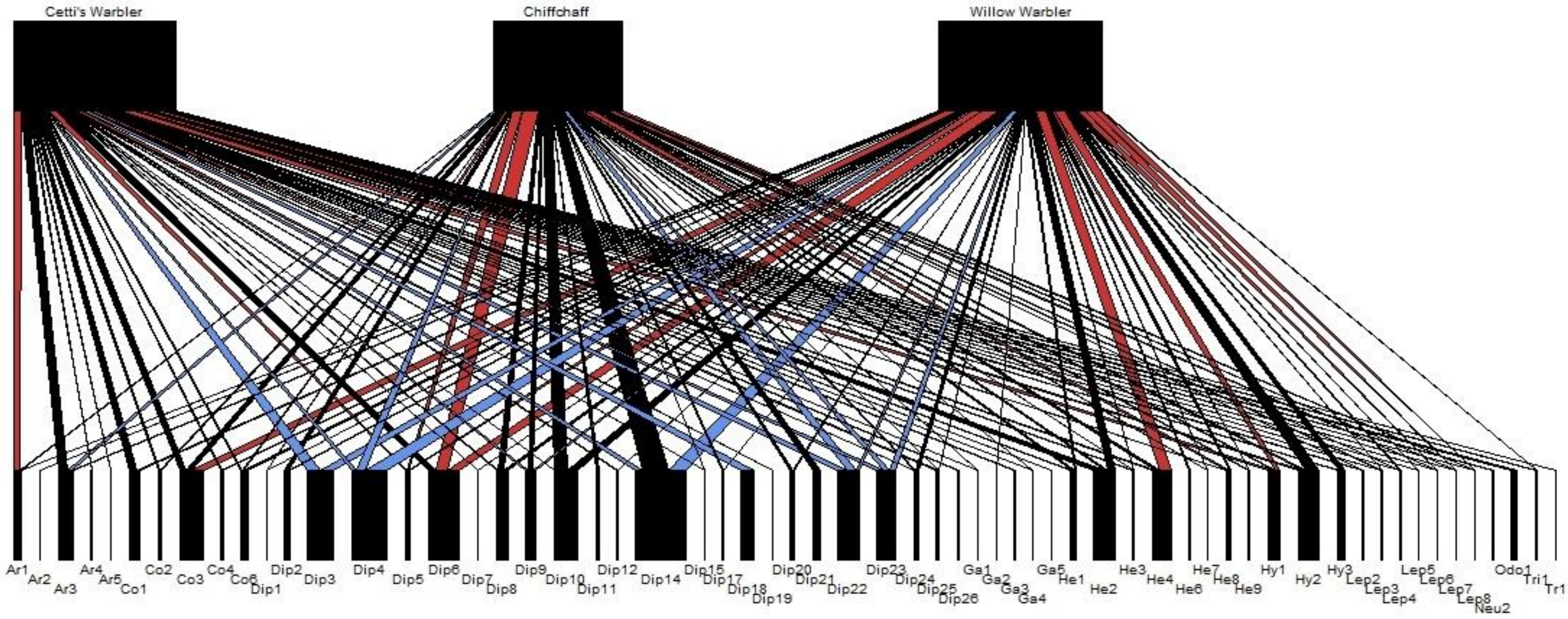
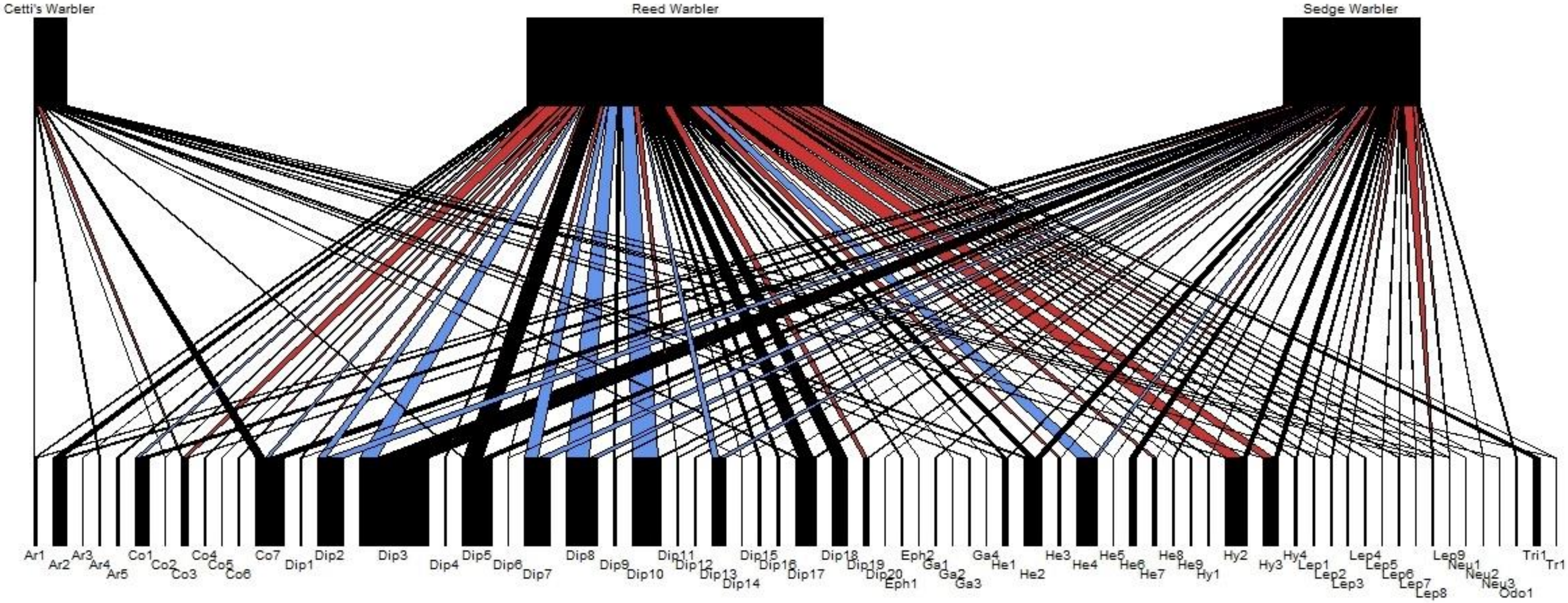


Figure 2.6. Bipartite plot showing positive and negative interactions between warbler species and invertebrate families in this study in 2017. For ease of interpretation rare prey families were removed from the plot but not the analysis (see Appendix 1.11 for a full list of interactions). Interactions are colour coded, and the arrow width indicates the strength of interaction. Blue = consumed at a frequency lower than expected based on the availability of the prey item, orange = consumed at a frequency higher than expected based on the availability of the prey item, white = consumed at a frequency not significantly different to the rate expected by the null model, i) reed and sedge warblers, ii) Cetti’s warblers, chiffchaffs and willow warblers.



Key to prey item codes: Ar1 = Clubionidae, Ar2 = Linyphiidae, Ar3 = Philodromidae, Ar4 = Tetragnathidae, Ar5 = Thomisidae, Co1 = Cantharidae, Co2 = Carabidae, Co3 = Chrysomelidae, Co4 = Coccinellidae, Co5 = Helophoridae, Co6 = Kateretidae, Co7 = Scirtidae, Dip1 = Calliphoridae, Dip2 = Cecidomyiidae, Dip3 = Ceratopogonidae, Dip4 = Chaoboridae, Dip5 = Chironomidae, Dip 6 = Culicidae, Dip 7 = Dolichopodidae, Dip8 = Hybotidae, Dip9 = Lauxaniidae/Drosophilidae, Dip10 = Muscidae/Fanniidae/Anthomyiidae, Dip11 = Opomyzidae/Tephritidae, Dip12 = Pipunculidae, Dip13 = Psychodidae, Dip14 = Ptychopteridae, Dip15 = Rhagionidae, Dip16 = Sarcophagidae, Dip17 = Scathophagidae, Dip18 = Sciaridae, Dip19 = Syrphidae, Dip20 = Tipulidae, Eph1 = Baetidae, Eph2 = Heptageniidae, Ga1 = Agriolimacidae, Ga2 = Arionidae, Ga3 = Lymnaeidae, Ga4 = Succineidae, He1 = Anthocoridae, He2 = Aphididae, He3 = Aphrophoridae, He4 = Cicadellidae, He5 = Coreidae, He6 = Delphacidae, He7 = Miridae, He8 = Nabiidae, He9 = Psyllidae/Triozidae, Hy1 = Aphelinidae, Hy2 = Braconidae, Hy3 = Ichneumonidae, Hy4 = Tenthredinidae, Lep1 = Coleophoridae, Lep2 = Crambidae, Lep3 = Erebidae, Lep4 = Gelechiididae, Lep5 = Geometridae, Lep6 = Glyphipterigidae, Lep7 = Lasiocampidae, Lep8 = Noctuidae, Lep9 = Tortricidae, Neu1 = Chrysopidae, Neu2 = Coniopterygidae, Neu3 = Hemerobiidae, Odo1 = Coenagrionidae, Tri1 = Hydroptilidae, Tr1 = Geoplanidae.

Figure 2.7. Bipartite plot showing positive and negative interactions between warbler species and invertebrate families in this study in 2018. For ease of interpretation rare prey families were removed from the plot but not the analysis (see Appendix 1.11 for a full list of interactions). Interactions are colour coded, and the arrow width indicates the strength of interaction. Blue = consumed at a frequency lower than expected based on the availability of the prey item, orange = consumed at a frequency higher than expected based on the availability of the prey item, white = consumed at a frequency not significantly different to the rate expected from the null model.

2.4.8. Prey characteristics: body size and habitat associations (Prediction 4)

Aquatic and terrestrial prey

Year (LRT = 12.63, p<0.001), season (LRT = 15.42, p<0.01), cumulative GDD for *H.pruni* (t = -3.09, p<0.01), and the interaction between warbler species and site (LRT = 40, p<0.001) were all significant predictors of the proportion of aquatic prey items consumed per individual bird (adjusted R-squared = 0.39, F = 11.79 on 25 and 391 degrees of freedom, p<0.001, Fig. 2.8). Age was marginally non-significant (LRT = 3.76, p=0.05).

Birds captured in 2017 consumed significantly more aquatic prey than birds captured in 2018 (z = 3.44, p<0.001). In addition, birds captured in the late summer, consumed significantly more aquatic prey than those in the early (z = 2.44, p<0.05) and mid-summer seasons (z = 3.72, p<0.001). However, there was a negative association with growing degree days and aquatic prey contribution (t = -3.09, p<0.01) suggesting that some of the variation in consumption of aquatic prey is explained by external temperature. Birds captured at Kenfig (z = 6.53, p<0.001), Chew (z= 12.2, p<0.001) and Wheldrake (z = 8.64, p<0.001) consumed a higher proportion of aquatic prey items than birds captured at Oxwich. Warblers at Chew and Wheldrake respectively, consumed a higher proportion of aquatic prey than Kenfig (z = 5.72, p<0.001, z = 2.76, p<0.05)) and Magor Marsh (z = 3.8, p<0.01, z = 2.76, p<0.05).

Cetti’s warblers consumed a significantly lower proportion of aquatic prey items than reed (z = -3.76, p<0.01) and sedge (z = -4.14, p<0.001) warblers. Moreover, differences between species varied according to site. Sedge warblers at Chew consumed a greater proportion aquatic prey than reed warblers (z = 2.58, p<0.01) but both consumed a higher proportion than Cetti’s warblers (z = 5.15, p<0.01, z = 4.0, p<0.05).

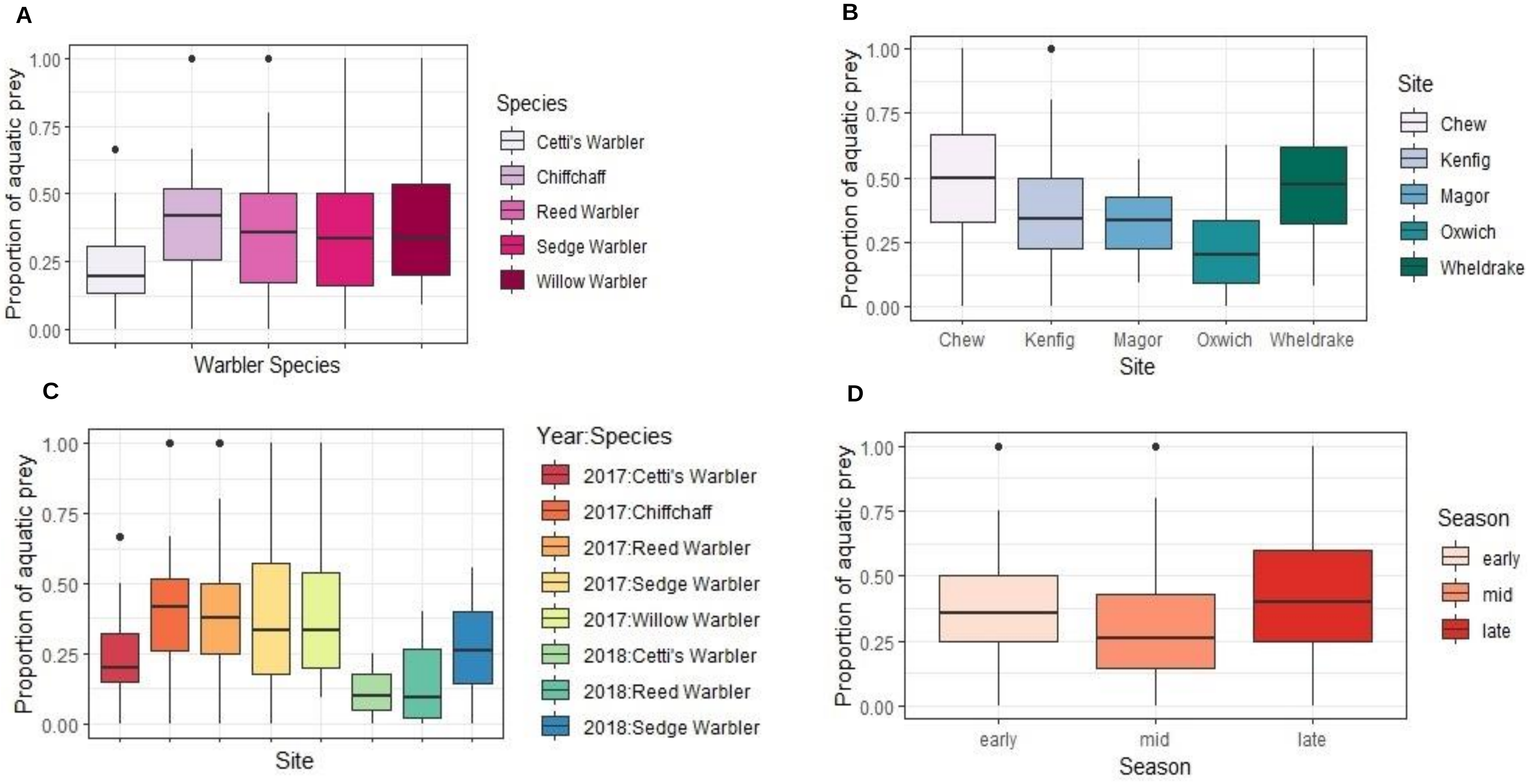


Figure 2.8. Boxplots of the proportion of aquatic prey detected per dietary sample in UK warblers across A) focal warbler species, B) the five study sites, C) warbler species according to year of capture and D) season (period of the breeding season/summer).

Prey body-size selection

The model examining the patterns of prey-size detected in diet samples was significant overall (adjusted R-squared = 0.09, F = 4.27 on 13 and 404 df, p<0.001). Site was not significant in explaining average prey body size (p = 0.08) but the interaction between warbler species and age class was significant (F = 3.35, p<0.05). Site was retained in the model to avoid pseudoreplication. The post-hoc Tukey test (averaged over the sites) of the age-species interaction revealed that Cetti's warbler juveniles consumed prey of a significantly larger body length than both reed warbler adults (z = 3.23, p<0.05) and juveniles (z = 3.90, p<0.01), as well as the juveniles of chiffchaffs (z = 3.71, p<0.01), sedge warblers (z = 3.97, p<0.01) and willow warblers (z = 4.01, p<0.01). Willow warbler adults also consumed significantly larger prey than willow warbler juveniles (z = 3.27, p<0.05). Warbler species as a term on its own was not significant in explaining variation in the consumption of prey of different sizes, and this was apparent in the average body lengths of different invertebrate orders across focal species (Table 2.8). Cetti's warblers consumed the largest prey on average when all orders were combined but this was not a great enough difference to be significant in the model and was largely explained by age (Fig. 2.9).

Table 2.8. Mean prey body lengths in millimetres of invertebrate species (± standard deviation) consumed by the warblers in this study, organised by taxonomic order. Where only one species from a given order was detected across the samples from a given focal warbler; standard deviation = 0.0.

	Mean body length (mm) ± SD							
Warbler Species	All Orders	Araneae	Coleoptera	Diptera	Gastropoda	Hemiptera	Hymenoptera	Lepidoptera
Cetti's Warbler	7.9 ± 2.3	5.2 ± 1.9	3.9 ± 1.5	5.9 ± 1.4	17.9 ± 7.2	2.9 ± 1.2	3.4 ± 1.2	13.84 ± 2.4
Chiffchaff	6.5 ± 1.4	4.1 ± 3.1	3.8 ± 1.1	5.7 ± 1.0	22.5 ± 10.6	4.3 ± 1.4	3.3 ± 1.0	13.9 ± 3.6
Reed Warbler	6.5 ± 1.5	5.5 ± 2.2	4.4 ± 1.3	5.8 ± 1.3	24.2 ± 7.2	4.4 ± 2.4	4.8 ± 1.9	13.1 ± 4.5
Sedge Warbler	6.6 ± 1.8	5.4 ± 2.2	4.3 ± 1.4	5.8 ± 1.7	22.6 ± 5.2	4.2 ± 2.9	8.9 ± 1.7	13.1 ± 3.8
Willow Warbler	6.9 ± 1.9	3.4 ± 0.7	3.7 ± 0.7	5.8 ± 1.4	30.0 ± 0.0	3.9 ± 1.4	4.3 ± 1.6	15.9 ± 2.8

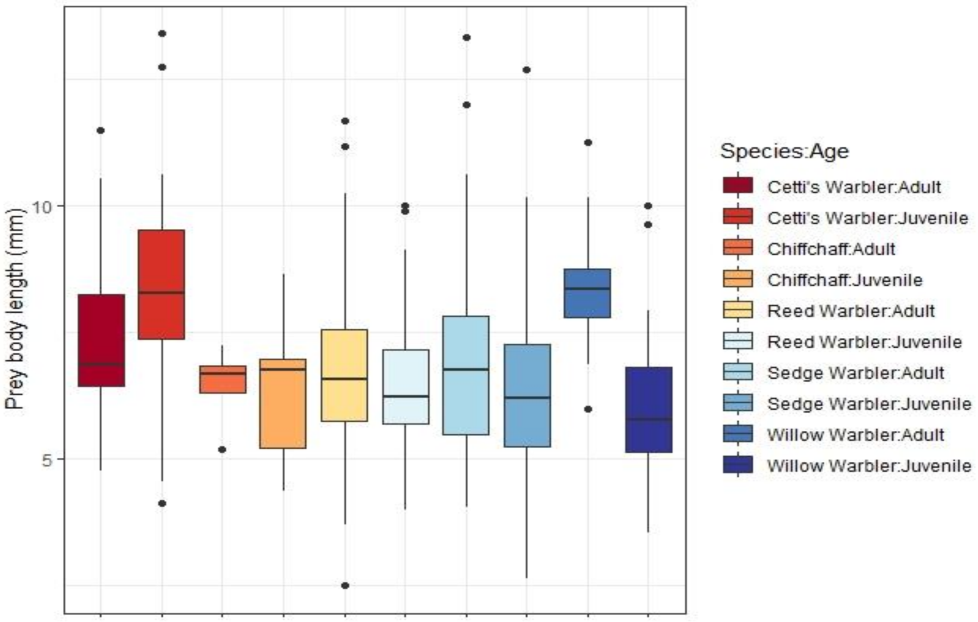


Figure 2.9. Box plot of the average body length of all prey items (in mm) detected in the diets of the focal warbler species subset by age (adults and juveniles).

2.5. Discussion

2.5.1. Summary

The results of this chapter demonstrate that warbler communities in UK wetlands show subtle diet differentiation at the family and species level, despite most species pairs showing moderate to high overlap. At least a portion of the dietary preferences held by the different warbler species can be explained by optimal foraging theory (Krebs and Davies 1991, Brodmann and Reyer 1999). Prey abundance and diversity in the field was variable across sites, seasons and years and this resulted in changes in the observed diets of warblers and patterns of diet overlap. Where a higher degree of partitioning of food resources by different warbler species did occur, it was associated with lower abundance and diversity of prey relative to other scenarios in time and space.

2.5.2. Dietary richness and prey availability

A high diversity of arthropods was consumed by warblers, consistent with past literature that describe warblers as opportunistic foragers (Bibby and Green 1983, Simms 1985, King et al. 2015). The Chao richness estimates imply that the birds may encounter and consume many more possible prey species (ranging from 400-600 additional species), highlighting both the generalist nature of the warblers and the spatio-temporal variation in availability of different prey species.

Dietary richness differed across UK sites, with each focal species diet often reaching its greatest diversity at differing sites to other species, indicating a degree of flexibility in dietary breadth. In addition, some sites may have been better for certain species than others in terms of food availability (Stephens et al. 2007, Naef-Daenzer 2012, Pagani-Núñez et al. 2015). At least one climate shift occurred between the northern and southern study sites in the UK. Wheldrake, the most northerly site, experienced lower summer temperatures, translated into lower GDD production, which may have delayed prey emergence times compared to further south. Prey abundance peaked in the middle of summer at Wheldrake suggesting there may be a shorter window of invertebrate abundance, shouldered between cooler spring and autumn temperatures.

Different populations or sub-populations of the same consumer species might utilise a specialist or generalist approach to feeding depending on the habitat experienced (Quevedo et al. 2009). This can occur once a "search image" is developed for specific taxa commonly encountered in the environment (Krebs et al. 1978, Tucker 1991, Symondson 2002). Reed and sedge warblers had the highest diet richness and niche breadth overall, whereas chiffchaffs showed the lowest, indicating a slightly more specialised diet. This species shows greater specialisation in its habitat preferences than the closely-related willow warbler (Gregory et al. 2005, Smart et al. 2007, Lerche-Jorgensen et al. 2019), however this is the first study to suggest that this is translated into a more specialised diet at a fine taxonomic resolution.

2.5.3. Patterns of dietary overlap

For the majority of warbler species pairs, dietary overlap ranged from moderate to high, (Pianka range - 0.36-0.77), and only one species pair showed significant dietary partitioning. The highest overlap was between reed and Cetti's warblers (0.77) and reed and sedge warblers (Pianka = 0.76). Even though the latter pair are documented as segregating their foraging habitats when in sympatry (Catchpole 1972), they nonetheless shared a large proportion of dietary items. In addition, sedge warblers and chiffchaffs (0.62) showed relatively high overlap, despite these two species differing in habitat occupation and presumably foraging technique (Simms 1985, Cramp and Brooks 1992).

If prey resources are limiting, high overlap indicates that each consumer is more restricted in their dietary choices (Clare et al. 2014*b*, Salinas-Ramos 2015). However, the diversity of dietary prey detected in our study was vast, and dietary overlap was usually highest in scenarios where prey abundance increased. This contradicts the earlier prediction made (prediction 2) that increased prey abundance and richness would be associated with lower dietary overlap in the focal warblers. Removal of the most frequently consumed prey items led to a decrease in Pianka's overlap index between pairs of consumers. These high-frequency prey items are likely very common in the field and are perhaps i) locally non-limiting and/or ii) widespread, associated with multiple habitats, both of which make them widely available and likely to be shared among all warblers. The less common prey items were not shared as extensively and may have been preferred by different bird species or taken by different birds using different foraging habitats or feeding techniques (Heithaus et al. 1975, Pagani-Núñez et al. 2015).

Our dietary overlap results are similar to those discussed in other recent studies of passerine diet (Orłowski and Karg 2013, Trevelline et al. 2018, Kent and Sherry 2020). Trevelline et al. (2018) found that the diets of riparian bird species overlapped greatly as a result of very high seasonal abundances of aquatic invertebrates. These supplemented the surrounding feeding habitats and were available to all birds. Kent and Sherry in 2020 detected high dietary overlap in neotropical warblers that masked subtle, species-specific dietary differences. The same pattern is further evidenced in this study in that the *manyglm* multivariate model indicated that diet composition was significantly different among the focal warbler species. The observed patterns of frequency of occurrence for each warbler species implied that although a common suite of species was consumed by most if not all warblers, different prey species, families and even orders were consumed at quite different frequencies by each warbler.

Spatio-temporal changes in overlap

In a study by Laursen (1978), large and rapid shifts of dietary overlap occurred in relation to changes in food abundance and bird numbers. Seasonal changes in overlap within the summer of 2017 were evident in our study, with lower than expected overlap detected among pairs captured during the early summer when prey abundance and richness was low compared to the remainder of the summer. Niche breadth was narrowest in the early summer, which may indicate that development of some prey groups is still temperature limited at this time (Barlow 1962). Most species pairs showed higher overlap and individual species showed highest dietary breadth in the middle and late summer, consistent with the theory that a combination of warmer temperatures and sufficient elapsed development time (Barlow 1962, Bryant et al. 2002), leads to higher invertebrate abundance and diversity and more mass emergences of aquatic prey (Sardiña et al. 2017) that become common, shared components of the diet of multiple species (Trevelline et al. 2018).

Dietary overlap decreased significantly, indicating partitioning of dietary items, between reed and sedge warblers from the 2017 breeding season to the 2018 breeding season, the latter coinciding with a much warmer and drier summer and lowered measured invertebrate abundance and lower prey diversity with respect to 2017. Partitioning between some of the other warbler species was already occurring in Oxwich in 2017 and both years at Oxwich showed lower invertebrate richness than the other sites, so differences in environmental conditions, invertebrate habitat composition and vegetation structure at Oxwich compared to Chew and Kenfig may be partly behind the patterns revealed.

Oxwich comprised generally drier, less diverse reedbed habitat even in 2017, compared to more complex reedbed flora and more widespread submerged zones at the remaining sites. A similar pattern of site differences was described by Bibby and Green in their 1983 study of three French marshlands. Vegetation structure and habitat heterogeneity differed between two of the sites, one with a monoculture of *Phragmites* reedbed and the other with a more diverse vegetation structure and richer flora within the reedbeds. These differences were mirrored in the measured invertebrate fauna and resulting diet of the warblers occurring there.

Dietary overlap and niche breadth

Dietary niche breadth in both reed and sedge warblers was highest at Oxwich in 2017 followed by 2018. Interestingly, the broadening of diet coincided with greater dietary niche partitioning in this pair, which was unexpected (Salinas-Ramos et al. 2015, Clare et al. 2014*b*). However, when prey is highly abundant, predators should specialise on a few prey types that are highly profitable, but when abundance decreases, predators are expected to select a greater range of prey, including items that are less profitable (Pyke et al. 1977, Krebs et al. 1977, Pulliam 1980a, Pulliam 1985). The lower abundance and diversity of prey at Oxwich may mean that preferred prey is not as easily available, and diets broaden to incorporate less profitable prey (Salinas-Ramos et al. 2015). Warblers are able to use multiple foraging habitat types so there was likely still a high enough prey diversity at Oxwich across habitats to permit both species to exhibit both a broader dietary niche alongside minimal sharing of prey types. Contrastingly at Chew and Wheldrake diets were narrowed (except in the *Phylloscopus* warblers), but this coincided with dietary overlap, which at Chew was very high. Prey abundance may have been high enough at Chew to allow birds to narrow their dietary niche and simultaneously show increased overlap with other competitors, since enough profitable prey such as large chironomids were present to support both species.

2.5.4. Diet composition and observed preferences

In the UK sites, despite the presence of overlap, each species showed a relatively distinct diet and species-specific preferences, perhaps due to individual feeding adaptations to capture specific prey types (Whitaker 2004, Pagani-Núñez et al. 2015). Feeding on different prey types minimises the risk of competition, which is evolutionarily advantageous (Whitaker 1994, 2004). Warbler traits and feeding behaviours may be selected to retain the ability to feed on a wide range of prey items, so that they can better capture locally abundant prey (that serve as a buffer when preferred groups are limiting), while also exhibiting species-specific traits to best capture the optimal prey available (Whitaker 2004).

In accordance with other long-standing studies on marshland warblers, birds at different sites differed considerably in diet composition. Chironomids and craneflies were consumed in especially high proportion at Wheldrake perhaps as a result of lower temperatures with respect to the southern sites. Milder temperatures coupled with increased moisture levels are known to be most favourable for Diptera with a northerly distribution, particularly cold-adapted groups which are negatively affected by drought and increased summer temperature (Bale et al. 2002, Pearce-Higgins et al. 2010, Carroll et al. 2011). Between-year differences (at Oxwich) were smaller than between site differences. This suggests that site level differences are not solely due to temperature and rainfall but are probably affected by other climatic factors as well as habitat composition, reed-bed management, topography and geographic location (Bibby and Green 1983, Bibby and Thomas 1985).

Chironomid consumption

Chironomid and chaoborid midges were the most frequently consumed invertebrates in all warbler species. This finding was not surprising given that midges, especially chironomids, are abundant in temperate zones and cited as important prey for warblers, (Bibby and Thomas 1985, Chernetsov and Manukyan 2000, Dyrcz and Flinks 2000, López-Iborra et al. 2005, King et al. 2015) and other passerines (McDade et al. 2011), especially during periods where other prey are limiting (Dyrcz and Flinks 2000). In this study, they were available throughout the breeding season, across a wide range of weather conditions, and in multiple habitats utilised by the different warblers. Chironomids fly in swarms which are mainly active during dusk and dawn but settle in the vegetation and remain relatively immobile during the day, making them easily captured prey (López-Iborra et al. 2005, Bell 2011). Accordingly, larger chironomid individuals on sticky traps, that more closely matched the average size of chironomid species consumed by the focal warblers (~5-7mm, see Appendix 1.10), were strongly associated with traps near deeper standing water or near permanent waterbodies, and with the presence of scrub vegetation (López-Iborra et al. 2005). Large chironomids were likely preferred by warblers over smaller ones as predicted by optimal foraging theory (Turner 1982, Robinson and Wilson 1998, Leisler et al. 2002). This is further evidenced by the fact that smaller nematocerans such as Cecidomyiidae and Sciaridae were consumed far less frequently by birds, despite having a similar abundance to chironomids on the sticky traps.

The recorded (sticky trap) chironomids were significantly smaller in both years at Oxwich than at the remaining sites and also generally smaller than the estimated average size for the chironomid species consumed by the warblers in this study. This could be due to the drier prevailing conditions, leading to limited aquatic productivity at Oxwich. This result explains why both frequency of occurrence and preferences (SES) for chironomids by warbler consumers were lower at Oxwich. In addition, dietary diversity and niche breadth was higher in Oxwich warblers, suggesting that birds might rely less on chironomids at this site and instead supplement the diet with other invertebrates. One caveat to this assumption is that the size of the chironomids in the diet was estimated from the average size for the species detected and significant variation in size within a species may occur in some invertebrate taxa. However, this finding is in accordance with a study on chiffchaff diet, which found only larger chironomids (>6mm) in diet samples, despite higher availability of medium and small chironomids in the environment (López-Iborra et al. 2005).

Consumption of the reed plum aphid

In this study there was mixed evidence for the importance of the reed plum aphid to migrating warblers. Sedge warblers with larger fat deposits sampled between July and late August consumed this aphid species at much higher frequency than by birds with smaller fat deposits or those captured earlier in the summer. The availability of this aphid is highly variable across sites and years (Ormerod et al. 1991*b*, Chernetsov and Manukyan 2000), and in our study *H. pruni* was largely consumed by the sedge warblers at just one site, Oxwich (most in 2017). In contrast, reed warblers consumed *H. pruni* at the three southern sites, but consumption was not related to body fat or period of the summer when the bird was captured. It should be noted that reed warblers do not use the same fuelling strategy as sedge warblers, since the former migrate with short steps, accumulating smaller fat reserves at each stopover site (Bibby and Green 1981, Katarzyna et al. 2018). Our results align with Chernetsov and Manukyan (2000) that reed plum aphids might be important fuel for migrating birds where available, but they can be substituted with alternative prey at least in the UK sites studied here. Sedge warblers sampled during the pre-migratory stage where *H. pruni* was not present or in low numbers, may simply travel further south and fuel on aphids there before departure (Bayly et al. 2007). No evidence was found for the so-called “aphid gap” in warmer years, as birds captured in Oxwich in 2018 consumed *H. pruni* on similar dates to 2017. It may be that the cooler temperatures experienced in the late winter of 2018 did not allow enough GDD to accumulate earlier in the year to promote advanced timing of aphid emergence in 2018 compared to 2017.

Diet selectivity

It was evident that different warbler species generally did not partition their diets through differential use of prey sizes. This aligns with what has previously been documented in warbler assemblages of a similar size and body morphology (Laursen 1978, Bibby and Green 1983, Marchetti et al. 1998). Nonetheless, both reed and sedge warblers showed a significant preference (SES > 2) for larger prey taxa, consuming larger prey families at a frequency disproportionately higher than expected from their recorded abundances. This may indicate a preference for larger and more nutritious prey items that is constrained by the need to optimise food intake while reducing handling times (Davies 1977, Marchetti et al. 1998), (i.e., optimal foraging (Turner 1982, Robinson and Wilson 1998, Leisler et al. 2002), since larger invertebrates are often difficult to capture (Moore and Simm 1985). While Cetti’s warbler juveniles consumed larger prey on average, they and the remaining warblers did not show a significant preference for this characteristic. Consumption of larger prey by this species may be a side-effect of them using habitats where larger taxa are more frequently encountered (Bibby and Green 1983, Cramp and Brooks 1992).

Lepidoptera and nematocerous flies were consistently targeted above all other prey orders by all warblers, alluding to their high value to birds (Robinson and Wilson 1998, Skipper and Kim 2013). Smaller prey items that are probably less profitable from an optimal foraging perspective were usually avoided by warblers. In addition, some of the more agile, mobile prey families were also consumed less than expected probably because of the high energy expenditure required to capture them (Turner 1982, Bibby and Green 1983, Pulliam 1985). Invertebrate families in the environment were more likely to be preferred as prey by warblers if their relative abundance was low. It is likely that some prey types are consumed at a higher frequency when they are less available simply because they are i) highly profitable prey that will always be targeted, ii) of an optimal body size, iii) because of a search-image formed from past interactions or iv) a combination of the above (Krebs et al. 1978, Turner 1982, Tucker 1991, Symondson 2002).

Dietary preferences differed with time and space suggesting that prey switching may be common in this system as patterns of invertebrates changed. This again alludes to high dietary flexibility and perhaps nutritional requirements that change over time.

Consumption of aquatic prey and the importance of cross-system subsidies

Numerous studies that demonstrate the great importance of aquatic subsidies (i.e., emerging aerial prey) to terrestrial insectivores, particularly birds and bats (Polis and Hurd, 1996, Nakano and Murakami 2001, Sanzone et al. 2003, Orłowski and Karg 2013, Trevelline et al. 2018, Lewis-Phillips et al. 2020). Aquatic insects are rich in highly unsaturated omega-3 fatty acids (HUFA), the availability of which is associated with reproductive success in adult birds and the likelihood of successful fledging in nestlings (Twining et al. 2018). These prey often emerge in swarms or insect chimneys, which may be profitable for birds to target as the energetic cost to capture multiple prey from the same patch is lower than searching for and capturing individual prey items (Bell et al. 2001, Ornes 2013, Sherry 2016).

All warblers in the study frequently consumed prey with an aquatic or semi-aquatic life stage. Chiffchaffs and willow warblers consumed a surprisingly high proportion of aquatic invertebrates despite having a stronger association with seasonal, terrestrial habitats than the reedbed warblers. Most of the aquatic fauna they consumed preferentially were generally abundant on sticky traps in both scrub and reed, suggesting high local availability and/or dispersal ability into *Phylloscopus* habitats. This may suggest that these two warblers largely forage in scrub or woodland vegetation but opportunistically feed on prey that cross the aquatic-terrestrial interface into these habitats (Nakano and Murakami 2001, Lewis-Phillips et al. 2020). Despite chironomids making up a substantial part of the diet, they were consumed less frequently by Cetti's warblers, and aquatic/semi-aquatic prey, on the whole, did not contribute as great a proportion to Cetti's warbler diet as it did in other species. This is likely to be a result of their feeding behaviour, with this species tending to forage at low vegetation heights and sometimes from the ground (Bibby and Green 1983).

Both consumption and strength of preference for some aquatic prey families fell between 2017 and 2018, due to drier conditions. The reduction in aquatic subsidies may be cause for concern from a conservation standpoint (Lewis-Phillips et al. 2020). Aquatic and semi-aquatic prey are locally very abundant during emergence windows, but if aquatic resource phenology and/or availability continues to be altered due to widespread habitat degradation or climate change, as implicated by our study, there may be a risk of future predator/prey mismatches in terrestrial insectivores that depend on such prey subsidies (Twining et al. 2018). If future climate change brings similar conditions to those at Oxwich in 2018 (i.e., drier, warmer conditions), prey availability – particularly of aquatic groups – may decrease and competition between songbirds might increase. This would have implications for future survival and reproductive success rates, eventually affecting longer-term population trends.

Several prey families associated with scrub habitat such as Lepidoptera and herbivorous Hemiptera were more frequent in reed warbler diets in the warmer year, which might suggest that scrub is more heavily utilised as an alternative feeding habitat when aquatic subsidies on the breeding grounds are reduced. The importance of vegetation heterogeneity may thus also be an important factor in habitat management for warblers.

This study advocates conservation approaches that improve landscape habitat heterogeneity, alongside careful, monitored reedbed management. This should be carried out with the aim of enhancing invertebrate biodiversity and availability to support the wider ecological community, including terrestrial insectivores.

2.5.5. Study limitations

Many of the challenges faced in this Chapter are also mirrored in future chapters. A comprehensive list of study limitations is given here that can be applied to the whole PhD project. General limitations to metabarcoding are discussed in Chapter 6.

Some sample sizes were uneven and small for certain study sites and species, so findings were interpreted cautiously in analyses where data were split by multiple factors, such as site and season. The proportions of samples for the three main warbler species; reed, sedge and Cetti's roughly matched the relative proportions of mist-netted birds (inferred from BTO ringing data for each site in 2017).

Measures of prey size were an average body length for each species based on the adult stage, from multiple sources and publications. Therefore, it is likely that some items selected by the birds may have been larger or smaller than indicated, either because of individual variation in body size within an invertebrate species or differences in size due to life stage sizes differing within a species (e.g., imago moth *versus* 1st instar caterpillar or even eggs).

Future studies should be cautious to avoid over-analysing single instance preferences for individual prey families by a single bird species. Moreover, some of the highly positive SES values seen in groups such as moths and gastropods could be a result of low measured abundance on sticky traps which might allude to sampling biases affecting some invertebrate orders (Romeis et al. 1998, Southwood and Henderson 2000, Thomson et al. 2004). Such biases may also have skewed estimations of overall arthropod abundance and richness. Nonetheless, yellow sticky traps were considered the most appropriate and effective sampling method available to passively capture both mobile, flying insects as well as a number of sessile prey groups (Thomson et al. 2004) that together formed the majority of warbler prey items.

Finally, and most importantly, confirmation of interspecific competition is only possible with experimental manipulation of populations in the field, such as removing one competitor and assessing the impact on niche breadth and dietary composition in the remaining birds (Navalpotro et al. 2016). This was not feasible in this study; however, I nonetheless provide limited evidence for the existence of mechanisms that prevent competition and allow coexistence in warblers cohabiting the same wetland ecosystems.

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Chapter Three - Age-class-specific variation in the diet and prey selectivity of Eurasian reed warblers at Chew Valley Lake.

Top left: the reedbeds at Chew Valley lake, bottom left: a reed warbler nest with eggs (Wikimedia Commons), top right: a juvenile (left) and adult (right) reed warbler (Photo creds: Owain Gabb; Gower ringing group), bottom right: an adult reed warbler with nestlings (Photo creds: Richard Nicholl 2013).

3.1. Abstract

Songbirds are subject to different selective pressures over the course of their lifespans. As a result, their dietary requirements are unlikely to remain static from nestling to juvenile to adult stage. Moreover, the abundance of prey is likely to fluctuate over the course of the breeding season, and birds might show flexibility when feeding to maximise optimal foraging. In this chapter, metabarcoding techniques were used to elucidate the diet of Eurasian reed warblers at different life stages (*Acrocephalus scirpaceus*). Prey remains were sequenced from the faeces of adult, juvenile and nestling birds at Chew Valley Lake in Somerset, UK, over the 2017 breeding season, alongside invertebrate monitoring in the field. Despite moderate to high dietary overlap between age classes, significant dietary differences were mediated through the selection of arthropod prey, i) from different taxonomic groups, ii) with different habitat associations (aquatic *versus* terrestrial prey) and iii) of different body sizes. Dietary prey richness also changed with age with adults consuming the greatest number of prey species. Nestling diet was distinguished by the presence of larger, softer-bodied prey items that reflected high value "breeding currency", i.e., relatively large, high-value prey items for nestling growth that optimise the overall reproductive success of parents. Juvenile diet indicated a stronger association with prey from aquatic zones that may have been locally abundant and easy to capture. Seasonal changes in prey abundance were reflected in the diet, particularly as prey-size selection and preferences for different prey families changed over the summer. Together, our results demonstrate the value of metabarcoding data for enhancing ecological studies, particularly those concerning generalist insectivores in dynamic environments.

3.2. Introduction

3.2.1. Food resources and fitness in birds

The acquisition of food resources is fundamental to fitness in animals, as a major driver of survival and reproduction. When foraging, individuals are under selective pressure to choose appropriate food resources for nutrition, while balancing the time and energy costs of obtaining the food resource (Krebs 1973, Davies 1977, Pyke et al. 1977, Meire and Ervynck 1986, Bautista et al. 1998). In songbirds, restricted food resources on the breeding grounds may lower the reproductive success of parents by reducing the body condition of nestlings, and their likelihood of survival to fledging (Rodenhouse and Holmes 1992, Sillett et al. 2000, Trevelline et al. 2016). Prey resources may vary substantially across the breeding season, and birds must adapt their foraging strategies to changing prey availability. In addition, as the bird ages from nestling, to fledgling, to adult, their nutritional requirements change to coincide with the new challenges they face. In this chapter I ask, does a generalist passerine, the Eurasian reed warbler (*Acrocephalus scirpaceus*) change its diet to meet these changing demands?

3.2.2. Expected dietary differences in birds of different life stages

Several studies have described acrocephalid diet at different stages of their life history, including growth and development, dispersal and territory formation, reproduction and chick-rearing and finally pre-migratory fuelling and passage migration (Bibby and Green 1983, Ezaki 1992, Chernetsov and Manukyan 2000, Dyrcz and Finks 2000, Kerbiriou 2011), but comparative studies focusing on variation in diet between age classes are mainly restricted to other avian groups such as seabirds (e.g. Navarro et al. 2010, Alonso et al. 2014).

In passerines, differences in adult and nestling diet might involve the consumption of i) different prey taxa ii) a greater or smaller diversity of prey groups or iii) different prey sizes. Juveniles form the bridge between the nestling and adult life stages, but little is known about how their diets may differ. Moreover, there are known age-dependent differences in the use of habitat by acrocephalid species which may in turn affect what prey items are encountered by adults and juveniles (Marchetti and Price 1989, Wunderle 1991, Preizner and Csorgo 2008). Several factors, discussed below, may drive differences in diet composition between nestlings, adults and juveniles.

Birds should be selected to optimise their diets by selecting prey resources on the basis of their nutritional content (Krebs 1973, Davies 1977, Pyke et al. 1977). Dietary differences between age groups may thus be driven by differences in prey selectivity for groups of invertebrates that satisfy specific nutritional requirements at a given life stage. In reed warblers, nestlings are fed by both parents until fledging at 11-14 days of age (Cramp and Brooks 1992). To achieve optimal reproductive success, adults need to provision their offspring accordingly and capture prey that will best benefit their offspring, allowing them to grow more rapidly, fledge earlier and ultimately gain a greater chance of surviving and successfully reproducing.

Like other altricial passerines, broods of the reed warbler require food that is both high in calories and protein-dense for growth and development (Krupa et al. 2004). Reed warbler nestlings are predominantly fed arthropods and molluscs, which are high in protein (Cramp and Brooks 1992, Grim 2006), but some invertebrate taxa may be more beneficial for the growth of passerine broods than others (Skipper and Kim 2013). “Breeding currency” is defined as high-value prey resources that are required for nestling development during the breeding season, and which, depending on their abundance, ultimately serve to improve overall breeding productivity (Greenberg 1995, Yard et al. 2004). The importance of particular types of prey as breeding currency is indicated by observations of adult birds feeding their brood a diet of predominantly caterpillars and other large, nutrient rich, soft-bodied prey (such as prey > 5mm, Araneae, soft-bodied Hemiptera, or the larvae of other insect orders) that are easily digested (Krupa 2004, Johnson et al. 2005, Orłowski et al. 2014, Xiong and Lu 2014, Ceia et al. 2016). However, these same prey characteristics may not be optimal for adult or juvenile birds. While the breeding productivity of adults may be related to the total abundance of breeding currency invertebrates, for growth of their nestlings, survival of adult (and juvenile) birds themselves may instead be regulated by the total abundance of all invertebrates, many of which are too small to be considered breeding currency but might offer other nutritional benefits or meet conditions for optimal foraging (Greenberg 1995, Johnson et al. 2005).

Foraging experience and learned behavior also affects what prey items are consumed. Newly fledged, first calendar year juvenile birds are inexperienced, and due to developmental constraints, may lack the dexterity and prey handling abilities needed to tackle certain prey groups (Marchetti and Price 1989). Moreover, adults which may still be breeding when juveniles are beginning to forage independently, are highly territorial and exhibit dominance over juveniles such that the latter may not have access to higher-quality foraging habitats (Wunderle 1991). Both of these mechanisms could cause a discrepancies between adult and juvenile diet.

Differential use of foraging habitats could be indicated by the habitat associations of invertebrate prey. For example, many insectivorous birds are reliant on aerial insects with an aquatic life stage (Baxter et al. 2005, Trevelline et al. 2016, Stanton et al. 2017). However, reed warbler feeding habitats are not limited to aquatic zones, rather they encompass a broader range of ecotypes, and this might lead to more encounters with terrestrial arthropods (Grim and Honza 1996) especially at times when preferred prey are limited (Turner 1982). Furthermore, adults may use several different foraging strategies when provisioning a nest full of hungry chicks (Root 1967). They may also take advantage of a broader range of foraging sites or forage differently for themselves and their offspring (Jedlicka et al. 2017).

Fledging birds are exploratory, making them more likely to encounter habitats that are less species-specific. In many acrocephalids, including the reed warbler, moustached warbler (*Acrocephalus melanopogon*), marsh warbler (*A. palustris)* and sedge warbler (*A. schoenobaenus*) juveniles show a wider range of habitat utilisation than the adults of the same species (Preiszner and Csorgo 2008, Leisler and Shulze-Hagen 2011). Habitats used by juveniles may be sub-optimal as a result of moving to areas where they can avoid competition with adults, evade predators, or capture prey more easily (Marchetti and Price 1989, Wunderle 1991, Preizner and Csorgo 2008).

Finally, the relative population densities of the different age classes are not constant over the summer. The first fledged juveniles are not foraging independently on the breeding grounds until the middle of the breeding season. Any intraspecific competitive pressure might intensify in the middle of the breeding season as more food resources are needed to sustain adults and juveniles. By the end of the breeding season fewer nestlings may remain within the breeding territories, once again reducing the energetic demands on adults that have completed brood rearing. These all have implications for dietary selectivity of the different age classes.

3.2.3. Additional drivers of diet differentiation

It is important to note that much of the diet of reed warblers may be unrelated to age or development. The main driver of diet composition in birds is usually prey availability, which is often highly variable both spatially and temporally (Aldridge and Rautenbach 1987, Whitaker 1994). Invertebrate emergence in reedbeds is temperature dependent, and the prey community present at the beginning of the breeding season in late April/May is often different to that at the end of summer in July/August (McKee and Richards 1996, Halupka et al. 2008, Vafidis et al. 2016). Thus, reed warblers might consume different prey as the composition and diversity of prey communities changes over the summer (Bibby and Thomas 1985, Vafidis et al. 2016).

Seasonal changes in prey availability are also tied to local weather patterns. The abundance of insects, especially those that develop in aquatic habitats, depends on precipitation (Janzen and Schoener 1968, Poulin et al. 2002, Rolfe 2011), and a combination of low precipitation and higher temperatures can result in declining arthropod richness and abundance, if wetland zones dry out (Frampton et al. 2000, Zhu et al. 2014).

3.2.4. Chapter aims and objectives

Until recently it has been difficult to compare the diets of reed warblers and other passerines at different life stages. The use of dietary metabarcoding has greatly improved the detection of prey in avian faecal samples (Jedlicka et al. 2013, 2017, Trevelline et al. 2016, 2018, da Silva et al. 2019, Moran et al. 2019, Rytokonen et al. 2019, Shutt et al. 2020). These new developments allow diets to be compared across distinct avian age classes.

In this chapter high-throughput sequencing (HTS) was used to compare the diets of reed warblers of three age classes – nestlings, fledged juveniles and adults, at a wetland site in the UK over the breeding season of 2017. The hypothesis of this study is that age will be a predictor of diet in reed warblers and that each age class will show dietary differences. These differences may be related to i) prey composition (i.e., the families or species of prey consumed), ii) prey size, iii) prey diversity/richness or iv) habitat associations of the prey; e.g., terrestrial prey *versus* prey with an aquatic or semi-aquatic life stage. The extent of dietary overlap between pairs of age-classes is also assessed and invertebrate prey availability is measured to determine dietary preferences between the age classes.

Furthermore, it is hypothesised that diet will differ over the breeding season, either as a result of birds tracking their diet to changes in prey availability, or because of changes in prey selectivity over time that are unrelated to prey availability. These seasonal effects are predicted to alter patterns of intraspecific dietary composition, dietary overlap, and prey selectivity between the age classes.

3.3. Methodology

3.3.1. Study site

Chew Valley Lake (Grid Ref: ST5659) is situated at the northern edge of the Mendip Hills in Somerset, England, UK. It is a large (approx. 486 ha) artificial lake surrounded by grassland, scrub and carr woodland with extensive reedbed habitat around the fringe of the lake. The reedbeds are dominated by stands of the common reed (*Phragmites australis*) but also includes other grasses such as *Typha* and *Carex* species. Extensive herbaceous vegetation such as nettles (*Urtica* spp.) and willowherbs (*Epilobium* spp.) form a boundary between the wetland and scrub habitats. Scrub and woodland habitats encroach the reedbeds in some areas and they are predominantly composed of willows (*Salix spp.*) but also feature oaks (*Quercus* spp.), birch (*Betula* spp.), poplar (*Populus* spp.), bramble (*Rubus* spp.) and hawthorn (*Crataegus* spp.).

The lake itself is an important site for wildlife and is a designated as a Site of Special Scientific Interest (SSSI) and a Special Protection Area (SPA). The site also comprises hedge-bounded pasture which is grazed by cattle. Chew Valley bird ringing station (CVRS) has been active on-site since the 1960s and bird ringing activities take place regularly year-round. At least one or more constant effort sites (CES) are monitored every summer between the end of April and August, as part of the British Trust for Ornithology’s constant effort ringing survey, with standardised bird ringing activities taking place at least once every 10 days.

3.3.2. Faecal sample collection

Adults and juveniles

Collection of reed warbler faecal samples took place throughout the breeding season at Chew Valley Lake. Visits were split between two ringing locations that were situated on two opposite sides of a small section of the west side of the lake; 51°19'57.76"N, 2°38'16.39"W and 51°19'48.76"N, 2°38'15.23"W. Bird ringing sessions took place approximately once a week between the end of April and the end of August 2017 as part of the British Trust for Ornithology (BTO) CES population monitoring programme. At both sites, mist nets were set up in a mixture of habitats, including through a section of reedbed and the surrounding scrub and woodland.

Faecal samples were collected from adult and juvenile warblers captured by mist netting, with the assistance of fully licensed ringers. The protocol for sample collection was identical to Chapter 2. Adults were classed as birds hatched at least one calendar year before, whereas juveniles were birds hatched in the current year. Birds were aged as juvenile or adult based on plumage, with adults showing signs of wear and bleaching from the sun on the primaries and tail, and juveniles showing very fresh flight and tail feathers and juvenile or moulting body feathers and/or aligned growth/fault bars on the tail.

Nestlings

Faecal samples were collected from reed warbler nestlings by a licensed ringer from the Chew Valley ringing group with a *pulli* ringing licence endorsement from the British Trust for Ornithology (BTO). Reed warbler nests were located using a combination of i) systematic searching through sections of reedbed surrounding the two CES sites, ii) following individual reed warblers to their nest sites (either by visual observation or following singing/calling birds by sound), and iii) re-visiting known nesting sites from previous years. Nests were visited regularly as part of the BTO nest monitoring scheme between May and early August 2017. At each monitored nest ($n = 40$), chicks were gently removed from the nest to be ringed and their estimated age (in days), and nest ID was recorded, along with the total number of nestlings present in the nest. Any faecal sacs produced by the nestling were collected directly with sterile forceps and immediately transferred to 100% ethanol in sterile 2ml Eppendorf tubes. To prevent premature fledging or disturbance of newly hatched young, only nestlings that were of a ringable age (i.e., between 7 and 10 days old) were handled. To avoid repeated disturbance, and to prevent predators from finding the nests, samples were only collected from an individual nest once over the summer period, even if the same nest had a second brood. Samples of adult, juvenile and nestling birds were initially stored in a domestic freezer (~-10°C) for up to two weeks before transfer to a laboratory freezer for long term storage at -80°C.

3.3.3. Weather data

Data for minimum temperature, maximum temperature and rainfall were obtained for each day from April to September 2017 from the nearest available weather station to Chew Valley, located in Bishop Sutton ~ 2km away (www.weatherunderground.com). Total rainfall for each week was calculated by taking the sum of each consecutive day of rainfall every 7 days. Maximum and minimum temperature for each week was represented by the average of the maximum and minimum daily temperature values, per 7-day period.

3.3.4. Invertebrate availability data

Prey availability was measured via sticky trap monitoring, as described in Chapter 2. Monitoring took place three times over summer to capture early, middle and late summer abundances of arthropods in reedbed and scrub habitats, with trap plots in close vicinity to the CES mist nets. Invertebrates captured on the sticky traps were identified to family level where possible using the same methods as described in Chapter 2.

3.3.5. Molecular analysis, bioinformatics and data processing

The entire metabarcoding methodology from sample extraction through to sequencing was performed as described in Chapter 2 with the following modifications. Nestling faecal samples were collected as a whole sac and as a result were encased in a higher amount of uric acid than adult and juvenile samples. Removal of uric acid was found to improve amplification success of prey DNA from bird faeces. This was validated by comparisons of PCR success (visualised by gel electrophoresis) from rounds where uric acid was not removed and rounds where uric acid was almost entirely removed. Thus, where possible, the surrounding uric acid was removed from the nestling samples before extraction, but due to the structure of the faecal sac, complete removal could not always be achieved. The same bioinformatics pipeline as outlined in Chapter 2 was used to clean the resulting diet dataset following assignment of sequences to taxonomic IDs. This process should have minimised artefacts from tag-jumping and contamination errors, providing a good baseline from which to begin statistical analyses.

Data for nestlings were not pooled by nest to avoid potential problems of uneven sampling effort between pooled nests and individual adults and juveniles, which could inflate estimates of dietary richness and frequency of occurrence of prey items in nestlings. Instead, diet data from only one individual nestling per nest, were selected at random for inclusion in the study. This served to avoid pseudo-replication in the next steps from the sampling of several nestlings from one nest. Diet data across samples were grouped into species, family, and order level. Frequency of occurrence (FOO) of a prey taxon was calculated by summing the number of instances that taxon occurred across all sampled individuals. % frequency of occurrence (% FOO) was calculated for each age class by dividing frequency of occurrence of all individuals per group by the number of samples per age class and converting to a percentage. Data were organised by “season”: either “early”, “middle”, or “late” which corresponded with the date the individual bird was captured and sampled. Early season was defined as May-early June when birds begin to breed (adults and nestlings present), middle season; mid-June to mid-July, in the height of the breeding season when all age groups are present, and late season; mid-July to the end of August when birds are ending their breeding and preparing for Autumn migration. These three periods matched the timescale of the three sampling periods during which the invertebrate monitoring occurred.

3.3.6. Statistical analysis

Dietary diversity estimates and age comparison

All analyses were carried out in R version 3.5.1. and RStudio version 1.1.463 (RStudio Team 2016, R Core Team 2018). To test whether species richness (total number of unique taxa in each sample) differed by age class, season and weather (the two-way interaction between the average daily maximum temperature and total rainfall over the week prior to sampling) data were fitted to an initial Poisson family general linear model, but an overdispersion parameter of >2 was revealed. The standard errors were corrected using a quasi-GLM model where variance was theta*mu, where mu was the mean of the dependent variable distribution and theta was the dispersion parameter of the quasi-model. Model validity was tested by plotting deviance residuals vs, each candidate independent variable, to determine which variables warranted inclusion in the model. Pairwise differences between factors were measured with post-hoc Tukey tests which adjust the p-value for multiple comparisons, using the package *emmeans* (Lenth 2020).

To test the assumption that Lepidoptera are valuable "breeding currency" for nestling diet (Greenberg 1995), a Mann-Whitney *U* test was used to test for significant differences in the proportion of dietary items that were from i) the order Lepidoptera and ii) dipteran suborder: Nematocera between each age pair. These two insect groups were chosen for this comparison because they represent some of the most available, diverse and heavily utilised prey resources for insectivores (Erwin 1982, Stork 2018), often forming much of the dietary diversity in molecular studies on bats (Vesterinen et al. 2018) and birds (Trevelline et al. 2016, 2018, Rytokonen et al. 2019, Shutt et al. 2020). Lepidoptera, while not the only example of breeding currency arthropods likely to be present at the study sites, are the most commonly cited representative arthropods that fulfil the requirements of the term, being uniformly soft-bodied (particularly larvae), generally of a large size (>5mm), high in calories and protein, and furthermore their larvae are known to be important components of nestling diet as evidenced by a large number of studies (e.g., Krupa 2004, Maziaz and Wesołowski 2010, Skipper and Kim 2013, Orłowski et al. 2015, Wesołowski et al. 2019). Contrastingly, Nematocera are generally small-bodied insects and are unlikely to be considered breeding currency for nestlings.

The diversity estimates Shannon's H and Simpson's diversity were calculated for each age class, based on frequency of occurrence data using the package *vegan* (Oksanen 2018). Levin's Index of niche breadth was also calculated using the package *spaa* (Zhang 2016). The Chao variant of extrapolated richness of adults, juveniles, nestlings and all ages combined was estimated using the specpool function in *vegan* with a small sample size correction *(N-1)/N*, where *N* is the number of age categories (=3) (Chao 1987, Palmer 1990, Oksanen 2018). The proportion of the total dietary diversity explained by our observed data was calculated from this value (observed species richness/Chao extrapolated estimate).

Diet composition

An analysis of the factors affecting the dietary composition at the family level was carried out using the package *mvabund* (Wang et al. 2012), using the same methodology (*manyglm*) as Chapter 2. The data were fitted with a binomial family *manyglm* with a complimentary log-log ("cloglog") link function to the data. The predictor variables, age and time of the breeding season captured (season) were tested with the function *anova* . The univariate test option within *anova* was applied to identify any significant relationships between the test variables and specific prey families within the diet matrix. Non-metric multi-dimensional scaling (NMDS) based on Jaccard dissimilarities was used to visualise the differences in diet among ages and season, in *vegan.* One extreme outlier was removed from the visualisation because it strongly impaired the visibility of the remaining data points in the plot. Although the "stress" value was high (stress = 0.23) a third axis was not shown, in order to ensure ease of interpretation.

Additional models for diet characteristics

For each dietary sample, prey items were divided into two classes; i) "aquatic" here encompassed both semi-aquatic or aquatic prey groups, identified by having one or more aquatic stage in the life cycle, and ii) "terrestrial" encompassed all terrestrial prey taxa. The proportion of aquatic invertebrate species detected in reed warbler diets was tested across ages and seasons using a two-way analysis of variance (ANOVA) and a post-hoc Tukey test. Entomological literature, specialist websites and invertebrate keys were systematically searched to find approximate body lengths in millimetres as a proxy for the body size of each of the detected prey species in the diet samples (as described in Chapter 2). The average prey size of items in each faecal sample for each bird was calculated and differences in mean prey size between adults, juveniles and nestlings was determined using a one-way ANOVA and a post hoc Tukey test.

Dietary overlap tests and prey choice model

Pairwise analyses were performed for adults, juveniles and nestlings using a null model simulation to calculate Pianka's measure of dietary overlap (Pianka 1973) using the *EcoSimR* package (Gotelli and Ellison 2013). A species matrix with frequency of occurrence data across each age class for each prey taxon was used in the analysis. This analysis was run for pairwise ages for all birds, to determine the extent of overlap. To determine the effect of season, separate analyses were run, subsetted for birds captured during early, middle and late summer.

Prey consumed in the diet was compared to the monitored prey abundance at Chew Valley Lake from sticky trap sampling (see Chapter 2 for detailed methodology). The R package *econullnetr*

(Vaughan et al. 2018) was used to characterise prey preferences among warblers of different ages and whether preferences changed across the seasons (early, middle and late summer visits). To determine what prey characteristics affected the feeding preferences of birds, the standard effect sizes (SES) of the consumer-resource interactions from the *econullnetr* analysis were log-transformed (after making all values positive) and used as the dependent variable in a Gamma GLM with an identity link function. Observed preferences were tested against the independent variables; prey size, reed warbler age class, total abundance of the prey from sticky trap data (coinciding with the time the specific age class was present on the breeding grounds), and prey taxonomic order. Prey families in the diet that were not recorded on sticky traps were omitted from the model because inclusion greatly skewed the model residuals plot and violated assumptions of the GLM. Several data points had very high influence in the residuals vs leverage plots, and they were removed from the model to improve fit, however their removal did not affect the significance of the model, or significance of individual predictor variables.

3.4. Results

3.4.1. High-throughput sequencing

In total, 140 reed warbler faecal samples were collected at Chew Valley, including 65 nestling samples collected from 42 nests. The remaining 75 samples were collected from adults and juveniles. After amplification, sequencing and bioinformatic processing, 60 samples (39 adult samples and 21 juvenile samples) contained dietary data giving a success rate of 80%. Of the 65 nestling samples, dietary items were identified in 42, giving a success rate of 64%. Thirty (n =30)samples from unique nests were selected from the 42 individual samples. In total, 102 samples contained dietary data, giving an overall success rate of 71.4% from DNA extraction to data acquisition. After selecting one nestling sample per nest, the total number of samples was 90 (30 nests, 21 juveniles and 39 adults).

3.4.2. Diet composition

Following taxonomic assignment, 258 prey species were detected from the total of 90 reed warbler faecal samples, comprising 94 invertebrate families and 11 orders (Table 3.1). Of these, 95 species were detected from nestlings, 103 species from juveniles, and 175 species were detected from the adult reed warbler samples. On average each sample contained a mean of 9.1 unique taxa ± 6.3 SD, ranging from 1 to 33 taxa (Fig. 3.1). Diptera was the most frequently detected order, present in 96.6% of samples. The remaining orders with the highest % frequency of occurrence were Lepidoptera (53.3%), Hemiptera (42.2%), Araneae (38.9%), Coleoptera (36.7%) and Hymenoptera 33.3%).

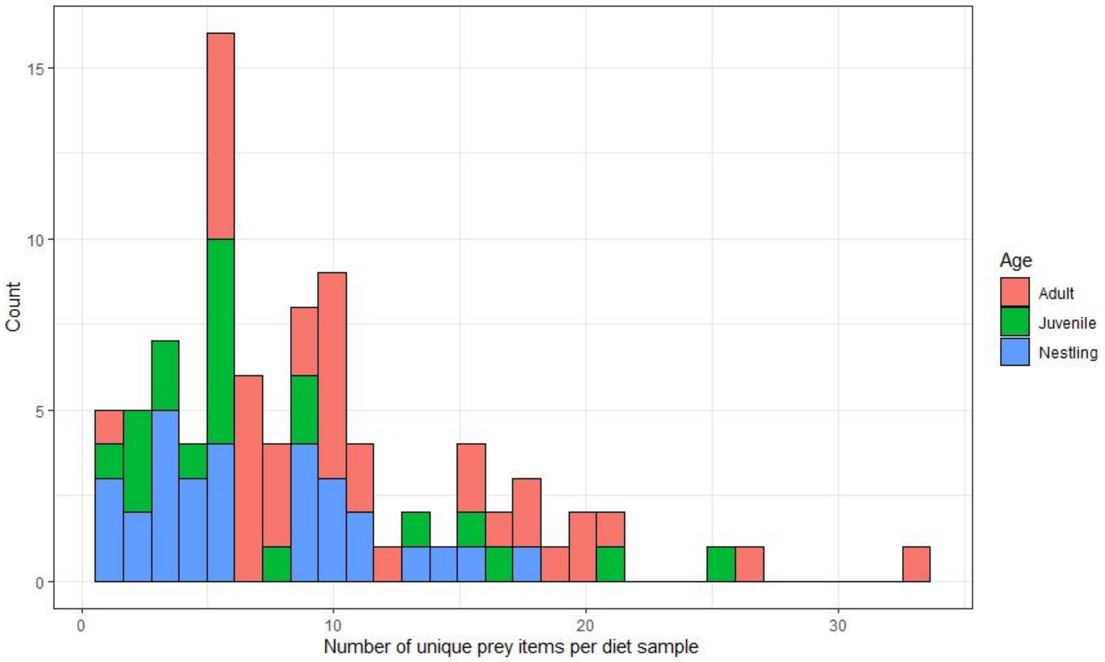


Figure 3.1. Histogram of the frequencies (counts) of the total number of unique prey items detected in each diet sample at Chew Valley according to age class.

By far the most abundant species detected was the phantom midge *Chaoborus flavicans*, present in 78% of samples, followed by two chironomid species; *Cladotanytarsus atridorsum* in 42% of samples and *Endochironomus albipennis* in 27% samples, a spider, *Clubiona phragmitis* in 24% of samples, a dungfly *Scathophaga stercoraria* in 21% of samples, a muscid fly *Helina* sp. in 17% of samples, and a caddisfly *Oecetis ochracea* in 16% of samples (Table 3.2). These taxa were detected in all age classes sampled. Lepidopteran species took up a significantly greater proportion of the total species present in each nestling sample on average, whereas this was significantly lower in adults (Mann-Whitney *U* test, w = 351.5, p<0.01), and in juveniles (w = 207.5, p<0.05). Contrastingly nematocerous Dipterans showed the reverse pattern, comprising a lower percentage of each sample in nestlings than in adult (w = 502.5, p<0.001) and juvenile reed warblers (w = 883, p<0.001, Fig. 3.2). Adults and juveniles did not differ significantly in the proportion of dietary species from either of these two groups.

Table 3.1. % Frequency of occurrence and species richness (number of unique prey items) of invertebrate orders detected in adult, juvenile and nestling reed warbler diet samples. Values are colour coded according to frequency of occurrence (darker colour = higher abundance).

	% Frequency of Occurrence			Species Richness		
Order	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)
Acari	0	4.76	0	0	1	0
Araneae	56.41	38.09	16.67	6	4	7
Coleoptera	48.71	33.33	23.33	14	10	4
Collembola	5.13	0	0	2	0	0
Diptera	100	100	90	91	51	47
Ephemeroptera	2.56	9.52	3.33	2	2	1
Gastropoda	25.64	4.76	26.67	3	2	5
Hemiptera	56.41	23.86	40	5	5	10
Hymenoptera	53.84	33.33	6.67	19	10	2
Lepidoptera	48.72	42.86	66.67	13	13	15
Neuroptera	5.12	9.52	6.67	2	2	2
Odonata	7.69	0	23.33	1	0	1
Psocoptera	7.69	9.52	0	1	1	0
Trichoptera	5.13	38.1	10	1	2	1

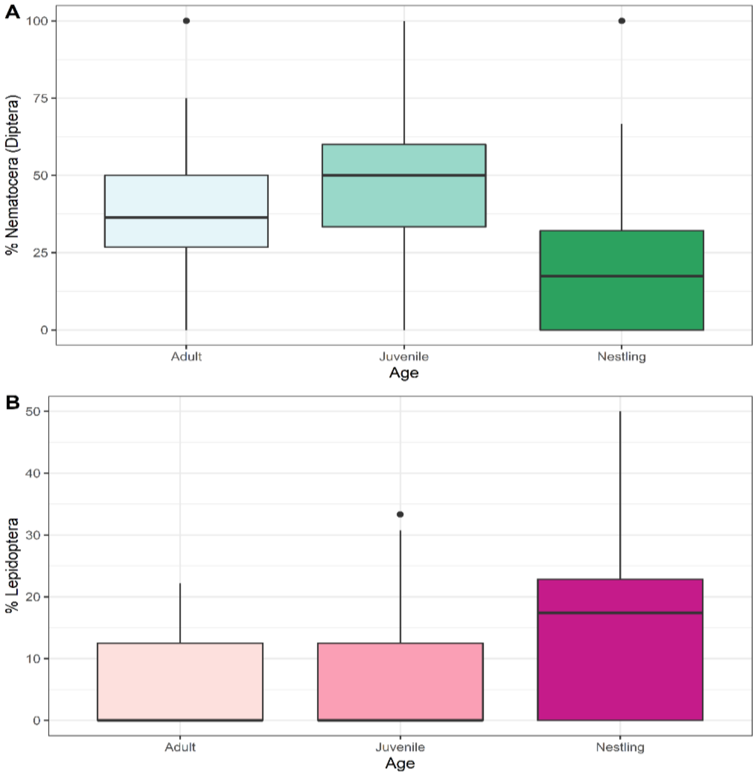
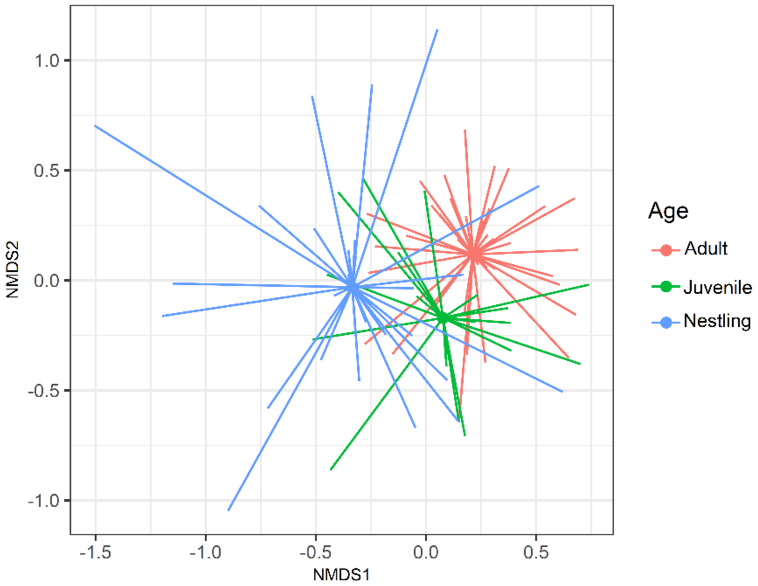


Figure 3.2. The percentage of dietary items per sample classified as A) nematocerous Diptera and B) Lepidoptera in adult, juvenile and nestling faeces.

Manyglm model

The final *manyglm* model in the *mvabund* multivariate analysis included the parameters age and season. Both age (LRT = 401, p<0.001) and season (LRT = 193.3, p<0.05) had a significant effect on the diet, with a different assemblage of prey families associated with different reed warbler age groups and different stages of the breeding season. Several individual prey families showed a significant association to a specific reed warbler age class. Erebidae were positively associated with nestlings (LRT = 14.26, p<0.05); Braconidae (LRT = 20.97, p<0.002), Tipulidae (LRT = 14.73, p<0.05) and Anthomyiidae (LRT = 17.79, p<0.01) with adults: and Hybotidae with both adults and juveniles (LRT = 13.21, p<0.05). Empididae was significantly associated with diet samples collected in the early breeding season (LRT = 14.73, p<0.05). The NMDS plots showed a degree of separation in the prey community with different age classes and seasons (Fig. 3.3).

A



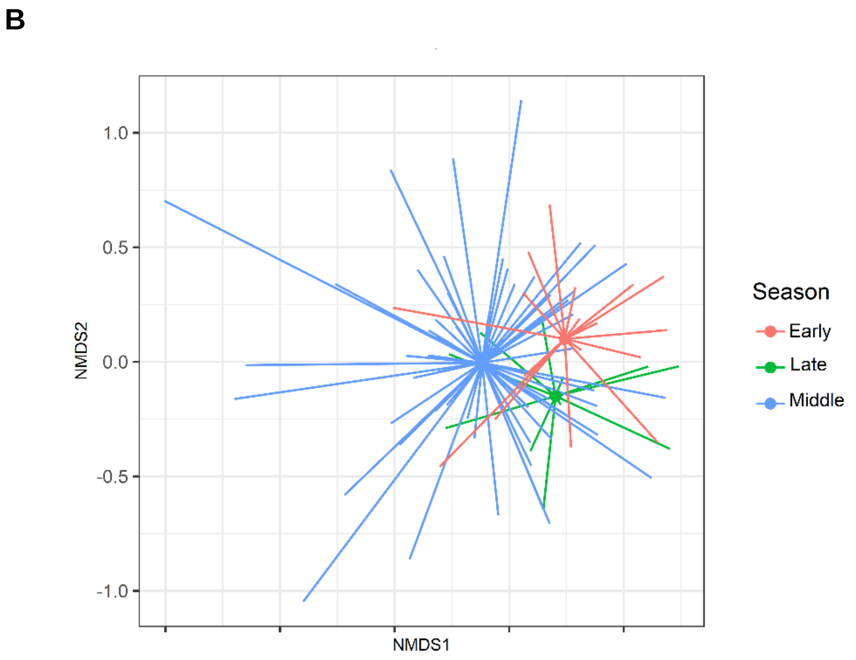


Figure 3.3. NMDS plot of the prey detected in reed warbler diet samples collected at Chew Valley according to the significant parameters from the *manyglm* model: A) age class, B) season (early summer, mid-summer, late summer).

Patterns in frequency of occurrence of diet items by age class

Adult and juvenile diet was characterised by both a high frequency of occurrence and high diversity of Diptera (100% of samples), particularly nematoceran families (midges) and Empidoidea: Dolichopodidae, Hybotidae and Empididae (Appendix 3.1).

Adults frequently consumed calyptrate flies such as dung flies (Scathophagidae), bean-seed flies (Anthomyiidae), houseflies (Muscidae), flesh flies (Sarcophagidae), blow flies (Calliphoridae) and lesser houseflies (Fanniidae). Aphids were by far the most frequent hemipteran family present in 36% of adult samples. Lepidopterans were highly prevalent in adult samples, predominantly noctuids but also notable were Geometridae, Crambidae, and Tortricidae. The damselfly *Enallagma cyathigerum* (Coenagrionidae: Odonata), Hemerobiidae (Neuroptera) and Leptocerid caddisflies (Trichoptera) were all consumed at a low frequency.

Coleoptera and Araneae were less frequently taken by juveniles compared to adults, although they did consume additional groups not taken by adults such as Carabidae, and Cantharidae in low frequencies. Hemiptera and Lepidoptera were generally consumed at a lower level than adults and nestlings, 14% of samples contained Noctuidae and Geometridae and Gelechiididae was unique to juveniles. They also consumed a higher frequency of Chrysopidae (Neuroptera) than nestlings. Juveniles were the only age group that did not consume damselflies, but they did consume a high frequency of the caddisfly *Oecetis ochracea* in the family Leptoceridae (38%).

Nestling diet appeared to be the most distinct of the three age groups. Coleoptera was restricted to two families and consumption of nematoceran flies was greatly reduced compared to the other age groups, only the families Chironomidae and Chaoboridae were above 5% in frequency. Hoverflies (Syrphidae) were in 10% of nestling samples, compared to a low presence in adults and absence in the diet of juveniles. The horsefly *Hybomitra lurida* (Tabanidae) was present in 6% of samples (n=2) and did not appear in adult or juvenile samples. Generally, calyptratae flies were less prevalent in nestling samples compared to in adults but almost half (53%) contained Muscidae, 23% contained Scathophagidae and 10% contained Calliphoridae. Spiders were less common in nestling diet, but gastropods were taken consistently, especially Succineidae (16%). In the Hemiptera, aphid presence was low compared to adults (10%), but the larger sized Gerridae (23%) and Notonectidae (26.6%) were detected at an elevated level. Froghoppers (Aphrophoridae) and water boatmen (Corixidae) also occurred in 3% of samples and were unique to nestling diet. Hymenoptera were not a significant group in nestling diet, with ichneumonids and braconids the only families consumed. Lepidopteran prey items were present in 70% of nest samples. 26% of nestlings consumed Erebidae (most incidences were *Scoliopteryx libatrix*). 10% consumed *Gelechia sororcullella* (Gelechiidae), 37% Noctuidae (mostly *Lenisa geminipuncta*, *Mythimna straminea*, *Phlogophora meticulosa* and *Orthosia cerasi*), 16% *Pandemis heparana* (Tortricidae) and 6% *Notodonta ziczac* (Notodontidae). Frequency of *Enallagma cyathigerum* (Coenagrionidae) was greatly elevated in nestling diet with a prevalence of 23%.

Table 3.2. The diet of the reed warbler in Chew Valley determined by metabarcoding The percentage of reed warbler samples testing positive for dietary items split by age and stage of the breeding season (early summer, mid-summer, late summer). Species that were only present in a single diet sample, and taxa identified to order level only, were removed from the table. Values are highlighted according to their detection rate. The family and order of each dietary item is also indicated.

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Diptera	Chaoboridae	*Chaoborus flavicans*	Aquatic/Semi-aquatic	77.78	76.92	85.71	50	57.89	73.33	72.73
Diptera	Chironomidae	*Cladotanytarsus atridorsum*	Aquatic/Semi-aquatic	42.22	61.54	33.33	16.67	73.68	26.67	54.55
Diptera	Chironomidae	*Endochironomus albipennis*	Aquatic/Semi-aquatic	26.67	25.64	42.86	10	10.53	28.33	27.27
Aranae	Clubionidae	*Clubiona phragmitis*	Terrestrial	24.44	38.46	23.81	3.33	36.84	13.33	54.55
Diptera	Scathophagidae	*Scathophaga stercoraria*	Terrestrial	21.11	20.51	9.52	20	10.53	18.33	27.27
Diptera	Muscidae	*Helina sp.*	Terrestrial	16.67	12.82	4.76	20	0	18.33	9.09
Trichoptera	Leptoceridae	*Oecetis ochracea*	Aquatic/Semi-aquatic	15.56	5.13	38.10	10	0	16.67	27.27
Lepidoptera	Noctuidae	*Lenisa geminipuncta*	Terrestrial	15.56	28.21	0	6.67	26.32	13.33	0
Hemiptera	Notonectidae	*Notonecta glauca*	Aquatic/Semi-aquatic	13.33	7.69	0	26.67	5.26	15.00	9.09
Odonata	Coenagrionidae	*Enallagma cyathigerum*	Aquatic/Semi-aquatic	13.33	7.69	0	23.33	10.53	13.33	0
Diptera	Empididae	*Empis stercorea*	Terrestrial	12.22	23.08	4.76	3.33	36.84	6.67	0
Diptera	Chironomidae	*Tanytarsus bathophilus*	Aquatic/Semi-aquatic	12.22	7.69	0	23.33	10.53	13.33	0
Gastropoda	Agriolimacidae	*Deroceras reticulatum*	Terrestrial	11.11	23.08	0	3.33	15.79	11.67	0
Diptera	Chironomidae	*Chironomus sp.*	Aquatic/Semi-aquatic	11.11	12.82	14.29	6.67	21.05	5.00	27.27
Diptera	Chironomidae	*Chironomidae sp.*	Aquatic/Semi-aquatic	11.11	17.95	4.76	3.33	10.53	8.33	18.18
Diptera	Anthomyiidae	*Delia florilega*	Terrestrial	10	20.51	4.76	0	15.79	8.33	9.09
Diptera	Tipulidae	*Prionocera subserricornis*	Aquatic/Semi-aquatic	10	20.51	4.76	0	15.79	10	0
Hemiptera	Gerridae	*Gerris odontogaster*	Aquatic/Semi-aquatic	10	2.56	0	23.33	5.26	11.67	0
Lepidoptera	Erebidae	*Scoliopteryx libatrix*	Terrestrial	10	2.56	0	23.33	5.26	11.67	0

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Coleoptera	Chrysomelidae	*Galerucella lineola*	Terrestrial	10	15.38	9.52	3.33	0	11.67	18.18
Coleoptera	Helophoridae	*Helophorus nivalis*	Terrestrial	8.89	15.38	9.52	0	26.32	5.00	0
Lepidoptera	Tortricidae	*Pandemis heparana*	Terrestrial	8.89	5.13	0	16.67	5.26	10	0
Coleoptera	Chrysomelidae	*Galerucella nymphaeae*	Terrestrial	8.89	2.56	4.76	16.67	0	11.67	0
Aranae	Linyphiidae	*Hypomma bituberculatum*	Terrestrial	8.89	12.82	9.52	3.33	10.53	8.33	9.09
Diptera	Dolichopodidae	*Chrysotus femoratus*	Aquatic/Semi-aquatic	8.89	10.26	19.05	0	0	10	18.18
Diptera	Ptychopteridae	*Ptychoptera contaminata*	Aquatic/Semi-aquatic	8.89	10.26	14.29	0	5.26	6.67	18.18
Diptera	Chironomidae	*Paraphaenocladius impensus*	Aquatic/Semi-aquatic	8.89	15.38	9.52	0	5.26	8.33	18.18
Diptera	Chironomidae	*Cricotopus sylvestris*	Aquatic/Semi-aquatic	8.89	10.26	9.52	3.33	10.53	5.00	18.18
Gastropoda	Succineidae	*Succineidae sp.*	Terrestrial	7.78	2.56	0	16.67	0	8.33	9.09
Diptera	Chironomidae	*Procladius sagittalis*	Aquatic/Semi-aquatic	7.78	15.38	4.76	0	10.53	8.33	0
Lepidoptera	Noctuidae	*Mythimna straminea*	Terrestrial	7.78	2.56	4.76	6.67	5.26	3.33	9.09
Diptera	Chironomidae	*Cricotopus laricomalis*	Aquatic/Semi-aquatic	6.67	7.69	0	10	10.53	6.67	0
Diptera	Muscidae	*Helina depuncta*	Terrestrial	6.67	0	0	16.67	5.26	6.67	0
Diptera	Muscidae	*Musca autumnalis*	Terrestrial	6.67	0	4.76	10	0	5.00	9.09
Hemiptera	Aphididae	*Pterocomma sp.*	Terrestrial	6.67	15.38	0	0	10.53	6.67	0
Diptera	Chironomidae	*Chironomus pallidivittatus*	Aquatic/Semi-aquatic	6.67	10.26	9.52	0	5.26	3.33	27.27
Diptera	Chironomidae	*Cryptochironomus psittacinus*	Aquatic/Semi-aquatic	6.67	10.26	4.76	3.33	10.53	1.67	27.27
Diptera	Chironomidae	*Cryptochironomus obreptans*	Aquatic/Semi-aquatic	6.67	12.82	0	3.33	10.53	5.00	9.09
Hemiptera	Aphididae	*Hyalopterus pruni*	Terrestrial	6.67	5.13	9.52	6.67	0	6.67	18.18
Hemiptera	Tingidae	*Physatocheila dumetorum*	Terrestrial	5.56	5.13	0	10	5.26	6.67	0
Lepidoptera	Crambidae	*Acentria ephemerella*	Aquatic/Semi-aquatic	5.56	7.69	9.52	0	5.26	5.00	9.09
Diptera	Muscidae	*Myospila meditabunda*	Terrestrial	5.56	5.13	0	6.67	5.26	5.00	0
Diptera	Chironomidae	*Prodiamesa olivacea*	Aquatic/Semi-aquatic	5.56	12.82	0	0	15.79	3.33	0

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Hymenoptera	Braconidae	*Aphidius urticae*	Terrestrial	5.56	12.82	0	0	10.53	5.00	0
Diptera	Chironomidae	*Tanytarsus mendax*	Aquatic/Semi-aquatic	5.56	10.26	4.76	0	10.53	1.67	18.18
Diptera	Muscidae	*Helina impuncta*	Terrestrial	5.56	5.13	4.76	6.67	5.26	6.67	0
Diptera	Hybotidae	*Platypalpus sp.*	Terrestrial	5.56	12.82	0	0	5.26	6.67	0
Lepidoptera	Noctuidae	*Phlogophora meticulosa*	Terrestrial	5.56	0	4.76	10	0	6.67	0
Hymenoptera	Braconidae	*Praon abjectum*	Terrestrial	5.56	10.26	4.76	0	0	5.00	18.18
Lepidoptera	Noctuidae	*Orthosia cerasi*	Terrestrial	4.44	0	0	10	10.53	1.67	0
Lepidoptera	Notodontidae	*Notodonta ziczac*	Terrestrial	4.44	5.13	0	6.67	0	6.67	0
Diptera	Dolichopodidae	*Dolichopus plumipes*	Aquatic/Semi-aquatic	4.44	7.69	0	3.33	10.53	3.33	0
Diptera	Sarcophagidae	*Sarcophaga carnaria*	Terrestrial	4.44	10.26	0	0	5.26	5.00	0
Lepidoptera	Gelechiididae	*Gelechia sororculella*	Terrestrial	4.44	0	4.76	10	0	6.67	0
Hemiptera	Aphididae	*Microlophium carnosum*	Terrestrial	4.44	10.26	0	0	15.79	1.67	0
Coleoptera	Chrysomelidae	*Psylliodes affinis*	Terrestrial	4.44	2.56	4.76	3.33	0	5.00	0
Lepidoptera	Noctuidae	*Agrochola lota*	Terrestrial	4.44	10.26	0	0	10.53	3.33	0
Hemiptera	Cicadellidae	*Empoasca luda*	Terrestrial	4.44	2.56	9.52	3.33	0	5.00	9.09
Diptera	Chironomidae	*Chironomus nuditarsis*	Aquatic/Semi-aquatic	4.44	2.56	9.52	3.33	5.26	5.00	0
Diptera	Dolichopodidae	*Dolichopus cilifemoratus*	Aquatic/Semi-aquatic	3.33	0	0	10	0	5.00	0
Ephemeroptera	Baetidae	*Cloeon dipterum*	Aquatic/Semi-aquatic	3.33	2.56	4.76	3.33	5.26	0	18.18
Diptera	Fanniidae	*Fannia lepida*	Terrestrial	3.33	2.56	4.76	0	0	1.67	9.09
Hymenoptera	Braconidae	*Ephedrus lacertosus*	Terrestrial	3.33	5.13	0	3.33	0	5.00	0
Diptera	Anthomyiidae	*Zaphne divisa*	Terrestrial	3.33	5.13	4.76	0	5.26	1.67	9.09
Diptera	Muscidae	*Neomyia cornicina*	Terrestrial	3.33	0	4.76	3.33	5.26	1.67	0
Gastropoda	Lymnaeidae	*Stagnicola fuscus*	Aquatic/Semi-aquatic	3.33	2.56	4.76	3.33	0	3.33	9.09
Diptera	Dolichopodidae	*Dolichopus longitarsis*	Aquatic/Semi-aquatic	3.33	2.56	0	3.33	0	3.33	0

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Diptera	Hybotidae	*Platypalpus candicans*	Terrestrial	3.33	2.56	9.52	0	0	5.00	0
Diptera	Scathophagidae	*Scathophaga suilla*	Terrestrial	3.33	2.56	4.76	3.33	5.26	1.67	9.09
Hymenoptera	Braconidae	*Praon gallicum*	Terrestrial	3.33	7.69	0	0	15.79	0	0
Diptera	Hybotidae	*Platypalpus calceatus*	Terrestrial	3.33	2.56	9.52	0	0	5.00	0
Lepidoptera	Geometridae	*Idaea biselata*	Terrestrial	3.33	2.56	4.76	3.33	0	3.33	9.09
Diptera	Ceratopogonidae	*Atrichopogon winnertzi*	Aquatic/Semi-aquatic	3.33	7.69	0	0	5.26	3.33	0
Aranae	Linyphiidae	*Porrhomma pygmaeum*	Terrestrial	3.33	7.69	0	0	10.53	1.67	0
Diptera	Scathophagidae	*Scathophaga furcata*	Terrestrial	3.33	7.69	0	0	5.26	3.33	0
Diptera	Chloropidae	*Elachiptera cornuta*	Terrestrial	3.33	2.56	9.52	0	0	1.67	18.18
Diptera	Opomyzidae	*Opomyza germinationis*	Terrestrial	3.33	2.56	0	6.67	0	5.00	0
Diptera	Culicidae	*Culiseta morsitans*	Aquatic/Semi-aquatic	3.33	7.69	0	0	5.26	3.33	0
Diptera	Chironomidae	*Polypedilum sordens*	Aquatic/Semi-aquatic	3.33	2.56	9.52	0	0	1.67	18.18
Hemiptera	Aphrophoridae	*Aphrophora sp.*	Terrestrial	3.33	0	0	3.33	0	1.67	0
Diptera	Chironomidae	*Glyptotendipes sp.*	Aquatic/Semi-aquatic	3.33	5.13	4.76	0	0	3.33	9.09
Coleoptera	Chrysomelidae	*Crepidodera fulvicornis*	Terrestrial	3.33	7.69	0	0	10.53	1.67	0
Aranae	Philodromidae	*Philodromus albidus*	Terrestrial	2.22	0	0	6.67	5.26	1.67	0
Diptera	Chironomidae	*Procladius rufovittatus*	Aquatic/Semi-aquatic	2.22	5.13	0	0	10.53	0	0
Diptera	Phoridae	*Megaselia sp.*	Terrestrial	2.22	2.56	4.76	0	0	0	18.18
Diptera	Tabanidae	*Hybomitra lurida*	Terrestrial	2.22	0	0	6.67	0	3.33	0
Lepidoptera	Choreutidae	*Anthophila fabriciana*	Terrestrial	2.22	2.56	0	3.33	0	3.33	0
Aranae	Theridiosomatidae	*Theridiosoma gemmosum*	Terrestrial	2.22	2.56	4.76	0	0	3.33	0
Diptera	Muscidae	*Muscina prolapsa*	Terrestrial	2.22	0	0	6.67	0	3.33	0
Diptera	Sepsidae	*Sepsis punctum*	Terrestrial	2.22	2.56	4.76	0	0	3.33	0
Diptera	Calliphoridae	*Lucilia caesar*	Terrestrial	2.22	2.56	0	0	0	1.67	0

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Diptera	Empididae	*Rhamphomyia crassirostris*	Terrestrial	2.22	5.13	0	0	10.53	0	0
Hemiptera	Aphididae	*Sitobion sp.*	Terrestrial	2.22	5.13	0	0	10.53	0	0
Hymenoptera	Braconidae	*Aphidius matricariae*	Terrestrial	2.22	5.13	0	0	5.26	1.67	0
Diptera	Dolichopodidae	*Campsicnemus vanduzeei*	Aquatic/Semi-aquatic	2.22	2.56	4.76	0	0	1.67	9.09
Hemiptera	Cicadellidae	*Empoasca decipiens*	Terrestrial	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Chironomidae	*Chironomus anthracinus*	Aquatic/Semi-aquatic	2.22	5.13	0	0	0	1.67	9.09
Diptera	Pipunculidae	*Chalarus latifrons*	Terrestrial	2.22	2.56	4.76	0	0	3.33	0
Hymenoptera	Ichneumonidae	*Diplazon tetragonus*	Terrestrial	2.22	2.56	0	3.33	0	1.67	9.09
Diptera	Anthomyiidae	*Delia platura*	Terrestrial	2.22	5.13	0	0	0	0	18.18
Coleoptera	Helophoridae	*Helophorus arvernicus*	Aquatic/Semi-aquatic	2.22	2.56	4.76	0	5.26	1.67	0
Diptera	Lauxaniidae	*Lauxaniidae sp.*	Terrestrial	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Limoniidae	*Rhipidia maculata*	Terrestrial	2.22	2.56	0	3.33	5.26	1.67	0
Lepidoptera	Noctuidae	*Archanara dissoluta*	Terrestrial	2.22	2.56	0	3.33	5.26	1.67	0
Coleoptera	Kateretidae	*Kateretes rufilabris*	Terrestrial	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Lonchopteridae	*Lonchoptera lutea*	Terrestrial	2.22	5.13	0	0	5.26	1.67	0
Hymenoptera	Ichneumonidae	*Campopleginae sp.*	Terrestrial	2.22	2.56	4.76	0	0	1.67	9.09
Lepidoptera	Geometridae	*Cabera exanthemata*	Terrestrial	2.22	0	0	6.67	0	3.33	0
Diptera	Tipulidae	*Tipula oleracea*	Terrestrial	2.22	5.13	0	0	0	3.33	0
Ephemeroptera	Baetidae	*Procloeon bifidum*	Aquatic/Semi-aquatic	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Muscidae	*Azelia cilipes*	Terrestrial	2.22	2.56	4.76	0	5.26	1.67	0
Diptera	Culicidae	*Coquillettidia richiardii*	Aquatic/Semi-aquatic	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Hybotidae	*Hybos femoratus*	Terrestrial	2.22	5.13	0	0	5.26	1.67	0
Diptera	Cecidomyiidae	*Cecidomyiidae sp.*	Terrestrial	2.22	2.56	0	0	0	1.67	0
Coleoptera	Scirtidae	*Contacyphon coarctatus*	Aquatic/Semi-aquatic	2.22	2.56	0	3.33	5.26	1.67	0

				Percentage of reed warblers testing positive for a dietary item						
Order	Family	Dietary Item	Habitat	All (n = 90)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)	Early (n = 19)	Middle (n = 60)	Late (n = 11)
Diptera	Culicidae	*Anopheles messeae*	Aquatic/Semi-aquatic	2.22	5.13	0	0	5.26	1.67	0
Hymenoptera	Braconidae	*Aphidius ervi*	Terrestrial	2.22	5.13	0	0	10.53	0	0
Neuroptera	Hemerobiidae	*Hemerobius humulinus*	Terrestrial	2.22	2.56	0	3.33	10.53	0	0
Diptera	Anisopodidae	*Sylvicola sp.*	Terrestrial	2.22	5.13	0	0	5.26	1.67	0
Coleoptera	Chrysomelidae	*Lochmaea capreae*	Terrestrial	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Rhagionidae	*Rhagio lineola*	Terrestrial	2.22	2.56	4.76	0	0	3.33	0
Diptera	Chironomidae	*Chironomus riparius*	Aquatic/Semi-aquatic	2.22	2.56	4.76	0	0	1.67	9.09
Diptera	Hybotidae	*Bicellaria vana*	Terrestrial	2.22	5.13	0	0	10.53	0	0
Lepidoptera	Geometridae	*Operophtera brumata*	Terrestrial	2.22	5.13	0	0	0	1.67	9.09
Diptera	Chironomidae	*Chironomus plumosus*	Aquatic/Semi-aquatic	2.22	5.13	0	0	10.53	0	0

3.4.3. Prey characteristics

Selection of aquatic and terrestrial prey items

Reed warbler age (F = 10.11, p<0.001) was a significant predictor of the proportion of aquatic prey consumed by an individual (adjusted R squared = 0.17, F = 5.41 on 4 and 84 degrees of freedom, p<0.001), whereas time of capture in the breeding season (early, middle or late breeding season) was not significant (p = 0.49). Adults and nestlings consumed a similar proportion of aquatic dietary items (0.45 and 0.39, respectively), whereas juveniles consumed aquatic prey more frequently (0.68) compared to adults (p<0.01) and nestlings (p<0.001) on average (Fig. 3.4).

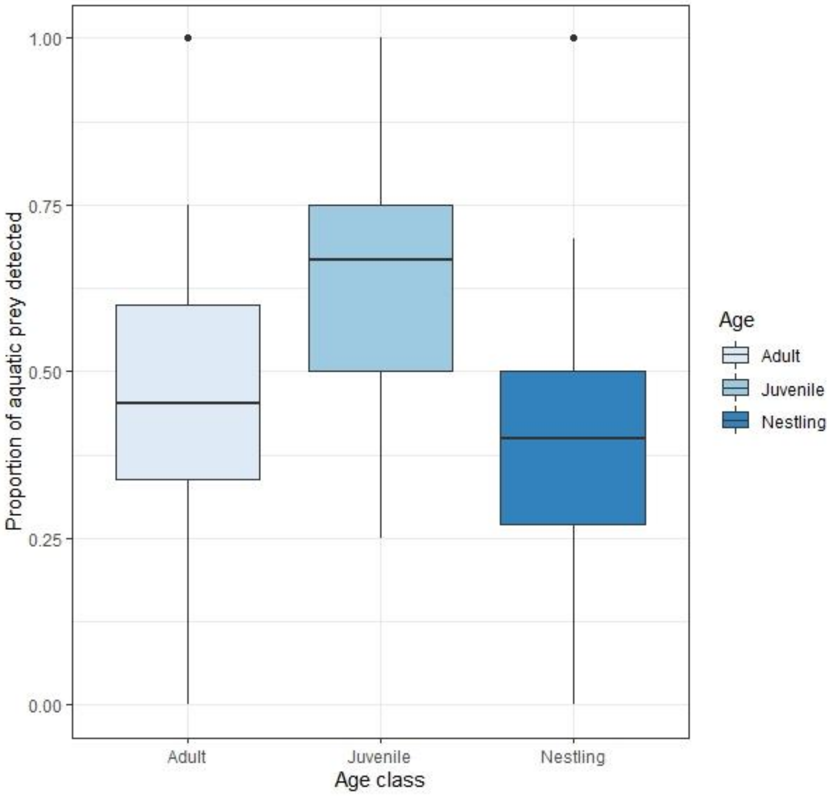


Figure 3.4. The proportion of prey items detected in the diet of Chew Valley reed warblers that were classed as either aquatic or semi-aquatic for all or part of their life cycle for adult, juvenile and nestling age classes.

Prey size selection

The average body length of prey across all age classes was 7.77 mm (± 2.7 SD). Age class (F = 30.27, p<0.001) was a highly significant predictor in explaining the size of prey consumed by reed warblers (adjusted R-Squared = 0.42, F=17.06 on 4 and 87 degrees of freedom, p<0.001).

Season was also significant (F = 3.84, $p<0.05$). Nestlings were fed larger prey on average (Fig 3.5), than the average size of prey consumed by adults (average size = 10mm in nestlings, 6.8mm in adults, $p<0.001$) and juveniles (juvenile average size = 6.3mm, $p<0.001$) but any size differences in prey between adults and juveniles were not significant ($p = 0.6$). The size of prey consumed by all reed warblers was on average greater in the middle of summer compared to early summer (Fig 3.5), but this difference was only marginally significant ($p<0.05$). Nestlings were fed larger Coleoptera, Diptera, Hemiptera and Lepidoptera compared to prey of these groups consumed by adult and juvenile reed warblers (Table 3.3.).

Mean prey size in the early part of the breeding season (April-May) was 6.9 mm, 8.2mm in the mid-breeding season (June-early July) and 7.3 mm in the late season (late July-August). It is likely that the effect of season is somewhat tied to the presence of more nestling samples during the middle part of the breeding season, however invertebrate availability data from sticky trap samples suggests that the larger body sizes measured by length (mm) are more frequent in the middle of the breeding season (Fig. 3.6). The most abundant prey was between 2 and 4.5mm in length, however the number of prey items in the range 9mm and over are highest in the June sampling round (mid-summer).

Table 3.3. Average body lengths in millimetres (± standard deviation) of prey species from all orders detected in adult, juvenile and nestling diet samples. Where only one species from a given order was detected across the samples from a given age class; standard deviation = 0.0.

	Average Body Length (mm)		
Prey Order	**Adults**	**Juveniles**	**Nestlings**
All Taxa	6.78 ± 1.7	6.33 ± 1.2	10.08 ± 2.8
Araneae	6.06 ± 2.5	5.84 ± 2.55	4.6 ± 1.5
Coleoptera	3.79 ± 1.2	4.42 ± 1.5	5.17 ± 1.4
Diptera	5.99 ± 1.0	5.64 ± 0.8	6.56 ± 1.4
Gastropoda	28.75 ± 3.9	17.5 ± 0.0	18.13 ± 5.3
Hemiptera	3.39 ± 2.3	4.5 ± 3.7	8.54 ± 3.2
Hymenoptera	3.56 ± 1.8	5.5 ± 2.2	5.5 ± 3.5
Lepidoptera	15.22 ± 3.4	12.17 ± 5.4	19.06 ± 5.6
Neuroptera	8.5 ± 0.0	11.5 ± 2.1	9.75 ± 1.8
Trichoptera	10 ± 0.0	9.88 ± 0.4	10 ± 0.0

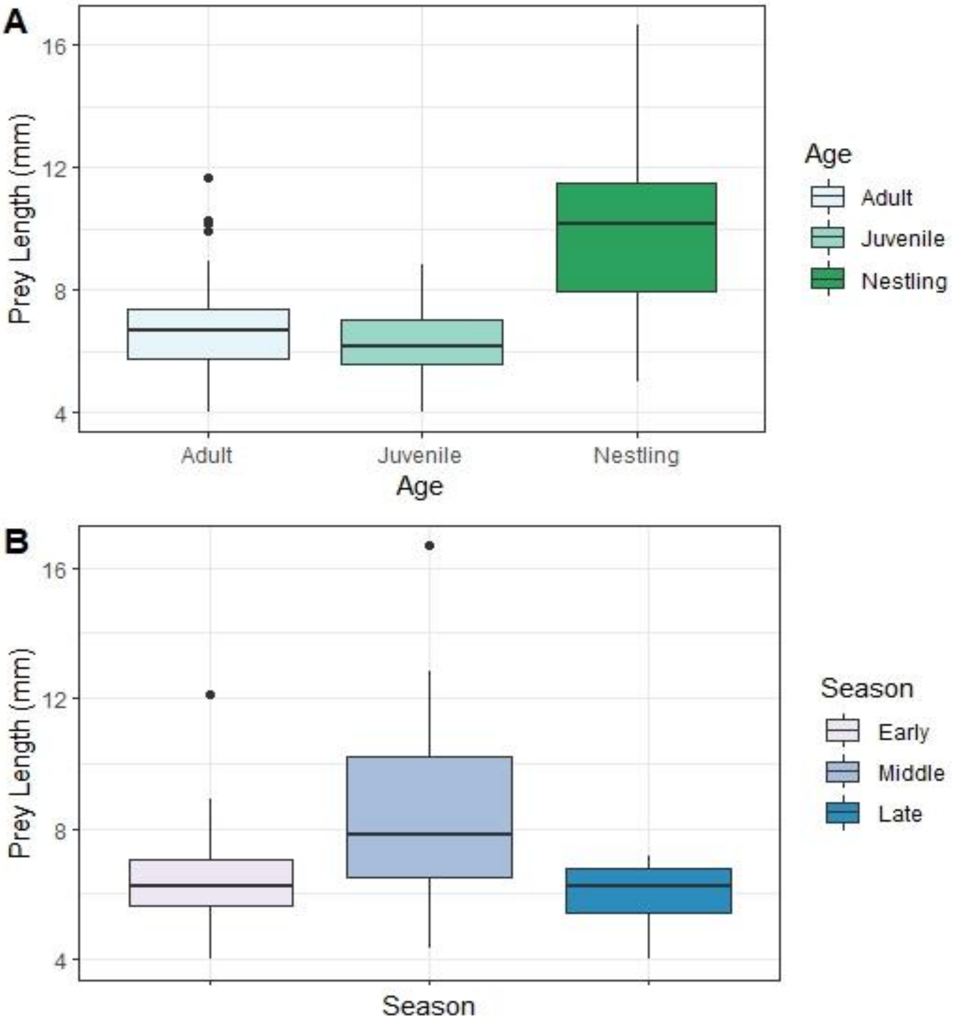
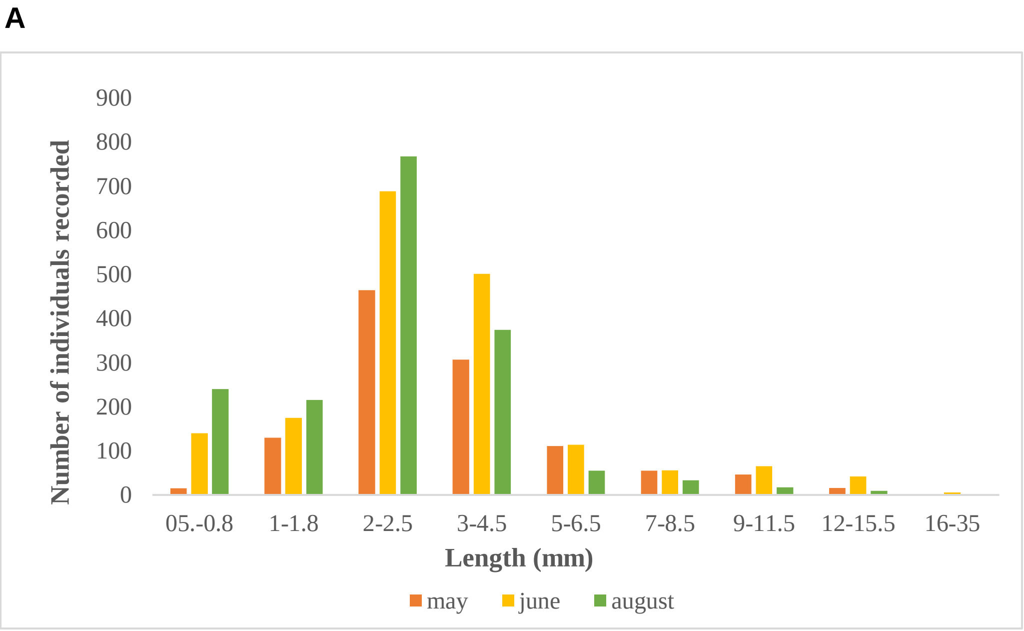


Figure 3.5. Boxplot of the average size of prey (body length mm) detected in the diet samples of reed warblers at Chew Valley. A) reed warblers of different age classes and B) reed warblers captured during different periods of the breeding season (early (n = 19), middle (n = 60) and late summer (n = 11)).



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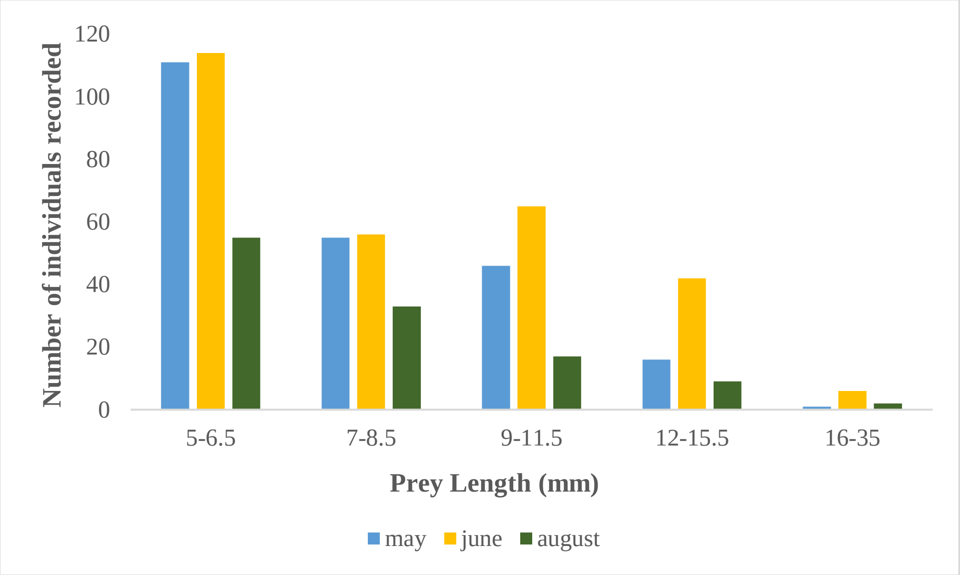


Figure 3.6. The number of arthropods recorded at Chew Valley on sticky traps according to body length (mm) classes in the three sampling periods: May (early summer), June (mid-summer) and August (late summer). A) All prey size classes, B) Prey items of 5mm in length and above.

3.4.4. Measures of dietary diversity and richness

All age groups showed high Shannon’s H diversity and Simpson’s diversity (Table 3.4). Species richness and Levin’s index was highest in adults, followed by nestlings and juveniles both within and across samples. The Chao estimate suggested that the total number of possible prey items was approximately 452 (± 46.58) prey species compared to our measure of 258 observed species. Thus, ~57% of possible dietary diversity estimated by the Chao method was described. This suggests that between 150 and 240 additional prey species may have been included in the diets of reed warblers at Chew.

Prey species richness measured by the number of species detected per faecal sample, was significantly associated with the age of the reed warbler (LRT = 10.21, p<0.01), but not the remaining variables (Fig. 3.7). The model was significant (adjusted R-Squared = 0.94, F= 202.3 on 7 and 81 degrees of freedom, p<0.001), and both juveniles (z = -2.36, p<0.05) and nestlings (z = 3.21, p<0.01), showed lower dietary richness than the adults, but differences between juveniles and nestlings were not significant (z = 0.68, p = 0.7).

Table 3.4. Diversity indices calculated for the reed warbler age groups. Shannon H, Simpson Diversity, Levin Index, Chao Estimate, species richness, average richness per sample and percentage of species diversity described (Chao estimated diversity/observed species richness) are given for adults, juveniles, pooled nestlings, and all ages combined.

Diversity Index	**Adult**	**Juvenile**	**Nestling**	**All ages**
Shannon H	4.67	4.26	4.19	4.94
Simpson's Diversity	0.98	0.97	0.97	0.98
Levin's Index	61.06	39.2	46.33	67.34
Chao Estimate (± S.E.)	381.6 ± 59.76	288.7 ± 63.94	240.0 ± 57.43	452.3 ± 46.58
Species richness	175	103	95	258
Average richness per sample	11.23	8.35	6.83	9.10
Percentage diversity described	45.86	35.68	39.58	57.04

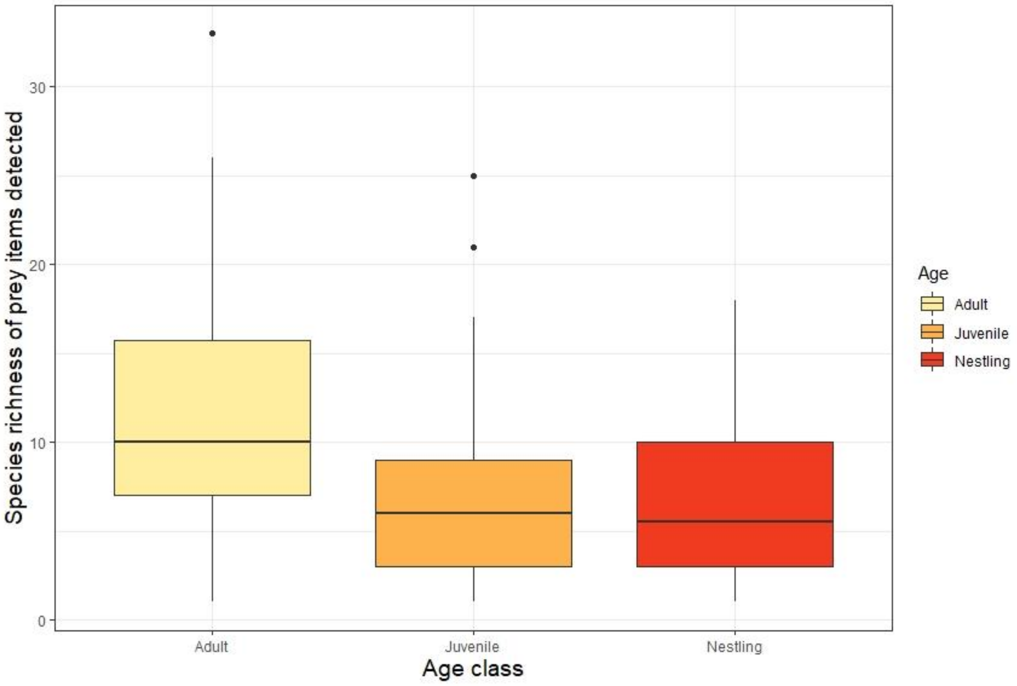


Figure 3.7. Box plot of species richness of prey items detected in the reed warbler diet samples at Chew Valley, organised by age class.

3.4.5. Dietary overlap

The Pianka Index showed that dietary overlap was greatest between adults and juveniles (0.74) and lower between adults and nestlings (0.57) and juveniles and nestlings (0.53). All pairwise combinations showed significantly greater dietary overlap than predicted by the null model ($p>0.05$, Table 3.5i).

A significantly higher level of overlap than expected under the null model was detected between all pairs at all time periods, except for adults and nestlings in the early season (Table 3.5ii). In the early part of the breeding season dietary overlap did not differ from the null model (p (obs = null) = 0.04), and the Pianka index was lower than middle and late season (Pianka index = 0.31). Nestlings and adults showed a significant increase in dietary overlap from early to mid-season (Pianka index early = 0.31, middle = 0.60). The strongest overlap was between adults and juveniles in the middle of the breeding season (Pianka index = 0.67) and in the late breeding season (Pianka index = 0.67). Juveniles and nestlings only coexisted in the middle of the breeding season, when they showed significant dietary overlap (0.51), but not as high as that between adults and juveniles or adults and nestlings.

Table 3.5. Pianka's index of niche overlap (O_{jk}) in observed diet i) between pairs of reed warblers of the three age classes i) for all seasons combined, ii) during different stages of the breeding season. Standard effect sizes (SES) are indicated in brackets. Cells are colour coded according to significance with respect to the null model (see notes below).

i)

Reed Warbler Age	Adult	Juvenile
Nestling	0.57***(6.5)	0.53**(4.7)
Adult		0.74***(9.6)

ii)

	Adult			Juvenile		
	early	middle	late	early	middle	late
Nestling	0.31 (0.001)	0.60*** (5.39)			0.51** (3.9)	
Adult					0.67*** (6.97)	0.67* (2.47)

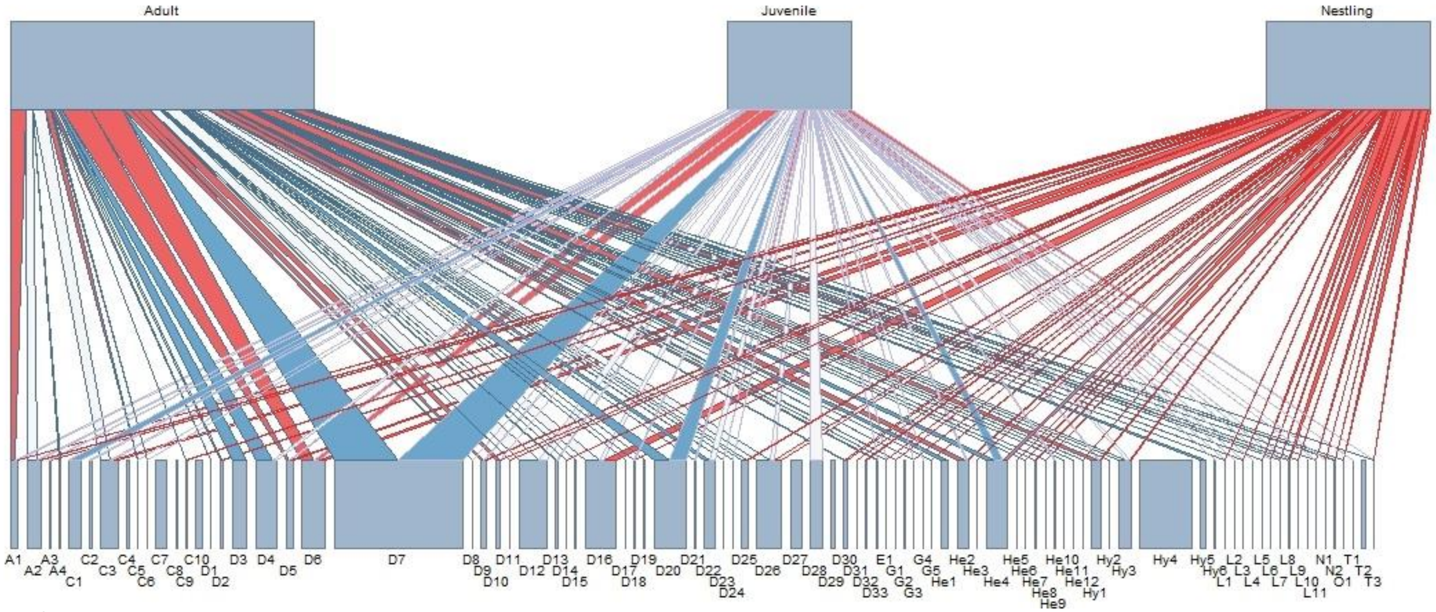
Notes: Asterisks denote significantly higher or lower overlap than predicted from the null model (* p<0.05, **p<0.01, ***p<0.001). Positive SES and a green shade indicate higher observed overlap than the null model. Yellow shade indicates observed overlap is not significantly different from the null model.

3.4.6. Prey choice

In the *econullnetr* analysis, 68 prey families showed either a significantly stronger or weaker association with the diet of reed warbler adults, juveniles and nestlings than expected from their relative abundance at Chew Valley Lake (Fig. 3.8, Table 3.6). Some families were universally preferred by all ages; the midge families; Chaoboridae and Chironomidae, the caddisfly family Leptoceridae, the reed spider family Clubionidae, the aphids; Aphididae and the geometrid and noctuid moths. Reed warblers of all ages showed significantly fewer detections of Cicadellidae, Pteromalidae/Torymidae, Chloropidae, Phoridae, Scatopsidae and Ceratopogonidae than expected from the abundance of these families in the field. Stronger associations between consumers and prey resources than expected from prey relative abundance were most frequent in the middle of the breeding season, however; Empididae, Dolichopodidae, Saldidae, Tipulidae and Hemerobiidae were preferred disproportionately more in the early summer and Baetidae, Scathophagidae, Crambidae and Aphididae in the late summer.

In nestling diet, 33 families were consumed at a level above that expected by the null model. The strongest interactions (standardised effect size > 10) were between nestlings and i) the aquatic hemipteran families Gerridae (water striders) and Notonectidae (backswimmers), ii) several moth families, iii) Succineidae (amber snails), and iv) the family Coenagrionidae (the blue damselflies). For adult reed warblers, stronger than expected interactions were recorded for 33 families. Families that were uniquely preferred by adult reed warblers included Anisopodidae, Micropezidae, Tipulidae, Braconidae and Mycetophilidae. The strongest interactions (SES >10) were between reed warbler adults and i) the beetle family Helophoridae, ii) the nematocerous dipterans; Chaoboridae, Culicidae, Ptychopteridae and Tipulidae and iii) noctuid moths. 30 prey families were consumed at a frequency higher than expected from their abundance by juveniles. Beraeidae and Pentatomidae preferences were unique to juvenile birds. The strongest interactions (SES > 10) were between juveniles and i) the dipteran families Chaoboridae (phantom midge) and Ptychopteridae (phantom crane flies), ii) the geometrid moths and iii) leptocerid caddisflies.

Feeding preferences by reed warblers were significantly affected by prey size, prey abundance and taxonomy (Adjusted R-squared = 0.4, F = 12.06 on 16 and 247 degrees of freedom, $p<0.001$). The strength of preference for a prey item (SES >2) by all age classes was significantly larger if the prey item was large-bodied (i.e., greater inferred body length) compared to small-bodied prey ($t = 3.78$, $p<0.001$). Recorded abundance of a prey item on the sticky traps during the periods that the three age classes were feeding on the breeding grounds was significantly negatively associated with preference for a prey item ($t = -11.19$, $p<0.001$). Taxonomic order also played a role in determining prey preference; families from Araneae were significantly more likely to be preferred by reed warblers than Coleoptera ($z = 3.56$, $p<0.05$), Hymenoptera ($z = 3.43$, $p<0.05$) and Mecoptera ($z = 3.94$, $p<0.05$). Mecoptera were also significantly less likely to be preferentially consumed than nematocerous flies ($z = 4.05$, $p<0.05$), Lepidoptera ($z = 3.65$, $p<0.05$) and Ephemeroptera ($z = 4.05$, $p<0.01$).



Key to family codes:

A1: Clubionidae A2: Linyphiidae A3: Philodromidae, A4: Tetragnathidae. Coleoptera families: C1: Cantharidae, C2: Carabidae, C3: Chrysomelidae, C4: Coccinellidae, C5: Helophoridae, C6: Kateretidae, C7: Nitidulidae, C8: Phalacrididae, C9: Scirtidae C10: Staphylinidae ,D1: Anisopodidae, D2: Calliphoridae, D3: Cecidomyiidae, D4: Ceratopogonidae, D5: Chaoboridae, D6: Chironomidae, D7: Chloropidae, D8: Culicidae, D9: Dolichopodidae, D10: Empididae, D11: Ephydridae, D12: Hybotidae, D13: Lauxaniidae/Drosophilidae, D14: Limoniidae, D15: Micropezidae, D16: Muscidae/Fanniidae/Anthomyiidae, D17: Mycetophilidae, D18: Opomyzidae/Tephritidae, D19: Pediciidae, D20: Phoridae, D21: Pipunculidae, D22: Psychodidae, D23: Ptychopteridae, D24: Rhagionidae, D25: Sarcophagidae, D26: Scathophagidae, D27: Scatopsidae, D28: Sciaridae, D29: Stratiomyiidae, D30: Syrphidae, D31: Tabanidae, D32: Tachinidae, D33: Tipulidae, E1: Baetidae, Gastropoda families: G1: Agriolimacidae, G2: Helicidae, G3: Lymnaeidae, G4: Physidae, G5: Succineidae. Hemipteran families: He1: Anthocoridae, He2: Aphididae, He3: Aphrophoridae, He4: Cicadellidae, He5: Corixidae, He6: Gerridae, He7: Miridae, He8: Notonectidae, He9: Pentatomidae, He10: Psyllidae, He11: Saldidae, He12: Tingidae, Hy1: Braconidae, Hy2: Cynipidae, Hy3: Ichneumonidae, Hy4: Pteromalidae/Torymidae, Hy5: Tenthredinidae, Hy6: Vespidae, L1: Choreutidae, L2: Coleophoridae, L3: Crambidae, L4: Depressariidae, L5: Eerebidae, L6: Gelechiidae, L7: Geometridae, L8: Noctuidae, L9: Notodontidae, L10: Nymphalidae, L11: Tortricidae,N1: Chrysopidae, N2: Hemerobiidae, Odonata families: O1: Coenagrionidae, Trichoptera families: T1 Beraeidae, T2: Leptoceridae.

Figure 3.8. Bipartite plot showing trophic interactions between warbler age classes and arthropod prey from the *econullnetr* analysis , that were stronger (red), weaker (blue), or equal (white) to that expected from the prey item's measured resource availability. Some prey taxa featured in Table 3.6 that were present on sticky traps but not detected in the diet were removed to improve plot interpretability.

Table 3.6. Results from the *econullnetr* model showing trophic interactions between consumers (warbler age classes) and resources (prey families). Values displayed are standard effect sizes (SES) of the strength of the interaction. Interactions with SES >2 are highlighted, with darker tone indicating a greater significance (red = stronger interaction than expected from null model, blue = weaker interaction than expected from the null model). Families are ordered by habitat association and body length (mm). Data for each age was subsetted by time of capture in the breeding season.

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Gastropoda	Agriolimacidae	Terrestrial	30	22.84	15.03	18.73	-0.17	-0.25	-0.23	-0.10	3.63	-0.14	4.34
Odonata	Coenagrionidae	Aquatic/Semi-aquatic	25	5.75	3.63	5.10	-0.14	-0.33	-0.29	-0.14	19.32	9.90	17.07
Gastropoda	Helicidae	Terrestrial	23.5	-0.44	-0.23	-0.33	-0.17	-0.19	-0.17	-0.14	-0.17	NA	-0.17
Lepidoptera	Erebidae	Terrestrial	20	2.98	-0.20	5.66	-0.17	-0.27	-0.20	-0.17	26.20	6.96	25.38
Lepidoptera	Notodontidae	Terrestrial	20	5.76	-0.17	7.53	-0.14	-0.27	-0.20	-0.17	6.30	-0.14	7.04
Mecoptera	Panorpidae	Terrestrial	20	-1.69	-1.53	-0.63	-0.63	-0.88	-0.52	-0.67	-0.94	-0.64	-0.67
Gastropoda	Lymnaeidae	Aquatic/Semi-aquatic	18.3	1.94	-0.25	-0.29	5.66	3.63	6.96	-0.23	6.01	-0.20	7.53
Lepidoptera	Noctuidae	Terrestrial	17.9	32.63	21.07	34.82	-0.17	11.43	11.49	4.87	55.65	NA	45.49
Lepidoptera	Depressariidae	Terrestrial	17	-0.54	-0.41	-0.34	NA	3.63	4.87	-0.17	-0.35	-0.14	-0.31
Diptera: Nematocera	Tipulidae	Aquatic/Semi-aquatic	16	8.33	9.62	6.14	-0.17	0.97	1.02	-0.17	-1.07	-0.10	-1.05
Gastropoda	Physidae	Aquatic/Semi-aquatic	15	-0.33	-0.17	-0.27	NA	3.94	6.96	-0.20	-0.37	-0.14	-0.33
Megaloptera	Sialidae	Aquatic/Semi-aquatic	15	-0.60	-0.54	-0.26	-0.10	-0.25	-0.20	-0.14	-0.29	-0.17	-0.23
Gastropoda	Succineidae	Terrestrial	15	2.37	-0.25	-0.25	5.66	-0.23	-0.14	-0.17	16.25	-0.14	18.04
Lepidoptera	Geometridae	Terrestrial	14.9	5.43	-0.42	7.53	4.87	17.32	14.07	9.90	11.27	-0.17	14.38
Hemiptera	Notonectidae	Aquatic/Semi-aquatic	14	9.62	-0.23	8.90	NA	-0.23	-0.14	-0.17	36.29	NA	31.73
Lepidoptera	Tortricidae	Terrestrial	14	5.76	-0.17	7.53	-0.14	3.37	4.34	-0.17	19.23	NA	15.33
Neuroptera	Chrysopidae	Terrestrial	11.3	-0.54	-0.23	-0.45	-0.14	5.33	6.30	-0.20	2.57	-0.10	2.69
Lepidoptera	Coleophoridae	Terrestrial	11	-0.45	-0.29	-0.29	-0.10	3.94	3.94	NA	-0.17	NA	-0.17
Lepidoptera	Crambidae	Terrestrial	11	4.35	4.34	2.89	-0.10	4.42	2.20	5.66	1.82	-0.10	1.88
Hemiptera	Pentatomidae	Terrestrial	11	-0.43	-0.34	-0.35	NA	3.37	4.87	-0.20	-0.20	-0.10	-0.17

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Diptera: Nematocera	Ptychoperidae	Aquatic/Semi-aquatic	10.5	9.36	3.63	8.13	4.90	15.03	14.07	6.96	-0.33	-0.14	-0.29
Diptera: Brachycera	Calliphoridae	Terrestrial	10.3	0.21	-0.98	0.58	1.37	-1.03	-0.92	-0.45	1.46	-0.34	1.75
Diptera: Brachycera	Tabanidae	Terrestrial	10.3	-0.35	-0.26	-0.17	-0.14	-0.27	-0.20	-0.17	5.48	-0.20	6.58
Hymenoptera	Apidae	Terrestrial	10	-1.27	-0.31	-1.24	-0.14	-1.09	-1.04	-0.20	-1.16	-0.17	-1.17
Julida	Julidae	Terrestrial	10	-0.40	-0.25	-0.25	-0.14	-0.20	-0.17	-0.10	-0.26	-0.14	-0.22
Trichoptera	Leptoceridae	Aquatic/Semi-aquatic	10	4.32	-0.22	2.57	NA	26.20	21.71	14.07	8.37	-0.14	9.07
Diptera: Brachycera	Syrphidae	Terrestrial	10	-1.18	-1.51	0.47	-0.56	-0.63	-0.45	-0.47	1.95	0.79	1.95
Diptera: Brachycera	Sarcophagidae	Terrestrial	9.5	-0.45	-1.04	0.42	-0.38	-1.27	-1.15	-0.42	-0.89	-0.67	-0.65
Hemiptera	Aphrophoridae	Terrestrial	9	-0.47	-0.25	-0.32	-0.17	-0.20	-0.10	-0.17	3.94	-0.10	4.34
Hemiptera	Gerridae	Aquatic/Semi-aquatic	9	2.13	-0.22	3.37	-0.14	-0.25	-0.20	-0.14	22.88	6.96	21.71
Coleoptera	Oedemeridae	Terrestrial	9	-1.70	-0.35	-1.59	-0.14	-1.00	-0.99	-0.14	-1.55	NA	-1.55
Lepidoptera	Choreutidae	Terrestrial	8.8	1.64	-0.42	3.63	-0.10	5.66	6.96	-0.10	3.16	NA	3.16
Diptera: Nematocera	Limoniidae	Aquatic/Semi-aquatic	8.8	1.23	1.52	-0.20	-0.14	-0.20	-0.17	-0.10	2.57	-0.29	4.34
Neuroptera	Hemerobiidae	Terrestrial	8.5	4.79	3.94	3.16	NA	-0.23	-0.20	-0.10	3.37	6.96	-0.25
Hymenoptera	Andrenidae/Halictidae	Terrestrial	8	-0.90	-0.85	-0.25	-0.20	-0.29	-0.20	-0.20	-0.42	-0.33	-0.23
Trichoptera	Beraeidae	Aquatic/Semi-aquatic	8	-0.37	-0.25	-0.23	-0.17	4.87	6.96	-0.14	-0.31	-0.17	-0.25
Hymenoptera	Crabronidae	Terrestrial	8	-0.64	-0.23	-0.53	-0.29	-0.49	-0.45	-0.20	-0.42	NA	-0.42
Diptera: Nematocera	Pediciidae	Terrestrial	8	0.57	-0.76	3.37	-0.17	-0.23	-0.23	NA	-0.37	-0.25	-0.25
Hymenoptera	Vespidae	Terrestrial	8	-0.75	-0.31	-0.54	-0.45	1.32	2.57	-0.41	-0.48	NA	-0.48
Diptera: Brachycera	Scathophagidae	Terrestrial	7.6	-1.22	-1.71	-0.81	1.74	-1.57	-2.69	2.88	-1.00	-1.32	-0.56
Diptera: Brachycera	Rhagionidae	Terrestrial	7.5	2.29	-0.21	4.87	-0.10	5.66	6.96	-0.10	-0.28	NA	-0.28
Coleoptera	Tenebrionidae	Terrestrial	7.5	-0.87	-0.27	-0.78	-0.10	-0.49	-0.46	-0.14	-0.58	-0.10	-0.57
Araneae	Araneidae	Terrestrial	7	-0.35	-0.14	-0.28	-0.14	-0.23	-0.14	-0.17	2.58	NA	-0.32
Ephemeroptera	Baetidae	Aquatic/Semi-aquatic	7	1.24	-0.38	-0.31	4.87	7.53	4.87	5.66	2.84	NA	-0.28
Coleoptera	Cerambycidae	Terrestrial	7	-0.45	-0.31	-0.27	-0.17	-0.19	-0.17	-0.14	-0.29	-0.14	-0.25
Hemiptera	Cercopidae	Terrestrial	7	-0.44	-0.34	-0.24	-0.10	-0.20	-0.20	NA	-0.10	NA	-0.10

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Lepidoptera	Gelechiididae	Terrestrial	7	-0.42	-0.27	-0.29	-0.14	5.70	5.95	-0.10	11.43	-0.10	12.32
Coleoptera	Meloididae	Terrestrial	7	-0.54	-0.28	-0.39	-0.14	-0.37	-0.31	-0.17	-0.35	-0.10	-0.33
Diptera: Brachycera	Musc/Fann/Anthomyiidae	Terrestrial	7	1.31	0.07	1.60	0.46	0.66	1.41	-1.13	5.01	2.00	4.75
Diptera: Brachycera	Otitidae/Ulidiidae	Terrestrial	7	-0.66	-0.53	-0.34	-0.10	-0.31	-0.20	-0.23	-0.35	-0.20	-0.27
Diptera: Brachycera	Tachinidae	Terrestrial	7	-0.87	-0.25	-0.78	-0.25	-0.70	-0.67	-0.14	0.39	-0.10	0.41
Hymenoptera	Ichneumonidae	Terrestrial	6.6	-0.92	-1.10	-1.23	1.60	0.16	0.87	-1.19	-1.58	-0.68	-1.37
Coleoptera	Cantharidae	Terrestrial	6.5	-2.77	-0.20	-2.22	-1.65	-1.55	-0.97	-1.34	-1.81	-0.10	-1.83
Coleoptera	Staphylinidae	Terrestrial	6.5	-2.34	-1.33	-1.44	-0.88	-1.26	-1.17	-0.67	-1.26	-0.49	-1.14
Araneae	Philodromidae	Terrestrial	6.3	-0.52	-0.23	-0.42	-0.13	3.16	3.94	-0.17	7.38	6.96	5.10
Diptera: Nematocera	Anisopodidae	Terrestrial	6	4.32	2.70	3.16	NA	-0.29	-0.23	-0.17	-0.31	-0.20	-0.23
Diptera: Nematocera	Chironomidae	Aquatic/Semi-aquatic	6	8.03	3.44	6.30	3.39	7.83	5.80	6.22	4.66	2.75	4.26
Hemiptera	Corixidae	Aquatic/Semi-aquatic	6	-0.44	-0.32	-0.27	-0.14	-0.14	-0.10	-0.10	4.34	-0.14	5.66
Diptera: Nematocera	Culicidae	Aquatic/Semi-aquatic	6	9.73	7.53	8.75	-0.20	2.47	-0.33	4.87	-0.26	-0.14	-0.22
Diptera: Brachycera	Heleomyzidae/Diastatidae	Terrestrial	6	-0.95	-0.23	-0.83	-0.38	-0.73	-0.71	-0.10	-0.86	-0.17	-0.83
Diptera: Brachycera	Micropezidae	Terrestrial	6	1.97	2.84	0.93	-0.14	-0.50	-0.49	-0.14	-0.60	-0.20	-0.56
Hemiptera	Nabiidae	Terrestrial	6	-0.61	-0.35	-0.42	-0.10	-0.39	-0.38	-0.14	-0.53	-0.17	-0.48
Hymenoptera	Tenthredinidae	Terrestrial	6	-1.87	-1.71	-0.25	-0.46	-0.65	-0.27	-0.59	-1.03	-0.99	-0.26
Diptera: Brachycera	Xylophagidae	Terrestrial	6	-0.60	-0.40	-0.35	-0.17	-0.33	-0.29	-0.14	-0.41	-0.20	-0.35
Araneae	Clubionidae	Terrestrial	5.7	5.53	6.45	1.51	2.87	1.95	0.30	3.24	0.42	2.57	-0.15
Coleoptera	Carabidae	Terrestrial	5	-0.97	-0.30	-0.56	-0.67	0.81	1.37	-0.47	-0.69	-0.17	-0.64
Diptera: Nematocera	Chaoboridae	Aquatic/Semi-aquatic	5	13.69	7.86	9.44	5.16	11.33	8.76	10.68	8.50	3.78	7.64
Coleoptera	Coccinellidae	Terrestrial	5	-1.54	-0.34	-1.58	-0.10	-1.12	-1.09	-0.17	-1.26	-0.17	-1.26
Diptera: Brachycera	Conopidae	Terrestrial	5	-0.51	-0.20	-0.46	-0.10	-0.33	-0.29	-0.14	-0.42	-0.10	-0.40
Coleoptera	Elateridae	Terrestrial	5	-0.52	-0.35	-0.35	-0.17	-0.23	-0.17	-0.14	-0.22	-0.14	-0.16
Diptera: Brachycera	Empididae	Terrestrial	5	3.77	7.67	-0.75	-0.10	-0.73	-0.73	-0.10	-1.20	-0.56	-1.12
Hemiptera	Miridae	Terrestrial	5	-0.55	-0.67	-0.17	-0.14	-0.96	-0.94	-0.17	1.13	-0.26	1.27

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Diptera: Brachycera	Sciomyzidae	Aquatic/Semi-aquatic	5	-0.12	-0.55	-0.77	1.32	-0.80	-0.57	-0.60	-0.67	-0.13	-0.65
Araneae	Tetragnathidae	Terrestrial	5	0.77	-0.20	0.87	NA	-0.48	-0.45	-0.14	-0.50	-0.14	-0.49
Diptera: Brachycera	Opomyzidae/Tephritidae	Terrestrial	4.6	-0.20	-0.70	0.59	-0.40	0.82	0.99	-0.33	1.64	-0.25	1.83
Coleoptera	Chrysomelidae	Terrestrial	4.4	0.36	-1.46	0.95	2.42	0.93	0.62	1.17	0.17	-1.43	0.85
Diptera: Brachycera	Dolichopodidae	Aquatic/Semi-aquatic	4.3	3.13	6.05	1.33	0.66	2.38	2.02	0.99	1.40	-0.20	1.43
Diptera: Nematocera	Bibionidae	Terrestrial	4	-1.41	-1.30	-0.25	-0.50	-0.41	-0.20	-0.37	-0.58	-0.55	-0.14
Hemiptera	Delphacidae	Terrestrial	4	-0.51	-0.27	-0.41	-0.10	-0.33	-0.31	-0.10	-0.48	-0.14	-0.48
Hymenoptera	Evaniidae	Terrestrial	4	-0.99	-0.37	-0.78	-0.56	-0.83	-0.68	-0.50	-0.75	-0.17	-0.71
Diptera: Brachycera	Lonchopteridae	Terrestrial	4	1.91	3.17	1.18	-0.45	0.89	2.12	-0.59	-0.55	-0.10	-0.53
Diptera: Brachycera	Pipunculidae	Terrestrial	4	4.94	2.84	4.87	-0.10	2.58	2.99	-0.17	-0.29	NA	-0.29
Hemiptera	Psyllidae	Terrestrial	4	1.19	3.94	-0.43	-0.31	-0.38	-0.33	-0.17	-0.40	-0.14	-0.36
Hemiptera	Saldidae	Aquatic/Semi-aquatic	4	2.13	5.66	-0.38	NA	-0.31	-0.29	-0.10	-0.35	-0.10	-0.34
Coleoptera	Scirtidae	Aquatic/Semi-aquatic	4	0.31	4.34	-0.74	-0.20	-0.63	-0.57	-0.20	0.73	NA	0.73
Diptera: Brachycera	Sepsidae	Terrestrial	4	-0.57	-0.77	0.06	-0.50	0.36	1.02	-0.54	-0.95	-0.35	-0.91
Diptera: Brachycera	Stratiomyidae	Terrestrial	4	-2.01	-0.28	-2.04	-0.14	-1.46	-1.45	-0.10	-1.90	-0.14	-1.89
Hemiptera	Anthocoridae	Terrestrial	3.5	-1.77	-0.65	-2.04	0.56	-1.52	-1.35	-0.88	-1.89	-0.31	-1.82
Coleoptera	Curculionidae	Terrestrial	3.5	-1.52	-0.30	-1.49	NA	-1.18	-1.20	-0.10	-1.40	-0.20	-1.37
Diptera: Brachycera	Ephydridae	Aquatic/Semi-aquatic	3.5	1.82	4.34	-0.27	-0.28	-0.17	-0.10	-0.14	4.34	-0.10	4.87
Diptera: Brachycera	Lauxaniidae/Drosophilidae	Terrestrial	3.5	0.60	-0.94	1.78	-0.27	2.65	1.10	4.34	-0.93	-0.44	-0.70
Coleoptera	Nitidulidae	Terrestrial	3.5	-3.48	-2.87	-1.93	-0.27	-1.26	-1.27	-0.23	-2.38	-1.39	-1.71
Coleoptera	Ptinidae	Terrestrial	3.5	-0.65	-0.22	-0.59	-0.17	-0.54	-0.53	-0.10	-0.70	-0.14	-0.67
Hemiptera	Cicadellidae	Terrestrial	3.3	-4.26	-1.86	-4.62	0.48	-2.31	-2.73	0.85	-3.62	-0.68	-3.66
Coleoptera	Helophoridae	Terrestrial	3.3	11.50	13.28	3.16	-0.17	5.70	6.58	-0.17	-0.23	-0.10	-0.20
Diptera: Brachycera	Hybotidae	Aquatic/Semi-aquatic	3.3	-1.25	0.27	-0.69	-2.41	-1.15	-0.17	-1.85	-2.90	-0.89	-2.88
Diptera: Brachycera	Anthomyzidae	Terrestrial	3	2.06	-0.27	-0.31	9.90	-0.35	-0.29	-0.17	-0.44	-0.14	-0.41
Coleoptera	Cleridae	Terrestrial	3	-0.39	-0.27	-0.20	-0.20	-0.25	-0.23	-0.10	-0.20	NA	-0.20

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Hymenoptera	Cynipidae	Terrestrial	3	2.06	3.37	-0.27	-0.14	-0.35	-0.27	-0.20	-0.31	-0.10	-0.29
Coleoptera	Dytiscidae	Aquatic/Semi-aquatic	3	2.28	-0.25	3.37	-0.14	-0.20	-0.20	NA	-0.25	-0.10	-0.23
Coleoptera	Haliplidae	Aquatic/Semi-aquatic	3	-0.55	-0.38	-0.28	-0.23	-0.28	-0.23	-0.20	-0.22	-0.14	-0.20
Trichoptera	Hydroptilidae	Aquatic/Semi-aquatic	3	-1.86	-0.60	-1.84	-0.23	-1.32	-1.30	-0.14	-1.30	-0.17	-1.29
Hymenoptera	Pteromalidae/Torymidae	Terrestrial	3	-5.87	-1.44	-4.14	-4.68	-4.48	-2.93	-3.14	-3.73	-0.51	-3.74
Hymenoptera	Braconidae	Terrestrial	2.9	5.93	4.97	4.06	0.21	0.38	0.28	0.34	-1.15	-0.59	-0.96
Coleoptera	Apionidae	Terrestrial	2.5	-1.78	-1.80	-0.24	-0.20	-0.24	-0.17	-0.20	-0.64	-0.64	-0.10
Hymenoptera	Diapriidae	Terrestrial	2.5	-1.01	-0.23	-0.52	-0.87	-0.91	-0.42	-0.80	-0.62	-0.14	-0.61
Araneae	Linyphiidae	Terrestrial	2.5	-0.14	-0.82	2.09	-1.55	-0.15	0.05	-0.27	-0.91	-1.17	-0.10
Diptera: Nematocera	Mycetophilidae	Terrestrial	2.5	3.16	-0.23	5.66	-0.10	-0.25	-0.20	-0.14	-0.39	-0.20	-0.35
Hymenoptera	Platygasteridae	Terrestrial	2.5	-2.15	-0.20	-0.52	-2.17	-2.04	-0.37	-1.95	-0.51	-0.17	-0.46
Hymenoptera	Pselaphidae	Terrestrial	2.5	-0.49	-0.45	-0.24	-0.10	-0.19	-0.19	NA	-0.10	NA	-0.10
Diptera: Nematocera	Sciaridae	Terrestrial	2.5	-3.45	-3.49	-1.15	-0.58	0.07	0.36	-0.49	-1.85	-1.68	-1.02
Coleoptera	Scraptiidae	Terrestrial	2.5	-0.67	0.07	-1.13	-0.13	-0.71	-0.71	NA	-1.00	-0.35	-0.93
Hemiptera	Tingidae	Terrestrial	2.5	3.82	-0.39	6.30	NA	-0.20	-0.20	NA	17.32	NA	11.49
Hemiptera	Triozidae	Terrestrial	2.5	-1.09	-0.33	-1.03	-0.35	-1.01	-0.77	-0.43	-0.77	-0.10	-0.76
Hemiptera	Aphididae	Terrestrial	2.3	2.28	1.91	0.93	2.69	-0.61	-1.24	2.20	-0.91	-0.81	-0.67
Diptera: Brachycera	Chloropidae	Terrestrial	2.3	-12.15	-8.01	-8.94	-6.32	-6.09	-5.70	-2.59	-8.58	-3.46	-7.94
Diptera: Nematocera	Psychodidae	Aquatic/Semi-aquatic	2.2	-2.39	-0.91	-2.66	1.46	-0.84	-0.67	-0.55	-2.45	-0.67	-2.47
Diptera: Nematocera	Ceratopogonidae	Aquatic/Semi-aquatic	2	-3.84	-2.78	-1.88	-1.34	-1.97	-1.46	-1.34	-3.13	-1.59	-2.63
Hymenoptera	Eulophidae	Terrestrial	2	-1.25	-0.45	-0.33	-1.06	-1.14	-0.17	-1.15	-0.27	-0.10	-0.25
Coleoptera	Kateretidae	Terrestrial	2	1.41	-0.31	-0.28	2.99	9.90	NA	-0.10	-0.25	-0.10	-0.23
Coleoptera	Lathridiidae	Terrestrial	2	-0.76	-0.31	-0.67	-0.23	-0.54	-0.53	-0.20	-0.63	-0.17	-0.65
Coleoptera	Phalacridae	Terrestrial	2	-0.92	-0.25	-0.83	-0.14	-0.71	-0.68	-0.20	-0.80	-0.14	-0.79
Diptera: Brachycera	Phoridae	Terrestrial	2	-3.93	-1.40	-3.60	-1.82	-3.32	-3.06	-1.24	-3.73	-0.54	-3.75
Diptera: Nematocera	Scatopsidae	Terrestrial	2	-3.46	-0.58	-3.61	-0.29	-2.80	-2.90	-0.25	-2.95	-0.20	-2.96

				Adult				Juvenile			Nestling		
Order	Resource	Habitat	Body Length (mm)	All	Early	Middle	Late	All	Middle	Late	All	Early	Middle
Diptera: Nematocera	Simuliidae	Aquatic/Semi-aquatic	2	-0.89	-0.78	-0.29	-0.14	-0.14	-0.14	NA	-0.37	-0.29	-0.20
Araneae	Theridiosomatidae	Terrestrial	2	1.70	-0.29	3.37	-0.10	NA	NA	NA	-0.32	-0.14	-0.28
Hemiptera	Aleyrodoidae	Terrestrial	1.5	-0.48	-0.27	-0.33	-0.20	-0.31	-0.20	-0.23	-0.26	NA	-0.26
Diptera: Nematocera	Cecidomyiidae	Terrestrial	1.5	-2.91	-1.58	-1.55	-1.57	-2.16	-1.77	-1.26	-2.01	-0.74	-1.80
Hymenoptera	Mymaridae	Terrestrial	1	-1.70	-0.67	-0.58	-1.35	-1.18	-0.43	-1.12	-0.52	-0.27	-0.46

3.5. Discussion

3.5.1. Summary

The species rich diet of reed warblers of three age classes (adults, juveniles, and nestlings) in Chew Valley Lake, Somerset, was successfully characterised with metabarcoding. Modifications made to the extraction protocol (Zeale et al. 2011, Nicholls 2017 pers. comm., Shutt et al. 2020) were beneficial in reducing large amounts of PCR inhibiting uric acid from the nestling faecal sacs, but complete removal could not always be achieved. This was reflected in the lower success rate for nestling samples. The dietary spectrum of reed warblers was strongly associated with age and season, with each age group occupying a subtly different dietary niche. Juvenile diet occupied an intermediate niche space between nestling and adult diet and was not simply a subset of adult diet.

3.5.2. Dietary richness and diversity

Reed warblers consumed a broad range of arthropods (258 species). All age groups consumed a relatively high diversity of prey items, and generalist diet was achieved at all age stages. The incompleteness of our approach in describing all possible diet items is partly due to large numbers of rare prey items (singletons and doubletons) that dominated the samples (Colwell et al. 2004). Achieving a mixed diet improves the fitness of generalists since they obtain a more complete range of nutrients (reviewed in Lefcheck et al. 2013). By consuming a diverse array of prey, they also limit the quantity of toxins ingested from any one resource (Pulliam 1975, Bernays et al.1994).

As expected, invertebrates from wet habitats and waterbodies were a major food resource. The significance of aquatic prey in sustaining insectivorous bird populations is well established in the literature (Newton 1998, Baxter et al. 2005, Bartels et al. 2012, Trevelline et al. 2016, Michelson et al. 2018). However, averaged across all ages, terrestrial groups formed roughly half of the diet of Chew reed warblers. This was at first surprising given the wetland habitat associations of reed warblers but confirms that reed warblers do forage in a wide range of vegetation types; from reeds and shrubs to herbaceous stands of vegetation (Grim and Honza 1996). Another insectivorous bird, the tree swallow (*Tachycineta bicolor*), shows a preference for aquatic aerial insects, but switches to terrestrial prey when the latter are higher in abundance (Turner 1982, Michelson et al. 2018). In this study the use of short-term resources is implied by the variation in diet across individuals and nests, and this may be a result of tracking other abundant prey between peaks of aerial insect emergence from the wetland environment.

The average species richness of prey measured by the number of unique taxa in each dietary sample was not affected by local weather. However, weather was recorded from a single locality over a single year, and likely requires further data collection for a deeper analysis. Weather patterns can differ drastically both year on year and between sites, causing dramatic changes to prey availability. This can be seen in the different dietary composition of the same songbird species in different years (Rotenberry 1980, Trevelline et al. 2018) and at different sites (Rotenberry 1980, Bibby and Thomas 1985).

Adult reed warblers showed greater dietary species richness than juveniles and nestlings. Since adults are the most experienced hunters, they may utilise a wider variety of feeding habitats so that a wider range of prey is available to them. The lower dietary diversity in juveniles could relate to their use of sub-optimal habitats. Adults are likely to be more dominant and might occupy the most productive feeding habitats to the competitive exclusion of juveniles. Alternatively, adults and juveniles may simply have divergent feeding strategies (Marchetti and Price 1989, Wunderle 1991). Nestling dietary richness may be lower than the remaining age classes because their requirements for growth might necessitate selection of dietary items richest in calories at the expense of variety, resulting in a higher prevalence of breeding currency arthropods in the diet (Greenberg 1995).

3.5.3. Diet composition and breeding currency arthropods

Optimal foraging theory dictates that common prey may become increasingly profitable for consumers as they form a search image and increase their capture and handling efficiency for the most frequently encountered prey (Krebs et al. 1978). Some commonly recorded prey items on sticky traps were also consumed at an elevated frequency by adults compared to the other reed warbler age groups. This may reflect optimal foraging by adults disproportionately feeding on more abundant prey groups that they have formed a search image for.

Differences between adult and nestling diet were found in other studies, for example by Durst et al. (2008) in their study on southwestern willow flycatchers (*Empidonax traillii extimus*). In our study, nestling diet was species rich for large, soft-bodied prey groups (that can be considered breeding currency), including both Lepidoptera and Hemiptera, and detections of Lepidoptera, Odonata and Gastropoda were most frequent in nestling samples. Jedlicka et al. (2017) detected a similar trend in their study on western bluebirds (*Siala mexicana*). The families Erebidae, Tortricidae and Noctuidae were the most frequent of the Lepidoptera fed to reed warbler nestlings. It is likely that these comprise caterpillars rather than adult imagoes, due to the reported high prevalence of larval Lepidoptera in the diet of many other species of passerine nestlings (Krupa 2004, Maziaz and Wesołowski 2010, Skipper and Kim 2013, Xiong and Lu 2014, Orłowski et al. 2015, Wesołowski and Neubauer 2017, Wesołowski et al. 2019).

Lepidoptera accounted for a greater proportion of the nestling faecal samples than adult and juvenile samples, aligning with the expectations of the breeding currency hypothesis. Lepidoptera are a high-quality, nutrient-rich food group, and many families comprising relatively large-bodied species were consumed by nestlings disproportionately to their availability. This alludes to preferential selection of this high-quality prey by their parents to enhance nestling growth and increase breeding productivity (Greenberg 1995, Yard 2004, Skipper and Kim 2013). The reverse pattern was found for nematocerous flies, which are a species-rich group, which comprised a greater proportion of the diet of adult and juvenile reed warblers than that of nestlings.

Surprisingly, an elevated consumption of spiders by nestlings was not observed in this study, as is often reported in passerine nestling diet (Pagani-Núñez et al. 2011, Navalpotro et al. 2016, Wesołowski et al. 2019). Spiders contain high concentrations of the amino acid taurine, which is essential for development of the central nervous system of young birds (Ramsay and Houston 2003, Arnold et al. 2007). However, philodromid crab spiders were disproportionately fed to nestlings at a rate higher than expected from their abundance, perhaps suggesting that this particular family is important for nestlings. A larger sample size of nestlings would help reveal additional contributions of spiders to nestling diet and allow us to better assess their importance for growth and development.

The caddisfly, *Oecetis ochracea* was the only prey species consumed by juveniles at a frequency higher than adults and nestlings. They also showed fewer preferences for specific taxa than adults foraging for themselves and foraging for broods in the *econullnetr* analysis. This suggests that juveniles are feeding less selectively than the other two age groups, most likely taking any groups encountered that could be captured, regardless of profitability. Ecologically this should suggest both naivety and exploratory feeding behaviour that would be expected from newly fledged birds (Marchetti and Price 1989). Some prey may have been difficult to capture, either due to their speed or large body size. One example is the damselfly *Enallagma cyathigerum* which was consumed by adults and nestlings but not juveniles, despite being present throughout the summer months.

Juvenile birds did show a greater proportion of aquatic prey per sample compared to either nestlings or adults, irrespective of the time in the breeding season when the bird was captured. To our knowledge this difference has not been described in reed warblers before and may be site or year dependent. Nonetheless, the different diet of the juveniles compared with adults and the higher incidence of semi-aquatic prey may be evidence of foraging habitat separation between juveniles and their adult counterparts. Juveniles may be more reliant on super-abundances of emerging aerial insects, which are largely semi-aquatic, showing a stronger association with wetter habitats. Consequently, juveniles might show lower dietary plasticity and be less able to adapt to the more seasonal prey resources found in terrestrial habitats. Analysis of a larger number of samples would certainly provide more information about juvenile diet and would potentially reveal additional preferences. Additionally, monitoring individual birds in the field would allow the tracking of juvenile bird movements and confirm patterns in habitat separation.

3.5.5. Prey size

There seemed to be a minimum threshold for prey size in the diet of all reed warbler age classes since no taxa of less than 2mm in length were detected at a high frequency. The average prey length of ~7mm is consistent with other warbler studies (Leisler et al. 2002, Marchetti et al. 1998, Kerbiriou et al. 2011). Although it might be expected that young birds would be fed more manageable, smaller items, in our study nestlings were fed the largest prey items. In accordance with our findings, Grim and Honza (1996) found that the average body length of prey fed to reed warbler nestlings was 8mm and ranged from 1.9 to 21mm. The larger prey items in our study largely represent softer bodied, high calorie taxa which are considered valuable nestling food (Skipper and Kim 2013). The adults of acrocephalids feed their nestlings larger than average sized prey in bundles, so that they can save energy by reducing the number of trips to the nest (Leisler et al. 2002). Those that inhabit more productive habitats and that can capture larger prey can provision their young in fewer flights to the nest and improve their reproductive success. In the great reed warbler (*Acrocephalus arundinaecus*), a close relative of the Eurasian reed warbler, females that lacked an assisting male compensated by increasing the frequency of feedings and bringing prey of a heavier weight (Sejberg et al. 2000).

Moreover, the nutritional requirements of a warbler brood will change from hatching to fledging as demonstrated in other passerine groups (Wesołowski et al. 2017, 2019, Jedlicka et al. 2017). All the faecal samples from nestlings in this study were collected from individuals that were 7-10 days old and by this age they were probably able to handle both larger and more chitinous prey compared to younger nestlings (Orłowski et al. 2015). In the latter study, the prey biomass of Hemiptera increased from 12% in 2-3-day old barred-warbler (*Curruca nisoria*) nestlings to 25% in 7-9 day old nestlings. Krupa et al. (2004) also showed in willow warblers (*Phylloscopus trochilus*) that the daily number of feeds, number of prey items received, and the biomass of food increased over time as the nestlings developed. Therefore, like willow warblers, reed warbler parents may be under selective pressure to bring ever-larger prey to the nestlings as they develop, to ensure their brood receives the optimal sustenance for growth.

3.5.6. Dietary overlap and diet preferences

Overlap among age classes

Although considerable differences in selected prey were found between age groups, the diet of each age group was always supplemented with commonly available and shared prey items, such as *Chaoborus flavicans*, *Clubiona phragmitis*, *Scathophaga stercoraria* and various chironomids. Dietary overlap among the three ages was significantly greater than expected by chance, and this is likely to be a consequence of very high abundances of these prey groups, providing an ongoing, plentiful food resource (Rosenberg et al. 1982), which may limit the potential for inter-age competition. Trevelline et al. (2018) suggested a similar mechanism for high levels of dietary overlap observed between nestlings of different riverine species coinciding with superabundances of emerging aquatic prey. This high degree of dietary similarity found in the present study is not surprising since the samples all came from the same species in one site during the same breeding season and year.

Seasonal changes in dietary overlap

In both the middle and late breeding season, pairwise diet comparisons showed dietary overlap that was significantly greater than expected. However, in the early breeding season, overlap between nestlings and adults was lower. This may reflect reedbed primary productivity being limited by lower temperatures earlier in the year, and many invertebrate groups not yet emerging in large numbers (McKee and Richards 1996, Halupka et al. 2008). When there are fewer prey items to choose from, adults might prioritise more nutritious prey for their offspring at the expense of their own needs, to ensure nestling growth is optimised.

3.5.7. Patterns of prey choice

Different aged birds showed some unique preferences for invertebrate families. However, aquatic Diptera such as Chironomidae and Chaoboridae and the caddisfly family Leptoceridae were universally preferred by all ages of reed warblers and exploited more frequently than expected from their measured abundance.

Pearson et al. (2018) found evidence that consumers disproportionately consumed the most abundant species, which supported the theory that the key determinants of prey choice are encounter rate, capture success, handling efficiency and nutritional quality (Symondson 2002). Our study provides some evidence of several common species being consumed at a higher rate than expected, especially by adult birds. However, some of the most abundant potential prey items were relatively underexploited compared with scarcer ones (Bibby and Thomas 1985). The total abundance of a prey family on sticky traps, was negatively associated with the strength of preference by warblers for that prey family, but this may be a result of sticky trap biases, where some very abundant groups of invertebrates attracted to the sticky traps were patchily distributed and would have needed to be consumed at an extremely high rate by warblers to be a classed as a proportional preference. This might reflect a trade-off between encounter rate and nutritional quality or prey size.

Disproportionate consumption of larger prey items was observed compared to their availability, and larger prey items were significantly more likely to be preferred by reed warblers. This suggests that many of the avoided prey families common to sticky traps were simply not of a sufficient size to be profitable to the birds.

Seasonal changes in diet selectivity

Preferences for some prey families were specific to one time period, suggesting that reed warblers might exhibit different dietary preferences over time, irrespective of prey abundance. Mid-season dietary samples (June-July) coincided with the peak of prey availability for many invertebrates. Prey size in the diet also appeared to peak in the middle of summer, coinciding with the peak of invertebrate availability for prey items 9mm or larger. Similarly, the number of stronger than expected, positive interactions between warblers and prey resources peaked in the middle of summer. Perhaps reed warblers show stronger preferences at this time of year because total prey abundance and diversity is at its peak, allowing individuals to be more selective, and target prey that give them optimal nutritional benefit (Francois et al. 2020). This period of greater selectivity coincides with the period of the greatest reed warbler abundance on the breeding grounds when each of the age classes are present together in higher densities and demand for food is greatest. Thus, if food supply is plentiful and if different age groups consume different prey groups disproportionately, any competitive pressure would be alleviated.

3.5.8. Limitations and caveats to interpretation

Just one nestling sample per nest was carried through to the frequency of occurrence calculation step prior to analyses to avoid pseudoreplication, especially since mixed model random effects cannot yet be incorporated into *manyglm* models. Although this may have risked the loss of some dietary information, it should have provided an unbiased method of comparison between individual birds.

As with Chapter 2, sticky trap biases may have skewed our measures of prey availability and prey choice analyses. When compared to their measured abundance, several lepidopteran families were consumed much more frequently than expected. Yellow traps have a lower success for Lepidoptera, causing them to be under-represented (Thomson et al. 2004). The same is true for the gastropod snails and slugs, which despite being a regular component of the reed warbler diet, are primarily found at ground level and were not captured frequently by traps. It is clear from the high % FOO values however, that Lepidoptera are important in the diet even if their availability is higher than estimates suggest, thus it is likely that the preferences for this group would still have been detected even with a higher and more accurate estimate of availability.

In addition, because reed warblers are an example of a dietary generalist capable of opportunism and dietary switching and they show a wide dietary breadth, a longer-term project monitoring the diet of this species over multiple years would be needed to better characterise reed warbler diet and identify additional temporal patterns. Sample sizes of juveniles and subsetted nestlings in the early season were very small and may have affected the power of some statistical analyses. Care should thus be taken when making broad generalisations based on these findings.

3.6. Acknowledgements

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Chapter Four - Dietary selectivity, opportunistic foraging and interspecific competition in a community of Mediterranean reedbed warblers in Catalonia, Spain

A great reed warbler being processed during a ringing session at Canal Vell Biological Station in the Ebro Delta. Bird ringing was undertaken as part of the SYLVIA scheme by the Catalan Ornithological Institute (ICO).

4.1. Abstract

The study of ecological interactions in songbirds is challenging. These species are often characterised by opportunistic life-histories, characterised by multiple habitats and prey resources that are spatially and seasonally variable (Sherry et al. 2016). To maintain coexistence in environments with fluctuating resources, sympatric species should differ along one or more ecological dimensions, but it is unclear how this is achieved in the generalist reedbed warblers that show overlaps in habitat use. A dietary metabarcoding approach was applied to study the dietary composition of sympatric reed-bed warblers on their breeding grounds in Catalonia, Spain. The findings together imply that a degree of dietary niche separation is occurring among the warblers which may explain their patterns of coexistence. Although dietary overlap was significant between most pairs of warblers, individual warbler species differed in prey resource use by consuming different taxa at differing frequencies, partitioning prey of different sizes and foraging on different proportions of aquatic and terrestrial prey species. Evidence of dietary opportunism and flexibility was indicated in the observed differences in dietary composition at the four study sites and at different stages of the breeding season. These effects were at least partially driven by local weather patterns and the effects of seasonality which are known to affect the abundance and availability of prey resources. The results have implications for future coexistence of warblers in Iberia as the climate warms.

4.2. Introduction

4.2.1. Resource partitioning in songbirds

Resource partitioning theory hypothesises that prey resource use should differ between sympatric species (Cody 1968, Schoener 1974*b*). MacArthur’s formative study (1958) showed that sympatric warblers coexisted by foraging at different heights in conifer trees, even while eating similar prey. Since then, similar proxies of resource use promoting coexistence have been demonstrated across a wide range of organisms including birds (Lack 1976, Hiron et al. 2006), lizards (Pianka 1974) and arthropods (Albrecht and Gotelli 2001). Competition between sympatric species can differ across a focal species’ range, either relaxing or strengthening as environmental conditions change (Schoener 1974*a*, Wiens 1977). Moreover, species may encounter new competitors across their range (Diamond 1973, Freeman et al. 2016). For example, the Eurasian reed warbler (*Acrocephalus scirpaceus*) (hereafter termed “reed warbler”), loses its competitor the sedge warbler at the limit of the latter’s natural range, but gains another, the great reed warbler (*A. arundinaceus*).

Amongst reedbed warblers, the partitioning of trophic resources is likely to be important in maintaining coexistence. Previous studies show that despite them occupying very similar breeding habitat niches, different reed-bed warblers can cohabit the same marshlands without exclusion (Martínez-Vilalta et al. 2002, Kennerly and Pearson 2010). This can be achieved by ecological segregation, either directly by differences in foraging behaviour and habitat use or indirectly by differences in morphology (e.g., body size or bill size and shape) that may make certain prey types or feeding strata more accessible (MacArthur 1958, Pigot et al. 2018).

This chapter will focus on the competitive relationships between interspecific reedbed warblers in southern Europe. In the reedbed ecotones of north-eastern Spain three warbler species commonly co-occur during the breeding season: the reed warbler, the great reed warbler and the Cetti's warbler (*Cettia cetti*). While the reed and great reed warbler are congeneric Afro-Palearctic migrants, breeding in Europe, Cetti's warblers are more distant relatives, and are non-migratory residents of southern Europe (Kennerly and Pearson 2010).

Habitat segregation between the three focal species is limited in extent during the breeding season. Cetti's warblers are generally more terrestrial in their nesting habitat, often residing in low bushes and dense vegetation. While these are often in close proximity to water, this species does not nest in dense reeds (Harvey 1977, Bibby and Green 1984, Balança and Schaub 2005). However, they frequently forage in aquatic habitats such as reedbeds and riparian zones, and as a result may consume aquatic prey (Molina et al. 1998, Araújo et al. 2016). Reed and great reed warblers are associated with dense reedbeds for breeding and foraging, even utilising open water zones (Cramp and Brooks 1992, Kennerly and Pearson 2010). Although they both inhabit the littoral vegetation of marshes, there is some horizontal and vertical separation in their habitat selection (Dyrcz 1981, Leisler 1981). Great reed warblers prefer the taller, thicker *Phragmites* stems in deeper water zones, often at the edge of open water zones whereas reed warblers occur more frequently in areas of dense, shorter reed in shallower water which are less productive (Kleindorfer et al. 1997, Poulin et al. 2002). These general preferences can mask a degree of flexibility in habitat choice when selecting nesting territories and when foraging (Grim 1999, Surmacki 2005). For example, the reed warbler can travel long distances from the nest territory to exploit richer food sources in a variety of habitats (Ezaki 1992) and may feed just as frequently in scrub vegetation as it does in reedbeds and aquatic zones (Thomas 1984, Simms 1985). This observed variation in foraging and breeding habitat utilisation challenges the idea that reed-bed warbler habitats are highly species-specific. Rather, a degree of habitat overlap between conspecific species may be commonplace, which provides ample opportunity for overlapping diet if similar prey items are consumed.

4.2.2. Competitive interactions in reedbed warblers

Access to resources may not be equal if one competitor is dominant over the others or has some competitive advantage. As a general rule, larger species are usually competitively superior to smaller species in asymmetrical, competitive interactions (Schoener 1983, Shelley et al. 2004). Great reed warblers are up to 20g heavier than reed warblers (Cramp and Brooks 1992) and are competitively dominant over the reed warbler in the breeding season (Dyrcz 1981). The two species are known to compete for enemy free space with the former often excluding the latter from nesting areas, delaying the onset of reed warbler breeding (Dyrcz 1981, Hoi et al. 1991, Honza et al. 1999, Schaefer et al. 2006). Later in the breeding season, reed warblers are at higher population density and can drive off breeding great reed warblers (Hoi et al. 1991). This species pair is a well-known example of birds that exhibit competition at several levels (Dyrcz 1981), with exploitation competition and predator-mediated apparent competition discussed as likely drivers of reproductive success in this species pair (Hoi and Winkler 1994, Schaefer et al. 2006).

It is not currently known if aggressive interactions are at all based on access to food resources, however habitat and food requirements of the species do broadly overlap (Dyrcz 1981, Dyrcz and Zdunek 1996). Moreover, high quality territory could be indicative of enhanced food availability if nest sites are close to productive feeding areas (Dyrcz 1986).

The differing life-history strategy of the resident Cetti’s warbler likely led to more distinct differences in their feeding ecology compared to the acrocephalids. Studies in the tropics and the Americas have shown that resident birds are able to monopolise the most profitable trophic resources year-round, whereas arriving migrants are forced to consume less optimal, smaller prey (Poulin and Lefebvre 1996). The resident Cetti’s warbler may be more selective than its migrant neighbours, and more likely to take larger, more profitable prey.

4.2.3. Mechanisms of partitioning

If habitat segregation between warblers is incomplete, a degree of niche separation must be present via other means, to avoid competitive exclusion. It has already been shown that the three warbler species show some differences in behavioural foraging strategies, but they too have been shown to overlap, especially in reed and great reed warblers. Prey capture methods include hawking, the taking of mobile insects from the air, which is used by reed and great reed warblers, whereas Cetti’s warblers tend to glean insects from foliage (Green and Davies 1972, Cramp and Brooks 1992, Kennerly and Pearson 2010). Competitors may also feed at different vegetation heights; for example, middle to upper heights of vegetation by reed warblers (Green and Davies 1972) and lower vegetation /ground level by the Cetti’s warbler (Cramp and Brooks 1992). Great reed warblers are less active foragers than reed warblers, taking more prey from the water surface and at a lower reed stratum than reed warblers (Leisler 1991, Cramp and Brooks 1992, Schaefer et al. 2006).

One limitation to foraging behaviour studies is that they often assume that differences in foraging technique translate to the consumption of different prey taxa, but this is not always the case (Poulin and Lefebvre 1996, Navalón 2019, Gordon 2019). Many warbler species do not regularly co-occur at finer spatial scales which indicates that differing foraging behaviours alone may not be enough to guarantee coexistence (Lovette and Hochachka 2006). Prey may be highly mobile and move between many microhabitats, making them available to many consumers (Wilson 2010). Differences in capture rate and capture efficiency may also dampen the effects of behavioural niche segregation (Drenner et al. 1978) and different foraging methods may target the same prey species, leading to greater overlap between two consumers than expected.

To understand how sympatric birds partition prey, better, detailed knowledge of the diet is required. Various studies have documented the diet of the reed warbler (Bibby and Thomas 1985) and great reed warbler (Dyrcz and Flinks 2000, Dyrcz 2016, Ferreiro-Carballal et al. 2017), but these have mostly been limited to hard-part analyses which may underestimate certain soft-bodied prey (Pompanon et al. 2012). In addition, dietary studies of populations in Iberia are relatively scarce (Cardenas et al. 1983, Ferreiro-Carballal et al. 2017).

Reed and great reed warblers are known to consume a wide array of aquatic and semi-aquatic prey, but little is known about Cetti's warbler diet in Spain (Molina et al. 1998, Araújo et al. 2016). If warblers within a shared site select prey that have different habitat associations (i.e., aquatic or terrestrial), this small effect of resource partitioning could reduce potential interspecific competition.

Diet overlap may not be as large as predicted if different size classes or life stages of the same prey species are consumed by the focal competitors (e.g., Martínez-Curci et al. 2015). Morphological differences in body and bill size between different warbler species and/or different dietary requirements, could lead to differential prey-size selection and reduced foraging competition (Marchetti et al. 1996). Existing studies suggest that reed and great reed warblers may take prey of different sizes, with great reed warblers preferring larger arthropods that are newly emerged from the water margin and stem-boring taxa at low vegetation levels, while reed warblers feed more opportunistically on smaller, usually winged, mobile prey such as dipterans and aphids, that occur higher in the reeds but may be highly variable in local availability (Leisler 1991, Schulze-Hagen 1991, Cramp and Brooks 1992).

4.2.4. Dynamic patterns of diet overlap

Since it is well established that prey resources fluctuate over time, it is not surprising that dietary overlap may vary (Wiens 1977, McMartin et al. 2009). Changes in the environment relating to shifts in local climate, stochastic weather events, or the effects of seasonality all alter the diversity and abundance of prey resources and/or foraging habitats available to birds. Fluctuations in weather conditions have been shown to change microhabitat selection and feeding intensity of birds by directly influencing the activity, abundance and distribution of arthropod prey (Petit et al. 1990). Marchetti et al. (1996) posited that subtle changes in passerine diet at their field site in Capo Caccia in 1990-1991 reflected differences in weather, with 1991 a wetter and cooler year than 1990. This change to a sub-optimal diet was reflected in lower and slower weight gains in 1991 (Marchetti et al. 1993). In addition, individuals may be constrained by age-related factors, such that adults and juveniles show different patterns in resource use (Olson et al. 2003, see Chapter 3).

Understanding how dietary generalist songbirds can coexist within dense breeding communities, during an energetically demanding period of the year (Holmes et al. 1979a, Martin 1987) may help us predict how they might adapt to the environmental changes brought on by climate change. One of the biggest barriers to understanding how passerines partition prey resources on the breeding grounds is the lack of taxonomic resolution from traditional dietary analyses (Pompanon et al. 2012). High-throughput sequencing allows genus and species level descriptions of diet to be achieved, which can potentially resolve subtle but important differences in prey utilisation between competitors (Krüger et al. 2014).

4.2.5. Chapter aims and objectives

The aim of this chapter is to use metabarcoding to characterise the diets of the three reedbed warbler species across four sites in Catalonia, north-eastern Spain, over the course of one breeding season in 2018. I hypothesise that the dietary composition of the three species will be differentiated in terms of: i) the consumption of different prey taxa at the species, family or order level, reflected by different frequency of occurrence patterns, ii) prey diversity and dietary overlap between consumers, iii) the body size ranges of prey selected, and iv) the composition of prey according to terrestrial and aquatic guilds. Critically, I extend i) to include species-specific dietary interactions indicated by positive selection or “preference” (i.e., disproportionate consumption of a prey item compared to its relative abundance in the environment) (Vaughan et al. 2018; Pearson et al. 2018).

4.3. Methodology

4.3.1. Study location

The sites chosen for the study were i) Mas del Matà, part of the large nature reserve Aiguamolls de l'Emporda (42°12'47.55"N, 3° 5'57.05"E) in northern Catalonia near Girona, ii) the Llobregat Delta (comprised of two connected reserves; Cal Tet and Remolar-Filipines: 41°17'44"N, 2° 6'43"E): a coastal, wetland south of Barcelona, iii) Sebes (41°14'15.57"N, 0°30'39.49"E): a wetland nature reserve near Flix along the Ebro river in western Catalonia and iv) Canal Vell (40°44'34.61"N, 0°47'10.25"E): a wetland reserve and biological research station situated on the Ebro Delta on the eastern coast of Catalonia (Fig. 4.1). Sites differed in topography, proximity to the coast, altitude, and sub-climate, but comprised similar warbler assemblages. Vegetation was composed predominantly of reedbeds dominated by *Phragmites australis*, surrounded by herbaceous vegetation, woodland or associated scrub. *Tamarix* and *Salix* largely dominated the scrub, but in Sebes olive trees (*Olea*) were common, and in Canal Vell the non-native *Myoporum*. Temperatures in the 2018 field season increased from May to August, with a rapid climb in maximum temperature between late May/early June and late/June-July. Daily Maximum temperatures did not generally fall below 20°C or above 35°C. Rainfall was low throughout the summer, although heavy rainfall events did occasionally take place. Rainfall events became less frequent as summer progressed and sites became very dry, with some aquatic habitats becoming increasingly desiccated.

Figure 4.1. Map of Spain and Catalonia, indicating the region covered by our study. Stars indicate the location of the four study sites.

4.3.2. Field methodology

Faecal samples were collected from mist-netted, ringed birds in the three main sites; Canal Vell, Sebes and the Llobregat Delta, between May and August 2018 as part of the Catalan Institute of Ornithology (ICO) monitoring scheme: SYLVIA. Ringing sessions took place every 10-14 days, an average of four visits to each site (range 3-6, total visits = 15). At Aiguamolls de l'Emporda, sample collection took place over two consecutive days of ringing during the end of the spring migration in early May (before the other sites had begun their ringing programs).

Mist nets were set up adjacent to the reedbeds or in surrounding scrub. Although mist-net captures could not be entirely standardised, no more than 15 x 40-60m nets were set at each site (range 8 – 15), and an average of 17.5 samples were collected per visit (Canal Vell = 18.3 (range 7-26), Llobregat Delta = 15.3 (range = 3-28), Aiguamolls de l'Emporda = 18.5 (range = 9-28), Sebes = 17.5 (range = 12-24)). Nets were opened at dawn (05:00-06:00) and checked every 20 minutes for captured birds. Birds were immediately extracted from the nets safely and placed into clean, cotton bags so that faeces could be collected. Birds were processed and released after 15 minutes in the bag regardless of if a sample was produced. Nets were taken down at midday (12:00-13:00) or when temperatures rose above 35°C.

The method for collecting faecal samples followed a protocol identical to the UK study in 2017 (see Chapter 2 for details). Samples were stored in a 2ml tube containing 100% ethanol in a ratio of 3-parts ethanol to 1-part faecal material. Upon returning from the field at the end of each day the samples were transferred to a domestic freezer (-4°C) until transfer back to the UK where they were stored in a -80 freezer at Cardiff University.

4.3.3. Monitoring invertebrate prey

To monitor available warbler prey in Catalonia, doubled-sided yellow "sticky traps" (as in Chapter 2) were deployed three times over the breeding season at the Llobregat delta, Canal Vell and Sebes. Due to the high numbers of invertebrates captured per trap in the UK (Chapter 2), traps were cut to 25% of the full-size trap (approx. 10 x 6.25 cm). For consistency this is the same percentage of the trap that was used in invertebrate identification as a sub-sample of the full-size traps in Chapter 2. The smaller traps were more environmentally friendly, easier to store and transport and more manageable for identification.

Traps were set across sites during three periods or "seasons": late May/early June, late June/early July, and late July/early August. Traps were deployed in reedbed and scrub vegetation for seven consecutive days at each site with little or no rainfall or up to 10 days with significant rainfall (intermittent rain for more than three consecutive days or one or more days of heavy rainfall). Where possible sites were visited for trap set-up within ten days of each other. Ten sticky traps per sampling round were set in the scrub vegetation and ten in the reedbeds at each site, at varying heights to capture the range of foraging heights utilised by the three warblers. The same trap locations were used in all three sampling rounds. Traps were set at heights between 0.5m and 1.7m in reeds (heights per sampling round = 0.5m (n = 5), 1m (n= 6), 1.3m (n=9), 1.5m (n=8), 1.7m (n=2)) and 0.5m and 2m in scrub (0.5m (n=2), 1m (n=4), 1.3m (n=4), 1.5m (n=9), 1.7m (n=8), 2m (n=3)).

Invertebrates were identified from the sticky traps with the aid of a microscope. Due to time restraints only half of the traps were identified, 10 per site (5 x reed and 5 x scrub) per sampling period (90 traps in total). Specimens were identified to family level where feasible, in some cases superfamily level was achieved, or very closely related and/or morphologically similar families were merged together (see Appendix 1.8 for details).

4.3.4. Weather and climate data

To assess the effects of weather on arthropod abundance, warbler diet and resulting competition, daily maximum temperature, minimum temperature, and precipitation from each site was generated between January and December 2018 using data from the nearest weather station to each study location (available at www.wunderground.com). Where possible, stations were chosen within 10km from the study sites (Llobregat Delta = El Prat de Llobregat 3.5km, Sebes = Riba-roja d'Ebre 9.5km, Aiguamolls de l'Emporda = Sant Pere Pescador, 4km) but at Canal Vell the nearest weather station was 15km away (L'Ampolla station).

To identify periods of prolonged drying, the number of days that had passed since the last rainfall event was recorded for each sampling day. At each site, growing degree days (GDD) were calculated for the common reed (*Phragmites australis*, T^{base}= 4°C) and a local invertebrate the reed plum aphid (*Hyalopterus pruni*, T^{base} = 8.65°C) which is dependent on the common reed for part of its life cycle. Daily GDD values were used to produce a running total of cumulative growing degree days at each site and provide a measure that combine the effects of seasonality and temperature.

4.3.5. Molecular analysis and bioinformatics

The metabarcoding and bioinformatics protocols were carried out as described in Chapter 2. The Catalan samples were pooled together and indexed separately to the UK samples but were run on the same sequencing chip. As with previous chapters, for each sample, the number of reads for each prey item was converted to presence-absence data. Frequency of occurrence (FOO) and % FOO for each prey item detected was calculated for each warbler species. Data were organised by "season": either "early", "middle", or "late" which corresponded with the date the individual bird was captured and sampled in the breeding season, and roughly coincided with the same three periods that the sticky trap monitoring took place. Early season was defined as May-early June, middle season as mid-June to mid-July, and late season as mid-July to August.

4.3.6. Statistical analysis

Statistical analyses were performed in R version 3.5.1. and RStudio version 1.1.463 (RStudio Team 2016, R Core Team 2018) and plots were created with the package *ggplot2* (Wickham 2016). The fits of all models to the data were checked using plots of the residuals, and checks made for overdispersion where appropriate (e.g., Poisson GLMs). Unless otherwise stated, stepwise model deletion using the "drop1" and "step" functions based on AIC was chosen to refine models and remove non-explanatory variables. Pairwise differences in each model were assessed with post-hoc Tukey tests (using the package *emmeans* (Lenth 2020).

Prey abundance and richness

Relative abundance of pooled families and the total number of invertebrate families present (family richness) was calculated for each sticky trap and compared across and within i) sites and ii) sampling rounds (corresponding to early, middle and late summer). In both cases a Gamma

GLM with a "log link" function was selected with site, season and the interaction between site and season as the independent variables.

Dietary composition

Factors affecting warbler diet were assessed using the *manyglm* function in the package *mvabund* (Wang et al. 2012). To avoid pseudoreplication from birds sampled more than once, retrapped birds were removed so that there was just one individual per sample point. The diet data were fitted with a binomial GLM with a "cloglog" link function. The explanatory variables were warbler species, site, age (juvenile or adult), the two-way interaction between species and age, number of cumulative growing degree days for *H. pruni* and the number of days since the last rainfall event. As in Chapter 2, the function *anova* with a Monte Carlo resampling method was used to determine the significance of each predictor on overall diet composition, and univariate tests were used to test for significant effects on individual prey families. Similar results were obtained if the *mvabund* analysis was run using the family- or species-level diet matrices, implying that similar dietary differences can be detected at different taxonomic resolutions. The significant variables were visualised by plotting an NMDS ordination with Jaccard dissimilarities in *vegan* (Oksanen 2018). Due to the high stress >0.2 when using family level data in the NMDS, the ordination was ran again using species level data with singletons removed. This gave us a lower stress of 0.14.

Dietary richness was calculated for each faecal sample by counting the total number of unique prey items (at the lowest taxonomic rank possible, usually species level), which was then averaged across each warbler species. A Poisson GLM with an identity link function was used to investigate differences between species, sites, and seasons. Shannon's and Simpson's diversity indices were calculated for each species and site were calculated in R using the *vegan* package. Levin's Index of niche breadth was also calculated using the package *spaa* (Zhang 2016).

Dietary sampling completeness was assessed by the Chao estimator of extrapolated richness, calculated using the *specpool* function in *vegan* with a small sample correction (N-1)/N, where N is the number individual birds (Chao 1987, Palmer 1990, Oksanen 2018). This was used to calculate the proportion of the total dietary diversity explained by the observed data (observed species richness/Chao extrapolated estimate).

Information from the available online literature, specialist websites and invertebrate keys was collated to estimate the body length in millimetres of each prey species detected in the bird diets (see Chapter 2). An average from the body lengths of all the invertebrate prey species detected for each individual bird was taken and invertebrates in the diet were also grouped according to whether they were terrestrial or had an aquatic life stage (classed as aquatic or semi-aquatic). For each sample, the proportion of prey species present from aquatic/semi-aquatic and terrestrial habitats was calculated. Two GLMs were fitted to examine the effect of site, species, age, season and local climate on i) the average size of prey species in the samples and ii) the proportion of aquatic/semi-aquatic prey in the diet samples. For the prey size GLM, a Gamma family and an identity link function gave the best model fit. For the proportion of aquatic prey GLM a binomial GLM with a logit function was used and this was weighted by total diet richness per sample.

Dietary overlap and prey choice

Dietary overlap was measured between pairs of warbler species with *EcoSimR* (Gotelli and Ellison 2013), using randomization algorithm 3 (RA3), for 10,000 simulations to test whether Pianka's overlap index was significantly greater or smaller than expected under the null model (Pianka 1973) (see Chapter 2 for details). Pianka's index was generated for each pair of species and also subsetted species pairs by season (early, middle, and late breeding season) and site. A sensitivity analysis was run as in Chapter 2, in which the shared families with the highest FOO were removed sequentially and overlap recalculated. A test for species pairs at Mas Matà, (Aiguamolls de L'Emporda) was not carried out as very few Cetti's warblers and great reed warblers were captured. No Cetti's warblers were captured at Canal Vell and only very small numbers of great reed warblers were captured at the Llobregat Delta, so these species were also omitted from respective pairwise dietary overlap tests at the site level.

Dietary preferences were investigated using the *econullnetr* package (Vaughan et al. 2018) using the function *generate_null_net* via the same method as Chapter 2. Data were split by warbler species, site and visit time (1: mid-May-early June, 2: mid-June-mid July, 3: late July-August). If a prey family was detected in the diet of one or more warbler but not present on the sticky traps, the value 0.5 as a small constant for abundance (i.e., half of an individual) was used. To investigate if prey size and prey availability had an effect on prey preference, the standard effect sizes (SES) of the interaction between each warbler species and prey item was log-transformed (after adding 22 to all values, so that all values became positive) and used as the response variable in a Gamma GLM with an identity link function. The interaction between prey size and warbler species, total abundance (for the respective prey item) and prey taxonomic order were chosen as the predictor variables. We did not include prey families not recorded on sticky traps (i.e., abundance = 0.5) in this GLM because they affected the assumptions of the model by skewing the model residuals plot. Two data points had high influence in the residuals vs leverage plot, but their removal did not affect the results or significance of the model or the individual variables.

4.4. Results

4.4.1. Patterns in seasonality, study sites and local weather

Sites varied in their weather patterns (Table 4.1). Aiguamolls de l'Emporda and Sebes had higher precipitation and an increased number of days with precipitation with respect to the Llobregat Delta and Canal Vell. Across all sites the temperature increased from May to July, but maximum temperatures were highest in Sebes and Canal Vell.

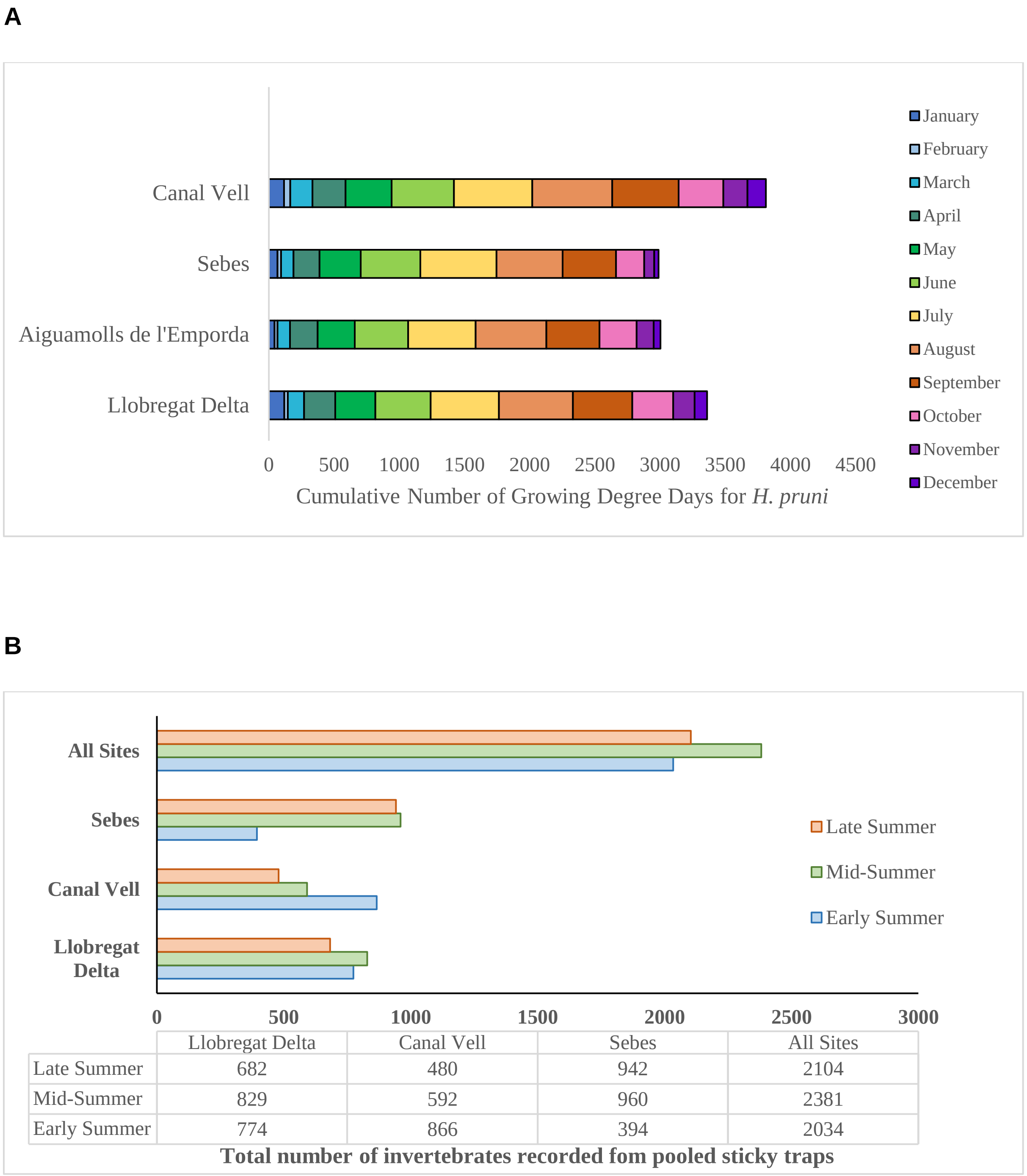
Canal Vell generated the greatest number of cumulative growing degree days for *H.pruni*, followed by the Llobregat Delta, Sebes and Aiguamolls de l'Emporda (Fig. 4.2A). This relates to the differences between daily minimum and maximum temperatures being consistently smaller at Canal Vell throughout the year due to the buffering effect of the Mediterranean Sea. Although maximum temperatures were greatest at Sebes by the end of summer, early season temperatures were cooler and its inland location and proximity to mountains were behind the greater discrepancy between minimum and maximum temperatures. Sebes fell behind the other sites in GDD production for the remainder of the summer.

The interaction between site and season was significant in influencing invertebrate abundance on sticky traps (LRT = 22.16, $p<0.01$) and the model was significant (F= 2.31 on 8 and 81 degrees of freedom, adjusted R-squared = 0.12, $p<0.05$). Invertebrate abundance on sticky traps was significantly higher at Canal Vell compared to Sebes in the early summer (z=3.17, $p<0.05$). This pattern was reversed in the late season with Sebes showing higher total invertebrate abundance when all traps were combined (Fig. 4.2B), but this was not significant for between-sticky trap differences. Within-site changes in abundance were also detected over the summer at Sebes, which saw higher abundance in the middle of summer (z= 3.45, $p<0.05$) and late summer (z = 3.52, $p<0.05$) compared to the early summer. Abundance at Llobregat Delta was relatively consistent and Canal Vell showed a general decline in total abundance (all families combined) from early to late summer (Fig. 4.2.B) but the abundance of prey families per trap did not change significantly over the summer.

For family level richness on sticky traps, site (LRT = 7.32, $p<0.05$), season (LRT = 6.66, $p<0.05$) and their interaction (LRT = 26.18, $p<0.001$) were significant in the GLM (F=6.52 on 8 and 81 df, adjusted R-squared = 0.33, $p<0.001$). Family level richness significantly rose between early summer and mid-summer sampling periods (z = 2.51, $p<0.05$). Sebes had higher richness than Canal Vell (z= 3.01, $p<0.01$) and this was more distinct in the late summer (z = 4.99, $p<0.001$). Sebes also showed higher richness than the Llobregat Delta in the late summer (z = 3.96, $p<0.01$). Within sites, diversity increased from early to mid-summer (z= 3.05, $p<0.05$) and early to late summer at Sebes (z = 4.99, p<0.001). Richness at Canal Vell and Llobregat Delta did not change significantly over the sampling periods.

Table 4.1. Temperature and rainfall data for each study site in Catalonia. Mean maximum, minimum and average daily temperature ± SD, total precipitation, and number of days with precipitation at the four study sites for the months, May, June, and July, and for the entire study period May-July.

Site	**Month**	**Max Temp (°C)**	**Min Temp (°C)**	**Average Temp (°C)**	**Total Precipitation (mm)**	**Days with Precipitation**
Sebes	May	24.8 ± 2.6	12.8 ± 2.9	18.8 ± 2.4	73.3	10
	June	29.9 ± 3.4	17.8 ± 2.6	23.9 ± 2.6	3.8	2
	July	34.4 ± 2.2	20.7 ± 1.7	27.5 ± 1.6	17	6
	All	**29.7 ± 4.7**	**17.1 ± 4.1**	**23.4 ± 4.2**	**94.1**	**18**
Llobregat Delta	May	22.2 ± 2.2	14.9 ± 3.1	18.6 ± 2.4	2.8	2
	June	25.9 ± 2.5	19.6 ± 2.4	22.8 ± 2.3	0	0
	July	29.2 ± 1.5	21.9 ± 1	25.5 ± 1.1	9.6	2
	All	**25.8 ± 3.6**	**18.8 ± 3.7**	**22.3 ± 3.5**	**12.4**	**4**
Canal Vell	May	25 ± 2.2	15.1 ± 2.3	20.1 ± 1.8	12.4	3
	June	29.2 ± 3	19.9 ± 1.8	24.6 ± 2.2	16.3	4
	July	33.3 ± 1.2	22.8 ± 1.2	28 ± 1	4	2
	Total	**29.2 ± 4.1**	**19.3 ± 3.7**	**24.2 ± 3.7**	**32.7**	**9**
Mas del Matà	May	22.9 ± 2	13.4 ± 2.7	18.2 ± 2.1	89.3	11
	June	27 ± 2.3	17.6 ± 2.4	22.3 ± 2.1	34.2	7
	July	30.7 ± 1.9	19.9 ± 1.4	25.3 ± 1.3	21.1	3
	All	**26.9 ± 3.9**	**16.9 ± 3.5**	**21.9 ± 3.5**	**144.6**	**21**



	Llobregat Delta	Canal Vell	Sebes	All Sites
Late Summer	682	480	942	2104
Mid-Summer	829	592	960	2381
Early Summer	774	866	394	2034

Figure 4.2. Plots of growing degree days and invertebrate abundance in Catalonia; A)The cumulative number of growing degree days accumulated for the reed plum aphid (*Hyalopterus pruni*) subset for each month of 2018 at each of the four study sites. B) The total number of invertebrates recorded from pooled sticky traps at the three main study sites for each of the three sampling periods (early summer, mid-summer, late summer).

4.4.2. HTS success rate

Across all sites, a total of 246 faecal samples were collected from all warbler species. The reed warbler samples were by far the most numerous (180 samples), followed by 39 great reed warbler samples and 27 Cetti's warbler samples, reflecting the relative capture rates of the three species. Dietary data was successfully obtained for 137 reed warblers and 26 each of great reed warblers and Cetti's warblers. A total of 2,637,259 paired-end (merged) reads were generated which corresponded to 357 unique prey items the vast majority of which were identified to species or genus level.

4.4.3. Species diversity

Pooled dietary species richness was greatest in reed warblers with 235 prey species detected overall. Despite the small number of samples analysed, Cetti's warblers consumed 164 species whereas great reed warblers only consumed 90 species (Table 4.2). This pattern was mirrored in the Shannon and Simpson diversity indices which were relatively high across all three species (Shannon's H range = 4.2-4.8, Simpson's diversity range 0.97-0.98). The total extrapolated dietary species richness across all species and sites (Chao Estimate) was 638 (± 57.49) suggesting that many more prey species (estimated between 223 – 338, considering standard error) are likely to be present in the diets of the warblers, as collected samples only equated to 55% of the estimated total.

Reed warblers and Cetti's warblers both showed a wide dietary breadth (Levin's index = 66.12, 63.18 respectively), with a narrower breadth in great reed warblers (46.14). At Canal Vell the difference in niche breadth between reed and great reed warblers was stark: whereas reed warbler dietary breadth was very high (62.37), it was very low in great reed warblers (17.18, Fig. 4.3). Great reed warbler niche breadth was much higher at Sebes than at Canal Vell however, where similar values to the reed warbler were attained. At the Llobregat Delta and Sebes, Cetti's warbler niche breadth exceeded that of reed warblers.

The niche breadth of warblers also differed across the three periods of the breeding season, but responses varied by focal species. Great reed warblers and Cetti's warblers showed elevated niche breadth in the middle of the breeding season when abundance of arthropods on the sticky traps was highest, while dietary breadth in the late season was narrowest. Reed warblers however showed consistently high niche breadth, but it was slightly lower in the middle of the breeding season compared to both earlier and later in the season.

Table 4.2. Diversity indices calculated for each focal warbler species in Catalonia among sites and seasons. Species richness (number of unique items detected) and the diversity indices Shannon H, Simpson's Diversity, Levin's Index, Chao Estimate and percentage of species diversity described (Chao estimated diversity/observed species richness) are given for each focal warbler species, and all warbler species combined, and split i) across three study sites and ii) across seasons.

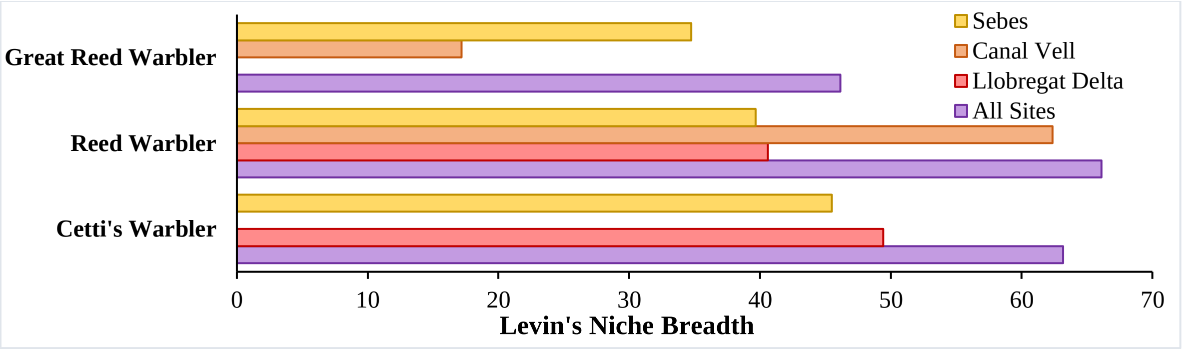
i)

Warbler Species	Site	Species Richness	Chao Estimator	% Diversity explained	Shannon H	Simpson's Diversity	Levin's Index
Reed	All sites	235	422.73 ± 47.06	55.59	4.8	0.98	66.12
Warbler	Canal Vell	82	180.28 ± 31.13	45.48	4.81	0.96	62.37
	Sebes	107	231.34 ± 45.35	46.25	4.15	0.97	39.65
	Llobregat Delta	70	196.71 ± 56.76	35.58	3.72	0.97	40.58
Great	All sites	89	200.57 ± 42.25	44.37	4.18	0.97	46.14
Reed	Canal Vell	33	82.61 ± 33.67	39.94	3.16	0.94	17.18
Warbler	Sebes	49	107.32 ± 27.86	45.65	3.75	0.97	34.75
Cetti's	All sites	164	440.01 ± 81.99	37.27	4.6	0.98	63.18
Warbler	Sebes	73	193.17 ± 51.72	37.79	4.12	0.97	45.49
	Llobregat Delta	104	249.2 ± 54.12	41.73	4.27	0.98	49.41
All	All sites	357	637.64 ± 57.49	55.98			
species	Canal Vell	101	224.09 ± 48.49	45.07			
	Sebes	156	272.32 ± 35.97	57.28			
	Llobregat Delta	150	337.16 ± 57.47	43.3			

ii)

Warbler Species	Season	Species Richness	Levin's Index
Reed	Early	119	53.23
Warbler	Middle	117	44.22
	Late	94	49.80
Great	Early	45	32.02
Reed	Middle	45	35.39
Warbler	Late	29	20
Cetti's	Early	96	50.27
Warbler	Middle	91	58.21
	Late	20	20

A



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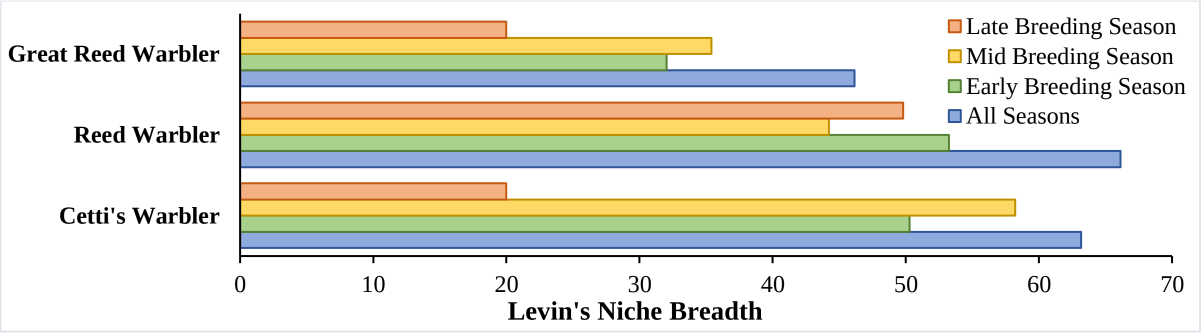


Figure 4.3. Levin's index of Niche Breadth calculated for each focal warbler species in Catalonia; A) for each of the three main sites and all sites combined B) for each season and all seasons combined.

Species (LRT = 95.82, $p<0.001$), site (LRT = 21.27, $p<0.001$) and season (LRT = 9.1, $p<0.05$) were significant in explaining species richness in the dietary samples, and the model was significant (F = 17.6 on 8 and 169 df, adjusted R-squared = 0.43, $p<0.001$). The number of GDD produced did not influence the dietary richness of warblers significantly but was retained in the model with stepwise deletion because its inclusion improved the overall model AIC. It is fair to conclude that there is a very small effect of temperature alongside seasonality (combined as growing degree days), but it was less important in shaping species richness compared to the other predictors.

Cetti's warblers consumed a diet that was significantly more species-rich than the diets of both reed (z = 8.87, $p<0.001$) and great reed warblers (z = 7.98, $p<0.001$) but reed and great reed warbler dietary richness did not significantly differ (Fig. 4.4). Birds captured at Canal Vell had a lower dietary richness than at Llobregat Delta (z = -3.12, $p<0.01$) and Sebes (z = -4.59, $p<0.001$). Moreover, early breeding season birds had a less diverse diet than those captured during the middle of the breeding season (z = -2.91, $p<0.05$), but early and late breeding season birds, and middle and late breeding season birds were not significantly different.

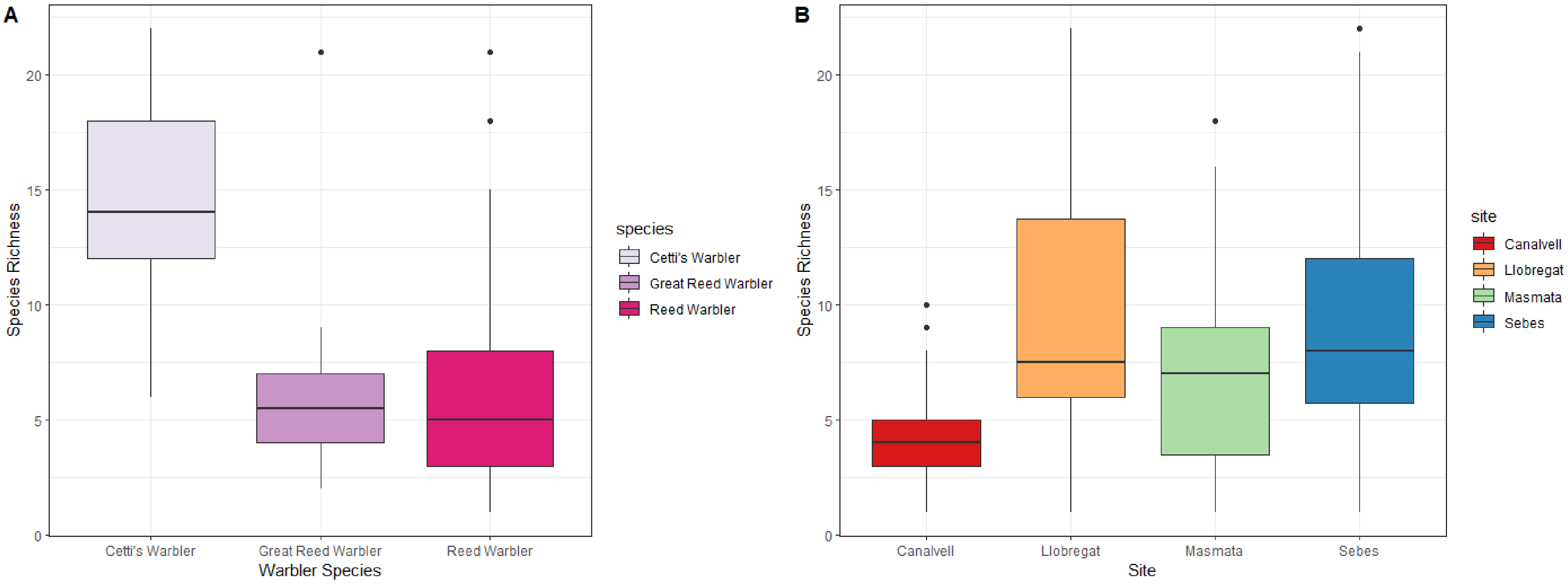


Figure 4.4. Boxplots of the species richness (the number of unique prey items detected per faecal sample) of diet samples in Catalonia; for A) the three focal warbler species, reed warbler, Cetti's warbler and great reed warbler and B) the four study sites, Canal Vell, Llobregat Delta, Mas del Matà (Aiguamolls de l'Emporda) and Sebes.

4.4.4. Dietary overlap

When data from all sites were combined, observed dietary overlap between reed and great reed warblers (Pianka = 0.56, p<0.001, SES = 7.5) and between reed and Cetti's warblers (Pianka = 0.35, p<0.01, SES = 2.7, Table 4.3i) was significantly greater than the null model predicted. There was neither significant overlap nor significant partitioning between Cetti's warblers and great reed warblers (Pianka = 0.29, SES = 0.47). Our sensitivity analysis, in which the shared families with the highest FOO were removed sequentially, generally led to a reduction in overlap and at times significant resource partitioning. This suggests that the most frequently consumed dietary items present in the diet of multiple warblers played an important role in the observed overlap.

Partitioning occurred between reed and Cetti's warblers, and between Cetti's and great reed warblers, in the early breeding season, where the observed Pianka index was significantly lower than the null model (Table 4.3ii). Reed and great reed warblers in the same period showed significant dietary overlap. In the mid-season, reed and great reed warblers again showed significant overlap. Between reed and Cetti's warblers, and between great reed and Cetti's warblers, overlap was not significantly different from the null model. In the late season there was significant partitioning between great reed and Cetti's warblers but all other pairwise combinations did not show significant overlap. However, the sample size for late season Cetti's warblers was very low at only two individuals.

Most pairwise comparisons among the different sites did not show significant dietary overlap (Table 4.3iii). At Sebes, dietary overlap was significantly greater than expected for both reed and great reed warblers and reed and Cetti's warblers.

Table 4.3. Pianka's index of niche overlap (*Ojk*) in observed diet , i) between pairs of warblers for all sites and seasons combined, ii) between pairs of warblers at three of the study sites, iii) between pairs of warblers during different stages of the breeding season. Standard effect sizes (SES) are indicated in brackets. Cells are colour coded according to significance with respect to the null model (see notes below).

i)

Treatment	RW	CW	All species
GRW	0.56*** (7.5)	0.29 (0.47)	
RW		0.35*** (2.7)	
All species			0.37*** (8.5)

ii)

	RW		CW	
Site	Canal Vell	Sebes	Sebes	Llobregat Delta
GRW	0.33 (1.7)	0.65 ***(5.7)	0.46 (0.99)	
RW			0.48** (3.03)	0.36 (1.15)

iii)

	RW			CW			All		
	early	middle	late	early	middle	late	early	middle	late
GRW	0.59*** (5.26)	0.45** (3.1)	0.27 (-0.11)	0.13** *(-2.83)	0.29 (-1.24)	0.13*** (-5.01)			
RW				0.19** (-1.91)	0.45 (1.91)	0.26 (-1.03)			
All							0.27 (1.65)	0.41*** (4.48)	0.26 (-0.15)

Notes: Asterisks denote significantly higher or lower overlap than predicted from the null model (* p<0.05, **p<0.01, ***p<0.001). Negative standard effect sizes (SES) and a red shade indicate lower observed overlap than the null model, whereas positive SES and a green shade indicates higher observed overlap than the null model. Yellow shade indicates observed overlap is not significantly different from the null model.

4.4.5. Multivariate analysis of diet

Diet composition varied between warbler species (LRT = 455.6, p<0.001), ages (LRT = 145.4, p<0.01) and their interaction (LRT = 123.8, p<0.001), and with site (LRT = 700.1, p<0.001), number of days since precipitation (LRT = 127.1, p<0.01) and cumulative number of GDD for *H.pruni* (LRT = 327.6, p<0.001). Cetti's and great reed warbler diets appeared more distinctly different from each other than they each were to reed warbler diet (Fig. 4.5). In the univariate tests several prey families significantly differed in the frequency of incidences in the diet between two or more warblers (Table 4.4). Differences in Acrididae consumption between all adults and juveniles was marginally significant (LRT = 12.75, p = 0.053). Generally, juveniles of a given warbler species were more similar to adult conspecifics than they were to juveniles or adults of other species. The only exception was great reed warbler adults who clustered closer to both adult and juvenile reed warblers than they did to great reed warbler juveniles. Several prey families differed significantly across sites, with chironomids consumed more frequently by birds in Sebes than at the remaining sites, mosquitoes (Culicidae) by birds from Mas del Matà and Canal Vell, and braconid wasps by birds from Sebes and Delta de Llobregat (Table 4.4). In the NMDS, sites appeared to cluster according to climatic similarity (e.g., Sebes and Mas del Matà). Canal Vell appeared to be more distinct from those collected at the remaining field sites.

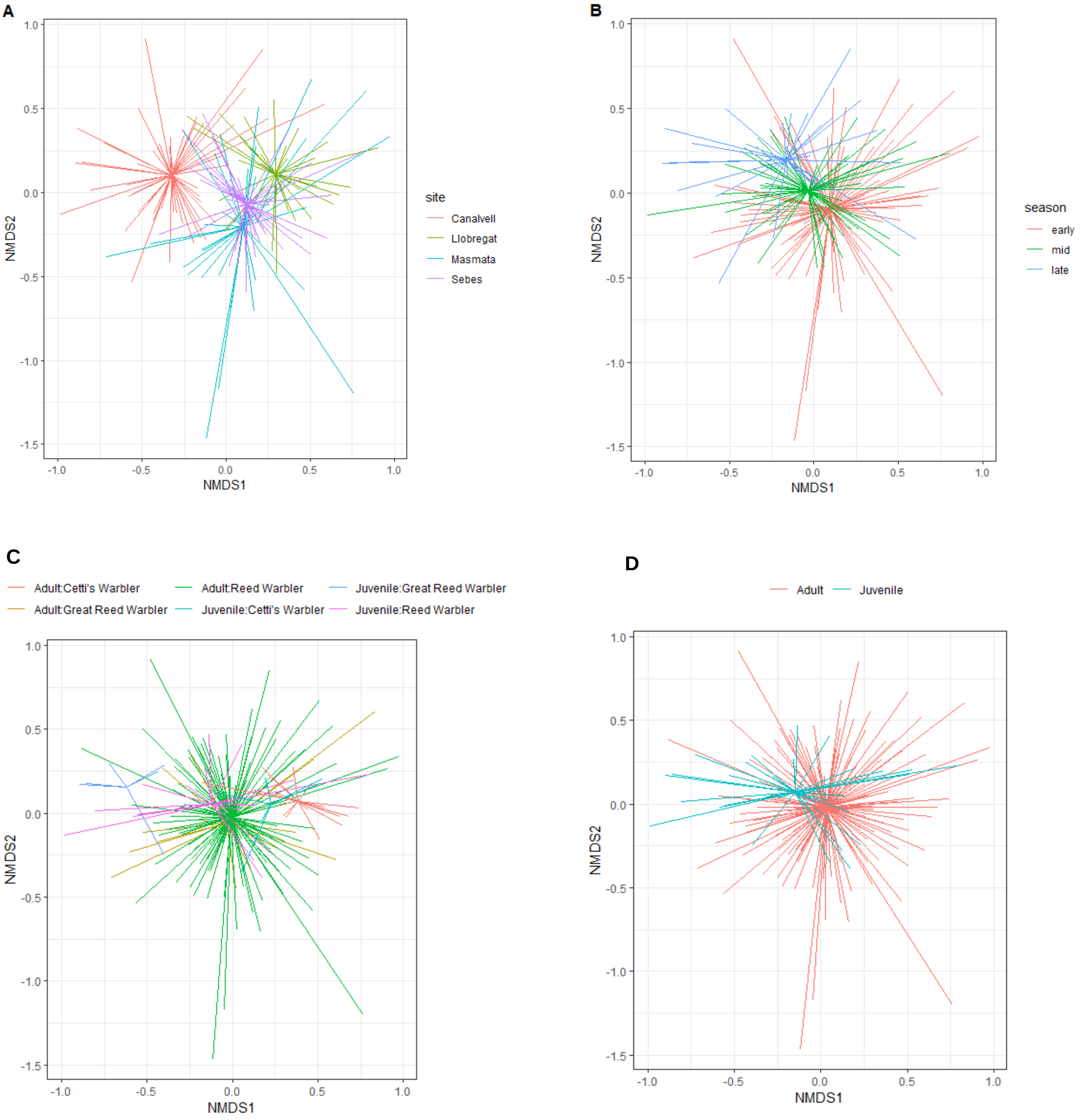
A gradual transition in diet occurred from early summer to late summer, with mid-summer samples clustering centrally. Gelechiididae (LRT = 31.12, p<0.001) and Psilidae (LRT = 30.58, p<0.001) were consumed significantly less frequently when GDD accumulation was high compared to when it was low. Although rainfall did not affect any individual family significantly, it had a general effect on diet composition. Birds captured when many days had passed since the last rainfall event clustered with Miridae, Aphrophoridae and Chrysomelidae whereas those captured on days where the last rainfall event was more recent were associated with Phalacrididae, Coccinellidae, Tortricidae, Agriolimacidae, Oedemeridae and Rhinophoridae.

Table 4.4. Results for the univariate *anova* test in the *manyglm* model. Prey families that were significantly affected (i.e., consumed more or less frequently) by one or more of the model test variables are shown with their respective likelihood ratio test values (LRT) and p-values. Percent frequency of occurrence values (% FOO) for each prey family across the factor levels are also indicated. Cells are colour coded with a darker tone indicating a greater % FOO.

Predictor Variable	Prey Order	Prey Family	LRT	p-value	% FOO			
					CW	GRW		RW
Species	Araneae	Philodromidae	18.16	0.003**	42.3	-		10.0
Species	Araneae	Tetragnathidae	18.49	0.003**	26.9	-		2.9
Species	Coleoptera	Carabidae	17.79	0.004**	38.5	3.8		8.8
Species	Diptera	Calliphoridae	27.13	0.001***	50.0	7.7		8.6
Species	Diptera	Culicidae	12.97	0.042*	11.5	11.5		36.4
Species	Diptera	Syrphidae	26.22	0.001***	57.7	15.4		10.0
Species	Gastropoda	Helicidae	17.26	0.005**	11.5	15.4		-
Species	Hymenoptera	Ichneumonidae	14.23	0.024*	15.4	50.0		13.1
Species	Lepidoptera	Erebidae	21.82	0.001***	53.8	11.5		10.7
Species	Lepidoptera	Geometridae	17.8	0.004**	46.2	-		15.0
Species	Lepidoptera	Noctuidae	32.73	0.001***	92.0	50.0		27.0
					Canal Vell	Llobregat Delta	Sebes	Mas Matà
Site	Diptera	Calliphoridae	16.33	0.067.	4.3	21.6	16.7	25.8
Site	Diptera	Culicidae	19.78	0.018*	45.7	11.1	14.8	41.9
Site	Hemiptera	Piesmidae	26.16	0.002**	15.7	-	-	3.2
Site	Hymenoptera	Braconidae	17.97	0.038*	10.0	47.2	25.9	12.9
Site	Hymenoptera	Halictidae	17.21	0.043*	2.9	-	18.5	3.2
Site	Diptera	Chironomidae	32.33	0.001***	47.1	55.5	83.3	38.7
					Adult		Juvenile	
Age	Orthoptera	Acrididae	12.75	0.053.	3.8		-	

					400-700	700-1000	1000-1500	1500-2000
GDD	Diptera	Psilidae	30.58	0.001***	27.5	1.8	-	-
GDD	Lepidoptera	Gelechiididae	31.12	0.001***	27.5	5.7	-	-

Notes: Asterisks denote the significance level of the test result (. p>0.05<0.08, * p<0.05, **p<0.01, ***p<0.001).



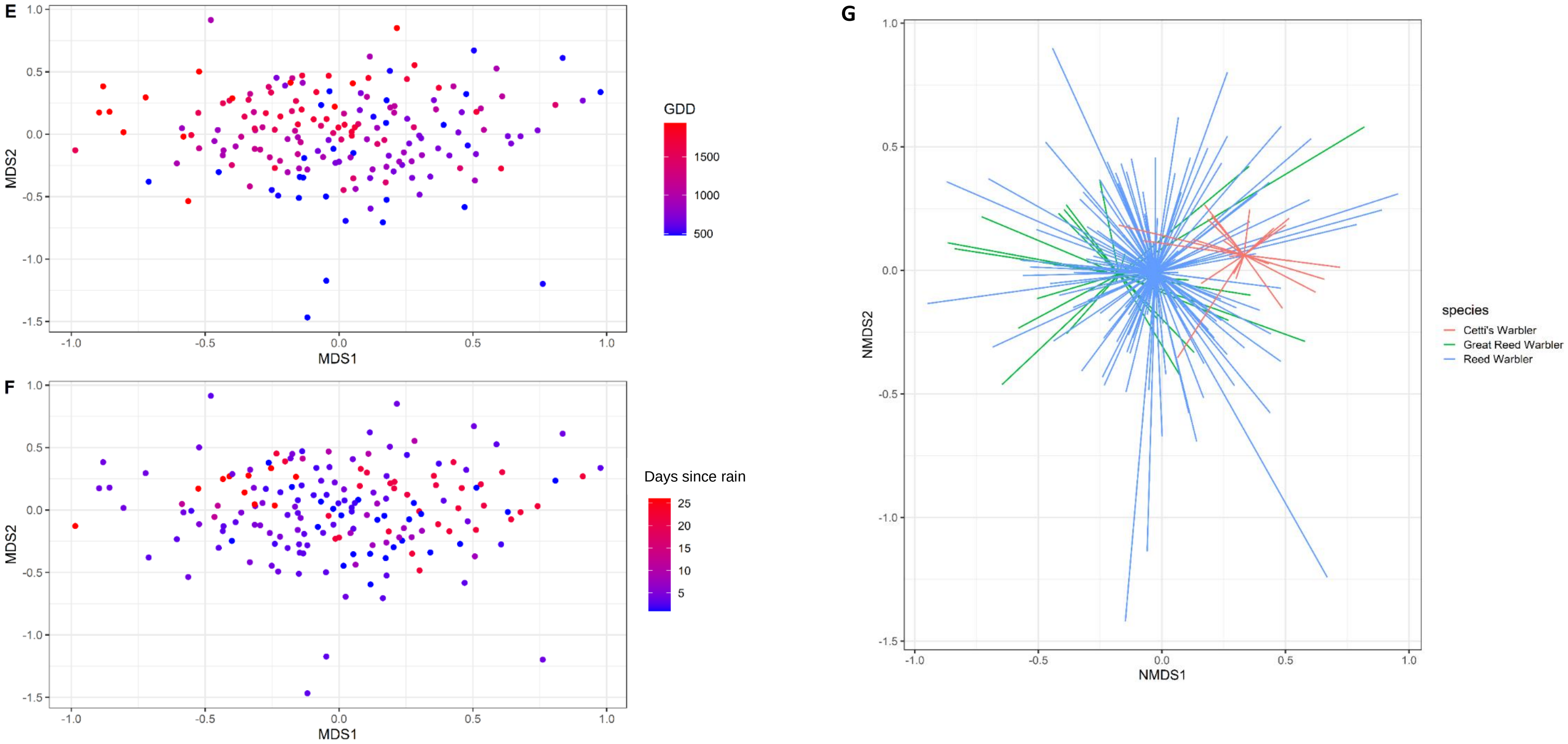


Figure 4.5. NMDS of prey detected in the Catalan warbler diet samples according to the significant predictor variables from the *manyglm* model; A) site, B) season and C) age D) age subsetted by species E) cumulative number of growing degree days GDD for *H. pruni*, F) number of days since the last rainfall event and G) focal warbler species

4.4.6. Dietary patterns

A wide range of invertebrate species were consumed by Catalan warblers. Across all warblers, sites and seasons, the most frequently consumed species was the sac spider *Clubiona phragmitis*, followed by a chironomid *Cladotanytarus atridorsum* and a mosquito *Aedes caspius* (Table 4.5). A suite of chironomid species, *Chironomus nuditarsis, C. plumosus, C. curabilis, C. riparius, Cryptochironomus obreptans*, *Cricotopus bicinctus*, *C. sylvestris*, were also very frequent dietary items. *Neophilaenus lineatus*, a froghopper was present in 73% of Cetti's warbler samples but neither the reed nor great reed warbler preyed on this species. *Episyrphus balteatus* was present in almost half of the Cetti's warbler samples and 11.5% and 2.9% of great reed and reed warbler samples, respectively. At the family level the most frequently detected group by far were the non-biting midge family Chironomidae, with a frequency of occurrence of 58.7% samples (Table 4.6). This was followed by the noctuid moths (39.2%) and clubionid spiders (35.4%).

Table 4.5. Top 20 species detected in the diets of focal warbler species, reed warbler, great reed warbler and Cetti's warbler in Catalonia, Spain. Values shown are percent frequency of occurrence = number of faecal samples testing positive for an order divided by the total number of samples (for each species). Values are colour coded according to frequency of occurrence (darker colour = higher abundance).

Order: Family	**Prey Species**	**Reed Warbler (n = 137)**	**Cetti's Warbler (n = 26)**	**Great Reed Warbler (n = 26)**	**All Warblers (n = 189)**
Araneae: Clubionidae	*Clubiona phragmitis*	**32.8**	**26.9**	**26.9**	**31.2**
Diptera: Chironomidae	*Cladotanytarsus atridorsum*	**27**	**19.2**	**26.9**	**25.9**
Diptera: Culicidae	*Aedes caspius*	**26.3**	**3.8**	**11.5**	**21.2**
Diptera: Chironomidae	*Chironomus plumosus*	**21.9**	**3.8**	**23.1**	**19.6**
Diptera: Chironomidae	*Chironomus nuditarsis*	**16.1**	**15.3**	**26.9**	**17.5**
Araneae: Philodromidae	*Philodromus cespitum*	**9.4**	**34.6**	**0**	**11.6**
Diptera: Chironomidae	*Cricotopus bicinctus*	**12.4**	**11.5**	**7.7**	**11.6**
Lepidoptera: Erebidae	*Polypogon plumigeralis*	**6.5**	**46.2**	**0**	**11.1**
Diptera: Syrphidae	*Episyrphus balteatus*	**2.9**	**46.2**	**11.5**	**10.1**
Hemiptera: Aphrophoridae	*Neophilaenus lineatus*	**0**	**73.1**	**0**	**10.1**
Diptera: Chironomidae	*Chironomus curabilis*	**10.2**	**7.7**	**7.7**	**9.5**
Hymenoptera: Ichneumonidae	*Diplazon laetatorius*	**7.3**	**15.4**	**15.4**	**9.5**
Diptera: Chironomidae	*Cricotopus sylvestris*	**9.4**	**11.5**	**0**	**8.5**
Lepidoptera: Noctuidae	*Chilodes maritimus*	**8.02**	**7.7**	**11.5**	**8.5**
Orthoptera: Tettigoniidae	*Ruspolia nitidula*	**2.2**	**7.7**	**38.4**	**7.9**
Diptera: Calliphoridae	*Pollenia leclercqiana*	**0.7**	**46.2**	**3.8**	**7.4**
Diptera: Chironomidae	*Chironomus riparius*	**6.5**	**19.2**	**0**	**7.4**
Diptera: Chironomidae	*Cryptochironomus obreptans*	**8.8**	**0**	**7.7**	**7.4**
Hemiptera: Aphididae	*Hyalopterus pruni*	**6.5**	**11.5**	**7.7**	**7.4**
Lepidoptera: Noctuidae	*Leucania obsoleta*	**6.5**	**7.7**	**11.5**	**7.4**

Table 4.6. The top 30 most frequently detected invertebrate families from the diets of focal warbler species in Catalonia, Spain. Values shown are percent frequency of occurrence = number of faecal samples testing positive for an order divided by the total number of samples (for each species). Values are colour coded according to frequency of occurrence (darker colour = higher abundance).

Order	**Suborder**	**Family**	**Reed Warbler (n = 137)**	**Cetti's Warbler (n = 26)**	**Great Reed Warbler (n = 26)**	**All Warblers (n = 189)**
Diptera	Nematocera	Chironomidae	**59.8**	**50**	**61.5**	**58.7**
Lepidoptera		Noctuidae	**27**	**92.3**	**50**	**39.2**
Araneae		Clubionidae	**35.7**	**42.3**	**26.9**	**35.4**
Diptera	Nematocera	Culicidae	**37.2**	**11.5**	**11.5**	**19.6**
Hymenoptera	Parasitica	Braconidae	**21.9**	**38.5**	**11.5**	**22.8**
Hemiptera		Aphididae	**18.2**	**30.7**	**15.4**	**19.6**
Hymenoptera	Parasitica	Ichneumonidae	**13.1**	**15.4**	**50**	**18.5**
Lepidoptera		Geometridae	**15.3**	**46.2**	**0**	**17.5**
Diptera	Aschiza	Syrphidae	**10.2**	**57.7**	**15.4**	**17.5**
Lepidoptera		Erebidae	**10.9**	**53.8**	**11.5**	**16.9**
Diptera	Brachycera	Calliphoridae	**8.8**	**50**	**7.7**	**14.3**
Araneae		Philodromidae	**10.2**	**42.3**	**0**	**13.2**
Coleoptera		Carabidae	**8.8**	**38.5**	**3.8**	**7.9**
Hemiptera		Aphrophoridae	**0**	**76.9**	**0**	**10.6**
Diptera	Nematocera	Cecidomyiidae	**8**	**11.5**	**7.7**	**8.5**
Orthoptera		Tettigoniidae	**2.2**	**7.7**	**38.5**	**7.9**
Lepidoptera		Crambidae	**8**	**7.7**	**3.8**	**7.4**
Lepidoptera		Gelechiididae	**8.8**	**7.7**	**0**	**7.4**
Coleoptera		Halictidae	**7.3**	**3.8**	**7.7**	**6.7**
Hymenoptera		Formicidae	**6.6**	**3.8**	**7.7**	**6.3**
Hemiptera		Piesmidae	**8**	**3.8**	**0**	**6.3**
Hemiptera		Pseudococcidae	**8**	**0**	**3.8**	**6.3**
Araneae		Salticidae	**8**	**3.8**	**0**	**6.3**
Gastropoda		Agriolimacidae	**0**	**42.3**	**0**	**5.8**
Araneae		Dictynidae	**2.2**	**30.8**	**0**	**5.8**
Diptera	Brachycera	Muscidae	**6.6**	**7.7**	**0**	**5.8**
Coleoptera		Ptinidae	**1.5**	**34.6**	**0**	**5.8**
Araneae		Tetragnathidae	**2.9**	**26.9**	**0**	**5.8**
Diptera	Brachycera	Sarcophagidae	**5.8**	**7.7**	**0**	**5.3**
Coleoptera		Scirtidae	**6.6**	**3.8**	**0**	**5.3**

Reed warblers had a strong affinity with Diptera (Table 4.7), with 24 families detected in total, and with 83% of sampled individuals consuming at least one species. Chironomidae, Culicidae, Syrphidae, Calliphoridae and Sarcophagidae were the top families consumed. Nine families of spiders were consumed, the most frequent of which was Clubionidae, but some families detected in Cetti’s warblers were missing in reed warblers. The most common moth families were less frequently consumed by reed warblers compared to Cetti’s warblers, but a more diverse array of moth families was recorded overall. A small percentage of samples contained ant species, and the larger wasp families Halictidae, Pompilidae and Crabronidae, while parasitic wasps (Ichneumonidae and Braconidae) were frequent. A third of reed warbler samples contained Hemiptera, of which aphids were both frequent (17.8%) and species rich in the diet. All 12 coleopteran families were detected in less than 10% of samples each.

The highest percent frequency of occurrence values for most invertebrate orders were from Cetti’s warbler samples. The most frequent prey items were Araneae, Lepidoptera and Diptera (all above 90% occurrence). Eleven spider families of 19 species were detected; aside from Clubionidae the most important were Philodromidae, Dictynidae and Tetragnathidae. Noctuid moths were extremely prevalent in 92% of samples, and several other moth families also showed high incidences. The most notable dipterans were Syrphidae (largely *Episyrphus balteatus*), Chironomidae and Calliphoridae. Hemipterans and Coleopterans were more commonly found in Cetti’s warblers than in reed and great reed warblers. Aphrophoridae and were detected at a surprisingly high frequency, as were Carabidae and Ptinidae (34%). Detections of Hymenoptera were mostly from Braconidae and Ichneumonidae but *Apis mellifera*, the European honeybee was present in two samples. Another notable distinction was a high prevalence of gastropods in the diet, over half of samples tested positive. Harvestmen (Opiliones), thrips (Thysanoptera) and millipedes (Julida) were also recorded in Cetti’s warbler samples, but not in samples of either of the other warblers. More detections of Psocoptera and green lacewing neuropterans (Chrysopidae) were recorded than in reed warblers.

Great reed warblers showed the lowest frequency of occurrence for Araneae and Diptera, but the highest frequency of the family Chironomidae and a relatively high frequency of both hoverflies and mosquitoes. Only two species of spiders were detected, including *Clubiona phragmitis* with a frequency of 26%. Aside from Oedemeridae beetles were scarce. Instead, this species consumed a higher frequency of odonates (15.4%), orthopterans (46%) and hymenopterans (65%). Tettigoniidae (bush crickets) were detected in 38% of great reed warbler samples and Acrididae (grasshoppers) in a further 11.5%. Hymenopteran families comprising fairly large bodied species such as Crabronidae, Halictidae, Pompilidae and Tenthredinidae were present in two samples each. Apart from Noctuidae and Erebidae, most moth families were present as single detections. Despite this, great reed warblers were unique in consuming a number of large-bodied species such as the ruby tiger moth *Phramatobia fuliginosa*, the elephant hawkmoth *Deilephila elpenor* and the hornet clearwing *Sesia apiformis*. Aphids were taken less frequently compared to the other warblers, but great reed warblers were the only species to consume the large-bodied hemipterans;

Coreidae and Pentatomidae. Aquatic groups consumed included caddisflies and an invasive crayfish: *Procambarus clarckii*.

Table 4.7. Percent frequency of occurrence and species richness (number of species) of invertebrate orders detected in the faecal samples of three warbler species. Background colour indicates highest (green), second highest (yellow) and lowest (orange) incidences of a given taxon across the different consumers. Percent frequency of occurrence = number of faecal samples testing positive for an order divided by the total number of samples (for each species).

	% Frequency of Occurrence				**Species Richness**			
Order	All spp. (n = 189)	Reed warbler (n =137)	Cetti's warbler (n = 26)	Great reed warbler (n = 26)	All spp. (n =192)	Reed warbler (n = 140)	Cetti's warbler (n = 26)	Great reed warbler (n = 26)
Araneae	**57.14**	**54.74**	**96.15**	**26.92**	**33**	**23**	**19**	**2**
Coleoptera	**32.28**	**24.82**	**76.92**	**26.92**	**25**	**14**	**13**	**6**
Collembola	**0.53**	**0**	**3.84**	**0**	**1**	**0**	**1**	**0**
Decapoda	**2.13**	**1.46**	**0**	**7.69**	**1**	**1**	**0**	**1**
Diptera	**82.54**	**83.94**	**92.31**	**61.54**	**103**	**79**	**42**	**27**
Gastropoda	**9.52**	**0**	**53.84**	**15.38**	**7**	**0**	**7**	**1**
Hemiptera	**39.15**	**27.20**	**84.61**	**38.46**	**34**	**15**	**19**	**10**
Hymenoptera	**49.74**	**45.26**	**57.69**	**65.38**	**61**	**43**	**17**	**19**
Isopoda	**2.13**	**1.46**	**7.69**	**0**	**1**	**1**	**1**	
Julida	**0.53**	**0**	**3.84**	**0**	**1**	**0**	**1**	**0**
Lepidoptera	**60.85**	**54.01**	**96.15**	**61.54**	**72**	**43**	**33**	**17**
Mecoptera	**1.09**	**1.46**	**0**	**0**	**1**	**1**	**0**	**0**
Neuroptera	**4.76**	**6.57**	**15.38**	**0**	**6**	**7**	**3**	**0**
Odonata	**2.65**	**0.73**	**0**	**15.38**	**2**	**1**	**0**	**2**
Opiliones	**0.53**	**0**	**3.84**	**0**	**1**	**0**	**1**	**0**
Orthoptera	**10.58**	**3.65**	**11.53**	**46.15**	**3**	**2**	**2**	**3**
Psocoptera	**8.47**	**5.11**	**34.61**	**0**	**4**	**3**	**3**	**0**
Trichoptera	**1.06**	**2.18**	**3.84**	**3.85**	**1**	**2**	**1**	**1**

Most resource use at the order level was relatively consistent across the breeding season, but subtle changes did occur (Appendix 3.1., Table A3.1.*a*). Detections of Araneae were elevated in faecal samples from birds captured at the Llobregat Delta and this matched the higher number recorded from the Llobregat Delta sticky traps (Appendix 3.1, Table A3.1.*b*). Detections of Diptera and Neuroptera were the highest at Sebes and Lepidoptera highest at Aiguamolls de l'Emporda. At Canal Vell, great reed warblers showed elevated frequency of occurrence for the orders that comprised the largest prey species. % FOO of chironomids was 90% in Sebes but compared to Canal Vell, Lepidoptera, Orthoptera and Odonata were reduced. At Canal Vell chironomid % FOO fell to 50% while Lepidoptera, Orthoptera and Odonata increased. Lepidoptera detections were lower in Canal Vell reed warblers but elevated in great reed warblers.

4.4.7. Prey choice

These results from the *econullnetr* analysis imply the existence of prey preferences among the three warbler consumers. Several families were disproportionately consumed by one species of warbler only, further confirming species-specific dietary preferences. Patterns were not solely explained by taxonomy, instead preferences were spread across invertebrate families relating to prey size and availability (Table 4.8). This was confirmed by the GLM investigating patterns in the *econullnetr* SES values. The final model was significant (F = 13.3 on 13 and 177 degrees of freedom, adjusted R-squared = 0.46, $p<0.001$). When the other variables were controlled for, warblers did not prefer any invertebrate orders significantly more than others. Although not significant, prey order was retained in the model because it improved the overall model fit. Taxonomic rank may thus have some associated importance in explaining dietary preferences. The interaction between prey size and warbler species was significant (F = 4.94, $p<0.01$), with a positive association between prey body size and SES (preference) in Cetti’s warblers (t = 2.5, $p<0.05$), reed warblers (t = 2.7, $p<0.01$) and great reed warblers (t = 3.8, $p<0.001$). Larger prey families had a greater SES value and were thus more preferred than smaller prey families. In addition, total abundance had a significant negative effect on SES with the most abundant families generally being less preferred (t = -14.3, $p<0.001$).

There was a trend of more strongly negative SES (i.e., avoidance) of families with high prey abundance on traps. Ceratopogonidae, Chloropidae, Hybotidae, Psychodidae, Cicadellidae and Formicidae were consistently avoided across all warbler species. Exceptions to this rule included Chironomidae and Clubionidae, which although abundant on sticky traps were generally positively selected by warblers. Only five of the preferred families were consumed at an elevated frequency to that expected from their abundance by all three focal warblers. These were Clubionidae, Syrphidae, Culicidae, Erebidae and Noctuidae.

Some of the prey preferences shown by the warblers were consistent throughout the summer, such as the most common moth families (Table 4.8). Hoverflies (Syrphidae) and mosquitoes (Culicidae) were consumed disproportionately in early and mid-summer, Cambaridae in the mid-summer and Calliphoridae and Muscoid flies in late summer. Many families were preferred by a focal warbler in only one part of the breeding season, suggesting preferences are dynamic. Most of the invertebrate families that were consumed less than expected were consistently avoided over the entire summer, but aphids and leafhoppers (Aphididiae and Cicadellidae) were only avoided in the middle of summer in great reed and Cetti’s warblers.

Some families such as Calliphoridae were preferred prey at all sites, by different consumers, for example by Cetti’s warblers in Sebes, reed warblers in Canal Vell and both species at the Llobregat Delta (Table 4.9). Chironomidae were consumed disproportionately by all species in Sebes, and by just reed warblers in the remaining sites. Syrphidae were eaten at rates greater than the null model at Sebes and the Llobregat Delta but were most abundant in Canal Vell, where they were consumed in proportion to their abundance. Feeding intensity on the spider families Tetragnathidae and Theridiidae switched between reed warblers in Canal Vell to Cetti’s warblers in the Llobregat Delta. Aphididae was consumed disproportionately less than expected in Sebes but more than expected by reed warblers in Canal Vell. A superabundance of aphids was recorded in Sebes, so warblers would have had to consume extremely high frequencies in order to consume them in proportion to their availability. Avoided groups were usually universal across sites, representing superabundant groups that were highly common on the sticky traps. Coenagrionidae was a very abundant family in Canal Vell but they were surprisingly not consumed by any warblers in the study. Ant activity density was inflated, due to their attraction to other insects on the sticky traps and subsequent colonisation and capture. This may explain why they were consumed at a much lower frequency than expected and avoided in the majority of scenarios.

Table 4.8. Results from the *econullnetr* model showing trophic interactions between focal warbler species and resources (prey families) subsetted by season (early, middle and late breeding season stages). Prey families are ordered by average body length in millimetres, with NA indicating a family not recorded in the diet of the warblers but present on sticky traps. Values displayed are standard effect sizes (SES) of the strength of the interaction. SES values >2 and <-2 are highlighted, with darker tone indicating a greater strength of interaction (orange = stronger interaction than expected from null model, SES >2, blue = weaker interaction than expected from the null model, SES<-2). Families are ordered by increasing average body length (mm). Some families are grouped together, and names shortened (see Appendix 1.8).

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Hymenoptera	Chalcidae	1.00	-0.44	-0.45	-0.37	-0.14	-0.35	-0.17	-0.23	NA	0.79	3.38	-0.50	-0.37
Hymenoptera	Platygasteridae	1.00	-4.59	-4.48	-0.30	-0.14	-1.21	-1.23	-0.14	-0.10	-1.78	-1.88	1.93	-0.47
Thysanoptera	Thripidae	1.00	1.78	2.47	-0.42	-0.10	-0.34	-0.20	-0.25	-0.10	-0.56	-0.38	-0.40	-0.27
Hymenoptera	Aphelinidae	1.20	-2.39	-0.29	-2.95	NA	-1.54	-0.14	-1.15	-0.71	-3.52	-0.25	-3.11	-1.50
Diptera: Nematocera	Cecidomyiidae	1.50	-3.01	-1.48	-2.47	-0.85	-0.89	0.50	0.28	-1.41	-3.35	-1.49	-1.96	-1.58
Coleoptera	Phalacridae	1.67	-1.12	-1.50	0.31	-0.48	-1.35	-0.82	-0.81	-0.63	-3.00	-1.09	-2.01	-1.93
Collembola	Entomobryidae	2.00	1.42	2.84	-0.31	-0.10	-0.31	-0.10	-0.17	-0.13	-0.62	-0.23	-0.44	-0.27
Diptera: Brachycera	Asteiidae	2.00	1.21	-0.40	4.34	-0.17	-0.25	-0.14	-0.25	-0.25	-0.52	-0.28	-0.33	-0.35
Diptera: Brachycera	Phoridae	2.00	-2.50	-0.74	-2.06	-1.51	-2.52	-0.55	-1.59	-1.06	-3.99	-0.93	-2.83	-2.26
Hemiptera	Triozidae	2.00	-0.80	-0.36	-0.78	-0.10	-0.59	-0.14	-0.69	-0.25	-1.24	-0.31	-1.10	-0.39
Hymenoptera	Eulophidae	2.00	0.83	-0.45	1.82	-0.10	-0.62	-0.40	-0.49	-0.27	-0.30	-0.57	0.14	-0.28
Hymenoptera	Figitidae	2.00	1.60	-0.29	3.63	-0.10	2.83	-0.17	-0.10	NA	0.91	2.15	-0.44	-0.34
Neuroptera	Coniopterygidae	2.00	-0.82	-0.34	-0.58	-0.24	-0.48	-0.14	-0.37	-0.31	-0.87	-0.20	-0.66	-0.52
Psocoptera	Stenopsocidae	2.00	3.56	-0.33	2.98	9.90	-0.20	-0.14	-0.23	-0.14	3.19	-0.25	6.16	-0.35
Diptera: Nematocera	Psychodidae	2.17	-7.56	-9.09	-0.91	-0.10	-2.47	-2.52	-0.74	-0.20	-3.94	-3.82	-0.29	-0.54
Araneae	Dictynidae	2.43	13.35	2.37	12.93	NA	-0.36	-0.20	-0.27	-0.14	0.91	-0.23	1.46	-0.19
Araneae	Corinnidae	2.50	-0.65	-0.29	-0.34	-0.14	-0.29	-0.29	-0.17	-0.14	0.76	-0.29	-0.35	2.70
Diptera: Brachycera	Chloropidae	2.50	-6.31	-3.25	-4.77	-1.01	-3.30	-1.81	-2.13	-0.92	-6.04	-2.83	-4.52	-2.00

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Diptera: Nematocera	Ceratopogonidae	2.50	-6.30	-2.95	-2.49	-1.79	-3.20	-2.21	-2.39	-1.42	-6.21	-2.78	-5.28	-2.85
Hemiptera	Cicadellidae	2.50	-5.28	-2.07	-3.79	-1.18	-2.64	-0.86	-2.20	-0.94	-4.93	-1.84	-4.30	-2.87
Hymenoptera	Diapriidae	2.50	-0.57	-0.37	-0.35	-0.10	-0.23	-0.14	-0.14	-0.10	2.72	3.37	2.00	-0.35
Hemiptera	Aphididae	2.53	-2.33	0.29	-3.18	-0.17	-0.71	1.59	-2.95	1.69	0.92	2.11	-1.22	4.02
Araneae	Linyphiidae	2.55	-0.06	-0.72	-0.19	2.69	-0.61	-0.25	-0.63	-0.27	1.91	-0.48	1.23	2.54
Coleoptera	Apionidae	3.00	-1.01	-0.48	-0.35	-0.14	-0.47	2.37	3.63	-0.17	-0.87	-0.67	-0.40	9.07
Coleoptera	Brentidae	3.00	-1.53	1.23	-1.14	-1.73	-0.76	-1.03	-0.92	-1.52	-2.78	-1.35	-1.66	-3.29
Coleoptera	Curculionidae	3.00	-0.33	-0.27	-0.92	2.47	-0.80	-0.17	-0.77	-0.34	-1.52	-0.31	-1.09	-0.84
Coleoptera	Scirtidae	3.00	-3.10	-2.50	-1.37	-0.20	-1.29	-0.77	-0.68	-0.38	2.22	-0.13	1.09	3.97
Diptera: Brachycera	Psilidae	3.00	-0.62	-0.32	-0.26	NA	2.99	-0.23	9.90	-0.10	1.09	2.59	-0.47	-0.27
Diptera: Nematocera	Simuliidae	3.00	-0.56	-0.37	-0.44	NA	2.47	9.90	-0.23	-0.10	3.83	-0.29	6.62	-0.35
Hemiptera	Pseudococcidae	3.00	-0.67	-0.31	-0.37	NA	2.69	4.34	-0.10	NA	0.83	2.98	-0.39	-0.23
Hymenoptera	Pteromalidae	3.00	-2.29	-0.95	-1.68	-0.57	-1.62	-0.71	-1.37	-0.54	-2.70	-1.09	-1.93	-1.09
Trichoptera	Hydroptilidae	3.00	0.99	-0.34	-0.37	NA	-0.34	-0.17	-0.23	-0.10	4.03	-0.42	-0.35	8.70
Hymenoptera	Braconidae	3.15	2.94	2.12	1.96	2.47	0.53	1.03	0.44	-0.40	10.83	10.59	6.56	2.25
Diptera: Brachycera	Hybotidae	3.25	-7.80	-5.35	-5.61	-0.82	-5.03	-2.71	-2.22	-1.94	-6.61	-4.17	-4.82	-3.16
Hemiptera	Piesmidae	3.30	-0.62	-0.29	-0.35	NA	-0.34	-0.20	-0.23	-0.17	18.52	7.04	21.91	2.28
Coleoptera	Coccinellidae	3.50	-1.56	-0.76	-1.08	-0.25	-0.97	-0.41	-0.76	-0.25	-1.46	0.78	-1.79	-0.80
Diptera: Nematocera	Sciaridae	3.50	-1.30	0.78	-2.00	-0.72	-1.76	-1.42	-0.91	-0.78	-3.53	-1.55	-2.04	-1.61
Hemiptera	Anthocor/Rhyparochromidae	3.50	-0.72	-0.43	-0.45	NA	-0.44	-0.25	-0.29	-0.14	-0.83	-0.37	-0.47	-0.35
Hemiptera	Lygaeidae	3.50	-0.67	-0.38	-0.38	-0.31	-0.44	-0.22	-0.28	-0.25	2.05	2.98	1.37	-0.58

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Hemiptera	Saldidae	3.50	1.24	-0.41	2.83	-0.10	-0.23	-0.25	-0.14	-0.17	-0.64	-0.37	-0.49	-0.30
Araneae	Therediidae	3.77	3.85	3.63	3.94	NA	-0.26	-0.20	-0.20	-0.14	5.12	2.57	5.53	2.28
Coleoptera	Ptinidae	3.80	14.86	18.00	11.43	NA	-0.32	-0.14	-0.25	-0.20	2.91	3.37	2.20	-0.33
Hymenoptera	Formicidae	3.83	-9.13	-3.12	-5.52	-2.62	-9.73	-4.63	-4.09	-6.93	-20.23	-8.33	-12.19	-10.42
Diptera: Brachycera	Pipunculidae	4.00	-0.66	-0.31	-0.39	-0.20	-0.44	-0.29	-0.14	-0.23	0.48	-0.41	-0.45	1.69
Hymenoptera	Chrysididae	4.00	-1.21	-0.39	-0.83	-0.48	-0.84	-0.48	-0.53	-0.45	-1.15	-0.65	0.35	-1.27
Araneae	Salticidae	4.04	-1.92	-1.16	-1.37	-0.50	-0.79	-0.53	-0.53	-0.52	0.91	2.13	1.33	-0.49
Coleoptera	Malachiididae	4.50	-0.55	-0.28	-0.33	-0.10	-0.31	-0.10	-0.20	-0.14	1.30	-0.28	1.78	-0.28
Diptera: Brachycera	Laux/Drosophilidae	4.50	-2.50	-1.23	-2.11	-0.55	-2.37	-1.53	-1.31	-0.60	-3.27	-2.01	-2.86	-1.43
Lepidoptera	Gracillariidae	4.50	-0.29	2.83	-0.33	-0.10	-0.60	-0.14	-0.13	-0.25	-0.76	-0.25	-0.41	-0.28
Diptera: Nematocera	Culicidae	4.60	4.78	5.70	2.00	-0.35	3.45	8.13	1.35	-0.27	28.67	21.14	21.44	13.95
Diptera: Brachycera	Ephydridae	4.75	-3.64	-3.56	-0.54	-0.17	-0.12	-0.96	2.47	-0.25	-0.79	-1.73	-0.66	6.30
Hemiptera	Miridae	4.75	0.68	-0.46	2.29	-0.14	-1.05	-0.73	-0.75	-0.27	0.14	0.00	0.65	-0.58
Coleoptera	Carabidae	4.80	8.89	0.90	15.55	5.66	-0.40	-0.20	-0.17	-0.20	9.43	8.05	11.26	-0.55
Araneae	Philodromidae	4.83	7.48	7.73	6.70	-0.43	-0.71	-0.35	-0.28	-0.53	6.82	2.03	11.04	0.18
Araneae	Gnaphosidae	5.00	-0.65	-0.40	-0.39	-0.17	2.28	-0.17	4.87	-0.10	-0.70	-0.30	-0.43	-0.33
Araneae	Thomisidae	5.00	1.02	2.57	-0.41	-0.10	-0.54	-0.20	-0.14	-0.27	-0.86	-0.33	-0.42	-0.58
Coleoptera	Bupestridae	5.00	-0.83	-0.28	-0.55	-0.25	-0.53	-0.20	-0.30	-0.17	0.24	-0.31	0.61	-0.38
Diptera: Brachycera	Acroceridae	5.00	-0.49	-0.29	-0.28	-0.10	-0.35	-0.17	-0.17	-0.20	0.94	2.99	-0.44	-0.44
Lepidoptera	Cosmopterigidae	5.00	1.33	-0.33	2.57	-0.14	-0.34	-0.17	-0.25	-0.10	7.13	8.90	5.33	2.83
Opiliones	Sclerosomatidae	5.00	1.20	-0.25	2.98	-0.14	-0.35	-0.23	-0.17	NA	-0.70	-0.35	-0.38	-0.43
Diptera: Nematocera	Chironomidae	5.38	1.56	-0.62	3.22	0.45	6.09	5.12	3.75	2.38	12.73	7.91	8.59	6.19

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Hemiptera	Aphrophoridae	5.75	18.38	15.96	9.32	1.77	-0.93	-0.73	-0.45	-0.45	-1.87	-1.40	-0.68	-0.97
Coleoptera	Chrysomelidae	6.00	-1.12	-0.77	-0.77	-0.31	-0.51	-0.37	-0.31	-0.17	-0.12	-0.50	1.13	-0.46
Diptera: Brachycera	Dolichopodidae	6.00	-0.84	-0.35	-0.24	-0.87	-2.15	-0.45	-1.90	-1.03	-4.18	-0.70	-4.03	-2.06
Hymenoptera	Adrenidae/Halictidae	6.00	0.75	-0.38	1.12	-0.17	1.15	-0.25	-0.47	2.99	8.86	-0.37	2.18	12.19
Neuroptera	Hemerobiidae	6.00	4.28	2.69	2.37	-0.10	-0.31	-0.22	-0.20	-0.17	3.03	3.16	1.73	-0.25
Araneae	Clubionidae	6.10	5.07	4.29	3.38	1.22	5.72	1.19	8.70	2.94	21.90	7.10	23.84	8.18
Lepidoptera	Gelechiidiidae	6.75	3.21	5.16	-0.33	-0.10	-0.37	NA	-0.10	-0.23	2.93	4.34	1.61	-0.20
Araneae	Anyphaenidae	7.00	-0.53	-0.42	-0.30	-0.10	-0.39	-0.20	-0.25	NA	1.04	2.99	-0.45	-0.28
Diptera: Brachycera	Stratiomyiidae	7.00	-0.66	-0.30	-0.29	NA	-0.23	-0.25	-0.14	-0.14	-0.73	-0.37	-0.41	-0.35
Gastropoda	Geomitridae	7.00	1.05	2.69	-0.37	-0.14	-0.42	-0.25	-0.20	-0.20	-0.59	-0.25	-0.48	-0.31
Hemiptera	Membracidae	7.00	1.29	-0.33	2.12	NA	3.16	-0.29	5.66	-0.20	-0.62	-0.20	-0.42	-0.32
Hemiptera	Reduviidae	7.00	1.49	-0.37	2.70	-0.14	-0.37	-0.20	-0.23	-0.17	-0.67	-0.34	-0.39	-0.42
Lepidoptera	Plutellidae	7.00	4.78	7.38	-0.43	NA	-0.29	-0.27	-0.23	-0.10	-0.60	-0.25	-0.45	-0.29
Diptera: Brachycera	Musc/Fann/Anthomyiidae	7.16	-0.73	0.83	-1.12	-0.61	0.28	-0.43	-0.94	3.18	2.00	1.66	0.19	2.74
Araneae	Tetragnathidae	7.25	8.89	-0.31	10.50	-0.10	-0.26	NA	-0.25	-0.10	4.97	3.37	-0.54	8.20
Hymenoptera	Tenthredinidae	7.25	-0.53	-0.38	-0.31	-0.10	3.63	-0.10	3.64	-0.17	-0.62	-0.23	-0.42	-0.33
Lepidoptera	Tortricidae	8.00	-0.46	-0.37	-0.27	-0.20	-0.27	-0.20	-0.17	-0.14	1.21	-0.17	-0.41	2.07
Diptera: Brachycera	Syrphidae	8.25	15.97	13.70	18.00	3.16	4.21	3.88	3.71	-0.35	6.34	5.40	5.61	-0.75
Diptera: Brachycera	Rhinophoridae	8.50	-2.24	-0.85	-1.46	-1.24	-0.22	1.73	-0.44	-1.21	-1.75	-0.73	-0.22	-1.57
Hymenoptera	Ichneumonidae	8.73	0.13	-0.87	1.93	-0.73	8.46	7.71	6.46	4.06	4.89	1.49	5.80	0.57
Araneae	Agelenidae	9.00	1.21	-0.25	2.83	-0.10	-0.31	-0.14	-0.17	-0.17	-0.63	-0.35	-0.36	-0.31
Araneae	Titanoecidae	9.00	1.11	-0.26	3.16	-0.10	-0.33	-0.17	-0.23	-0.17	-0.73	-0.25	-0.40	-0.20

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Coleoptera	Scarabeidae	9.00	-0.55	-0.28	-0.25	-0.14	3.37	-0.17	3.94	-0.20	-0.62	-0.28	-0.40	-0.30
Diptera: Brachycera	Tachinidae	9.17	1.01	-0.25	3.63	-0.25	4.42	NA	3.63	2.57	3.60	-0.29	7.19	0.91
Diptera: Brachycera	Sarcophagidae	9.50	-0.62	-0.70	0.19	-0.72	-1.17	-0.54	-0.76	-0.59	1.03	0.64	1.03	0.02
Coleoptera	Oedemeridae	9.67	0.44	-0.49	2.57	-0.10	9.97	15.03	3.16	-0.17	0.43	-0.41	1.09	-0.42
Hymenoptera	Apidae	9.75	2.76	2.57	2.13	-0.17	-0.45	-0.20	-0.43	-0.17	0.50	-0.27	-0.67	2.47
Diptera: Nematocera	Limoniidae	10.00	-0.48	-0.27	-0.37	-0.10	2.28	-0.20	6.96	NA	-0.51	-0.25	-0.37	-0.26
Hymenoptera	Crabronidae	10.00	-2.15	-0.90	-1.75	-0.72	-0.70	-0.79	-0.50	0.76	-2.00	-0.94	-1.55	-0.84
Diptera: Brachycera	Calliphoridae	10.07	13.21	4.94	10.61	3.64	0.55	-0.27	-0.40	6.96	5.48	-0.26	2.45	10.04
Neuroptera	Chrysopidae	10.60	2.48	-0.38	3.94	9.90	-0.37	-0.17	-0.20	-0.14	5.40	-0.50	7.02	5.70
Lepidoptera	Crambidae	10.78	1.32	2.38	-0.29	NA	2.69	-0.23	5.66	-0.10	16.20	5.76	13.63	10.55
Hemiptera	Gerridae	11.00	1.35	-0.33	-0.29	-0.14	2.00	-0.10	4.87	-0.20	-0.60	-0.30	-0.47	-0.37
Lepidoptera	Nolidae	11.00	1.16	2.20	-0.29	-0.10	-0.32	-0.17	-0.20	-0.10	-0.73	-0.37	-0.50	-0.31
Lepidoptera	Pyralidae	11.25	1.41	2.98	-0.26	NA	2.84	3.95	-0.29	-0.20	5.66	4.34	7.02	-0.40
Hemiptera	Coreidae	11.50	-0.61	-0.32	-0.29	-0.14	6.64	5.66	4.87	-0.14	1.15	-0.28	2.37	-0.33
Gastropoda	Hygromiidae	12.50	2.93	2.37	-0.38	NA	-0.17	-0.20	-0.25	-0.19	-0.60	-0.40	-0.47	-0.37
Trichoptera	Hydropsychidae	12.50	-0.55	-0.26	-0.31	-0.10	2.57	-0.27	3.38	-0.17	0.99	-0.20	-0.47	2.10
Lepidoptera	Geometridae	12.71	19.44	20.74	8.82	-0.14	-0.25	-0.14	-0.20	-0.20	25.29	8.90	10.19	27.50
Araneae	Zoropsidae	13.00	1.18	1.88	-0.27	NA	-0.38	-0.14	-0.10	-0.14	-0.54	-0.31	-0.46	-0.29
Hemiptera	Pentatomidae	13.75	-0.48	-0.39	-0.26	-0.14	5.48	-0.10	-0.17	NA	-0.71	-0.23	-0.52	-0.43
Lepidoptera	Erebidae	14.64	19.47	5.16	22.54	6.96	4.38	3.64	3.94	-0.10	24.13	9.95	15.56	15.55
Gastropoda	Lymnaeidae	15.00	1.30	-0.35	-0.31	-0.14	-0.30	-0.17	-0.14	-0.14	-0.66	-0.28	-0.42	-0.31
Diptera: Nematocera	Tipulidae	16.00	-0.47	-0.35	-0.31	-0.10	-0.35	-0.14	-0.23	-0.10	0.93	-0.22	-0.43	3.16

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Hymenoptera	Pompilidae	16.00	-1.16	-0.37	-0.84	-0.27	2.16	3.63	-0.60	2.47	-0.16	-0.44	-0.18	0.40
Isopoda	Armadillididae	16.00	1.55	1.60	1.60	-0.20	-0.35	-0.20	-0.29	-0.17	1.53	-0.29	-0.61	5.33
Lepidoptera	Noctuidae	17.29	45.37	32.26	32.83	5.66	36.37	28.29	23.16	23.16	57.60	20.55	42.61	30.25
Lepidoptera	Cossidae	20.00	-0.51	-0.45	-0.38	NA	-0.31	-0.20	-0.23	-0.20	0.97	-0.31	1.64	-0.27
Gastropoda	Helicidae	24.00	4.28	4.99	4.34	NA	8.49	4.87	14.07	-0.10	-0.67	-0.25	-0.31	-0.42
Lepidoptera	Nymphalidae	24.00	1.44	2.99	-0.29	NA	-0.22	-0.10	-0.14	-0.14	-0.56	-0.38	-0.30	-0.34
Gastropoda	Agriolimacidae	25.00	13.94	4.76	14.17	NA	-0.37	-0.17	-0.17	-0.14	-0.67	-0.35	-0.42	-0.29
Lepidoptera	Sessidae	35.00	-0.66	-0.28	-0.34	NA	2.70	5.66	-0.17	-0.17	-0.61	-0.37	-0.39	-0.33
Lepidoptera	Pieridae	37.50	1.25	1.88	-0.35	NA	-0.31	-0.14	-0.20	-0.20	0.99	-0.31	2.07	-0.31
Odonata	Libellulidae	44.00	-0.59	-0.29	-0.22	NA	11.45	-0.27	11.49	8.90	1.03	-0.27	-0.35	3.94
Decapoda	Cambaridae	45.00	-0.55	-0.31	-0.32	-0.10	6.64	-0.14	14.07	-0.14	2.36	-0.40	5.28	-0.27
Orthoptera	Tettigoniidae	46.00	2.08	2.37	1.33	-0.23	24.37	3.63	11.45	25.18	3.48	3.94	2.54	-0.29
Lepidoptera	Sphingidae	48.00	-0.61	-0.35	-0.26	NA	2.14	NA	2.98	-0.10	-0.63	-0.25	-0.51	-0.42
Orthoptera	Acrididae	56.00	0.85	-0.47	3.63	-0.20	7.71	-0.22	7.53	5.66	1.99	-0.40	2.37	1.05
Coleoptera	Cantharidae	NA	-0.81	-0.40	-0.48	-0.30	-0.82	-0.63	-0.35	-0.35	-1.16	-0.74	-0.56	-0.67
Coleoptera	Ciidae	NA	-0.55	-0.34	-0.32	-0.14	-0.35	-0.13	-0.23	-0.10	-0.64	-0.27	-0.58	-0.30
Coleoptera	Elateridae	NA	-0.57	-0.31	-0.35	-0.10	-0.28	-0.20	-0.26	-0.17	-0.73	-0.24	-0.31	-0.30
Coleoptera	Erotylidae	NA	-0.69	-0.25	-0.23	-0.17	-0.29	-0.10	-0.30	-0.10	-0.72	-0.33	-0.50	-0.30
Coleoptera	Lathridiidae	NA	-1.08	-0.45	-0.94	NA	-0.66	-0.38	-0.45	-0.14	-1.21	-0.45	-0.82	-0.55
Coleoptera	Meloididae	NA	-0.68	-0.30	-0.60	-0.10	-0.41	-0.17	-0.45	NA	-0.84	-0.30	-0.69	-0.23
Coleoptera	Melyridae	NA	-0.68	-0.33	-0.37	-0.25	-0.53	-0.20	-0.23	-0.25	-1.03	-0.35	-0.37	-0.81
Coleoptera	Pselaphidae	NA	-0.61	-0.30	-0.39	-0.14	-0.37	-0.10	-0.19	-0.14	-0.70	-0.33	-0.47	-0.27

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Coleoptera	Scraptiidae	NA	-1.33	-0.83	-0.93	-0.17	-0.79	-0.29	-0.62	-0.14	-1.45	-0.41	-1.16	-0.45
Coleoptera	Staphylinidae	NA	-0.76	-0.29	-0.74	-0.10	-0.50	-0.10	-0.38	-0.27	-0.97	-0.29	-0.74	-0.39
Diptera: Brachycera	Lonchopteridae	NA	-0.54	-0.35	-0.38	NA	-0.58	-0.20	-0.23	-0.20	-0.74	-0.35	-0.50	-0.49
Diptera: Brachycera	Micropezidae	NA	-0.49	-0.37	-0.42	-0.14	-0.35	-0.20	-0.25	-0.14	-0.63	-0.35	-0.43	-0.41
Diptera: Brachycera	Milichiididae	NA	-0.49	-0.35	-0.35	-0.10	-0.55	-0.59	-0.17	-0.23	-0.77	-0.64	-0.41	-0.41
Diptera: Brachycera	Opomyz/Tephritidae	NA	-0.73	-0.67	-0.35	-0.23	-0.35	-0.23	-0.23	-0.14	-0.70	-0.44	-0.34	-0.33
Diptera: Brachycera	Ulidiidae	NA	-1.19	-0.37	-1.06	-0.61	-0.79	-0.10	-0.20	-0.66	-1.86	-0.17	-0.75	-1.40
Diptera: Brachycera	Rhagionidae	NA	-0.60	-0.34	-0.31	-0.17	-0.37	-0.22	-0.20	-0.10	-0.73	-0.23	-0.47	-0.40
Diptera: Brachycera	Scathophagidae	NA	-0.92	-0.70	-0.57	NA	-0.44	-0.17	-0.55	-0.17	-1.04	-0.52	-0.79	-0.35
Diptera: Brachycera	Sciomyzidae	NA	-0.57	-0.50	-0.37	NA	-0.27	-0.23	-0.10	-0.14	-0.71	-0.37	-0.43	-0.25
Diptera: Brachycera	Xylomyiidae	NA	-0.67	-0.33	-0.31	-0.10	-0.47	-0.34	-0.20	-0.14	-0.67	-0.59	-0.42	-0.42
Diptera: Nematocera	Scatopsidae	NA	-0.73	-0.59	-0.31	NA	-0.28	-0.26	-0.14	-0.10	-0.67	-0.42	-0.31	-0.29
Hemiptera	Aeopophilidae	NA	-0.53	-0.48	-0.33	-0.10	-0.39	-0.17	-0.20	-0.23	-0.70	-0.39	-0.41	-0.29
Hemiptera	Aleyroididae	NA	-1.29	-0.34	-1.04	-0.17	-0.88	-0.14	-0.75	-0.20	-1.73	-0.24	-1.43	-0.92
Hemiptera	Cercopidae	NA	-0.53	-0.28	-0.29	NA	-0.27	-0.14	-0.14	-0.17	-0.62	-0.17	-0.33	-0.23
Hemiptera	Delphacidae	NA	-2.24	-0.46	-1.72	-0.69	-1.89	-0.89	-1.17	-0.76	-3.15	-1.10	-2.06	-1.53
Hemiptera	Hebridae	NA	-0.47	-0.35	-0.27	-0.10	-0.51	-0.33	-0.27	-0.14	-0.72	-0.64	-0.40	-0.29
Hemiptera	Psyllidae	NA	-0.87	-0.27	-0.77	NA	-0.70	-0.10	-0.42	-0.20	-1.13	-0.29	-0.85	-0.37
Hymenoptera	Evaniidae	NA	-1.07	-0.29	-0.99	-0.20	-0.48	-0.23	-0.32	-0.14	-1.18	-0.22	-0.70	-0.48
Hymenoptera	Mymariidae	NA	-2.59	-1.55	-1.55	-0.47	-1.65	-0.61	-0.81	-0.58	-2.62	-0.95	-2.05	-1.25
Hymenoptera	Prototrupidae	NA	-0.75	-0.63	-0.35	NA	-0.29	-0.25	-0.20	-0.17	-0.77	-0.41	-0.55	-0.31
Hymenoptera	Vespidae	NA	-0.60	-0.37	-0.30	-0.19	-0.37	-0.17	-0.23	-0.27	-0.73	-0.35	-0.39	-0.65

			Cetti's Warbler				Great Reed Warbler				Reed Warbler			
Order	Family	Average Length (mm)	All	Early	Middle	Late	All	Early	Middle	Late	All	Early	Middle	Late
Odonata	Coenagrionidae	NA	-1.25	-0.64	-1.00	-0.10	-1.25	-0.22	-0.71	-0.96	-2.25	-0.43	-1.49	-1.45
Orthoptera	Gryllidae	NA	-0.76	-0.51	-0.28	-0.17	-0.40	-0.14	-0.22	-0.25	-0.72	-0.29	-0.40	-0.45

Table 4.9. Results from the *econullnetr* model showing trophic interactions between focal warbler species and resources (prey families) subsetted by the three main sites. CW = Cetti's warbler, GRW = great reed warbler and RW = reed warbler). Values displayed are standard effect sizes (SES) of the strength of the interaction. Interactions with an SES>2 or <-2 are highlighted, with darker tone indicating a more positive or negative interaction orange = stronger interaction than expected from null model, blue = weaker interaction than expected from the null model). Families are ordered alphabetically by their respective taxonomic orders.

		Llobregat Delta			Sebes			Canal Vell	
Order	Family	CW	GRW	RW	CW	GRW	RW	GRW	RW
Araneae	Agelenidae	1.73	NA	-0.32	-0.29	-0.25	-0.43	-0.31	-0.35
Araneae	Anyphaenidae	-0.45	NA	3.16	-0.23	-0.14	-0.29	-0.17	-0.42
Araneae	Clubionidae	5.00	-0.27	7.84	2.42	2.64	13.26	5.70	19.09
Araneae	Corinnidae	-0.41	-0.10	-0.33	-0.35	-0.25	2.69	-0.20	-0.47
Araneae	Dictynidae	5.92	-0.10	2.84	18.03	-0.17	-0.41	-0.25	-0.38
Araneae	Gnaphosidae	-0.59	-0.14	-0.37	-0.31	6.96	-0.37	-0.17	-0.41
Araneae	Linyphiidae	-1.08	-0.14	0.34	2.10	-0.62	1.62	-0.41	0.60
Araneae	Philodromidae	9.39	-0.29	10.99	2.05	-0.37	1.72	-0.64	1.05
Araneae	Salticidae	-1.78	-0.33	0.95	-0.73	-0.65	-1.31	-0.64	1.81
Araneae	Tetragnathidae	3.65	-0.14	-0.33	12.37	-0.17	-0.48	-0.25	6.53
Araneae	Theridiidae	5.28	-0.10	-0.28	-0.28	-0.14	-0.37	-0.23	11.27
Araneae	Thomisidae	1.88	-0.14	-0.27	-0.27	-0.25	-0.42	-0.33	-0.58
Araneae	Titanoecidae	1.77	-0.17	-0.31	-0.27	-0.23	-0.40	-0.20	-0.34
Araneae	Zoropsidae	1.44	NA	-0.31	-0.27	-0.20	-0.26	-0.27	-0.31
Coleoptera	Apionidae	-0.50	NA	-0.25	-0.90	-0.41	-0.66	-0.25	-0.29
Coleoptera	Brentidae	1.45	-0.20	-0.40	-2.16	-0.82	-2.14	0.17	-1.34
Coleoptera	Bupestridae	-0.53	NA	4.87	-0.65	-0.48	-0.60	-0.27	-0.50
Coleoptera	Cantharidae	-0.47	NA	-0.37	-0.64	-0.33	-0.78	-0.55	-0.82
Coleoptera	Carabidae	3.16	-0.23	16.58	13.94	-0.39	1.30	-0.23	2.20
Coleoptera	Chrysomelidae	-1.04	-0.17	-0.72	-0.62	-0.33	-0.70	-0.47	1.15
Coleoptera	Ciidae	-0.42	-0.14	-0.22	-0.38	-0.20	-0.38	-0.17	-0.57
Coleoptera	Coccinellidae	-1.76	-0.14	-1.44	-0.04	-0.85	-1.50	-0.32	0.77
Coleoptera	Curculionidae	-0.73	-0.14	-0.41	0.22	-0.90	-1.24	-0.24	-0.57
Coleoptera	Elateridae	-0.45	-0.17	-0.54	-0.32	-0.27	-0.40	-0.23	-0.27
Coleoptera	Erotylidae	-0.46	-0.14	-0.33	-0.37	-0.10	-0.30	-0.33	-0.46
Coleoptera	Lathridiidae	-0.97	-0.10	-0.84	-0.65	-0.43	-0.89	-0.27	-0.49
Coleoptera	Melyridae	-0.51	-0.17	-0.33	-0.45	-0.40	-0.81	-0.29	-0.33
Coleoptera	Oedemeridae	0.72	5.66	2.38	-0.37	2.37	-0.44	6.01	-0.55
Coleoptera	Phalacridae	-2.16	-0.35	-2.63	3.21	-0.55	-1.11	-1.36	-1.95
Coleoptera	Pselaphidae	-0.50	-0.20	-0.33	-0.41	-0.27	-0.48	-0.28	-0.42
Coleoptera	Ptinidae	19.85	-0.10	-0.25	2.98	-0.14	-0.42	-0.27	4.79
Coleoptera	Scarabaeidae	-0.45	-0.17	-0.27	-0.31	3.17	-0.28	-0.19	-0.43
Coleoptera	Scirtidae	-2.91	-0.73	-1.04	-0.88	-0.62	-1.19	-0.79	5.53
Coleoptera	Scraptiidae	-0.95	-0.20	-0.61	-0.78	-0.71	-1.15	-0.31	-0.38
Coleoptera	Staphylinidae	-0.55	NA	-0.47	-0.60	-0.44	-0.73	-0.24	-0.35
Collembola	Entomobryidae	1.99	-0.14	-0.23	-0.23	-0.14	-0.36	-0.23	-0.37

		Llobregat Delta			Sebes			Canal Vell	
Order	Family	CW	GRW	RW	CW	GRW	RW	GRW	RW
Decapoda	Cambaridae	-0.54	NA	-0.33	-0.31	6.58	4.79	-0.19	-0.37
Diptera	Acroceridae	-0.37	-0.10	-0.44	-0.26	-0.27	2.20	-0.26	-0.42
Diptera	Asteiidae	1.36	-0.14	-0.31	-0.27	-0.23	-0.35	-0.29	-0.29
Diptera	Calliphoridae	10.19	-0.14	10.12	8.90	1.30	1.33	-0.33	4.21
Diptera	Chloropidae	-4.73	-0.85	-2.46	-3.48	-2.41	-4.92	-2.01	-2.82
Diptera	Dolichopodidae	0.37	-0.10	-1.33	-1.37	-1.12	-2.00	-2.14	-4.17
Diptera	Ephydridae	-3.95	-1.00	-1.76	-0.63	2.07	1.05	-0.37	0.96
Diptera	Hybotidae	-8.65	-1.37	-3.94	-3.01	-2.00	-3.76	-3.30	-5.39
Diptera	Lauxan/Drosophilidae	-2.13	-0.42	-1.71	-1.56	-1.00	-1.76	-2.08	-2.92
Diptera	Lonchopteridae	-0.54	-0.17	-0.35	-0.23	-0.17	-0.25	-0.43	-0.44
Diptera	Micropezidae	-0.48	-0.17	-0.27	-0.38	-0.23	-0.43	-0.33	-0.42
Diptera	Milichiididae	-0.48	NA	-0.34	-0.34	-0.14	-0.37	-0.70	-0.82
Diptera	Musc/Fann/Anthomyiidae	-0.22	-0.23	1.72	-1.01	0.69	1.84	-0.56	0.05
Diptera	Opomyz/Tephritidae	-0.71	-0.23	-0.46	-0.28	-0.14	-0.51	-0.25	-0.39
Diptera	Phoridae	-0.98	-0.20	-0.89	-2.45	-1.88	-3.87	-1.47	-1.84
Diptera	Pipunculidae	-0.54	-0.14	-0.34	-0.38	-0.25	1.49	-0.33	-0.44
Diptera	Psilidae	-0.52	NA	-0.30	-0.29	4.34	1.82	-0.23	-0.50
Diptera:	Rhagionidae	-0.53	NA	-0.25	-0.30	-0.28	-0.48	-0.23	-0.39
Diptera	Rhinophoridae	-1.54	-0.17	-1.22	-1.98	-1.13	-1.21	3.37	-0.38
Diptera	Sarcophagidae	-1.04	-0.20	0.84	-0.19	-0.92	1.71	-0.60	-1.08
Diptera	Scathophagidae	-0.84	-0.17	-0.48	-0.73	-0.43	-0.78	-0.27	-0.32
Diptera	Sciomyzidae	-0.49	-0.14	-0.31	-0.32	-0.25	-0.28	-0.20	-0.35
Diptera	Stratiomyiidae	-0.45	-0.17	-0.31	-0.31	-0.23	-0.36	-0.25	-0.39
Diptera	Syrphidae	15.25	14.07	6.64	8.69	4.90	8.84	-0.72	0.50
Diptera	Tachinidae	-0.37	-0.10	-0.26	2.06	4.34	3.74	3.16	3.37
Diptera	Ulidiidae	-1.07	NA	-0.78	-0.77	-0.65	-1.42	-0.38	-0.75
Diptera	Cecidomyiidae	-2.43	-0.64	-1.73	-1.72	-1.22	-1.75	0.01	-2.03
Diptera	Ceratopogonidae	-4.52	-0.87	-3.05	-2.39	-2.15	-3.23	-2.78	-4.17
Diptera	Chironomidae	0.39	2.26	4.17	2.47	8.15	13.85	1.17	4.29
Diptera	Culicidae	4.79	-0.14	8.13	2.20	4.34	7.07	2.81	24.49
Diptera	Limoniidae	-0.45	-0.14	-0.29	-0.31	-0.29	-0.29	4.34	-0.42
Diptera	Psychodidae	-8.94	-2.43	-3.55	-1.01	-0.83	-1.19	-0.47	-0.61
Diptera	Scatopsidae	-0.61	-0.10	-0.35	-0.43	-0.27	-0.45	-0.27	-0.41
Diptera	Sciaridae	-0.53	-0.45	-1.39	-1.79	-1.18	-2.10	-1.39	-1.47
Diptera	Simuliidae	-0.41	-0.17	-0.35	-0.28	4.87	8.49	-0.33	-0.48
Diptera	Tipulidae	-0.35	-0.10	-0.33	-0.38	-0.17	2.57	-0.29	-0.16
Gastropoda	Agriolimacidae	9.28	NA	-0.40	9.28	-0.23	-0.32	-0.29	-0.40
Gastropoda	Geomitridae	1.49	-0.14	-0.31	-0.25	-0.29	-0.34	-0.20	-0.38
Gastropoda	Helicidae	5.51	-0.10	-0.34	-0.23	-0.25	-0.34	12.32	-0.41
Gastropoda	Hygromiidae	1.28	-0.10	-0.23	3.63	-0.16	-0.41	-0.20	-0.41
Gastropoda	Lymnaeidae	-0.48	-0.10	-0.25	5.66	-0.20	-0.31	-0.20	-0.35
Hemiptera	Aeopophilidae	-0.45	-0.10	-0.27	-0.33	-0.16	-0.37	-0.33	-0.37

		Llobregat Delta			Sebes			Canal Vell	
Order	Family	CW	GRW	RW	CW	GRW	RW	GRW	RW
Hemiptera	Aleyrodoidae	-0.39	NA	-0.82	-1.39	-0.93	-1.44	-0.17	-0.40
Hemiptera	Anthocoridae	-0.39	-0.10	-0.20	-0.50	-0.25	-0.51	-0.25	-0.45
Hemiptera	Aphididae	0.41	1.86	0.65	-5.28	-3.14	-4.38	1.55	6.51
Hemiptera	Aphrophoridae	32.95	-0.10	-0.36	3.19	-1.36	-1.71	-0.30	-0.45
Hemiptera	Cercopidae	-0.36	-0.14	-0.29	-0.33	-0.20	-0.34	-0.14	-0.28
Hemiptera	Cicadellidae	-3.12	-0.54	-1.97	-3.99	-2.32	-4.57	-0.54	-1.04
Hemiptera	Coreidae	-0.51	9.90	-0.33	-0.27	3.94	2.21	-0.19	-0.35
Hemiptera	Delphacidae	-1.16	-0.14	-0.81	-1.90	-1.29	-2.29	-1.12	-1.66
Hemiptera	Gerridae	-0.38	-0.14	-0.29	2.37	6.96	-0.47	-0.23	-0.39
Hemiptera	Hebridae	-0.35	-0.14	-0.23	-0.23	NA	-0.42	-0.48	-0.50
Hemiptera	Lygaeidae	-0.43	-0.17	-0.30	-0.42	-0.23	-0.51	-0.23	7.53
Hemiptera	Membracidae	1.50	-0.10	-0.33	-0.30	6.96	-0.44	-0.17	-0.41
Hemiptera	Miridae	1.19	-0.42	2.45	-0.75	-0.72	-1.15	-0.75	-1.08
Hemiptera	Pentatomidae	-0.33	-0.10	-0.40	-0.23	11.49	-0.34	-0.23	-0.55
Hemiptera	Piesmidae	-0.54	NA	-0.35	-0.29	-0.31	-0.34	-0.20	32.16
Hemiptera	Pseudococcidae	-0.55	-0.10	-0.24	-0.40	-0.23	-0.38	5.66	3.37
Hemiptera	Psyllidae	-0.57	NA	-0.39	-0.63	-0.62	-0.76	-0.25	-0.48
Hemiptera	Reduviidae	2.28	-0.17	-0.39	-0.17	-0.17	-0.40	-0.29	-0.44
Hemiptera	Saldidae	-0.45	-0.17	-0.22	3.37	-0.23	-0.40	-0.23	-0.47
Hemiptera	Triozidae	-0.48	-0.10	-0.38	-0.59	-0.50	-0.94	-0.48	-0.75
Hymenoptera	Andren/Halictidae	-0.47	-0.10	-0.30	1.93	2.13	10.84	-0.33	3.00
Hymenoptera	Aphelinidae	-2.96	-0.14	-2.05	0.44	-0.62	-1.09	-1.56	-2.69
Hymenoptera	Apidae	4.18	-0.10	-0.37	-0.48	-0.37	1.24	-0.22	-0.48
Hymenoptera	Braconidae	3.00	1.01	6.44	1.30	1.02	8.49	-0.74	4.66
Hymenoptera	Chalcidae	-0.45	-0.17	2.98	-0.35	-0.23	-0.43	-0.23	-0.43
Hymenoptera	Chrysididae	-0.74	-0.10	-0.59	-0.87	-0.78	-0.73	-0.47	-0.68
Hymenoptera	Crabronidae	-1.80	-0.27	-1.89	-1.23	0.49	-0.29	-0.99	-1.73
Hymenoptera	Diapriidae	-0.51	-0.14	3.17	-0.35	-0.25	2.06	-0.25	-0.40
Hymenoptera	Eulophidae	-0.47	-0.25	-0.38	1.88	-0.10	2.13	-0.49	-0.91
Hymenoptera	Evaniidae	-0.86	-0.10	-0.58	-0.55	-0.48	-0.74	-0.25	-0.45
Hymenoptera	Figitidae	-0.37	-0.10	-0.41	3.37	3.94	2.13	-0.20	-0.34
Hymenoptera	Formicidae	-6.38	-1.05	-3.95	-3.91	-2.03	-4.95	-24.37	-27.60
Hymenoptera	Ichneumonidae	-0.69	1.87	2.69	1.38	5.24	4.49	7.45	-0.04
Hymenoptera	Mymaridae	-2.50	-0.38	-1.60	-1.03	-0.87	-1.48	-0.94	-1.91
Hymenoptera	Platygasteridae	-4.62	-0.93	-1.13	-0.25	-0.17	-0.48	-0.48	-0.68
Hymenoptera	Pompilidae	-0.79	-0.14	-0.80	-0.57	-0.56	-0.78	2.93	0.91
Hymenoptera	Prototrupidae	-0.74	-0.27	-0.54	-0.27	-0.14	-0.42	-0.20	-0.40
Hymenoptera	Pteromal/Torymidae	-1.51	-0.33	-1.62	-1.62	-1.29	-2.08	-0.88	-0.95
Hymenoptera	Tenthredinidae	-0.45	-0.10	-0.35	-0.23	5.66	-0.32	-0.19	-0.40
Hymenoptera	Vespidae	-0.45	-0.10	-0.42	-0.44	-0.29	-0.65	-0.19	-0.35
Isopoda	Armadillidiidae	1.86	-0.23	1.95	-0.23	-0.23	2.98	-0.29	-0.42
Lepidoptera	Cosmopterygidae	2.47	-0.10	5.76	-0.31	-0.17	-0.31	-0.23	6.12

		Llobregat Delta			Sebes			Canal Vell	
Order	Family	CW	GRW	RW	CW	GRW	RW	GRW	RW
Lepidoptera	Cossidae	-0.42	-0.10	-0.37	-0.24	-0.23	-0.33	-0.29	2.70
Lepidoptera	Crambidae	1.69	-0.14	3.94	-0.31	6.96	12.50	-0.26	13.28
Lepidoptera	Erebidae	8.04	-0.10	24.89	31.73	4.34	8.44	4.87	4.66
Lepidoptera	Gelechiidae	3.97	NA	-0.29	-0.28	-0.29	5.10	-0.17	-0.37
Lepidoptera	Geometridae	21.15	-0.14	7.04	3.37	-0.25	11.07	-0.27	24.93
Lepidoptera	Gracillariidae	1.56	NA	-0.25	-0.22	-0.27	-0.37	-0.25	-0.25
Lepidoptera	Noctuidae	31.14	6.96	21.71	22.88	11.49	37.83	52.32	32.16
Lepidoptera	Nolidae	-0.51	-0.14	-0.45	4.34	-0.27	-0.45	-0.20	-0.47
Lepidoptera	Nymphalidae	2.00	-0.14	-0.45	-0.23	-0.10	-0.38	-0.17	-0.33
Lepidoptera	Pieridae	1.83	NA	3.37	-0.29	-0.16	-0.37	-0.27	-0.38
Lepidoptera	Plutellidae	7.19	-0.10	-0.28	-0.32	-0.17	-0.36	-0.20	-0.35
Lepidoptera	Pyralidae	1.73	NA	-0.33	-0.35	-0.31	4.38	6.96	5.16
Lepidoptera	Sessidae	-0.51	9.90	-0.30	-0.35	-0.25	-0.29	-0.23	-0.40
Lepidoptera	Sphingidae	-0.43	-0.10	-0.40	-0.23	4.34	-0.41	-0.20	-0.31
Lepidoptera	Tortricidae	-0.40	NA	-0.25	-0.27	-0.19	-0.36	-0.20	1.61
Neuroptera	Chrysopidae	-0.37	-0.10	-0.33	3.17	-0.20	9.65	-0.20	-0.35
Neuroptera	Coniopterygidae	-0.57	-0.20	-0.33	-0.49	-0.53	-0.70	-0.27	-0.33
Neuroptera	Hemerobiidae	2.06	-0.14	-0.31	3.37	-0.14	-0.44	-0.27	4.54
Odonata	Coenagrionidae	-1.30	-0.17	-0.93	-0.35	-0.24	-0.47	-1.37	-2.31
Odonata	Libellulidae	-0.50	-0.14	-0.19	-0.37	-0.23	-0.45	16.51	1.88
Opiliones	Sclerosomatidae	1.69	NA	-0.29	-0.26	-0.25	-0.36	-0.31	-0.40
Orthoptera	Acrididae	-0.44	NA	-0.32	1.88	6.64	3.10	4.34	-0.38
Orthoptera	Gryllidae	-0.64	-0.14	-0.42	-0.29	-0.27	-0.50	-0.10	-0.38
Orthoptera	Tettigoniidae	1.78	-0.17	-0.20	1.14	4.94	-0.76	29.05	8.49
Psocoptera	Stenopsocidae	1.70	-0.10	6.64	2.98	-0.14	-0.38	-0.27	2.06
Thysanoptera	Thripidae	2.21	-0.14	-0.38	-0.30	-0.25	-0.31	-0.17	-0.31
Trichoptera	Hydropsychidae	-0.56	-0.10	-0.24	-0.19	4.87	2.37	-0.20	-0.34
Trichoptera	Hydroptilidae	-0.39	-0.14	-0.37	3.94	-0.10	7.94	-0.17	-0.49

4.4.8. Prey size differentiation

The GLM explaining prey body size was significant (Adjusted R-squared = 0.28, F = 9.65 on 8 and 168 df, $p<0.001$). Site was not a significant predictor of size, but it was retained in the model to avoid pseudoreplication. The average prey sizes consumed by individual birds differed significantly among warbler species (F = 61.18, $p <0.001$, Fig. 4.6); 15.9 mm for great reed warblers, 9mm for Cetti's warblers and 7.4 mm for reed warblers (Table 4.11). Great reed warblers consumed prey of a significantly greater size than both reed warblers (z= 7.12, $p<0.001$) and Cetti's warblers (z = 4.65, $p<0.001$) and reed warbler prey was smaller than Cetti's warbler prey (z = -2.89, $p<0.01$). Great reed warblers also consistently consumed larger species per order when compared to the other warblers (Table 4.10). This was particularly marked for Hemiptera, Hymenoptera, Lepidoptera and Trichoptera. Also significant were warbler age (F = 14.71, p<0.001) and season (F = 6.93, p<0.01). Juveniles consumed smaller prey items than adults (t = -3.89, p<0.001) In addition, prey size did not significantly differ between any periods of the breeding season, when all bird species were considered.

Consumption of large prey items by the great reed warbler was also related to spatial (site) and temporal (seasonal) differences. Early season great reed warblers consumed smaller prey on average compared to those captured in the middle (z = -3.45, p<0.01) and late summer (z = -3.28, p<0.01). At Canal Vell the average prey size consumed by great reed warblers was larger than at the remaining sites, but post-hoc pairwise site differences were not significant.

Table 4.10. Mean prey body lengths (±SD) of invertebrates consumed by the warblers in this study arranged by taxonomic order. Where only one species from a given order was detected across the samples from a given focal warbler; standard deviation = 0.0.

	Mean body length (mm) ± SD		
	Great Reed Warbler	Cetti’s Warbler	Reed Warbler
All Orders	15.9 ± 6.8	9.0 ± 2.3	7.4 ± 3.3
Araneae	7.8 ± 0.6	5.3 ± 1.6	6.4 ± 1.7
Coleoptera	7.6 ± 1.9	4.2 ± 1.1	4.0 ± 1.1
Diptera	7.6 ± 2.7	6.9 ± 2.1	6.8 ± 0.1
Gastropoda	23 ± 0.0	22.9 ± 3.2	-
Hemiptera	19.7 ± 8.5	10.2 ± 3.5	8.8 ± 6.8
Hymenoptera	8.1 ± 3.4	4.8 ± 2.7	4.9 ± 2.8
Lepidoptera	20.1 ± 6.9	15.4 ± 2.7	12.2 ± 4.6
Neuroptera	-	8.0 ± 2.3	8.6 ± 3.2
Orthoptera	48.1 ± 12.5	49.3 ± 5.7	50 ± 5.5
Trichoptera	12.5 ± 0.0	7.8 ± 0.0	4.6 ± 2.7

4.4.9. Proportion of aquatic prey

The GLM testing for differences in the proportion of aquatic prey detected in the bird diets study was significant (Adjusted R-squared = 0.37, F =13.98 on 8 and 171 df, p<0.001). The effect of warbler species was significant (LRT = 43.28, p<0.001) where reed warbler samples had a significantly higher proportion of aquatic prey compared to Cetti’s warblers (z = 5.65, p<0.001) and great reed warblers (z = 2.57, p<0.05, Fig. 4.7). Cetti’s warbler samples had a significantly lower proportion of aquatic prey compared to great reed warblers (z = 2.45, p<0.05). Site was also significant (LRT = 41.74, p<0.001). Birds sampled at Canal Vell (z = 4.87, p<0.001) and Sebes (z = 5.63, p<0.001) had a significantly higher proportion of aquatic prey than birds sampled at Mas del Matà but not the Llobregat Delta. Season was also significant (LRT = 13.47, $p<0.01$) as late season birds had a significantly lower proportion of aquatic prey than early season birds ($z = -3.15$, $p<0.01$) but differences between early and middle season birds and middle and late season birds were not-significant. The number of days since the last rainfall event was not significant but was retained in the model after model refinement based on AIC.

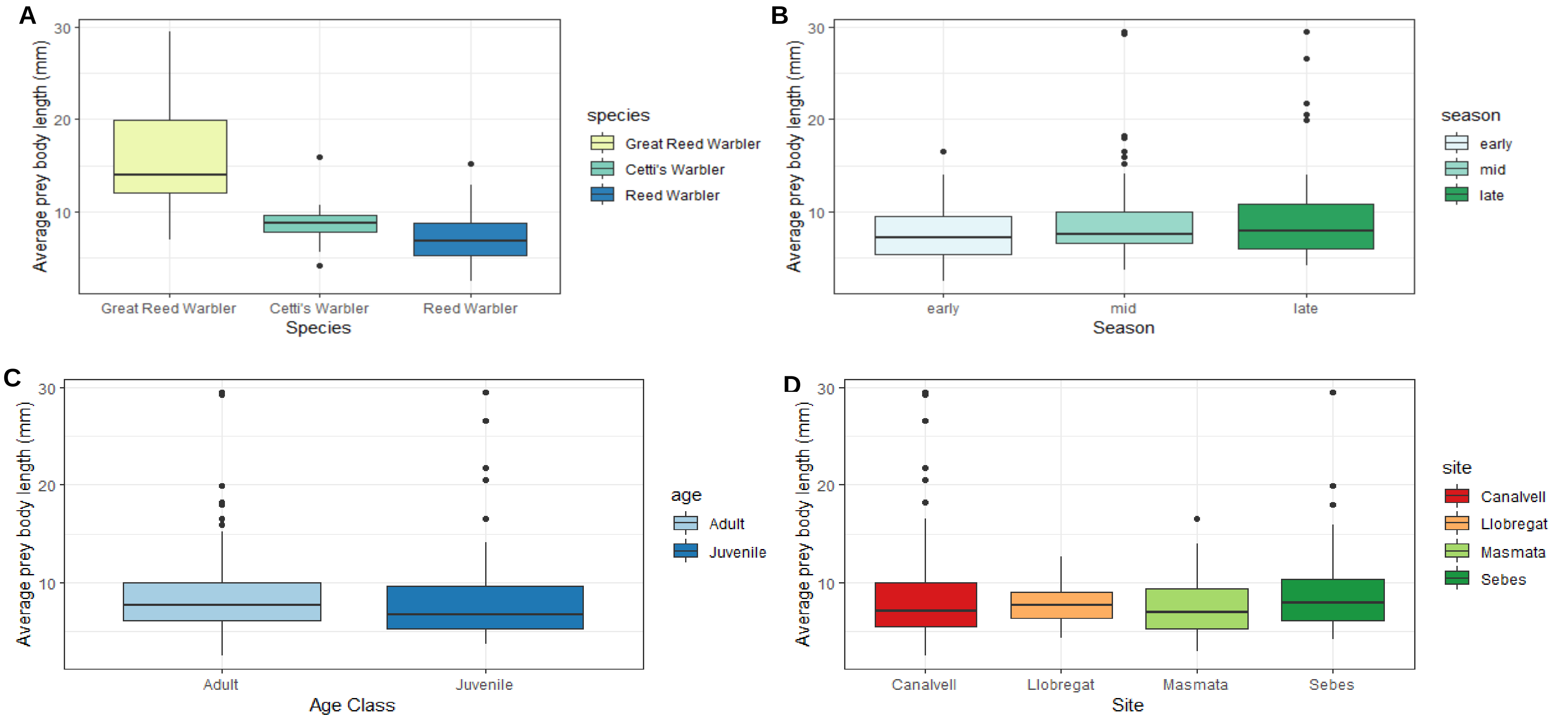


Figure 4.6. Boxplots of the average body length (mm) of prey items detected in diet samples in Catalonia; A) the three focal warbler species; reed warblers, great reed warblers and Cetti’s warblers, B) birds captured at different stages of the breeding season, early, middle and late, C) adult and juvenile birds of all species combined and D) birds captured at the four study sites, Canal Vell, Llobregat Delta, Mas del Matà (Aiguamolls de l’Emporda) and Sebes.

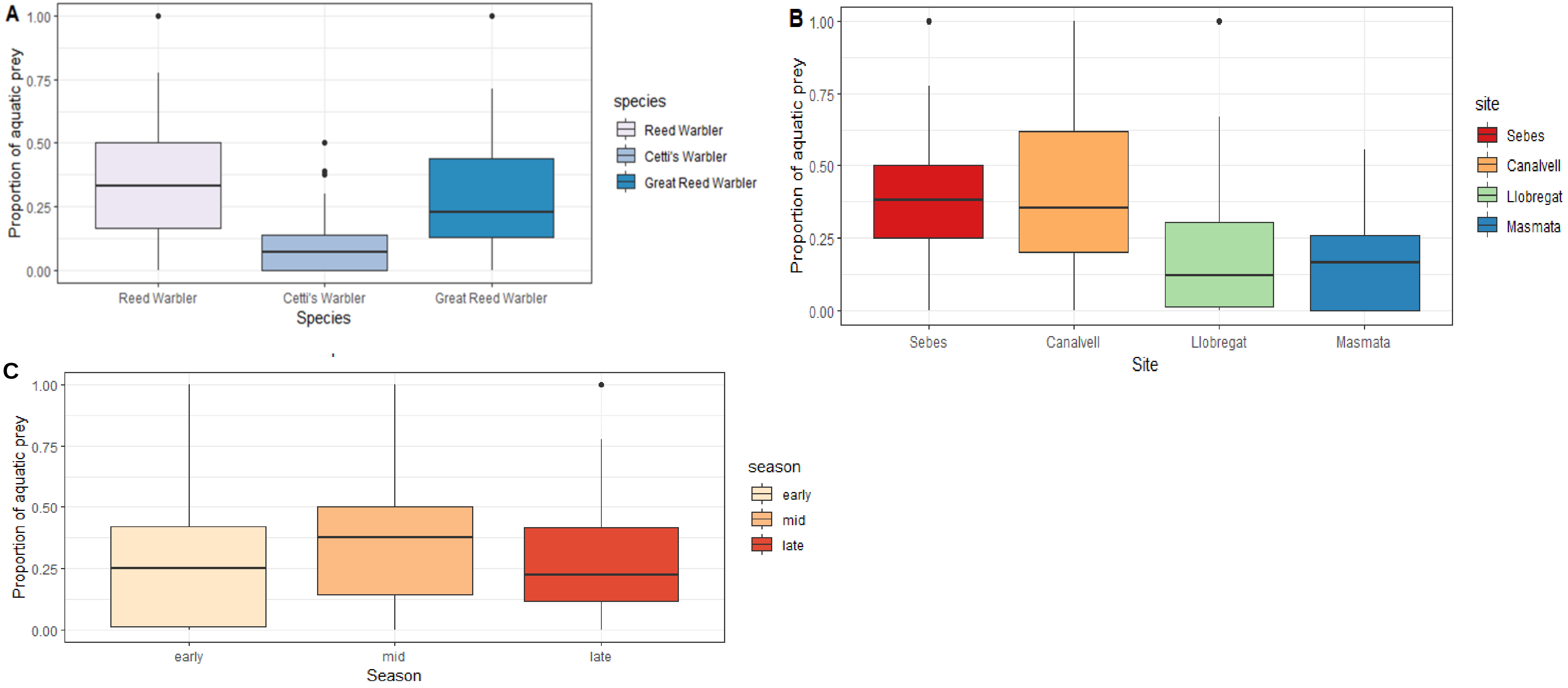


Figure 4.7. Boxplots of the proportion of aquatic/semi-aquatic prey out of the total number of unique prey species detected in Catalan diet samples; for A) the three focal warbler species, reed warbler, Cetti’s warbler and great reed warbler B) study sites Sebes, Canal Vell, Llobregat Delta and Mas del Matà (Aiguamolls de l’Emporda) and C) early, middle and late breeding season birds.

4.5. Discussion

4.5.1. Summary of findings

The findings of this chapter clearly demonstrate that a metabarcoding approach can help to unravel complex patterns in competition ecology between generalist consumers. There was ample evidence that the three warblers exhibited differences in prey resource use. Although competitive pressure could not be directly measured, dietary partitioning is likely to be one of the main mechanisms permitting coexistence during periods of competition. Significant overlap and a high degree of diet similarity between two of the three species pairs was largely explained by the widespread consumption of common prey items found at all study sites such as chironomids and clubionid spiders, which were abundant and available to all birds. It was possible to identify clearly, differences in the main prey resources selected by the focal warbler species, associated with 1) differences in dietary diversity and niche breadth in the three warblers, 2) differential consumption of prey with different habitat associations (i.e., aquatic or terrestrial), 3) differences in the body sizes of selected prey and 4) differences in positive or negative selection of prey groups related to their relative abundance.

4.5.2. Dietary overlap and competition

In cases of severe resource limitation, the diets of different consumers should converge on the few prey available, leading to stronger competition as resources become depleted (Clare et al. 2014*b*, Salinas-Ramos et al. 2015). It is unlikely that the overlap detected in our study reflects food shortage, because pairwise overlap values rarely exceeded 0.6, and warbler diets were significantly different in their composition. Significant incidences of overlap in our study were between 0.39 and 0.65, falling into the category of 'moderate' overlap (Novakowski et al. 2008). Each warbler species showed unique patterns of prey choice, consumed prey taxa of different sizes and, in the case of the Cetti's warbler, different proportions of aquatic and terrestrial prey compared to their competitors. The warblers in our study also showed high dietary plasticity, with dietary composition and niche breadth often changing between sites and seasons.

Optimal foraging theory postulates that predators should specialise on a few highly profitable prey types when those prey are abundant, but when preferred resources become rare they should broaden the prey types taken (Pulliam 1985, Martínez-Curci et al. 2015).The dietary overlap observed in our study system was, for the most part, a result of the widespread sharing of common, abundant prey occurring across sites and seasons. At least some of the observed overlap in our study could also be explained by optimal foraging theory however, since all three warblers showed similar consumption of several larger-sized, high-quality prey items such as moths, hoverflies and spiders (Robinson and Wilson 1998), and often shared a strong preference for families with these characteristics (e.g., Clubionidae, Calliphoridae, Syrphidae).

In addition, some of the shared prey families represent those which are highly mobile and may move between microhabitats, so that warblers may encounter them even when foraging in different places (Wilson 2010, Kent and Sherry 2020). Chironomids in particular were detected very frequently in both the diet of the birds and on the sticky traps (which were placed in different microhabitats). Due to their high availability and wide emergence window, chironomid species form an important component of the diet of many passerines (Dyrcz and Flinks 2000, López-Iborra et al. 2005, King et al. 2015). Aerial groups such as nematocerous flies, often exhibit swarming behaviour (Bell 2011, Sherry et al. 2016). Opportunistic feeding strategies may benefit from the patchiness of invertebrates that occurs with aggregations, and optimal foraging models show that once a patch or swarm of prey is discovered by a consumer, search times are rapidly reduced, and foraging efficiency is improved (Ornes 2013, Sherry et al. 2016).

Dietary overlap between Cetti’s warblers and great reed warblers was not significant, suggesting that a higher degree of dietary partitioning was occurring between them. This is perhaps due to stronger pairwise divergences in other niche dimensions such as foraging habitat, morphology, and life history traits. This pair represented the least similar diets of the triad; the great reed warbler fed on the largest prey items, a greater proportion of which were aquatic compared to the Cetti’s warblers, and the literature documents them as foraging from the water’s surface and using aerial hawking (Kennerly and Pearson 2010). Cetti’s warblers are smaller bodied, consumed medium-sized prey items and although they forage in the reedbed (Araújo et al. 2016) they are otherwise the most terrestrial of the triad in the breeding season, preferring areas of marshy scrub and willow-carr (Harvey 1977, Bibby and Thomas 1984) where they forage by gleaning in lower vegetation layers (Cramp and Brooks 1992). It is also possible that competition events in the past have driven the partitioning seen here, and the two species may have subsequently been under strong selection to avoid competing with each other (Poulin and Lefebvre 1996). In addition, the extent of overlap is likely to be variable across years, and as shown in our study, across sites and seasons (Petit et al. 1990, Marchetti et al. 1996).

Finally, high dietary overlap can sometimes hide individual consumer preferences, especially if they are also feeding opportunistically on abundant prey. The sensitivity analysis performed within the *EcoSimR* program made the selectivity of each warbler more apparent. Subtle dietary differences can exist despite dietary overlap, even if they serve only to supplement the main diet of shared prey that are accessible to all birds.

4.5.3. Mechanisms of diet differentiation

The potential effects of competition may be limited via partitioning prey in more subtle ways, i) by maintaining small but distinct differences in consumed prey taxa, ii) consuming different proportions of prey species obtained from aquatic and terrestrial environments, iii) consumption of prey within a distinct body size range and iv) exhibiting diverging foraging behaviours. This study provides evidence for the first three mechanisms. Foraging behaviour was not measured here but has been documented in the literature (Green and Davies 1972, Dyrcz 1981, 2016, Kennerly and Pearson 2010, but see Ezaki 1992). A recent study found that differences in foraging behaviour helped to explain subtle variation in the diets of neotropical sympatric wood warblers (Kent and Sherry 2020), even though dietary overlap between pairs of species were high. The findings uncovered by this study are consistent with studies that span across the animal kingdom including birds (Orłowski and Karg 2013), bats (Razgour et al. 2011, Kruger et al. 2014), amphibians (Steel and Brammer 2006) and reef fish (Nagelkerken et al. 2009), suggesting that even very subtle dietary differences in the diet of individual species may support coexistence.

The three species showed significant differences in the prey sizes consumed, with the largest species consumed by great reed warblers, intermediate-sized prey species by Cetti's warblers and the smallest prey species by reed warblers. In Western France, great reed warblers were documented feeding on larger size classes of prey, compared to six other warbler species that were of a smaller size (Bibby and Green 1983). Partitioning of prey body sizes has also been observed in sympatric aerial insectivores (common swift (*Apus*), barn swallow (*Hirundo rustica*), and house martin (*Delichon urbicum*)) with swifts consuming prey items more than twice as small as those consumed by both swallows and house martins (Orłowski and Karg 2013). Because the consumers foraged at different heights and in different habitats, the authors surmised that partitioning may have been a result of separation of the focal species on some ecological dimension (Dyrcz and Flinks 2003, Vieira and Port 2007, Privitera et al. 2008, Whitfield et al. 2013). In addition, a recent molecular study on sympatric bats also detected differences in the average prey size of species consumed between consumers (Vesterinen et al. 2018). In our study, the average body size of prey items was relatively constant across sites, suggesting that birds had access to a similar prey size range across the study sites. Juvenile birds consumed smaller prey than their adult conspecifics, perhaps due to them lacking the handling skills required to capture large prey (Marchetti and Price 1989).

4.5.4. Patterns of prey choice by different warbler species

Cetti’s warblers showed a highly generalist pattern of invertebrate selectivity, consuming many prey orders at high frequency, and upwards of 10 unique species were present per faecal sample. Ecological separation in foraging heights and feeding substrates may explain the size differences in taxa consumed by Cetti’s warblers compared to reed warblers, as large spiders, beetles and larger flies (e.g., Calliphoridae) were likely foraged from low vegetation layers and air spaces (Orłowski and Karg 2013). Many of the Cetti’s warblers most frequently consumed prey families such as aphids, froghoppers and other phytophagous hemipterans likely gleaned from vegetation.

It has been argued that residents such as the Cetti’s warbler are very well integrated in their environments and have a competitive advantage over migrants because they are only subject to selective pressures in one location (Herrera 1978). This allows development of ecological specialisms that can better exploit the largest and most profitable prey species at their location (Herrera 1978, Leisler 1992, Poulin and Lefebvre 1996). Contrastingly, migrants in the same ecosystem have been reported to feed more extensively on small arthropods, that are often low quality but easy to capture (Poulin and Lefebvre 1996, Orłowski and Karg et al. 2013, Trevelline et al. 2018). Migrant birds may also preferentially target groups that are easily digested and can be foraged efficiently, such as aquatic Diptera, over those that are large and difficult to handle (Raley and Anderson 1990, Trevelline et al. 2018). However, many of the existing studies on migrant passerines are from tropical locations in the winter breeding grounds of migrants (Poulin and Lefebvre 1996, Sherry et al. 2016, Kent and Sherry 2020), or temperate North American breeding grounds (Yard 2004, Trevelline et al. 2018) and factors such as prey availability, competitive forces and foraging pressures in these localities may be very different to those over the breeding season in southern Europe.

Migrants like the great reed warbler and reed warbler encounter many different habitats with fluctuating resources during their annual cycle, so it would be expected that they are under selection to remain plastic in their dietary selectivity (Toms 2011). Studies on foraging behaviour often report that palearctic migrants use a wider range of foraging strategies and foraging substates than residents, showing broader feeding behaviours in line with adaptations to fluctuations in the availability in food resources that might occur at stopover sites (Leisler 1992, Salewski et al. 2003). Their ability to partition diet through morphological or behavioural foraging may be constrained in order to maintain this flexibility (Toms 2011). In addition, different breeding, migration stopover and wintering locations entail different communities where competition for resources is likely to differ, so interspecific niche overlap likely involves several species (Toms 2013).

Reed warbler diets were extremely varied, showing the greatest dietary breadth, though this may partly be attributed to the larger sample size achieved. They selected a higher proportion of aquatic/semi-aquatic prey than their closely related neighbour, the great reed and Cetti's warblers. Diet similarity was greatest between the more closely related *Acrocephalus* warblers. This is perhaps not surprising given their similar use of habitat and overlapping foraging behaviours. Arguably, this similarity may be a result of the relatively more recent shared ancestry (Wiens et al. 2010), but it should be noted that rather than being sister species, this pair are from different basal clades within their monophyletic group, the former in the "smaller bodied," group and the latter the "larger bodied" group of the *Acrocephalus* genus (Leisler et al. 1997, Helbig and Seibold 1999). Thus, our assumption that these two closely related species should share more similarities in habitat and behavioural traits than less closely related species, due to ancestry alone, should be taken with some caution.

Dietary opportunism can enhance fitness in generalists if it confers the ability to exploit new, abundant prey resources (Greenberg 1995, Yard et al. 2004). When comparing the frequency of occurrence of invertebrate orders across the breeding season (Appendix 3.1, Table A.3.1.*a*), reed warblers seemed to track prey more in accordance with availability, compared to its competitors. One explanation is that reed warblers are simply best able to deal with fluctuations in the abundance of different prey, switching their diet over the season to exploit the most abundant groups. The highly opportunistic foraging of reed warblers confirmed by their high degree of dietary breadth is explained by their feeding on variably abundant prey groups (Cramp and Brooks 1992). However, seasonal changes in prey selection by birds are not always based on prey abundance and may simply reflect different feeding intensities or a change in nutritional requirements over the breeding season (Yard et al. 2004, Rytkonen et al. 2019). No doubt dietary flexibility has assisted reed warblers in colonising numerous habitats as they are often the dominant warbler in terms of density and population at wetland breeding grounds (Cramp and Brooks 1992). Eurasian reed warblers in southern Europe are often most common in territories that are sub-optimal for other warblers and their highly plastic habitat associations may reduce competition with interspecifics through access to resources avoided by others (Ceresa et al. 2016).

Reed warblers also consumed the smallest prey items, much smaller than prey selected by great reed warblers. This finding is backed up by existing literature on prey size differences between reed and great reed warblers (Leisler 1991, Schulze-Hagen 1991). It may be that great reed warblers prevent reed warblers from accessing foraging territories with the largest prey items through interspecific aggression (Dyrcz 1981, Hoi et al. 1991, Honza et al. 1999, Schaefer et al. 2006). Size-specific foraging by reed warblers may be a mechanism that helped to avoid competition with other closely related or similar reed-bed warblers (such as the great reed warbler) in the past (Lack 1976, Connell 1980), or may have evolved separately in reed warblers to increase foraging efficiency (Robinson and Holmes 1982), since smaller prey items are also often more abundant than larger items, even if they are less nutritious (Leisler et al. 2002). However, all three warblers showed stronger preferences for the largest and least abundant prey families, suggesting that the smaller prey consumed by the reed warblers is not positively selected or optimal, but consumed opportunistically, in proportion to availability. Thus, while taking larger prey may be more efficient in terms of resource acquisition, the largest, most valuable items may be less available, causing reed warblers to switch to more abundant prey (Pulliam 1985).

Great reed warblers certainly had the narrowest dietary niche of the triad, consuming a smaller suite of species from most of the arthropod orders. Niche breadth did change across sites however, suggesting that this species can exhibit some dietary flexibility. At Sebes, where there were two competitors present, niche breadth was wider than at Canal Vell where only one competitor was present. This could also be explained by differences in the available prey at Canal Vell compared to Sebes. Despite them showing a significantly lower dietary richness than the other focal species (considering they had the same sample size as Cetti’s warblers) great reed warblers did consume more species of odonates and orthopterans and consumed them disproportionately to their abundance. Great reed warbler diet also included larger species not consumed by the other two warblers, the Egyptian locust (*Anacridium aegyptium*, Acrididae, Orthoptera) and the white-tailed skimmer (*Orthetrum albistylum*, Libellulidae, Odonata). Many of the larger lepidopterans such as the elephant hawkmoth (*Deilephila elpenor*) and hornet clearwing (*Sesia apiformis*) were only found in great reed warbler diets. Since these prey species have wingspans of approximately 43-50mm, for smaller reed and Cetti’s warbler the energetic cost of capture and handling time may exceed the profit gained from consuming them (Turner 1982, Pulliam 1985), a point discussed by Bibby and Green (1983). In contrast, if the larger body size of the great reed warbler infers greater calorific requirements and an increased ability to handle large prey, then selecting larger prey items would better achieve optimal foraging (Turner 1982, Wilson and Robinson 1998).

Our finding that great reed warblers consumed the largest prey species is consistent with those of other studies. For example, great reed warblers brought only the largest female orb-web spiders to their chicks, whereas the Eurasian reed warbler in the same location only provisioned their nestlings with smaller spiders of the same species (Leisler and Schulze-Hagen 2011). Unfortunately, it is unclear whether great reed warblers in our study also took the largest individuals of each prey species, since our molecular approach cannot provide information about the sizes of individual invertebrates or their life stage. The effects of within-species size selection, whereby larger representatives of a common species are predominantly consumed by one predator, leaving the smaller individuals for another predator, may be masked by high dietary overlap at the species level.

4.5.5. Effects of site and season on diet

Potential competitive interactions between consumers can vary with season and habitat in the same locality (Korb and Linsenmair 2001, Salewski et al. 2003). Diet differed with site, season, age (within and across species) and local climate. Climate is a known major driver of prey resources, affecting phenology, abundance and availability of invertebrates (Poulin et al. 2002, Schaefer et al. 2006, Halupka et al. 2008). In temperate areas productivity is limited primarily by temperature which is linked to seasonality (Taylor 1974). In localities with Mediterranean climates, however, it is usually the availability of water that is limiting (Mooney and Parsons 1973). The period of peak prey availability for birds in the summer is typically truncated by the hot and dry conditions experienced in the late summer months whereby food availability is reduced until the rainy season (Herrera 1978).

Canal Vell and Sebes had a higher proportion of aquatic prey in warbler diets compared to Mas del Matà but not the Llobregat Delta. This may be confounded by season, as Mas del Matà was only sampled in May when temperatures may have been limiting for some aquatic prey groups (Halupka et al. 2008, Vafidis et al. 2016). Late season birds consumed a lower proportion of aquatic prey than early season birds which could be linked to fluctuations in the relative availability of prey groups with an aquatic life stage. The late summer was characterised with low rainfall and high daily maximum temperature. In Mediterranean reedbeds, elevated maximum temperatures alongside reduced rainfall reduces water levels. Food availability for warblers in the south of France was found to be negatively correlated with the length of time between June and December where there was no surface water in the reed habitats (Poulin et al. 2002).

Elevated dietary overlap in the middle of the breeding season, and at certain sites (e.g., Sebes) was probably due to prey availability increasing, allowing overlap without competition (Dhondt 2011, Trevelline et al. 2018). Prey assemblages were significantly more species rich at Sebes compared to Canal Vell, and there was also a greater proportional contribution of aquatic prey to the bird's diets. Sticky trap records at Sebes suggest that prey was limiting in the early season, possibly due to lower temperatures, but arthropod abundance peaked in the middle of summer. This was different from the prey patterns seen at Canal Vell and Llobregat Delta where insect abundance and richness were more consistent. Diet differentiation among Neotropical warblers changed over the breeding season due to an outbreak of budworm larvae, which relaxed competition for prey and allowed a high degree of dietary overlap (McMartin et al. 2009). Similarly, a molecular study of the diet of cryptic bats found high overlap during the summer coinciding with peak Lepidoptera availability (Razgour et al. 2011). Resource partitioning was elevated in autumn when Lepidoptera become more limiting, and the bats respond by switching to a diet of predominantly Diptera (Jones 1990).

Dietary species richness was significantly lower at Canal Vell compared to the Llobregat Delta and Sebes, with prey detected in the diet matching the patterns detected on the sticky traps. Dietary species richness increased from early to mid-summer suggesting a potential peak in prey richness or abundance during the mid-summer, before a late-summer decline consistent with the temperature patterns at Catalonia that year, which suddenly increased in mid-June – July. However, extremely high overlap between reed and great reed warblers, which is often observed in resource limited environments, was not observed here (Clare et al. 2014*b*, Salinas-Ramos et al. 2015). Instead, a degree of partitioning was evident between the diets, consistent with niche differentiation (Chesson 2000). Possibly, prey numbers were still high enough to promote dietary selectivity, but there was reduced sharing of common prey items, probably a result of drier conditions limiting the emergence of locally abundant reed-associated invertebrates.

Although they always consumed larger prey on average than the other species, size selection of prey in great reed warblers appeared to be flexible. For example, in the early season, average prey size in great reed warbler samples was significantly lower than in the middle and late season. Some of the largest prey species consumed were grasshoppers, crickets and large moths and these groups may have been limiting in the early summer. It has been suggested that when larger prey is in short supply, outbreaks of chironomids could form a replacement in great reed warbler diet (Dyrcz and Flinks 2000). A number of large prey species were consumed at highest frequency at Canal Vell (e.g., Tettigoniidae, Acrididae, large lepidopteran species) or consumed exclusively at Canal Vell (Libellulidae) whereas chironomid consumption was reduced compared to the other sites, with the exception of Mas del Matà (50% of Canal Vell great reed warblers, compared to 90% at Sebes and 100% at Canal Vell, but 0% Mas del Matà) despite being consistent in reed warblers. Dietary breadth was very narrow at Canal Vell, possibly as a result of the consumption of a smaller subset of prey that were large, nutritionally dense and more strongly associated with the warmer and drier weather experienced at Canal Vell. Niche breadth broadened at Sebes where a more diverse assortment of orders was consumed, including increased frequency of occurrence of Hemiptera and Coleoptera in the diet. Reed warblers broadened their niche at Canal Vell with respect to the other sites, which could indicate an increase in diet flexibility, perhaps permitted by the narrower food niche of its competitor. Later in the summer, prey abundance at Canal Vell fell (albeit non-significantly) and prey richness was lower compared to Sebes, so mechanisms of dietary partitioning that reduce overlap between these two warblers might serve to alleviate competition.

4.5.6. Study limitations

Studying the diets of warblers at a southern European location, where climates are elevated with respect to the UK, provided a new insight into how competitive exclusion is avoided in different environmental conditions. Nonetheless, this study was prone to the same limitations discussed in Chapters 2 and 6. In addition, the study design did not consider the migratory stopover periods for the migratory species which may have entailed different competitive scenarios (e.g., Sherry et al. 2016). Some of the individual reed and great reed warblers captured at Mas del Matà may have been stopping over briefly as part of spring migration and were under different foraging pressures than the breeding birds that had already established breeding territories. Similarly, the birds captured at the end of July/August may have been on passage, shortly before autumn migration.

Our sample sizes were uneven and smaller for both the Cetti's warbler and great reed warbler. Thus, caution was given in interpreting our results for analyses where data was split by site and season. For example, only two Cetti's warblers were captured in the late season and very few great reed warblers were captured in the Llobregat Delta during our study. The proportions of samples of the different warbler species matched that of the relative proportions of captures in mist nets (inferred from ICO ringing data for each site in 2018), however. Reed warblers dominated the catches suggesting high population density, whereas great reed and Cetti's warblers were likely less abundant or were not captured as easily. Collection of a larger number of samples at each of the sites across the summer and if possible, over multiple years would improve the reliability of this dataset and allow more accurate characterisation of the diet.

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Chapter Five - The effect of climate on prey availability and dietary competition along a temperature gradient: a comparison of reedbed warblers in the UK and in Catalonia, Spain.

The warbler species studied in the UK and Catalonia. Top left: the Eurasian reed warbler (photo credit: Pallotta Luigi), top middle: the great reed warbler (photo:treknature.com), top right: the Cetti's warbler (photo: David Tipling), bottom left: the sedge warbler (photo: Colin Brown), bottom middle: the chiffchaff (photo: Ferran Pestaña) and bottom right: the willow warbler (photo: Michael Flowers).

5.1. Abstract

Climate change may alter species interactions in a myriad of ways. Changes in food resources resulting from climate shifts have the potential to alter dietary competition between sympatric species. One approach to assessing the future impacts of warming on current communities of songbirds is to study the same communities in different conditions along a latitude-climate gradient, using a “space for time” approach. Here, the potential impacts of warming on prey abundance and richness, warbler diet composition and overlap, and the body condition of individual birds, were assessed over a latitude-climate gradient, using study sites in Catalonia as a climate analogue for future conditions in the UK. The diet of warblers differed significantly across the climate gradient, and this appeared to be related to the local abundance and richness of prey. Diet preferences remained consistent for the prey orders that were likely the most profitable, such as Araneae and Lepidoptera, but were flexible for alternative prey types. As with the previous chapters, different warbler species differed significantly but subtly in their diet composition. The body condition of birds (BMI) was influenced by climate, prey richness, species, sex, age, time of year, and (for reed warblers), the density of great reed warblers. The latter effect, while requiring more research, suggests possible negative consequences of interspecific competition in Catalonia which may be mediated via interference competition by great reed warblers on **re**ed warblers. Diet overlap was strongly influenced by both prey abundance and prey richness across the gradient, with increases in prey availability driving increases in Pianka’s index of overlap. The response of overlap to temperature and rainfall variation differed depending on the country studied, with overlap increasing in Catalonia in conditions of lower maximum temperatures and elevated rainfall in relative terms, but the opposite pattern was seen in the UK. These differential responses have implications for competitive scenarios under future climate change, notably the negative consequences of reedbed drying on potential food resources for birds and highlights the importance of protecting aquatic habitats through conservation practises.

5.2. Introduction

5.2.1. Climate change and species interactions

Biological responses to climate change reflect a combination of both abiotic and biotic factors (Walther et al. 2002). The abiotic effects of climate may directly influence the many biotic relationships that underpin species interactions, including interspecific competition for resources (Harmon et al. 2009, Traill et al. 2010). Climate change may alter current interactions within and among species through a diverse set of mechanisms, resulting in numerous responses by animal and plant communities (Walther et al. 2002, Parmesan 2006), some of which may be unexpected (Rosenzweig et al. 2007, Traill et al. 2010). In the case of insectivores, the major impact of changing climate may be via alterations to their arthropod food supplies driven by changes in temperature or rainfall patterns (Stenseth et al. 2002, Pearce-Higgins et al. 2009, Walther et al. 2010, Arbeiter et al. 2016). This in turn may alter the dietary overlap and competition between sympatric species utilising similar resources (Chesson 2000, Walther et al. 2002, Trail et al. 2010, Wittwer et al. 2015).

Breeding productivity is the main driver of population trends in many bird species (Newton 1998, Eglington et al. 2015). Accordingly, changes in prey availability, diet and competition between bird species under climate change will have far reaching and mounting consequences if they influence the production of offspring and survival of future generations (McCarty 2001*b*, Walther et al. 2002, Parmesan 2006). The impact of changing climate on fitness in songbirds is little studied however, and in migratory species attention has mostly focused directly on life history traits such as the timing of migration and reproductive output (Winkle and Hudde 1997, Sillet et al. 2000, Stevenson and Bryant 2000, Schaefer et al. 2006).

Dietary competition and climate change

Future warming is likely to affect the distribution and relative abundance of many invertebrate populations (Bale et al. 2002, Walther et al. 2002). The patterns of dietary competition between sympatric species are likely to be strongly affected by climate change if i) warming drives an increase or decrease in overall prey abundance or ii) if the availability of one or more important prey groups changes substantially (Marshall et al. 2002, Pearce-Higgins et al. 2009). During times of environmental change, selection should favour dietary generalists if they are more resilient to changes in food resources (Colles et al. 2009, Mallord et al. 2017). Interspecific competition for access to prey could either increase or decrease via bottom-up forces, leading to either dietary partitioning, whereby species consume different prey, or dietary overlap, where species share a higher proportion of prey groups in their diets (Chesson 2000).

Climate associated changes in diet composition across species ranges

Patterns of prey availability and resulting diet are often distinct in different parts of a consumer’s geographical range (e.g., Clare et al. 2014*a*, Pagani-Núñez et al. 2015, Eitzinger et al. 2019). In Iberia, songbird populations often utilise alternative prey types compared to conspecific populations present further north (Pagani-Núñez et al. 2015). Variation in foraging strategies by birds is expected in response to a more diverse and variable distribution of food resources that are more typical of Mediterranean/Iberian habitats (Blondel et al. 2010). For example, in temperate woodlands, the lack of alternative, top-ranked prey types to the preferred prey (caterpillars), limits the breeding output of tits (Paridae) (Rytkönen and Krams 2003), while in southern populations at the range margins, parids feed on alternative prey types, particularly spiders (Blondel et al. 1991, Ziane et al. 2006, Pagani-Núñez et al. 2011). In great reed warblers (*Acrocephalus arundinaceus*), milder winters and higher summer temperatures and precipitation in Japan were reflected in higher chironomid abundance, high occurrence of chironomids in nestling diet and a 10-fold higher population density compared to Poland (Dyrcz and Flinks 2000).

5.2.2. Reedbeds and prey availability

Wetlands in Northern Europe are host to abundant prey resources for breeding songbirds (McKee and Richards 1996, Lewis-Phillips et al. 2020) and are likely to be highly responsive to changes in climate (Enlonger 2009). In reedbeds and associated habitats, prey resources are available throughout the summer as a succession of taxa emerge (Paerzold et al. 2011, Hambäck et al. 2016). Superimposed on this succession, climate can adjust the timing and magnitude of prey availability (Chernetsov and Manukyan et al. 2000), and differential weather patterns can affect the phenology of relationships between host plants and insect groups, and either advance or delay their emergence (Sardiña et al. 2017).

In temperate northern Europe, prey is likely to be limiting early in the breeding season, as spring temperatures remain lower than thresholds for invertebrate development and emergence (Schaefer et al. 2006, Vafidis 2014). This limitation is short-lived, as climbing temperatures in the middle and later part of summer exceed these thresholds, driving mass emergences of prey with an aquatic larval stage (Schaefer et al. 2006, Halupka et al. 2008). Vafidis (2014) experimentally demonstrated that future climate change in the UK should increase the overall abundance of insects in reedbeds further, with resources becoming available to birds earlier in the summer. If, as predicted, warming increases the abundance of invertebrate groups by increasing development rates and the number of generations per year, generalists may benefit from higher levels of potential prey, enhancing fecundity and reproductive success (Halupka et al. 2008, Vafidis 2014, Vafidis et al. 2016).

In Iberian reedbeds the length of wetland hydroperiods (where reedbeds are inundated), is decreasing in response to climate change, as elevated temperatures coupled with lowered rainfall result in substantial reductions in water level and drive shifts in invertebrate community composition (Bedford and Powell 2005, Jiménez et al. 2018, Aspin 2019). Seasonal patterns exist, and loss of invertebrates due to drought is likely to occur in mid to late summer (e.g., July and August) when maximum temperatures in the environment are very high (Poulin et al. 2002). This may lead to an overall reduced number and potentially species-poor assemblage of prey for birds, which may serve to increase competition between the bird species using the wetland.

Changes to the warbler assemblage

Species assemblages are determined by both environmental conditions at the regional scale and habitat and resource quality at local scales (Kissling et al. 2012). Range limits can be determined by climate, mediated via plant productivity and arthropod abundance, but also by competitors (Gross and Price 2000). Even if prey abundance is high near the range limit, the presence of competitors can prevent access to prey resources, through interspecific aggression.

In southern Europe, the Eurasian reed warbler (*Acrocephalus scirpaceus*) and Cetti's warbler (*Cettia cetti*) are joined by several other species which are not present in their UK communities, including the larger bodied great reed warbler. In contrast, the sedge warbler (*A. schoenobaenus*), a common species of UK reedbeds, is largely absent. These changes in assemblage could alter competitive interactions between reedbed warblers in more southerly European climates. The Cetti's warbler is a recent colonist in the UK, with a limited current range and a small population (Robinson et al. 2010), but this species occupies the marshlands of southern Europe in higher numbers (Cramp and Brooks 1992). The higher population density of Cetti's warblers in Catalonia could result in a greater competitive pressure on sympatric Eurasian reed warblers for access to resources (Svensson et al. 2018). Perhaps more importantly, the great reed warbler is competitively dominant over the reed warbler (Dyrcz 1981, Hoi et al. 1991, Honza et al. 1999, Schaefer et al. 2006; discussed in Chapter 4), but population densities of the former species are usually far lower than that of the latter. Despite this, competition for nest sites could prevent reed warblers from accessing i) habitats with preferred, or important prey groups or ii) habitats rich in prey.

5.2.3. Body condition in birds

A high-quality diet can lead to better physical condition, and higher reproductive output in birds (Gonzalez-Medina et al. 2018). Any abiotic driver that can cause an increase or a reduction in prey availability, can have an indirect impact on an individual's body condition and fitness (Schekkerman and Boele 2009, Vafidis et al. 2016). The flight activity of invertebrate prey is crucial to the foraging success of vertebrates that rely on mobile, aerial insect groups (both captured in the air and on substrata), but their activity and availability is highly dependent on local weather (Taylor 1963, Vicens and Bosch 2000, Arbeiter et al. 2016). These insect groups are far less active during prolonged or heavy rainfall and at low temperatures. Since local weather is often subject to rapid change, aerial prey is often an unpredictable source of food (Cucco and Malacarne 1996*b*, Grüebler et al. 2008). Consequently, insectivore body reserves are often depleted during long periods of adverse weather conditions, with knock on effects on reproductive output (Grassel et al. 2016, Arbeiter et al. 2016).

Food limitation and potential knock-on effects of resource competition during energetically demanding periods such as the chick rearing stage, might lead to reduced nestling growth, smaller clutch sizes and reduced fledgling survival, affecting overall breeding success (McCarty 2001*a*, Winkler et al. 2013). Prolonged adverse weather could lead to a marked decrease in adult body condition during times of chick provisioning, with potential carry-over consequences for offspring quality and the ability of newly fledged juveniles to gain weight. Young birds may be subordinate to adult rivals, limited by sub-optimal feeding habitats, and/or may not be as adept at capturing prey as their adult counterparts (Marchetti and Price 1989). The sex of the bird may also play a role in determining body condition, as breeding males exert a great deal of energy advertising to future mates and establishing and maintaining breeding territories, whereas breeding females invest a great deal of energy in egg production, incubation and brooding (Williams 1966).

The body condition and productivity of Eurasian reed warblers might be lower in areas where the density of the great reed warbler is greater, compared to areas where this competitor is at lower density or does not occur at all. In Chapter 4, great reed warbler diet was distinguished by the consumption of larger prey than the other warblers. It is thus worth considering whether Cetti's and reed warblers experience competitive release due to the absence of the great reed warbler in the UK, perhaps consuming larger prey than they are able to in Catalonia.

5.2.4. Study background and location of climate gradient

Recently there has been a shift in focus in the scientific community from studying the direct effects of climate change on a single study species, towards examining impacts on multiple study species interacting within a location (e.g., Singer et al. 2013, Wittwer et al. 2015), or across a series of locations (e.g., Gross and Price 2000, Harley 2011, Engler et al. 2013). It is possible to measure climate-driven changes in species interactions in controlled experiments, but these are often oversimplified and specific to a limited set of environmental conditions or localities. Environmental gradients encompass a wider range and variation of conditions and can be examined to provide a broader view on ecological and biotic processes (Blois et al. 2013, Sundqvist et al. 2013, Eitzinger et al. 2019).

Some climate models suggest that UK climate will mirror that of southern Europe towards the end of the century (Kopf et al. 2008). The latest Met Office UKCP model predictions forecast further increases in spring temperatures, coupled with rises in summer maximum temperatures (+3.6-5°C) and increasingly dry summers (+16-46%) by 2060-80, under the high emissions scenario (Lowe et al. 2018, Kendon et al. 2019), suggesting that a gradual shift towards southern European conditions may indeed be the general trend in the coming decades.

To predict how climate change might affect competition for food in warblers via the cascading effects of warming, I will compare the diets of European warblers at study sites in two distinct climatic zones within their breeding ranges: the UK and Spain. Catalonia in north-eastern Spain was chosen as the study region for the climate comparison with the UK due to the similarity in coastal position, the occurrence of comparable levels of rainfall and humidity (albeit lower in Catalonia compared to the UK), and the high availability and suitability of wetland study sites. The higher summer temperatures and less frequent precipitation in Catalonia with respect to the UK, make it a useful location from which to project the general direction of associated changes to prey resources and warbler diet resulting from changes in climate.

5.2.5. Chapter aims and objectives

Future changes in diet composition and dietary overlap between sympatric warblers can be inferred by examining both invertebrate prey composition and warbler diets (elucidated by metabarcoding) along a north-south gradient across the UK and in Catalonia.

The existence of interspecific competition cannot be confirmed with certainty without experimental manipulation of study species in the field, through the use of exclusion experiments (Navalpotro et al. 2016). In this study system, such an approach was not feasible, however it is possible to use dietary overlap as an indicator of the potential for competition, by comparing warbler communities i) under current UK climates, and ii) under analogues of future climates, to identify the likely mechanisms driving dietary patterns and dietary overlap in sympatric warblers.

Based on the above assumptions, I aim to test four predictions:

i) Invertebrate abundance and diversity will change along the climate gradient and this can at least partially be accounted for by i) climate and ii) habitat characteristics. Increasingly hot and dry weather will lead to a decrease in invertebrate abundance and diversity.

ii) Warbler species' diets and prey preferences will change over the gradient as an indirect result of climate.

iii) Changes in prey abundance will lead to changes in dietary overlap and the possibility of future competition between warbler pairs in the UK and Catalonia. An increase in prey abundance will be associated with higher pairwise overlap, whereas a decrease will be associated with partitioning - but in conditions of extremely low prey availability diets will converge on the few remaining prey items (high overlap).

iv) There will be a positive relationship between prey abundance/richness and body condition in warblers, and an associated change in body condition in different climates with different prey availabilities and with different warbler assemblages (i.e., different potential competitors). Body condition will also differ as a result of age and sex characteristics of warblers.

To address these predictions, prey availability data from invertebrate monitoring were analysed across seasons, sites and regions to infer changes in the arthropod prey base for warblers as a result of climate. The diet composition and dietary preferences in both climate regions of the two species that co-occurred in both the northern and southern part of the climate gradient; the reed warbler and the Cetti's warbler (using data from Chapter 2 and 4) were compared, and this approach determined whether any shifts occur along the gradient. The diet composition of all the UK and Spanish warbler species studied in Chapter 2 and 4 were also broadly examined, to determine how patterns of prey consumption vary with temperature across the gradient.

Dietary overlap was assessed between two pairs of *Acrocephalus* warbler species across sites in both regions: i) reed and great reed warblers in Catalonia, and ii) the reed and sedge warblers in the UK, using climate and prey availability measures to identify patterns in overlap. Cetti's warblers were not included in these dietary overlap analyses because of their absence at two sites; Canal Vell in Catalonia and Wheldrake Ings in the UK. Finally, biometric data from bird ringing activities were incorporated into analyses spanning both countries, and body condition scores were calculated and used to compare birds of difference ages, sexes across sites and seasons in differing weather conditions.

This study is, to my knowledge, the first of its kind that considers how the impacts of climate on arthropod availability is translated, not only into the diet of an insectivore, but the contribution to i) individual body condition on a finer scale, and ii) patterns of overlap between neighbouring species on a broader scale. Addressing the above predictions will answer an important question; how might competition between warbler species change under future climate change?

5.3. Methods

5.3.1. Study sites

Data from warbler faecal samples, invertebrate sticky traps and weather stations from all sites visited in Chapters 2 and 4 were combined to form the climate gradient (location of sites indicated in Fig. 5.1; see Chapters 2 and 4 for site details). Within the UK, two climate zones were studied, i) southwest England and Wales, (mean daily maximum temperatures = 9-21°C, from Met Office UK climate averages 1981-2010) and northern England (6-20°C). In Catalonia average daily maximum temperatures are much higher at 14-29°C (Met Office). Data from Oxwich Marsh (S Wales) and Wheldrake (N England) were included in both study years; 2017 and 2018, as the weather over the summer of 2018 was warmer and drier with respect to 2017. Data from supplementary sites that were not studied over the entire breeding season, Magor Marsh (S Wales) and Mas del Matá (Catalonia), were included for general dietary comparisons but were excluded from comparisons involving climate, invertebrate availability, and prey choice.

5.3.2. Data assimilation

Data were collated from the following research activities (described in Fig. 5.2);

Invertebrate Data and Warbler diet Data

Invertebrate availability data (from sticky trap monitoring) and dietary information from the high-throughput sequencing data, were both obtained from the methods described in the previous chapters. Biometric data for individual birds captured and ringed (including birds from which a diet sample could not be collected) over the course of the PhD was obtained in collaboration with local ringers and ringing groups under licences issued by the British Trust for Ornithology (BTO, UK) and Institut Català d'Ornitologia (ICO, Catalonia). Data were collated for all warbler species in our study that were captured over the summer of 2017 in the UK (and 2018 at Oxwich Marsh and Wheldrake, UK) and 2018 in Catalonia. Capture dates of birds and sticky trap data were organised into three distinct periods of the breeding season: “early summer” (late April to early June), “mid-summer” (mid-June to late July), and “late summer” (late-July to September).

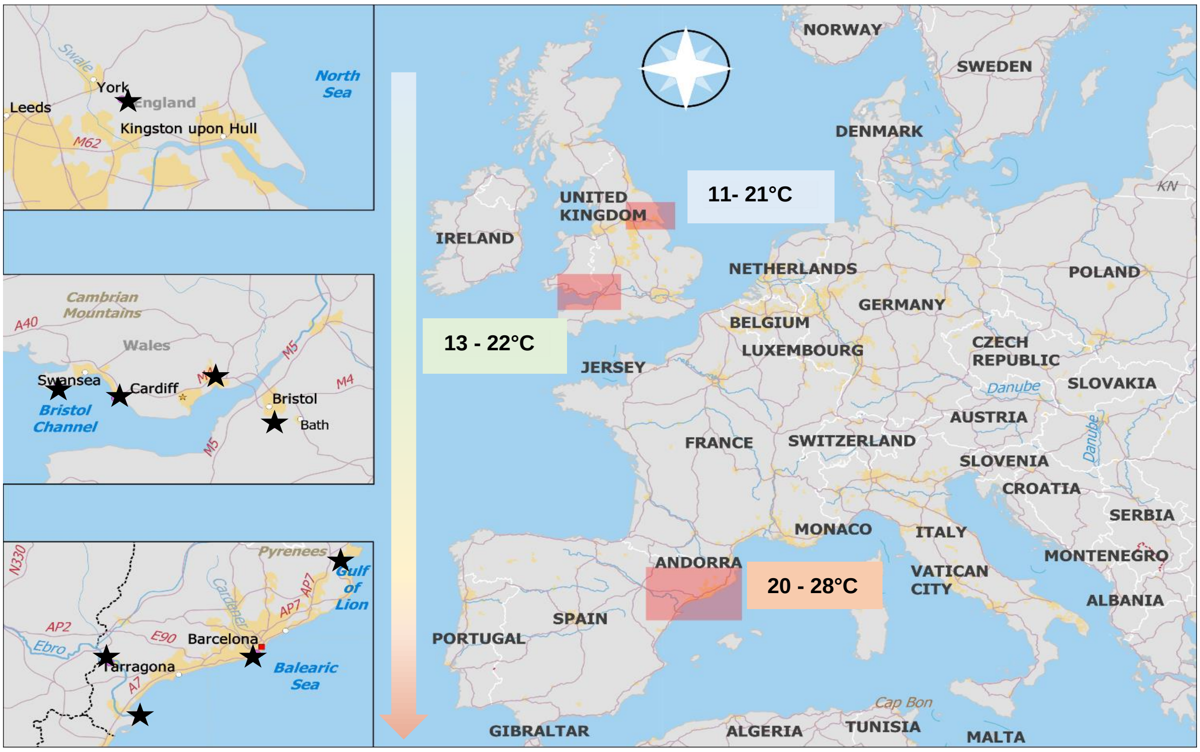


Figure 5.1. Map of the climate transect from northern to southern Europe. Areas highlighted in red indicate the regions covered by our study within the UK and Catalonia, Spain, shown in greater detail on the smaller maps to the left. Black stars indicate the location of all study sites covered in northern England (Wheldrake), South-west England (Chew Valley) and Wales (Kenfig, Oxwich and Magor) and Catalonia (Mas del Matá, Llobregat Delta, Sebes and Canal Vell). Average July minimum and maximum temperatures in degrees Celsius (°C) are shown within each climate zone.

Environmental conditions at invertebrate trapping plots

During invertebrate monitoring, the following was recorded at each sticky trap location: habitat type (reed or scrub), % humidity measured by a humidity probe, % shade estimated from the vegetation cover, approximate depth of any standing water or waterbodies within 10m, and habitat type. In addition, the percentage of surrounding vegetation that was not composed of dominant vegetation – *Phragmites/Glyceria* for the reed traps or the dominant tree species for the scrub traps – was estimated at each trap plot. A higher percentage indicated a greater vegetation heterogeneity and can be viewed as a proxy for within-habitat diversity. Over the seven days the traps were set, the average daily maximum temperature and total precipitation for the site as a whole were recorded using data from weather stations as outlined in previous chapters.

Climate and growing degree day data

Growing degree day calculations (see Chapter 2) for common reed (*Phragmites australis*) and the reed plum aphid (*H. pruni*) ((daily minimum + maximum temperature/2) – T^{base} for *P. australis*/*H. pruni*) and daily rainfall and maximum temperature records from both countries and years were combined. The average daily maximum temperature (in °C) and total precipitation (in mm) was also recorded over the 7 days preceding capture of an individual bird at a given site, or for invertebrate data over the 7-10 days that a sticky trap was set at a given site. These terms are hereafter referred to as “total weekly rainfall” and “maximum temperature”. The described measure of temperature was chosen for the analysis rather than the daily maximum temperature or mean temperature, because the activity of invertebrates available on a given day, and thus the diet of birds, was likely influenced by temperatures experienced during the preceding several days, not only the day on which the sample was taken. In addition, maximum temperatures rather than average daily temperatures are likely to have had greater and longer-lasting effects on flying invertebrate prey, especially if periods of hot weather are prolonged.

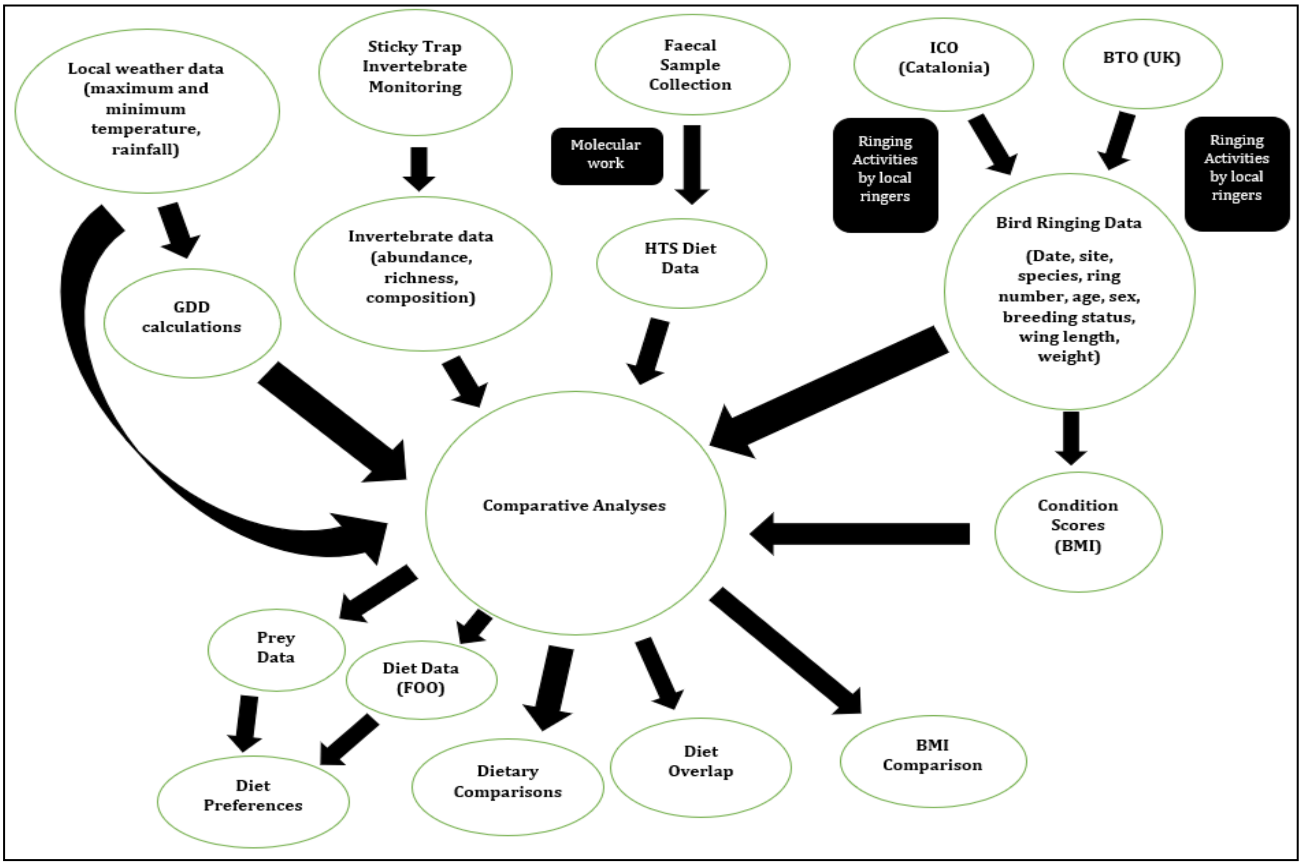


Figure 5.2. Flow diagram showing how data across the climate gradient was collated from various research activities at each study site in the UK and Catalonia.

5.3.3. Data organisation and calculations prior to statistical modelling

Measures of Invertebrate Availability

For each invertebrate monitoring sampling round at each site, the total number of invertebrate specimens recorded from pooled sticky traps was used as a measure of relative abundance.

At several sites in Catalonia, a very high number of ants was attracted to the sticky traps, sometimes covering the entirety of the traps in several layers. Accurate counts of these ants were difficult to achieve and their inclusion greatly inflated the overall prey abundance measures. Since the ants were i) clumped in distribution, rather than uniform across the environment, ii) attracted to the other invertebrates on the traps as a source of food, and iii) only consumed at very low frequency by birds, any instances of >20 ants per trap were removed from the dataset, to allow a fair comparison between sites and countries.

The number of unique prey families on the pooled sticky traps was used as a measure of prey diversity; deemed “prey richness”. Rarefied prey richness was calculated for each sampling round at each site by pooling abundance data from all traps. The function *rarefy* in the *vegan* package was then used to estimate the expected number of species in a subsample of 100 individuals.

Organisation of diet data

The frequency of occurrence (FOO) of prey items in faecal samples was calculated at the species, family and order level, by counting the number of incidences a prey item was detected across i) each warbler species (split by country where applicable), ii) sites, iii) seasons and iv) years (where applicable). In each case this value was expressed as a percentage (% FOO) by dividing FOO by the total number of samples and multiplying by 100. Prey body length was used as an indicator of prey body size, and values were assigned to each prey item detected in the diet following the methodology described in Chapters 2, 3 and 4.

Each diet sample and sticky trap was assigned to its respective maximum temperature i) for the week leading up to the capture of the individual bird (for diet samples) or ii) over the period of time the sticky trap was set for. A range of several of the more common invertebrate prey were ranked according to i) the % frequency of occurrence of the species in the diet of reedbed warblers (reed, Cetti’s, sedge and great reed warbler) and ii) % frequency of occurrence of the prey family (that the species in the diet belonged to) on sticky traps, in both cases in three temperature ranges (<20°C, 21-25°C and 26-35°C). Invertebrate species and/or families were grouped as occurring more often in cool (<20°C) medium (21-25°C) or warm (26-35°C) temperatures, if their frequency of occurrence in warbler diet was elevated at a given temperature range.

Body Condition

The condition of individual birds was scored using a body mass index (BMI) based on measured morphometrics. Negative values indicated birds in below average condition and positive values indicated above average condition. The condition index chosen used the residuals from an ordinary least squares (OLS) regression of body mass against a linear morphometric measure (here wing length), representing relative body size (Jakob et al. 1996, Hayes and Shonkwiler 2001). Retrapped birds were removed from the ringing dataset so that only one data point per individual bird was included in the regression (Table 5.1). This ensured that the index was calculated from independent measurements. Before performing the regression, histograms of wing lengths and body masses for each focal warbler species were checked for a normal distribution, and where appropriate, data were transformed with a log or square root transformation to improve linear regression fit. The regression was performed separately for each warbler species, and residuals (hereafter referred to as "BMI") were calculated and assigned to each individual bird.

Table 5.1. Breakdown of bird species included in the condition index analysis with the country and site origin of captured birds from ringing records. Year indicates the breeding season in which ringing data was obtained from (April – October). The total number of birds captured at each site over the study period is stated and the number of birds that were sampled and the number where dietary information was successfully obtained from the HTS analysis is indicated.

	Site	Year	Country/Region	Total captured	Total sampled for diet study	Total with diet data successfully obtained
Cetti's Warbler	Canal Vell	2018	Catalonia	3	0	0
	Chew Valley	2017	UK, SW England	42	20	14
	Kenfig	2017	UK, S Wales	7	5	4
	Llobregat Delta	2018	Catalonia	52	15	15
	Mas Matà	2018	Catalonia	64	2	2
	Oxwich	2017	UK, S Wales	37	13	12
	Sebes	2018	Catalonia	13	10	8
	Total			218	65	55
Chiffchaff	Chew Valley	2017	UK, SW England	196	9	9
	Kenfig	2017	UK, S Wales	11	7	5
	Oxwich	2017	UK, S Wales	82	5	3
	Wheldrake	2017	UK, N England	118	9	9
	Total			407	30	26
Great Reed Warbler	Canal Vell	2018	Catalonia	52	14	10
	Llobregat Delta	2018	Catalonia	16	5	2
	Mas Matà	2018	Catalonia	50	4	4
	Sebes	2018	Catalonia	17	16	10
	Total			135	39	26

	Site	Year	Country/Region	Total captured	Total sampled for diet study	Total with diet data successfully obtained
Reed Warbler	Canal Vell	2018	Catalonia	739	80	60
	Chew Valley	2017	UK, SW England	848	75	60
	Kenfig	2017	UK. S Wales	70	62	51
	Llobregat Delta	2018	Catalonia	158	24	20
	Mas Matà	2018	Catalonia	821	31	25
	Oxwich	2017, 2018	UK, S Wales	437	83	71
	Sebes	2018	Catalonia	112	42	35
	Wheldrake	2017, 2018	UK, N England	68	20	19
	Total			3253	417	341
Sedge Warbler	Chew	2017	UK, SW England	187	38	30
	Kenfig	2017	UK, S Wales	27	25	19
	Oxwich	2017, 2018	UK, S Wales	313	75	66
	Wheldrake	2017, 2018	UK, N England	70	23	18
	Total			597	161	133
Willow Warbler	Chew	2017	UK, SW England	20	0	0
	Kenfig	2017	UK, S Wales	69	16	13
	Oxwich	2017	UK, S Wales	65	7	7
	Wheldrake	2017	UK, N England	103	9	9
	Total			257	32	29

5.3.4. Statistical analyses

All statistical analyses were performed in R version 3.5.1. and RStudio version 1.1.463 (RStudio Team 2016, R Core Team 2018, Appendix S3). A combination of base R and *ggplot2* (Wickham 2016) were employed to create figures and plots. Retrapped birds, sampled more than once, represented 2.9% of the successful diet samples (18 samples out of a total of 620). These were removed before proceeding with GLM and GAM modelling, to avoid pseudoreplication. For all models we followed the same model refinement and validation methodology and performed appropriate checks on residuals plots and fitted data, as outlined in Chapter 2. Post-hoc Tukey tests from the package *emmeans* (Lenth 2020) measured pairwise differences between different levels of categorical variables.

Invertebrate availability and prey assemblage composition

Generalized linear models (GLMs) were fitted to examine i) the total abundance of invertebrates recorded on sticky traps in relation to site, season, % shade, maximum temperature and the two-way interaction between maximum temperature and country and ii) the total family level richness of sticky trap invertebrates in relation to season, % of non-dominant surrounding vegetation (heterogeneity), the two way interaction between habitat type (reed or scrub) and country, and the two way interaction between maximum temperature and country. In both models a “Gamma” GLM with an “identity” link function was selected. Data from Oxwich in 2018 were omitted because environmental measurements were not recorded at the sticky trap plots.

Canonical correspondence analysis (CCA) was performed using the package *vegan* (Oksanen et al. 2018) to assess how environmental variation related to overall changes in the invertebrate composition of the reedbeds and surrounding scrub. CCA was chosen ahead of the *manyglm* approach (*mvabund*) because only changes to the whole community were of interest (cf. looking at individual prey families as well), allowing CCA’s simpler and more effective results plots to be used. The abiotic variables selected were % non-dominant vegetation, % shade, % humidity, water depth, total rainfall and average daily maximum temperature over the trapping period, and the categorical variables were habitat (reed and scrub) and season (early, middle and late). Prey abundance was log+1 transformed, and collinearity checked using variance inflation factor (VIF) scores. Permutation tests were applied to check for the significance of the CCA model overall, and to obtain the significance of each of the environmental variables on structuring the invertebrate community.

Patterns of diet composition across the gradient

The R package *mvabund* was applied to create a *manyglm* multivariate GLM model, explaining both global differences in overall dietary composition across all of the warblers sampled in the UK and Catalonia, and revealing factors affecting the prevalence of individual prey families in the diets (Wang et al. 2012). The prey matrix (at the family level) and multivariate data for each individual bird were fitted to a binomial *manyglm* model with a “cloglog” link function. The candidate independent variables were warbler species, average daily maximum temperature in the week prior to capture, season, country and the interaction between species and country. To determine the significance of each predictor variable on the diet composition, the *anova* function with a Monte Carlo method was run to resample the data, and univariate tests revealed significant effects of the predictor variables on individual prey families. All relevant explanatory variables were retained if they were significant to the $p<0.05$ threshold.

Significant predictors were visualised in space with NMDS ordination implemented in the *vegan* package using “Jaccard” dissimilarities. The stress statistic was high (>2), indicating that an additional axis may have aided visualisation. To allow ease of interpretability only two axes were used but the interpretation of patterns was carried out with due caution.

Dietary overlap

In Chapters 2 and 4 dietary overlap was measured between i) reed warblers and sedge warblers in the UK, and ii) reed warblers and great reed warblers in Catalonia, at each respective site subset by the three seasons, using *EcoSimR* (Gotelli and Ellison 2013, see previous Chapters for details). Three combinations of sites and seasons were omitted due to insufficient sample sizes (Wheldrake in the early and middle of summer, and Llobregat Delta in the late summer).

Pianka overlap values between reed and sedge warblers in the UK, and between great reed and reed warblers in Catalonia were tested for changes relating to climate and prey availability using a generalized additive model (GAM) implemented in the *mgcv* package (Wood 2011). The beta regression family “betar” was used with a “logit” link function for the mean model (mu) and an “identity” link function for the precision model (phi) (Cribari-Neto and Zeileis 2010). The effects of prey abundance, rarefied prey richness, maximum temperature and rainfall on the Pianka overlap index were tested for significance in the model. These effects were subset by country to test whether the relationship varied between a temperate and a Mediterranean climate. A smoothing function (k=3) was applied to each variable in the model to incorporate non-parametric relationships, where k sets the number of knots (degree of smoothing). Since there were <30 observations, three knots were recommended (Thomas et al. 2017). The model was weighted using the inverse of the 95% Pianka confidence interval widths, so that high weighting was given to values with a narrow confidence window. Model predictions were generated to create plots for each independent variable against the Pianka overlap value.

Prey size comparison

Differences in average prey sizes consumed by reed and Cetti’s warblers across both regions were tested using a Gamma GLM with an “identity” link function. Age, maximum temperature, and the interaction between species and country were included as independent terms in the model. To investigate whether the body size of prey items differed by country, another Gamma GLM with an “identity” link function was applied to pooled unique dietary items detected in the UK and Catalonia. Several data points had undue influence in the Cook’s distance test. These were removed from the analysis after checking the model terms before and after removal to ensure the overall results did not change. Prey order was included as a variable to avoid psuedoreplication.

Examining differences in prey selectivity between the UK and Catalonia

In Chapter 2 and 4, dietary preferences of warblers were investigated using the function *generate_null_net* in the *econullnetr* package (Vaughan et al. 2018). These results were used to compare the dietary preferences of reed and Cetti’s warblers both in the UK and in Catalonia.

Comparing the body mass index (BMI) of warblers as a proxy for condition

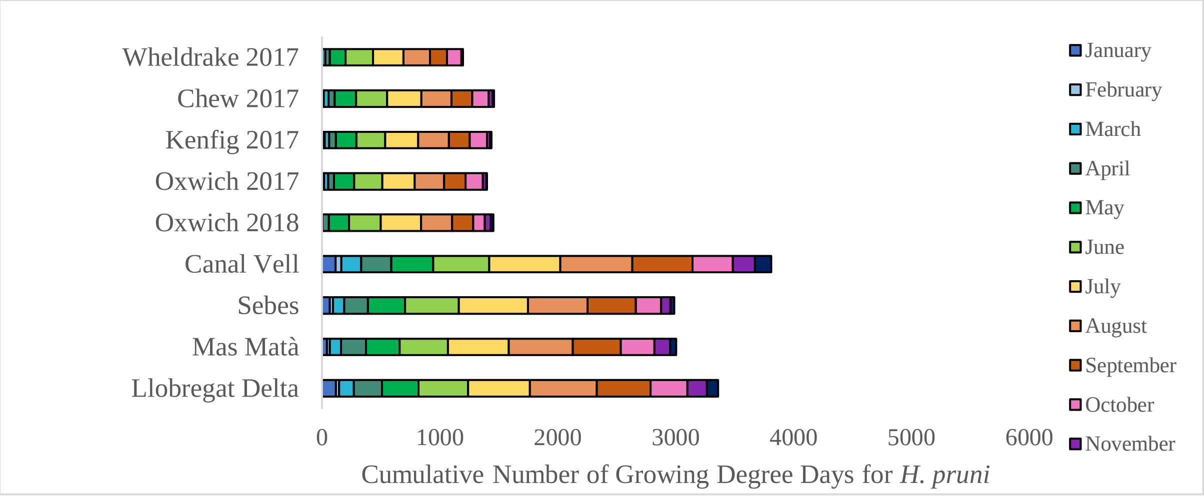
Pearson's correlation was used to test whether dietary richness (number of unique prey items detected in faecal samples) in reed warblers was correlated with the richness of invertebrate families captured on sticky traps, using data from all reed warblers where dietary data were reported. Two GLMs, with a "gaussian" family and "identity" link function were fitted to test the effects of local climate, prey availability and richness, seasonality, sex and age on the body mass index (BMI) as a proxy for body condition in i) all reed warblers captured and ringed/retrapped at all study sites in the UK in 2017 and 2018 and Catalonia in 2018, regardless of whether a sample was collected and ii) as in i) but for all warbler species combined. In the second model the number of great reed warblers captured (as a percentage of the total number of *Acrocephalus* warblers captured in total for each site and season) was included in the model to determine if presence and/or increased density of great reed warblers negatively affects body condition in reed warblers.

5.4. Results

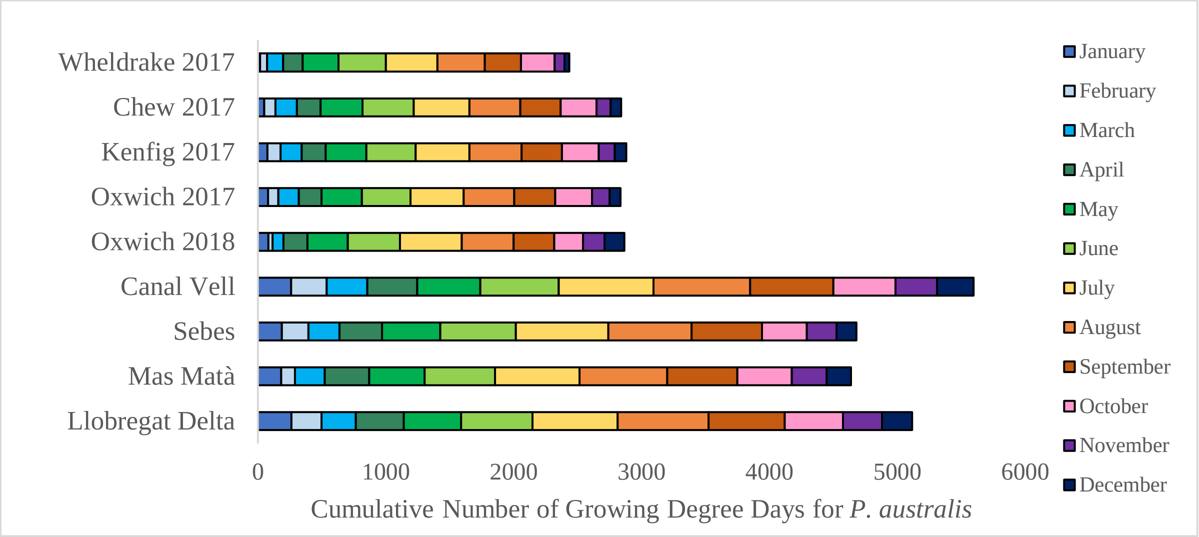
5.4.1. Climate and reed / invertebrate growing degree days (GDD) in the UK and southern Europe.

Sites in Catalonia accumulated substantially more daily growing degree days (GDDs) for *H. pruni* than UK sites (Fig. 5.3A). July was the month in which the largest number of GDDs occurred, indicating a mid-late summer peak in growth. Within the Catalan region, Canal Vell accumulated the most GDDs (Fig. 5.3B). Within the UK sites, a difference in GDD could be seen between i) the north and the south and ii) between 2017 and 2018.

A i)



ii)



B

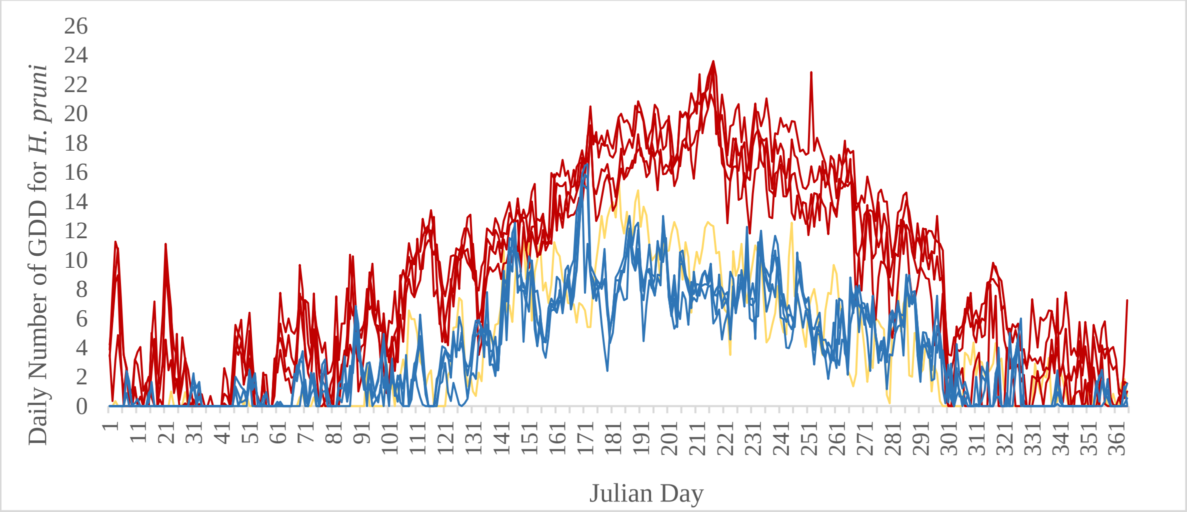
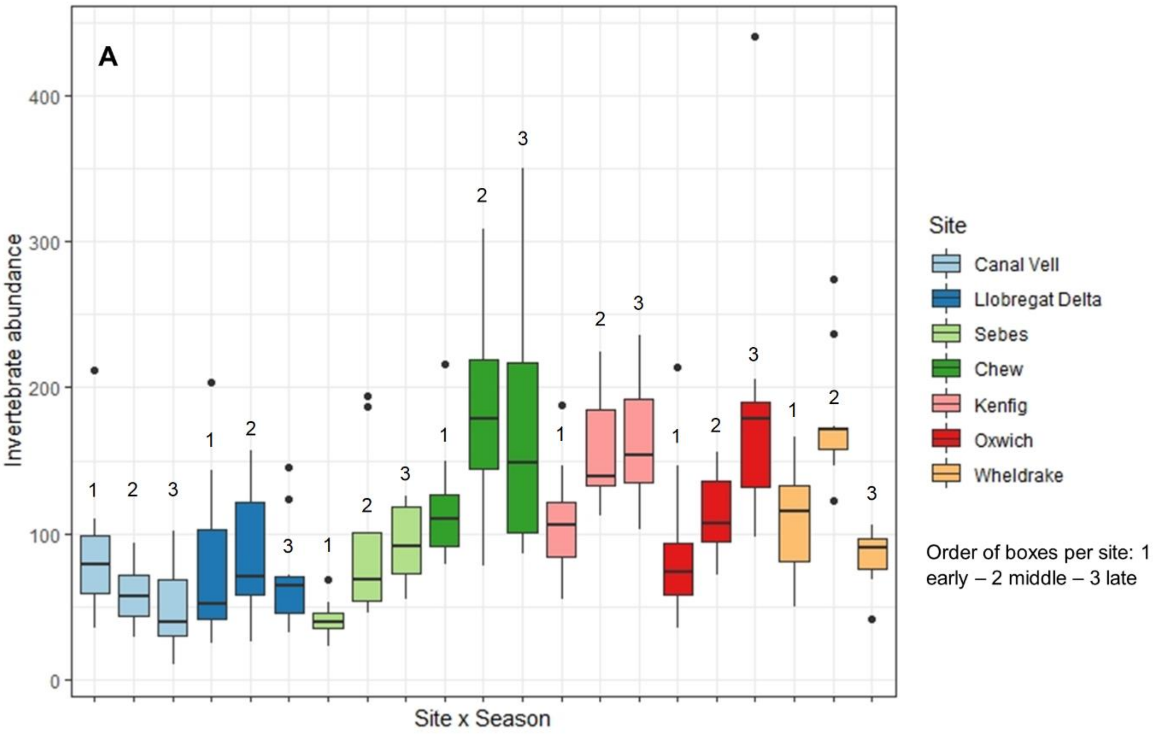


Figure 5.3. Growing degree day calculations across all sites in the UK and Catalonia, Spain in their respective years of study. Plot A) Cumulative growing degree day totals for each calendar month for the sites and years indicated for i) the reed plum aphid (*Hyalopterus pruni*) and ii) the common reed (*Phragmites australis*). Plot B) Daily number of growing degree days . Blue coloured lines = UK sites in 2017, yellow lines = UK sites in 2018 (Oxwich), red lines = Catalan sites in 2018.

5.4.2. Invertebrate abundance and richness

The invertebrate abundance model was significant ($p<0.001$, Adjusted R-squared = 0.36, F = 11.7 on 11 and 198 degrees of freedom). Abundance declined with increasing shade (t = -5.14, $p<0.001$) and traps set in the early summer had lower abundance than those set in the late summer (z = -2.54, $p<0.05$) and middle summer (z = -2.61, $p<0.05$) but middle and late summer traps did not significantly differ (Fig. 5.4). In the UK, the relationship between maximum temperature and abundance was positive (t = 3.52, p<0.001) but in Catalonia it was negative (t = -3.2, p<0.001). Overall, the UK had significantly higher invertebrate abundance than Catalonia (z = -3.5, p<0.001). Canal Vell had significantly lower prey abundance than both Chew (z = -3.68, p<0.01) and Kenfig (z = -3.07, p<0.05). The Llobregat Delta had significantly lower prey abundance than all the UK sites (z-value range = -4.87 to -3.60, p<0.001-p<0.01).

Invertebrate family level richness was explained by season, the interaction between country and trap habitat type (reed or scrub), habitat heterogeneity, and the interaction between average maximum temperature during the time the trap was deployed and country (all p < 0.05; overall model adjusted R-squared = 0.53, F = 30.84 on 8 and 201 df, p<0.001). Traps deployed in mid-summer had higher richness than in the early summer (z = -2.67, p<0.05), and those in the UK were richer than in Catalonia (z = 8.17, p<0.001). Greater vegetation heterogeneity had a positive effect on prey diversity (t = 2.45, p<0.05). Scrub habitats in the UK (z = 2.93, p<0.05) and Catalonia (z = 4.35, p<0.001) were more diverse than reed traps, and both reed (z = 5.31, p<0.001) and scrub (z = 9.55, p<0.001) habitats in the UK were more diverse than the same habitats in Catalonia. In the UK there was a significant positive effect of maximum temperature on invertebrate diversity (t =4.32, p<0.001), but in Catalonia the relationship between temperature and prey diversity was not significant (Fig. 5.5).



Order of boxes per site: 1 early – 2 middle – 3 late

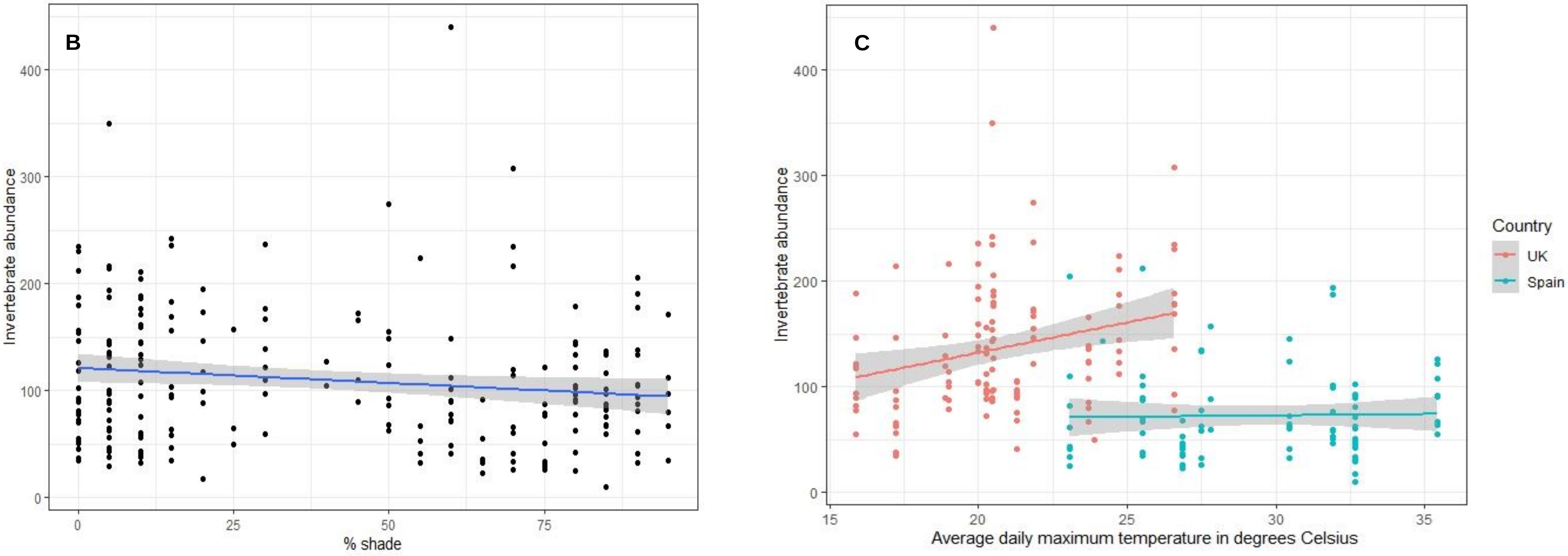


Figure 5.4. Plots from the GLM model investigating biotic and abiotic effects on invertebrate abundance in the UK and Catalonia. The relationship between invertebrate relative abundance (number of individual invertebrates) measured on sticky traps and A) the site location for each sampling round/season (boxes ordered by early / middle / late season), B) percentage of the trap plot that was in shade and C) mean maximum temperature over the period that the trap was set in each country (UK and Catalonia (Spain).

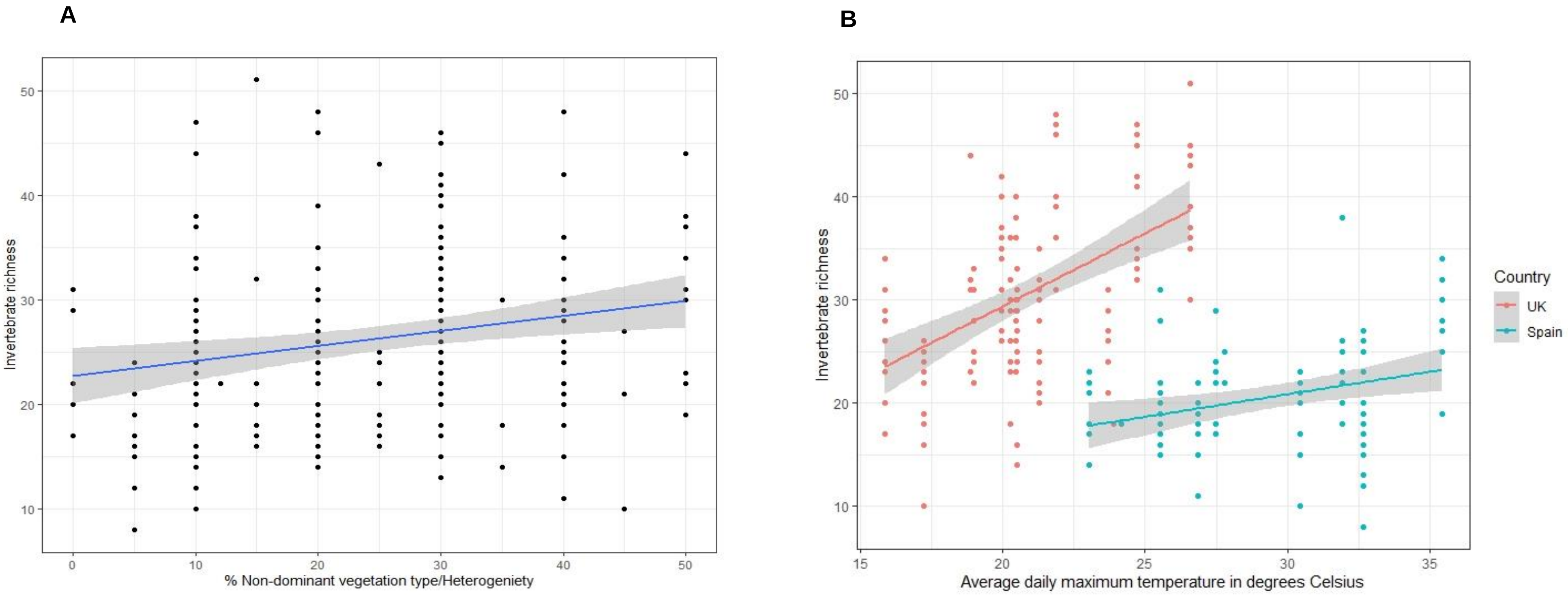


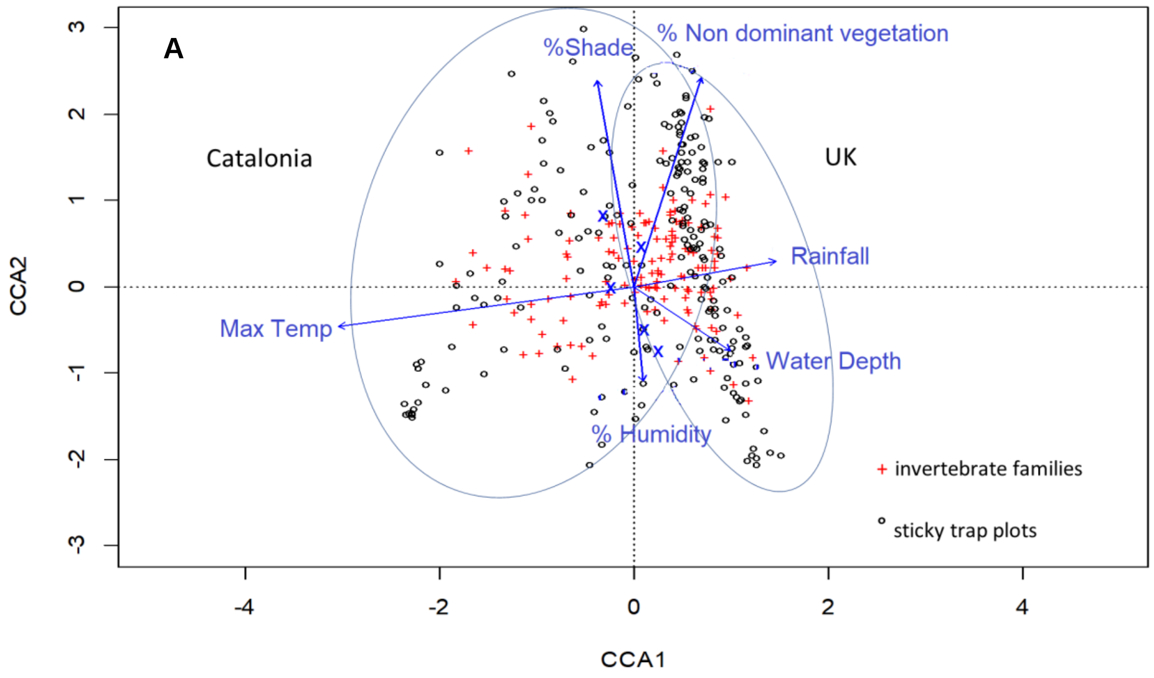
Figure 5.5. Plots from the GLM model investigating biotic and abiotic effects on invertebrate richness in the UK and Catalonia. The relationship between sticky trap invertebrate richness and A) percentage of the trap plot that comprised vegetation that was not the dominant vegetation type (e.g., reed, dominant tree/shrub) and B) mean maximum temperature over the period that the trap was set in each country (UK and Catalonia (Spain)).

5.4.3. Invertebrate community structure across European reedbeds

Permutation tests confirmed that i) our CCA model was significant in explaining patterns of invertebrate composition among sites in both countries (F = 3.9 on 9 and 203 df, $p<0.001$), ii) the environmental variables measured were all significant at the $p<0.001$ threshold, except for humidity and water depth which were both significant at the $p<0.05$ threshold, and iii) CCA axes 1-5 were significant, with axis 1 and 2 significant at the $p<0.001$ level. CCA axis 1 was strongly associated with maximum temperature and rainfall, with invertebrate family composition structured strongly by these two variables (Fig. 5.6).

Families that were recorded in greater numbers in warmer conditions and low rainfall e.g., Acrididae (grasshoppers), Philodromidae (crab spiders) and Formicidae (ants) fell to the left hand side of the plot, and those present most often in cooler conditions with higher rainfall (e.g., Leptoceridae (caddisflies), Empididae (dance flies) fell to the right. The second axis was strongly correlated with habitat variables: scrub, % non-dominant vegetation and shade (increasing towards the top) reedbed habitat, humidity and water depth (increasing towards the bottom). Assemblages of invertebrate families were also highly structured by these characteristics, such as aquatic families (e.g., Haliplidae) towards the bottom and terrestrial (e.g., Panorpidae) towards the top.

The Catalan invertebrate traps were used under hotter, drier conditions than the UK ones, and were thus more dispersed on the CCA plot, indicating greater variation in composition. Season was a significant driver of invertebrate community composition but did not have as strong a structuring effect as the other independent variables on the invertebrate community. However, many invertebrate families were common to all sites and seasons and were present in a wide range of environmental conditions (e.g., Scirtidae (marsh beetles), Limoniidae (craneflies), Muscidae (house flies and allies) and these tended to cluster centrally.



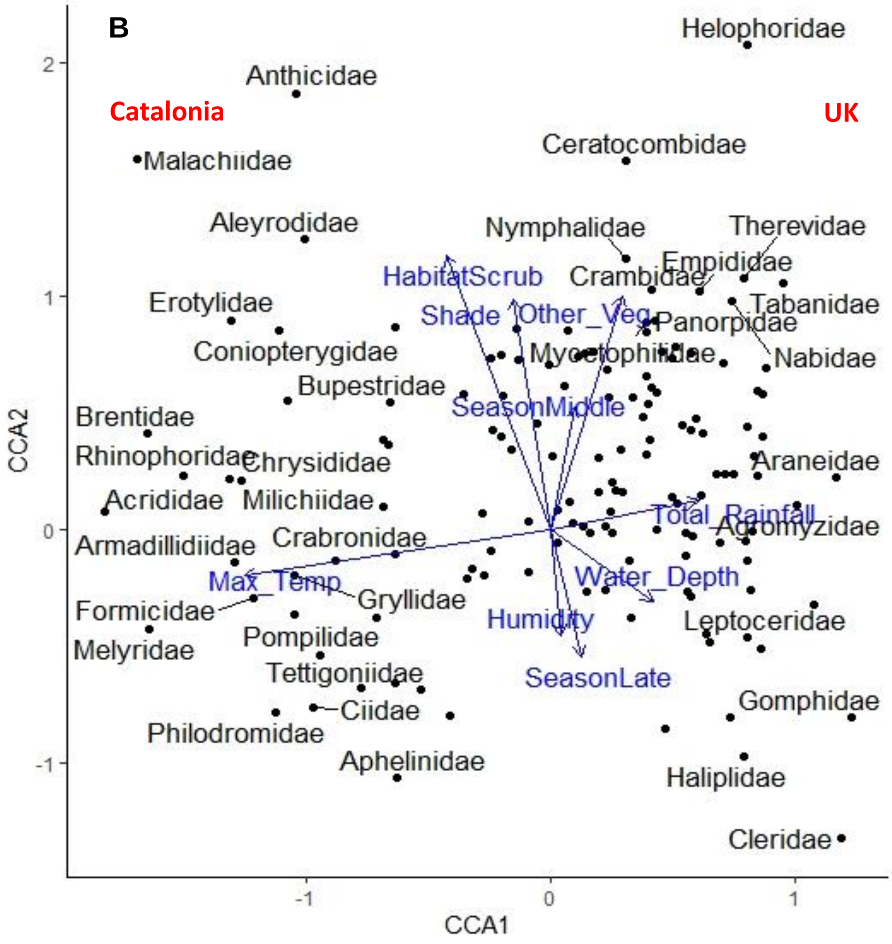
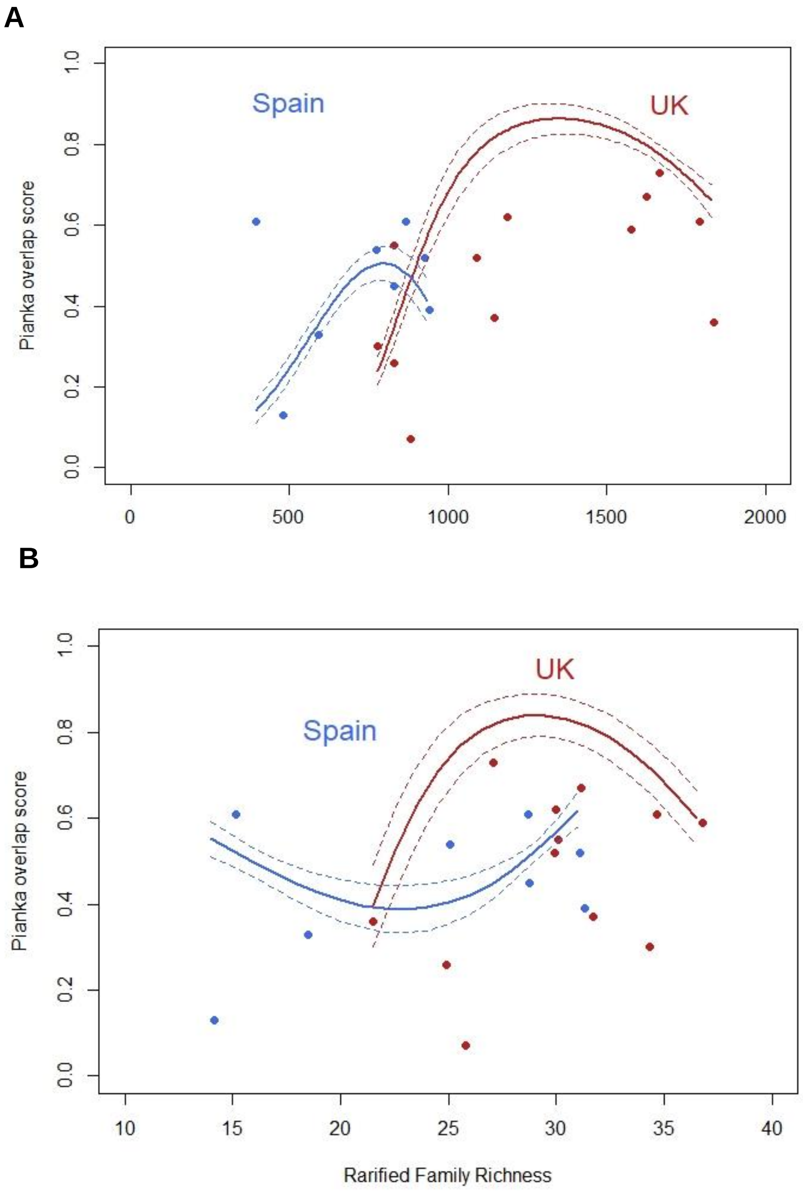


Figure 5.6. Canonical correspondence analysis (CCA) plots for combined UK and Catalonia invertebrate data, showing; A) sticky trap plots, environmental variables and invertebrate families and B) notable families and their association to the environmental variables.

5.4.4. The effects of prey abundance, diversity and climate on dietary overlap in Acrocephalus warblers

All smoothed independent terms were significant in the diet overlap GAM model: thus Pianka's overlap was significantly influenced by the non-linear effects of; prey abundance in Catalonia (chi-squared = 67.63, $p<0.001$) and the UK (chi-squared = 132.81, $p<0.001$), rarefied prey richness in Catalonia (chi-squared = 6.52, $p<0.05$) and the UK (chi-squared = 125.03, $p<0.001$), rainfall in Catalonia (chi-squared = 28.86, $p<0.001$) and the UK (chi-squared = 43.34, $p<0.001$) and maximum temperature in Catalonia (chi-squared = 17.71, $p<0.001$) and the UK (chi-squared = 30.77, $p<0.01$). These variables explained 88.3% of the model deviance (adjusted R-Squared = 0.61, restricted maximum likelihood (REML) = -90.25, scale estimate = 1). Plots of the model predictions suggest that in both the UK and Catalonia there is a general increase in dietary overlap with increasing prey abundance until a threshold is reached, after which overlap begins to show a slight decrease (Fig. 5.7A). The peak of overlap was reached at a lower abundance threshold in Catalonia compared to the UK where maximum overlap values were also generally lower.

In the UK, there was a humped relationship with dietary overlap and rarefied prey family richness, with a general increase in prey richness resulting in an increase in overlap, until a richness of around 30 species per trapping round was reached, after which dietary overlap started to decrease (Fig. 5.7B). In Catalonia, the relationship was inverted, with very low richness indicating high dietary overlap, medium richness seeing a reduction in overlap, followed by a second increase in overlap when richness was at its peak. In the UK increases in maximum temperature corresponded to an increase in dietary overlap, but overlap peaked at 22-25°C, before showing signs of a decline (Fig. Fig. 5.7C). This decline can then be seen in Catalonia where maximum temperatures above 22°C had an almost linear negative relationship with dietary overlap. In the UK rainfall had a non-linear negative relationship with dietary overlap, but in Catalonia the reverse was seen (Fig. 5.7D).



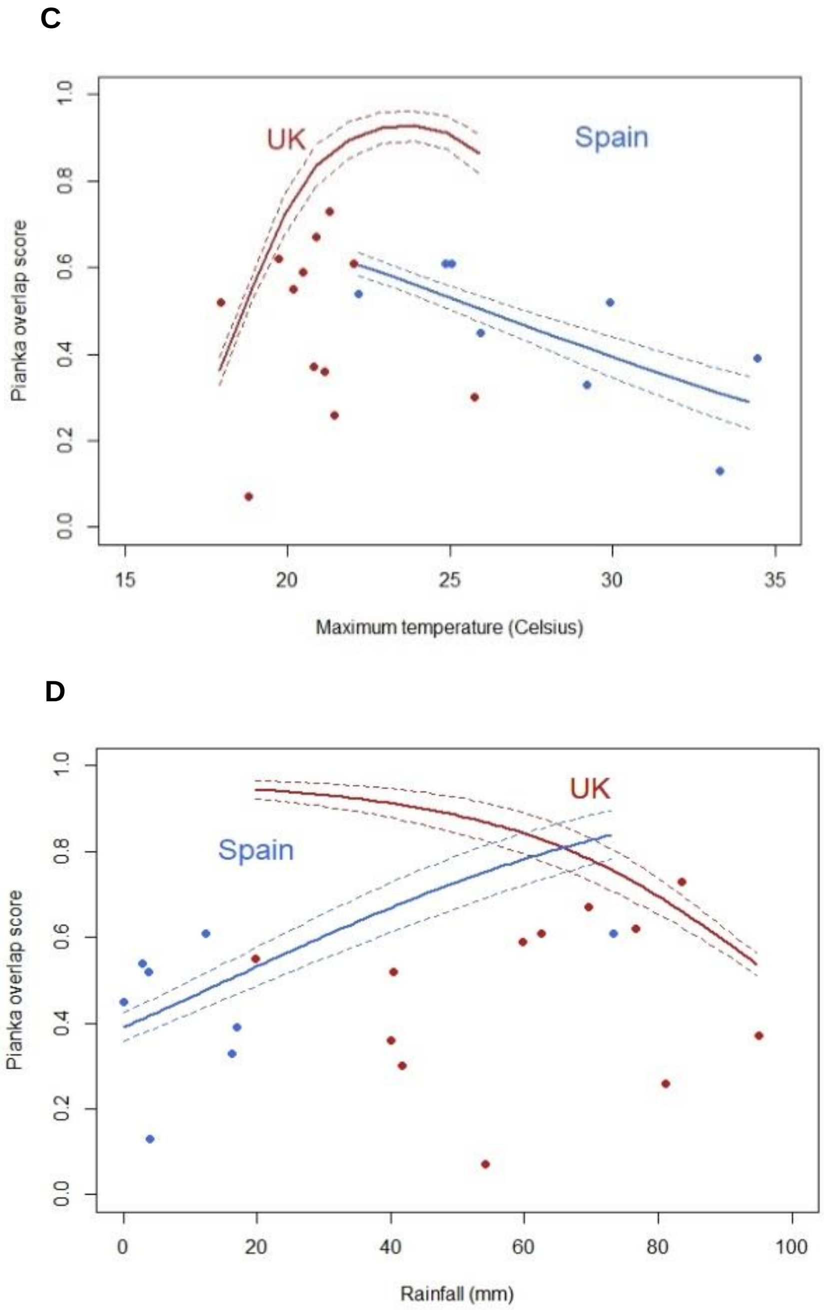
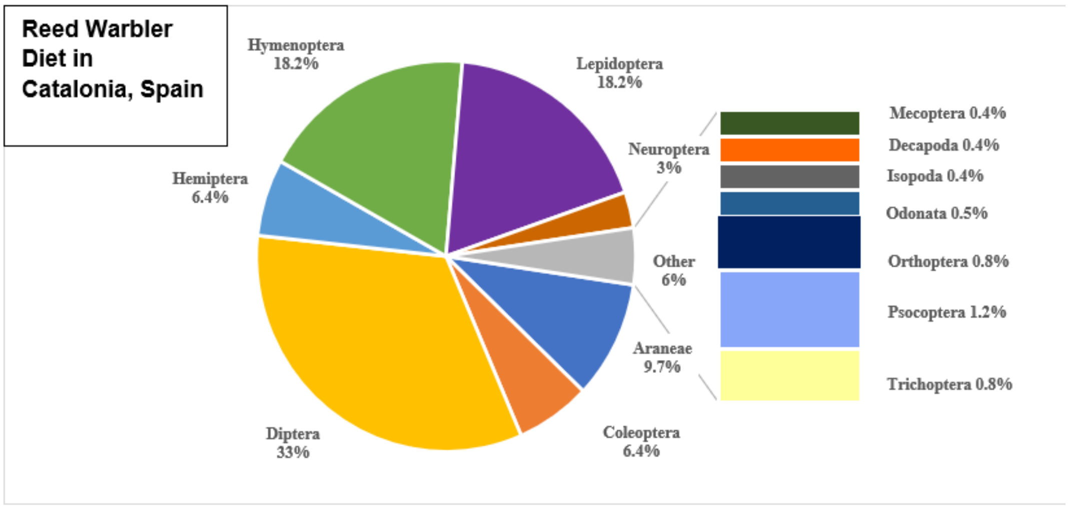


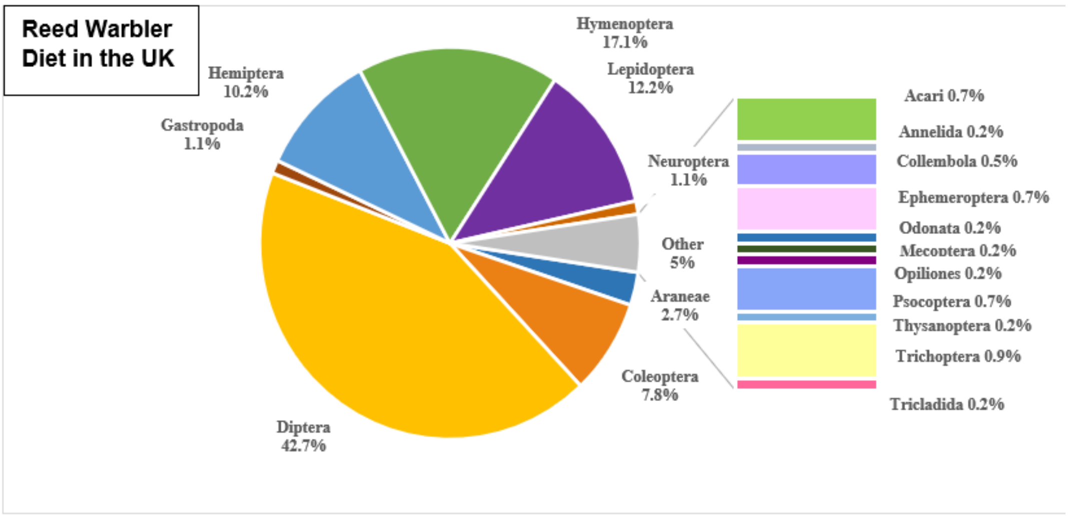
Figure 5.7. Prediction plots of the final GAM model explaining expected Pianka overlap scores between pairs of sympatric warblers across the climate gradient; (*A. scirpaceus* vs. *A. schoenobaenus* in the UK and *A. scirpaceus* vs. *A. arundinaceus* in Catalonia) with changing values of A) prey abundance, B) prey rarefied richness, C) mean maximum temperature and D) total rainfall for both countries in the study. Unbroken lines indicate predicted trends for both the UK (red) and Catalonia (blue), with dashed lines indicating +/-1 standard error.

5.4.5. Dietary richness in reed and Cetti's warblers in the UK and Catalonia, Spain

A total of 1116 prey items (majority at species or genus level – rarely at family level) from ~230 invertebrate families and 28 orders were recorded in the diets of the six warbler species across the gradient (Appendix 4.1). In reed and Cetti's warbler diets, 715 and 311 prey species were recorded in total. Among reed warblers in the UK (n = 206), 550 unique prey species were detected and among reed warblers in Catalonia (n = 137), 235 prey species were detected, whereas among Cetti's warblers in the UK (n=31), 172 prey species were identified and 164 among Cetti's warblers in Catalonia (n = 26). Reed warblers and Cetti's warblers in Catalonia both showed a similar dietary niche breadth (Levin's index = 66.12 and 63.18 respectively), but in the UK the niche breadth of reed warblers was much wider than that of Cetti's warblers living in sympatry (Levin's index = 154.8 and 77.3 respectively).

The main components of the diet (i.e., most prey orders) remained relatively consistent between the UK and Catalonia, but subtle differences were observed. For both species, the contribution of Araneae to the dietary richness was greater in Catalonia relative to the UK (Fig. 5.8). The proportion of richness that was formed by Lepidoptera was also greater for reed warblers in Catalonia but was marginally lower for Cetti's warblers in Catalonia. In reed warblers, Neuroptera formed a greater percentage of the dietary richness in Catalonia compared to the UK, whereas in Cetti's warblers Trichoptera made up a larger percentage of the UK diet.


Reed Warbler Diet in Catalonia, Spain
Hymenoptera 18.2%
Lepidoptera 18.2%
Neuroptera 3%
Other 6%
Araneae 9.7%
Coleoptera 6.4%
Diptera 33%
Hemiptera 6.4%
Mecoptera 0.4%
Decapoda 0.4%
Isopoda 0.4%
Odonata 0.5%
Orthoptera 0.8%
Psocoptera 1.2%
Trichoptera 0.8%


Reed Warbler Diet in the UK
Hymenoptera 17.1%
Lepidoptera 12.2%
Hemiptera 10.2%
Gastropoda 1.1%
Neuroptera 1.1%
Other 5%
Araneae 2.7%
Coleoptera 7.8%
Diptera 42.7%
Acari 0.7%
Annelida 0.2%
Collembola 0.5%
Ephemeroptera 0.7%
Odonata 0.2%
Mecoptera 0.2%
Opiliones 0.2%
Psocoptera 0.7%
Thysanoptera 0.2%
Trichoptera 0.9%
Tricladida 0.2%

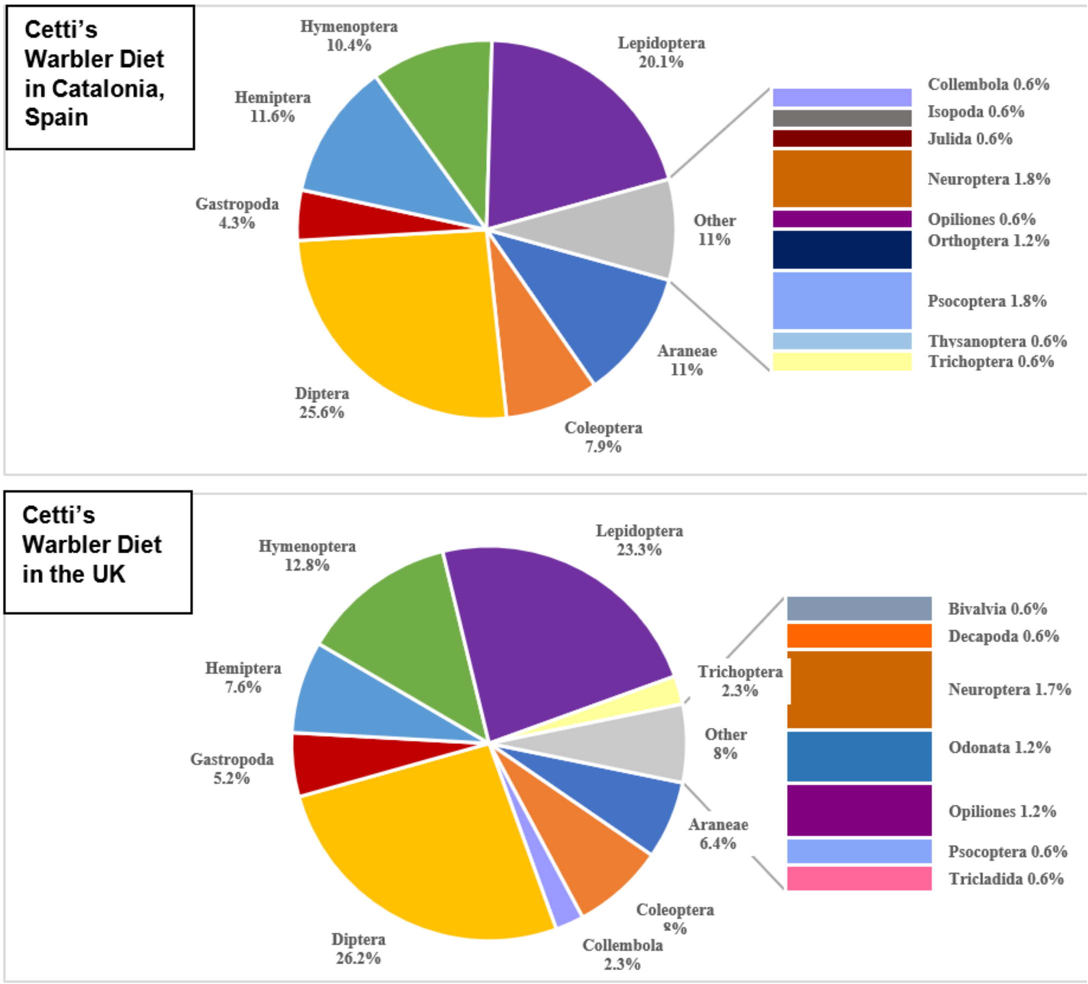


Figure 5.8. Pie charts comparing the percentage of dietary richness formed by each taxonomic order of invertebrates detected in reed warbler and Cetti's warbler diet samples in both the UK and Catalonia. Orders representing a smaller portion of the diet are classed as "other" in the main pie chart and are displayed in full with their respective percentages in the panel to the left of the pie chart.

5.4.6. Multivariate effects of diet composition: comparing the diets of all sampled warbler species in the UK and Catalonia

All tested variables were significant in explaining the variation in diet; country (LRT = 1820.4, p<0.001), season (LRT = 840.4, p<0.001), average daily maximum temperature (LRT = 445.4, p<0.001), warbler species (LRT = 1691.1, p<0.001) and the interaction between country and warbler species (LRT = 193.7, p<0.001). In addition, a suite of prey families in the diet showed compositions that were significantly influenced by the independent variables in the univariate tests (Table 5.2).

There was a high turnover of prey families between Catalonia and the UK (Fig. 5.9C, 5.9E). Diets showed a transition from those containing the largely Catalan families (Salticidae, Tettigoniidae, Halictidae, Membracidae and Simuliidae) to those containing families more prevalent in the UK such as Chaoboridae, Leptoceridae, Rhagionidae, Cicadellidae and Gracillariidae. Some overlap did occur in the consumption of very common prey items in both regions (e.g., Chironomidae), those of high nutritional value to birds (e.g., moth families, Noctuidae, Geometridae and Erebidae) or both (e.g., Clubionidae).

A total of 25 prey families were consumed differentially by different warbler species (Table 5.2), and an additional three families were significantly influenced by the interaction between the effect of warbler species and country of origin (Calliphoridae, Agriolimacidae and Syrphidae: more frequent in Cetti's warblers in Catalonia). Reed warbler diet was very broad, overlapping with all other warbler species (Fig. 5.9D). Diets of the remaining warblers appeared to be on a continuum, from warbler populations sampled in the UK to warblers sampled in Catalonia (from upper right to lower left; chiffchaff, willow warbler, sedge warbler, Cetti's warbler). Great reed warbler diets were most distinct from all the other species, clustering most closely with Eurasian reed warbler. In reed and Cetti's warblers, dietary differences between countries were greater than the differences between the two species as a whole (Fig. 5.9F). The position of the centroids in the NMDS plots suggested that reed and Cetti's warbler diets were more similar in the UK than in Catalonia. Differences in diet between reed and Cetti's warblers were always in the same direction in both countries (i.e., Cetti's warblers always cluster to the left of reed warblers), which suggests that some species-specific components of the diet remained consistent across countries.

Changes in diet also correlated with maximum temperature and stage of the breeding season (Fig. 5.9A, B). Average daily maximum temperature over the preceding week was a good predictor of the frequency of Philodromidae and Nabiidae (more frequent during warmer periods), and Syrphidae (more frequent in the diet during cooler periods) in warbler diets. Aphids and empids featured in the diet significantly more frequently in early summer, Ichneumonidae and Choreutidae in mid-summer, and Halictidae and Hydroptilidae in late summer.

Table 5.2. Results of the univariate anova tests within the multivariate *manyglm* model. Prey families that were significantly (p<0.05) associated with one or more of the candidate independent variables in the final model are shown, in taxonomic order. Likelihood ratio test values (LRT) and p-values are given for each univariate test. Percent frequency of occurrence values (% FOO) for each prey family across the factor levels and/or with increasing/decreasing values of the numeric predictors are indicated and colour coded, with a darker tone indicating a larger % FOO. Codes: CW = Cetti's warbler, CC = chiffchaff, RW = reed warbler, SW = sedge warbler, WW = willow warbler.

Predictor Variable	Prey Order	Prey Family	LRT	p-value	Notes on % FOO	
					UK	Catalonia (Spain)
Country	Opiliones	Phalangiidae	15.30	0.028*	4.91	-
Country	Diptera	Anisopodidae	16.05	0.014*	5.14	-
Country	Diptera	Rhagionidae	17.55	0.006**	5.61	-
Country	Hymenoptera	Pompilidae	17.58	0.003**	-	2.08
Country	Diptera	Pipunculidae	18.24	0.003**	9.58	1.04
Country	Collembola	Entomobryidae	17.64	0.003**	7.94	0.52
Country	Diptera	Psilidae	19.48	0.002**	0.47	6.25
Country	Diptera	Muscidae	20.13	0.002**	19.63	5.73
Country	Diptera	Empididae	18.29	0.002**	6.07	-
Country	Trichoptera	Leptoceridae	31.29	0.001***	10.28	-
Country	Orthoptera	Tettigoniidae	34.66	0.001***	-	7.81
Country	Lepidoptera	Tortricidae	40.54	0.001***	17.99	1.56
Country	Hymenoptera	Halictidae	32.13	0.001***	-	6.77
Country	Hymenoptera	Braconidae	29.06	0.001***	44.39	22.40
Country	Hemiptera	Piesmidae	27.09	0.001***	-	6.25
Country	Hemiptera	Cicadellidae	52.74	0.001***	14.72	0.52
Country	Diptera	Scathophagidae	48.86	0.001***	15.19	-
Country	Diptera	Ptychopteridae	34.43	0.001***	11.21	-
Country	Diptera	Hybotidae	55.74	0.001***	21.26	1.04

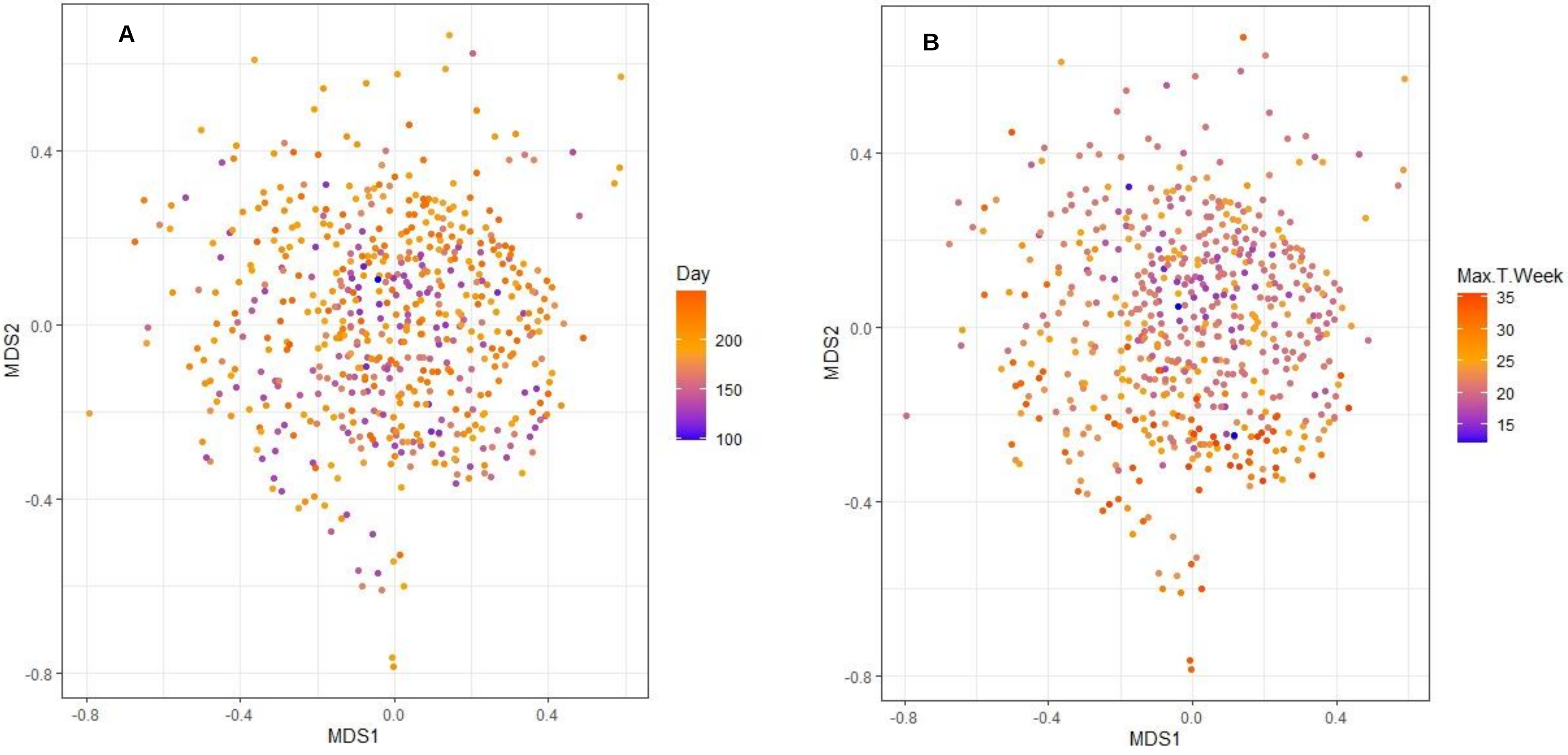
Predictor Variable	Prey Order	Prey Family	LRT	p-value	Notes on % FOO					
Country	Diptera	Dolichopodidae	33.64	0.001***	10.98	0.52				
Country	Diptera	Culicidae	24.91	0.001***	13.08	29.69				
Country	Diptera	Chaoboridae	175.42	0.001***	46.26	-				
Country	Diptera	Ceratopogonidae	22.33	0.001***	10.98	1.04				
Country	Diptera	Anthomyiidae	38.36	0.001***	16.12	1.04				
Country	Collembola	Tomoceridae	20.56	0.001***	6.54	-				
Country	Coleoptera	Ptinidae	24.59	0.001***	-	5.73				
Country	Coleoptera	Chrysomelidae	105.3	0.001***	35.98	1.56				
Country	Araneae	Salticidae	29.61	0.001***	-	6.25				
Country	Araneae	Philodromidae	27.89	0.001***	1.40	13.02				
Country	Araneae	Linyphiidae	23.51	0.001***	16.82	3.65				
					Early	Middle	Late			
Season	Hemiptera	Aphididae	33.54	0.001***	36.42	27.63	13.92			
Season	Diptera	Empididae	18.41	0.017*	8.61	3.27	2.06			
Season	Hymenoptera	Halictidae	18.14	0.018*	0.66	1.09	4.64			
Season	Trichoptera	Hydroptilidae	17.47	0.023*	-	-	3.09			
Season	Lepidoptera	Choreutidae	17.10	0.027*	-	4.00	-			
Season	Hymenoptera	Ichneumonidae	16.14	0.045*	11.92	29.82	24.74			
					12-20°C	20-25°C	25-30°C			
Max T Week	Araneae	Philodromidae	17.05	0.004**	-	4.65	8.72			
Max T Week	Hemiptera	Nabiidae	15.37	0.018*	-	0.78	1.34			
Max T Week	Diptera	Syrphidae	14.04	0.037*	17.86	14.47	14.09			
					CW	CC	GRW	RW	SW	WW
Species	Araneae	Clubionidae	29.08	0.001***	38.60	3.70	26.92	29.48	22.96	3.45
Species	Araneae	Tetragnathidae	30.47	0.001***	19.3	-	-	4.34	2.96	-

Predictor Variable	Prey Order	Prey Family	LRT	p-value	Notes on % FOO					
Species	Coleoptera	Chrysomelidae	32.48	0.001***	26.32	33.33	-	24.28	20.74	72.41
Species	Diptera	Hybotidae	38.29	0.001***	1.75	44.44	-	16.76	8.89	34.48
Species	Diptera	Muscidae	34.47	0.001***	5.26	29.63	-	20.23	8.15	10.34
Species	Diptera	Ptychopteridae	35.91	0.001***	21.05	-	-	7.51	7.41	-
Species	Diptera	Tachinidae	32.85	0.001***	19.30	3.70	7.69	2.31	2.22	6.90
Species	Gastropoda	Helicidae	29.70	0.001***	10.53	-	15.38	-	1.48	-
Species	Hymenoptera	Ichneumonidae	28.40	0.001***	15.79	7.41	50	25.14	20.74	31.03
Species	Lepidoptera	Crambidae	42.86	0.001***	35.09	7.41	3.85	8.09	14.07	-
Species	Lepidoptera	Gelechiidae	29.95	0.001***	5.26	-	-	12.14	2.96	6.90
Species	Lepidoptera	Noctuidae	39.02	0.001***	70.18	14.81	50	27.46	38.52	41.38
Species	Neuroptera	Hemerobiidae	39.78	0.001***	22.81	-	-	2.02	2.96	-
Species	Hemiptera	Miridae	25.88	0.005**	12.28	40.74	-	6.65	7.41	24.14
Species	Lepidoptera	Geometridae	25.82	0.005**	29.82	40.74	-	17.34	17.78	41.38
Species	Collembola	Tomoceridae	24.29	0.006**	12.28	-	-	2.02	10.37	-
Species	Diptera	Anthomyiidae	24.12	0.007**	3.51	33.33	3.85	13.29	6.67	13.79
Species	Diptera	Culicidae	23.21	0.008**	8.77	-	11.54	24.57	11.85	13.79
Species	Gastropoda	Agriolimacidae	23.38	0.008**	21.05	3.70	-	3.47	2.22	-
Species	Hemiptera	Psyllidae	22.58	0.009**	-	18.52	-	1.45	2.22	17.24
Species	Hymenoptera	Braconidae	22.56	0.009**	61.40	22.22	11.54	33.53	43.70	48.28
Species	Coleoptera	Ptinidae	21.68	0.017*	15.79	-	-	0.58	-	-
Species	Diptera	Chloropidae	21.13	0.02*	-	-	-	7.80	6.67	-
Species	Hemiptera	Aphididae	21.23	0.02*	45.61	11.11	15.38	19.65	34.81	34.48
Species	Coleoptera	Carabidae	20.45	0.025*	24.56	11.11	3.85	8.96	4.44	-

CW Spain CW UK CC GRW RW Spain RW UK SW WW

Predictor Variable	Prey Order	Prey Family	LRT	p-value	Notes on % FOO							
Country: Species	Diptera	Calliphoridae	19.79	0.001***	50	-	18.52	3.85	10	11.65	5.93	6.89
Country: Species	Diptera	Syrphidae	23.31	0.001***	57.69	3.22	7.41	15.38	10	15.53	15.56	10.34
Country: Species	Gastropoda	Agriolimacidae	25.15	0.001***	42.31	3.23	3.70	-	-	5.83	2.22	-

Notes: Asterisks denote the significance level of the test result (* p<0.05, **p<0.01, ***p<0.001).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B
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0.4
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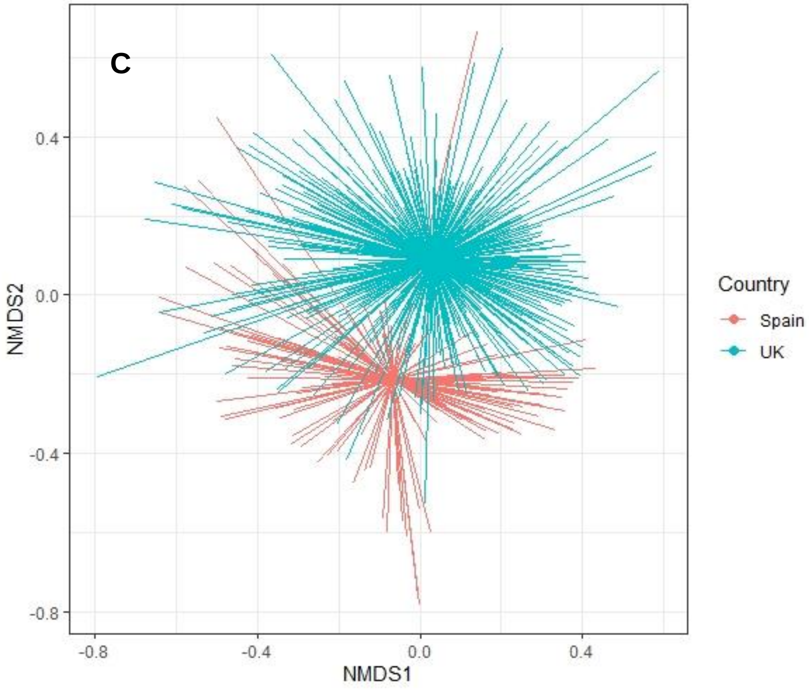
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Country
Spain
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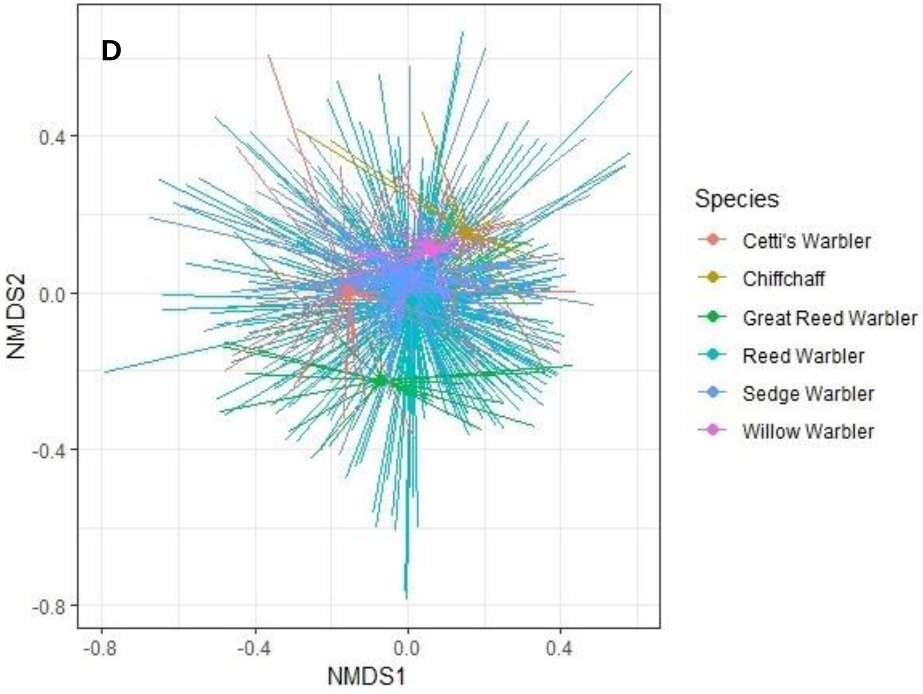
D
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0.0
-0.4
-0.8
NMDS2
-0.8
-0.4
0.0
0.4
NMDS1
Species
Cetti's Warbler
Chiffchaff
Great Reed Warbler
Reed Warbler
Sedge Warbler
Willow Warbler

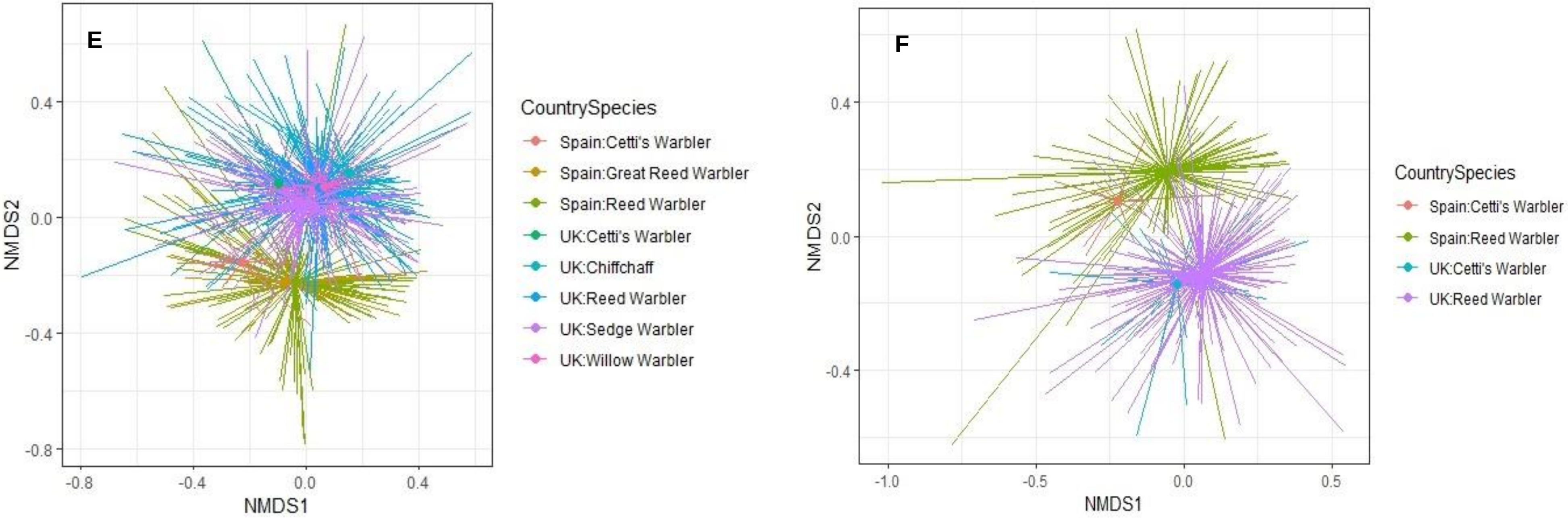


Figure 5.9. Non-metric multidimensional scaling (NMDS) plots of prey detected in warbler diets across the climate gradient according to the significant predictor variables of the *manyglm* model: A) Julian day for both breeding seasons (as a proxy for season), B) Mean maximum temperature of the week prior to capture C) country: UK and Catalonia (Spain), D) warbler species from both countries combined, E) warbler species subset by country and F) warbler species subset by country for reed and Cetti's warbler only.

In Catalonia, the most frequently consumed prey species were different in reed and Cetti's warblers (Table 5.3, see Chapter 4 for a list of top prey species), but in the UK, the top prey species in each of the focal warbler species comprised many of the same groups (Chapter 2). Generally, preferences for prey families were consistent between the UK and Spain, but preferences for some groups were specific to either one country or one warbler species (Table 5.3).

In both of these warbler species the frequency, species richness and strength of preference for spiders in the diet was higher in Catalonia compared to the UK (Table 5.3, see Chapter 2 and 4 for regional breakdowns of frequency of occurrence). Chironomids were the most frequently consumed prey family overall, in all cases >50% of warblers consumed them but Chaoboridae (midges), Leptoceridae (caddisflies) and Limoniidae (craneflies) did not feature at all in the diets of birds in Catalonia. Large aquatic or semi-aquatic taxa were preferred by UK reed warblers in particular; both Dipteran and non-Dipteran groups (see Chapter 2 *econullnetr* analysis), but Spanish reed warblers showed preferences for a smaller set of non-Dipteran aquatic groups (see Chapter 4). In Spain, both bird species disproportionately selected Calliphoridae and Syrphidae but in the UK these were preferred solely by reed warblers. Cetti's warblers appeared to partition Lepidoptera (particularly Noctuidae) more strongly in Catalonia (96% of Cetti's warbler diets) while Catalan reed warblers consumed this group less than in the UK (Table 5.4). Erebidae, Geometridae and Noctuidae were preferred across species and regions but preferences for several families were tied to specific countries or warbler species.

Table 5.3. Percent frequency of occurrence (% FOO) of notable prey families detected in dietary samples from reed (RW) and Cetti's warblers (CW) in the UK and Spain (taken from Chapter 2 and 4 respectively). Families are given in order of overall highest % FOO. Cells are colour coded based on whether they were preferred (orange), avoided (blue) or consumed in proportion to their abundance (no highlight) on sticky traps in the *econullnetr* analysis. The most frequently consumed prey taxa for some prey families are given (highlighted in yellow) along with their % FOO in each country and warbler species subset, with deeper yellow tone with increasing prevalence for that species/country. Families and species not detected in a country and warbler species subset are highlighted in grey.

		% Frequency of Occurrence			
Order	Family	RW UK (n = 206)	CW UK (n = 31)	RW Spain (n = 137)	CW Spain (n = 26)
Diptera	Chironomidae	71.84	51.61	59.8	50
	Cladotanytarsus atridorsum	27.67	22.58	27	19.23
Lepidoptera	Noctuidae	28.16	54.84	27	88.46
	Mythimna straminea	5.34	32.26	0	0
	Axylia putris	0	0	0	46.15
Hymenoptera	Braconidae	41.75	80.65	21.9	38.46
Araneae	Clubionidae	25.73	35.48	35.77	42.31

		% Frequency of Occurrence			
Order	Family	RW UK (n = 206)	CW UK (n = 31)	RW Spain (n = 137)	CW Spain (n = 26)
	Clubiona phragmitis	24.27	35.48	32.85	26.92
Hemiptera	Aphididae	20.87	58.06	18.25	30.77
Lepidoptera	Geometridae	18.93	16.13	15.33	46.15
Diptera	Culicidae	16.50	6.45	37.23	11.54
Diptera	Syrphidae	15.53	3.23	9.51	57.69
	Episyrphus balteatus	7.76	0	2.92	46.15
Hemiptera	Aphrophoridae	8.25	9.68	0	76.92
Lepidoptera	Erebidae	8.74	6.45	10.95	53.85
	Polypogon plumigeralis	0	0	6.56	46.15
Diptera	Calliphoridae	11.65	0	8.76	50
Lepidoptera	Crambidae	7.77	58.06	8.03	7.69
	Chilo phragmitella	1.46	45.16	0	3.85
Coleoptera	Chrysomelidae	39.32	48.39	2.19	0
Coleoptera	Carabidae	9.22	12.90	8.75	38.46
Diptera	Chaoboridae	50.49	38.71	0	0
Araneae	Philodromidae	2.43	0	10.22	42.31
	Philodromus cespitum	2.42	0	9.49	34.62
Diptera	Ptychopteridae	12.62	38.71	0	0
Neuroptera	Hemerobiidae	2.43	35.48	1.46	7.69
Diptera	Scathophagidae	17.48	29.03	0	0
Diptera	Tachinidae	1.94	32.26	2.92	3.85
Trichoptera	Leptoceridae	10.19	19.35	0	0
Diptera	Limoniidae	8.74	12.90	0	0

Table 5.4. The species richness and family richness of each of the main invertebrate orders recorded in the pooled diet samples of both reed (RW) and Cetti's warblers (CW) in the UK, Catalonia, and across both locations. The turnover of species is indicated as a percentage of the total number of species consumed against the number of possible species consumed. Values are highlighted by increasing richness, with deeper tone indicating increasingly large values. Values in brackets indicate the average number of dietary items (species level) per sample ± SD.

	Species Richness							
Order	Catalonia		UK		Total		% Turnover	
	CW (14.6 ± 4.7)	RW (6.0 ± 3.7)	CW (12.9 ± 8.1)	RW (12.2 ± 7.7)	CW (13.6 ± 6.8)	RW (9.8 ± 7.1)	CW (13.6 ± 6.8)	RW (9.8 ± 7.1)
Lepidoptera	**33**	**43**	**40**	**66**	**71**	**106**	**97.26**	**97.25**
Diptera	**42**	**78**	**45**	**235**	**83**	**289**	**95.40**	**92.33**
Araneae	**19**	**23**	**11**	**16**	**27**	**32**	**90**	**82.05**
Hemiptera	**19**	**15**	**13**	**60**	**28**	**67**	**87.50**	**89.33**
Coleoptera	**13**	**15**	**13**	**41**	**25**	**56**	**96.15**	**100**
Hymenoptera	**17**	**43**	**22**	**95**	**37**	**127**	**94.87**	**92.03**

All species	164	236	172	550	320	735	95.24	93.51
Family Richness								
	CW (11.3 ± 3.6)	RW (4.8 ± 2.7)	CW (10.2 ± 5.1)	RW (9.1 ± 4.9)	CW (10.7 ± 4.5)	RW (7.4 ± 4.7)	CW (10.7 ± 4.5)	RW (7.4 ± 4.7)
Araneae	11	9	4	8	12	15	80	88.24
Coleoptera	8	11	6	14	11	20	78.57	80
Diptera	16	24	24	45	30	49	75	71.01
Gastropoda	5	0	5	5	7	5	70	100
Hemiptera	10	7	5	17	11	19	73.33	79.17
Hymenoptera	9	16	5	15	11	23	78.57	74.19
Lepidoptera	12	13	12	17	18	23	75	76.67
All families	82	93	72	146	119	207	77.27	86.61

5.4.7. Temperature dependent patterns of prey availability and frequency in the diet

Clubiona phragmitis (Clubionidae), *Episyrphus balteatus* (Syrphidae) and *Cladotanytarsus atridorsum* (Chironomidae), were consumed by warblers at a high frequency at all three temperature ranges (Table 5.5). On sticky traps, Aphididae, Clubionidae, Calliphoridae, Chironomidae and Syrphidae showed a consistently high abundance, and featured across all the temperature ranges.

Invertebrate families on sticky traps were generally captured at their highest frequency within the same temperature range that species from that same family were most frequently consumed by warblers. However, several mismatches also occurred. The damselfly family Coenagrionidae was most frequently captured on warmer traps (>25°C) but consumed more frequently at lower temperatures. Species level differences within families were apparent, with some being more prevalent in the diet in cooler temperatures and others in warmer ones. In some of the most abundant families, (e.g., Chironomidae) high family-wide availability across all temperature ranges may be masking local peaks of species abundances within a single temperature range.

Table 5.5. Percent frequency of occurrence (% FOO) of i) prey species in the diet of warblers and ii) prey families, recorded on sticky traps at three temperature ranges. % FOO of prey items in the reedbed warblers; Cetti's warbler, reed warbler, great reed warbler and sedge warbler sampled during the temperature ranges <20°C, 20-25°C and 25-35°C are given with the taxonomic order and family of the prey species indicated, and percent frequency of occurrence of prey families recorded on sticky traps in the same temperature ranges are shown alongside. Values are colour coded by a heat-map method with higher values increasingly orange-red. Species and families are also colour coded according to temperature associations as "cool" = light blue, where most instances occur at temperatures <20°C, "medium" = yellow, where most instances occur between 20-25°C and "warm" = red, where most instances occur between 25-35°C. Species and families shaded in green were present in similar frequency across all temperatures.

			% FOO in reedbed warblers						% FOO on sticky traps		
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Aranae	Clubionidae	*Clubiona phragmitis*	17.50	18.26	31.97	Araneae	Clubionidae	Terrestrial	48.57	35.48	27.16
Aranae	Linyphiidae	*Porrhomma pygmaeum*	7.50	1.50	0	Araneae	Linyphiidae	Terrestrial	71.43	53.23	41.98
Aranae	Linyphiidae	*Hypomma bituberculatum*	10	4.79	2.72						
Aranae	Linyphiidae	*Gnathonarium dentatum*	3.75	4.79	2.72						
Aranae	Philodromidae	*Philodromus cespitum*	0	4.49	8.16	Araneae	Philodromidae	Terrestrial	0	8.06	11.11
Aranae	Tetragnathidae	*Tetragnatha pinicola*	0	2.10	0	Araneae	Tetragnathidae	Terrestrial	0	8.06	4.94
Aranae	Tetragnathidae	*Tetragnatha sp.*	0	0.30	4.76						
Aranae	Tetragnathidae	*Pachygnatha clercki*	3.75	1.50	1.36						
Aranae	Theridiidae	*Theridion hemerobium*	0	1.80	2.72						
Coleoptera	Cantharidae	*Crudosilis ruficollis*	1.25	1.80	0.68	Coleoptera	Cantharidae	Terrestrial	45.71	35.48	17.28
Coleoptera	Carabidae	*Paradromius longiceps*	0	1.20	3.40	Coleoptera	Carabidae	Terrestrial	30	37.10	8.64
Coleoptera	Carabidae	*Paradromius linearis*	6.25	4.49	1.36						
Coleoptera	Chrysomelidae	*Psylliodes affinis*	11.25	2.69	0	Coleoptera	Chrysomelidae	Terrestrial	58.57	56.45	16.05
Coleoptera	Chrysomelidae	*Galerucella lineola*	12.50	4.49	0						
Coleoptera	Chrysomelidae	*Galerucella nymphaeae*	2.50	3.89	0						
Coleoptera	Chrysomelidae	*Lochmaea capreae*	1.25	5.39	0						
Coleoptera	Helophoridae	*Helophorus nivalis*	2.50	2.40	0						
Coleoptera	Oedemeridae	*Nacerdes melanura*	0	0	1.36	Coleoptera	Oedemeridae	Terrestrial	1.43	8.06	14.81

			% FOO in reedbed warblers						% FOO on sticky traps	
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Coleoptera	Pentatomidae	*Troilus luridus*	1.25	1.50	0						
Coleoptera	Pentatomidae	*Rhaphigaster nebulosa*	0	0	1.36						
Coleoptera	Ptinidae	*Anobium punctatum*	0	1.50	4.08	Coleoptera	Ptinidae	Terrestrial	0	4.84	3.70
Coleoptera	Scirtidae	*Contacyphon hilaris*	1.25	6.59	0.68	Coleoptera	Scirtidae	Aquatic	42.86	58.06	37.04
Collembola	Tomoceridae	*Tomocerus minor*	6.25	4.19	0.68						
Collembola	Tomoceridae	*Tomocerus vulgaris*	1.25	5.09	2.04						
Decapoda	Cambaridae	*Procambarus clarkii*	0	0	2.72						
Diptera	Anthomyiidae	*Delia florilega*	7.50	1.50	0						
Diptera	Anthomyiidae	*Pegomya solennis*	1.25	2.10	0						
Diptera	Calliphoridae	*Lucilia caesar*	2.50	0.30	0	Diptera	Calliphoridae	Terrestrial	22.86	27.42	13.58
Diptera	Calliphoridae	*Hemipyrellia ligurriens*	0	0	1.36						
Diptera	Chaoboridae	*Chaoborus flavicans*	47.50	38.92	0	Diptera	Chaoboridae	Aquatic	35.71	22.58	9.88
Diptera	Chironomidae	*Procladius rufovittatus*	2.50	0	0	Diptera	Chironomidae	Aquatic	87.14	77.42	77.78
Diptera	Chironomidae	*Procladius sagittalis*	16.25	2.99	4.08						
Diptera	Chironomidae	*Cricotopus sylvestris*	16.25	7.19	8.84						
Diptera	Chironomidae	*Chironomus curabilis*	0	0.30	11.56						
Diptera	Chironomidae	*Chironomus riparius*	1.25	3.89	8.84						
Diptera	Chironomidae	*Chironomus transvaalensis*	0	0	4.76						
Diptera	Chironomidae	*Cricotopus bicinctus*	0	0.60	13.61						
Diptera	Chironomidae	*Orthocladiinae sp.*	0	0	10.20						
Diptera	Chironomidae	*Cladotanytarsus atridorsum*	35.00	22.46	28.57						
Diptera	Culicidae	*Ochlerotatus detritus*	0	5.99	0	Diptera	Culicidae	Aquatic	1.43	6.45	12.35
Diptera	Dolichopodidae	*Chrysotus femoratus*	0	6.29	1.36	Diptera	Dolichopodidae	Aquatic	28.57	40.32	48.15
Diptera	Empididae	*Rhamphomyia crassirostris*	3.75	0	0	Diptera	Empididae	Terrestrial	24.29	25.81	11.11
Diptera	Hybotidae	*Bicellaria vana*	6.25	5.39	0	Diptera	Hybotidae	Terrestrial	48.57	79.03	71.60
Diptera	Limoniidae	*Rhipidia maculata*	6.25	0.30	0	Diptera	Limoniidae	Terrestrial	8.57	3.23	0

			% FOO in reedbed warblers						% FOO on sticky traps		
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Diptera	Muscidae	*Azelia cilipes*	1.25	2.99	0	Diptera	Musc/Fann/Anthomyiidae	Terrestrial	95.71	69.35	45.68
Diptera	Muscidae	*Helina impuncta*	2.50	3.89	0						
Diptera	Muscidae	*Musca domestica*	0	0	4.08						
Diptera	Mycetophilidae	*Exechia similis*	2.50	0	0	Diptera	Mycetophilidae	Terrestrial	0	3.23	0
Diptera	Pediciidae	*Ula sylvatica*	3.75	0.90	0	Diptera	Pediciidae	Terrestrial	8.57	0	0
Diptera	Pipunculidae	*Cephalops varipes*	0	1.50	0	Diptera	Pipunculidae	Terrestrial	1.43	6.45	2.47
Diptera	Pipunculidae	*Chalarus fimbriatus*	3.75	2.40	0.68						
Diptera	Ptychopteridae	*Ptychoptera minuta*	10	2.69	0						
Diptera	Ptychopteridae	*Ptychoptera contaminata*	6.25	8.08	0						
Diptera	Rhinophoridae	*Stevenia deceptoria*	0	0.30	2.72	Diptera	Rhinophoridae	Terrestrial	1.43	3.23	25.93
Diptera	Sarcophagidae	*Sarcophaga carnaria*	6.25	2.10	0	Diptera	Sarcophagidae		20	29.03	35.80
Diptera	Sarcophagidae	*Sarcophaga tibialis*	0	0	2.04						
Diptera	Scathophagidae	*Scathophaga stercoraria*	18.75	6.89	0.68	Diptera	Scathophagidae	Terrestrial	64.29	61.29	16.05
Diptera	Scathophagidae	*Scathophaga furcata*	3.75	2.40	0						
Diptera	Simuliidae	*Simulium erythrocephalum*	0	0.30	2.04						
Diptera	Syrphidae	*Meliscaeva auricollis*	6.25	0.30	1.36	Diptera	Syrphidae	Terrestrial	35.71	29.03	18.52
Diptera	Syrphidae	*Eupeodes luniger*	0	0.60	3.40						
Diptera	Syrphidae	*Episyrphus balteatus*	6.25	10.18	6.80						
Diptera	Tipulidae	*Tipula luna*	3.75	0	0	Diptera	Tipulidae	Terrestrial	21.43	19.35	3.70
Diptera	Tipulidae	*Prionocera subserricornis*	5.00	2.40	0						
Diptera	Tipulidae	*Tipula oleracea*	5.00	1.50	0						
Ephemeroptera	Baetidae	*Procloeon bifidum*	1.25	2.69	0	Ephemeroptera	Baetidae	Aquatic	4.29	4.84	0
Gastropoda	Helicidae	*Theba andalusica*	0	0.60	2.72						
Gastropoda	Succineidae	*Succineidae sp.*	1.25	2.40	0						
Hemiptera	Aphididae	*Sitobion sp.*	16.25	2.69	0	Hemiptera	Aphididae	Terrestrial	54.29	62.90	56.79

			% FOO in reedbed warblers						% FOO on sticky traps		
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Hemiptera	Aphididae	*Amphorophora rubi*	6.25	2.99	0.68						
Hemiptera	Aphididae	*Microlophium carnosum*	8.75	4.19	0.68						
Hemiptera	Aphididae	*Hyalopterus pruni*	5.00	10.48	4.76						
Hemiptera	Aphrophoridae	*Neophilaenus lineatus*	0	4.19	6.80	Hemiptera	Aphrophoridae	Terrestrial	12.86	19.35	22.22
Hemiptera	Cicadellidae	*Empoasca luda*	6.25	3.89	0	Hemiptera	Cicadellidae	Terrestrial	58.57	66.13	64.20
Hemiptera	Coreidae	*Coreus marginatus*	0	1.20	4.08						
Hemiptera	Miridae	*Heterotoma planicornis*	0	0.90	2.72	Hemiptera	Miridae	Terrestrial	27.14	27.42	14.81
Hemiptera	Piesmidae	*Parapiesma quadratum*	0	0	7.48						
Hemiptera	Psyllidae	*Baeopelma foersteri*	1.25	1.50	0	Hemiptera	Psyllidae	Terrestrial	8.57	8.06	9.88
Hemiptera	Rhyparochromidae	*Pachybrachius fracticollis*	2.50	3.89	0.68						
Hymenoptera	Braconidae	*Aphidius rhopalosiphi*	7.50	4.19	1.36	Hymenoptera	Braconidae	Terrestrial	64.29	61.29	37.04
Hymenoptera	Braconidae	*Praon abjectum*	6.25	7.78	6.12						
Hymenoptera	Halticidae	*Lasioglossum malachurum*	0	0	4.08	Hymenoptera	Halictidae	Terrestrial	2.86	1.61	7.41
Hymenoptera	Halticidae	*Lasioglossum politum*	0	0	3.40						
Hymenoptera	Ichneumonidae	*Woldstedtius biguttatus*	1.25	1.20	0	Hymenoptera	Ichneumonidae	Terrestrial	80	56.45	43.21
Hymenoptera	Ichneumonidae	*Diplazon laetatorius*	3.75	3.89	13.61						
Hymenoptera	Pompilidae	*Anoplius caviventris*	0	0	1.36	Hymenoptera	Pompilidae	Terrestrial	0	0	23.46
Hymenoptera	Tenthredinidae	*Birka cinereipes*	1.25	0.90	0	Hymenoptera	Tenthredinidae	Terrestrial	45.71	14.52	0
Hymenoptera	Tenthredinidae	*Empria pallimacula*	0	0.90	0.68						
Isopoda	Armadillidiidae	*Armadillidium vulgare*	0	0.30	2.04	Isopoda	Armadillidiidae	Terrestrial	0	3.23	8.64
Lepidoptera	Crambidae	*Chilo phragmitella*	11.25	4.19	2.04	Lepidoptera	Crambidae	Terrestrial	2.86	0	1.23
Lepidoptera	Crambidae	*Cataclysta lemnata*	2.50	5.09	0.68						
Lepidoptera	Crambidae	*Palpita vitrealis*	0	0	2.04						
Lepidoptera	Erebidae	*Thumatha senex*	1.25	3.89	1.36	Lepidoptera	Erebidae	Terrestrial	0	6.45	0
Lepidoptera	Erebidae	*Polypogon plumigeralis*	0	1.80	10.20						
Lepidoptera	Gelechiididae	*Brachmia blandella*	1.25	3.89	0						

			% FOO in reedbed warblers						% FOO on sticky traps		
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Lepidoptera	Gelechiididae	*Scrobipalpa costella*	1.25	2.10	0						
Lepidoptera	Geometridae	*Operophtera brumata*	16.25	3.29	0	Lepidoptera	Geometridae	Terrestrial	2.86	0	0
Lepidoptera	Geometridae	*Peribatodes rhomboidaria*	0	1.80	2.04						
Lepidoptera	Geometridae	*Phaiogramma faustinata*	0	0	3.40						
Lepidoptera	Lasiocampidae	*Euthrix potatoria*	0	1.80	0.68						
Lepidoptera	Noctuidae	*Conistra ligula*	5.00	0.30	0	Lepidoptera	Noctuidae	Terrestrial	2.86	0	0
Lepidoptera	Noctuidae	*Agrochola lota*	8.75	3.89	0						
Lepidoptera	Noctuidae	*Lenisa geminipuncta*	8.75	5.69	0.68						
Lepidoptera	Noctuidae	*Ochropleura plecta*	5.00	1.80	0						
Lepidoptera	Noctuidae	*Phlogophora meticulosa*	7.50	3.59	2.72						
Lepidoptera	Noctuidae	*Mythimna straminea*	8.75	9.58	0.68						
Lepidoptera	Noctuidae	*Chilodes maritima*	1.25	1.50	7.48						
Lepidoptera	Noctuidae	*Leucania obsoleta*	0	2.10	8.16						
Lepidoptera	Noctuidae	*Sesamia nonagrioides*	0	0.30	4.08						
Lepidoptera	Noctuidae	*Simyra nervosa*	0	0	6.12						
Lepidoptera	Pyralidae	*Endotricha flammealis*	0	0	3.40						
Lepidoptera	Tortricidae	*Epinotia sp.*	6.25	0.30	0						
Lepidoptera	Tortricidae	*Gypsonoma dealbana*	3.75	0	0						
Mecoptera	Panorpidae	*Panorpa communis*	2.50	0	0	Mecoptera	Panorpidae	Terrestrial	27.14	24.19	2.47
Neuroptera	Chrysopidae	*Cunctochrysa albolineata*	0	3.59	0	Neuroptera	Chrysopidae	Terrestrial	0	3.23	6.17
Neuroptera	Hemerobiidae	*Hemerobius humulinus*	11.25	2.40	0						
Odonata	Coenagrionidae	*Enallagma cyathigerum*	6.25	3.89	0	Odonata	Coenagrionidae	Aquatic	12.86	24.19	29.63
Odonata	Libellulidae	*Orthetrum albistylum*	0	0	2.04						
Odonata	Libellulidae	*Sympetrum fonscolombii*	0	0	2.04						
Opiliones	Phalangiidae	*Paroligolophus agrestis*	5.00	4.49	0						
Orthoptera	Acrididae	*Anacridium aegyptium*	0	0	2.72	Orthoptera	Acrididae	Terrestrial	0	0	2.47

			% FOO in reedbed warblers						% FOO on sticky traps		
Order	Family	Species	<20°C	20-25°C	25-35°C		Family	Habitat	<20°C	20-25°C	25-35°C
Orthoptera	Tettigoniidae	*Ruspolia nitidula*	0	0.30	9.52	Orthoptera	Tettigoniidae	Terrestrial	0	0	3.70
Psocoptera	Stenopsocidae	*Lachesilla pedicularia*	0	0	3.40						
Trichoptera	Beraeidae	*Beraea pullata*	1.25	0.90	0						
Trichoptera	Leptoceridae	*Triaenodes bicolor*	3.75	3.59	0	Trichoptera	Leptoceridae	Aquatic	15.71	9.68	1.23
Trichoptera	Leptoceridae	*Oecetis ochracea*	5.00	6.29	0						

5.4.8. Prey size comparison: reed and Cetti's warblers in the UK and Catalonia

The average prey size consumed for nearly all orders was larger in Catalonia than it was in the UK (Table 5.6i). This coincides with the larger prey items detected in the diets of warblers in Catalonia, compared to the UK (Table 5.6ii, Catalan average = 8.55, range = 1-56mm, UK average = 6.44, range = 0.5mm-50mm). Overall, there were no significant differences between the average size of prey items in diet samples between the countries in either warbler species, but there was an effect of maximum temperature, with the average size of prey in a diet sample being higher on warmer days (t = 2.39, $p<0.05$). Reed warblers also consumed significantly smaller prey items on average than Cetti's warblers, regardless of the effect of country. The strongest differences in sizes consumed were between Cetti's warblers and reed warblers in Catalonia (z = 1.83, $p<0.01$) followed by Cetti's warblers and reed warblers in the UK (z = 1.46, $p<0.01$), but UK Cetti's warblers also had significantly larger prey items in their diet than reed warblers in Catalonia (z = 3.31, $p<0.01$). In addition, juvenile birds consumed smaller species on average than adult birds (t = -2.80, $p<0.01$). The independent variables of age, maximum-temperature of the week of capture, and the interaction between species and country were retained in the model (Adjusted R-squared = 0.09, F = 8.65 on 5 and 375 df, $p<0.001$). Prey size was significantly influenced by country (F = 14.12, $p<0.001$) and order (F = 58.79, $p<0.001$) in our GLM investigating prey size across pooled samples (Adjusted R-squared = 0.44, F = 44.14 on 22 and 1207 df, $p<0.001$). When all samples from all species were pooled, prey species in the diet that were detected in the Spanish birds were often larger in size than those present in the diets of UK birds (z = 3.41, $p<0.001$).

Table 5.6. i) Mean body lengths (± SD) of prey species detected in the diets of reed and Cetti's warblers in the UK and Catalonia, Spain , arranged by taxonomic order and ii) percentages of dietary items detected in all warbler diets arranged by prey body size categories.

i)

	Mean body length (mm) ± SD						
Cetti's Warbler	All	Araneae	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera
UK 2017	7.9 ± 2.3	5.2 ± 1.9	3.9 ± 1.3	5.9 ± 1.4	2.9 ± 1.2	3.4 ± 1.2	13.8 ± 2.4
Catalonia 2018	9.0 ± 2.3	5.4 ± 1.6	4.2 ± 1.1	6.9 ± 2.1	10.2 ± 3.5	4.8 ± 2.7	15.4 ± 2.7
Reed Warble							
UK 2017	6.5 ± 1.5	5.2 ± 2.21	4.4 ± 1.3	5.8 ± 1.3	4.4 ± 2.38	4.8 ± 1.9	13.1 ± 4.5
Catalonia 2018	7.4 ± 3.3	6.4 ± 1.7	4.0 ± 1.1	6.8 ± 0.1	8.8 ± 6.8	4.9 ± 2.8	12.2 ± 4.6

ii)

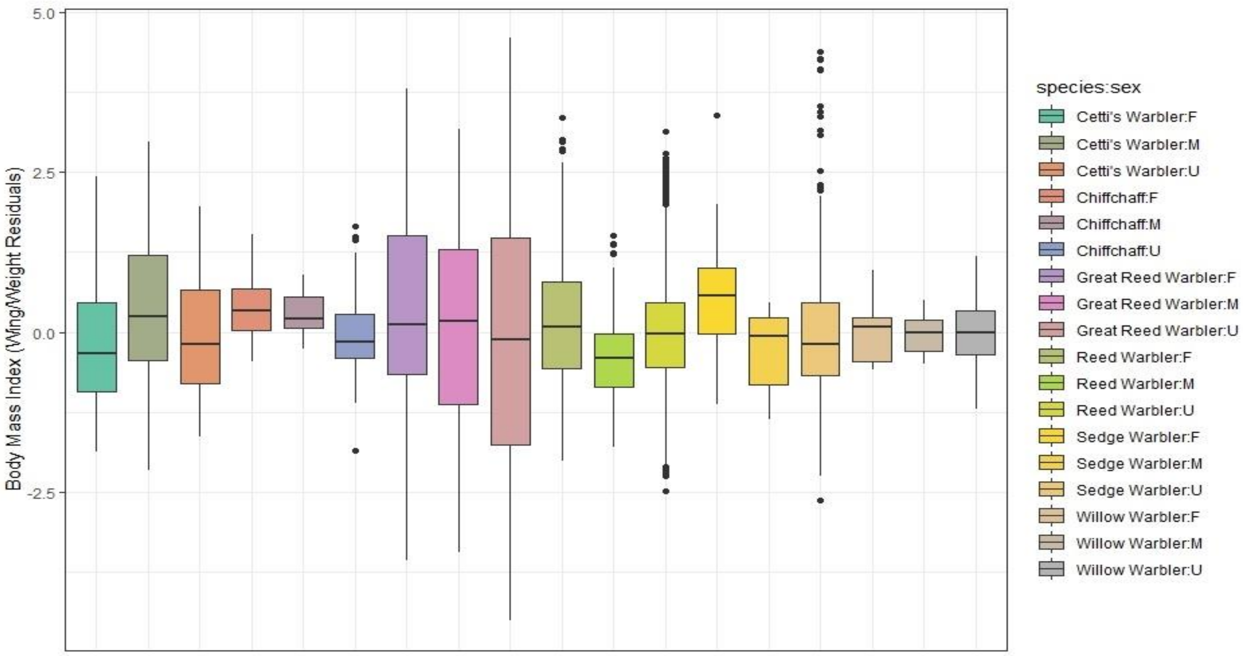
	% of total detected prey items	
Size Category	UK	Catalonia
>40mm	0.11%	1.97%
>30mm	0.22%	2.82%
>20mm	2.03%	5.65%
>10mm	12.54%	23.73%
>5mm	45.42%	55.65%
>2mm	89.15%	90.11%
2-5mm	51.52%	40.40%
<2mm	3.05%	4.24%

5.4.9. The effects of life history, climate and prey availability on body condition in warblers

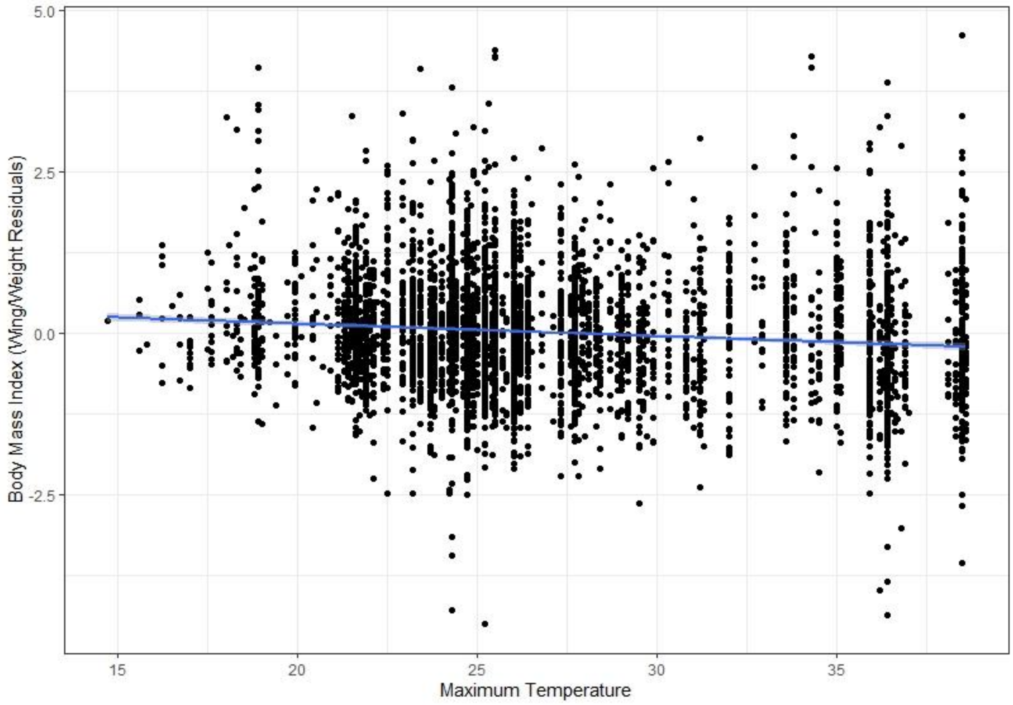
The final model investigating the effects on warbler BMI included the variables species, sex, age, maximum temperature, the interaction between rainfall and country, site, Julian day and the interaction between prey abundance, country and season (adjusted R-squared = 0.060, F = 11.05 on 25 and 3902 df, $p<0.001$). Increasing maximum temperature was associated with a general decrease in BMI ($t = -3.05$, $p<0.01$), while Julian day was positively associated with BMI, suggesting that the condition of birds improves over time ($t = 3.30$, $p<0.001$, Fig. 5.10). In Catalonia, total rainfall during the week of capture did not significantly change body condition, but in the UK, there was a negative effect of rainfall on body condition ($t = -3.1$, $p<0.01$). In the UK, prey abundance had a positive effect on body condition in early-summer ($t = 3.44$, $p<0.001$) and mid-summer ($t=2.65$, $p<0.01$).

Birds captured in Catalonia in the early summer had lower body condition than birds captured in the UK in early summer ($z = -3.48$, $p<0.01$). Birds captured in the early summer in the UK had higher body condition than those in the late ($z = 3.27$, $p<0.05$) and middle summer ($z = 2.91$, $p<0.05$). Breeding females were in better condition than breeding males ($z = 4.73$, $p<0.001$), as were unsexed birds ($z = 4.9$, $p<0.001$), but there was no difference between breeding females and unsexed birds. Adults were in better condition than juveniles ($z = 7.99$, $p<0.001$). Great reed warblers were also in better condition than the remaining warbler species (z-value range = 5.35 – 6.4, $p<0.001$). Birds at both Kenfig ($z = 3.08$, $p<0.05$) and the Llobregat delta ($z = 3.33$, $p<0.05$) were in better condition than birds at Sebes.

A


Body Mass Index (Wing/Weight Residuals)
5.0
2.5
0.0
-2.5
species:sex
Cetti's Warbler:F
Cetti's Warbler:M
Cetti's Warbler:U
Chiffchaff:F
Chiffchaff:M
Chiffchaff:U
Great Reed Warbler:F
Great Reed Warbler:M
Great Reed Warbler:U
Reed Warbler:F
Reed Warbler:M
Reed Warbler:U
Sedge Warbler:F
Sedge Warbler:M
Sedge Warbler:U
Willow Warbler:F
Willow Warbler:M
Willow Warbler:U

B


Body Mass Index (Wing/Weight Residuals)
5.0
2.5
0.0
-2.5
15
20
25
30
35
Maximum Temperature

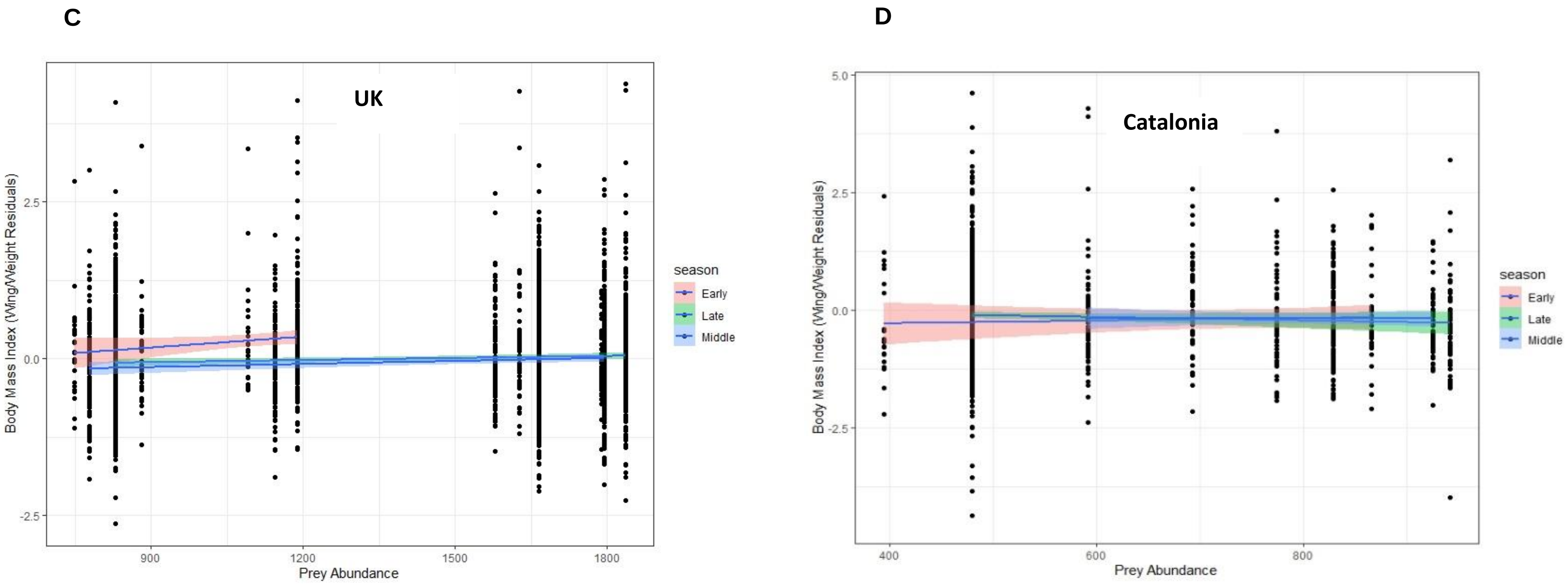


Figure 5.10. Box and scatterplots showing results of the GLM investigating biotic and abiotic effects on the body condition of warblers across the climate gradient. The relationship between A) species identity (subset by sex; F = female, M = male, U = unsexed), B) maximum temperature, C) prey abundance on sticky traps in the UK subset by sampling round (early, middle or late) and D) prey abundance on sticky traps in Catalonia subset by sampling round, on the body mass index (BMI) - a measure of body condition, for all birds captured and ringed over the study period based on wing/weight residuals calculated for each species independently. Lines on scatter plots indicate linear regression lines.

Where the diet had been ascertained in reed warblers by metabarcoding, there was a significant positive correlation between the number of unique dietary items consumed per individual bird (dietary richness) and the richness of prey families on sticky traps (Pearson's r = 0.21, t = 3.60, df = 281, p<0.001). Reed warbler individuals captured during periods where the richness of invertebrates had increased, consumed a greater number of different prey species/genera, than birds captured when invertebrate richness was lower (Fig. 5.11).

Our model on reed warbler BMI, was highly significant (adjusted R-squared = 0.072, F = 12.7 on 16 and 2411 df, p<0.001). The interaction between rainfall and country (F = 6.15, p<0.01), between Julian day and site (F=8.15, p<0.001), and the categorical variables; sex (F=10.26, p<0.001) and age (F=16.51, p<0.001) were all significant, as were the included continuous variables (Fig. 5.12). Trap invertebrate family level richness had a significant positive effect on warbler BMI (t = 4.05, p<0.001) whereas maximum temperature (t = -3.52, p<0.001) and the percentage of great reed warblers captured with respect to all captured *Acrocephalus* warblers (t = -4.08, p<0.001) had a negative effect on BMI. In the UK, increased rainfall led to a decrease in BMI (t = 3.34, p<0.001). At all sites BMI increased over the breeding season with Julian day. Females had significantly higher BMI than males (z = 4.37, p<0.001) but not unsexed individuals, and males had a significantly lower BMI than unsexed individuals (z = -4.14, p<0.001). Adults were in better body condition than juveniles (z = 5.68, p<0.001). Spanish birds were in better condition than birds in the UK (z = 2.42, p<0.05), but this can be largely attributed to site differences due to large differences in BMI between the Llobregat Delta and Sebes (z = 2.96, p<0.05) and Canal Vell (z = 3.47, p<0.01) in Catalonia and the Llobregat Delta and Chew Valley (z = 4.35, p<0.001), Oxwich (z = 4.63, p<0.001) and Wheldrake (z = 3.83, p<0.01) in the UK.

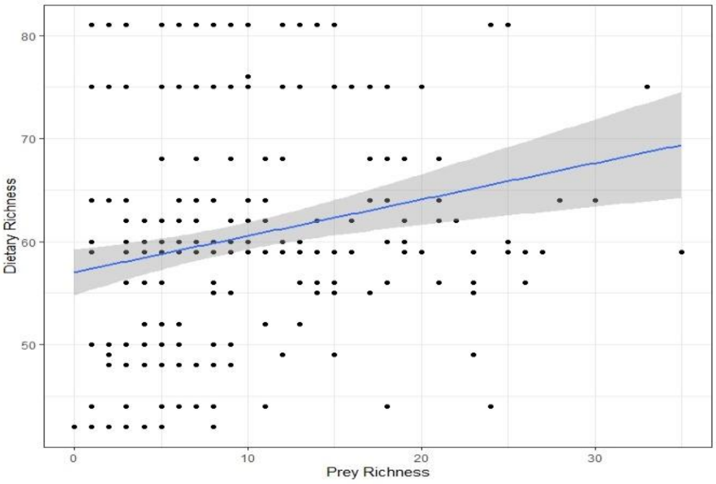
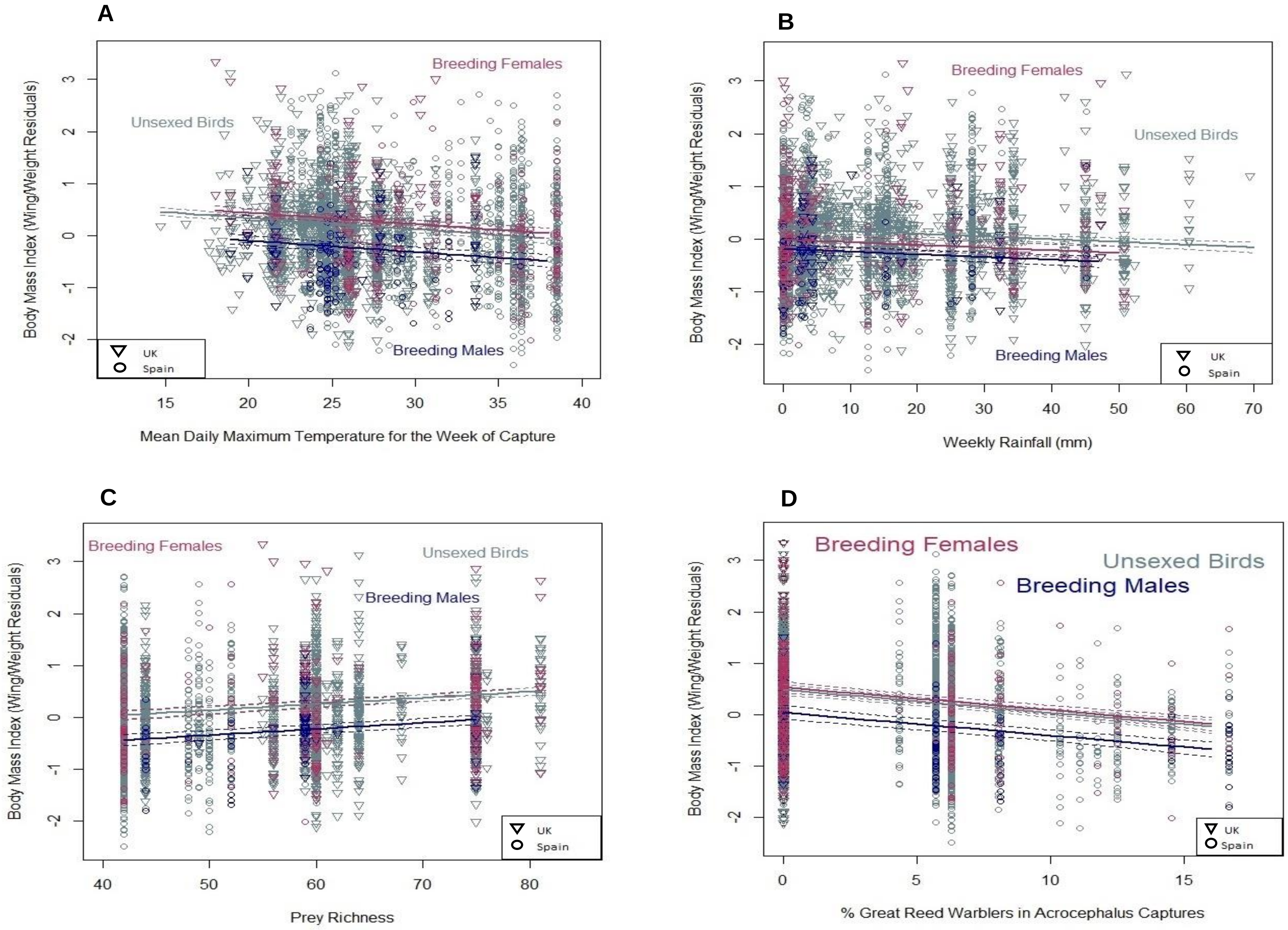


Figure 5.11. The relationship between prey family richness on sticky traps and dietary richness (i.e., the number of unique prey species detected) in reed warblers across the climate gradient.


A
Body Mass Index (Wing/Weight Residuals)
-2
-1
0
1
2
3
Breeding Females
Unsexed Birds
Breeding Males
UK
Spain
15
20
25
30
35
40
Mean Daily Maximum Temperature for the Week of Capture
B
Body Mass Index (Wing/Weight Residuals)
-2
-1
0
1
2
3
Breeding Females
Unsexed Birds
Breeding Males
UK
Spain
0
10
20
30
40
50
60
70
Weekly Rainfall (mm)
C
Body Mass Index (Wing/Weight Residuals)
-2
-1
0
1
2
3
Breeding Females
Unsexed Birds
Breeding Males
UK
Spain
40
50
60
70
80
Prey Richness
D
Body Mass Index (Wing/Weight Residuals)
-2
-1
0
1
2
3
Breeding Females
Unsexed Birds
Breeding Males
UK
Spain
0
5
10
15
% Great Reed Warblers in Acrocephalus Captures

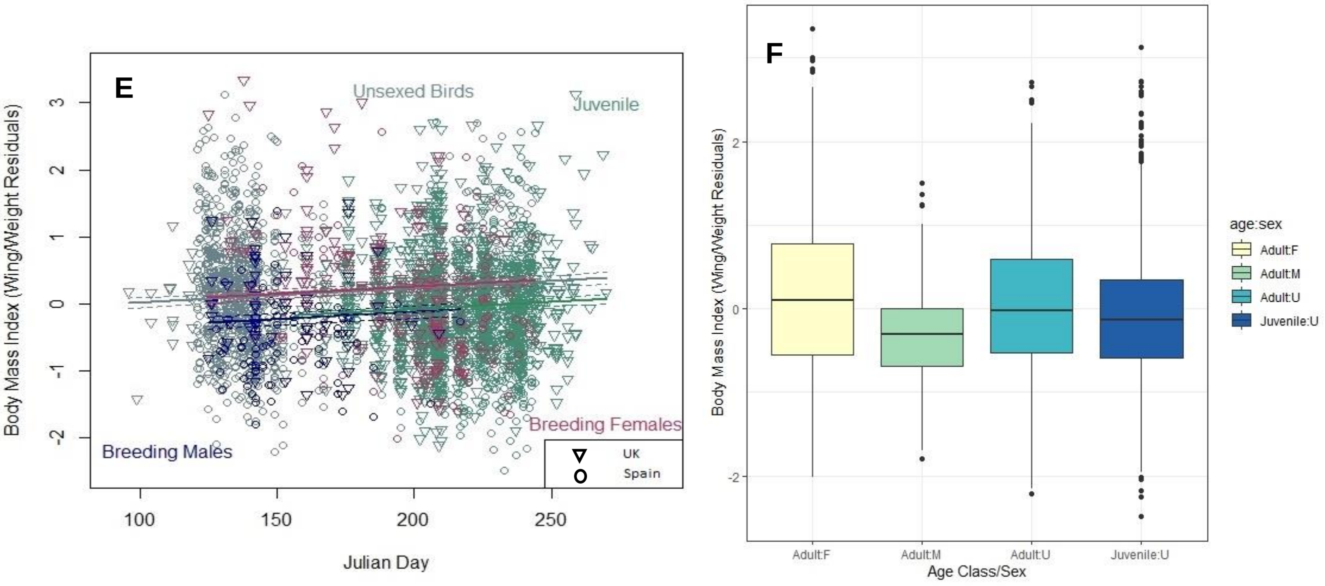


Figure 5.12. Prediction scatterplots and boxplots showing results of the GLM investigating biotic and abiotic effects on the body condition of ringed reed warblers across the climate gradient. The relationship between A) mean daily maximum temperature for the week leading up to capture, B) total rainfall for the week leading up to capture, C) rarefied family richness of invertebrates on sticky traps set during the period coinciding with capture, D) the % of captures of *Acrocephalus* warblers that were identified as great reed warblers during the period coinciding with capture, E) Julian day over the summer for both 2017 and 2018, and F) age class subset by sex, and the body mass index (BMI) of reed warblers in both the UK and Catalonia over the two breeding seasons studied (2017 and 2018). For prediction plots, unbroken lines indicate the predicted trend for subsets of breeding females, breeding males, unsexed individuals (split into unsexed adults and juveniles for plot E) and standard errors around each line are indicated by dashed lines. Data points from the UK and Catalonia are indicated by different symbols.

5.5. Discussion

5.5.1. Summary

This chapter used study sites in Catalonia as a climate analogue for a future, warmer UK to assess the potential impacts on the diets of reedbed warblers. Climatic conditions in Catalonia during the summer were several degrees warmer and generally drier than the UK climate, in line with the predicted direction of summer temperature and precipitation change expected to occur in the UK by the end of the century. Using this “space for time” approach, each of the predictions was addressed. Distinct changes to the composition, abundance and richness of prey resources with the change from the cooler UK climate to the warmer, drier Catalan climate were associated with shifts in diet composition and dietary overlap in warblers. Variation in the body condition of warblers was explained by both biotic and abiotic factors, notably with a role for prey richness and – for Eurasian reed warblers – the density of a larger competitor, the great reed warbler.

5.5.2. Climatic gradient

Just as climate models predict that under future warming, multiple climate variables will change in concert (IPCC 2013), gradient studies consider environmental complexity and feature many abiotic and environmental characteristics together. Gradients are a good option for “space for time” studies, as observed differences in a whole suite of measurements across a geographic, climatic or altitudinal range can act as proxies for future conditions (Körner 2007). “Space for time” studies have been shown to be 72% as accurate in predicting climate change effects on groups of organisms as “time for time” predictions (Blois et al. 2013). While not a perfect approach (Chuine 2010), many of the same assumptions used in gradient studies are used to create species distribution models based on future climate change predictions and known data on species ranges (Araújo and New 2007, Pereira et al. 2010, Thuiller et al. 2005). Arguably, using the same approach to predict species interactions is simply an extension of these same ideas (Eitzinger et al. 2019).

Although Catalonia (compared to other regions of Spain) is not so far inland or so far south that a comparison with the UK is unfeasible, it is not advised to view Catalonia as a direct climate analogue to southern UK, due to the uncertainty of current climate change projections. It would also likely take multiple decades of warming for the UK to reach temperatures comparable to Catalonia throughout the year, particularly in the summer, and multiple abiotic factors might differ between the two study regions. Nonetheless, current climatic conditions in Catalonia could provide a broad representation of a future UK climate, or at the very least represent the direction of change for UK climates under future climate change.

Study sites in both regions varied in habitat composition, topography, proximity to the coast and local climate measures. Combining the data from each site captured a high degree of the within-region variation to better understand patterns climate, prey availability and warbler diet.

5.5.3. Patterns of invertebrate prey availability

In the UK sites, higher maximum temperatures generally drove higher abundance and richness of prey resources. Cooler spring temperatures coupled with lower invertebrate abundance in the first sampling round in May, suggest that in the early summer in temperate northern European climates such as the UK, temperatures are limiting for *Phragmites* reed growth which has knock on effects for prey availability (McKee and Richards 1996) and thus bird productivity, aligning with the findings of earlier studies (Halupka et al. 2008, Vafidis 2014, Vafidis et al. 2016). This effect appeared to be particularly marked in more northerly latitudes within the UK, such as Wheldrake, where seasonality is likely exerting a stronger influence, as it is known to do elsewhere in Europe (Halupka et al. 2008, Schaefer et al. 2006).

Elevated temperatures in Catalonia were associated with a decrease in prey abundance, particularly in the late summer. Together these results suggest an overall humped relationship with temperature for prey availability in European reedbeds overall. Moreover, they allude to an important role for phenology which may be mediating a lot of the influence of climate by lengthening or shortening the growing season or development period of arthropods. Overall prey abundance and richness were both higher in the UK which can be attributed at least partially to the milder climate, more regular rainfall and a longer hydroperiod within aquatic habitats. These factors help ensure the continuous emergence of aerial insects that form “invertebrate chimneys” defined here as newly emerged adult stages of aquatic insects that form dense aggregations or swarms around the water surface and disperse to the wider landscape (Gee et al. 1997, Richardson et al. 2010, Sayer et al. 2012, Lewis-Phillips et al. 2020).

The CCA analysis underscored the importance of both maximum temperature and rainfall in structuring the invertebrate community across the two countries. Nonetheless, the importance of habitat characteristics such as shade, vegetation heterogeneity and water depth in altering the assemblages of invertebrates should also be emphasized. The two countries showed high turnover of invertebrate prey families, from the transition from high rainfall and lower temperatures (UK) to low rainfall and high temperatures (Catalonia). Some overlap did occur in the form of the most common invertebrates recorded on sticky traps, which were able to tolerate a wide range of conditions. These groups might be reliable prey for birds along the climate gradient in future, providing they meet the nutritional needs of the birds.

The species richness of plants and structural diversity of vegetation is a known driver of invertebrate abundance (Haddad et al. 2001, Grüebler et al. 2008). Sites such as Kenfig and Chew had very high structural diversity both in reedbeds and scrub, whereas large swathes of reedbeds at Oxwich were almost monocultures of *Phragmites*. In addition, reed habitats at Kenfig and Chew both surrounded large, permanent bodies of water, whereas at Oxwich the reedbed was divided by small streams and ditches, which were at times desiccated. The provision of more diverse, structurally complex habitat may also explain why the more heterogeneous sites harboured higher richness. This same pattern was observed in Catalan sites, with Sebes harbouring a richer fauna than Canal Vell as temperatures rose in the middle and late summer. Part of the reason for this could be the more diverse tree and vegetative flora fringing the reedbeds at Sebes, which may provide shaded areas and refuge from prolonged drying (Strachan et al. 2016).

Invertebrate richness between tree species can vary by more than one order of magnitude (Kennedy and Southwood 1984). In the UK, dominant tree species in scrub habitats surrounding the reedbed were often willows (*Salix* spp.), which alongside oaks (Quercus) and birches (Betula), generally harbour the highest invertebrate richness of all UK tree genera (Kennedy and Southwood 1984, Shutt et al.2019). In Catalonia, *Tamarix* was equally common, and understories of nettle, bramble and willowherb less commonplace. Wiesenborn and Hayden (2007) found that southwestern willow flycatcher (*Empidonax traillii extimus*) diet was very similar to the invertebrate composition sampled in native willow habitats, and higher than in invasive *Tamarix* habitats, confirming that willow habitats are richer in prey. Moreover, scrub in the UK often consisted of a diverse understory, attracting herbivorous and frugivorous insects and pollinators (Vicens and Bosch 2000). Having more structural elements in an otherwise monoculture of *Phragmites* reed stands provides calm air for invertebrate swarming, resulting in greater abundance of aerial insects (Whitaker et al. 2000). Thus, reedbed warblers, such as the reed warbler, feeding on mobile, aerial insects, should benefit from breeding in areas with generally high vegetative habitat heterogeneity.

5.5.4. Diet composition and frequency of occurrence of prey groups across the climate transect

Over 1000 prey species were recorded in the diets of the six warbler species: 715 in reed warblers alone. This highlights the opportunistic nature of European warblers, conferring them the ability to consume a wide variety of arthropod prey. At the same time, warblers (perhaps with the exception of the reed warbler) focussed the majority of their feeding on a smaller set of prey families, evidenced by the finding that frequency of occurrence across samples was high for a limited suite of prey, and low for many other prey items (that were probably consumed opportunistically). This pattern of selective feeding has been demonstrated in other generalist insect predators such as passerines, bats, and spiders (e.g., Clare et al. 2014*a*, Pagani-Núñez et al. 2015, Eitzinger et al. 2019).

In many generalist invertebrate consumers, the abundance, distribution and activity of prey resources is likely to influence foraging behaviour and diet (Holmes and Schultz 1988, Durst et al. 2008). As has been demonstrated in birds and other insectivores, plasticity in the diets of birds in the different regions was expected, as a result of changes in the most abundant food groups. For example, Clare et al. (2014*a*) found substantial changes in species richness of different orders in the diet of *Myotis* bats across Canada. In our study, the most obvious change in reed and Cetti’s warbler diet across regions was the greater number of spider species consumed by both species in Catalonia, and the higher frequency of occurrence of Araneae generally. This was mirrored in the preferences for spider families in the *econullnetr* analyses (see Chapter 2 and 4 for detailed breakdown of preferences). Araneae contain high concentrations of amino acids compared to other invertebrates (Ramsay and Houston 2003) and in a study by Pagani-Núñez et al (2011) they were important as alternative food in Mediterranean populations of tits, where their preferred prey (Lepidoptera) were less available. It is highly probable that they play a similarly important role in warbler diets, either targeted in their own right for nutrient optimization or as alternative prey in Catalonia when other prey groups are less abundant.

Reed and Cetti's warblers might also be partitioning different prey in different climates across the gradient. A number of taxa in the diet were switched in their relative % FOO between reed and Cetti's warbler samples in the change from UK to Catalonia; (i.e., consumed more by reed warblers in one country and Cetti's warblers in the other). Notably, there were contrasting differences in Lepidoptera richness between the two species in the two countries. This suggests high spatial plasticity and flexible preferences for prey as demonstrated in other insectivores with wide geographic ranges (e.g., Clare et al. 2014*a*). These changes could also allude to a change of feeding habitat, due to different habitat composition in the two countries. The turnover of species in the diet between the UK and Catalonia was very high (93.5%), although it should be noted that most species were only recorded in the diet of less than 5% of individuals and a suite of species and families were very common in the diets of birds from both the UK and Catalonia.

Several dipteran families such as craneflies and chaoborid midges were most often consumed and preferred by warblers in the UK, likely due to their association with milder temperatures and dependence on aquatic habitats (see % frequency of occurrence in Chapter 2 vs. Chapter 4). While Diptera always made the greatest contribution to warbler diet in terms of species richness, they become more abundant further north in latitude and show declines with warming (Høye et al. 2013, Loboda et al. 2018), which explains the greater dominance of several groups in the diet of northern birds (Pearce-Higgins et al. 2009). Furthermore, predaceous Diptera are richer in protein than herbivorous arthropods, and tend not to show antipredator behaviour, so they may be positively selected under optimal foraging (Fagan et al. 2002, Rickers et al. 2006, Schmidt et al. 2012). Some large brachyceran Diptera were consumed less frequently than expected, which is probably due to them being agile in flight and difficult to capture (Eitzinger et al. 2019).

Positive selection of several other aquatic insect families was observed, notably selection by UK reed warblers of Ephemeroptera (mayflies) and Trichoptera (caddisflies) which often emerge in swarms or "insect chimneys", providing an aggregated, profitable supply of food (Ornes 2013). Fewer preferences for aquatic groups in Catalonia may be a result of the drier summers there, perhaps constraining dependable mass emergences.

5.5.5. The effect of temperature on dietary patterns

Eitzinger et al. (2019) examined wolf spider (*Pardosa glacialis*) diet along an environmental gradient and found that predators were choosing similar prey despite the changing availability of these groups associated with increasing or decreasing elevation. In contrast, the findings of this study suggest that warbler diet does change significantly across a temperature gradient, although some prey taxa were always disproportionately selected by warblers.

Warbler diets were clearly structured by climate and location, as both maximum temperature and country of origin caused the composition of diets to diverge. Groups that are available in a wide range of temperatures may be important food groups for warblers as the climate changes as they are dependable during times of temperature fluctuation or extreme weather conditions such as drought or prolonged cold spells (Dyrcz and Flinks 2000). On sticky traps Aphididae, Clubionidae, Calliphoridae, Chironomidae and Syrphidae were present at relatively consistent availability across the temperature ranges, either suggesting wide temperature tolerances, or that they consist of species that are locally adapted (e.g., Chironomidae). Since sticky traps measure activity density, these groups (with the exception of aphids which tend to be sessile) may also simply be particularly active across the study sites.

Sometimes closely related prey species were consumed at a higher or lower frequency in different temperature ranges. This may be due to local adaptation or plastic responses of invertebrates in their thermal tolerance or development rates, or of birds in their foraging strategies (Davis et al. 2005), particularly in species unique to just one of the two countries studied. Lepidoptera showed wide variation in the temperatures where they were consumed most frequently. Some of this variation might be explained by identifying the life stage of the prey item. For example, caterpillars are more likely to emerge and be consumed in late spring, when temperatures are still relatively low, but adult imagoes might be more active in warm temperatures.

Mismatches where birds consumed a prey item more frequently at a temperature range where they are less available in the environment may suggest that supply and demand for invertebrate prey groups do not always match up perfectly, but nonetheless, predators may augment their diet with valuable prey that are limiting in the environment (Yard et al. 2004, Durst et al. 2008, Rytkönen et al. 2019, Eitzinger et al. 2019). Nonetheless the mismatched prey groups were still present in the environment in sufficient numbers during peak demand to be preyed on by birds, even if their abundance was higher during other periods. For example, damselflies were captured on sticky traps at all temperature ranges but were consumed most frequently in the cooler temperature range, perhaps as they are first emerging from aquatic zones and easily captured by birds, whereas they were more active in the environment in the warmer temperatures.

5.5.6. Prey size variation along the climate gradient

Reed and Cetti’s warblers did not consume larger prey in the UK as a response to release from possible competition with great reed warbler (Leisler 1991, Schulze-Hagen 1991, Cramp and Brooks 1992, our study; see Chapter 4 for details). Smaller warblers might be less well-adapted to regularly capture large, mobile prey groups without high energetic cost. Thus, focusing on medium-sized prey may simply be advantageous for Reed and Cetti's warblers (Robinson and Holmes 1982, Pulliam 1985). The average body length of prey consumed increased with maximum temperature; prey consumed by both species were overall larger in Catalonia than the UK. This might suggest local adaptation, enabling warblers to capture larger prey in Catalonia. Another explanation is that in the UK, smaller prey are more numerous and it is more economical for the birds to consume patches of aggregated small prey than to capture rarer but more nutritious large-bodied prey (Poulin and Lefebvre 1996, Raley and Anderson 1990, Trevelline et al. 2018), whereas in Catalonia the greater diversity of sizes encountered is increased, so that encounter rates and capture success rate for larger prey is improved (Robinson and Holmes 1982, Leisler et al. 2002). Although our traps did not capture a greater number of large prey groups in Catalonia compared to the UK, larger invertebrates were less likely to get captured as frequently as small prey, with the exception of very agile, mobile groups such as damselflies and muscoid flies.

5.5.7. Patterns of dietary overlap along the climate gradient

The effect of prey availability

Many studies suggest that both dietary overlap and niche breadth should increase when prey availability is decreasing, as a result of the broadening of the diet in response to nutritional constraints (e.g., Tebbich et al. 2004, Clare et al. 2014*b*, Salinas-Ramos et al. 2015, Cutting et al. 2016). This did appear to occur in Catalonia during periods of very low prey richness, where there was an associated increase in dietary overlap, suggesting that the diets of reed and great reed warblers were converging on a smaller subset of prey. Although it cannot be determined without exclusion experiments, this scenario shows potential for greater inter-specific competition. In contrast, in the UK dietary overlap decreased non-linearly with a decrease in rarefied prey richness and abundance in the field. Further investigation into pairwise species dietary overlap at low prey richness and/or abundance with a larger sample size is required to confirm this trend.

For most scenarios in our study system higher prey abundance and richness was associated with higher dietary overlap. An increase in the relative abundance and diversity of prey in environments utilised by songbirds can at times allow a broader suite of items to be shared among an insectivore community (i.e., allowing high dietary overlap) without an associated increase in competitive pressure, particularly if there is sustained, high availability of several preferred invertebrate groups (Razgour et al. 2011, Trevelline et al. 2018). When prey availability decreased below this level, both warbler pairs partitioned their diets to a greater extent, as has been previously demonstrated in wetland warblers (Bibby and Green 1983). It is widely acknowledged that increased dietary partitioning may decrease the likelihood of intense interspecific competition when prey become limiting, allowing continued coexistence of similar species in the same location (Chesson 2000, Pigot et al. 2018, Kent and Sherry 2020).

I propose that the effect of prey availability (i.e., richness and abundance) on dietary overlap can be viewed as a continuum (Fig. 5.13). Very low prey availability coincides with high dietary overlap as diets converge on the remaining prey, medium (but increasing) prey availability with high partitioning as birds become less limited in the prey they can select, and show individual preferences or foraging differences, and finally, high prey availability where overlap increases once again as birds are able to share a greater subset of highly abundant groups that may be locally widespread e.g. chironomids and other aquatic taxa. In this last scenario the high dietary overlap masks subtle diet differentiation and individual preferences by birds (indicative of partitioning) that were detected in the *manyglm* analysis.

It is important to mention that the species pairs examined differed in Catalonia (great reed warbler *vs.* reed warbler) and the UK (sedge warbler *vs.* reed warbler), and this may have driven a portion of the differences observed between countries; however, given the similar ecology and life history of the three *Acrocephalus* warblers, as well as evidence that these species pairs compete for access to resources (Brown and Davies 1949, Catchpole et al. 1972, Hoi et al. 1991, Honza et al. 1999), our study suggests that this difference should be given more importance than the overarching effects of prey availability and climate.

The effects of climate; temperature and rainfall

Differential effects of both temperature and rainfall on overlap were reported in the UK and Catalonia. When rainfall in Catalonia increased, it coincided with increased dietary overlap between reed and great reed warblers, whereas the reverse was seen in UK sedge and reed warblers. Overlap showed a humped relationship with increasing temperature where an optimum temperature range coincided with high overlap in the warbler pairs. Beyond this threshold zone (around 27°C in the UK and 23°C in Catalonia), overlap decreased, and this could be a result of prey abundance or richness decreasing under heightened temperatures. The reason that the threshold appeared to occur at a lower temperature in Catalonia may be due to the earlier loss of aquatic habitats and subsidies of aquatic/semi-aquatic prey to the birds (Poulin et al. 2002). These results indicate that rainfall is the limiting factor in Catalonia as with elsewhere in Spain (Mooney and Parsons 1973, Herrera 1978), but temperature is currently the limiting factor in the UK (Taylor 1974). Elevated rainfall as a result of climate change in the UK could be associated with a potential for increased resource competition, as persistently high rainfall or sudden extreme rainfall events both reduce the availability of flying prey and change the composition of invertebrates in the aquatic zones (Taylor 1963, Bedford and Powell 2005, Arbeiter et al. 2016, Aspin et al. 2019). However, in Spain, a moderate increase in rainfall might prevent or alleviate drying or drought in reedbeds and surrounding waterbodies, leading to a greater provision of aquatic prey and increased overlap between bird species consuming the same abundant prey groups.

Consequences for warblers

Our findings imply that currently in the UK, birds in scenarios of lowered prey abundance can coexist by partitioning their diets. In Catalonia, partitioning is also possible during periods of lowered prey abundance, provided that prey richness is not severely reduced. From these results, it is inferred that reedbed warbler species feed opportunistically on locally abundant prey items when they begin to emerge in high numbers. Certainly, birds are able to change the breadth of prey consumed in different scenarios, as evidenced by changes in dietary breadths, measured by Levin's Index, at different sites by the same species (see Chapter 2 and 4). One possible limitation to current bird productivity in some localities in both the UK (particularly in 2018) and Catalonia could be the loss of aquatic subsidies in warmer, drier environments, which are important for supplementing the diets of bird populations and those of other organisms in wetlands and associated fringing habitats (Polis and Hurd, 1996, Nakano and Murakami 2001, Sanzone et al. 2003, Orłowski and Karg 2013, Sardiña et al. 2017, Lewis-Phillips et al. 2019, 2020). For example, in Japan, local superabundance of chironomids inflated total prey abundance, compensating for the smaller size classes of prey available and less frequent occurrences of alternative prey such as beetles and dragonflies (Drycz and Flinks 2000). Continued emergences of semi-aquatic, mobile prey may provide a baseline of abundant prey that birds can depend on, and can be shared by a variety of warblers, permitting high dietary overlap (Trevelline et al. 2018). Reductions in aquatic prey emergence could have been associated with lowered prey abundance and richness, so that birds no longer consume these prey opportunistically and instead show species-specific feeding patterns. It is expected that such partitioning should reduce interspecific competition for prey.

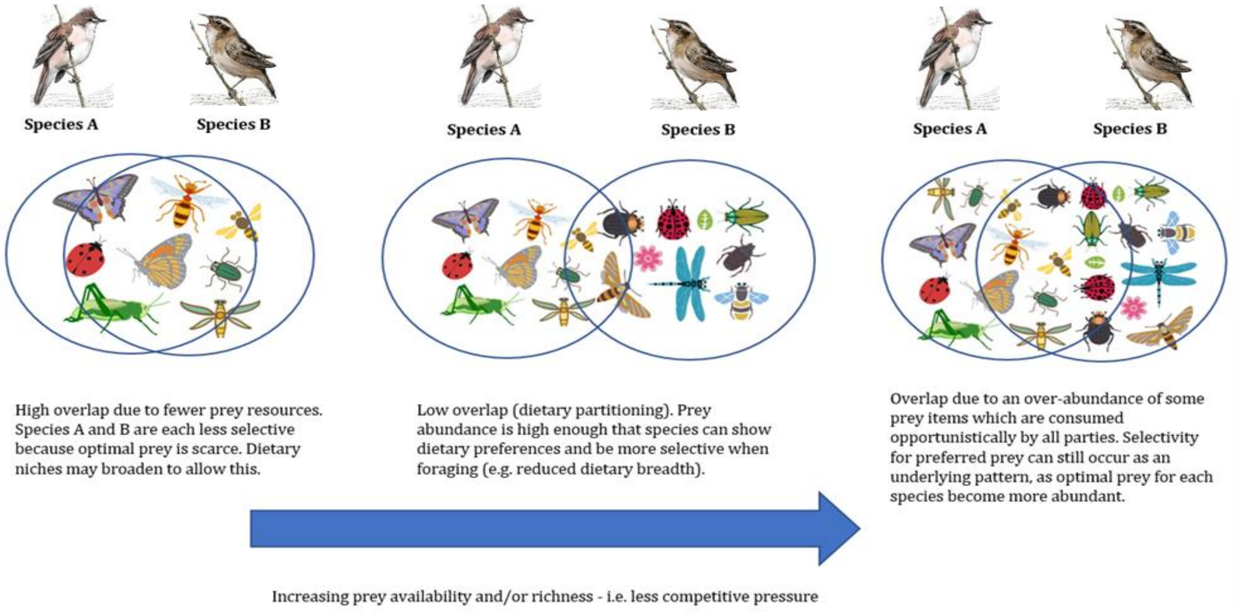


Figure 5.13. Flow diagram describing the proposed relationship between dietary overlap in pairs of sympatric warblers and increasing availability and richness of prey with a possible associated decrease in competitive pressure. Warbler drawings by Ulf Andersson/Artmagenta.com.

5.5.8. Drivers of body condition in warblers

The richness of prey had a positive association with warbler body condition. There was also further evidence that richness of invertebrate prey in the environment might be translated into dietary richness in the consumer, as the two variables were positively correlated in sampled reed warblers. Laboratory and behavioural studies on generalist animals have shown that when suitable combinations of food resources are available, (where individual resources comprise different nutrients), organisms will broaden the range of food consumed, selecting a diet that is balanced in macronutrients, but which meets the required amounts of the most important nutrients, to optimise fitness (Raubenheimer and Simpson 1997, Simpson et al. 2004, Jensen et al. 2012, Cui et al. 2018). It may be important for warblers to obtain a variety of different prey and optimise prey quality rather than sheer quantity of prey items.

When data from ringed birds were analysed, adults showed a higher BMI than juveniles. This again aligns with the findings of previous chapters, suggesting that newly fledged individuals may be inexperienced foragers and/or have reduced access to the best foraging areas compared to dominant adults (Marchetti and Price 1989, Wunderle 1991). The body condition of birds increased over time which is most likely a result of birds beginning to build fat reserves at the end of summer prior to autumn migration. However, it could also be a direct result of prey increasing over the summer, or alternatively could imply that individual condition improves in adults once the energetically demanding period of breeding is completed.

Male passerines exert a great deal of energy in the early breeding season when defending territories and displaying and searching for mates, which can lower their body condition over time, inferring higher risk of mortality (Liker and Székely 2005). Females with brood patches may appear to be in better condition if they are carrying eggs, which would increase their overall body mass with respect to their wing length, and this was not corrected for in our analysis. Given this bias, it is advised that future studies calculate BMI separately for males and females. However, females were not in significantly better condition than unsexed birds (suggesting that this is a minor effect) and at least a proportion of females were in the later stages of breeding where eggs have already been laid, at which point weights should no longer be inflated by egg mass, with respect to their wing length.

The negative relationship between rainfall and body mass in the UK supports the notion that either prolonged wet weather or sporadic adverse downpours is detrimental to foraging warblers (Grassel et al. 2016, Arbeiter et al. 2016). Increasing maximum temperature had a negative effect on body condition in both models, perhaps due to its effect on prey abundance or richness discussed above. An alternative explanation is that high temperatures coincide with the peak of breeding. At this time adult birds may see a drop in condition as they provision their broods, termed "the cost of reproduction" (Williams 1966). Simultaneously, juvenile birds begin to emerge on their natal breeding grounds, and density dependent effects could cause an overall reduction in the body condition of birds (Frederiksen and Bregnballe 2000, Lok et al. 2013, Norman and Peach 2013).

Reed warblers in Catalonia showed higher body condition than birds in the UK, but this was largely a result of the very high BMI of birds in just one Catalan site, the Llobregat Delta. In the UK, high BMI of birds at Kenfig might be explained by the greater diversity of habitats, resulting in high invertebrate richness. Similarly, prey availability was high in Sebes with respect to the other Catalan sites, likely as a result of enhanced habitat heterogeneity, so the lowered condition of reed warblers there requires explanation. However, the BMI of reed warblers fell during periods when the ratio of great reed warblers to reed warblers in mist-net captures increased. Great reed warbler densities were higher in Sebes than they were in the Llobregat Delta throughout the breeding season. Perhaps additional competitive interactions contributed to the lower weight gains seen in the reed warblers at Sebes. Similarly, in another study, high abundances of chironomids at a wetland in Japan were associated with 10 times higher great reed warbler densities than in a site in Poland (Dyrcz and Flinks 2000). Likewise, in Sebes the higher density of great reed warblers breeding locally could be as a direct result of the higher food availability, particularly from June onwards, compared to the other Spanish sites.

During times of low prey abundance, the additional pressures of competition from great reed warblers could affect feeding rates, weight gain and fitness of this species. Interference competition could be preventing reed warblers from accessing high quality prey areas, or exploitation competition could serve to limit the prey available for reed warblers, both of which could explain the lower BMI of reed warblers (Petren and Case 1996, Jaentti et al. 2007). Aside from the reproductive advantages afforded, one additional benefit of exhibiting dominance over neighbouring warbler species (particularly the reed warbler), is that the great reed warblers can optimise breeding in areas that encompass prey-rich foraging sites (Dyrcz 1986, Hoi et al. 1991), which may be reflected in the higher body condition scores, compared to the remaining warblers. Great reed warblers are also much larger bodied than the remaining warblers in our study, which better equips them to regularly capture and consume larger prey items (Bibby and Green 1983, Leisler et al. 1992). In the absence of the largest prey groups, this warbler was also able to consume smaller-bodied prey (see Chapter 4). Utilising a greater range of prey size classes is a flexible strategy which may buffer this species against shortages of large-bodied prey, while also taking full advantage of them when they do become available. It is possible that this provides a fitness advantage over the other species. Finally, population densities of great reed warblers have seen a gradual decrease in Europe (del Hoyo et al. 2016) which has served to reduce intraspecific competitive pressure on the breeding grounds, allowing most birds to occupy higher quality breeding territories where food quality may be enhanced (Schaefer et al. 2006).

Our models only explained a small proportion of the variation in warbler body condition, suggesting a role for additional factors such as overall health, parasite load, infection, carry-over effects from the nestling stage (e.g., poor growth), genetic factors and environmental factors such as habitat quality, presence of predators, intraspecific competition and the level and frequency of aggressive interactions from conspecifics. Nonetheless, the variables in our study do implicate the diversity of prey types available, which is mediated via local weather and climate. In addition, other measures of condition may be preferable to BMI, such as plasma metabolites (e.g., Cutting et al. 2016) which consider energy gain, the direction of body-mass change and physiological state in an integrative manner (Jenni-Eiermann and Jenni 1994, MacDade et al. 2011). However, if results are interpreted with caution, BMI is a useful and non-invasive proxy for condition in the absence of such methods.

5.5.9. Predictions for warbler communities under future climate change

Small changes in body condition over time are not necessarily detrimental, unless lowered body condition is consistent over long time spans and comes at the expense of reduced survival, lower reproductive output or lower nestling condition and survival (Williams et al. 1966). In Poland, climate change has improved the overall fitness of reed warblers, since earlier emergence of prey and advanced reed growth for nest concealment provides optimum conditions for birds to commence breeding earlier (Halupka et al. 2008). Due to the country's central continental location, Polish climates are more seasonal than UK climates, inducing colder springs but warmer summers, and thus responses to climate change may be different.

UK predictions

Our cross comparison of the UK and Catalonia suggests that future climate change could have a negative effect on warbler populations in both regions, but such consequences in the UK may not be observed for some time. Current warming in the UK has so far increased reed warbler productivity (Vafidis 2014, Eglington et al. 2015), but the productivity of other warbler species using similar habitats has not changed (Eglington et al. 2015). Initially, warming in the UK might release birds from the limitations posed by low temperatures at the beginning of the breeding season, in a similar way to that observed in Poland (Halupka et al. 2008), by increasing prey abundance in aquatic habitats (Vafidis et al. 2016). Over the coming decades, however, daily maximum temperatures in the UK are projected to increase further, and UK reedbed habitats may be in danger from the same localised and widespread drying or drought over the summer that currently presents a great challenge to wetland conservation in the Mediterranean (e.g., Poulin et al. 2002, Schröter et al. 2005, Jiménez et al. 2018). For example, the latest edition of the "Climate Change Adaptation Manual" by Natural England and the RSPB (2019) state that projected drier summers may lead to the drying out of reedbeds and the loss of aquatic species, and a decline in reedbed specialist invertebrate and bird species.

At Oxwich Marsh, an already dry reedbed site, the warmer breeding season of 2018 caused birds to partition prey to a greater extent and the site experienced a decrease in prey availability by the end of summer (see Chapter 2). Although it was not possible to show that this was translated into lowered body condition in birds, this site may already be experiencing some of the negative impacts of warming associated with drying, as shifts in invertebrate communities may occur before the surface water is completely dried up (James and Suren 2009, Aspin et al. 2019).

In addition, climate models predict increased adverse weather, higher frequency of storm events and more intense downpours in the UK (Kendon et al. 2014), particularly in northern England (Chan et al. 2018). This is expected to have a negative effect on both prey availability, and the foraging success of birds (Arbeiter et al. 2016). Heavy rainfall is also a leading cause of mortality in the young of reed nesting warblers, by direct flooding of nests and resulting nestling hypothermia and starvation (Dyrcz and Halupka 2009), which has implications for reproductive output and future productivity.

Southern Europe predictions

The ranges of many European warblers reach their southern limit in the Mediterranean (Eglington et al. 2015) where population growth rates are lower than in the northern limit of the range (Jiguet et al. 2010, Morrison et al. 2013). July and August temperatures in the Mediterranean are already very high, and many wetlands suffer from periodic drought, reducing the hydroperiod length and decreasing local arthropod abundance and availability (Frampton et al. 2000). Jiménez et al (2018) found that exacerbated drying and drought in a Spanish reedbed over several years led to reductions in recruitment in the local reed warbler population. Future warming is expected to exacerbate this trend, potentially causing some species ranges to contract on their southern limit (Huntley et al. 2007). This is thought to be particularly likely in migratory species, and examples of southern range contractions in neotropical migrant bird species breeding in eastern North America are beginning to emerge (Rushing et al. 2020). The implications for our study sites in Catalonia are that further temperature rises are likely to be damaging for reedbed habitats. Currently prey availability at our field sites in Catalonia are still sufficiently high, and dietary partitioning is sufficient to allow populations of warblers to coexist. More intense competition for food resources between warbler species may be expected in future, which could eventually lead to local extirpation of one or more species in some sites.

5.5.10. Conservation Implications

The results of this study highlight the importance of wetland habitats as a source of aquatic prey for warblers. It is likely that sites with a greater availability of permanently flooded zones or standing bodies of water as well as greater habitat heterogeneity will be better buffered against the effects of climate change and will continue to provide refugia for invertebrates that form the diet of the warblers in this study (Poulin et al. 2002, Haddad et al. 2001, Grüebler et al. 2008, Strachan et al. 2016).

The study sites with the richest invertebrate fauna; Chew Valley and Kenfig in the UK and Sebes in Catalonia, benefited from the presence of nearby, permanent waterbodies, comprised a rich and varied understory flora and harboured a mixture of habitat types throughout the reedbed and scrub areas, providing structure and complexity for invertebrates as well as areas of shade (Vicens and Bosch 2000, Whitaker et al. 2000). Oxwich Marsh comprised more homogenous reedbed, with central sections composed of old, dry stands with thin stems which showed evidence of desiccation. At Canal Vell, the number of different scrub and tree species surrounding the reedbed was low (formed mainly of Tamarix), and understory vegetation was sparse.

Conservation at the sites most prone to the negative impacts of climate change could involve improving habitat heterogeneity for arthropods through habitat management to include the planting of native tree species (Strachan et al. 2016) and creating or restoring additional wetland habitats (e.g., ponds/ditches) within or near the reedbed zone, to provide a greater subsidy of emerging insects (Lewis-Phillips et al. 2019, 2020). If required, careful management of water levels can be implemented to extend the hydroperiod in seasonally flooded reedbeds, which should serve to alleviate drying (Carroll et al. 2011).

Conclusion

The use of climate gradients as analogues of future climate change allows researchers to forecast areas where conservation action may be needed in future. The predictions of this study have implications for the future conservation of both reedbeds and their fauna across Europe in the light of current and future climate change. Measures to prevent reedbed drying or drought in northern Europe, including the UK (discussed in Chapter 6), are necessary at this current stage of warming, to best buffer habitats against future cascades of negative impacts from climate change on birds and their invertebrate prey, which although are likely to lag behind, may nonetheless soon mirror those experienced in southern Europe.

5.6. Acknowledgements

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Chapter Six - General discussion

Top left: myself with a great reed warbler at Canal Vell Biological Station, top right: damselflies, such as the Banded demoiselle pictured, are examples of aerial insects dependent on aquatic habitat for part of their life cycle (stock photo from Getty Images; photographer: Sophia Spurgin Photography 2017), bottom left: an inundated section of reedbed at Kenfig Nature reserve, UK (photo credit: Dave Carrington), bottom right: a once inundated mist net ride through a reedbed at Sebes Nature Reserve, Catalonia, in late July showing signs of desiccation.

6.1. Project aims

The main aim of this PhD research was to assess the current and future impacts of warming on sympatric reedbed warblers, using a "space for time", climate gradient approach. The project aimed to explore the effects of i) climatic conditions on the breeding grounds and ii) the abundance and richness of invertebrate prey, on the diet composition of warblers and the degree of dietary overlap among warblers across sites and seasons, giving an indication of potential current, and projected future, interspecific competition.

Specific objectives were to: i) set up a network of reedbed sites in the UK and Catalonia, Spain; ii) use metabarcoding to characterise the diet of warblers in both regions, and determine their dietary preferences; iii) monitor the invertebrate prey base present at each location over the breeding season, to assess how warming changes the invertebrate composition of reedbeds; iv) analyse the extent of dietary overlap between pairs of species at each site along the climate gradient, and investigate how different types of resource partitioning (e.g. prey taxa, prey size, terrestrial/aquatic prey origin) might prevent intense competition and/or competitive exclusion; v) assess the effects of climate, prey availability and density of a competitor on body condition in individual birds, and vi) use the above findings to predict how future climate change will alter dietary overlap and competitive interactions between sympatric birds.

6.2. Completion of research objectives:

6.2.1. Main findings

A general invertebrate primer pair targeting ~ 400bp region of the COI barcode region, was used to amplify and sequence DNA from a wide range of invertebrate prey items present in warbler faeces using high-throughput sequencing (HTS) and compare the output sequences to those held within the barcode of life database (BOLD) to identify prey items. Even though the chosen primer pair targeted a longer region of DNA than previous studies have used (e.g., King et al. 2008 , Zeale et al. 2011, Jedlicka et al. 2013, Trevelline et al. 2016, 2018) a wide range of dietary items were successfully detected, and the longer target length permitted successful identification of most invertebrate prey items to genus or species level. This primer pair can thus be recommended for future studies on avian diet.

The diet of six European warbler species were described across the European network, over multiple sites and years. Changes in diet composition were assessed in birds captured over the breeding season and with changing rainfall and temperature patterns. A major finding was that although all the warblers in the study can be described as generalist insectivores, an individual warbler's diet is highly variable between and within species, sites, countries, years and over the breeding season. The majority of prey species and families detected were consumed by less than 10% of individual birds, whereas a smaller suite of families were detected at very high frequency in the diet. This emphasizes how predators of arthropods may focus on a small selection of beneficial prey groups while also opportunistically feeding on a wider spectrum of alternative prey (e.g., Eitzinger et al. 2019). Moreover, climatic differences between sites and countries drove differences in dietary composition and dietary overlap. Together these findings have implications for our understanding of the effects of future climate changes on reedbed warblers in particular, and insectivores in general.

6.2.2. PhD Chapter summaries

In Chapter 2 and 4 the diet of birds across several sites, i) in the UK (Chapter 2) and ii) in Catalonia, Spain (Chapter 4) were studied, and dietary overlap was measured between pairs of species. Diets were found to differ across sites and seasons, likely as a response to changes in weather, prey availability and the composition of the warbler communities present. In the UK, availability of prey taxa at Oxwich Marsh differed in a cooler, wetter summer (2017) compared to a warmer, drier summer (2018). This was mirrored in the diets of the birds, resulting in increased resource partitioning in the warmer year, when prey was less abundant and diverse, particularly by the end of summer. In addition, the cooler climate of Wheldrake Ings in Yorkshire was associated with a mid-season peak in prey abundance, and a lag in growing degree day (GDD) accumulation suggesting later emergence of prey groups compared to the more southerly sites, and this was mirrored in slight apparent shifts in warbler diet, although this change was perhaps obscured by a relatively small sample size.

In Chapter 3, Eurasian reed warblers (*Acrocephalus scirpaceus*) were the focus of a case study of age differences in diet of reedbed warblers at Chew Valley in Somerset. The main finding was the discovery of significant dietary differences between nestling, juvenile and adult birds across the breeding season. In accordance with other songbird studies (Krupa 2004, Orłowski et al. 2014, Rytkönen et al. 2019), a high frequency and species richness of Lepidoptera was reported in nestling diet, and larger and softer-bodied prey species were preferred. Provisioning of nestlings by parents likely maximised the allocation of the most nutritious arthropods that enable more rapid growth and development (Krupa et al. 2004, Skipper and Kim 2013). In accordance with previous studies, (Marchetti and Price 1989, Wunderle 1991) juveniles appeared to be inexperienced, or restricted to certain habitats and they consumed less optimal prey groups perhaps as a result of these factors. However, on average they consumed a higher proportion of aquatic prey than the other age-classes, perhaps relying on mass emergences of mobile adult stages of aquatic invertebrates, that are easy to capture when swarming (Sherry et al. 2016) and represent dependable prey in the warmer summer months when juveniles fledge and disperse.

In Chapter 5, a direct comparison of the diets of both reed and Cetti's warblers (*Cettia cetti*) in the UK with their conspecifics in Catalonia elucidated that distinct dietary changes occurred along the geographical gradient from a cooler maritime climate (UK) to a warmer, Mediterranean climate (Catalonia). Some changes to the diet were subtle, with many of the same prey orders and families consumed across the climate gradient, but changes in the composition of prey in the environment were more substantial. Although many similar arthropods were retained in the diets of birds of both countries, particularly spiders, aphids, moths and chironomids, there was a shift in Catalonia towards crickets, large hymenoptera and a wider range of spiders, whereas the UK diet featured a broader suite of aquatic and semi-aquatic prey. This might be attributed to the wider availability of prey groups emerging from aquatic habitats in the UK, as a direct result of both regular rainfall and milder temperatures, and habitat heterogeneity and aquatic habitat availability.

In the same chapter it was revealed that differences in body condition were partially explained by local weather and availability of prey, among other drivers such as age, sex and site, and in Catalan reed warblers, the ratio of captured great reed warblers (*Acrocephalus arundinaceus*) to reed warblers. These findings implicated direct consequences of reduced prey richness on the fitness of passerines. In addition, these results imply that great reed warblers were dominant over reed warblers and may be causing lowered fitness in the latter (Dyrcz 1981, Hoi and Winkler 1994, Schaefer et al. 2006). The mechanism by which this occurs is unclear, but it was speculated that great reed warblers may be preventing reed warblers from nesting in, or near to, high-quality feeding areas as has previously been documented (Hoi et al. 1991 Honza et al. 1999). These high-quality areas might harbour abundant, high quality food resources, or contain the preferred prey of great reed warblers, explaining why great reed warbler diets appeared to be more selective than the diet of reed warblers.

6.2.3. Patterns across the climate transect

From the findings of Chapter 2 and 4 it can be surmised that sympatric warbler species partition their prey via several mechanisms, the most common being taxonomic group e.g., order, family and species, but also via the consumption of different prey size classes (in Catalonia), and feeding on fewer or more aquatic prey groups (in both regions), which may be a result of using different foraging habitats. Prey size as well as taxonomy was important in determining the preferences of warblers, and ample evidence was uncovered showing that birds use an optimal foraging approach when selecting prey.

Dietary overlap between sympatric bird species increased non-linearly with an increase in both prey abundance and prey richness across the climate transect, but the effects of maximum temperature and rainfall on dietary overlap were very different in the two climatic regions studied.

This is due to their differential effects in influencing productivity; water is limiting in Iberia (Mooney and Parsons 1973, Herrera 1978) but temperatures are limiting in the UK (Taylor 1974).

In Chapter 5 it was proposed that the relationship between prey availability (richness and abundance) and dietary overlap fell along a continuum whereby both very low and very high availability of prey was associated with high dietary overlap, and intermediate availability of prey was associated with low dietary overlap. In scenarios of both intermediate and high prey availability, enough prey types exist to allow birds to be selective and partition their diets, but in the case of high availability, this partitioning is masked by widespread opportunistic, consumption of locally abundant prey shared by all birds (see Chapter 5, Fig. 5.13). This indicates high flexibility and plasticity in warbler diet, but also species-specific preferences as a result of adaptation and optimal foraging constraints. Since the real level of competition cannot be accurately ascertained by the methods used here, more work is needed to determine these patterns experimentally, and to determine whether the realised level of competition changes across this continuum.

In all chapters, when warbler diets were compared to the availability of prey groups (from sticky traps), many items were consumed more or less in proportion to their abundance, but the most common prey groups captured on the traps were not always consumed at a frequency expected from their high abundance. Although this might suggest they were simply too numerous to be consumed at the predicted rate, it has been shown that bird species do not always switch to consuming the most abundant prey in the area (Rytkönen et al. 2019). However, at least part of this pattern is a result of the relatively low nutritional value to birds of some of these highly abundant invertebrate groups, particularly ants and small insects generally, such as small parasitic wasps (e.g., Chalcidoidea), and small acalyptratae flies (e.g., Chloropidae) (Poulin and Lefebvre 1996, Sherry et al. 2016). It might be expected then that birds will switch to feeding on locally abundant prey in space and time, only if they fulfil optimal foraging requirements, i.e., they afford a good balance of energy gain per energy expended (Krebs and Davies 1991). A number of prey items were consistently consumed more than expected based on their relative abundance. The finding that a relatively wide range of preferences did exist, suggests that birds do not simply consume all available prey encountered, known as a “meet and eat” generalist foraging strategy (Ferrer et al. 2008, Layman and Allgeier 2012, Pagani-Núñez et al. 2015), but rather that they actively select prey types with certain traits that render them more nutritionally valuable, or make them worth the costs of capturing (e.g., reduced mobility, patchiness, and ease of capture). In addition, prey encounter rates are likely to be important, and although sticky traps are accurate at measuring activity-density, some prey items may be more active at night (when the birds are not foraging), and some warbler species may be better at locating day-inactive or sessile insects than others.

The literature has shown that both predator and prey body mass can affect predator-prey links and other interactions (Gravel et al. 2013, Brose 2010, Brosseau et al. 2018). In both regions, a relationship between prey body size and the strength of preference for a given prey item by multiple warbler species was discovered. The strongest relationship was in great reed warblers, who given their larger body size, are most adapted to consume larger prey items, and indeed they consumed the largest prey classes in higher frequency, with prey species consumed per bird on average being larger bodied species. Although larger prey items are often nutritionally valuable, the largest classes may be difficult to handle or capture, and thus they might be costly to obtain regularly for the smaller warblers. This difference between warblers in the optimal foraging trade-off will likely lead to the partitioning of prey sizes that occurred between warbler species, and this appeared to be particularly important in maintaining coexistence in Catalonia where prey availability was overall lower.

These findings underscore the difficulty of attributing prey abundance and availability to the observed diet composition of generalist birds that display a range of foraging strategies and that furthermore may encounter invertebrate prey that occur in a variety of microhabitats (Durst et al. 2008). However, it is also true that generalist insectivores living in dynamic systems such as reedbeds have evolved to take advantage of variable and fluctuating prey resources, and the observed discrepancies between available and consumed prey could be indicative of the relative plasticity of these birds in selecting prey. If the latter case is true it confirms the assumption that the productivity of generalist passerines is strongly driven by events that influence overall abundance and diversity of invertebrates, such as habitat destruction, degradation and loss, regional drought, and extreme weather events. Conservation measures aiming to alleviate these impacts, such as habitat restoration to improve floristic diversity and management to enhance wetland connectivity will have the added benefit of improving the resilience of wetland ecosystems to warming in future years.

6.3. Future research directions

6.3.1. Metabarcoding limitations

DNA metabarcoding is an effective tool for dietary analysis, generating information about a broad range of items in the diet, at a finer taxonomic resolution than traditional morphological methods can usually achieve (Soininen et al. 2009, Alonso et al. 2014). Although reliable, metabarcoding has several known limitations. Firstly, although the chosen primer set had very wide utility, no primer pair alone can provide a completely unbiased and comprehensive account of animal diet, particularly for insectivores such as the warblers studied. A proportion of prey items in the diet may have been under-represented or undetected due to i) highly degraded DNA that was not amplified successfully in PCR reactions, ii) primer bias, iii) differences in mitochondrial copy numbers per cell and iv) PCR inhibition of certain prey groups (reviewed in Pompanon et al. 2012, Clare 2014).

Metabarcoding reveals which taxa are present in the diet, but does not provide any information about life stage, nutritional value, quantity consumed, or size of prey items detected. Researchers are currently unable to fully quantify prey remains in insectivore consumer faeces, since there are unaccountable differences in prey digestion rates, the size of prey consumed and biases in PCR amplification (Pompanon et al. 2012, Deagle et al. 2013). Deagle et al. (2019), Thomas et al. (2016) and Cavallo et al. (2020) have had some success in trialling approaches to quantify the diet of animals, such as harbour seals (*Phoca vitulina*) and little penguins (*Eudyptula minor*), using the relative read abundance (RRA), defined as the number of sequences produced for a particular prey taxon, and prior information about known diet, that are used to generate correction factors for estimating consumed prey biomass. Such methods are not yet suitable for quantifying the diet of highly generalist insectivores such as reedbed warblers, which can potentially consume hundreds of different species. In addition, since it is not possible ascertain what life stages of prey are consumed, it was assumed, based on observations of foraging warblers, and morphological identification of prey remains in diet samples (Henry 1978, Bibby and Thomas 1985, Cramp and Brooks 1992, Leisler and Shulze-Hagen 2011), that adult stages were taken by birds in the majority of instances (with exceptions such as Lepidoptera). Differential amplification of adult and larval stage may have confounded results, and any partitioning of prey age classes that could have been occurring was not possible to measure.

Metabarcoding may also pick up i) secondary predation and ii) parasitism which are often not distinguishable from primary diet (Pompanon et al. 2012). Some dietary species in this study such as aphids, sawflies and syrphids may have contained parasitoids, which might explain the wide diversity of Hymenoptera detected in warbler faeces. Birds have been shown to consume parasitic Hymenoptera, especially larger groups such as Ichneumons, so an effort to separate likely parasitism of prey/secondary predation, from actual consumed prey was not attempted.

Solutions for many of these caveats are becoming rapidly available and it should be stressed that future studies that analyse each of these limitations experimentally will be of great importance, in order to shed light on possible options to minimise error and misinterpretation of results. In the meantime, the following describes ways that I attempted to reduce generating false-positive results in the diet datasets for each chapter.

Firstly, the post-HTS clean-up protocol removed zOTUs from PCR/extraction negatives and unused MID-tag combinations across all samples and then filtered out all sequences with less than 10 reads. This methodology used should have removed the majority of artefacts caused by cross-plate contamination and tag-jumping. Some expected (known) prey items were not recovered in the mock community and/or repeated warbler samples however, alluding either to possible primer biases or loss of a small number of prey taxa in some samples from the conservative clean-up step. Drake (2020) highlights further steps than can be taken to further reduce the risk of errors from artefacts, and better detect recovery of mock community species. These steps involve adjusting thresholds for sequence removal based on read counts for mock community species and positive controls. Although contamination and tag-jumping were likely to be present in the dataset at very low levels, these errors should not be significant enough to undermine any of the main conclusions of this PhD project.

Moreover, much of the analyses and diet comparisons (especially between prey consumed and available prey recorded in the environment) were carried out at the prey family level which should have reduced the potential for misidentification of dietary species from errors in the COI barcode library. While at lower resolution than for some other metabarcoding studies, this approach is more accurate than simply using order level classifications but also allows the inclusion of prey items in the diet that could not be identified to genus or species level. In addition, most prey items within a family are generally of a similar size range and shape, which birds may use in their prey choice decisions.

6.3.2. Recommendations for dietary studies

Several recommendations can be made for future work. Firstly, studies aiming to characterise diet should make use of long-term data wherever possible, especially within single study areas. The vast majority of conservation management plans are based on data from a single year or season or multiple years pooled together (Marzluff and Sallabanks 1998) which may be too simplistic or misleading, since annual variation in resource availability and the resulting diets of consumers is likely (Hejl and Vermer 1990, Miles 1990, Szaro et al. 1990, Miller and McEwen 1995, Durst et al. 2008). Future studies on warblers could also combine tracking techniques to identify foraging events by birds and record the feeding habitats used. Aside for allowing a more detailed understanding about horizontal and vertical separation of feeding, which might contribute to dietary partitioning, this approach should help pinpoint important foraging habitats for breeding birds which can be promoted through conservation and habitat management.

The use of a more integrative approach that combines metabarcoding data with traditional methods such as direct observations of feeding, morphological detection of items in faecal samples and stable isotope analysis is recommended, to fill the knowledge gaps about life stage consumed, and to allow more accurate measurements of prey size to be analysed. This dual approach has been used for example to analyse faecal samples of seabirds (Alonso et al. 2014, Waap et al. 2017). Nutrient content of different prey types could also be analysed to give a clearer measure of the contribution of each dietary item to the dietary choices and fitness of the bird, as has been recently demonstrated in spider predators (Cuff 2020). This would allow us to test further theories regarding optimal foraging.

I also recommend using a combination of multiple primer pairs in the molecular methodology that can detect a wider range of prey, closer to the maximum prey items present (Pompanon et al. 2012, Jusino et al. 2016). When characterising the diets of other insectivorous migratory songbirds that exhibit frugivory at certain times of the year (e.g. blackcaps (*Sylvia atricapilla*), whitethroats (*S. communis*) or omnivorous birds that capture a very wide range of prey taxa (e.g. fish and invertebrates) the use of multiple barcoding markers (e.g. COI alongside ITS2 or 16S or multiple COI primer sets), allows multiple components of diets (e.g. plants, invertebrates and vertebrates) to be ascertained simultaneously (e.g. Giebner et al. 2020). Great reed warblers occasionally feed on small vertebrates such as amphibians, reptiles and fish, (Cramp and Brooks 1992) but due to financial and time constraints it was only possible to report invertebrate diet for the bird species studied in this project. As well as providing additional nutritional advantages, incorporation of vertebrates into avian diet may provide another layer of resource partitioning that this project was unable to investigate. Thus, for future studies analysing the diet of the great reed warbler, it is advised that multiple primer sets, or barcode regions are used, in combination (e.g., Deagle et al. 2009, Rayé et al. 2011), to elucidate both invertebrate and vertebrate prey items taken.

Some of the discrepancies observed between the invertebrates captured on sticky traps and the invertebrates consumed by birds may be due to limitations in the trapping methods used to measure the abundance of prey items available to insectivorous passerines (Hutto 1990, Wolda 1990, Durst et al. 2008, discussed in data Chapters). I therefore recommend the combined use of several monitoring techniques that can target a wider array of prey taxa and compensate for the biases of each method alone. Trapping methods could combine passive sampling (such as water traps, malaise traps or sticky traps) with active sampling methods (e.g., suction sampling, netting, beating).

Finally, this PhD project compared diets across a series of sites in two main climate zones, the UK with its temperate, maritime climate and Catalonia with its Mediterranean climate. Gaps still exist in our knowledge of prey availability and the dietary choices of birds in the intermediate zones between these two regions. Thus, to generate a more comprehensive set of predictions about future climate change impacts on bird diet using “space for time”, in any continuing studies, effort should be focused on collecting samples from warblers in central and northern France. These extra sampling points on the “space for time” climate transect, will allow the advancement of finer predictions about how climate change will impact birds in the UK. The importance of replicates at each location wherever economically and practically feasible, is also stressed, to better infer local *versus* regional dietary changes due to shifts in climate.

6.4. Implications for warblers and their competitive interactions under future climate change

Undoubtably climate change will cause significant changes to ecosystems, communities and interactions between species. Few studies have considered the effects of climate change on species interactions, and the research conducted here provides a novel insight into future scenarios of food availability, diet selectivity and possible interspecific competition in communities of wetland warblers. The results of this PhD concur with Halupka et al. (2008) and Vafidis (2014) in that spring temperatures in the UK are thermally limiting for reedbed warbler productivity. Enhanced spring temperatures as a result of ongoing climate change will continue to drive earlier emergences of prey (Vafidis 2014, Vafidis et al. 2016, Sardiña et al. 2017). Initially this should benefit reedbed warblers, as warmer conditions on the breeding grounds permit earlier nesting and supply a greater abundance of prey during reproduction, when prey for nestlings is in high demand. Recently, reed warbler reproductive success and productivity has already increased both locally (Halupka et al. 2008) and across much of central and northern Europe (Eglington et al. 2015). Increases in prey abundance as a result of warmer summers might allow continued sharing of abundant prey resources by sympatric warblers, with high overlap masking resource partitioning.

However, future changes in climate are likely to be much more complex and extreme than simply a gradual increase in mean temperatures (Jiménez et al. 2018). It is forecast that high variation in mean maximum temperatures will be experienced worldwide, which might exceed the optimum temperature tolerance for many invertebrates (Johansson et al. 2020). Stochastic fluctuations in environmental conditions can drive species to extirpation from an area, and thus are viewed as a risk factor for populations (Lande 2003).

Future productivity of warblers may be subject to seasonal and annual fluctuations, as hot dry spells become more common, but also as extreme weather events such as storms and flooding alter the prey community and increase nestling mortality. In southern Europe, the impacts of long-lasting drought on more vulnerable marshland birds that rely on continuously flooded habitats are likely to be even more severe (Jiménez et al. 2015), and an overall loss of biodiversity of wetland specialist bird species is a real possibility (Filipe et al. 2013, Kingsford et al. 2016).

As inferred from the findings of this work, future warming in the coming decades is expected to drive stronger resource partitioning in sympatric warbler species in European wetlands. With ever warmer conditions, and increasing reedbed drought, the prey base may become depleted, leading to greater dietary overlap between birds which could result in stronger competition for access to prey at least during periods where recruitment from insects is low. Birds may be able to continue to avoid or reduce the intensity of competition by becoming more selective in their prey choices, but they may lose the advantage of plasticity that is afforded by being generalist with a broad dietary niche. It is likely then that selection will continue to favour generalists that can consume different prey groups opportunistically as they become available.

Responses to competition could result in extirpation of one or more warbler species locally or the movement of ranges further north. Patterns of dominance may also reveal why some species fare better than others under climate change. For example, reed warblers are known to oust sedge warblers (*Acrocephalus schoenobaenus*) from breeding habitats (Brown and Davies 1949, Catchpole 1972), but their northern range limit is further south than that of sedge warblers. Climate change has caused a northwards range expansion and increased productivity by the reed warbler (Eglington et al. 2015). Possibly they have moved into areas previously only occupied by the sedge warbler and this might increase competitive pressure and have adverse effects on sedge warblers.

The RSPB “Climate Atlas” report by Huntley et al. (2007) states that conditions in the UK are likely to become favourable for colonisation from southern European passerine species, which may establish new breeding populations. The great reed warbler has the potential to colonise the UK in future years (Huntley et al. 2007), which could disrupt current patterns of dominance in UK warbler communities. The added competitive pressure of coexisting with a larger competitor, may have a detrimental effect on the smaller warblers in this study. However, reed warblers can form dense populations in UK wetlands and might be constraining the productivity of other warblers, notably sedge warblers (Cramp and Brooks 1992). Thus, an alternative outcome, if great reed warblers are able to suppress the productivity of reed warblers in the Mediterranean as the results of Chapter 5 suggest, the establishment of great reed warblers in the UK might facilitate competitive release, and improve the productivity of sympatric warbler species, i.e., sedge and Cetti’s warblers. Further work would be needed to fully investigate this theory, by combining dietary competition work with comparative observations, population trends and productivity estimates.

6.5. Conservation implications for breeding songbirds in wetlands

Although the majority of warbler species in this study are not currently classed as vulnerable or red-listed, the possibility that under future climate change many populations of these birds could suffer declines, particularly in the southernmost part of their ranges cannot be ruled out. Future northwards expansion of distributions is thus expected, which may bring with it new competitive scenarios.

Aside from mediating prey availability and indirectly driving competition, climate may have more direct impacts on survival rates, leading to changes in population dynamics. The sedge warbler is extremely sensitive to rainfall patterns on its wintering grounds in the Sahel and has suffered recent declines due to severe drought and erratic rainfall, as a consequence of climate change (Foppen et al. 1999). Similar declines have also been documented in willow warblers (*Phylloscopus trochilus*) and other trans-Saharan migrants (Morrison et al. 2013, 2016).

Much of the literature on climate change adaptation practises by conservationists advocate accommodating irreversible changes that are likely to occur, to best enable species and habitats to shift their ranges either in a pole-wards direction, or to higher altitudes as they respond to warming (Vos et al. 2008, Willis et al. 2009). This approach is unlikely to be beneficial for species whose ranges are already at high-latitude or high-elevation, as they are constrained by available space to shift their distributions onto. Careful management to improve the resistance of these species and habitats to future changes is likely to be the only available option (Pearce-Higgins et al. 2011, Carroll et al. 2011). Local strategies should aim to promote the biodiversity and abundance of invertebrate populations by improving existing habitat or creating new, varied habitats, while regional and nationwide responses should implement policy to better protect ecosystems from inappropriate land management that results in degradation, fragmentation and loss.

By prioritising invertebrate conservation, a supply of abundant and diverse prey in ecosystems will continue to be available, dispersing outside of their immediate habitat to benefit insectivores across entire landscapes (Baxter et al. 2005). With proper management intervention, freshwater habitats may act as refuges from climate change and promote high landscape biodiversity (Chester and Robson 2013). Thus, a greater focus on maintaining and where necessary, enhancing the quality, heterogeneity and connectivity of aquatic habitats will be required for management strategies to be effective in alleviating the effects of fragmentation and loss of standing water zones with future drought coupled with ongoing habitat loss and destruction of wetlands (Natural England and RSPB, 2019).

Conservation efforts that focus on aquatic habitat restoration such as the creation of ponds (Sayer et al. 2012, Sayer 2013, Sayer et al. 2014, Davies et al. 2016, Lewis-Phillips et al. 2019, 2020), planting of buffer strips around aquatic habitats (Josefsson et al. 2013, Davies et al. 2016) and careful adaptive management of water levels e.g. by blocking drainage ditches (Carroll et al. 2011) will become more important in future years and deserve greater attention in both research and application. Care should be taken when manipulating reedbed water levels, due to the risk of sudden inundation negatively affecting communities of invertebrates (Kirby 1992, Burgess et al. 1995, Bedford and Powell 2005). The effectiveness of these measures should be assessed regularly via monitoring schemes to ensure practises are beneficial to the wider ecological community.

6.6. Advances in understanding the impact of climate change on species-interactions

To my knowledge, this PhD research is the first to predict the effects of climate change on a whole assemblage of invertebrates and insectivorous birds across a climate gradient and infer future potential competitive interactions between sympatric consumers, mediated by changes to prey resources, using the precision and fine-scale resolution of dietary metabarcoding, alongside field methods to monitor invertebrates. I promote the use of dietary metabarcoding to ecological studies and highlight the utility of such applications for studies across environmental gradients using “space for time”. Such approaches could be applied across a wide range of systems, enabling broad predictions to be made about the interacting responses of species to future climate change.

Specifically, these findings demonstrate that an improved insight into drivers of diet composition, selectivity and overlap of songbird species in one location (i.e., the UK) can be achieved by studying multiple sites across space (and time) to predict future patterns under changing environmental conditions. The results have implications for understanding the productivity of reedbed warblers under future climate change in both the UK and Catalonia, highlighting the importance of overall habitat quality and heterogeneity and also the provision of aquatic habitats within sites to provide a dependable and abundant food supply that can support multiple species of insectivores from the wider landscape.

Appendix One – Supplementary information relating to Chapter 2

Appendix 1.1. COI General Invertebrate Primer testing

Table A.1.1. Results of primer testing on a wide array of invertebrate taxa. Amplification success of extracted DNA from invertebrates by multiplex PCR using the COI primer pair MICO1intF and Nancy, with an annealing temperature of 55°C. Testing during this PhD project (of all samples shown below) was carried out by Sarah Davies (SD). Previous testing on a wider range of invertebrates was carried out by Jennifer Stockdale (see Stockdale 2018). Results were visualised on a 2% agarose gel and successful amplification was confirmed by the presence of a strong band under UV light proceeding electrophoresis. N = no amplification, S = slight amplification, Y = strong amplification.

Sample ID	Order	Family (if known)	Description	Amplification
RW1	Passeriformes	Acrocephalidae	Reed warbler feather 1	N
SW2	Passeriformes	Acrocephalidae	Sedge warbler feather 2	N
Helix A	Gastropoda	Helicidae	Freshwater snail	S
CY2	Neuroptera	Chrysopidae	Lacewing	Y
INV5	Aranae	-	Spider	N
INV1	Diptera	-	Fly	Y
INV4	Hymenoptera	Apidae	Bee	Y
Physidae	Gastropoda	Physidae	Freshwater snail	Y
Scritidae	Coleoptera	Scritidae	Water beetle	Y
L.terr	Oligochaeta	Lumbricidae	Worm	Y
INV23	Dermaptera	-	Earwig	Y
INV20	Orthoptera	-	Cricket	Y
LG1	Hemiptera	Aphididae	Aphid	Y
104B1	Ephemeroptera	Baetidae	Baetis mayfly	Y
Chiron	Diptera	Chironomidae	Chironomid	Y
Dinocras	Plecoptera	Perlidae	Stonefly	S
OV1	Collembola	Entomobryidae	Springtail	Y
Damsel1	Odonata	Coenagrionidae	Common blue/ azure damselfly	Y
Moth1	Lepidoptera	Erebidae	White moth	Y
Bug1	Hemiptera	Cicadellidae	Leaf hopper	S
Harvest1	Opiliones	-	Harvestman	N
Hover1	Diptera	Syrphidae	Syrphid hoverfly	Y
Brachy1	Diptera	-	Brachyceran fly	Y
Wasp1	Hymenoptera	Ichneumonidae/ Braconidae	Large parasitic wasp	Y
Spider1	Araneae	-	Medium sized spider	Y
Caddis1	Trichoptera	-	Caddisfly	N
Black slug	Gastropoda	-	Slug	S
S.olens	Coleoptera	Staphylinidae	Devils coach horse beetle	Y
Spider 3	Aranae	Lycosidae	Lycosid spider	Y

Spider 4	Aranae	-	Spider	Y
Spider 5	Aranae	Clubionidae	Clubionid spider	S
Spider 6	Aranae	Dysteridae	Dysterid spider	N
Spider 7	Aranae	Philodromidae	Crab spider	Y
Beetle 1	Coleoptera	-	Large beetle	Y
Beetle 2	Coleoptera	Coccinellidae	Ladybird larvae	Y
Beetle 3	Coleoptera	-	Elongated beetle with large abdomen	Y
Beetle 4	Coleoptera	Cantharidae	Soldier beetle	Y
Beetle 5	Coleoptera	Elateridae	Click beetle	Y
Brachy2	Diptera	-	Brachyceran fly	Y
Brachy3	Diptera	-	Large fly	Y
Brachy4	Diptera	Tabanidae	Large tabanid	Y
Nemo1	Diptera	Bibionidae	St Mark's fly	Y
Nemo2	Diptera	Chironomidae	Medium chironomid	Y
Dip1	Diptera	-	Small-medium fly	Y
Moth2	Lepidoptera	Crambidae	Reed boring moth larvae	Y
Arth1	Orthoptera	Tettigoniidae	Bush cricket	Y
Mec1	Mecoptera	Panorpidae	Scorpionfly	N
Moth 3	Lepidoptera	-	Green caterpillar	Y
Nemo3	Diptera	Chironomidae	Chironomid	Y
Snail1	Gastropoda	-	Small-medium snail, striped	Y
Wasp 2	Hymenoptera	Ichneumonidae/Braconidae	Parasitic wasp	Y
Wasp 3	Hymenoptera	Ichneumonidae/Braconidae	Parasitic wasp	S
Hydropsyche	Trichoptera	Hydropsychidae	Caddisfly	Y
Beetle 7	Coleoptera	Carabidae	Medium black carabid beetle	Y
Beetle 10	Coleoptera	Cantharidae	Medium red soldier beetle	Y
Bug 5	Hemiptera	Lygaeidae	Lygaeid bug	S
Bug 2	Hemiptera	Aphididae	Aphid	Y
Arth 3		-	Elongated arthropod, unknown order	Y
Dip2	Diptera	-	Small-medium fly	Y
Brachy 5	Diptera	-	Large brachyceran	Y
Brachy6	Diptera	Chloropidae/Anthomyzidae	Small brachyceran	S
Bug6	Hemiptera	Aphididae	Aphid	Y
INV2	Diplopoda	-	Millipede	Y
Pico 1:2:1	Annelida	-	Worm	Y

Appendix 1.2. Modifications to the QIAGEN QIAmp® DNA Stool Mini Kit protocol

DNA extraction from warbler faecal material was carried out following the standard protocol, including all recommended steps with modifications by Zeale et al. 2011, Nicholls 2017 (pers comm) and Sarah Davies (myself). The following modifications were used;

i) Uric acid was removed from each stool sample by scraping the sides of the faecal pellet. Either the whole pellet, or up to 220mg of the pellet (if sample is large) was used in the extraction. Samples were removed from ethanol and left to air dry before being added to a new microcentrifuge tube.

ii) 500µL of InhibitEx Buffer was added to each stool sample, then the sample was mixed manually using a pestle for 20 seconds before adding a further 500µL of InhibitEx Buffer. The samples were then homogenized by vortexing for 3-4 minutes and incubating for 10-15 minutes in a water bath at 70°C. Samples were vortexed again for 2 minutes to resuspend.

iii) 20µL of proteinase K, 400µL of supernatant and 400µL Buffer AL was added to a new tube in step 5 and samples were incubated at 70°C for 20 minutes before adding 400µL molecular grade (96-100%) ethanol.

iv) 80µL of Buffer ATE (or AE) was added to each spin column membrane, or 50-60µL for samples with small amounts of faecal material. Samples were incubated at room temperature for 5-10 minutes and then centrifuged at full speed for 1 min to elute DNA. To increase DNA yields we repeated this step by transferring the eluate back into the original spin column within a new tube and centrifuged at full speed for another 1 min before eluting DNA.

Appendix 1.3. List of forward and reverse MID-tag oligos used for metabarcoding

FF02	ACGCTCGACA	RR01	ACTAGCAGTA
FF03	AGACGCACTC	RR04	TGTGAGTAGT
FF04	AGCACTGTAG	RR05	TGACGTATGT
FF05	ATCAGACACG	RR07	TCTAGCGACT
FF06	ATATCGCGAG	RR08	TCGCACTAGT
FF07	CGTGTCTCTA	RR09	TCGATCACGT
FF08	CTCGCGTGTC	RR10	TAGTGTAGAT
FF10	TCTCTATGCG	RR11	TACGCTGTCT
FF11	TGATACGTCT	RR12	TACAGATCGT
FF13	CATAGTAGTG	RR13	TACACGTGAT
FF15	ATACGACGTA	RR14	TACACACACT
FF16	TCACGTACTA	RR15	CGACGTGACT
FF17	CGTCTAGTAC	RR16	CAGTAGACGT
FF18	TCTACGTAGC	RR17	CACGCTACGT
FF19	TGTACTACTC	RR18	ATAGAGTACT
FF20	ACGACTACAG		
FF21	CGTAGACTAG		
FF22	TACGAGTATG		
FF23	TACTCTCGTG		
FF24	TAGAGACGAG		

FF25	TCGTCGCTCG
FF26	ACATACGCGT
FF27	ACGCGAGTAT
FF30	AGACTATACT
FF31	AGCGTCGTCT

Appendix 1.4. Mock community composition

Table A.1.4. Mock community mixes included in the HTS run. Communities were composed of DNA samples from tissue extracts from the invertebrates listed below. All invertebrate DNA sample concentrations were standardised at 0.1 ng/µL^{-1} by diluting the DNA in DNase-free water. Values indicate the volume in microlitres (µL) of each DNA sample added to the mock community mix. The bottom row indicates the final total volume of each mix. Green background indicates species from each mix that were subsequently detected with high-throughput sequencing (after all bioinformatics and data clean-up steps), red indicates species that were not detected.

i) Composition of mock community mixes 1-5: DNA extracts from invertebrates collected from farmland.

		Volume added to mix (µL)				
Taxon Description	ID	Mix1	Mix2	Mix3	Mix4	Mix5
Springtail	Unknown sp.	1	1	3	1	2
Assassin bug	*Anthocoris nemorum*	1	2	3	1	1
spider	*Erigone dentipalpis*	1	1	3	1	2
wasp	*Trichopria sp.*	1	2	3	1	1
spider	Unknown sp.	3	1	1	1	2
aphid	*Utamphorophora sp.*	3	2	1	1	1
parasitoid	*Promethes sulcator*	3	1	1	1	2
springtail	*Orchesella villosa*	1	2	3	1	1
	Total	14	12	18	8	12

ii) Composition of mock community mixes 6-10; DNA extracts from reedbed invertebrates collected from field sites.

		Volume added to mix (µL)					
Taxon Description	ID	Mix6	Mix7	Mix8	Mix9	Mix10	Notes
Hoverfly	*Platycheirus clypeatus*	1	1	3	1	2	
Moth (imago)	*Euproctis similis*	1	2	3	1	1	
Wasp	*Aphelinus sp.*	1	1	3	1	2	
Chironomid	*Tvetentia clavenscens*	1	2	3	1	1	

Brachyceran Fly	*Sphaeroceridae sp.*	3	1	1	1	2	Present in one positive control
Crab spider	*Philodromus aureoles*	3	2	1	1	1	
Wolf spider	*Pardosa palustris*	3	1	1	1	2	Present in one positive control
Aphid	*Metopolophium dirhodum*	1	2	3	1	1	
	Total	14	11	18	8	12	

Appendix 1.5. Repeated samples

Table A.1.5. List of dietary items detected in the same faecal samples duplicated with new MID-tag combinations (repeats) after bioinformatic screening and data clean-up. Only original samples were included in subsequent data analyses.

Sample ID	MID-tag combination	No. Prey Items	No. Shared Prey Items	Shared Species	Unique Species
S483225 (original)	F2 R16	12	5	*Scathophaga sp.* *Clinohelea unimaculata* *Culiseta morsitans* *Culicoides halophilus*	*Lenisa geminipuncta* *Aphidius urticae* *Pterocomma sp.* *Deroceras reticulatum* *Prionocera subserricornis* *Sepsis punctum* *Allodia ornaticollis* *Prodiamesa olivacea*
S483225 (repeat)	F15 R16	8	5	*Scathophaga sp.* *Arion sp.* *Clinohelea unimaculata* *Culiseta morsitans* *Culicoides halophilus*	*Succineidae sp.* *Stagnicola fuscus* *Chrysoperla carnea*
S534204 (original)	F25 R13	11	10	*Tetragnatha pinicola* *Clubiona phragmitis* *Oulema melanopus* *Cladotanytarsus atridorsum* *Neria commutata* *Helina impuncta* *Ilione albiseta* *Notus sitka* *Brachmia blandella* *Psocoptera sp.*	*Bicellaria vana*
S53204 (repeat)	F25 R14	13	10	*Tetragnatha pinicola* *Clubiona phragmitis* *Oulema melanopus* *Cladotanytarsus atridorsum* *Neria commutata* *Helina impuncta* *Ilione albiseta* *Notus sitka* *Brachmia blandella* *Psocoptera sp.*	*Tenuiphantes tenuis* *Sylvicola sp.* *Alysiinae sp.*

Sample ID	MID-tag combination	No. Prey Items	No. Shared Prey Items	Shared Species	Unique Species
R425264 (original)	F6 R12	2	2	*Polypedilum sordens* *Triaenodes bicolor*	
R425264 (repeat 1)	F15 R17	4	2	*Polypedilum sordens* *Triaenodes bicolor*	*Pseudoperichaeta nigrolineata* *Succineidae sp.*
R425264 (repeat 2)	F2 R18	1	1	*Triaenodes bicolor*	
JVD054 (original)	F24 R13	12	9	*Dictyna arundinacea* *Crepidodera fulvicornis* *Cryptocephalus pusillus* *Galerucella lineola* *Chaoborus flavicans* *Endochironomus albipennis* *Helina setiventris* *Lomaspilis marginata* *Orthosia incerta*	*Empoasca sp.* *Operophtera brumata* *Archips rosana*
JVD054 (repeat)	F24 R14	11	9	*Dictyna arundinacea* *Crepidodera fulvicornis* *Cryptocephalus pusillus* *Galerucella lineola* *Chaoborus flavicans* *Endochironomus albipennis* *Helina setiventris* *Lomaspilis marginata* *Orthosia incerta*	*Lochmaea sp.* *Oncopsis speciosa*

Appendix 1.6. Shell and perl scripts for metabarcoding data used in the bioinformatics pipeline

The following scripts were written by Drake 2020 (modified from Helen Hippterson at NBAF, University of Sheffield). The entire pipeline was repeated for each indexing pool (pool 1 shown).

Script 1 – Trimming and aligning paired reads to generate complete amplicon sequence

```
## we will do FastQC quality check, merge the paired end reads and trim the sequences in one go using FastP to get the complete amplicon sequence
```

```
/mnt/data/GROUP-sabwocs/c1618864/fastp -i SD-CO1-1-290519_S1_L001_R1_001.fastq -I SD-CO1-1-290519_S1_L001_R2_001.fastq -l 200 -m --discard_unmerged -o pool1merged.fastq
```

```
## next convert the fastq file to fasta format
```

```
module load fastx_toolkit/0.0.14
```

```
fastq_to_fasta -i pool1merged.fastq -Q 33 -o pool1merged.fa
```

Script 2 – Allocate MID-tag combinations to their respective samples and remove primer sequences

```
## we will identify the sequences that match the oligos used, allowing for 1 mismatch. oligos =
text file where the first column reads #‘primer’, the second and third columns are the forward and
#reverse primer and MID-tag combinations for a particular #sample, and the
fourth column is the sample ID annotated with #an additional ‘a’ or ‘b’. ‘a’ is used when the
forward primer #is in column 2 and the reverse is in column 3. ‘b’ is used #when this order is
reversed. This means that the total number #of rows should be twice the number of samples.


#Run Mothur

module load mothur/1.39.5

mothur "#trim.seqs(fasta=merged.fa,oligos=oligo1.txt,checkorient=t,pdiffs=1)"


#split .groups file into A and B

grep 'a$' merged.groups > mergedA.groups

grep 'b$' merged.groups > mergedB.groups


#remove 'a' and 'b' labels

sed -i 's/a//g' mergedA.groups

sed -i 's/b//g' mergedB.groups
```

Script 3 – Demultiplexing

Part 1. Perl script

```
#!/usr/bin/perl

# fastalist1.txt is a text file that is identical to the fourth column of the oligos file described in
Script 3


unless ($#ARGV == 0)


{


   print "Usage: 3_Demultiplex.pl fastalist1.txt";


die;
```

```
}


open (INLIST, "<$ARGV[0]") || die;

# replace 'XXX' with your username, and if you want to put the output into another directory you can add that to the 'outdir' path here
$indir = "/mnt/data/GROUP-sabwocs/c1618864/pool1/deplexed";
$outdir = "/mnt/data/GROUP-sabwocs/c1618864 /pool1/deplexed";

# Loops through the list for your samples ('SampleList') and performs the commands for each one
while (<INLIST>) {
$lib = $_;
chomp($lib);

# A shortcut to read or write a file for each of your samples, each file having the same extension
$readidsa = $lib . "_a_ids.txt";
$readidsb = $lib . "_b_ids.txt";
$readidsab = $lib . "_ab_ids.txt";

$fa1 = $lib . ".fa";
$fa2 = $lib . ".fasta";

# split fasta read IDs into files grouped by sample ID. Replace 'XX' with the name of you '.groups' file (output from mothur)
system("grep -w $lib $indir/mergedA.groups | awk '{print \$1}' > $outdir/$readidsa");
system("grep -w $lib $indir/mergedB.groups | awk '{print \$1}' > $outdir/$readidsb");

# combine the list of sequence names for 'a' and 'b' matches
system("cat $outdir/$readidsa $outdir/$readidsb >> $outdir/$readidsab");

# split the trimmed fasta file into reads specific to each sample. Replace 'XX' with the name of your trimmed fasta file (output from mothur)
```

```
my $command1 = 'perl -ne'."'".'if(/^>(\S+)/){$c=$i{$1}}$c?print:chomp;$i{$_}=1 if'."
@ARGV'"." $outdir/$readidsab $indir/merged.trim.fasta > $outdir/$fa1";
system ($command1);


system("awk '{print \$1}' $indir/$fa1 > $indir/$fa2");


}


exit;
```

Part 2. Shell script

```
perl 3_Demultiplex.pl fastalist1.txt
```

Script 4. Editing header information for each sample

Part 1. Perl script.

```
#!/usr/bin/perl


unless ($#ARGV == 0)
{


  print "Usage: 4_Edit_Headers.pl fastalist1.txt";


die;
}


open (INLIST, "<$ARGV[0]") || die;


$indir = "/mnt/data/GROUP-sabwocs/c1618864/pool1/deplexed/fastafiles";


$outdir = "/mnt/data/GROUP-sabwocs/c1618864/pool1/deplexed/fastafiles";


while (<INLIST>) {
$lib = $_;
```

```
chomp($lib);

$fa1 = $lib . ".fasta";
$fa2 = $lib . "_edit.fasta";

system( qq(sed "s/^>/>$lib;/g" "$indir/$fa1" > "$indir/$fa2"));

}

exit;
```

Part 2. Shell script

```
perl 4_Edit_Headers.pl fastalist1.txt
```

Script 5 - Concatenate all sequences into one file

```
cat *edit.fasta > Allmerged.fasta
```

Script 6 – USEARCH

```
# removes identical replicates from the fasta input, output for next step =SampleName_rc_uniques.fasta
/mnt/data/GROUP-sabwocs/c1618864/pool1 -fastx_uniques pool1.fasta -fastaout Unique.fasta -sizeout -strand both -relabel Uniq -threads 4

# sort by size
/mnt/data/GROUP-sabwocs/c1618864/pool1 -sortbysize Unique.fasta -fastaout Sorted.fasta

# Cluster OTUs
/mnt/data/GROUP-sabwocs/c1618864/pool1 -cluster_otus Sorted.fasta -otus OTU.fasta -relabel Otu

# denoise and cluster using unoise3 to make zOTUs
/mnt/data/GROUP-sabwocs/c1618864/pool1 -unoise3 Sorted.fasta -zotus zOTU.fasta
```

```
# make matrix of zOTU's and the number of sequences per zOTU (size)

/mnt/data/GROUP-sabwocs/c1618864/pool1 -otutab pool1.fasta -zotus zOTU.fasta -otutabout zOTUtable_COI.txt -strand both -threads 4


# make matrix of OTU's and the number of sequences per OTU (size)

/mnt/data/GROUP-sabwocs/c1618864/ pool1 -otutab pool1.fasta -otus OTU.fasta -otutabout OTUtable_COI.txt -strand both -threads 4
```

Script 7. BLAST

```
# blast the clusters from usearch


module load blast/2.7.1


export BLASTDB=/mnt/data/GROUP-sabwocs/c1618864/pool1/BLAST-DB


blastn -query zOTU.fasta -db nt -num_threads 4 -evalue 0.00001 -perc_identity 97 -outfmt 6 -out zOTU.blastOutput.txt


blastn -query OTU.fasta -db nt -num_threads 4 -evalue 0.00001 -perc_identity 97 -outfmt 6 -out OTU_blastOutput.txt
```

Script 8. Filter the BLAST results

```
# only keep results with over 95% identity and remove and sequences with less than 100bp in length


awk ‘$3 >= 95’ OTU_blastOutput.txt | awk ‘$4 >= 100’ > OTU_blast_filtered.txt.

awk ‘$3 >= 95’ zOTU_blastOutput.txt | awk ‘$4 >= 100’ > zOTU_blast_filtered.txt.
```

Script 9. Add taxon information to diet zOTU matrix (R-script)

```
#Add in taxon information to your zOTU and OTU tables: Open R and run the following code on your blast output to get only the top hit for each motu based on bitScore (combination of e-value and percentage identity):


>blast <- read.table("zOTU_blastOutput.txt")

>summary(blast)
```

```
>library(dplyr)

>blast_filter <- blast %>%

 group_by(V1) %>%

filter(V12 == max(V12))

>write.table(blast_filter, "pool1_zOTU_TopHit_blastOutput.txt")
```

```
#Next use the program MEGAN to assign ids to each zOTU from the BLAST top hit output.
#Use VLOOKUP in Excel to add taxon ids to each zOTU in the diet matrix.
#Calculate maximum contamination/tag jumping from NAs and negative controls and remove the same value from all reads along each row. Convert negative values to 0.
#Remove all reads with a read count of less than 10.
#Remove zOTUs that are at their highest read count in positive controls and mock communities from the remaining diet matrix.
#Remove non-dietary data
#Convert matrix to csv file for aggregating in R.
```

Script 10. Aggregate zOTUs in diet matrix based on taxon id (R-script)

```
>pool1_to_Agg <- read.csv(“zOTUtable_COI.csv”, header = T)

>Agg <- aggregate(.~Taxon, data=pool1_to_Agg, sum)

>write.table(Agg, “pool1_Aggregated.csv”)
```

Appendix 1.7. Taxa removed from the metabarcoding datasets

Table A.1.7. Species removed from the dietary datasets during the data clean-up steps subsequent to sequence identification with BLAST against reference sequences on BOLD. The taxon species name, common name is given along with the respective reasons for removal and GenBank accession code.

Taxon	Common Name	Reason for removal	Accession Code
Arthrodermataceae sp.	Fungi	Not dietary	MG592681.1
Cladosporium sp.	Fungi/mould	Not dietary	FJ590524.1
Cercospora sojina	Fungal plant pathogen	Not dietary	KC888822.1
Penicillium chrysogenum	Pennicilin fungus	Not target taxa	AM920464.1
Penicillium cinnamopurpureum	Penicillin fungus	Not target taxa	FJ004561.1
Penicillium citrinum	Penicillin fungus	Not target taxa	EF180187.1
Penicillium janthinellum	Penicillin fungus	Not target taxa	FJ004537.1
Penicillium nordicum	Penicillin fungus	Not target taxa	KR952336.1
Penicillium polonicum	Penicillin fungus	Not target taxa	KU530219.1
Penicillium sp.	Penicillin fungus	Not target taxa	FJ004537.1, KU530219.1
Saprolegnia ferax	Water mould	Not target taxa	KM361513.1
Bremia sonchicola	Brown algae	Not dietary	MF687314.1
Pernospora aparines	Oomycete	Not target taxa	HM033187.1
Pernospora romanica	Oomycete	Not target taxa	KJ654123.1
Achlya hypogyna	Oomycete	Parasite, not target taxa	KF226724.1
Pythium sp.	Oomycete	Not target taxa	JN660054.1
Oomycetes sp.	Oomycete	Parasite, not target taxa	HQ708212.1
Leptolegnia sp.	Oomycete	Not target taxa	HQ708212.1
Proctophyllodes sylviae	Feather mite	Not dietary	KU203163.1
Trouessartia trouessarti	Feather mite	Not dietary	KP193817.1
Steinernema intermedium	Nematode of invertebrates	Not dietary	JN808126.1
Arenicola marina	Sandworm	Present only in positive controls	HQ691225.1
	Invertebrate environmental sample	Unclear identification	LC222761.1
Tetrastemma candidum	Ribbon worm	Not dietary	AY791973.1
Eratigena duellica	Giant house spider	Known lab contaminant - present in positive controls only	LT970989.1
Pterostichus melanarius	Strawberry ground beetle	Known lab contaminant – present in positive controls only	DQ063219.1
Blattella germanica	German cockroach	Known lab contaminant	EU854321.1
Pacifastacus leniusculus	Signal crayfish	Present only in positive controls	KY947333.1
	Zooplankton environmental sample	Not dietary	KC732416.1
Acanthamoeba sp.	Amoeba	Pathogen, not dietary	MG924682.1
Nitzschia palea	Marine diatom	Not dietary	AP018512.1

Appendix 1.8. Invertebrate families that were combined in the *econullnetr* analysis

Table A.1.8. List of invertebrate families identified from sticky traps that were combined in the *econullnetr* analysis. An abbreviated name for each merged taxa is shown with the families included and the taxonomic order indicated. Reasons for merging are given under morphological characteristics.

Abbreviation	Families combined	Order	Morphological characteristics
Anthocor/Rhyparochromidae	Anthocoridae Rhyparochromidae	Hemiptera	Similar body plan, coloration, difficult to separate
Adrenidae/Halictidae	Adrenidae Halictidae	Hymenoptera	Both members of Anthophila, similar morphology and wing venation
Heleomyz/Diastatidae	Heleomyzidae Diastatidae	Diptera	Both acalyptratae flies, colouration similar, superficial resemblance
Lauxanii/Drosophilidae	Lauxaniidae Drosophilidae	Diptera	Both acalyptratae flies, similar coloration, superficial resemblance
Musc/Fann/Anthomyiidae	Muscidae Fanniidae Anthomyiidae	Diptera	Calyptratae flies of the superfamily Muscoidea – similar wing venation and body plan
Opomyz/Tephritidae	Opomyzidae Tephritidae	Diptera	Both acalyptratae flies, similar wing markings, superficial resemblance
Pteromalid/Torymidae	Pteromalidae Torymidae	Hymenoptera	Both members of the Chalcidoidea, metallic green colouration, similar wing venation and antennae

Appendix 1.9. % Frequency of occurrence tables for dietary items detected in the five warblers in this chapter.

Table A.1.9.*a*. Top 30 most frequently detected dietary items (prey species) in diet samples for each warbler species in the UK. (RW = reed warbler, SW = sedge warbler, CW = Cetti's warbler, CC = chiffchaff, WW = willow warbler) and all warbler species combined. Values shown are percent frequency of occurrence (% FOO). Cells are colour coded based on % FOO with a gradient from low (paler tone) to high (deeper tone).

Order	Family	Prey Species	All	RW	SW	CW	CC	WW
Diptera	Chaoboridae	*Chaoborus flavicans*	79.84	50.49	38.52	38.71	55.56	51.72
Diptera	Chironomidae	*Cladotanytarsus atridorsum*	45.97	27.67	24.44	22.58	40.74	20.69
Araneae	Clubionidae	*Clubiona phragmitis*	34.27	24.27	17.78	35.48	0	0
Diptera	Chironomidae	*Chironomidae sp.*	32.66	24.76	8.15	9.68	22.22	34.48
Diptera	Chironomidae	*Endochironomus albipennis*	27.02	13.59	17.78	6.45	29.63	17.24
Coleoptera	Chrysomelidae	*Crepidodera fulvicornis*	20.16	12.14	7.41	0	11.11	41.38
Diptera	Scathophagidae	*Scathophaga stercoraria*	17.74	10.19	6.67	29.03	14.81	3.45
Diptera	Chironomidae	*Chironomus cingulatus*	16.94	8.25	9.63	6.45	11.11	24.14
Diptera	Chironomidae	*Cricotopus sylvestris*	16.94	11.65	7.41	6.45	18.52	3.45
Lepidoptera	Noctuidae	*Mythimna straminea*	16.13	5.34	14.07	32.26	0	0
Diptera	Chironomidae	*Paraphaenocladius impensus*	15.73	10.68	6.67	3.23	11.11	13.79
Lepidoptera	Geometridae	*Operophtera brumata*	15.73	6.80	7.41	3.23	29.63	20.69

Order	Family	Prey Species	All	RW	SW	CW	CC	WW
Diptera	Chironomidae	*Glyptotendipes sp.*	**14.92**	**11.17**	**5.93**	**3.23**	**7.41**	**10.34**
Diptera	Hybotidae	*Bicellaria vana*	**14.11**	**10.19**	**2.22**	**0**	**22.22**	**17.24**
Collembola	Entomobryidae	*Entomobrya nivalis*	**13.31**	**11.17**	**5.19**	**6.45**	**0**	**3.45**
Diptera	Ptychopteridae	*Ptychoptera contaminata*	**12.90**	**10.68**	**5.93**	**6.45**	**0**	**0**
Hemiptera	Aphididae	*Hyalopterus pruni*	**12.90**	**6.80**	**11.85**	**6.45**	**0**	**0**
Hymenoptera	Braconidae	*Praon abjectum*	**12.90**	**9.71**	**8.89**	**0**	**0**	**0**
Diptera	Chironomidae	*Cryptochironomus obreptans*	**12.50**	**6.31**	**8.89**	**3.23**	**14.81**	**3.45**
Coleoptera	Scirtidae	*Contacyphon hilaris*	**12.09**	**3.39**	**10.37**	**9.68**	**3.7**	**17.2**
Diptera	Syrphidae	*Episyrphus balteatus*	**12.09**	**7.77**	**10.37**	**0**	**0**	**0**
Diptera	Dolichopodidae	*Chrysotus femoratus*	**11.68**	**9.22**	**2.96**	**0**	**7.41**	**13.79**
Coleoptera	Chrysomelidae	*Galerucella lineola*	**11.29**	**9.71**	**3.70**	**3.23**	**0**	**6.89**
Lepidoptera	Crambidae	*Chilo phragmitella*	**10.89**	**1.47**	**5.93**	**45.16**	**7.41**	**0**
Diptera	Chironomidae	*Chironomus sp.*	**10.48**	**5.34**	**8.15**	**0**	**11.11**	**3.45**
Diptera	Chironomidae	*Procladius sagittalis*	**10.48**	**7.77**	**4.44**	**3.23**	**3.70**	**6.89**
Diptera	Chironomidae	*Chironomus pallidivittatus*	**10.08**	**4.85**	**8.89**	**0**	**3.70**	**6.89**
Hemiptera	Cicadellidae	*Empoasca luda*	**10.08**	**4.85**	**5.93**	**3.23**	**7.41**	**13.79**
Lepidoptera	Noctuidae	*Agrochola lota*	**10.08**	**7.77**	**2.22**	**6.45**	**11.11**	**3.45**
Trichoptera	Leptoceridae	*Oecetis ochracea*	**10.08**	**4.85**	**7.41**	**16.13**	**0**	**0**

Table A.1.9.*b*. Percent frequency of occurrence of notable taxa in the diets of reed and sedge warblers in the UK at different stages of fat accumulation : i) fattening at the middle and end of summer (autumn migrants), ii) birds with little to no fat deposit and iii) birds with fat deposits early in the summer (spring migrants) in both sedge warblers and reed warblers.

	% Frequency of Occurrence					
	Sedge Warbler			Reed Warbler		
Taxon	Fat Score 0-2 (n = 91)	Fat Score 3-5 (spring migrant) (n = 7)	Fat Score 3-5 (autumn migrant) (n = 13)	Fat Score 0-2 (n =143)	Fat Score 3-5 (spring migrant) (n = 1)	Fat Score 3-5 (autumn migrant) (n = 9)
Hemiptera	52.8	28.6	84.6	55.2	100	55.6
Diptera	94.5	100	92.3	96.5	100	100
Hyalopterus pruni	6.59	0	25	6.3	0	0

Appendix 1.10. The average size of chironomid species in diet samples

Table A.1.10. The average body length (mm) ± SD of chironomid species detected in the diets of UK warblers. Data are split by the four main study sites for each of the UK warbler species.

Site	Species	Mean ± SD
Chew Valley	Cetti's Warbler	5.81 ± 0.31
	Chiffchaff	5.52 ± 1.03
	Reed Warbler	6.17 ± 1.28
	Sedge Warbler	6.71 ± 1.99
	All	6.24 ± 1.48
Kenfig	Cetti's Warbler	5.01 ± 1.44
	Chiffchaff	5.30 ± 0.45
	Reed Warbler	5.46 ± 1.59
	Sedge Warbler	5.60 ± 1.08
	Willow Warbler	5.44 ± 2.41
	All	5.45 ± 1.35
Oxwich Marsh	Cetti's Warbler	5.67 ± 2.66
	Chiffchaff	5.67 ± 0.94
	Reed Warbler	4.98 ± 2.07
	Sedge Warbler	5.91 ± 2.54
	Willow Warbler	6.11 ± 1.78
	All	5.99 ± 2.25
Wheldrake Ings	Chiffchaff	6.23 ± 0.98
	Reed Warbler	6.66 ± 1.35
	Sedge Warbler	6.15 ± 0.98
	Willow Warbler	6.82 ± 1.45
	All	6.24 ± 1.19
All	Cetti's Warbler	5.56 ± 1.67
	Chiffchaff	5.73 ± 0.93
	Reed Warbler	5.71 ± 1.69
	Sedge Warbler	6.14 ± 1.95
	Willow Warbler	6.12 ± 1.49
	All	5.86 ± 1.72

Appendix 1.11. *econullnetr* outputs showing the strength of trophic interactions between warblers and prey resources.

Table A.1.11.*a* Results from the *econullnetr* model highlighting all trophic interactions between focal warbler species and prey resources (invertebrate families) in the UK subsetted by season (early, middle and late summer). “NA” indicates instances where sample sizes were too small to calculate an effect size. Prey families are ordered by average body length in millimetres. Families absent in the diet of the warblers but present on sticky traps are not shown (these were also not included in the GLM of prey preferences). Values displayed are standard effect sizes (SES) of the strength of the interaction. SES values >2 and <-2 are highlighted, with darker tone indicating a greater strength of interaction (red = stronger interaction than expected from null model, SES >2, blue = weaker interaction than expected from the null model, SES<-2). Families are ordered by increasing average body length (mm). Key to species codes: CW = Cetti’s warbler, CC = chiffchaff, RW = reed warbler, SW = sedge warbler, WW = willow warbler. See attached Excel file, Appendix 1.11, first tab.

Table A.1.11.*b* Results from the *econullnetr* model showing trophic interactions between focal warbler species and prey resources (invertebrate families) in the UK subsetted by the four main sites in 2017 and for Oxwich in 2018. Values displayed are standard effect sizes (SES) of the strength of the interaction. Interactions with an SES>2 or <-2 are highlighted, with darker tone indicating a more positive or negative interaction (red = stronger interaction than expected from null model, blue = weaker interaction than expected from the null model). “NA” indicates instances where sample sizes were too small to calculate an effect size.Families are arranged by their respective taxonomic orders. Key to species codes: CW = Cetti’s warbler, CC = chiffchaff, RW = reed warbler, SW = sedge warbler, WW = willow warbler. See attached Excel file, Appendix 1.11, second tab.

Appendix Two - Supplementary information relating to Chapter 3

Appendix 2.1. % Frequency of occurrence and species richness of invertebrate families detected in reed warbler faecal samples for adults, juveniles and nestlings.

Table A.2.1. % Frequency of occurrence and species richness of all invertebrate families detected in the faecal samples of adult, juvenile and nestling reed warblers at Chew Valley.

% Frequency of Occurrence				**Species Richness**		
Family	Adult (n = 39)	Juvenile (n = 21)	Nestling (n =30)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)
Humerobatidae	0	4.76	0	0	1	0
Araneidae	0	0	3.33	0	0	1
Clubionidae	38.46	23.81	10	1	1	3
Linyphiidae	17.95	9.52	3.33	3	1	1
Philodromidae	0	4.76	16.67	0	1	2
Tetragnathidae	2.56	0	0	1	0	0
Theridiosomatidae	2.56	4.76	0	1	1	0
Carabidae	0	4.76	0	0	1	0
Cantharidae	0	4.76	0	0	1	0
Chrysomelidae	30.77	23.81	20	7	5	3
Coleophoridae	0	4.76	0	0	1	0
Dytiscidae	2.56	0	0	1	0	0
Helophoridae	15.38	9.52	0	3	2	0
Kateretidae	2.56	4.76	0	1	1	0
Saldidae	2.56	0	0	1	0	0
Scirtidae	2.56	0	3.33	1	0	1
Scraptiidae	2.56	0	0	1	0	0
Entomobryidae	2.56	0	0	1	0	0
Tomoceridae	2.56	0	0	1	0	0
Anthomyzidae	2.56	0	0	1	0	0
Chloropidae	5.13	9.52	0	2	1	0
Drosophilidae	2.56	4.76	0	1	1	0
Ephydridae	2.56	0	3.33	1	0	1
Lauxaniidae	2.56	4.76	0	1	1	0

% Frequency of Occurrence				Species Richness		
Family	Adult (n = 39)	Juvenile (n = 21)	Nestling (n =30)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)
Lonchopteridae	5.13	4.76	0	1	1	0
Micropezidae	5.13	0	0	1	0	0
Opomyzidae	2.56	0	6.67	1	0	2
Sciomyzidae	2.56	0	0	1	0	0
Sepsidae	2.56	4.76	0	1	1	0
Tephritidae	0	4.76	0	0	1	0
Dolichopodidae	20.51	19.05	13.33	5	2	4
Empididae	28.21	4.76	3.33	2	1	1
Hybotidae	23.08	19.05	0	5	2	0
Phoridae	2.56	4.76	0	1	1	0
Pipunculidae	5.13	4.76	0	2	1	0
Rhagionidae	2.56	4.76	0	1	1	0
Syrphidae	2.56	0	10	1	0	4
Tabanidae	0	0	6.67	0	0	1
Anthomyiidae	33.33	14.29	0	5	3	0
Calliphoridae	7.69	0	10	3	0	3
Fanniidae	5.13	9.52	0	2	2	0
Muscidae	17.95	23.81	53.33	5	6	11
Sarcophagidae	10.26	0	3.33	1	0	1
Scathophagidae	28.21	14.29	23.33	3	2	3
Tachinidae	0	0	3.33	0	0	1
Anisopodidae	5.13	0	0	1	0	0
Cecidomyiidae	2.56	0	0	1	0	0
Ceratopogonidae	7.69	0	0	1	0	0
Chaoboridae	76.92	85.71	50	2	1	1
Chironomidae	84.61	76.19	50	24	15	11
Culicidae	17.95	9.52	0	4	2	0
Limoniidae	2.56	0	3.33	2	0	1
Mycetophilidae	2.56	0	0	1	0	0
Pediciidae	2.56	0	0	1	0	0
Psychodidae	5.13	9.52	0	3	2	0

% Frequency of Occurrence				**Species Richness**		
Family	Adult (n = 39)	Juvenile (n = 21)	Nestling (n =30)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)
Ptychopteridae	10.26	14.29	0	1	2	0
Sciaridae	0	4.761904762	0	0	1	0
Tipulidae	25.6	4.76	0	2	1	0
Baetidae	2.56	9.52	3.33	2	2	1
Agriolimacidae	23.07	0	3.33	1	0	1
Lymnaeidae	2.56	4.76	6.67	1	1	3
Physidae	0	4.76	0	0	1	0
Succineidae	2.56	0	16.67	1	0	1
Anthocoridae	5.13	0	0	2	0	0
Aphididae	35.89	9.52	10	8	1	2
Aphrophoridae	0	0	3.33	0	0	1
Cicadellidae	5.13	9.52	3.33	2	3	1
Corixidae	0	0	3.33	0	0	1
Gerridae	2.56	0	23.33	1	0	1
Miridae	2.56	0	6.67	1	0	2
Notonectidae	7.69	0	26.67	1	0	1
Pentatomidae	0	4.76	0	0	1	0
Psyllidae	2.56	0	0	1	0	0
Tingidae	5.129	0	10	1	0	1
Braconidae	46.15	14.29	3.33	12	3	1
Cynipidae	2.56	0	0	1	0	0
Ichneumonidae	12.82	14.29	3.33	5	6	1
Tenthredinidae	2.56	0	0	1	0	0
Vespidae	0	4.76	0	0	1	0
Choreutidae	0	4.76	0	0	1	0
Coleophoridae	0	4.76	0	0	1	0
Crambidae	7.69	9.52	3.33	1	1	1
Depressariidae	0	4.76	0	0	1	0
Erebidae	2.56	0	26.67	1	0	2
Gelechiididae	0	9.52	10	0	2	1
Geometridae	7.69	14.29	13.33	3	3	3

% Frequency of Occurrence				Species Richness		
Family	Adult (n = 39)	Juvenile (n = 21)	Nestling (n =30)	Adult (n = 39)	Juvenile (n = 21)	Nestling (n = 30)
Noctuidae	41.03	14.29	36.67	6	3	5
Notodontidae	5.13	0	6.67	1	0	1
Tortricidae	5.13	4.76	16.67	1	1	1
Chrysopidae	0	9.52	3.33	0	2	1
Hemerobiidae	5.13	0	3.33	2	0	1
Coenagrionidae	7.69	0	23.33	1	0	1
Beraeidae	0	4.76	0	0	1	0
Leptoceridae	5.13	38.09	10	1	1	1

Appendix Three - Supplementary information relating to Chapter 4.

Appendix 3.1. % Frequency of occurrence of dietary items detected in Catalan reedbed warblers.

Table A.3.1.*a*. Percent frequency of occurrence of invertebrate orders detected in the diets of Catalan warblers at different times in the breeding season (early summer, mid-summer, late summer). For each period, the total number of invertebrates of each taxonomic order recorded from sticky trap monitoring (from all sites combined) is indicated in the recorded abundance row. Values are colour coded according to the highest (green), middle (yellow) and lowest (orange) percent frequency of occurrence for a given species at each of the three time periods.

		% Frequency of occurrence in diet samples											
Season	Species	Araneae	Coleoptera	Diptera	Gastropoda	Hemiptera	Hymenoptera	Lepidoptera	Neuroptera	Odonata	Orthoptera	Psocoptera	Trichoptera
Early	Cetti's Warbler	100	76.92	84.6	100	92.3	61.5	100	7.7	0	7.7	23.1	0
	Reed Warbler	46.4	32.1	85.7	0	30.4	41.1	55.4	5.3	0	1.8	3.6	0
	Great Reed Warbler	18.1	36.4	63.6	18.2	36.4	63.6	72.7	0	0	9.1	0	0
	Recorded Abundance	**36**	**123**	**1009**	**1**	**229**	**2039**	**0**	**3**	**3**	**4**	**32**	**1**
Middle	Cetti's Warbler	90.9	72.7	100	100	81.8	45.5	100	18.2	0	18.2	45.5	0
	Reed Warbler	57.1	19.6	82.1	0	32.1	42.9	46.4	7.1	0	5.4	7.1	0
	Great Reed Warbler	37.5	37.5	75	25	37.5	75	50	0	25	62.5	0	12.5
	Recorded Abundance	**51**	**190**	**1016**	**0**	**379**	**1509**	**1**	**4**	**21**	**4**	**37**	**0**
Late	Cetti's Warbler	100	100	100	100	50	100	50	50	0	0	50	50
	Reed Warbler	60.7	25	75	0	17.9	50	71.4	7.1	3.6	3.6	3.6	10.7
	Great Reed Warbler	28.6	0	42.9	0	42.9	57.1	57.1	0	28.6	85.7	0	0
	Recorded Abundance	**82**	**218**	**985**	**0**	**162**	**1868**	**2**	**4**	**35**	**4**	**13**	**0**

Table A.3.1.*b*. Percent frequency of occurrence of invertebrate orders detected in the diets of Catalan warblers at each study site. For each site, the total number of invertebrates of each taxonomic order recorded from sticky trap monitoring (from all three sampling periods combined) is indicated in the recorded abundance row. No abundance information is available for Aiguamolls de l'Emporda. Values are colour coded according to the highest (green), middle (yellow) and lowest (orange) percent frequency of occurrence for a given species at each of the sites.

		% Frequency of occurrence in diet samples										
Site	Species	Araneae	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Neuroptera	Odonata	Orthoptera	Psocoptera	Trichoptera
Llobregat Delta	Cetti's Warbler	100	73.3	93.3	100	73.3	100	6.7	0	6.7	53	0
	Reed Warbler	80	40	75	30	65	55	0	0	0	25	0
	Great Reed Warbler	0	50	100	100	100	50	0	0	0	0	0
	Recorded Abundance	**71**	**189**	**1028**	**166**	**811**	**0**	**2**	**16**	**3**	**59**	**0**
Mas del Matà	Cetti's Warbler	100	100	66.7	66.7	33.3	100	0	0	0	0	0
	Reed Warbler	60	40	92	16	40	72	8	0	0	0	0
	Great Reed Warbler	25	25	0	25	75	100	0	0	0	0	0
Sebes	Cetti's Warbler	87.5	75	100	62.5	37.5	87.5	37.5	0	25	12.5	12.5
	Reed Warbler	51.4	11.4	94.3	17.1	68.6	71.4	14.3	0	5.7	2.9	8.6
	Great Reed Warbler	20	30	90	50	60	20	0	0	40	0	10
	Recorded Abundance	**51**	**232**	**950**	**506**	**636**	**1**	**8**	**1**	**9**	**17**	**0**
Canal Vell	Cetti's Warbler	-	-	-	-	-	-	-	-	-	-	-
	Reed Warbler	43.3	23.3	73.3	40	23.3	38.3	3.3	1.7	5	1.7	0
	Great Reed Warbler	40	20	50	20	60	90	0	40	80	0	0
	Recorded Abundance	**47**	**110**	**1032**	**98**	**3969**	**2**	**1**	**42**	**0**	**6**	**1**

Appendix Four - Supplementary information relating to Chapter 5.

Appendix 4.1. Tables of % frequency of occurrence of each unique dietary item detected in the warbler faecal samples. Attached as a supplementary Excel file with thesis.

Table A.4.1.*a*. Percent frequency of occurrence of each unique dietary item detected in the faecal samples of all warblers included in the study subset by species and country (UK and Catalonia). Items are ordered by taxonomic order and family. Excludes nestling samples and dietary items that could only be identified to order level (see attached Excel file, Appendix 4.1, first tab).

Table A.4.1.*b*. Percent frequency of occurrence of each unique dietary item detected in the faecal samples of adult, juvenile and nestling reed warblers sampled at Chew Valley Lake, Somerset. Items are ordered by taxonomic order and family (see attached Excel file, Appendix 4.1, second tab).

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