Insect life history responses
to global change

Thesis submitted for the degree of Doctor of Philosophy
by
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Abstract

1. Understanding life history variation in insects requires an assessment of how resources are allocated between reproductive and somatic traits. Associated data are not only a potential indicator of species adaptation, but also of environmental influences on fitness. So far, however, there is a dearth of data on factors affecting resource allocation, in particular in real environments.
2. Using an integrative nutrient assay protocol, this study assessed the effects of body size and environmental conditions on resource allocation strategies in terrestrial and aquatic semelparous insects: the indian mealmoth, *Plodia interpunctella* under laboratory conditions, and two caddisfly species (*Hydropsyche siltalai* and *Rhyacophila dorsalis)* in the field.
3. In *Plodia interpunctella,* larger females had higher potential lifetime fecundity due to their prolonged lifespan, while smaller females maintained greater initial egg loads and a shorter life-span. Assays revealed that current reproduction requires greater nutrient allocation in somatic and reproductive tissue, illustrating potentially fundamental factors underlying reproduction-longevity trade-offs.
4. Field studies showed how trichopteran larvae have species-specific responses to physicochemical conditions located along an altitudinal gradient (20 m - 230 m asl) in the River Usk.. Nutrient allocation in *Rhyacophila dorsalis* did not differ between upstream and downstream sites. In contrast, upstream *Hydropsyche siltalai* emerged with greater nutrient reserves consistent with early reproduction while those in warmer downstream environments must reallocate resources for somatic maintenance reducing available resource for reproduction. Consistent with this result, translocation of late instar *H. siltalai* over a thermal gradient (1.5 - 4.5 °C) showed how thermal exposure can affect adult fitness by reducing body size and increasing nutrient allocation for somatic maintenance.
5. These results show how insects adjust nutrient balance for reproduction and survival under different circumstances. The most significant result is that global warming might reduce body size and advance reproduction in some species with consequences for fitness. This is proposed as an area ripe for further study to understand the evolutionary consequences of global change.

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List of abbreviations

ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
GLM	General Linear Model
GLMM	General Linear Mix Model
HSD	Honest Significant Difference
LSM	Least Square Mean
PCA	Principle Component Analysis
PERMANOVA	Permutational Analysis of Variance
SE	Standard Error of the Mean
SD	Standard Deviation

1. General Introduction

1.1 Insect life history evolution

Understanding the factors affecting an organism's life history has been an important focus in evolutionary ecology. Recent progress in unravelling these factors has provided insights into how organisms allocate resources to life history components to optimise overall fitness within the constraints of developmental and physiological limits. These constraints cause life histories to vary in response to environmental conditions thereby giving rise to different phenotypic characteristics at both individual and population levels. Different phenotypic characteristics arise, in turn, from various combinations of key reproductive and demographic traits shaped by selective pressures. A recently developed conceptual framework on life histories has provided an integrated view of the processes of resource acquisition, allocation and utilization (Jervis *et al.,* 2007, 2008,2012). In empirical terms, however, relatively little is known of how these processes are affected by environmental conditions (Boggs, 2009), and how alterations in key components of life history (reproduction and survival) alter physiological traits and resource allocation patterns.

All organisms are generally limited by resource availability such that investment into one trait invariably occurs at the expense of another. This has been referred to as the 'Principle of Allocation' (Cody, 1966), and its clearest expression is in a negative correlation between reproduction and growth/survival (Roff, 1992; Stearns, 1992) - otherwise referred to as the "Y" model of resource allocation trade-offs (Van Noordwijk & De Jong, 1986). As an example, increased resource investment into early reproduction (e.g. initial egg load) should be correlated with reduced survival (e.g. life-span).

Understanding the physiological mechanisms underlying life history diversity demands information on the resource allocated to various correlates of fitness, for example, as reflected in morphological characteristics (Sibly *et al.,* 2013). Such character metrics are a good proxy for life history traits, particularly among metamorphic organisms with deterministic growth (e.g. Stevens *et al.,* 1999, 2000). The life history of holometabolous insects, in particular, presents an ideal system in which to examine the outcome of ontogenic allocation trade-offs within the newly eclosed adult. This is because pre-emergence allocation of resources takes place within a closed (non-feeding) system, the pupal stage (Nijhout & Emlen, 1998). While there is a significant body of evidence implicating differential allocation of limited resources as the process that underlies the cost of pre-adult investment in reproduction (Zera & Harshman, 2001; Karlsson & Johansson, 2008), far less is known of how these processes are affected by physiological traits or environmental stresses (Boggs, 2009). An additional gap arises in understanding how alterations in key components of life history - survival versus reproduction - affect overall fitness.

The present study was designed to address these gaps in two phases. First, to develop the methodological approach needed to assess nutrient allocation and fitness indicators, assays were carried out on the Indian mealmoth, *Plodia interpunctella* Hubner. Second, stream caddisflies (Trichoptera) were used as study species to investigate the effects of altitude and thermal regime variations on nutrient allocation. Streams are considered particularly vulnerable to global change, but there is a dearth of studies examining consequences for insect fitness.

1.2 Aims and Hypothesis

The overall aim of this study was to investigate the mechanism of insect life history trade-offs, specifically resource allocation between reproduction and survival, under different phenotypic characteristics (e.g. variation of body size) and environmental variables (e.g. temperature). The following predictions were tested:

i. If the cost of reproduction acts as a constraint on current reproduction, then females that allocate more resources into initial egg load will invest less resources into survival (**Chapter 4**).
ii. If environmental conditions influence resource allocation trade-offs, under stressful physicochemical environmental conditions, females will invest more resource into somatic functions while reducing allocation towards reproduction (**Chapter 5**).
iii. If the thermal environment influences resource allocation trade-offs, warmer conditions will be reflected in an increased resource investment into insect survival upon maturation (**Chapter 6**).

Chapter 2 reviews the literature to identify the array of resource allocation patterns that shape insect life histories. This is central to appreciating how natural selection modifies traits to produce diversity. In general, insects are faced with allocation choices because current and future reproductive rates cannot be maximised simultaneously with survival. Both reproduction and survival are costly in terms of resources and will compete for a limited supply, frequently under a changing environment. This chapter explores (i) factors that influence insect life histories and decisions on resource allocation between reproduction and somatic maintenance, and (ii) highlights the importance of individual resource allocation between reproductive and somatic components in life history trade-offs.

Studies to determine insect resource allocation requires an integrative approach. In **Chapter 3,** quantification of resource investment between insect survival and reproduction combined different experimental protocol such as insect dissections, female reproductive strategy and macronutrient assays. These methods were used throughout the study and given particular highlight in this chapter. **Chapter 3** provide detailed information regarding (i) insect dissection between reproductive and somatic traits, (ii) ovarian development of study species and (iii) insect nutrient assay protocol using colourimetric approach.

Chapter 4 explores experimentally the relationship between individual body size, life history, and current versus future reproduction in a representative holometabolous insect, *Plodia interpunctella*, an important pest of stored products. The study addresses two research questions: (i) does resource allocation change with female body-size? It is predicted that different reproductive traits between small and large females would translate into different resource allocation patterns, with smaller female moths investing more of their resources into current reproduction because of their short life-span; (ii) does resource allocation vary between initial egg and oocyte load as a trade-off between current and future reproduction? Resource investment into current reproduction is predicted to lead to higher nutrient allocation in reproductive tissue.

In **Chapter 5**, resource allocation patterns of free-living caddisflies (Trichoptera) are explored in eight sites located along an altitudinal gradient (20 m - 230 m asl) in the River Usk. Members of the family Hydropsychidae (*Hydropsyche siltalai* Döhler) and Rhyacophilidae (*Rhyacophila dorsalis* Curtis) were chosen for the study as they can be found in both reaches of the river. The Trichoptera are well-suited for study as they are abundant, diverse and typically ubiquitous in freshwater systems. The present study aims to enhance understanding of how individual species adapt to changes in environmental conditions. A central question was whether changes in stream physico-chemistry altered resource trade-off between survival and reproduction. Individuals collected upstream were predicted to invest more resource into reproduction than downstream individuals as the warmer, eutrophic environments downstream require greater somatic maintenance.

Thermal stress during juvenile development might determine the allocation of energy resources upon maturation. The final larval moult in holometabolous insects represents an important life history transition that provides an unequalled opportunity to consider patterns of resource allocation. Previous studies investigating the effects of temperature on stream insects have shown that increased temperatures may increase insects' respiratory rates at the expense of growth and fecundity, and that changes in growth rate, adult size and onset of adult emergence occur as a result of a warming environment (Hodkinson, 2005). **Chapter 6** examines the effect of varying thermal regimes during the fifth larval instar of the net-spinning *H. siltalai* on adult resource allocation using stream transplanted mesocosms. Specifically, the work tests the hypothesis that changes in thermal regime during the penultimate instar stage affect the balance in resource use and the rates of physiological processes.

Chapter 7 provides a general discussion on the implications of the study's findings to insect life history theory. Changes in insect resource allocation patterns may represent an early adaptation by species to a given environment. Strengths and limitations in the experimental design and methods are critiqued.

2. Literature Review: Insect Life History and Resource Allocation Trade-offs

2.1 Abstract

1. Variation in an organism's life history represents a diverse array of adaptations in response to different environmental conditions under which nutrient limitations can result in trade-offs between life history components. This review explores (i) factors that influence insect life histories and decisions on resource allocation between reproduction and somatic maintenance, and (ii) the importance of individual resource allocation between reproductive and somatic components in life history trade-offs.
2. In general, trade-offs are induced by different phenotypic characteristics that link to reproductive and somatic functions. Phenotypic trade-offs are influenced by extrinsic factors such as nutrient availability and physicochemical conditions that also influence juvenile development and adult condition. Insect body size can be correlated with timing of reproduction, predator avoidance and warmer conditions. Exposure to stress during immature stages can also reduce adult body size by shortening development. In addition, larger thoracic flight muscles may divert resource away from reproduction in wing dimorphic insects.
3. Such findings reveal an important research avenue that can enhance the understanding of insect evolutionary process under a changing environment. While most studies have highlighted the plasticity of insect responses to environmental variation, information on resource allocation between reproductive and somatic components is still scarce. Using data from various laboratory and field investigations, this review highlights the major differences between insect growth and reproduction, contextualising the remainder of the work reported in this thesis.

2.2 Introduction

Insect life history traits are governed by a range of trade-offs. Life history studies investigating phenotypic correlations provide a substantial body of empirical evidence indicating trade-offs between different physiological characteristics in response to environmental conditions. Such processes have been clearly illustrated in various insect species providing excellent examples of reproduction-longevity and dispersal-fecundity trade-offs. In this chapter, examples of insect life history trade-offs across different taxa are discussed, highlighting the fundamental influence of these important processes for reproduction and survival at individual, population and community scales. This review also highlights how adaptive plasticity changes across different environments, affecting both insect reproduction and survival. Many studies have documented the diversity in insect life history. There is, however, a lack of data on phenotypic plasticity and subsequent life history trade-offs. This review aims to provide insight into insect plastic responses that promote individual optimal fitness under different physiological and environmental conditions.

2.3 Insect life history: a brief definition

Numerous studies have documented the diversity of life history strategies (e.g. growth, reproduction and survival) in animal taxa, including mammals (Weiner *et al.*, 1992), birds (Ryder *et al.*, 2012), fish (Parra *et al.*, 2014), amphibians (Altwegg, 2002), lizards (Jordan & Snell, 2002) and insects (Bergland *et al.*, 2005). This diversity highlights the array of strategies that can determine an organism’s success within its environment. Most life history studies have focused on patterns in the evolution of reproductive investment, growth and survival, which in turn determine attributes such as age or size at first reproduction, number and size of offspring, and juvenile or adult survival rates (Atkinson & Hirst, 2007; Flatt *et al.*, 2013). Variations in these trait characteristics affect individual overall fitness under contrasting environmental conditions, for example, nutrition, temperature or photo-period. When environmental conditions change, an organism's life history may change accordingly (Ghalambor *et al.*, 2007).

Among insects, life history traits are directly related to two major components of fitness, survival and reproduction (Braendle *et al.*, 2011). These traits include adult size on eclosion, developmental pattern, age and size at maturity, number and size of offspring, and life-span (Stearns, 1992; Zera & Harshman, 2001). Morphological, physiological and behavioural traits are considered to contribute indirectly to insect fitness (Roff, 1992, Jervis *et al.*, 2005; Berger *et al.*, 2012).

2.3.1 Insects as model organism for evolutionary ecology studies

Insects have played a major role in the development of the fundamental principles of life history evolution by focusing attention on the variation and interaction of traits that determine insect reproduction and survival across individuals, populations and species (e.g. Allman *et al.*, 1998; Ahnesjo & Forsman, 2003; Amdam *et al.*, 2004; Elliott & Evenden, 2012). Studies on butterflies (Colasurdo *et al.*, 2009), parasitoids (Casas *et al.*, 2003; Jervis *et al.* 2008, Moiroux *et al.*, 2010), caddisflies (Steven *et al.*, 2000; Alvarez & Pardo 2005; Jannot, 2009), mayflies (López-Rodríguez *et al.*, 2009; Bottová *et al.*, 2012), stoneflies (Peckarsky *et al.*, 2000; López-Rodríguez & de Figueroa, 2012), orthopterans (Ahnesjo & Forsman 2003; Taylor *et al.*, 2010), phasmids (Trueman *et al.*, 2004; Maginnis, 2006) and ants (Kaspari & Vargo, 1995; Mcglynn *et al.*, 2012) have shown that adaptation affects individual life history traits by physiological changes. Such plasticity does not, however, only occur in different species but also between different populations of the same species, and even between different individuals of the same populations (Pelosse *et al.*, 2007).

Holometabolous insects provide a particularly useful model system for studying resource allocation (Nestel *et al.*, 2003; Jervis *et al.*, 2006; Pelosse *et al.*, 2007; Lann *et al.*, 2011; Vuarin *et al.*, 2012). During the final larval moult, these insects undergo complete metamorphosis in which larvae develop into adults through an intermediate pupal stage (**Figure 2.1**). Because pupae do not feed, a fixed amount of resources, gained during the larval stage and stored in various tissues during the pupal stage, must be divided between various life history functions (Emlen, 2001). Resources gained from larval feeding can be used to support both pupal diapause and adult development. For some species, these resources also support dispersal and reproduction. Semelparous insect species that do not feed during their adult stage use larval-acquired resources for egg production (Jervis & Boggs, 2005). Lepidoptera species display this segregated resource acquisition and allocation patterns between adult and immature stages, thereby allowing adults and immature stages to occupy different habitats and hence reduce competition for resources (Chown & Nicolson, 2004). Most Lepidoptera larvae feed on diverse compounds present in leaves, including carbohydrates and nitrogen (Boggs, 1987), and allocate large volumes of resource to protein production for rapid tissue growth (Chown & Nicolson, 2004). Adults, by contrast, are mostly nectarivores, with a diet that is very high in the carbohydrates that are essential for fuelling high energy-demanding flights (Boggs, 2009). Hemimetabolous insects, in contrast, require higher levels of resources to sustain activity as they develop into later instars. For example, the early instars of the grasshopper *Dosiostaurus maroccanus* need to acquire carbohydrate to sustain their high activity levels and feed on tender leaves of the grass *Poa bulbosa*. Later instars feed on many grasses and some forbs (Uvarov, 1977; Waldbauer & Friedman, 1991). This level of variation in the resource acquired during an insect's life stage can significantly affect resource investment into subsequent life history processes. Any decreases in the resources acquired will have a negative impact on growth, survival and fecundity (Boggs, 2009).

2.4 The "Y" model of resource allocation

Resource acquisition and allocation into subsequent life history functions is complex. When adult feeding is limited, nutrients stored during the larval stage are used to support biological functions during the adult period (Boggs, 1992), but increased investment in one fitness component will cause a reduced investment in another. Such trade-offs are seen as co-variation between two traits, where a change in one trait that increases fitness is accompanied by a change in a second trait that might decrease fitness. Van Noordwijk and De Jong (1986) describe this relationship as the “Y” model of resource allocation. Roff and Fairbairn (2007) expanded this concept by commenting on how partitioning of finite resources among functions has distinct consequences on both morphology and life history traits.

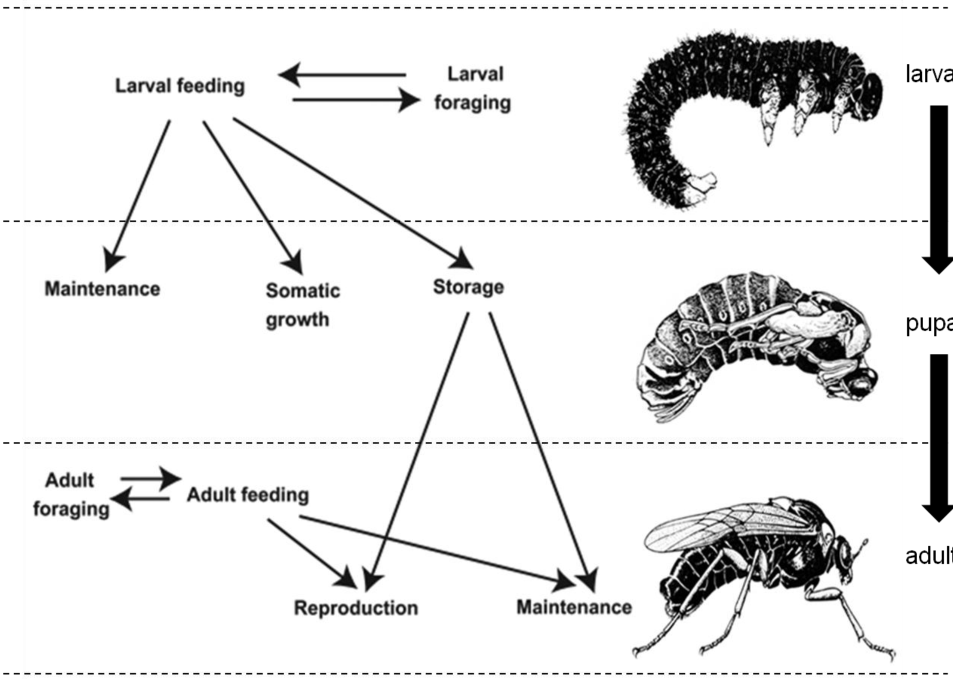


Figure 2.1: The holometabolous insect life-cycle. Resources acquired during the larval stage support reproduction and somatic maintenance during later stage when feeding is limited (Allocation Framework Theory; Boggs, 1981, 2009).

The "Y" model predicts that if variation in resource acquisition exceeds that of resource allocation, then there will be positive correlations between insect life history traits linked to reproduction (e.g. egg load) and survival (e.g. life-span) (Glazier, 1999). It is not, however, possible to maximise both reproduction and survival; increases in reproductive effort diverts essential resources away from survival functions, resulting in life history trade-offs (**Figure 2.2**). During development, allocation trade-offs may arise when competing morphological structures and functions compete for resources necessary to sustain growth (Moczek & Nijhout, 2004). For example, different body parts may compete for the same resources pool as shown in wing dimorphic insects (Roff & Gélinas, 2003; Ikeda *et al.,* 2008). Physiological studies of life history trade-offs have generally focused on the differential allocation to growth, reproduction and somatic maintenance within single species, or to variation in these processes among population or species (Smith & Fretwell, 1974; Stearns, 1989; Scheiner *et al.,* 1989; Glazier, 1999).

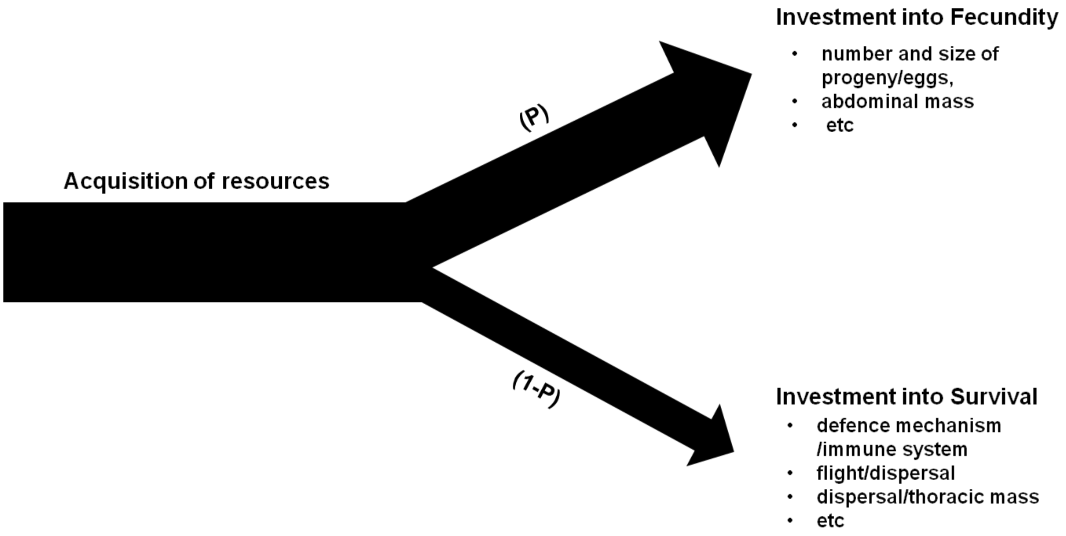


Figure 2.2: The "Y" model of resource allocation trade-offs. In this example, "P" as a limited resource (e.g. a nutrient) is acquired and competitively allocated into reproductive traits diverting nutrient reserves away from survival (Fabian & Flatt, 2012).

2.5 Insect life history trade-offs: comparing reproduction and survival

To date, empirical findings have explained resource trade-offs between reproduction and survival in insects in terms of physiological traits (Nijhout & Emlen, 1998; Zera *et al.*, 1998, Maginnis, 2006; Thaler *et al.*, 2012). Some of the best examples include fruit flies, wing-polymorphic crickets and parasitoids. The fruit fly, *Drosophila* sp., has been an important model system in the study of life history trade-offs, with most focus being given on the trade-offs between extended longevity and early reproduction (Flatt & Heyland, 2011). A high reproductive rate in the early life of *D. melagonaster*, for example, often results in a reduced life-span (Partridge, 1992). In a later study by Partridge *et al.* (1999), late-life breeding in *D. melagonaster* increased survival rates but reduced lifetime reproduction. These findings were observed in *Drosophila* sp. reared under laboratory conditions showing trade-offs between early age reproduction and adult longevity (Flatt, 2011; Klepsatel *et al.*, 2013). In another study on *D. melagonaster*, a restricted diet led to a positive correlation between longevity and fat reserves (Zwaan *et al.*, 1991). Female *D. melagonaster* on diet restriction invest more resources into somatic functions and have longer life-span (O'Brien *et al.*, 2008). Low food quality in *D. melagonaster* larvae can, however, shorten larval development causing smaller adult size with lower life-span and fecundity (Kolss *et al.,* 2009). This implies that under chronic nutritional stress, *D. melagonaster* may also exhibit enhanced larval development but with a subsequent impact on adulthood. Such trade-off patterns represent a shift of resources from reproduction into somatic maintenance. Resource allocations into somatic maintenance will, however, only extend the life-span if nutrient reserves allocated for somatic functions exceeds reproductive allocation (O'Brien *et al.,* 2008). Studies of this nature highlight how these insects are capable of adjusting their resource allocation under different conditions to maximise overall fitness (Flatt & Schmidt, 2009).

In the wing-polymorphic crickets, *Gryllus firmus*, short-winged morphs that cannot fly have greater egg production capabilities than long-winged morphs with higher flight-capability (Roff & Gélinas, 2003; Zera, 2005; Nespolo *et al.*, 2008). This suggests that reproductive effort is reduced in long-winged morphs as a trade-off between lifetime reproductive success and dispersal capacity. Flightless *G. firmus* also exhibit enhanced ovarian growth and a reduction in somatic triglyceride reserves compared with flight-capable morphs (Mole & Zera, 1993). In another study, by Saglam *et al.* (2008), on male *G. firmus*, individuals with higher gonad mass had reduced dorsal longitudinal muscles, indicating trade-offs between reproductive function and flight capability in male crickets.

Insect parasitoids have limited egg supplies, and oviposition is constrained by the time available to locate an appropriate oviposition site or host (Thompson, 1924). When female parasitoids are unable to use available hosts because of egg depletion, they approach ‘egg limitation’. ‘Time limitation’ is approached when females are unable to deposit all the eggs that they carry due to low survival rate or reduced somatic functions (Rosenheim, 1996). Reproductive life history in parasitoids is largely determined by the number of hosts encountered by females and the rate of egg maturation over their life-span (Jervis *et al.,* 2001). Time and egg limitation determine the parasitoid’s reproductive behaviour as well as influencing clutch size (Rosenheim *et al.,* 2008). Comparing pro-ovigenic and synovigenic (see Section 2.6 for definitions) parasitoids on the basis of their ovigeny index (expressed as a proportion of the initial egg load / fully mature eggs to the lifetime potential fecundity), most pro-ovigenic parasitoids have shorter life-spans than synovigenic species, a consequence of increased reproductive effort in early life (Jervis *et al.,* 2001). Ellers (1996, 2000) provided examples of trade-offs between reproduction and survival in the *Drosophila* parasitoid species, *Asobara tabida*, by comparing female investment into egg load and female lipid content. Longer-lived females had higher lipid content in their bodies compared to shorter-lived females with higher egg loads upon adult emergence. In addition, Amat *et al.* (2012) compared flight performance in another parasitoid, *Venturia canescens,* and showed that carbohydrates provide essential nutrient reserves to fuel flights in longer-lived adults. Casas *et al.* (2005), working on the synovigenic ectoparasitoid, *Eupelmus vuilletti*, found the parasitoid capable of shifting strategies between current and future reproduction depending on dietary conditions. More lipids in the diet led to early egg maturation while sugar prolonged life-span, increasing lifetime fecundity. Parasitoids provide some of the best examples of insect life history trade-offs as different resource acquisition and allocation affect both adult longevity and reproductive output.

For insect species that do not feed during adulthood (semelparous), different life history traits compete for resources from the same pool. This has significant impact on the more physiological traits as allocation involves the partitioning of limited resources for developing different body parts that have major influence on the evolution of both insect morphology and life history strategies (Trumbo, 1999). Semelparous insects often display different life history trade-offs as the result of physiological stress imposed during the immature stage. This was shown by Stevens *et al.* (1999) where increased investment in larval defence of the caddisfly, *Odontocerum albicorne*, resulted in reduced investment to the thorax tissues resulting in reduced flight capability, while preserving reproduction. In another study by Mondy *et al.* (2011), on larvae of the caddisfly, *Limnephilus rhombicus*, increased investment in the adult caddisfly in defence mechanisms linked to silk production resulted in a loss of protein content, causing a drastic impact on individual fitness. Reduction in protein levels can lead to a reduction in adult caddisfly thoracic mass. This led to a significantly reduced flight capability (Stevens *et al.*, 2000). In addition, Jannot *et al.* (2007) showed that removal of the case of the caddisfly *Agrypnia deflate,* shifted resources away from somatic maintenance; thoracic mass was reduced to mitigate impact on clutch size. In the chironomid *Echinocladius martini* (Orthocladiinae), where larvae also construct silk tubes for protection as in the Trichoptera, tube destruction resulted in retarded oocyte development and size in the female insect (McKie, 2004). Finally, in semelporous

Silphinae beetles (Coleoptera: Silphidae), changes in food habit from necrophagous to predation gave rise to reduced flight capability with higher fecundity in flightless adults (Ikeda *et al.,* 2008). Together, these examples illustrate how insects are capable of adjusting phenotypic characteristics under specific conditions to maximise overall fitness. This gives rise to specific life history trade-offs that influence adult dispersal rates, immune functions, life-span and reproductive output (**Table 2.1**).

2.6 Trade-offs between insect reproduction and survival

Extensive studies have demonstrated trade-offs between major life history strategies across numerous taxa (Gustafsson *et al.,* 1994; Sinervo & Denardo, 1996; Zuk, 1996). Stearns (1992) listed trade-offs that included current reproduction versus survival, future reproduction, parental growth and parental condition, as well as number of offspring versus offspring size. Some of the best evidence comes from entomological studies; in general, these tend to show strong correlations between survival and reproduction (Jervis *et al.,* 2005). That insects provide excellent examples of these types of trade-offs is the consequence of their life histories being generally explained by demographic parameters such as age and size at maturity, number and size of offspring, and length of life. More recent studies on insect resource allocation strategies have also been developed as a means of estimating fitness (Casas *et al.,* 2005; Boggs, 2009).

Insect resource allocation is often competitive. Boggs (1981) postulated that, at least among holometabolous insects, within-body patterns of resource allocation during metamorphosis in female insects should be correlated with both life-span and with the expected lifetime intake of dietary nutrients contributing to egg manufacture. With this understanding, Jervis *et al.* (2005, 2007) hypothesised that species that emerge with all their eggs mature should invest resources in reproduction at the expense of assembling a sturdy body. That is, these insects should increase the proportion of resources allocated to reproduction at the expense of allocation to somatic maintenance. Such trade-offs in insect reproductive strategies are usually visualised in terms of the investment in ‘current’ and ‘future’ reproduction (Jervis *et al.,* 2001; Pexton & Mayhew, 2002). The two terms, 'current' and 'future' reproduction, are derived from the earlier terms of 'capital' and 'income' breeding used to describe egg-laying and clutch-size decisions in birds (Drent & Daan, 1980). Thomas (1988) further described a ‘capital breeder’ as an “organism that accumulated nutrient reserves in the body prior to egg-laying and used it to provide the materials comprising eggs,” while “'income breeders' ingest food and immediately divert a fraction into egg formation without great reliance on stored reserves”. Many studies (see Bonnet *et al.,* 1998) have applied the 'current' and 'future' reproduction terminology to physiological differences between endotherms and ectotherms.

Many studies have described trade-offs between reproductive strategies in Hymenoptera and Lepidoptera. Within these insect orders are species that, on adult emergence, have the full, developed egg complement. Other species emerge with only immature eggs (Jervis & Ferns, 2004). The number of mature eggs carried by female insects early and late in their adult life-time represent a significant fitness variable (Papaj, 2000). Females whose lifetime potential complement of eggs is mature upon adult emergence are termed as being ‘pro-ovigenic’; those that invest into future reproduction, and mature and generate eggs throughout their lifetime, are classified as ‘synovigenic’. Pro-ovigenic insects generally allocate their limited reserves to early reproduction at the expense of somatic maintenance and survival. While females with a high proportion of mature eggs at emergence are able to maximise the number of eggs that can be laid during their early lifetime, they have considerably reduced ability to match egg supply to variation in host availability. Thus, while females may increase reproductive effort by spending more time and resource on oviposition, limited feeding reduces the resource available for survival. In contrast, in synovigenic species, females can minimize the risks of time- or egg-limitation (Rosenheim *et al.,* 2000). While only being able to lay a small fraction of their lifetime potential egg number at any one particular time point may disadvantage females with a low proportion of mature eggs, these insects are advantaged by an increased lifetime reproductive success responding to variation in host availability (Ellers & Jervis, 2003). Synovigenic insects may also acquire more resources by feeding. This provides nutrients for egg maturation and an extended life span (Casas *et al.,* 2000). In some parasitoid species, egg resorption enables females to reallocate resources initially dedicated to reproduction to somatic maintenance (Hegazi, *et al.,* 2013). Variation in reproductive strategies between current and future reproduction has adaptive significance, affecting fitness between insect early reproduction and life-span. Within the Lepidoptera, for example, the winter moth, *Operophtera brumata*, emerges with all its eggs mature and lives for several days, whereas the zebra longwing butterfly, *Heliconius charitonius*, has no mature eggs upon emergence and can live for several months (Boggs, 1986; Miller, 1996).

Table 2.1: Examples of physiological trade-offs between reproductive and somatic components during insect development.

Conditions	**Family**	**Species**	**Reproductive Investment**	**Survival/ Somatic Investment**	**Reference**
Dietary Restrictions	Neriidae	*Telostylinus angusticollis*	Egg-load	Life-span	Adler *et al*., 2013
		Anastrepha ludens	Egg-load	Life-span	Aluja *et al*., 2011
		Bactrocera tryoni	Egg-load	Life-span	Fanson & Taylor 2012
	Drosophilidae	*Drosophila melanogaster*	Egg-load	Life-span	Min *et al*., 2006
		D. melanogaster	Egg-load	Life-span	O'Brien *et al*., 2008
	Lygaeidae	*Oncopeltus fasciatus*	Egg-load	Life-span	Attisano *et al*., 2012
Wing dimorphism	Gryllidae	*Gryllus firmus, G. rubens, G. assimilis*	Gonad mass, testis size, ovarian tissue	Wing morphology, Dorso-longitudinal muscle	Roff *et al*., 2003; Saglam *et al*., 2008; Nespolo *et al*., 2008; Mole & Zera, 1993; Zera & Brink, 2000, Zera *et al*., 1998
	Silphidae	*Silphinae* sp.	Egg-load	Flight muscles	Ikeda *et al*., 2008
	Figitidae	*Leptopilina heterotoma*	Egg-load	Dispersal	Vuarin *et al*., 2012
Early Reproduction	Ichneumonidae	*Venturia canescens*	Egg-load, Clutch size	Life-span	Pelosse *et al*., 2007; Pelosse *et al*., 2011
	Drosophilidae	*Drosophila buzzati*	Initial egg	Life-span	Norry *et al*., 2006 Wajnberg *et al*., 2012
Increased Defence/Immune functions	Odontoceridae	*Odontocerum albicorne*	Abdominal mass	Thoracic mass	Steven *et al*., 1999
		Limnephilus rhombicus	Nutrient reserves	Diapause	Mondy *et al*., 2011
	Drosophilidae	*Drosophila melanogaster*	Egg-load	Life-span	Bashir-Tanoli & Tinsley, 2014
	Silphidae	*Nicrophorus vespilloides*	Egg-load	Cellular Immunity	Reavey *et al*., 2014
	Orthocladiinae	*Echinocladius martini*	Egg size	Diapause	Mckie, 2004

2.7 The cost of reproduction in insects

Individual investment by insects into current reproduction is inversely correlated with expected future fecundity (**Figure 2.3**; Creighton *et al.,* 2009). In particular, current reproduction frequently acts as a constraint on future reproduction, with higher investment into current reproduction leading to lower future reproductive effort and survival. Investment into early reproductive effort has the potential to be costly, as initial egg load requires allocation of fat body reserves. As most insects utilize their fat body reserves for specific life history adaptations, such as extended longevity and increased locomotion (e.g. dispersal) (Casas *et al.,* 2005; King *et al.,* 2012), higher resource investment into current reproduction may reduce available resources for somatic functions.

The relative energetic cost and benefits of insect reproductive strategies highlight the range of resource allocation patterns that can occur between somatic and reproductive aspects of an insect's life history (Boggs, 1997; Rosenheim, 1999; Kivelä *et al.,* 2009). Resource allocation to reproduction can be used as a quantitative indicator of reproductive traits such as age-specific realised fecundity or lifetime reproductive success. As all these traits require optimal conditions to meet their metabolic growth requirements, the higher cost of reproduction (e.g. the cost of mating and egg production) will reduce the resource available for somatic allocation (Yanagi & Miyatake, 2003). Harshman and Zera (2007) highlight those components that affect reproductive cost: hormonal regulation, allocation trade-offs, immune function, reproductive proteins, and defences against stress and toxicity. Allocation trade-offs are considered the primary factors that contribute to the cost of insect reproduction (Zera, 2005; Zera & Zhao, 2006). Studies on insect energetic reserves highlight that different nutrient allocation patterns in insect life history are governed by resource uptake (Llandres *et al.,* 2015). This is exemplified well in the flightless and flight-capable females of the wing polymorphic cricket, *Gryllus firmus.* Here, investment to early-age fecundity often results in reduced dispersal capability. Both morphs assimilate equivalent amount of resources but they vary in the level of internal allocation between reproduction and survival (Zhao & Zera, 2002). These same authors also showed that energy reserves are used differentially by insects to support specific physiological traits as a trade-offs between survival and reproduction. The cost of reproduction arising from current and future reproduction trade-off is likely to differ between species. Burying beetles, Silphidae species, for example, provide biparental care to their offspring and cost of reproduction involved preserving food source for their offspring (Scott & Traniello, 1990; Creighton *et al.*, 2009). In Lepidoptera and Hymenoptera, larval- and adult-derived resources influence the trade-off between offspring number and quality (Pöykkö, 2009; Innocent *et al.*, 2010; Javoiš *et al.*, 2011; Harvey *et al.*, 2012). Reproduction costs may also change depending on individual responses under different environmental conditions. Different environmental conditions affect metabolic processes and change the way insects utilize energy reserves. This would change reproductive cost in individuals and within populations.

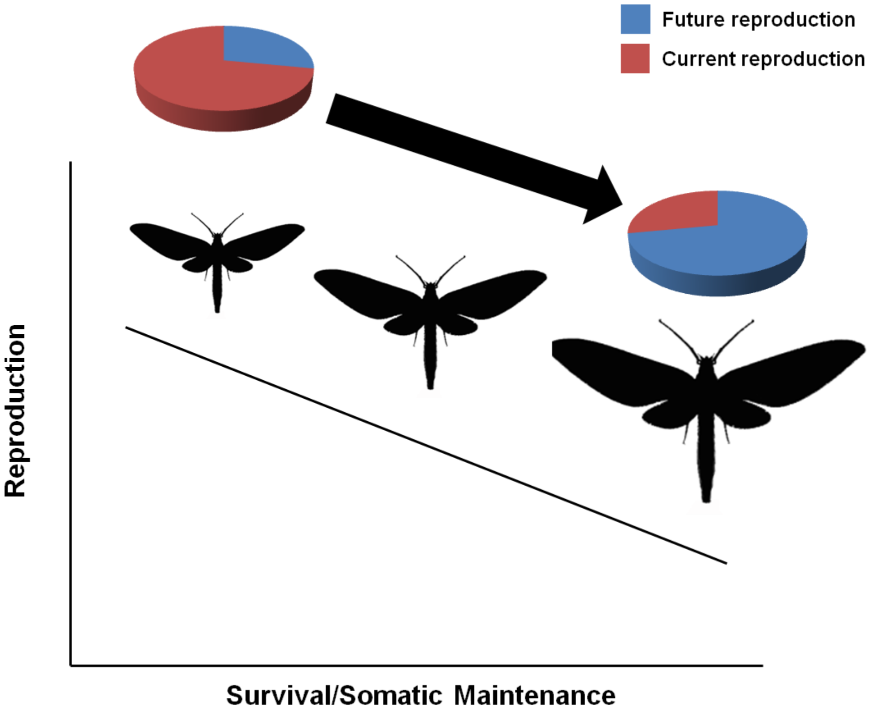


Figure 2.3: The cost of reproduction to an insect. The negative correlation implies a trade-off between current and future reproduction. As insects invest more into future reproduction, adults tend to have larger bodies or longer life-spans.

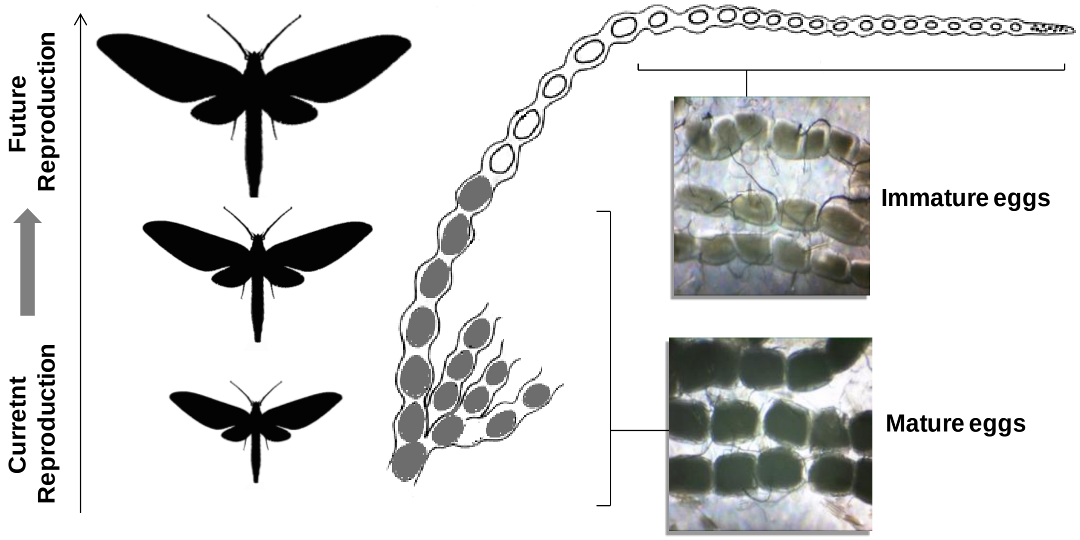


Figure 2.4: Insect reproductive strategies for current and future reproduction. Larger female insects invest more into initial oocyte load for future reproduction while smaller female tend to invest more into mature eggs for early reproduction.

2.8 Insect phenotypic plasticity

Insect life history varies with environmental conditions; demographic traits are also limited to within a defined range of tolerable environments (Glazier, 2002; Boyce *et al.*, 2006). Insects express alternative phenotypes during ontogeny and frequently provide an adaptive solution in a variable environment (Nylin & Gotthard, 1998; Reed *et al.,* 2010). ‘Phenotypic plasticity’ provides an insect with the ability to adjust its phenotype to support specific life history functions for increased survival and reproduction rates (Gotthard & Nylin, 1995). It also may be adaptive or non-adaptive depending on whether the induced phenotypes can optimize fitness (Ghalambor *et al.*, 2007; 2015). Whether plasticity is adaptive or not depends on an organism’s reaction norms; these are shown in specific phenotypic traits under various environmental conditions before the process of adaptive evolution takes place (e.g. genetic assimilation) (West-Eberhard, 2003). In general, adaptive plasticity refers to the production of phenotypes that support population persistence in the new environment (Pigliucci, 2001), while non-adaptive plasticity refers to reaction norms relating to stresses in the new environment that reduces fitness (Hoffman & Parsons, 1997). Expressing alternative phenotypes as being adaptive or non-adaptive is, however, complicated as phenotypic traits may perform differentially across environments (West-Eberhard, 2003).

Phenotypic plasticity in insects is illustrated in individual physiology, behaviour and allocation of resources that influence growth and development (Moczek, 2010). These traits are influenced by many factors including an individual's relationships with food resources (Jervis & Boggs, 2005), physicochemical variations (Hawkins, 1995; Kingsolver *et al.,* 2007), competitors and predators (Steiner & Pfeiffer, 2007; Janssens *et al.,* 2013), as well as life history variables such as developmental time, fecundity and dispersal behaviour (Thorne *et al.*, 2006; Jervis & Ferns, 2004). Insect plastic responses are shown in individual changes in development and allocation of resources to competing demands that influence reproductive (e.g. number and size of eggs) and somatic (e.g. life-span, dispersal rates) traits (Moczek, 2010). Examples include different horn expressions of Scarabaeidae species, that give rise to alternative male reproductive behaviours in response to variation in larval nutrition (Moczek & Emlen, 2000), and increased body size and fecundity in *Drosophila melanogaster* at higher temperature (Nunney & Cheung, 1997).

2.8.1 Plasticity in insect resource allocation trade-offs

Plasticity in insect life history trade-offs is determined by a set of priority rules shaped by ecological factors (Zera & Harshman, 2001). Insect plasticity can be evaluated when fitness trade-offs associated with survival and reproduction are realized in accordance with individual responses to environmental factors (Ghalambor *et al.*, 2007). Upon maturation, insect body parts linked to somatic and reproductive components are determined by growth and duration of the larval and pupal stages. Nutritional variation during larval development can influence adult body size and life history traits (Mirth *et al.,* 2016). For example, increased food intake by larval *Drosophila melanogaster* can lead to increases in ovary size and ovariole number (Hodin & Riddiford, 2000). In the tobacco hornworm, *Manduca sexta*, starvation of the larva resulted in wing size variation due to changes in wing disc growth during the pupal and larval period (Nijhout & Grunert, 2010). In braconid parasitoids, larvae that fed on hosts of different quality showed different lifetime fecundity and egg size upon emergence as adults (Cicero *et al.*, 2011). In addition, Saeki and Crowley (2013) compared feeding by the larvae of the parasitoid wasp, *Copidosoma bakeri*, in early and late instar hosts, and found that parasitoid larvae that fed on early instar host gave rise to larger adult body sizes. This was considered a consequence of faster development and resource acquisition in the early instar host stages. Studies on the damselfly, *Lestes* sp., on the other hand, revealed that when food was abundant, immature stages shorten their developmental times in the presence of predators, leading to a smaller adult (Johansson *et al.,* 2001; Stoks *et al.,* 2006). Thus, higher food abundance may contribute to increased lifetime reproduction, but when linked to other ecological factors such as predator presence, developmental plasticity can occur causing phenotypic changes.

Trade-offs between life history traits become apparent as a result of developmental plasticity (i.e. environmentally-caused variation within a single genotype during development). Developmental plasticity is influenced by physicochemical variations that affect metabolic activity throughout an insect's life history mostly during immature development with subsequent impact on adult life history (Roff, 1992; Gotthard *et al.,* 2007). Previous studies have shown that physicochemical variables such as temperature (Karl *et al.,* 2011), photoperiod (De Block & Stoks, 2003), acidification (Schartau *et al.,* 2008) and chemical pollutant (Alexander *et al.,* 2008; Magbanua *et al.,* 2013) can all reduce the development time of immature stages, further reducing adult body size upon emergence. This has a negative impact on reproduction. In addition, the interaction between multiple physicochemical elements can act as a constraint during larval development affecting survival in both immature and adult stages. This was clearly shown in, for example, the increased mortality rate at low pH conditions (the result of increased acidification and metal concentrations) in the mayfly, *Baetis rhodani* (see Ormerod *et al.,* 1987). In the damselfly, *Ischnura elegans*, pesticide and heat stress during the immature stages led to emerging adults with lower fat content and weaker immune functions (Janssens *et al.,* 2014). Verberk *et al.* (2016) demonstrated that oxygen limitations, coupled with heat stress, led to hypoxia in two mayfly species, *Ephemera danica* and *Serratella ignita*. This reduced individual somatic functions affecting the insect's survival. An insect's ability to express different phenotypic responses under temporal stress events shows that adaptation comes with a fitness cost resulting in retarded growth and development linked to survival and reproductive functions (Boggs & Ross, 1993; Zera *et al.*, 1998).

Insect plasticity in physiological characteristics reflect their different responses, at various life stages, in environmental conditions (Chown & Gaston, 2010). Thus, individual physiological responses are complicated by how different species persist within their environment. Individuals that are exposed to different selective pressures from the ambient conditions of their habitat may exhibit different resource allocation patterns that give rise to specific trade-off components between survival and reproduction.

2.9 Insect resource allocation trade-offs under climate change

Climate change is of global primary concern in ecological systems because of its impact on community composition, distribution and ecosystem functioning (e.g. Walther, 2002; Durance & Ormerod, 2007; Arribas *et al.*, 2012). Increased temperature can also be expected to alter individual life history, affecting both physiology and development (IPCC, 2007; Musolin, 2007; IPCC, 2014). Insect evolution and development, in particular, are closely linked to temperature. Higher resource allocation in an insect's body growth during the juvenile stage at lower temperature generally leads to a larger body size at maturity (Atkinson, 1994; Hance *et al.*, 2006). This has already been demonstrated for insect populations adapted to different climatic regions where populations from warmer regions are smaller than populations from colder regions (Bochdanovits & De Jong, 2003; Vuarin *et al.*, 2012).

Temperature constitutes an important ecological and climate change factor, and an insect's ability to tolerate temperature fluctuations is of prime importance for both individual and population survival (Overgaard & Sorensen, 2008). Increased temperature is predicted to modify insect phenotypic characteristics leading to variation in body size, fecundity and dispersal (Musolin, 2007; Hering *et al.*, 2009; Chown *et al.*, 2010). Changes in temperature are predicted to have an impact on both insect reproductive and somatic functions (Denis *et al.*, 2012; Le Lann *et al.*, 2014). As temperature governs the rate of an insect's metabolic processes, this, in turn, influences important cellular functions such as the rate of enzymatic reactions and membrane stability (Angilletta, 2009). Thus, temperature determines the rates of various biological processes ranging from growth and development, to feeding and dispersal (Chown & Nicolson, 2004; Brown *et al.,* 2004). Insect populations from different thermal regimes present different phenotypic characteristics, for example, in adult body size. Such differences are innate. This is evidenced by laboratory monitoring of development among insect populations collected from different geographic origins. For example, populations of the fruitflies, *Drosophila melanogaster* (see Bochdanovits & De Jong, 2003) and *D. buzzatii* (see Sarup *et al.*, 2006), and the parasitoid, *Leptopilina heterotoma* (see Vuarin *et al.*, 2012), from different climates vary in adult body size when kept under standard laboratory conditions. Local adaptation for insects from warmer environmental conditions often results in smaller individuals with shorter developmental time due to their faster growth rate. This affects levels of both voltinism and population density (Blanckenhorn & Demont, 2004).

Insect life history responses under a warming climate are complex as adaptation can influence one or more physiological traits that, in turn, affect the balance of resource allocation trade-offs (Parmesan, 2006; Karl *et al.,* 2011). Under elevated temperatures, insects shorten their development time (Temperature-Size Rule; Atkinson, 1994) resulting in reduced weight upon maturation (Kollberg *et al.*, 2013). The Temperature-Size Rule (TSR) explains how the relationship between environmental temperature and insect body size is a result of phenotypic plasticity (Angiletta & Dunham, 2003). Early insect emergence at smaller body size is frequently associated with reduced fitness (Kingsolver & Huey, 2008). Shorter development at increased temperature may, however, also increase fitness and reproductive rates. For insect herbivores (Bale *et al.,* 2002), shorter development times, coupled with an extended growing season and increased crop yield under warming climate scenarios, may favour adult overall fitness. This has been exemplified by the European pine sawfly, *Neodiprion sertifer* (see Kollberg *et al.*, 2013), the European spruce bark beetle, *Ips typographus* (see Marini *et al.*, 2012) and the potato tuber feeding moths, *Tecia solanivora*, *Symmetrischema tangolias* and *Phthorimaea operculella* (see Dangles *et al.,* 2013). Most insect herbivores benefit from a warming climate by increased population growth rate mainly due to higher feeding activity and higher level of energy intake (Cannon, 1998). Under these climatic conditions, insects may also mature their eggs earlier as seen in the satyrine butterfly, *Pararge aegeria*. Female *P. aegeria* oviposition, for example, requires an ambient temperature of 18 ºC for egg maturation (Gotthard *et al.*, 2007). Such examples indicate that under warming environments, insects experience time-limitation effects that may influence their future reproduction. With increased temperatures, for example, *P. aegeria* eggs matured earlier, increasing realised fecundity (Berger *et al.*, 2008). In another study on the herbivorous copepod, *Calanoides acutus*, individuals shifted their reproductive strategies from capital to income breeding, depending on seasonal environments (Varpe *et al.*, 2009). All these examples illustrate how insects show considerable potential for change in reproductive strategies under different environmental variations.

In contrast, shorter developmental time under warming environment scenarios may also reduce overall insect fitness. During the larval stage, an insect may increase resource allocation to somatic maintenance as a measure to cope with increased temperature. Karl *et al.* (2011) showed that the tropical butterfly, *Bicyclus anynana*, lowered its immune response (decrease in phenoloxidase activity and haemocyte numbers) at increasing temperatures. Such a response is related to increased cost of somatic maintenance; maintaining immune responses is considered energetically expensive due to the increased production of heat shock proteins (HSPs) in insect tissue at higher temperature (Fittinghoff & Riddiford, 1990; Robertson, 2004; Karl *et al.*, 2008). Increased somatic investment and shorter larval duration further leads to reduced resource acquisition and resource transition into the adult stage (Boggs, 2009). Insects that currently invest in a current reproduction/capital breeding strategy depend, in general, on the resource base that is transferred from larva to adult. This is mainly due to limited adult feeding or no feeding at all and has been shown to be prevalent in some Lepidoptera and Trichoptera species (Jervis *et al.*, 2005). Thus, energy storage accumulated for egg provision may be ‘shifted’ to fuel somatic maintenance and disrupt egg maturation strategy during the adult stage (Roux *et al.*, 2010).

2.9.1 Aquatic insect response to climate change and its influence on resource allocation trade-offs

Climate change can affect freshwater ecosystems by modifying water temperature, changing discharge and increasing the frequency of events such as floods and droughts (Milly *et al.,* 2006; Clarke, 2009; Ormerod, 2009; Woodward *et al.,* 2010). Extended dry periods can have a significant impact on invertebrate population and communities (Woodward *et al.,* 2016). Under these climate change scenarios, freshwater communities have to adapt *in situ* to maintain population levels due mainly to the narrow dispersal and thermal tolerance ranges of the majority of aquatic invertebrates (Sáinz-Bariáin *et al.,* 2015). In addition, the adaptive responses of freshwater communities to changes in temperature and other physicochemical variables may be interpreted as micro-evolutionary responses due to changes in phenotypic characteristics (e.g. body size) (Gienapp *et al.,* 2008; Hering *et al.,* 2009). Many stream invertebrates pass through a complex life cycle where egg and larval (nymphal) stages are typically aquatic, but adults emerge as terrestrial organisms (sub- and mature imago). Aquatic nymphal stages spend most of their time foraging for food. By doing this they ensure that sufficient resources are stored for growth and development in later stages. During adulthood, most of the life-span is dedicated to reproduction and dispersal. Several studies have shown that stream characteristics such as temperature (Durance & Ormerod, 2007), flow variability (Durance & Ormerod, 2009), stream oxygenation (Verberk *et al.,* 2016), nutrient dynamics (Pearson & Connolly, 2000), riparian vegetation (Clews & Ormerod, 2010; Thomas *et al.,* 2015), streambed substrates (Barnes *et al.,* 2013) and connectivity to permanent waters (Drummond *et al.,* 2015) can influence aquatic insect assemblage and stream colonization. In addition, different environmental conditions in the stream during the nymphal stage can influence metabolic activity and behaviour, resulting in phenotypic plasticity response in adult life history (Verberk *et al.,* 2008).

The responses of aquatic insects to climate change are also illustrated by the shifts of life cycle events that lead to changes in adult emergence and developmental times (Ward & Stanford, 1982). Aquatic insects show flexibility in the number of generations produced (voltinism) and hence fitness (Kivelä *et al.,* 2009). Even within species, populations from different habitat may exhibit uni-, bi-, tri- and poly-voltine life histories depending on ambient temperature. Corbet (1980), for example, showed that the damselfly *I. elegans*, shifted from univoltine to trivoltine life history at higher thermal regimes. Glossomatid caddisflies also exhibited different voltinism between temporary (Álvarez & Pardo, 2005) and permanent (Jin, 1994) streams. In general, aquatic insects show a pattern of increased voltinism along a thermal gradient from cold to warm, explained by increased growth and development (Chown & Gaston, 1999). Braune *et al.* (2008) showed that a dragonfly (*Gomphus vulgatissimus*) population increased the number of generations per annum across a latitudinal gradient as a consequence of increased temperature. Elevated stream temperatures can lead to low water levels, mainly because of increased evaporation (Stoks *et al.,* 2014). A shortened hydroperiod is expected to reduce growth and developmental rates in aquatic insects and even increase mortality in some species, particularly those that live in temporary waters (Juliano & Stoffrege, 1994). Aquatic insects that require gill respiration as juveniles (Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera) cannot exit shrinking water bodies in search for other aquatic locations to avoid dehydration. They may, however, be able to synchronize emergence into a desiccation-resistant stage (terrestrial adults or diapausing juveniles) (Delucchi & Peckarsky, 1989; Brock *et al.,* 2003). These shorter hydroperiods impose time constraints where nymphs are forced to switch into early adulthood, and nymphal development is accelerated as water volume decreases. Several studies have illustrated such responses in mosquitos (Schäfer & Lundström, 2006), caddisflies (Jannot, 2009) and damselfies (De Block & Stoks, 2005). Aquatic insect responses to shorter hydroperiods are expected to increase under warming environments, potentially causing extinction of local populations when adaptive evolution is absent.

Early emergence upon maturation under higher temperatures often results in reduced adult body size. This, in turn, can lead to reduced lifetime reproduction and life-span (Dallas & Ross-Gillespie, 2015). Smaller body sizes can also influence adult fitness due to trade-offs between size and age at maturation (Berger *et al.,* 2012). Harper *et al.* (2006) showed that the mayfly species, *Baetis bicaudatus*, emerged earlier in warmer water temperature and had smaller body size compared to conspecifics developing in ambient water temperature. Accelerated emergence is predicted to shift timing in mayfly metamorphosis. This is likely to have negative impacts on populations due to their reduced overall fitness. Harper *et al.* (2006) did, however, point out that early emergence may provide the benefit of finding more ovipositional sites during a summer drought period. The energetic cost to mayflies of finding ovipositional sites can be expensive and time-limiting due to their shorter adult life-span (Peckarsky *et al.,* 2000). In another study, there was a significant reduction in fourth and fifth instar overall mass in the caddisfly, *Brachycenturs occidentalis*, under conditions of increased water temperature. This arose from an inability to meet metabolic demands at a faster developmental rate (Miller *et al.*, 2011). Temperature effects may differ in different species. In the dragonfly, *Pachydiplax longipennis,* for example, early adult emergence at warmer water temperature does not reduce adult body size (Mccauley *et al.,* 2015). This may be explained by different responses to temperature between short- and long-lived aquatic insects. Stevens *et al.* (1999) demonstrated that short-lived caddisfly, *Odontocerum albicorne*, compensate lost resources from larval stage by shifting resources away from somatic (thoracic) into reproductive (abdominal) mass. In contrast, in a long-lived caddisfly, *Glyphotaelius pellucidus,* such trade-offs do not occur (Stevens *et al.*, 2000).

The effects of climate change may be amplified in combination with other potential environmental stressors. For instance, elevated temperatures may exacerbate the impacts of agri-pesticide contaminant concentrations. Insecticides have, for example, been recorded as inducing physiological changes in *Epeorus* spp. and *Baetis* spp., with adults emerging with smaller heads and shorter thorax lengths after exposure to the insecticide, imidacloprid (Alexander *et al.,* 2008). In addition, exposure to agriculture pesticide at warmer temperatures during the nymphal stage was found to increase susceptibility to pesticide stress, while reducing survival, growth rate and food intake (Dinh Van *et al.*, 2014). A similar finding of increased susceptibility to metal concentration at warmer temperatures has also been recorded in the damselfly, *I. elegans* (see Dinh Van *et al.,* 2013). Different environmental stressors in freshwater ecosystems also alter individual resource allocations leading to life history trade-offs (De Block & Stoks, 2005). Verberk *et al.* (2008) provide an overview of various biological traits relating to development, reproduction, dispersal and synchronisation in different freshwater macroinvertebrates species. In summary, they found that aquatic insects exhibit diverse strategies to maintain their populations in streams, reflecting plasticity in reproductive strategies, dispersal capability and developmental period. Using the wing dimorphic water strider, *Gerris odontogaster*, Vepsäläinen (1978) showed that the length of developmental period differed between small- and large-wing morphs. Small-wing morphs have rapid juvenile growth with limited dispersal capability. They also exhibited increased voltinism compared to large-wing morphs. The cost of early reproduction, extensively reported for terrestrial insects (**Table 2.1**), is negatively correlated with life-span in aquatic insects. For example, upon emergence, the short-lived caddisfly, *Triaenodes bicolor*, deposits eggs in a single batch and dies after reproduction, indicating semelparity (Higler, 2005). In long-lived aquatic insects such as the waterbug, *Iybius ater*, eggs are often deposited on several occasions and adults have longer life-spans (Verberk & Esselink, 2005). Aquatic insects are expected to represent clear trade-offs between early reproduction and adult longevity. This is explained by a female's lifetime potential egg complement that is mature at the time of adult emergence and is seen in many Ephemeroptera and Trichoptera species (Sweeney & Vannote, 1982).

Most adult Ephemeroptera and Trichoptera exhibit limited feeding. This is, at least in part, the result of morphological restrictions imposed by their reduced mouthpart structure (Crichton, 1957). Thus, adult dispersal and reproduction are highly dependent on resources acquired during the juvenile stages. For Trichoptera, adult feeding was only recorded in widely dispersed species such as Limnephilidae and Phryganeidae (Crichton, 1957). Few studies have emphasized the reproductive behaviour of aquatic insects; their ovipositional behaviours are highly specialized by order (Lancaster *et al.*, 2010). As an example, most Ephemeroptera deposit their eggs at the water surface with different ovipositional patterns such as either releasing the eggs from air into a fast velocity stream (Ephemerellidae) or by dipping abdomens into the water (Heptageniidae, Baetidae) (Encalada & Peckarsky, 2007). Trichoptera, on the other hand, attach the entire egg clutch as a single mass, to a hard substrate such as a rock or piece of wood (Reich & Downes, 2004). The cost of oviposition in aquatic insects is expensive as active flight and early egg maturation are prevalent as reproductive strategies upon emergence. Any stressors during the juvenile stage are therefore expected to reduce the volume of resources carried-over into the adult stage.

2.10 Nutritional physiology in insect life history trade-offs

The distribution of nutrients within an insect's body and organs provides a means by which to understand how resources are allocated to ensure increased fitness (Boggs, 1992, 2009). Empirical evidence suggests that insect body parts should respond similarly to environmental variables; phenotypic integration indicates similar reaction norms to environmental factors leading to "environmental static allometry" (Shingleton *et al.,* 2007). The allometric relationship between thorax and wing size, for example, should, if it generates the same response to a particular environmental variable, have the same isometric scaling. Such a response leads to similar nutrient allocation for body parts development. Phenotypic integration, however, should be differentiated between somatic and reproductive components as both components may respond in different ways to maximise overall fitness. Insect somatic components are comprised of body structures other than the ovaries, and mature and immature oocytes, but including the entire exoskeleton and musculature (of the head, thorax, wing and abdomen, and of all appendages) together with the gut, nervous and associated tissue. Insect reproductive tissue comprises the ovaries and oocytes, together with nutrient reserves (see below; **Figure 2.5**).

Numerous, previous studies have highlighted the importance of insect fat bodies as an important nutrient reserves to sustain life and reproduction (Arrese & Soulages, 2010). Insect fat bodies exist as a loose tissue that largely surround the gut and reproductive organs within the abdominal segment. Although stored energy reserves present in the fat body are known to be used for both reproduction (egg production) and somatic maintenance (immune system) (Min *et al.,* 2006; Arrese & Soulages, 2010), many studies have employed abdominal segment fat body as an approximate measure of reproductive allocation (Steven *et al.,* 1999; 2000; Colasurda *et al.,* 2009; Kivelä *et al.,* 2012). In this current study, and for logistical reasons, fat bodies within abdominal segment have been considered as part of reproductive allocation. In addition, capital breeding insects depend totally on stored energy reserves for egg production as they exhibit minimal adult feeding (Tammaru & haukioja, 1996; Jervis *et al.,* 2008). It is important, however, to note that insects may divert nutrient reserves from fat bodies for somatic maintenance, such as enhancing immune system, under stressful environment (Cotter *et al.,* 2011; Reavey *et al.,* 2014).

Protein along with lipids and carbohydrates form the major component of organic compounds in an insect's body (Agosin, 1978; Nation, 2008). Each major component is essential in regulating and maintaining insect life processes during growth and development from juvenile to adult stage. Nutrient reserves are allocated to support insect reproduction (eggs, spermatophores), somatic (muscle, cuticle) and storage (fat body) reserves. Nutrient allocations may change as a trade-off between reproductive and somatic function under different environmental conditions (Socha & Sula, 2008).

Many studies have addressed the importance of protein as an indicator for insect reproduction and survival (Zera & Brink, 2000; Lorenz *et al.,* 2004; Nestel *et al.,* 2004). A major component in insect reproductive and somatic tissues, protein, other than chitin, is also found in the insect cuticle where it provides a strong exoskeleton (Chapman, 2013). Within the insect's body fluid (hemolymph), proteins play an important role in binding and circulating important nutrient components throughout the insect body. Insect reproduction is highly dependent on protein influencing oocyte production and development (Fanson, *et al.,* 2012). Oocyte development is influenced by vitellogenin (egg yolk precursor protein) that increases oocyte maturation rates (Judd *et al.,* 2010). Insect oocytes consist primarily of protein; a lower protein allocation can hinder yolk synthesis. A strong positive association has been shown between protein reserves and egg production rates in the adult fruit flies, *Ceratitis capitata* (see Nestel *et al.,* 2005) and *D. melanogaster* (see Fanson & Taylor, 2012). Higher protein reserves are, however, associated with decreased life-span as increased egg production has a detrimental effect on insect longevity (Fanson *et al.,* 2012). Many studies have, for example, shown that in *Drosophila* species adding protein into the diet maximises lifetime fecundity but shortens adult life-span (Lee *et al.,* 2008; Grandison *et al.,* 2009). In addition, studies on Lepidoptera species have also shown that protein-biased diets represent individuals with higher reproductive tissue (Colasurda *et al.,* 2009; Roeder & Behmer, 2014). Protein also provides the necessary energy reserves during respiratory metabolism, and development during insect diapause (Hahn & Denlinger *et al.,* 2007). Insects produce heat shock proteins (HSPs) when exposed to sub-lethal hyperthermia (Fittinghoff & Riddiford, 1990; Robertson, 2004), which may enhance their thermal tolerance but also divert metabolic energy from reproduction (Rank & Dahlhoff, 2002). Increased

HSPs concentration, however, is a key strategy employed by ectotherms to survive stressful temperature environments, helping to maintain membranes functioning at higher temperature (Sarup *et al.,* 2006; Dahlhoff & Rank, 2007; Kingsolver *et al.*, 2016).

As with proteins, lipids also perform an important role in insect reproduction and somatic functions (Zera & Harshman, 2001). Lipids are an essential component of insect membrane and metabolic tissue structure (Hazel & Williams, 1990; Haunerland & Shirk, 1995); they can be found in the insect fat bodies that primarily support energy demands during high metabolic activity (Arrese & Soulages, 2010). Lipid reserves showed strong positive associations with specific insect life history variables such as extended longevity, increased locomotion (e.g. dispersal), exposure to environmental stressors (e.g. starvation) and physiological development (e.g. diapause) (Ellers, 1996; Ellers & Jervis, 2003; Winkelmann & Koop, 2007). Lipids consist of phospholipids and triglycerides that contribute up to 90% of the total lipid component in insects' bodies (Beenakkers *et al.,* 1985). Together with triglycerides, phospholipids or structural polar lipids form the major part of insect fat bodies. They are stored in an anhydrous form. Triglycerides are comprised of intracellular droplets surrounded by phospholipids and can be immediately released to the haemolymph when required (Chino, 1985). Thus, mobilization of lipid droplets for energy provides faster metabolic pathways compared to other macronutrient such as carbohydrates (Arrese & Soulages, 2010). Lipids are also used to provision eggs; lipid reserves in the fat body can be re-allocated for embryonic development when necessary (mostly for semelporous insects) (Lorenz & Anand, 2004). Most female insects tend to have higher lipid contents than male; oocytes are comprised of 15-40 % lipid. This may be even higher in some species (e.g. *Manduca sexta*; see Troy *et al.,* 1975, Kawooya & Law, 1988). In the insect cuticle, lipids also provide protection; a lipid membrane forms a free-wax layer that prevents water loss or dehydration at higher temperature (Chapman, 2013). Insect membrane-associated proteins also require a particular fluidity of lipid composition to maintain optimal functioning at higher temperature (Hazel & Williams, 1990; Hazel, 1995; Van Dooremalen *et al.,* 2013). Increased lipid allocation may also indicate increased allocation into reproductive traits or adaptive response for survival under stressful thermal environment.

Carbohydrates provide an important energy reserve that influences insect survival and reproduction (Behmer, 2009; Clark *et al.,* 2016). Including both monosaccharides (e.g. glucose, fructose, galactose) and disaccharides (e.g. trehalose, sucrose, lactose) along with the polysaccharide glycogen, most insects can convert mono- and disaccharide sugars into the latter for energy (Van Handel, 1985a; Olson *et al.,* 2000). Insects acquire carbohydrates through adult feeding; this is what sustains energy-demanding behaviours such as active flight (Amat *et al.,* 2012). In Hymenoptera, adult parasitoids acquire carbohydrates from floral nectars or pollen, sustaining flight when finding hosts for oviposition and maintaining oogenesis (Jervis *et al.,* 1996; Harvey *et al.,* 2012). Carbohydrates also enhance adult life-span and egg production as shown in the fruit fly, *Bactrocera tryoni* (see Fanson & Taylor, 2012). Insufficient carbohydrate acquisition reduces reproductive success in the parasitoid *Gelis agilis* (see Harvey *et al.,* 2012), retards growth in the locust *Chortoicetes terminifera* (see Clissold *et al.,* 2006), and also reduces survival in honeybee colonies (Winston, 1987; Suarez *et al.,* 2005).

Insects may utilize different nutrient reserves to fuel energy-demanding behaviours; carbohydrates for Hymenoptera (Rivero *et al.,* 2001) and lipids for Lepidoptera (Angelo & Slansky Jr, 1984). This, however, is influenced by adult feeding behaviour as non-feeding adults depend totally on resources, stored as lipid in fat bodies, carried over from larvae (Jervis *et al.,* 2005). Some Lepidoptera and Diptera, have been observed to use more than a single nutrient component to support their energetic and metabolic demands (Arrese & Soulages, 2010). Nutrient allocation between somatic and reproductive tissue is expected to change, along with insect physiological responses, under different environmental conditions. Thus, nutrient allocation into insect body parts linked to somatic or reproductive functions may provide an important indicator for resource allocation choices especially in semelparous insects that totally depends on resource acquired from the larval stage.

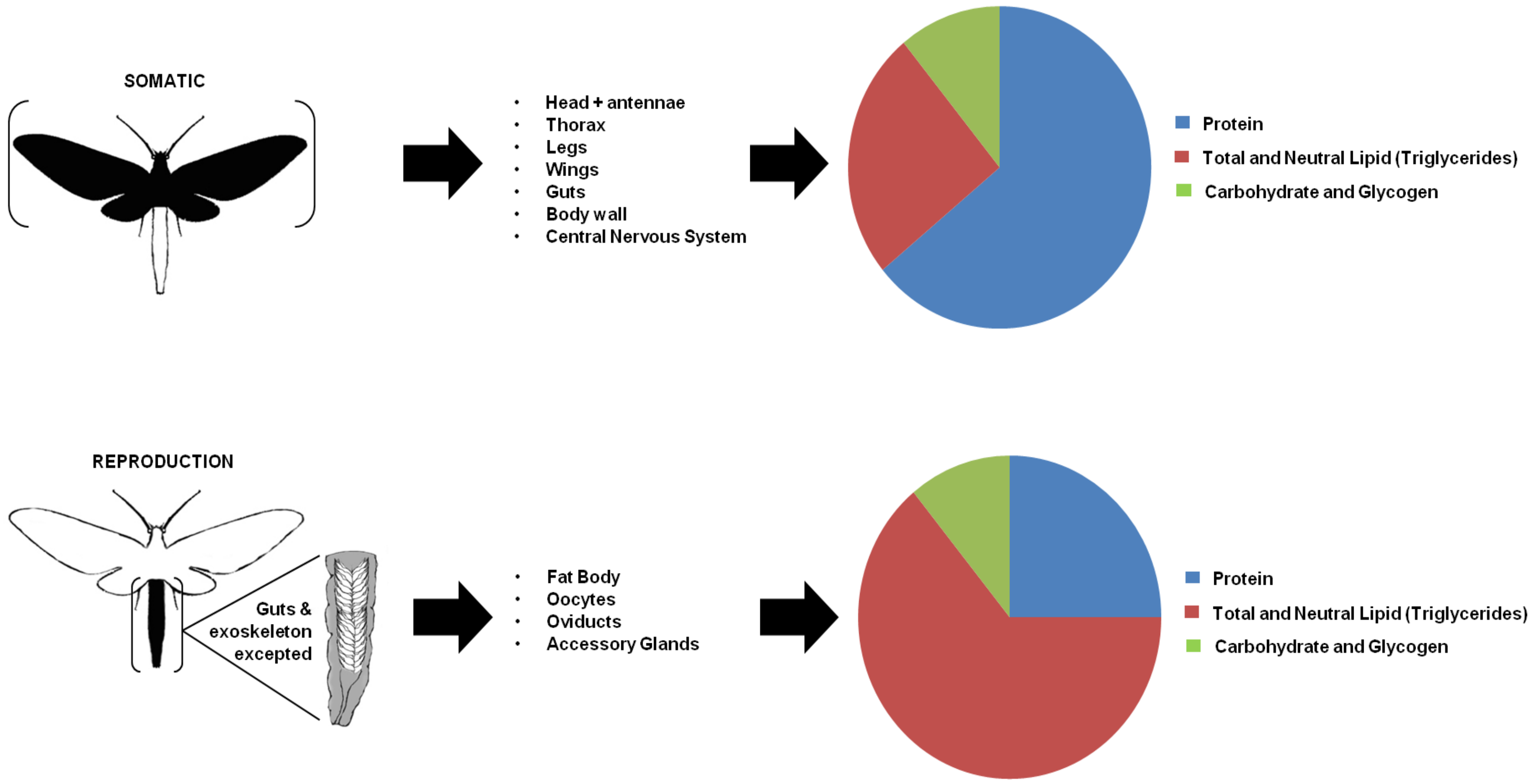


Figure 2.5: Diagrammatic representation showing the different insect body parts linked to reproduction and somatic functions. Individual investment into different body parts represent different nutrient components that are used to support energetic and metabolic demands throughout insect life history. See text above for discussion of why fat body is considered under reproductive allocation (section 2.10).

2.11 Perspectives and implications for the current study

This chapter has focused on the evolutionary aspects of insect trade-offs from the perspective of physiological characteristics and life history traits. Many studies have highlighted the importance of these trade-offs, in terms of nutrition, physiological development and environmental stressors. Insect life history traits are directly linked to reproduction and survival. This is shown in insect physiological traits (e.g. body size, wing dimorphism, abdominal and thoracic mass) that represent individual resource investment towards reproduction (e.g. lifetime fecundity, oocytes) and survival (e.g. life-span). In addition, recent biochemical studies also provide similar findings when considering an insect’s ability to allocate resources to meet energetic demands for survival and reproduction (i.e. biochemical composition of body parts). Insect nutrient allocation illustrates the fundamental aspects of life history that have critical consequences for fitness. Changes in nutrient allocation patterns may also represent early responses of insect adaptation under changing environmental conditions. Although these studies have advanced our understanding, the pattern of energy reserve distribution between reproductive and somatic components has been largely overlooked.

Detailed studies on allocation tendencies and nutrient levels have illustrated the physiological cost of specific traits throughout an insect’s life history. Under stressful environments, insects increase their resource investment into somatic maintenance and this alters resource allocation choices affecting, in turn, fitness. This is difficult to assess as insect adaptation may differ between individuals and populations depending on their physiological response. Most studies on life history trade-offs highlight the differences between physiological characteristics rather than comparing nutrient allocation into reproductive and somatic components. By measuring nutrient allocation into insect body regions linked to reproductive and somatic functions, detailed information of physiological differences can be obtained. Further studies on insect nutrient allocation may also explain the cost of reproduction; different nutrient allocation patterns are expected between current and future reproduction. In addition, insects that are exposed to different environmental conditions may allocate resources differently to maintain their survival. This again will result in trade-offs between reproduction and somatic maintenance. Studies on insect nutrient allocation also present the opportunity to test the "Y-model of Resource Allocation". Specific nutrient components may influence insect life-span and reproduction, and individual resource allocation choices into specific life history traits. As insects have the ability to alter allocation of nutrients to different tissues, studies on allocation theory may add further details on insect life history trade-offs.

This chapter has highlighted how insects may modify their allocation and utilization of nutrients to various body regions depending on energetic demands under differing environmental conditions. This raises a critical question of how insects achieve this and allocate energy reserves to maximise fitness. More studies on insect nutrient allocation between reproductive and somatic components, and across different environmental conditions may help to predict insect adaptation and evolutionary responses, and provide detailed information for an early conservation effort.

3. General Insect Methodology and Assays

3.1 Abstract

1. Insect are important organisms in which to determine resource allocation between reproductive and somatic functions, but there have been few investigations of insect resource allocation processes for ecological and evolutionary purposes, particularly where field and laboratory approaches are combined.
2. This chapter details the general dissection and experimental protocols used throughout the study on a range of species to determine ovarian development and apply macronutrient assays. The intention was that these methods would be used in each experimental study as a reliable and repeatable protocol to estimate insect resource allocation between major life history traits.
3. Trade-offs between reproductive and somatic traits are expressed primarily in individual physiological characteristics and egg production. Selected insect species were dissected into different body parts chosen to represent reproductive and somatic functions. Resource investment into organ masses were determined using multi nutrient insect protocols measuring protein, carbohydrate and lipid content.
4. The methods were apparently able to reveal resource allocation between somatic and reproductive functions in individual insects, and this chapter highlights the importance of using integrative nutrient assay protocols as the basis for insect ecophysiological investigations. The methods described here were subsequently applied in Chapters 4, 5 and 6.

3.2 Introduction

All the experimental chapters in the thesis depend on a common set of methods designed to assess how individual insects allocate resources between reproductive and somatic tissue. The purpose of this chapter is to provide some detail on these methods to underpin the methodological overview sections in the subsequent chapters. The overall methods were derived from similar procedures previously developed by Rivero *et al.,* (2001) and Foray *et al.,* (2012) and applied elsewhere by Jannot *et al.,* (2007), Amat *et al.,* (2012), Hidalgo *et al.,* (2016).

3.3 Correlations between reproductive traits and insect body size

Correlations between insect body size and reproductive traits were assessed by categorising individual insects according to body mass. Each female insect from all study species was weighed for whole-body wet mass to the nearest 0.1 mg using a digital balance. Morphological traits (e.g. wing and hind tibia length) were measured to the nearest 0.01 mm in a standard procedure using a digital calliper. Left forewing and hind tibia were carefully dissected from the thorax (ventral view) under a stereomicroscope (x20 magnification). Forewing measurement was taken as the distance from the axillary area to the wing apex (M_1) (**Figure 3.1**), while hind tibia length were measured under stereomicroscope. Measurements of the forewing and hind tibia could subsequently be used as proxy estimates for female body mass, but measurement of hind tibia length provided a better indicator of female body mass ($F_{1,48} = 883.5$, $P < 0.001$) than wing length ($F_{1,48} = 6.22$, $P = 0.016$) (**Figure 3.2**).

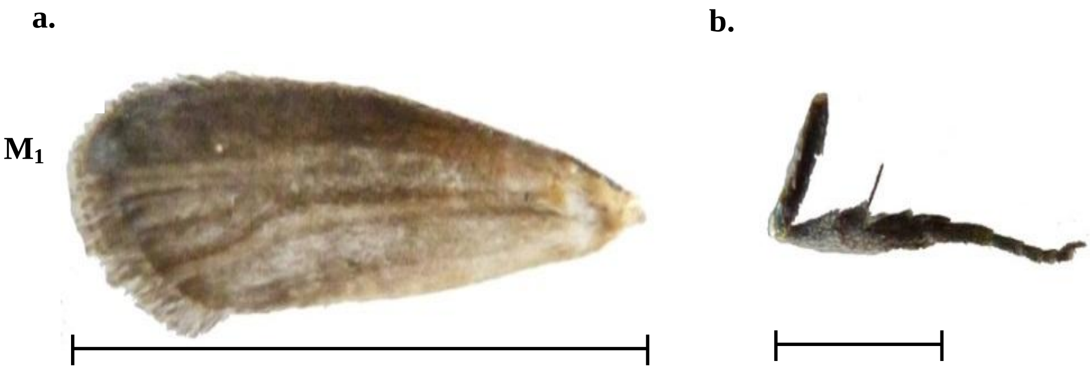


Figure 3.1: Image showing the measurement used to estimate a. forewing and b. hind tibia length for all study species.

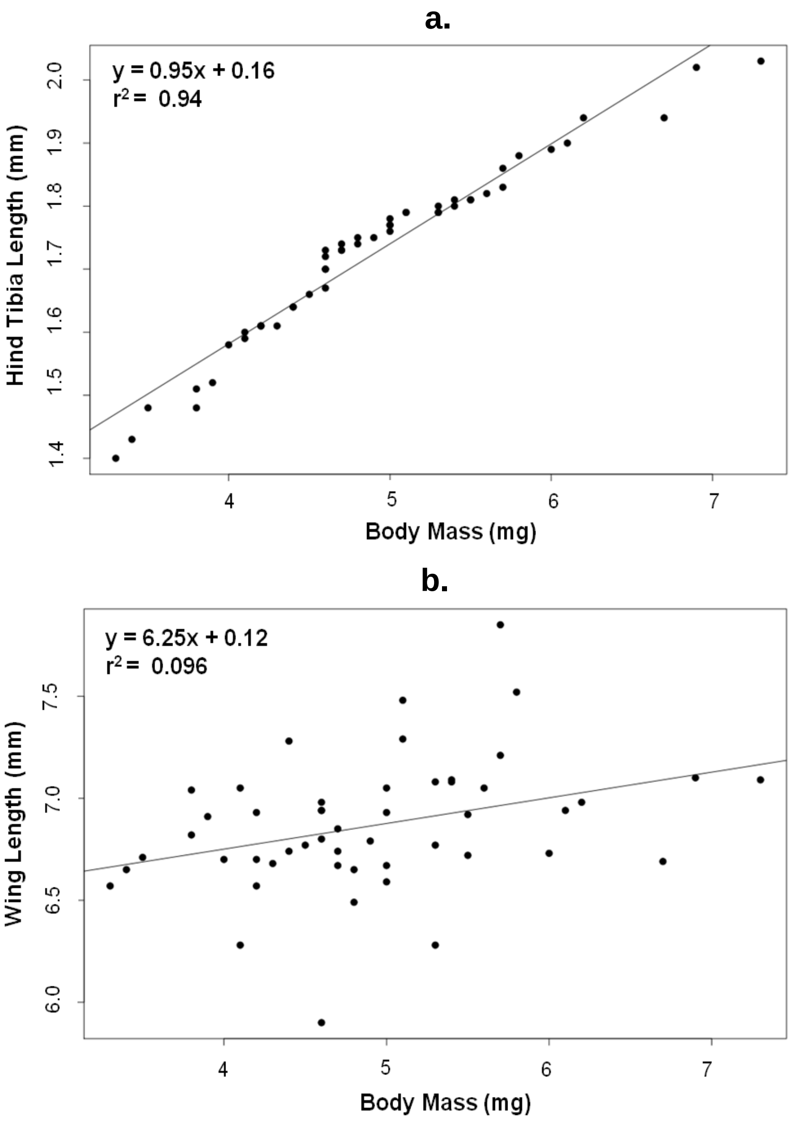


Figure 3.2: The relationship between a. hind tibia length ($P < 0.001$) and b. wing length ($P < 0.05$) plotted against female body mass.

3.4 Female Dissection into Somatic and Reproductive Components

Dissection of female insect to appraise division of tissue between somatic and reproductive components was carried out under a stereomicroscope (x20 magnification). The allocation of mass between somatic and reproductive tissues was then assumed to be an indicator for somatic and reproductive traits. Somatic allocation comprises body structures other than the ovaries, oviducts, and mature or immature oocytes together with initial nutrient reserves (refer to **Figure 3.3**). This somatic component basically consists of the entire exoskeleton and musculature (of the head, thorax, wings and abdomen, and of all appendages), together with the gut, nervous and associated tissue (Jervis, 2001).

The procedure to identify these components involved, firstly, labelling and weighing cover slips individually to 0.1mg using a digital balance. The insect abdomen was then split laterally along the ventral, anterior end and pulled down its length to the ovipositor. Somatic and reproduction components were weighed precisely using the same procedure, but on a different cover slip. The reproductive system (ovaries and oviducts) was further dissected to assess ovarian dynamics as identified in the ratio between mature and immature eggs. Dissection was done on cover slips placed on ice to prevent nutrient degradation (**Figure 3.3**).

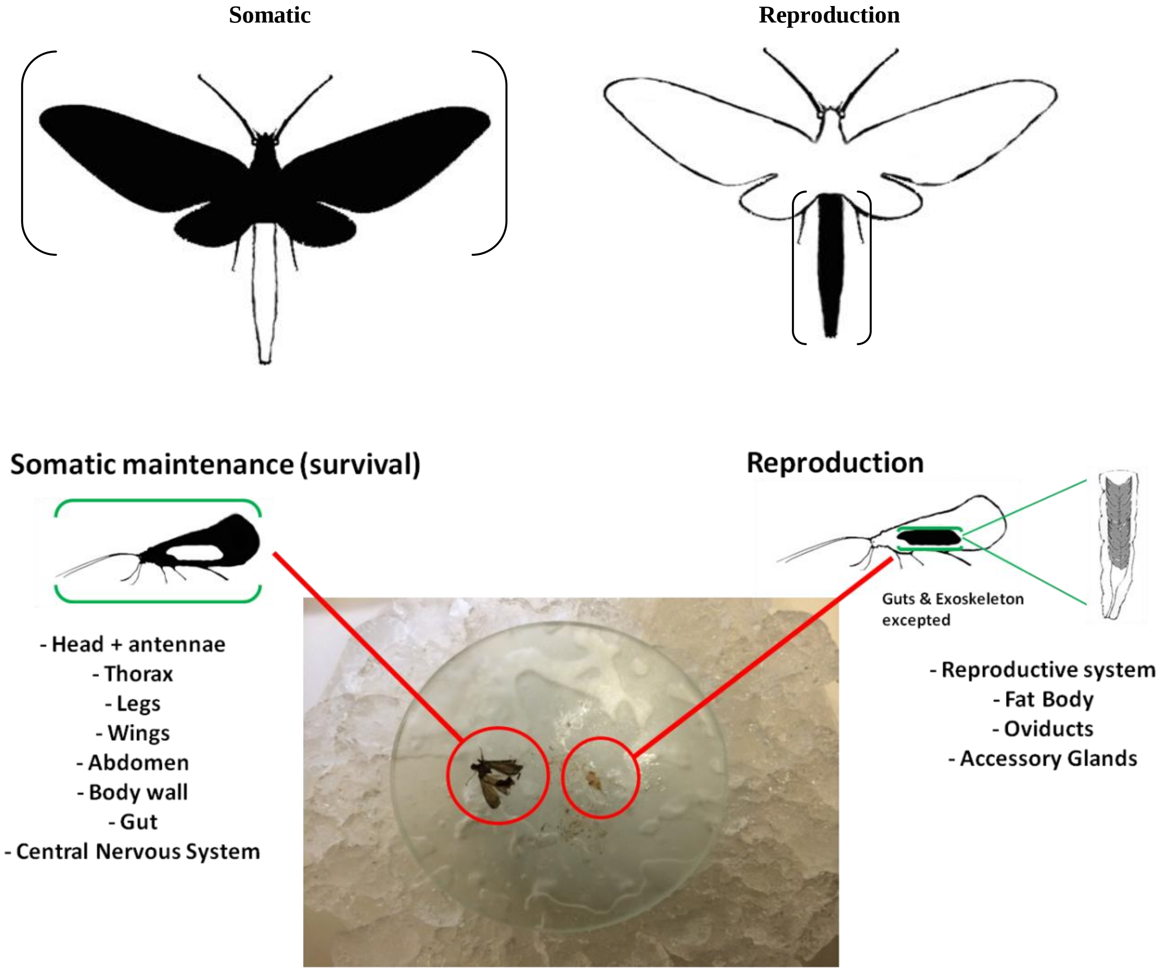


Figure 3.3: Diagram showing insect body parts link to somatic and reproductive components. Dissection for all study species with division of tissues between somatic and reproductive components under stereomicroscope (x20 magnifications), on cover slips with ice.

3.5 Ovarian Dynamics

3.5.1 The Indian mealmoth, P. interpunctella

For *P. interpunctella*, ovarioles were separated on the basis of possession of chorionated and unchorionated oocytes. Mature eggs were determined by having a full chorion while immature eggs had incomplete yolk contents (**Figure 3.4**). The numbers of mature and immature eggs were counted under a stereomicroscope (x20 magnification). As oocytes develop progressively along the ovarioles, it was not possible to count all oocytes, some being too small. Thus, such counts were limited to the fraction of clearly visible oocytes. Mature eggs also had a very regular shape with sculpturing that was absent from the immature ones (**Figure 3.4**). Inspection of oocytes was carried out in glycerol, thus preserving the sample and allowing the determination of the number of oocytes in each individual female.

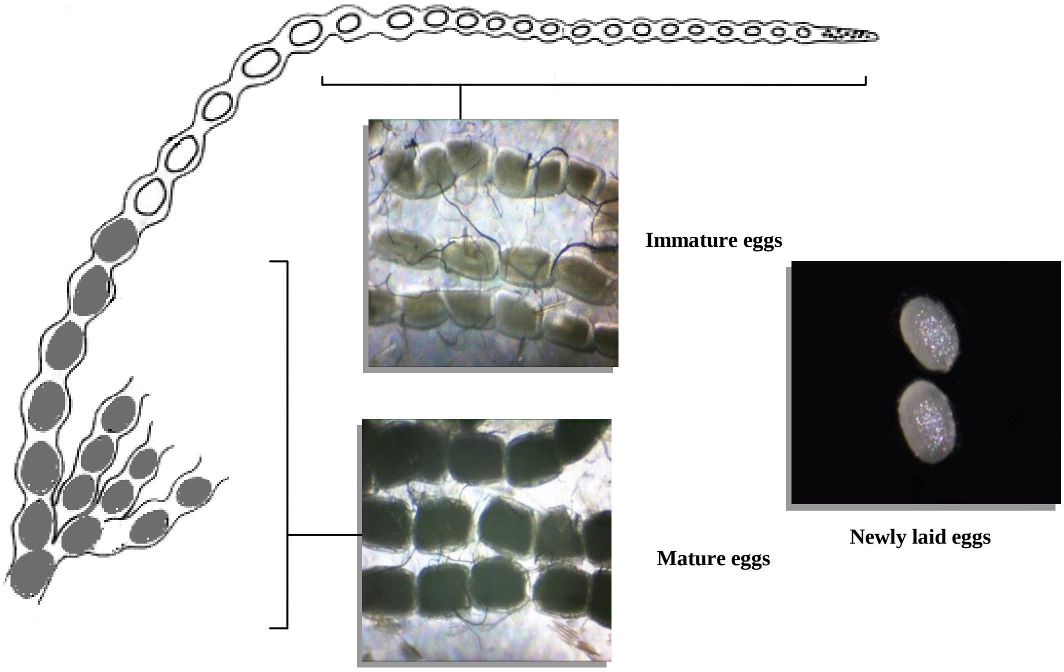


Figure 3.4: Diagram showing ovarioles with image of immature, mature and newly laid eggs under stereomicroscope (x20).

3.5.2 Free living caddisflies, Hydropsyche siltalai *and* Rhyacophila dorsalis

Reproductive traits for the female caddisflies, *Hydropsyche siltalai* and *Rhyacophila dorsalis* were not determined. Upon emergence, it was difficult to determine immature and mature caddisfly oocytes (**Figure 3.5**) due to the gelatinous materials, known as spumaline, that form a layer between and over the oocytes that attach caddisfly eggs to the substrate in streams.

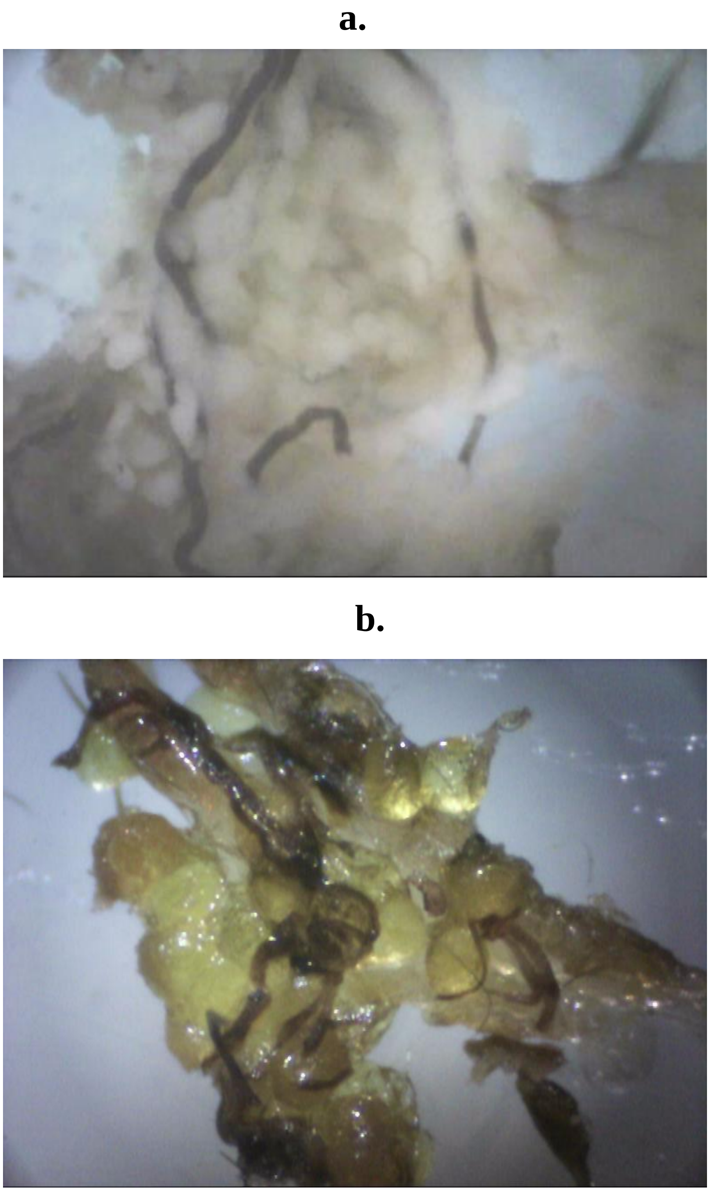


Figure 3.5: Image showing (a) immature and (b) mature egg mass in caddisfly. Egg yolk are clearly shown in immature eggs while mature eggs were covered with chitinous endocuticle that surrounds entire egg cell. This layer were form later during egg development.

3.6 Insect Nutrient Assay Protocol

The nutrient assay protocols used throughout these studies were modified from those of Foray *et al.* (2012). Assays were carried out on the reproductive and somatic tissues of individual insects. Individual body parts linked to somatic and reproductive functions were ground with a micro-pestle in an Eppendorf tube containing 180 uL aqueous buffer solution (100 mM potassium phosphate monobasic (KH2PO4), 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4). This volume was considered appropriate because in the next step more solution needed to be added into the tubes. Each sample was subjected to low spin centrifugation (180 *g* at 4º C) for 15 min. which allows gentle sedimentation of cuticle and cell debris that otherwise would alter the clarity of the sample.

3.6.1 Protein Determination

The supernatant from the centrifugation (2.5 uL) was transferred into a 96-well microplate, together with 250 uL of Bradford micro-assay reagent. Samples were incubated for 15 min at room temperature to ensure that the protein-dye complex was stable (maximum duration less than 50 min in accordance with manufacturer instructions). Samples were gently shaken (10 Hz for 3 s) to disrupt protein-dye aggregates and concentrations were determined spectrophotometrically at 595 nm (Infinite® 200 PRO series) using a dilution series of bovine serum albumine into the same buffer as the standard (5 uL for each well) (**Figure 3.6**).

3.6.2 Total Carbohydrate and Glycogen Determination

Sodium sulphate (Na_2SO_4) solution (20 uL) was added to the supernatant to dissolve all carbohydrates. To this solution, 1500 uL of a chloroform-methanol solution (1:2 v:v) was then added to solubilise the total lipids as well as the water soluble carbohydrates. The whole solution then underwent vigorous vortexing and centrifuging for 15 min (180 *g* at 4ºC) to remove glycogen. Glycogen remained at the bottom of the Eppendorf tube and the remaining supernatant was transferred into a new tube to separate between soluble carbohydrates and glycogen. In the first step, soluble carbohydrates were measured. The supernatant (150 uL) was transferred into a microplate well and evaporated for 30 min at room temperature until a volume of 10 uL was reached. Then, 240 uL of anthrone reagent was added to each well and incubated for 15 min at room temperature. The microplate was carefully covered and heated for 15 min at 90º C in a water bath. Absorbance of samples was read at 625 nm using D-glucose as the standard

(25 uL for each well) (**Figure 3.6**). In the second step, glycogen was washed twice using 400 uL methanol (80%) followed by vortexing and centrifuging for 5 min (1600 *g* at 4ºC). The remaining supernatant was removed and 1 ml of anthrone reagent was added to the pellet, followed by 15 min incubation at 90ºC in a water bath. Samples were cooled on ice and filtered using syringe and low-protein membranes (polyvinylidene fluoride; d-0.45 uM). Supernatant (250 uL) was added to each well and determined at 625 nm with D-glucose as the standard. In carbohydrate and subsequent assays, we used a flat-bottom 96-well borosilicate microplate because the organic assay used are incompatible with standard polystyrene microplate.

3.6.3 Total and Neutral Lipid Assay Determination

Lipid measurement involved two analyses; one for total lipid content and the other for neutral lipid content. For total lipids, 100uL of supernatant from the previous assay was transferred into the borosilicate microplate well and heated up at 90 ºC until complete solvent evaporation had occurred. Sulphuric acid (10uL of 98%) was then added to each well and the microplate incubated at 90 ºC for 2 min in a waterbath. After cooling on the microplate on ice, 190 uL of vanillin reagent was added to each well. Vanillin reagent has been recognized to provide accurate data for lipid measurement in insect (Williams *et al.,* 2011). The microplate was then homogenized and incubated at room temperature for 15 min and its absorbance was measured spectrophotometrically at 525 nm. For neutral lipids, 500 uL from the remaining supernatant was transferred into a new tube and heated dry at 90 °C until complete evaporation. One millilitre of chloroform was added to re-solubilise the lipids. In each tube, 200 mg of dry silicic acid was in excess specifically to bind all the polar lipids present (Zera & Larsen, 2001). The mixture was shaken and centrifuged for 10 min (180 *g* 4ºC). Phospolipids become bound and sink to the bottom of the tube. Measurement of neutral lipids, after removing the polar lipids, provides a reliable estimate for triglyceride content (Zera & Larsen, 2001). Final supernatant (100 uL) was measured using the same protocols. Absorbance of samples was read at 525 nm using two distinct standardized solutions of chloroform–methanol (1 : 2 v:v), one containing a mix of phospholipids (azolectine, 18811145; Sigma) and the other one containing triglycerides (triolein, 92860; Sigma) (**Figure 3.6**).

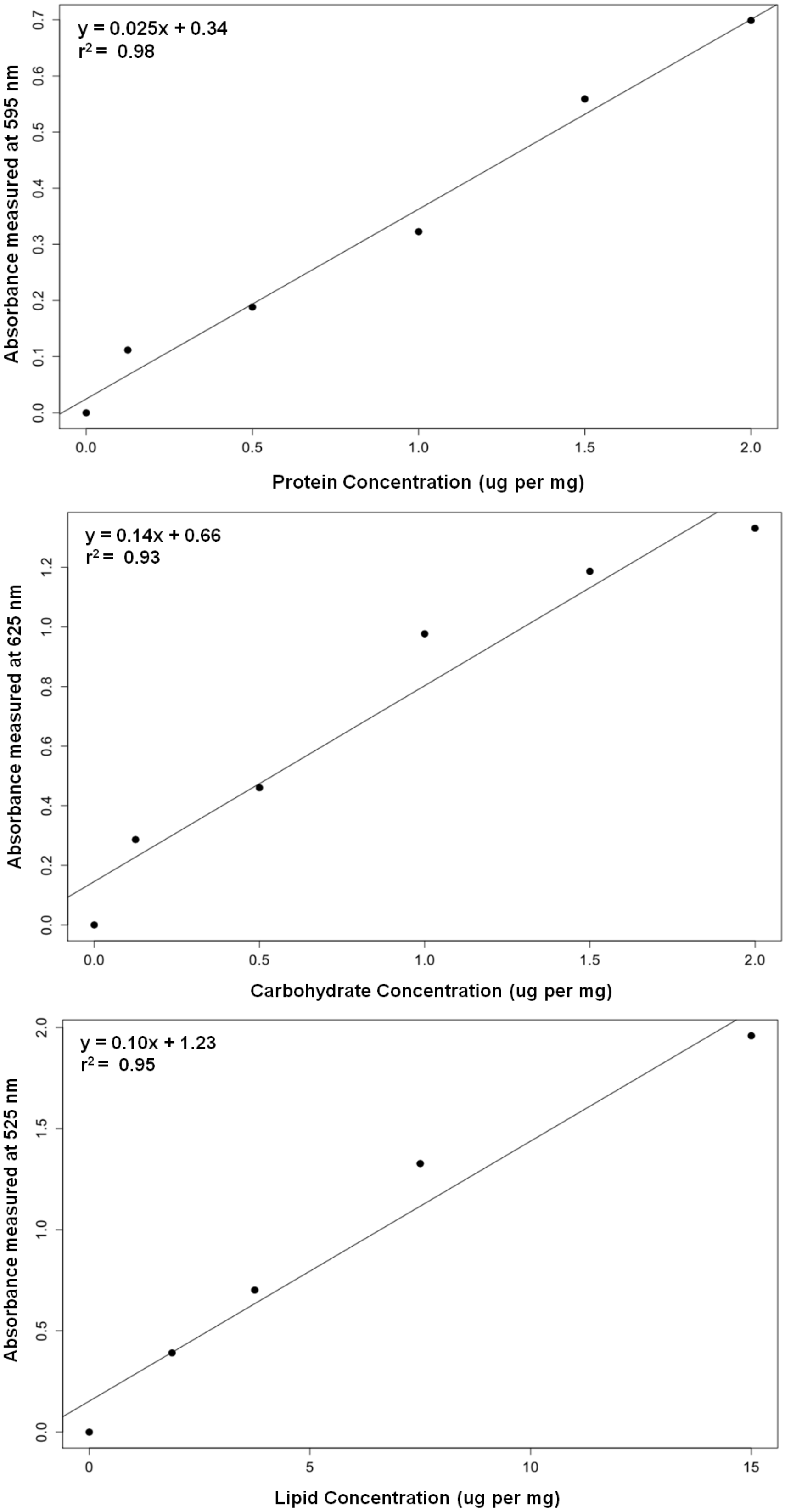


Figure 3.6: Standard curves for protein, carbohydrate and lipid using the modified insect assay protocol (from Foray *et al.*, 2012).

4. Individual Body Size and its Role in Determining Reproductive Fitness

4.1 Abstract

1. Quantifying how insects of different body size maximise overall fitness is key to understanding how resources are allocated into life history functions such as reproduction and survival. Female body size is predicted to influence life history trade-offs between current and future reproduction through prolonged survival in larger individuals. Data on intraspecific variation in individual lifetime fecundity and relative physiological costs are, however, scarce despite their importance in understanding trade-offs in reproductive strategies.
2. Females of the Indian mealmoth, *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae), were reared under laboratory conditions (25 ± 2 °C and 70-80 % relative humidity) to appraise the effects of body size on resource allocation in terms of lifetime potential fecundity (number of eggs that can be potentially laid by females), initial egg-load, oocyte load and the ‘ovigeny index’ (the proportion of the lifetime egg complement of a female individual that is mature upon emergence). Resource investment into reproduction and survival was also determined from macronutrient (e.g. protein, carbohydrate, glycogen and lipid) allocations in the tissues of newly-emerged females.
3. The ‘ovigeny index’ declined significantly with body size in female *P. interpunctella*. Increasing body size increased initial oocyte load, but not initial egg load. Smaller females had shorter life-spans compared to larger females, with higher allocations of protein, carbohydrate, glycogen and neutral lipids in reproductive and somatic tissue than larger females, with the exception of total lipids.
4. These data illustrate how the costs of reproduction in insects give rise to trade-offs between life-span and reproductive timing. Reproductive costs in *P. interpunctella* appear to reduce life-span in smaller females. Initial egg-load appears to require major resource investment for both somatic and reproductive tissues.

4.2 Introduction

A core theme in life history studies involves untangling the extent to which different traits affect reproductive output and, ultimately, reproductive fitness. For example, variations in reproductive output can arise from offspring size (Saeki *et al.,* 2013), female age or size upon maturation (Ross *et al.,* 2011), and lifetime reproductive success (Jervis *et al.,* 2001; Ellers & Jervis, 2004). Potentially more important, insect body size affects overall fitness by increasing potential reproductive effort (Roff, 1992, 2002; Henry *et al.*, 2009). Larger-sized females are expected to acquire more resources, and consequently produce more offspring (Belovsky & Slade, 2009). This prediction follows from the allometric relationship in which fecundity and offspring number (Honek, 1993) are directly dependent on female body size (Berger *et al.,* 2008; Chown & Gaston, 2010). The latter can also influence individual success in mating by enhancing longevity (Filin & Ovadia, 2007).

The array of potential life history influences on insect fitness means that no single strategy is guaranteed to be the most effective, and a range of trade-offs and permutations are possible. The number of mature eggs, for example, carried by a female at any given moment in her lifetime represents a trade-off between current and future reproduction (Jervis *et al.,* 2003). Strategies favouring current reproduction require greater resource investment, but this could incur costs such as reduced longevity and reduced future reproduction (Gotthard *et al.,* 2007). The converse involves maximising longevity at the potential cost of deferred reproduction, and size may play an important role in determining reproductive strategies. Thus, larger individuals tend to invest a greater proportion of their resources into future reproduction through extended life-spans (Ellers & Jervis, 2003), whereas smaller females with short life-spans are essentially investing into current reproduction. This trade-off between current and future reproduction in insects implies a species-specific optimal allocation of resources that yields maximal fitness (Angelo & Slansky Jr, 1984).

As well as body size, resource investment in insects reflects the need to match available nutrients to trait requirements, and strategies again vary within and between species (Mole & Zera, 1993; Congdon *et al.,* 2001; Carsten-Conner *et al.,* 2010; Wajnberg *et al.,* 2012). Endopterygota insects - those with complete metamorphosis - need to store sufficient resources during larval feeding to support pupal diapause and adult development. In species that do not feed as adults, resources available on emergence must support both future dispersal and reproduction (Jervis & Boggs, 2005). In general, however, most resources carried over to adulthood by the Endopterygota are allocated to manufacturing new tissues as most larval tissue is reconstructed during metamorphosis (Chapman, 2013). At this stage, resources are allocated either to the development of adult soma, or are invested into reproduction at adult eclosion (Kivela *et al.,* 2012). Resource allocation to the head and thorax - locations for the majority of the body's exoskeleton and musculature - closely approximates resource allocations to somatic function. In contrast, resource allocation to the abdomen approximates that given to reproductive function because the female abdomen is largely filled by ovaries and the major energy reserve, the fat body (Boggs, 1981; Jervis *et al.,* 2007, 2008). Boggs (1981) postulated that resource allocation during metamorphosis in female holometabolous insects should correlate with life-span and with the expected lifetime intake of dietary nutrients contributing to egg manufacture. On this basis, Jervis *et al.* (2005, 2007), in studies on Lepidoptera, hypothesised that species emerging with all their eggs mature should invest resources in reproduction at the expense of somatic growth. In contrast, species emerging with immature eggs should invest relatively more resources in body-building at the expense of allocation to reproduction. Greater investment into somatic function, coupled with a potentially longer life-span, would thus provide female insects with the opportunity to supplement or replenish, via their diet, the nutrient reserves needed for bringing the full lifetime potential egg complement to maturity. Jervis *et al.* (2001) developed this concept into an 'ovigeny index', expressing the ratio of initial egg load (fully mature eggs) to lifetime potential fecundity.

Conceived in this way, the 'ovigeny index' reflects not only the initial reproductive effort of the female insect but also the presumed relative allocation of resources to reproduction by both juvenile and adult stages (Jervis & Ferns, 2004). One possible route to appraising such relative allocations would be an assessment of how major biochemical compounds (i.e. proteins, lipids, carbohydrates and glycogen) are allocated within the insect body. While there have been previous attempts to quantify these biochemical components of individual insects, using methods proposed by Bradford (1976), Van Handel (1985a, 1985b) and Van Handel and Day (1988), it is only relatively recently that the colorimetric approach of Foray *et al.* (2012) has allowed such assays to be carried out in the same individual insects for different biochemical compounds. Changes in individual insect life history may correspond with shifts in nutrient storage; different nutrient reserves indicate a specific life history function. Nutrient allocation between reproduction and survival represents resource trade-offs under physiological constraints such as reduced reproduction for somatic maintenance (e.g. initial egg, flight capability). Examples of this are shown in several insect species such as the fruit fly, *Ceratitis capitata* (see Nestel *et al.,* 2005), the cricket, *Gryllus firmus* (see Mole & Zera, 1994), the parasitoid wasp, *Macrocentrus grandii* (see Olson *et al.,* 2000) and the beetle, *Coccinella septempunctata* (see Zhou *et al.,* 1995). Most studies, however, are limited to one or two biochemical compounds. The multi-nutrient assay protocol of Foray *et al.* (2012) allows investigation of different resource allocation into body parts associated with reproduction and survival. So far, experimental studies using this approach are surprisingly few (exceptions being Amat *et al.,* 2012; Hidalgo *et al.,* 2016).

This chapter provides a detailed analysis of reproductive life history and resource investment between reproduction and survival in the Indian mealmoth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). This species is a serious pest of stored food products, causing considerable losses to cereal grains, grain legumes and other high-value crops, such as cocoa beans and dried fruits (Na & Ryoo, 2000; Adarkwah & Schöller, 2012). Found globally (Rees, 2004), *P. interpunctella* was first described by Hübner in 1827 as *Tinea interpunctella* before being placed by Guénéé (1845) in the genus *Plodia*. It is the larval stages that cause the most damage; infested materials are typically found webbed or spun together with silken masses containing larval excreta which severely reduces food quality (Tzanakakis, 1959; Hobza *et al.,* 1972).

Plodia interpunctella is a semelparous moth where female reproduction largely depends on the resources acquired from the larval stage (Gage, 1995). More importantly in the present context, *Plodia* offers a valuable study species for understanding resource investment into potentially different phenotypes, and experimental data on reproductive life history would provide a baseline from which to evaluate rules of resource allocation between current and future reproduction. Two questions were posed. Firstly, does resource allocation change with female body size? It was predicted that different reproductive traits between small and large females would translate into different resource allocation, with smaller female moths investing more of their resources into current reproduction because of their shorter life-span. Secondly, does resource allocation vary between initial egg and oocyte load as a trade-off between current and future reproduction? Resource investment into current reproduction was predicted to lead to higher nutrient allocation in reproductive tissue, leading to a trade-off between resources. To test these hypotheses, the study set-out to (i) establish a baseline measure of reproductive life history traits (e.g. lifetime potential fecundity, initial egg load, initial oocyte load and lifetime realised fecundity), and (ii) measure the allocation of nutrients (e.g. proteins, lipids, sugars and glycogen) into the female moth's body components associated with somatic and reproductive functions.

4.3 Materials and Methods

4.3.1 Insect culture

The *Plodia interpunctella* population used in this study was obtained from a laboratory colony maintained at the University of Liverpool (UK). Larvae were reared under standard conditions at 25±2 °C and 70-80 % relative humidity with L16:D8 h photoperiod in an environmentally-controlled cabinet (incubators KBWF-720, BINDER GmbH, Tuttlingen, Germany). Larvae were housed in rearing containers (21 x 11 x 9 cm) with five net-covered holes for air-exchange and fed with *ad libitum* access to a 300 g mixture of bran, glycerol and yeast (ratio of 10:5:1 and based on Boles and Marzke (1966)). A total of 50 larvae was maintained in the rearing container, ensuring maintenance of conditions of optimal resource and minimal competition. During daily monitoring, any pupa formed was collected and transferred individually into Petri dishes containing moist filter paper to develop and emerge. Newly-emerged *P. interpunctellla* (less than 3 h old) were collected from the same rearing cage, thus individual insects were not independent.

4.3.2 Experiment 1: female reproductive life histories at different body size

Individual females were observed throughout their lifetime; potential fecundity, initial egg and oocyte load were recorded. Lifetime realised fecundity was determined from 50 pairs of adult moths maintained separately in small rearing containers (5 x 5 x 4 cm) lined with black filter paper. Male insects were assigned randomly to each female in the rearing containers. The study, however, did not observe the effects of male body size on female reproduction. Observation on egg production was initiated 24 h after set-up. Eggs were removed daily and the black filter paper replaced. Freshly-laid eggs were measured for length and width to determine egg volume (Blackburn, 1991). Data on female longevity and fecundity were collected until mortality had reached 100 %. In addition, 50 newly-emerged female moths were dissected under dissecting microscope at x20 to assess the complement of mature and immature eggs in the ovarian tracts. Following dissection, oocytes within the ovarioles were classified as chorionated or unchorionated; mature eggs were characterised by having a full chorion while immature eggs had incomplete yolk content visible. Mature eggs also had a very regular shape with sculpturing that is absent from the immature ones. Some difficulty was encountered in counting some of the very small oocytes, and these were excluded from analysis. Only a small proportion of eggs (2-5 %) were affected in this way. From these data the 'ovigeny index' could be determined and related to female body size. Selection for different female body sizes was made using individual hind tibia length and body mass.

4.3.3 Experiment 2: resource allocation between reproduction and survival

The allocation of resources between reproductive and somatic tissue was quantified using a multi-nutrient assay for protein, carbohydrate, glycogen and lipid (Foray *et al.*, 2012) (refer Chapter 3). In contrast to other work using stable isotopes to track nutrients in different body regions (e.g. thorax versus abdomen; Stevens *et al.*, 1999) or tissues (e.g. fat reserves, in comparison with egg load; Judd *et al.*, 2010), the multi-nutrient approach: (i) recognizes that several nutrients are likely to be involved in each of the supposedly competing physiological processes of reproduction and survival, and (ii) has the potential to reveal whether the hypothesized patterns of allocation apply to nutrients as a whole, or only to specific nutrients (Strand & Casas, 2008). All nutrient assays were undertaken using 50 newly-emerged moths (<1 h post-emergence, unfed). Individual females were killed by freezing to arrest metabolic processes and then weighed, measured for their hind tibia length and dissected. Separation to somatic and reproductive components was carried out on cooled Petri dishes. Tissues allocated as somatic included body structures other than the ovaries, oviducts, mature and immature oocytes, together with fat bodies (refer Chapter 2, p. 29). This represents the entire exoskeleton; musculature of the head, thorax, wings and abdomen, and of all appendages; and the gut, nervous system and associated tissues (Jervis *et al.*, 2001). The abdomen was split laterally along the ventral, anterior end and opened down its length to the ovipositor. Each component associated with the abdominal cavity was then placed individually into 2 ml Eppendorf tubes placed on ice for subsequent biochemical analyses. Quantification of the amounts of nutrient followed a procedure adapted from Foray *et al.* (2012) (refer Chapter 3) using modified colorimetric techniques originally developed for mosquitos that have since been applied successfully to a diverse array of insect taxa, including parasitoids (Amat *et al.*, 2012) and flies (Nestel *et al.*, 2003).

4.3.4 Data analysis

For statistical analysis, each individual *P. interpunctella* was considered a replicate. Reproductive life history and newly-emerged female nutrient content were determined on 50 individuals, and values of the ‘ovigeny index’ were arcsine-transformed prior to further analysis to normalise distributions and reduce heterogeneity. For female reproductive life history, linear regression was used to relate reproductive traits, including egg volume, to female body size. Variations between different macronutrient allocation into reproductive and somatic tissue were determined on 50 newly emerged females using linear regression. In addition, nutrient content (ug per insect) between reproductive and somatic functions were expressed as percentage to compared resource investment between major life history traits. Principle Components Analysis (PCA) was performed on female nutrient content (protein, carbohydrate, glycogen and total and neutral lipid) and compared using Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2006). This is a non-parametric test which compares groups in multi-variate space based on Bray-Curtis dissimilarities; p-values are generated via a permutational procedure. Although PERMANOVA does not require multivariate normality (Anderson, 2001), results can be confounded by unequal dispersion among groups. PERMANOVA tests were carried out using the 'Adonis' function, based on 4999 permutations (Oksanen *et al.*, 2012). All analyses were computed using the R statistical software (R Development Core Team, 2011).

4.4 Results

4.4.1 Experiment 1: female reproductive life history at different body size

Lifetime realised fecundity (hind tibia length: $F_{1,48} = 5.18$, $P = 0.027$; **Fig. 4.1a**) (body mass; $F_{1,48} = 9.98$, $P = 0.003$; **Fig. 4.1g**), lifetime potential fecundity (hind tibia length: $F_{1,48} = 13.62$, $P < 0.001$; **Fig. 4.1b**) (body mass: $F_{1,48} = 12.85$, $P = 0.007$; **Fig. 4.1h**), initial oocyte load (hind tibia length: $F_{1,48} = 20.4$, $P < 0.001$; **Fig 4.1c**) (body mass: $F_{1,48} = 21.8$, $P < 0.001$; **Fig. 4.1i**), egg volume (hind tibia length: $F_{1,41} = 4.10$, $P = 0.044$; **Fig. 4.1f**) (body mass: $F_{1,48} = 4.26$, $P = 0.045$; **Fig. 4.1l**) and life-span (hind tibia length: $F_{1,48} = 6.79$, $P = 0.012$; **Fig. 4.2c**) (body mass: $F_{1,48} = 4.71$, $P = 0.035$; **Fig. 4.2f**) in *Plodia interpunctella* females all significantly increased with relation to body size. There was no significant relationship between initial egg load and body size (hind tibia length: $F_{1,48} = 0.0012$, $P = 0.97$; **Fig. 4.1d**) (body mass: $F_{1,48} = 0.03$, $P = 0.85$; **Fig. 4.1j**). The 'ovigeny index' was negatively correlated with body size (hind tibia length: $F_{1,48} = 5.18$, $P = 0.027$; **Fig. 4.1e**) (body mass: $F_{1,48} = 5.56$, $P = 0.02$; **Fig. 4.1k**). Over the period of a female's life-span, mean (± SE) lifetime realised fecundity and potential fecundity were 123.02 ± 8.56 and 151.40 ± 5.61 eggs, respectively. During the early stage of the oviposition period, there was a steep increase in the number of eggs laid (until Day 4; **Fig. 4.1h**), followed by a steep decrease (until Day 9), before a slow decrease in fecundity that then dominated later in life. Female *P. interpunctella* had a mean (± SE) ovipositional and pre-ovipositional period of 6.80 ± 0.24 and 2.80 ± 0.35 days, respectively, with a mean life-span of 10.88 ± 0.40 days.

4.4.2 Experiment 2: resource allocation between reproduction and survival

Reproductive and somatic mass increased with female body size (hind tibia length: reproductive mass, $F_{1,48} = 195.8$, $P < 0.001$; somatic mass, $F_{1,48} = 4.25$, $P = 0.044$; **Fig. 4.2a & 4.2b**) (body mass: reproductive mass, $F_{1,48} = 385.1$, $P < 0.001$; somatic mass, $F_{1,48} = 2.81$, $P = 0.10$; **Fig. 4.2d & 4.2e**). Nutrient content analysis revealed negative correlation with relation to somatic (protein $F_{1,48} = 52.81$, $P < 0.001$; carbohydrate $F_{1,48} = 133.7$, $P < 0.001$; glycogen $F_{1,48} = 49.41$, $P < 0.001$; total, $F_{1,48} = 3.71$, $P = 0.06$, and neutral, $F_{1,48} = 24.89$, $P < 0.001$, lipid; **Figure 4.3a - 4.3e**) and reproductive (protein $F_{1,48} = 226.5$, $P < 0.001$; carbohydrate $F_{1,48} = 126.4$, $P < 0.001$; glycogen $F_{1,48} = 31.49$, P

< 0.001; total, $F_{1,48} = 17.58$, $P < 0.05$, and neutral, $F_{1,48} = 31.08$, $P < 0.01$, lipid; **Figure 4.3f - 4.3j**) mass for all the compounds assessed. When compared between reproductive and somatic tissue, females allocated greater protein (68%) carbohydrate (58%), glycogen (64%) and neutral lipid (67%) in reproductive tissue and greater total lipid (65%) in somatic tissue, **Fig. 4.4**).

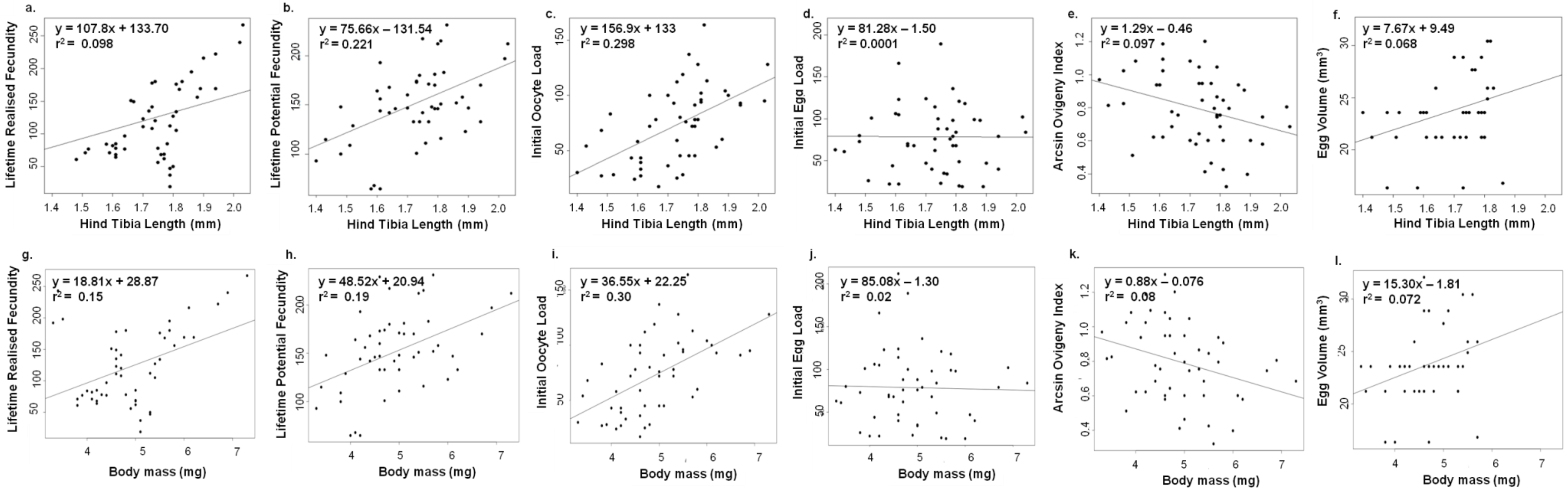


Figure 4.1: Variations in female reproductive traits with hind tibia length (a. - h.) and body mass (g. - l.) in *Plodia interpunctella*: lifetime realised fecundity (a., g.); lifetime potential fecundity (b., h.); initial oocyte load (c., i.); initial egg load (d., j.); ovigeny index (e., k.) and egg volume (f., i.). See text for statistical data.

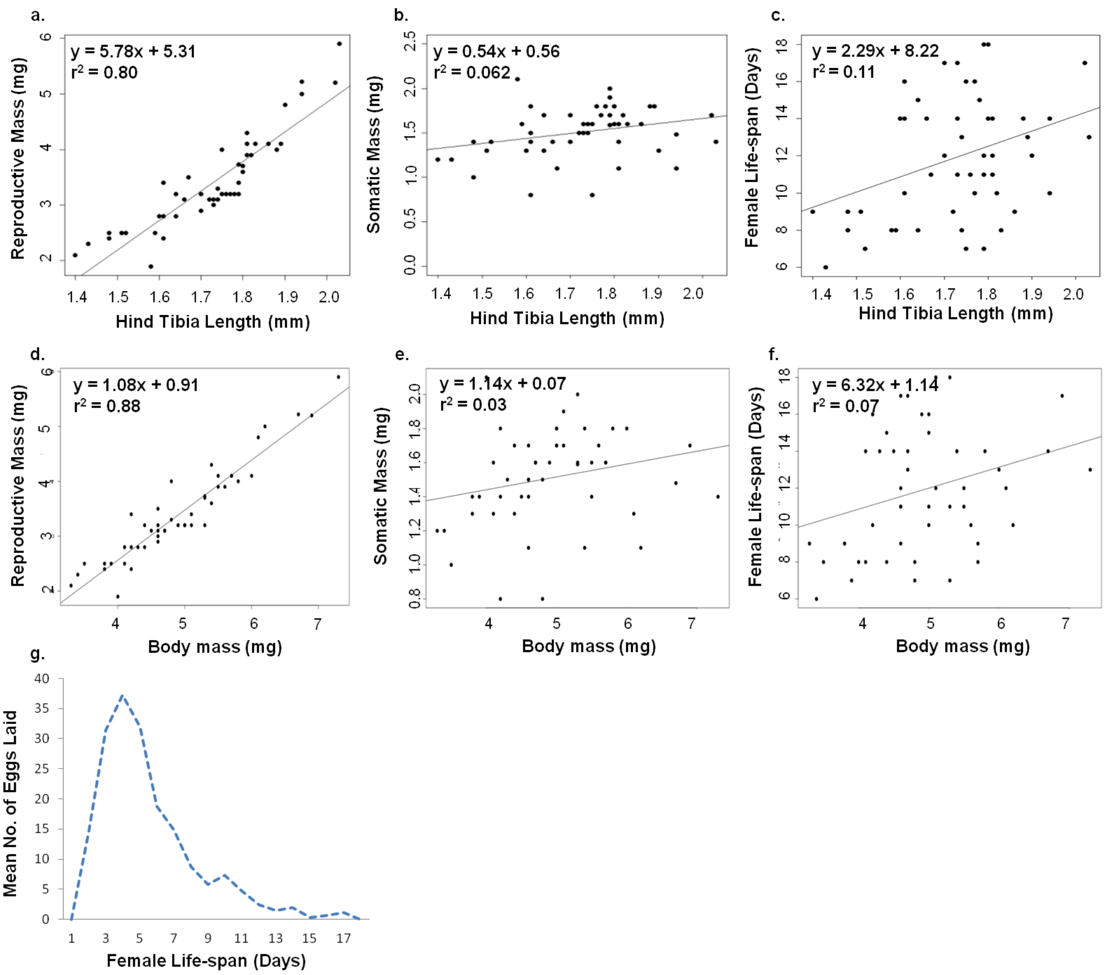


Figure 4.2: Changes in reproductive and somatic tissue mass with hind tibia length (a. & b.) and body mass (d. & e.) together with female life-span (g.) and mean fecundity curve (h.).

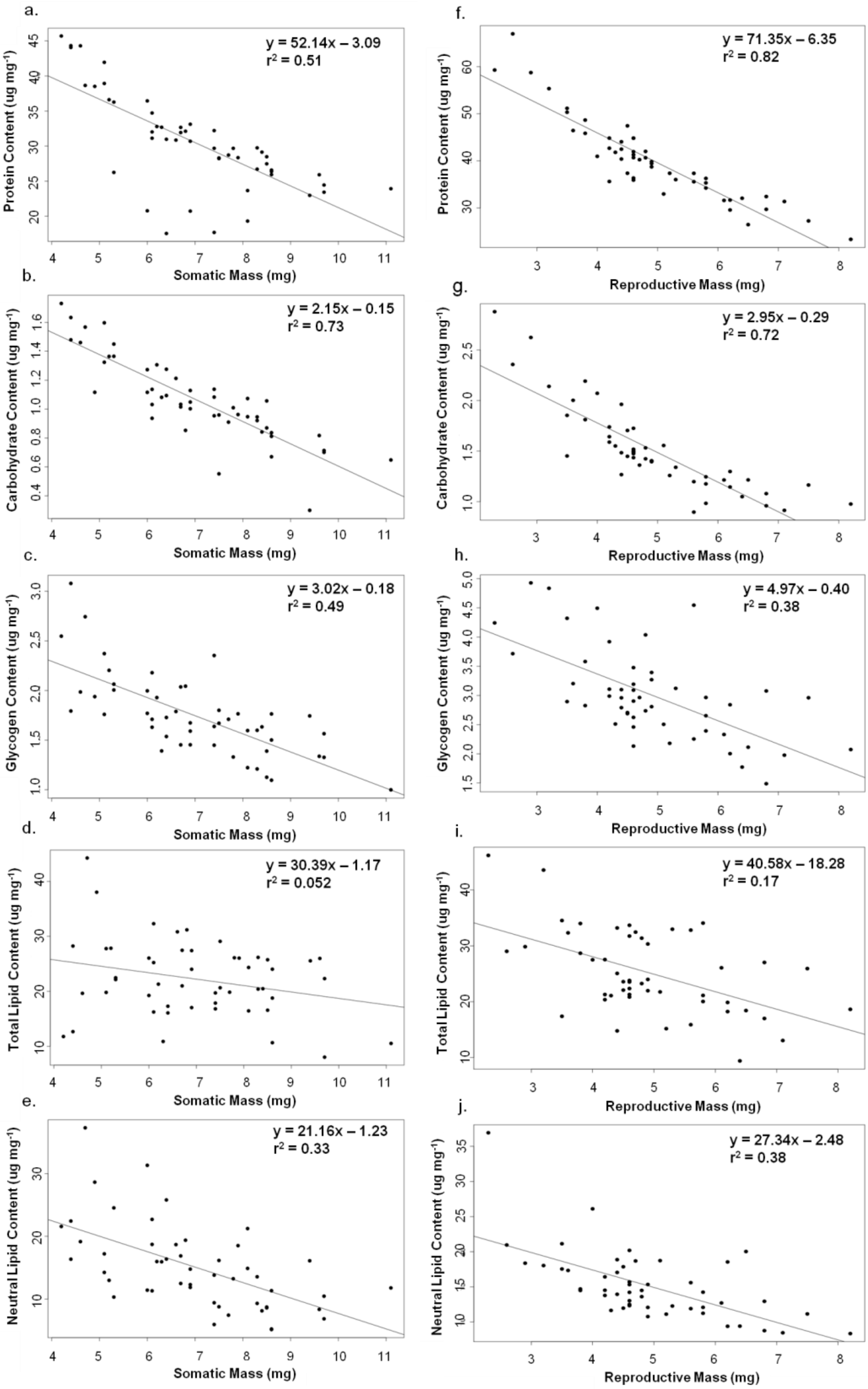


Figure 4.3: Nutrient allocation in somatic (a. - e.) and reproductive (f. - j.) mass for all macronutrients measured in newly emerged female, *Plodia interpunctella*.

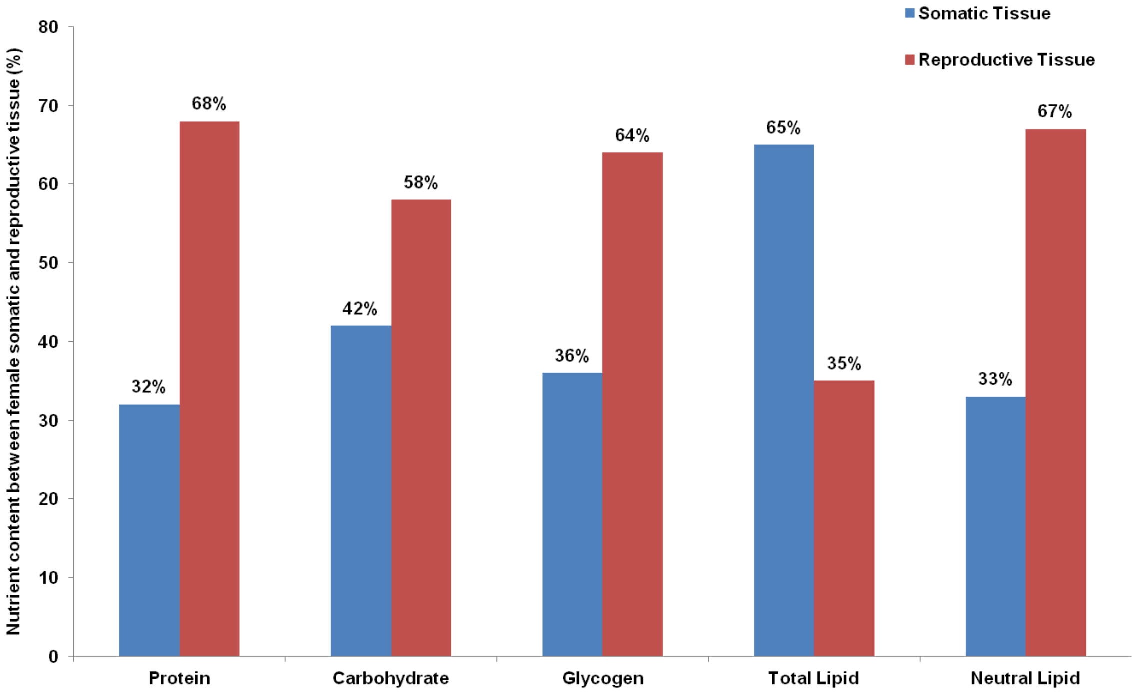


Figure 4.4: Nutrient content interpreted as percentage between somatic and reproductive tissue in newly emerged female, *Plodia interpunctella*.

Principle Components Analysis coupled with PERMANOVA revealed how overall nutrient content in somatic and reproductive tissue varied between small and large female *P. interpunctella*. For reproductive tissues, two principal components represented over 82% of the variation among individuals in nutrient allocation, and both represented a general gradient of nutrient content from small to large females. Protein, carbohydrate, glycogen, and both total and neutral lipid all decreased along PC1, while PC2 represented increasing protein and carbohydrates but decreasing glycogen and lipids (**Figure 4.5a**). For somatic tissue, two PCs explained 75% of the variance in nutrient allocations, with PC1 reflecting a decrease in protein, carbohydrate, glycogen, and total and neutral lipid (**Figure 4.5b**). PC2 again increased with protein and carbohydrates while decreasing with glycogen, and both total and neutral lipid (**Figure 4.5b**). PERMANOVA on these synoptic PCA data confirmed that smaller females allocated greater concentrations of nutrients than large females to both reproductive ($F_{1,48}$ = 13.86, P < 0.001) and somatic ($F_{1,48}$ = 16.14, P < 0.001) tissue (**Figure 4.5c, 4.45**).

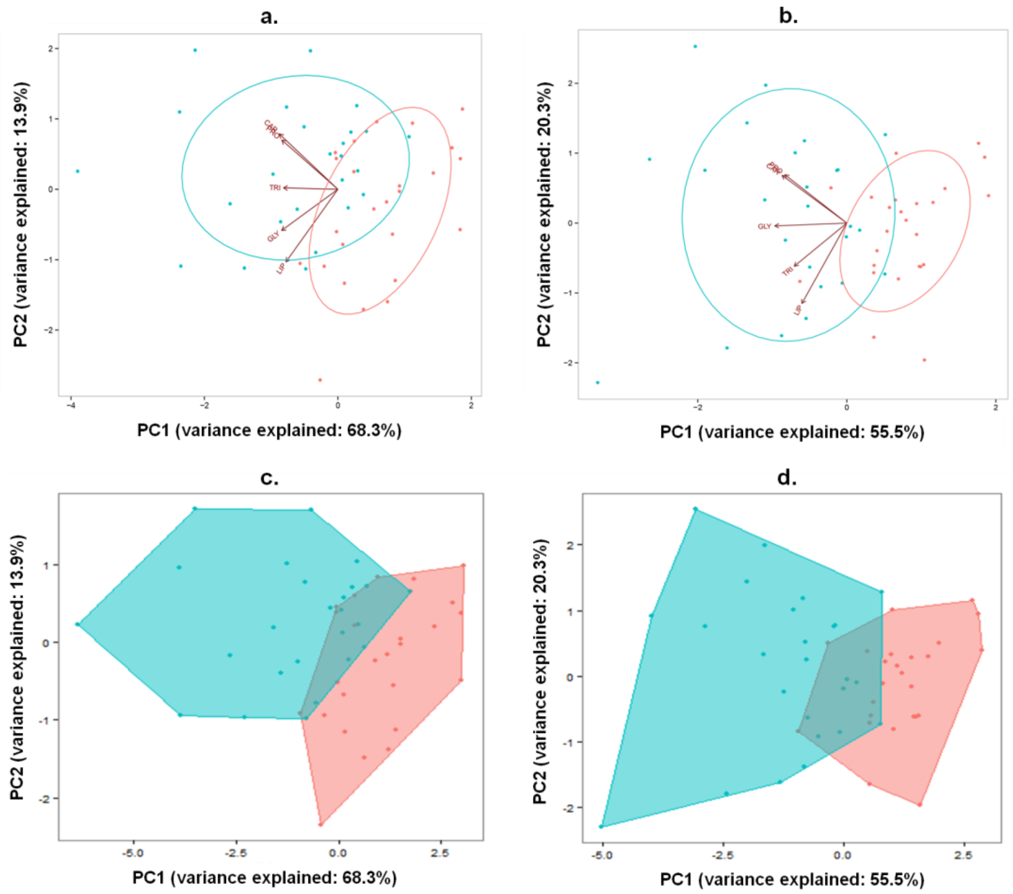


Figure 4.5: Principle Components Analysis (PCA) of different nutrients in reproductive (a.) and somatic (b.) tissue from newly-emerged female *Plodia interpunctella*; Permutational Multivariate Analysis of Variance (PERMANOVA) was used to estimate overall nutrient content between reproductive (c.) and somatic (d.) tissue. (Red = large females; Blue = small females).

4.5 Discussion

Higher correlation between hind tibia length and body size in *Plodia interpunctella* (**Figure 4.1a and 4.2d**) for reproductive mass represent less variation in female resource acquisition under controlled rearing conditions. Upon emergence, female *Plodia interpunctella* can sustain egg maturation using stored larval nutrients (i.e. capital reserves). This investment into egg maturation is, however, costly; thus smaller females that complement early reproduction with fully mature eggs upon emergence have reduced future reproduction (initial oocyte load) and survival. Current reproduction appeared to require greater resource investment in both somatic and reproductive tissue in smaller female *P. interpunctella* thereby supporting the first hypothesis tested. Thorne *et al.* (2006) reported similar observations in the parasitoid *Aphaereta genevensis*, where an increase in initial egg load came at a cost of reduced survival. The results presented in this study are also consistent with wider observations: adult longevity across a range of insect species (Hymenoptera, Trichoptera and Lepidoptera) is negatively associated with an increase in the 'ovigeny index' (Jervis *et al.*, 2003). The negative correlation between the 'ovigeny index' and body size recorded here could be explained by female investment into initial oocyte load for future reproduction. Theoretical examination of ovarian dynamics in synovigenic parasitoids by Ellers and Jervis (2003, 2004) suggested that smaller females allocate more resources into initial egg-load than larger individuals to compensate for their short life-span due to various environmental factor (e.g. resource acquisition, temperature, increased immune function).

4.5.1 Individual patterns of reproductive life history

In *P. interpunctella*, the data collected revealed a positive relationship between body size and reproductive traits (e.g. lifetime realised and potential fecundity, and initial oocyte load). The 'ovigeny index' of *P. interpunctella* was consistent with that determined by Jervis *et al.* (2001), where ovigeny values increased at lower body mass because of changes in both initial egg load and lifetime potential fecundity. In the present study, decline in ovigeny index (**Figure 4.1e and 4.1k**) was the result of a change in only the initial oocyte load (**Figure 4.1c and 4.1i**). The current finding, however, differs from that of Wajnberg *et al.* (2012) where the increase in the 'ovigeny index' of a parasitoid wasp was due to female investment in initial egg load. While an inverse correlation between the 'ovigeny index' and body size seems to be a general phenomenon, the mechanisms through which it occurs may vary across taxa. Lifetime potential fecundity, the maximum number of eggs that can be potentially laid by females during a lifetime, can be compared with the number of eggs actually laid over the life-span. Female *P. interpunctella* lifetime realised fecundity was lower than that estimated for potential fecundity. Lifetime potential and realised fecundity measures in this study were comparable to aphids, *Rhopalosiphum padi* (see Leather, 1988) where realised fecundity was lower in smaller females. This measure of fecundity is expected to increase intra-specifically with body size (Jervis & Ferns, 2004); larger females are capable of maturing more eggs throughout their lifetime due to higher energy reserves

(Thorne *et al.,* 2006). In addition, ovipositional success increases with larger body size. For example, in the parasitoid, *Anagrus sophiae* (see Segoli & Rosenheim, 2015), larger females may have better opportunities for foraging, thus surviving longer and ovipositing more. In contrast, smaller females are expected to perform better under warmer environments due to higher ovipositional rates during early reproduction. This is shown in the butterfly *Pararge aegeria*, where larger females exposed to higher temperature during late reproduction fail to realize fully their egg maturation rates (Gotthard *et al.*, 2007).

Results from the current study illustrate that *P. interpunctella* investment into future reproduction (initial oocyte load) increases with body size, supporting Blanckenhorn (2000)'s review that insect reproductive potential should increase with body size. The relationship between body size and fecundity in Lepidoptera may, however, vary between field and laboratory experiments (Gotthard *et al.*, 2007). Weight-fecundity data present strong positive linear relationships in capital breeding/current reproduction Lepidoptera species (Tammaru & Haukioja, 1996). In some Lepidoptera species, for example, *Orgyia antiqua*, wingless females oviposit all fully-mature eggs upon emergence (Tammaru *et al.,* 2002). Insects tend to adopt one of two egg maturation strategies. These determine egg production timing and are classified as 'synovigeny' (Flanders, 1950) and 'pro-ovigeny' (Heimpel & Rosenheim, 1998). Synovigenic insects incorporate nutrients from both larval and adult origin into their eggs, having a longer life-span and larger body size arising from higher resource allocation to somatic function and nutrient reserves (Jervis & Boggs, 2005). Synovigenic insects also acquire more resources throughout their lifetime for egg maturation (Casas, *et al.*, 2000). On the other hand, pro-ovigenic insects allocate resources into current reproduction at the expense of somatic maintenance. Pro-ovigenic insects do not manufacture eggs during their lifetime so egg number decreases with successive oviposition (Casas *et al.,* 2000; Rosenheim *et al.,* 2000; Jervis *et al.,* 2008). Female *P. interpunctella* appear to employ a strict pro-ovigeny (ovigeny index = 1) at smaller body size. Higher resource investment to initial oocyte load in larger female *Plodia* further leads to lower ovigeny indices. Resource investment into initial oocyte for future reproduction cannot be linked to synovigeny, as larger female *Plodia* do not replenish eggs during adulthood. Larger female *Plodia* survive to mature their initial oocyte load and lay more eggs over their life-span. Changes in the ovigeny index do not necessarily depend on initial egg load. In addition, lower ovigeny values might also reflect female longevity (Jervis *et al.*, 2005). The results from *P. interpunctella* in this study suggest that a higher ovigeny index comes with reduced survival and future reproduction, as expected in most Lepidoptera (Jervis *et al.,* 2005). Larger females are typically expected to invest in larger eggs in response to time-limited oviposition (Rosenheim, 1996); here, *P. interpunctella* egg size was positively correlated with female body size. Adaptive co-variation between egg and body size may be expensive in terms of reproductive strategy as a trade-offs between offspring size and number. *Ad-libitum* feeding did show that larger females may produce larger eggs due to higher resource accumulations. Egg size variation in species that experience time-limitation on oviposition are also modified by extrinsic mortality rates. Species adaptation under high extrinsic mortality rates (e.g. predation, immunity) should invest energy reserves into survival as offspring survival may increase with egg size (Berger *et al.,* 2012; Stahlschmidt *et al.,* 2013).

4.5.2 Resource investment into current and future reproduction

Reproductive investment is directly tied to the availability of resources and consequently the energy available for an individual to invest in reproduction (Reznick *et al.,* 2000). When resource availability is high, individuals will have sufficient energy to invest in egg production. The trade-off between current and future reproduction, known as the cost of reproduction, predicts that allocation to current reproduction reduces potential for future reproduction (Williams, 1966). Females that over-allocate resources to reproduction are predicted to have reduced survival or future fecundity; the cost of reproduction acts as a constraint on current reproductive effort. Results from the current study are consistent with this prediction as smaller females have reduced life-span and future fecundity (i.e. initial oocyte load) with relation to their investment into current reproduction (i.e. initial egg load). In addition, the importance of this trade-off shows that reproduction is costly for *P. interpunctella* and may contribute to shorter life-span in smaller female. Reproductive allocation over a female Lepidoptera's lifetime can be measured by exploring the relationship and trade-offs between adult and egg size, and larval development (García-Barros, 2000). In the present study, the costs of reproduction trade-offs were also measured by comparing the total amount of resources in reproductive and somatic tissue. Smaller females allocate more resources into both somatic and reproductive components compared to larger female. This leads to higher metabolic activity upon emergence which leads to shorter life-span as an adverse late-life consequence for current reproduction. Smaller *P. interpunctella* may reproduce early upon emergence but larger female moth reproduce more throughout a lifetime with higher quality offspring due to larger egg size.

4.5.3 Resource allocation into reproduction and somatic maintenance

An organism should be able to reach the adult stage at any given body size given an optimal allocation of resources to reproduction and survival (Berger *et al.,* 2012). Resource allocation patterns are expected to express the energetic demands of the female arising from the presence of a reproductive strategy allocating nutrients between current and future reproduction. The present study identified protein and neutral lipid as the major components for resource allocation upon emergence. As discussed previously (**Section 4.5.1**), female *P. interpunctella* invest more resources into current reproduction at smaller body size thus allocating more resources for mature eggs and reducing their future fecundity or life-span. Female *P. interpunctella* allocated higher protein, carbohydrate, glycogen and neutral lipid resources, at smaller body size, in both somatic and reproductive tissue. Different levels of nutrient concentrations represent allocation choices to support life history functions (e.g. flight capability and reproduction) (Arrese & Soulages, 2010). The nature of the primary energy sources that influence insect performance may, however, vary. Boggs (1997) reported that Lepidoptera use carbohydrate and glycogen as the primary resource for somatic maintenance and locomotion. Similarly, the ichneumonid parasitoid, *Venturia canescens,* also depends on glycogen as the essential energetic resource during adult flight (Amat *et al.,* 2012). Some insect species do, however, appear to fuel flight with carbohydrates only, with both carbohydrates and lipids, or with primarily proteins (Beenakkers *et al.,* 1984). Meanwhile, lipid content can vary with insect physiological state and life history stage (Lease & Wolf, 2011), with approximately 15% of total lipid weight being represented by ovaries and eggs (Grapes *et al.*, 1989). Lipids serve as an important energy reserve to support insect metabolic demands for prolonged life-span (Canavoso *et al.,* 2001) and periods of flight (Zera *et al.,* 1999; Arrese & Soulages, 2010). While being considered as an important component for somatic energy reserves, lipids also supply energy requirements during embryonic development (Wheeler, 1996; Zhou *et al.*, 1995).

Egg maturation in insects requires both triglycerides (Arrese & Soulages, 2010) and proteins; a lack of one, or both, of these compounds can disrupt insect egg maturation (McKie, 2004). In this present study, most all nutrients (proteins, carbohydrate, glycogen and neutral lipid) were higher in smaller female *P. interpunctella*. Protein and neutral lipid, however, represented the highest nutrient concentrations upon emergence. Proteins have been reported to be an important energetic source that fuels insect activity during flight (Suarez *et al.,* 2005), egg production (Bernstein & Jervis, 2008) and diapause (Hahn & Denlinger, 2007). Low protein levels have been reported to reduce dispersal ability, this being caused by a reduction in thoracic mass (Stevens *et al.*, 2000) or wing length (Mckie, 2004). Neutral lipids are mostly triglycerides and are stored in the insect fat body (Foray *et al.*, 2012). Smaller females allocate higher levels of triglyceride to sustain their flight and oviposition at early adult life. The remaining phospholipids, which form a negligible part of total lipid, are the major structural components of the biological membranes (Canavoso *et al.*, 2001). Higher nutrient allocation in reproductive components for smaller *P. interpunctella* represents higher resource investment to mature all eggs upon emergence. Meanwhile, higher nutrient allocation into somatic components indicate active flight activity for mating and oviposition upon emergence. During development, resource allocation to reproduction and survival may arise from resource investment between current and future reproduction. This study is limited to the intraspecific level in an attempt to illustrate how different reproductive investment at increasing body size influence rules of resource allocation pattern. Reproductive allocation in a female's lifetime for *P. interpunctella* indicates higher investment into current reproduction leading to reduced future reproduction and survival in smaller females.

The present study has demonstrated a clear trade-off between female body size and life-span in *P. interpunctella*, with larger females surviving longer and becoming more fecund, but the higher fecundity is represented by female investment into future reproduction and ‘better’ offspring. In conclusion, *P. interpunctella,* as a major pest of stored products, exhibits considerable variation in ovigeny strategies. This study demonstrates that female reproductive fitness is governed by the relationship between body size, current and future reproduction. This may, in turn, support their population growth as semelparous species; *P. interpunctella* depend totally on the resources acquired as larvae to support their adult life history (Gage, 1995). Increased understanding of factors affecting individual life history, as well as resource allocation between survival and reproduction, will require detailed studies on variations in nutrient availability and environmental conditions during larval development. Because nutrient acquisition and environmental conditions can strongly affect allocation trade-offs (Jervis *et al.*, 2007), they could have pivotal effects on both reproductive performance (i.e. oocyte development) and survival (i.e. flight capability and life-span).

5. Differential Resource Allocation to Survival and Reproduction in Stream Caddisflies along an Altitudinal Gradient

5.1 Abstract

1. Environmental influences on resource allocation to survival and reproductive aspects of insect life histories are poorly understood. Here, two species of stream caddisflies (Trichoptera) were used as study species to investigate variations in resource investment in newly-emerged adults along an altitudinal gradient of the temperate River Usk (Wales, UK). Physicochemical differences between upstream and downstream locations were predicted to influence adult maintenance costs and reduce resources available for reproduction: determined from measurements of macronutrients (protein, carbohydrate, glycogen and lipid) in somatic and reproductive tissues.
2. Upstream (> 200m asl) locations had significantly lower pH, conductivity, chloride, sulphate and nitrate concentrations than downstream sites (< 100 m asl), and in general were also cooler. *Hydropsyche siltalai* from downstream locations allocated less carbohydrate and neutral lipid to reproductive and somatic tissue than those upstream. Site-to-site variations also showed that *H. siltalai* had more glycogen and lipid in somatic tissue in warmer, nutrient-rich waters, indicating increased somatic maintenance. There was no gross difference in nutrient allocation in *Rhyacophila dorsalis* between upstream and downstream sites, although individuals from warmer, nutrient-rich sites had more nutrient in both somatic and reproductive tissue.
3. These data illustrate how physiological adaptations in some stream insects can allow re-adjustment for increased somatic maintenance, but at a potential cost to fitness. In particular, emerging *H. siltalai* from cooler headwaters appear able to invest into early reproduction while those in warmer, nutrient-rich waters must reallocate resources into somatic maintenance. The data also show how nutrient trade-offs between reproduction and survival differ between species, suggesting a potential mechanism of inter-specific sensitivity and plasticity to environmental change.

5.2 Introduction

Insect life history characteristics are widely interpreted through consideration of individual resource allocation (Chown & Gaston, 2008; Mawdsley, 2011; Llandres *et al.*, 2015). Through this perspective, insect physiological adaptation to environmental variation can be appraised as resource investment to different life history traits (Boggs, 2009), with response seen through varying allocation to development, reproduction and dispersal (Bale *et al.*, 2002; Rosenheim, 2010). For example, Jannot (2009) showed that pond-drying affected the juvenile life history of the caddisfly *Limnephilus indivisus* leading to post-metamorphic consequences for adult fitness such as reduced investment in adult thorax and wing development, while maintaining investment to the abdomen. This was interpreted as a trade-off between the physiological traits associated with survival and those with reproduction. Environmental variation can induce similar trade-offs between major life history traits relating to survival and/or reproduction (Stearns, 1992; Boggs, 2009), and, for example, Jannot (2009) showed how pupae of the caddisfly *Limnephilus indivisus,* stressed by pond-drying and warming, allocated more resource upon emergence into somatic functions. Similarly, Denis *et al.* (2012) showed that in pro-ovigenic parasitoids adult females tended to have lower initial egg load and higher concentrations of lipids in their body tissues when ambient temperature was increased - this was interpreted as the greater cost of habitat exploitation at higher temperature and the inability of these insects to synthesize lipids as adults. Earlier (Chapter 4), these concepts were applied to studies of the Indian Moth, *Plodia interpuntella* to show how female body size mediated variations among life span, reproductive strategies and resource allocations.

While the above studies highlight how insects can adopt different resource allocation patterns as a means of adapting to environmental variations, the number of examples from freshwater environments remain somewhat limited (Southwood, 1977; Townsend & Hildrew, 1994; Verberk *et al.*, 2008). This is despite the importance of this group to ecosystem function and biodiversity, and as bioindicators of freshwater ecosystems (Contador *et al.,* 2012, Thomas *et al.*, 2016), where insect research tends to focus instead on the effects of anthropogenic stressors on community structure or species composition (Benstead *et al.*, 2003; Hodkinson & Jackson, 2005; Ormerod *et al.*, 2010). Freshwater studies of life history traits and trade-offs linked to reproduction and survivorship can, however, provide exemplars of evolutionary ecology (Trumbo, 1999; Stevens *et al.*, 1999, 2000). In these environments, conditions can exert strong selection pressure on a wide range of species traits, and freshwater insects use various combinations of life history strategies to maintain viable populations (Verberk *et al.*, 2008; Storey & Quinn, 2013). For example, fundamental strategies such as oviposition behaviour range from those in the waterbug, *Hesperocorixa sahlbergi*, which lays single eggs on several occasions and has a prolonged adult longevity (Verberk & Esselink, 2005) to the dragonfly, *Libellula depressa*, which lays eggs in patches over a much shorter adult life-span (Sternberg & Buchwald, 1999). Freshwater organisms are also known to employ various physiological adaptations under different physical, thermal or flow conditions.

The Disposable Soma Hypothesis (Kirkwood, 1977) suggests that early life performance affects trade-off components between reproduction and survival, proposing that individuals should optimize resource allocation towards somatic maintenance rather than reproduction following exposure to environmental constraints. Aquatic insects are vulnerable to such constraints in their natural environments, individuals could be expected to increase resource investment into somatic functions to increase survival when maturing under stressful conditions. Such conditions are now widespread in freshwaters, with streams and rivers among the most intensely modified of all the Earth's ecosystems, and vulnerable to a wide range of global changes (Allan, 2004). Streams vary, for example, in water quality, habitat character, sedimentation, flow pattern and thermal regime as a consequence of human influence at scales ranging from local to global (Anderson *et al.*, 2006; Durance & Ormerod, 2009; Gutiérrez-Cánovas *et al.*, 2013; Cooper *et al.*, 2013). The resulting stressor effects in aquatic environments include organic pollution (Dohet, 2002), hydromorphological degradation (Lorenz *et al.*, 2004), acidification (Sandin *et al.*, 2004; Ormerod & Durance, 2009) and insecticide run-off (Schulz, 2004; Stoks *et al.*, 2015). In some cases, as along upstream-downstream gradients, several such putative stressors co-occur, for example as temperature, eutrophication and sedimentation effects increase with downstream progression, oxygen concentrations tend to fall creating a multiple stressor context (Arienzo *et al.*, 2001; Larsen & Ormerod 2010; Hrovat *et al.*, 2014).

The aquatic Order Trichoptera is a useful insect group in which to examine life history trade-offs as predicted by the Disposable Soma Hypothesis. Prior to terrestrial adult phases, most species have egg, larval and pupal stages spent in springs, streams, rivers, pond, lakes and wetlands, where feeding strategies include the breakdown of leaves, surface grazing, the construction of filtration nets and direct predation (Elliot, 1970; Ward 1992; Wallace *et al.*, 2003). As such a species-rich and ecologically diverse order, different caddisfly species offer opportunities for understanding stressor effects, and have already served in numerous investigations of life-history trade-offs (Stevens *et al.*, 1999; Jannot *et al.*, 2007).

This chapter investigates how changing environmental conditions along an upstream-downstream gradient shape resource allocation patterns between life history traits within two free-living, caseless Trichoptera species, *Hydropsyche siltalai* (Hydropsyche) and *Rhyacophila dorsalis* (Rhyacophilidae). Unlike previous chapter (Chapter 4), reproductive life history for caddisfly were not determined as ovarian characteristics of female caddisfly are difficult to distinguished (see Chapter 3). Physicochemical variables were measured along a gradient, and caddisfly resource allocation patterns determined. It was predicted that individuals collected upstream would invest more resource into reproduction than downstream individuals as the warmer, eutrophic environments downstream would require more resource investment into somatic maintenance. Resource allocation into early reproduction is costly as a major resource investment is required to mature eggs (Jervis & Ferns, 2004). This leads to a resource trade-off between reproduction and survival.

5.3 Materials and Methods

5.3.1 Study species

The two caddisfly species used in the study occur in both the upper and lower reaches of the River Usk (20 - 230 m asl) (**Figure 5.1**). Members of the Family Hydropsychidae are net-spinning caddisflies, constructing silk nets in rapidly flowing water where they occur in large numbers in spring and early summer. Rhyacophilid larvae are active foraging predators; important prey species include chironomid larvae, as well as the trichopteran larvae of *Baetis* sp. and the dipteran larvae of *Simulium* sp. (see Edington

& Hildrew, 1995). Both *Hydropsyche* sp. and *Rhyacophila* sp. are free-living caddisflies throughout their larval stage and only build cases prior to pupation. The two species used in this study were selected based on their: (1) diverse distribution with different species dominating different sectors of the riffle watercourse; (2) ability to tolerate high variations of water quality; and (3) phenotypic changes to changes in water physicochemical variable (Hildrew, 1979; Vuori 1995; Bonada & Williams 2002; Solà & Prat, 2006). These characteristics makes *Hydropsyche* sp. and *Rhyacophila* sp. suitable candidates for looking at changes in insect physiology and resource allocation pattern between survival and reproduction.

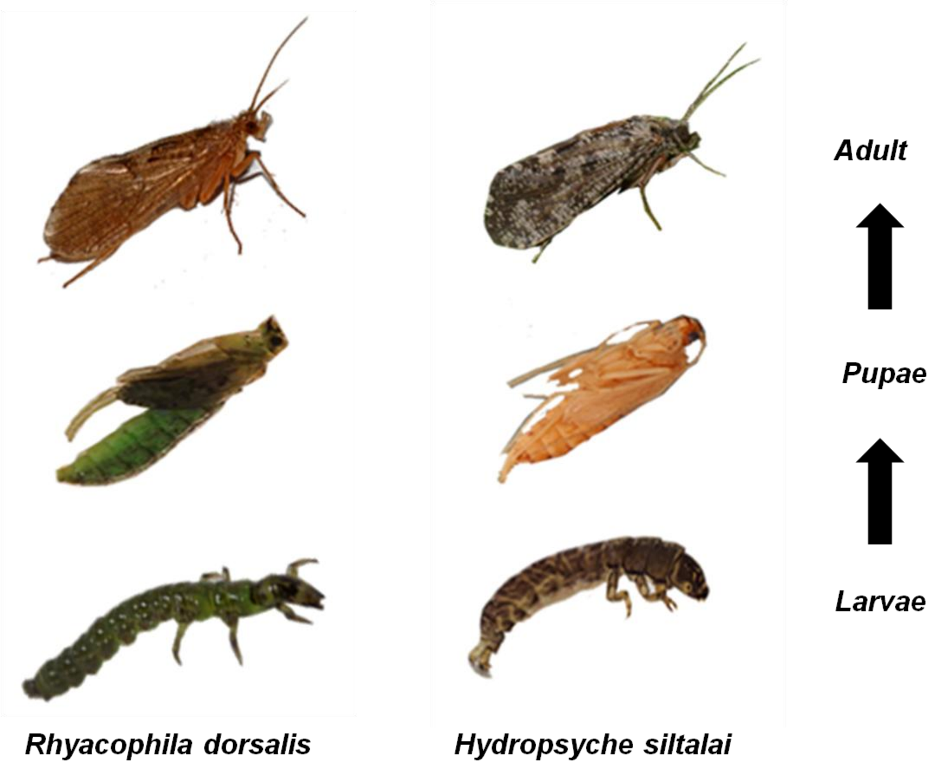


Figure 5.1: Image showing caddisfly life stages from (larvae to adult) for *Hydropsyche siltalai* and *Rhyacophila dorsalis*.

5.3.2 Study areas

Sampling occurred between June and December 2014 in the River Usk, Wales (Grid Reference: SO375005). This is one of the largest rivers in Wales and flows along 125 km with a catchment of 1,358 km^2 to join the Severn Estuary at Newport, Gwent (Barnes *et al.*, 2013). The main river and tributaries have been recognised as a Special Area of Conservation (SAC) under the EU Habitats Directive on the basis of its role as an important wildlife corridor and key area for species conservation (Environment Agency, 2007; Aprahamian *et al.*, 2010). The Usk system provides a suitable model for a study that is centred on studying changes in environmental factors along a stream gradient as land-use varies from semi-natural upland vegetation to increasing intensive pastoral grassland downstream (Larsen & Ormerod, 2010).

Eight tributary streams were selected (**Figure 5.2**), respectively divided between four (S1 - S4) at 'high' (> 200 m) altitude and four (S5 - S8) at 'low' (< 100 m) altitude. While the upstream (Cilieni (S2), Grid Reference: SN920303; Senni (S3), Grid Reference: SN930268; and Tarrell (S4), Grid Reference: SN984240) and downstream (Rhiangoll (S6), Grid Reference: SO185210; Rhyd-y-meirch (S7), Grid Reference: SO322089; and Berthin Brook (S8), Grid Reference: SO368019) streams are classified as second order tributaries (**Table 5.1**), the two remaining streams, S1 (Grid Reference: SN880287) and S5 (Grid Reference: SO151202) represent third order streams. The high and low altitude tributaries also varied in physicochemical properties (see Section 5.3.2 below), with means (± SE) for S1, S2, S3 and S4 of water temperature (11.66 ± 0.21 ºC), conductivity (105.9 ± 5.63 µS cm^{-1}), pH 7.95 ± 0.03), ionic composition (nitrate 2.45 ± 0.19 mg L^{-1}, chloride 9.3 ± 0.52 mg L^{-1}, sulphate 4.1 ± 0.25 mg L^{-1}) differing generally from the temperature (12.95 ± 0.20 ºC), conductivity (165.7 ± 11.94 µS cm^{-1}), pH (8.15 ± 0.07) and ionic composition (nitrate 7.47 ± 0.90 mg L^{-1}, chloride 13.45 ± 1.55 mg L^{-1}, sulphate 8.8 ± 1.23 mg L^{-1}) of S5, S6, S7 and S8. Streamside vegetation was abundant and land use across the watershed consisted of residential housing area with pastoral grassland from S1 to S6. Downstream sites, S7 and S8 consisted mainly of agriculture land. Stream substratum majorly consisted of gravel, small pebbles and larger rocks at all the sites.

5.3.3 Water physicochemical measurement

Water temperatures were measured between June and December 2014 using a data-logger (HOBO Pendant® Data Logger Model 8K-UA-002-64, Measurement System Limited, Berkshire, UK). Temperature loggers were attached to bricks and placed in all the streams to log temperatures at 15 min intervals. Streams were monitored on a weekly basis for two specified set of physicochemical variables at each site: pH and conductivity (HI 98204 Water Test Portable, Hanna Instrument). Additionally, water samples were taken from each site and stored in polyethylene bottles (250 ml) for transport back to the laboratory to measure concentrations of chloride, nitrate and sulphate using an ion analyser (Dionex DX-80). Anion concentrations were monitored bimonthly, three times throughout the study period. Water samples were kept in a freezer to prevent any metabolic activity within the sample.

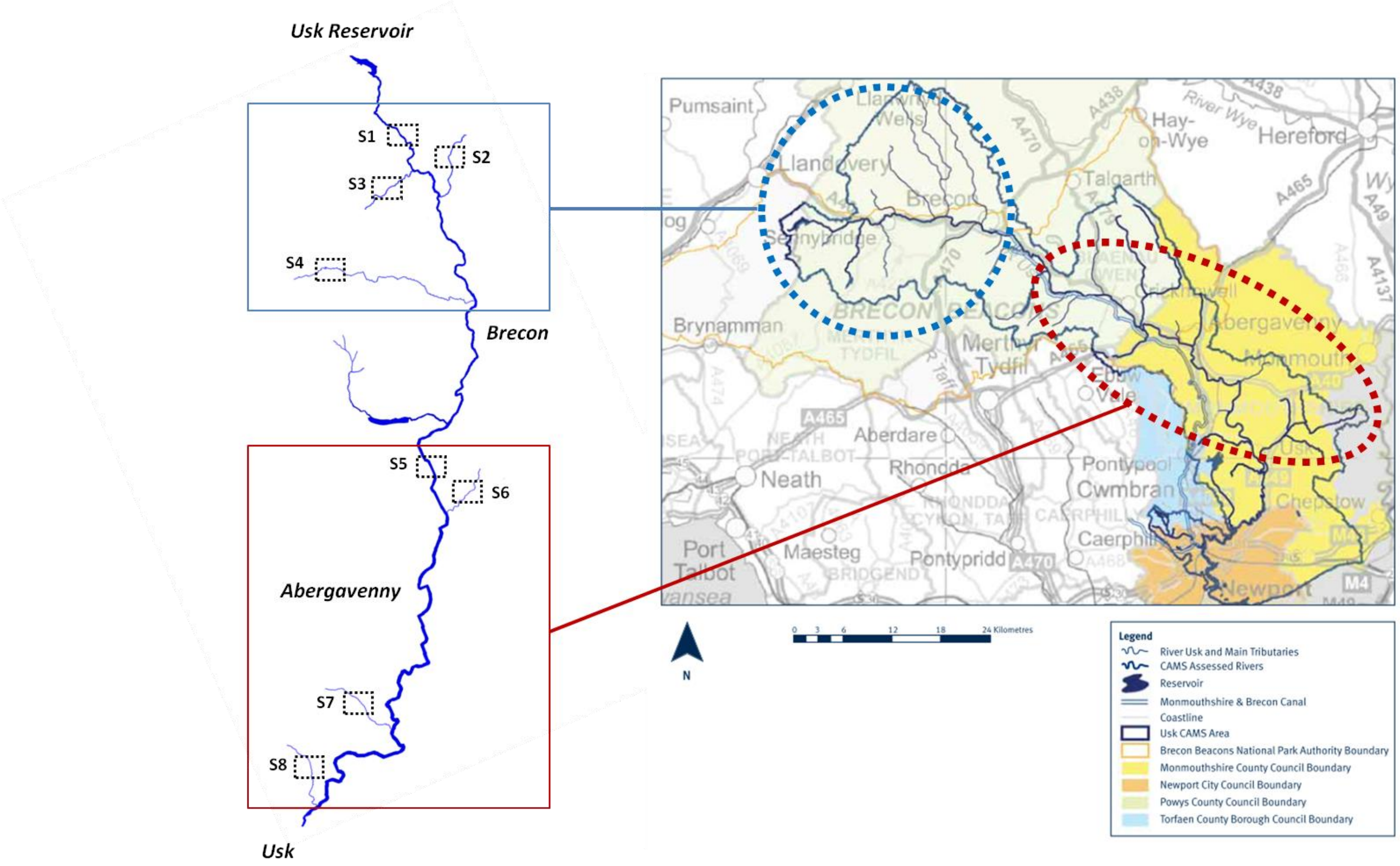


Figure 5.2: The location of each stream along the River Usk: upstream (S1-S4) and downstream (S5-S8) sites (Source: Environment Agency Wales, 2007) **(refer to Table 5.1 for more details).**

Table 5.1: Mean values and ranges (temperature, conductivity, pH and anion concentration) of some physicochemical characteristics of the eight streams (S1 - S8) along the River Usk (June to Dec 2014).

Stream		Location		Altitude asl (m)	Width (m)	Temperature (°C)	Conductivity (µS cm^{-1})	pH	Anion Concentration (mg L^{-1})		
									Nitrate	Chloride	Sulphate
Upstream											
S1	Usk	Trecastle	51º56'43"N	230	9.6	11.40	94.4	7.9	2.3	9.3	4.4
			3º37'48"W			(4.50-16.46)	(82-144)	(7.6-8.1)	(1.95-2.70)	(8.50-9.75)	(3.36-4.99)
S2	Cilieni	Pentre'r-Felin	51º57'39"N	210	5.8	11.94	103.1	8.0	2.1	7.6	3.7
			3º34'23"W			(4.60-17.65)	(106-136)	(7.7-8.2)	(1.40-2.66)	(4.26-9.35)	(2.04-5.11)
S3	Senni	Sennybridge	51º55'46"N	210	5.32	12.03	125.1	8.0	3.3	9.3	4.2
			3º33'26"W			(5.00-17.50)	(80-186)	(7.8-8.3)	(2.90-3.61)	(8.84-10.21)	(3.70-4.62)
S4	Tarrell	Libanus	51º54'22"N	230	3.67	11.29	101.3	7.9	2.1	11.0	4.0
			3º28'38"W			(4.42-15.98)	(65-143)	(7.6-8.2)	(1.84-2.52)	(10.92-11.15)	(3.45-4.44)
Downstream											
S5	Usk	Llangynidr	51º52'28"N	100	32.16	14.70	124.3	8.0	5.1	12.5	5.4
			3º14'01"W			(6.59-20.72)	(80-185)	(7.8-8.2)	(4.29-5.86)	(11.23-13.28)	(4.28-6.46)
S6	Rhiangoll	Tretower	51º52'56"N	90	3.29	13.00	164.4	8.2	4.3	9.8	5.5
			3º11'04"W			(6.59-19.49)	(71-249)	(7.8-8.5)	(3.46-5.75)	(7.02-11.26)	(3.75-7.16)
S7	Rhyd-y-meirch	Llanover	51º46'30"N	40	1.68	11.75	152.4	8.1	9.5	10.1	9.4
			2º58'59"W			(5.18-16.34)	(119-195)	(8.0-8.3)	(9.05-9.81)	(9.87-10.22)	(8.18-10.58)
S8	Berthin Brook	Rhadyr	51º42'47"N	20	3.46	12.36	221.9	8.3	11.0	21.4	14.9
			2º54'54"W			(5.40-16.74)	(121-305)	(8.2-8.5)	(9.31-12.58)	(17.91-26.44)	(12.51-16.80)

5.3.4 Laboratory rearing

During Summer 2014, caddisfly pupae, together with their cases, were collected, using forceps, from riffles in all stream over a period of 60 days. On collection, they were placed individually in capped 50 ml plastic centrifuge tubes filled with stream water. *Hydropsyche siltalai* and *R. dorsalis* pupae were identified by their morphological characteristics. Even though both species are free-living caddisflies, the last instar larva of both species builds a case with a translucent protective layer at the bottom. Through this layer the pre-pupa was examined to allow initial taxonomic identification in the field before further identification under laboratory conditions (Edington & Hildrew, 1995). The centrifuge tubes were transported on ice back to the rearing facility at Cardiff University. Pupae from individual streams were housed in aerated rearing containers (370 x 220 x 250 mm), covered with mesh and allowed to develop until adult emergence. Rearing containers were kept in an environmentally-controlled cabinet (incubators KBWF-720 BINDER) at 18 - 20 °C and a photoperiod of 16:8 h (L:D) with constant water depth of 4 cm (**Figure 5.3**). Throughout the study, water temperature were recorded to range between 14 - 16 °C in environmentally-controlled cabinet. The emergence of adults was monitored twice daily, and newly-emerged females identified using the checklist of Barnard and Ross (2012). Each was transferred into a Petri dish, logged and placed in a deep freeze for dissection later.

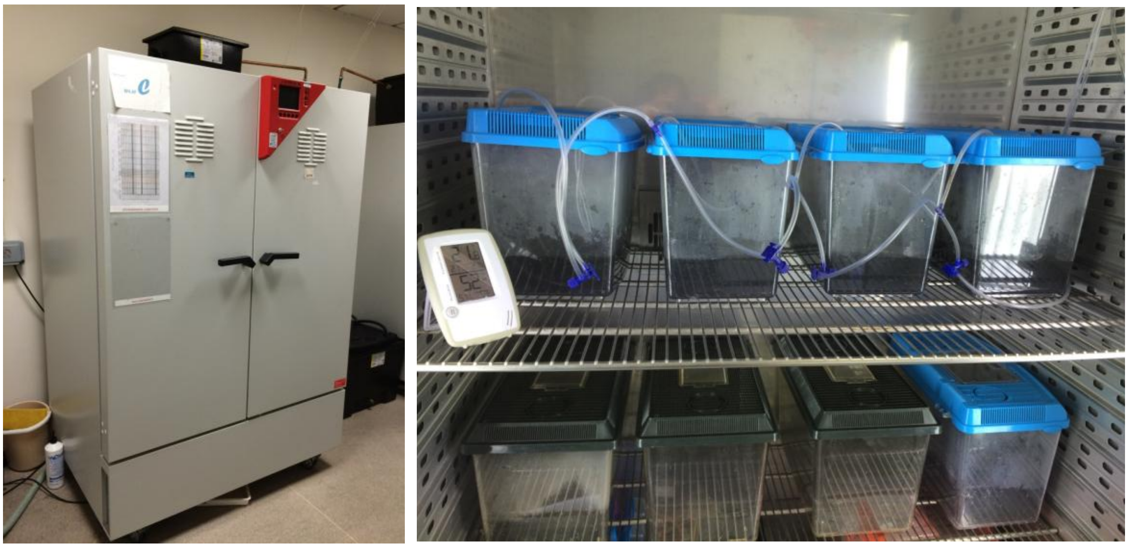


Figure 5.3: Environmentally-controlled cabinet used to keep rearing containers for caddisfly pupae under laboratory conditions.

5.3.5 Extraction of macronutrients in adult caddisfly

Newly-emerged females were dissected on cooled Petri dishes. Body components linked to reproduction and somatic function were separated to enable caddisfly resource allocation upon emergence to be assessed. Dissection of the somatic tissue included the head, the thorax and gaster appendages. The reproductive tissue was defined as the ovaries and the fat bodies (**refer to Section 2.10**). Each tissue fraction was placed separately into 2 ml Eppendorf tubes placed on ice ready for biochemical analyses. Insect nutrient assays adopted from Foray *et al.* (2012) (**refer to Chapter 3; Section 3.6**) were used to determine the total amounts of macronutrients (protein, carbohydrate, glycogen and lipid); these protocols have been used widely across different insect orders including Hymenoptera, Coleoptera, Diptera and Lepidoptera (Amat *et al.,* 2012; Louis *et al.,* 2014; Colinet & Renault, 2014), including the Indian mealmoth, *Plodia interpunctella* (**see Chapter 4**). It was assumed that the biochemical assay would provide precise measurements for caddisfly macronutrient allocation as both Trichoptera and Lepidoptera (butterflies and moths) together constitute the Superorder Amphiesmenoptera, a sister group relationship that share similar physiological characteristics in morphological and molecular data (Whiting, 2002; Beutel *et al.,* 2011). All nutrient assays were undertaken using 80 newly-emerged female caddisfly (40 for each species).

5.3.6 Data Analysis

Data analysis was performed using ‘R’ statistical software (‘R’ Development Core Team, 2011). Physicochemical variables (temperature, pH, conductivity, anion concentrations) were compared between different altitude from upstream to downstream sites using generalized mixed models (GLMMs), using the 'lme4' package (Bates *et al.,* 2012) with stream sites included as a random factor. In GLMMs, physicochemical variables and altitude variations were used as dependent and independent variables, respectively. Due to small sample size, allocation of mass between reproductive and somatic function was compared between upstream and downstream caddisfly populations using Analysis of Variance (ANOVA). *Post-hoc* comparisons were performed using Tukey's test. Macronutrient contents (ug per insect) for upstream and downstream caddisfly were expressed as percentages to compare nutrient allocation between somatic and reproductive traits. Water physicochemical variables related to warming and eutrophication events and their effects on caddisfly nutrient assay data were analysed using Generalised Linear Models (GLMs); using the 'Effects' function (Fox, 2003). It is important to note that water physicochemical variables were multi-collinear (**Table 5.2**). Multicollinearity among predictor variables were determined based on coefficient correlation and Wald statistics using Genstat, version 15 (VSNI Hemel, UK). In the study, temperature and nitrate were selected as the predictor variables to estimate the effects of water physicochemical variables on different macronutrient in reproductive and somatic tissue. Strong correlation among predictor variables (lrl > 0.7) suggested that studies looking into pH and interaction effects can lead to distortion in model estimation (Dormann *et al.,* 2013). Caddisfly nutrient content in somatic and reproductive tissue were combined using Principle Component Analysis (PCA) and the overall nutrient content were compared between upstream and downstream caddisflies using Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001). These PERMANOVA tests were carried out using the 'Adonis' function, based on 4,999 permutations (Oksanen *et al.,* 2012).

Table 5.2: Correlations among water physicochemical variables, water temperature (temp) and water chemistry (pH and nitrate (Nitr)) used in the correlation analyses (Pearson's r). Wald statistics represent the best predictor variables to estimate effects on caddisfly nutrient content.

Water Physicochemical Variables	**Nitr**	**pH**	**Temp**	**Nitr * pH**	**Temp * Nitr**	**Wald statistics**
Nitr						7.19
pH	0.85					2.13
Temp	0.78	0.99				8.90
Nitr * pH	0.98	-0.90	-0.84			2.03
Temp * Nitr	0.96	0.92	0.87	-0.99		0.07
Temp * pH	0.81	0.99	0.99	0.86	-0.89	2.38

5.4 Results

5.4.1 Physicochemical variables between upstream and downstream sites

Water physicochemical variations along an altitudinal gradient (20 - 230 m asl) represented significant difference in pH ($t_{1,\ 62}$ = 7.89, P = 0.031; **Figure 5.5b**), conductivity ($t_{1,62}$ = -2.81, P = 0.04; **Figure 5.5c**), chloride ($t_{1,22}$ = -1.48, P = 0.19; **Figure 5.5d**), sulphate ($F_{1,22}$ = -1.36, P = 0.22; **Figure 5.5e**) and nitrate ($F_{1,22}$ = -2.99, P = 0.024; **Figure 5.5f**). The thermal regimes approached formal significance ($t_{1,1462}$ = -1.11, P = 0.31; **Figure 5.5a**), with mean (± SE) daily temperature over the study period lower in upstream (4.63 ± 0.13 °C to 16.90 ± 0.40 °C) than downstream (5.97 ± 0.39 °C to 18.31 ± 1.05 °C) sites.

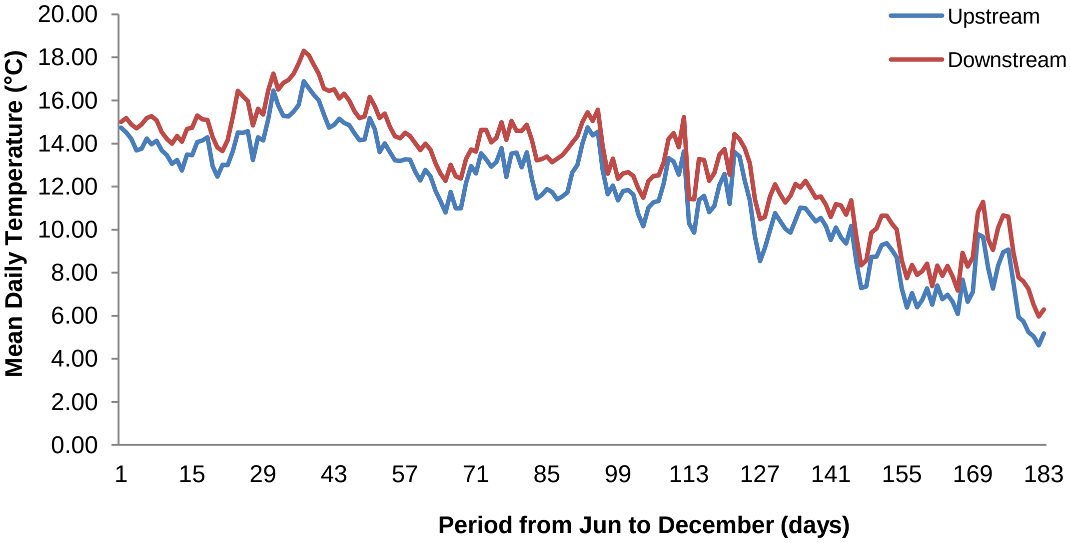


Figure 5.4: Mean daily temperature for upstream and downstream sites for the period between June and December 2014.

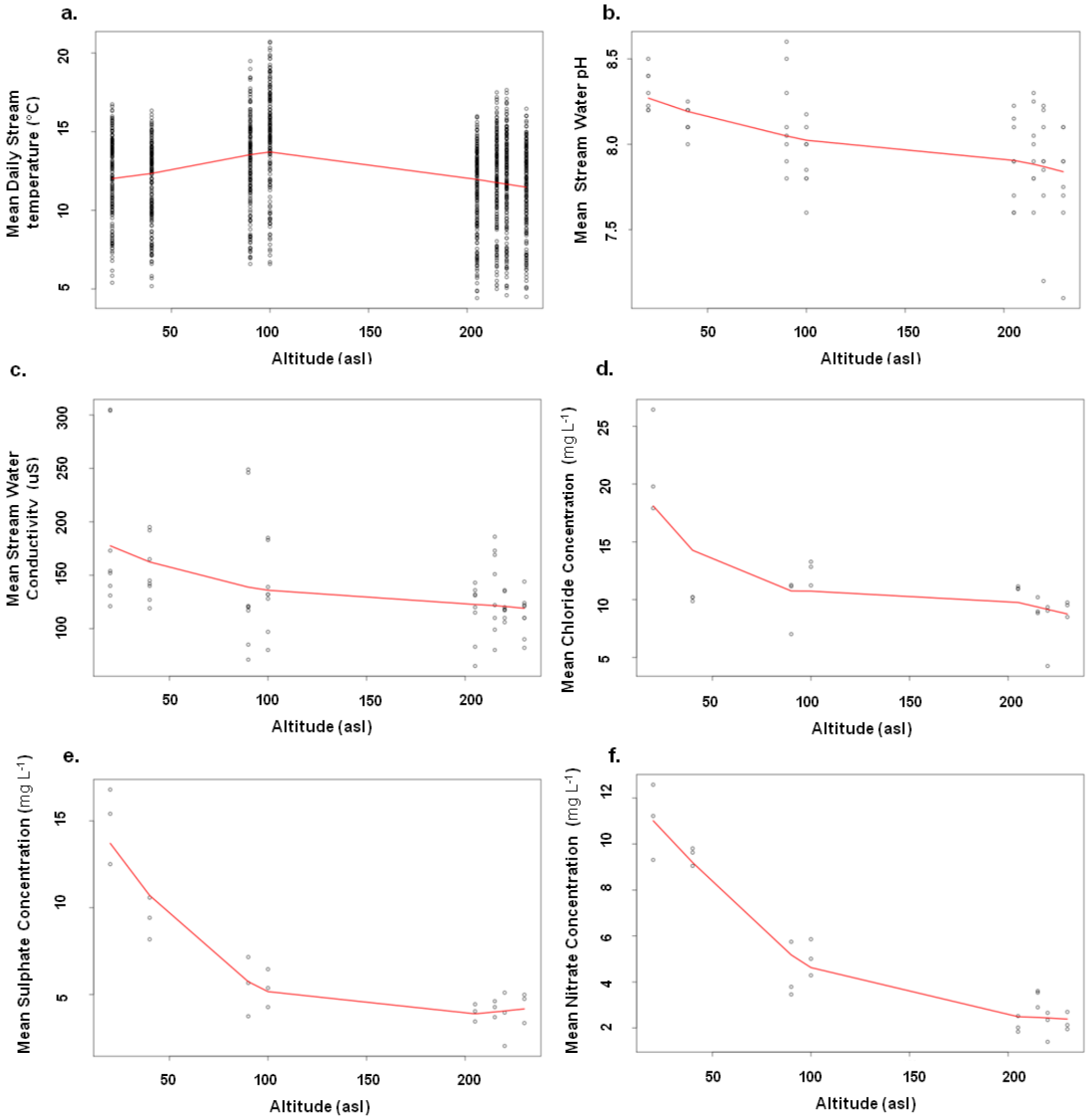


Figure 5.5: Scatterplots showing physicochemical variables along altitudinal gradient (June to December 2014) with best line of fit predicted by Linear Mixed Effects models. Measurements are given for (a.) mean daily stream temperature; (b.) mean stream water pH; (c.) mean stream water conductivity; (d.) mean chloride concentration; (e.) mean sulphate concentration; and (f.) mean nitrate concentration.

5.4.2 Resource allocation between reproductive and somatic tissue

Neither *Hydropsyche siltalai* nor *Rhyacophila dorsalis* newly-emerged females exhibited any differentiation between reproductive (*H. siltalai;* $F_{1,38} = 6.37$, P = 0.74; *R. dorsalis*; $F_{1,38} = 0.41$, P = 0.52; **Figure 5.6a, 5.6d**) and somatic (*H. siltalai;* $F_{1,38}$ =

0.005, P = 0.94; *R. dorsalis*; $F_{1,38}$ = 0.16, P = 0.68; **Figure 5.6a**) mass (mg) in upstream vs downstream locations.

In *R. dorsalis,* there were no differences in the allocation of protein ($F_{1,38}$= 0.35, P = 0.55), carbohydrate ($F_{1,38}$ = 0.44, P = 0.51), glycogen ($F_{1,38}$ = 0.37, P = 0.54), total ($F_{1,38}$ = 0.03, P = 0.85) and neutral ($F_{1,38}$ = 1.70, P = 0.20) lipid to reproductive tissue between upstream and downstream individuals. Nor did upstream and downstream individual *R. dorsalis* differ in allocations of protein ($F_{1,38}$ = 0.41, P = 0.52), carbohydrate ($F_{1,38}$ = 2.79, P = 0.10), glycogen ($F_{1,38}$ = 1.86, P = 0.17), total ($F_{1,38}$ = 1.70, P = 0.20) or neutral ($F_{1,38}$ = 0.85, P = 0.36) lipid to somatic tissues. Downstream *R. dorsalis*, however, allocate greater nutrient content (%) for protein (58%), carbohydrate (56%) and neutral lipid (54%) in somatic tissue compared to upstream individuals (**Figure 5.6e**). Meanwhile, for reproductive tissue, downstream *R. dorsalis* allocate greater glycogen (55%) and total lipid (48%) (**Figure 5.6f**).

Differences between upstream and downstream individuals were, however, detected in *H. siltalai*. Individuals collected from upstream sites allocated more carbohydrate ($F_{1,38}$ = 4.96, $P < 0.05$) and neutral lipid ($F_{1,38}$ = 11.37, $P < 0.01$) to reproductive tissue than downstream individuals, but not more protein ($F_{1,38}$ = 1.20, P = 0.28), glycogen ($F_{1,38}$ = 0.20, P = 0.65) and total lipid ($F_{1,38}$ = 0.55, P = 0.46). Upstream *H. siltalai* also allocated more neutral lipid ($F_{1,38}$ = 11.53, $P < 0.001$) to somatic tissue, but not more protein ($F_{1,38}$ = 1.44, P = 0.23), carbohydrate ($F_{1,38}$ = 0.13, P = 0.72), glycogen ($F_{1,38}$ = 0.65, P = 0.42) and total lipid ($F_{1,38}$ = 0.16, P = 0.69). Upstream *H. siltalai* showed greater nutrient allocation (%) for carbohydrate (46%), glycogen (59%), total (37%) and neutral (61%) lipid in reproductive tissue compared to downstream individuals (**Figure 5.6c**). Meanwhile, for somatic tissue, downstream *H. siltalai* showed greater nutrient allocation (%) for carbohydrate (59%), glycogen (54%), total (74%) and neutral (58%) lipid (**Figure 5.6b**).

5.4.3 Overall nutrient content between upstream and downstream individuals

Overall nutrient content in newly-emerged caddisflies was assessed using Principle Component Analysis (PCA). Measurements of the different macronutrients were combined to enable estimation for total accumulated nutrient in both somatic and reproductive tissue. Coupled with with PERMANOVA, PCA revealed how overall nutrient content in reproductive and somatic tissue varied between upstream and downstream locations. In female *H. siltalai*, two principle components (PCs) represented over 79% of the variation among individuals in nutrient allocation for reproductive tissue. Protein, carbohydrate, glycogen, and both total and neutral lipid increased varied along PC1, while PC2 represented increasing total and neutral lipid, but decreasing protein, carbohydrates and glycogen (**Figure 5.7a**). For somatic tissue, two PCs explained 76% of the variance in nutrient allocations, with PC1 reflecting a decrease in protein, carbohydrate, glycogen, and total and neutral lipid (**Figure 5.7b**). Principle Component 2 increased with carbohydrate, and total and neutral lipid, while decreasing with protein and glycogen. In female *R. dorsalis*, two principle components represented over 74% of the variation among individuals in nutrient allocation for reproductive tissue. Protein, carbohydrate, glycogen, and both total and neutral lipid increased along PC1, while PC2 represented increasing carbohydrate and neutral lipid, but declining protein, glycogen and total lipid (**Figure 5.7c**). For somatic tissue, two PCs explained 73% of the variance in nutrient allocations, with PC1 represented increasing protein, and total and neutral lipid, but decreasing carbohydrate and glycogen (**Figure 5.7d**). PC1 showed a similar pattern in nutrient allocation for *H. siltalai* and *R. dorsalis* in reproductive tissue. In somatic tissue, however, a different pattern was observed. There was a significant (PERMANOVA; $P<0.001$) difference between overall nutrient content in reproductive (**Figure 5.7e**) tissue between upstream and downstream *H. siltalai* but no difference in somatic tissue ($P=0.098$; **Figure 5.7f**). Overall nutrient content in *R. dorsalis* showed no difference in either reproductive ($P = 0.879$; **Figure 5.7g**) or somatic ($P = 0.446$; **Figure 5.7h**) tissue between upstream and downstream individuals.

5.4.4 Relationships between stream physicochemistry and nutrient content in reproductive and somatic tissue

For *H. siltalai*, nutrient in somatic tissue were positively correlated with increasing temperature (total lipid; $t_{3,36} = 2.58$, $P = 0.014$) (**Figure 5.8b**) and increasing nitrate (glycogen: $t_{3,36} = 2.19$, $P = 0.035$) (**Figure 5.8c**). In contrast, nutrients in somatic tissue declined with increasing temperature (protein; $t_{3,36} = -2.06$, $P = 0.047$) (**Figure 5.8a**). In contrast, there were no significant relationship between nutrients and water physicochemical variables (temperature and nitrate) (**Table 5.3**).

For *R. dorsalis*, nutrients in somatic tissue were positively correlated with increasing nitrate (carbohydrate: $t_{3,36} = 4.46$, $P < 0.001$) (**Figure 5.8e**). Nutrients in somatic tissue were negatively correlated with increasing nitrate (glycogen: $t_{3,36} = 4.46$, $P < 0.001$). In *R. dorsalis* reproductive tissue, nutrients increased with increasing nitrate (total lipid: $t_{3,36} = 2.19$, $P = 0.035$) (**Figure 5.9c**). Nutrients in reproductive tissue were negatively correlated with increasing temperature (carbohydrate: $t_{3,36} =-3.00$, $P = 0.004$; neutral lipid; $t_{3,36} = 2.44$, $P = 0.019$) (**Figure 5.9a, 5.9b**).

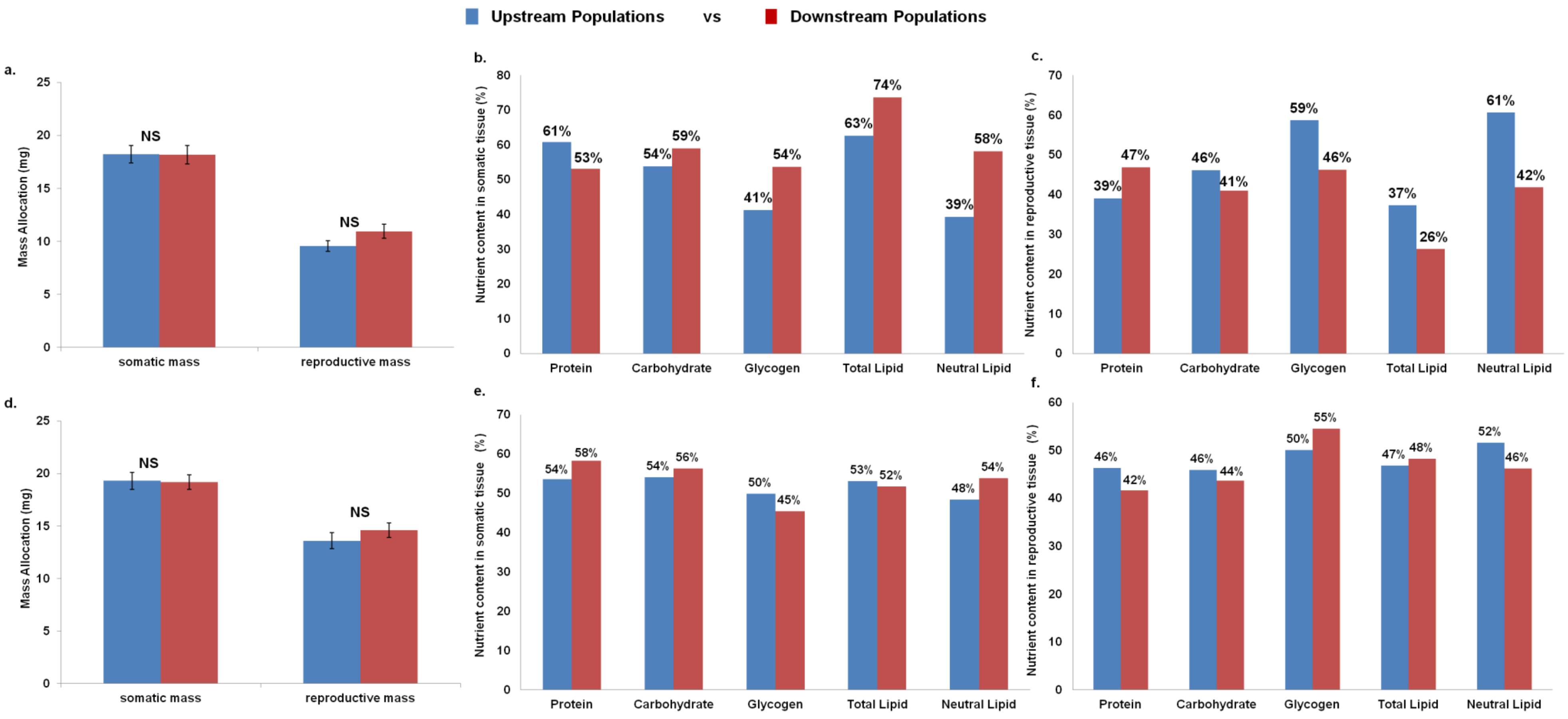


Figure 5.6: Allocation of mass and nutrient content (%) between upstream and downstream populations of *Hydropsyche siltalai* - a.) allocation of mass between somatic and reproduction; b.) nutrient allocation in somatic tissue; and c.) nutrient allocation in reproductive tissue; and *Rhyacophila dorsalis* - d.) allocation of mass between somatic and reproduction; e.) nutrient allocation in somatic tissue; and f.) nutrient allocation in reproductive tissue) (NS non-significant).

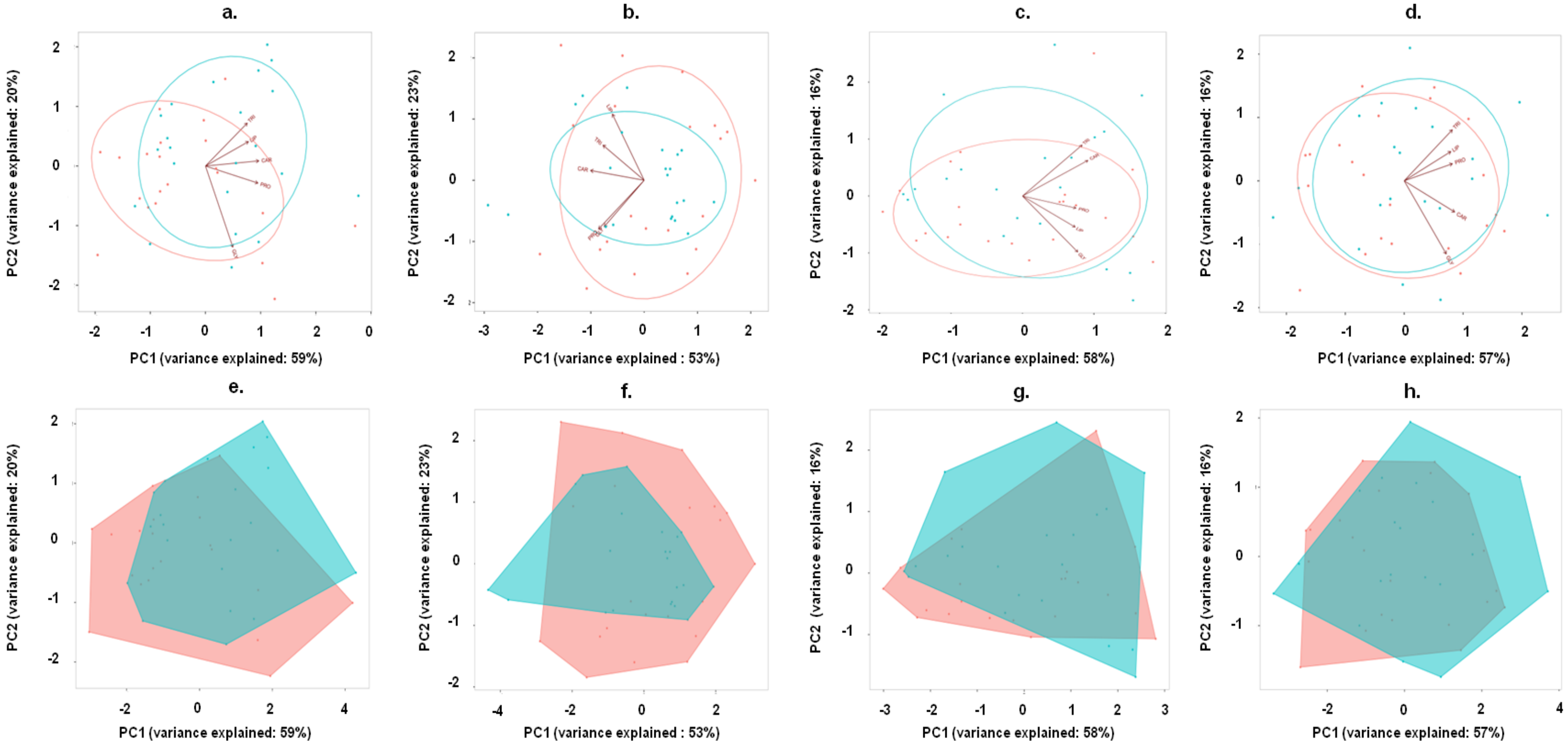


Figure 5.7: Principle Component Analysis (PCA) for different nutrient content in newly emerged caddisfly for *Hydropsyche siltalai* reproductive (a) and somatic (b), and *Rhyacophila dorsalis* reproductive (c) and somatic (d) tissues between upstream and downstream populations; Permutational Multivariate Analysis of Variance (PERMANOVA) was used to estimate overall nutrient content between upstream and downstream populations for *H. siltalai* reproductive (e) and somatic (f), and *R. dorsalis* reproductive (g) and somatic (h) tissues (Red = downstream individual ; Blue = upstream individuals.

Table 5.3: Relationship between physicochemical variable (temperature and nitrate) and caddisfly nutrient content in somatic and reproductive tissue. Significant P values (< 0.05) are in bold.

	Hydropsyche siltalai				*Rhyacophila dorsalis*			
	Somatic		**Reproductive**		**Somatic**		**Reproductive**	
Physicochemical Variables	$t_{3,36}$ value	P value	$t_{3,36}$ value	P value	$t_{3,36}$ value	P value	$t_{3,36}$ value	P value
Temperature								
Protein	-2.056	**0.047**	-1.105	0.276	0.473	0.639	-0.631	0.532
Carbohydrate	0.950	0.349	-0.160	0.874	-0.745	0.460	-3.004	**0.004**
Glycogen	-0.686	0.496	-0.827	0.414	-2.892	**0.006**	-0.401	0.690
Total Lipid	2.578	**0.014**	-0.972	0.337	-0.766	0.448	-1.260	0.215
Neutral Lipid	-0.855	0.398	-1.304	0.200	-0.066	0.948	-2.438	**0.0198**
Nitrate								
Protein	1.097	0.28	0.34	0.74	0.457	0.650	1.084	0.285
Carbohydrate	0.526	0.602	0.626	0.535	4.463	**<0.001**	1.568	0.125
Glycogen	2.189	**0.035**	1.472	0.150	1.926	0.062	1.675	0.103
Total Lipid	-0.009	0.993	-0.355	0.725	-0.172	0.860	2.190	**0.035**
Neutral Lipid	-0.033	0.974	-0.478	0.636	0.598	0.553	1.350	0.185

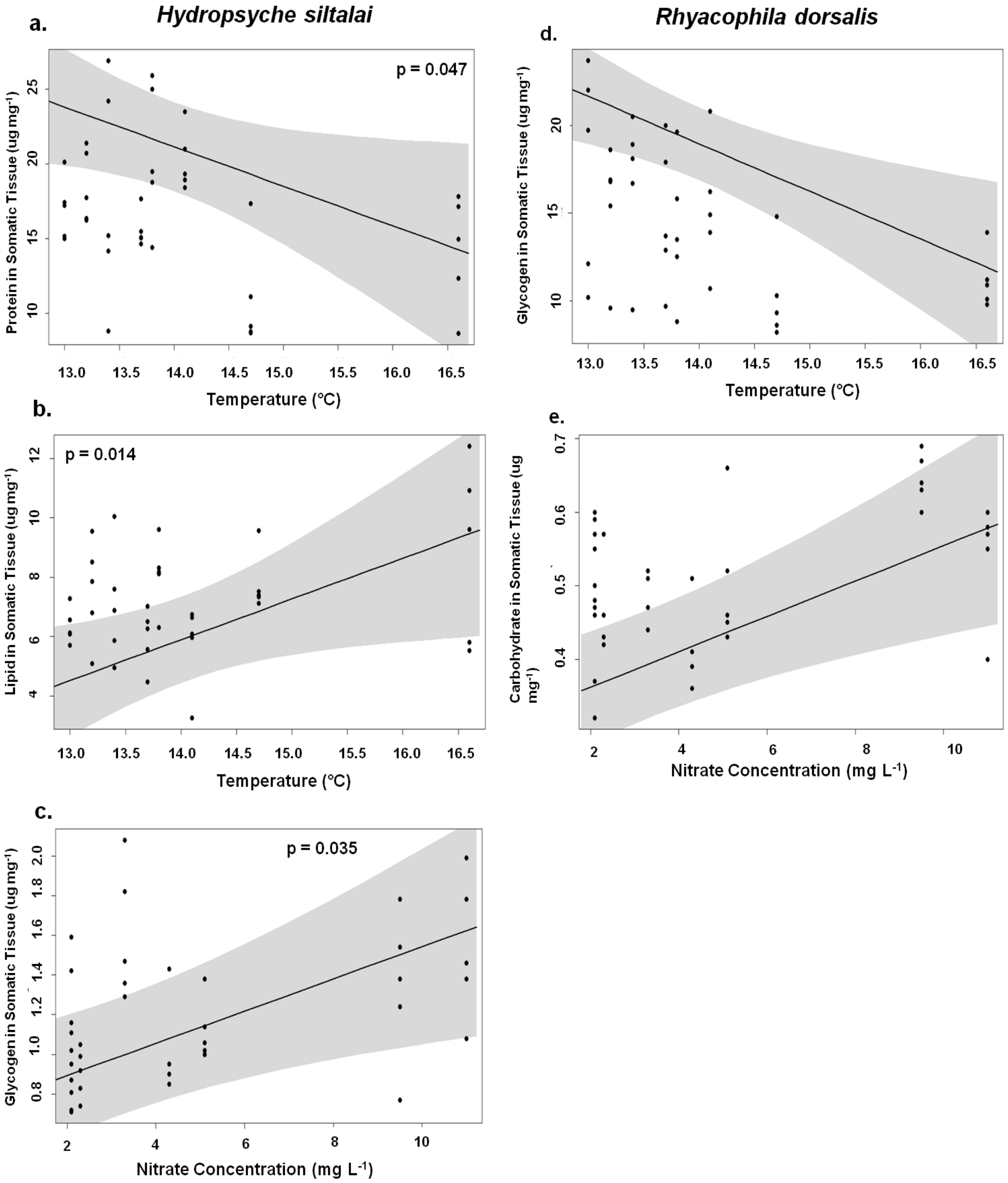


Figure 5.8: GLM showing significant relationships between nutrient content in somatic tissue with temperature and nitrate in *Hydropsyche siltalai* and *Rhyacophila dorsalis* with temperature (a., b., d.) and nitrate concentration (c., e.). The grey lines represent 95% confidence intervals.

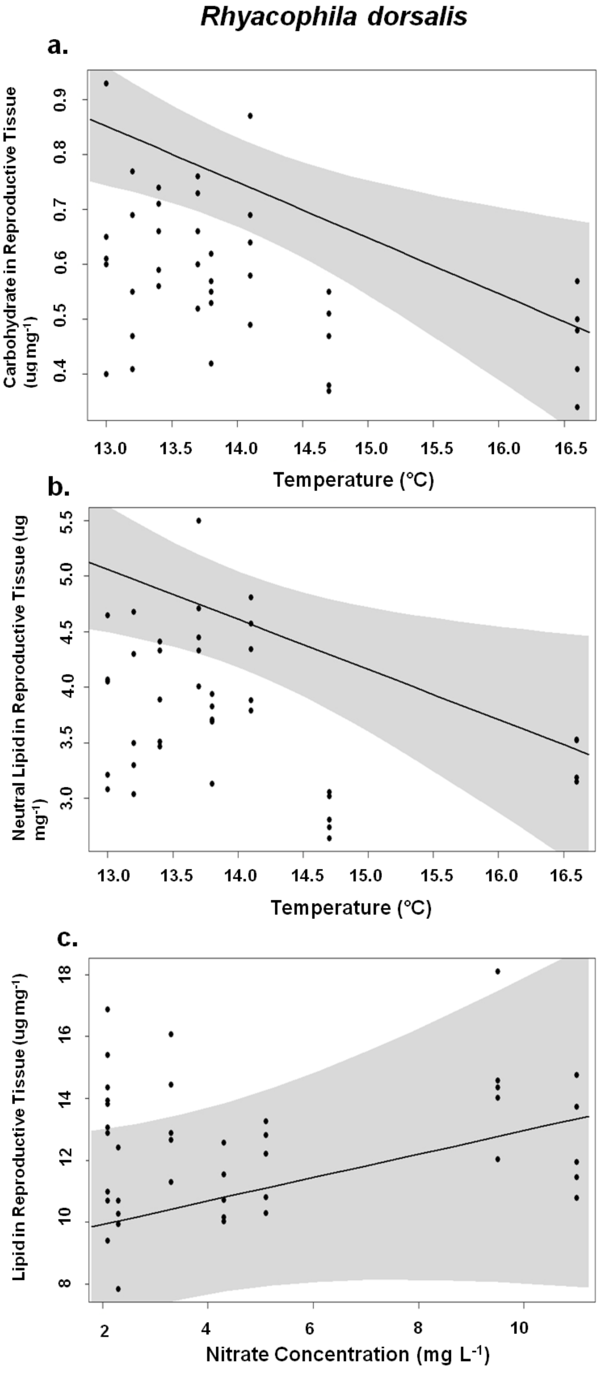


Figure 5.9: GLM showing significant relationships between nutrient content in reproductive tissue for *Rhyacophila dorsalis* with temperature (a., b.) and nitrate concentration (c.). The grey lines represent 95% confidence intervals.

5.5 Discussion

5.5.1 Resource allocation trade-offs between upstream and downstream caddisfly

Life history studies suggest that trade-offs between different life history functions occur when allocation to two or more competing functions comes from a common resource pool (De Jong & Van Noordwijk 1992; Barnes & Partridge, 2003). This is exemplified by holometabolous insects such as members of the Trichoptera, where resource accumulated during the larval stage is fully utilized at adult eclosion by allocation to different life history function; most adult caddisfly do not feed and totally depend on capital resources (resource derived from larval feeding; refer to Jervis *et al.* 2008). Consequently, any stressful events experienced during the larval stage, such as changes in environmental conditions, that may influence the resource acquisition process by, for example, increasing larval metabolic activity, will affect resource allocation in adulthood (Gauplae *et al.*, 2012). In this study, neither upstream nor downstream *Hydropsyche siltalai* and *Rhyacophila dorsalis*, regardless of provenance differed in overall somatic and reproductive mass. Higher physicochemical variables in sites at lower altitudes did, however, lead to different nutrient allocation in somatic and reproductive tissue between *H. siltalai* and *R. dorsalis*. Different resource allocation patterns under stressful environments may be indicative of species-specific responses at the higher physicochemical variables measured throughout the study. During the transition from pupa to adult insect, larval stress may be disguised as the stress of metamorphosis may override the stress experienced during the larval stage (Stoks *et al.*, 2015). One such example of this is the damselfy, *Coenagrion puella*, where pesticide stress increased asymmetry in larval stage but was not evident in the adult (Campero *et al.*, 2008). This might explain why, even though low altitude individuals are exposed to marked changes in physicochemical conditions, newly-emerged adults do not express differences in reproductive and somatic mass. Admittedly, in the present study, this finding was somewhat unexpected as physicochemical variables differed significantly with altitude; such variability was expected to change reproductive and somatic mass in newly-emerged caddisfly.

Physicochemical variables indicated that throughout the study period (June to December 2014), temperature, together with pH, conductivity and anion concentrations (chloride, sulphate, nitrate), in downstream sites were significantly higher than in the upstream sites. The lower carbohydrate, and total and neutral lipid in downstream *H. siltalai* may represent reduced reproduction with increased somatic investment in stressful environmental conditions. In general, water quality, as measured from source to river mouth, is likely to be reduced due to land-use impact influencing downstream water physicochemical characteristics (Frissel *et al.,* 1986). In the present study, upstream sites were heavily dominated by both improved pasture and pastoral grassland. In downstream sites, land-use intensification was higher and varied from pastoral grassland to crop cultivation. More vulnerable physicochemical conditions in downstream sites, can be caused by accumulation of anthropogenic contaminants from terrestrial runoff that are transferred along the stream gradient (Power *et al.,* 1992; Relyea & Hoverman, 2006; Floury *et al.*, 2012). Coupled with application of herbicide for crop cultivation in downstream catchments, physicochemical variables may affect lotic system by lowering periphyton abundance causing reduction in invertebrate herbivore biomass (Rohr & Crumrine, 2005). Warmer temperatures in downstream sites in the River Usk reflect the fact that temperature differences do occur as a result of stream physicochemical variables. Warming in lotic systems usually covaries with respect to topography and climatic conditions (Jacobsen *et al.*, 1997). The present study reflects that temperature differences do not only apply at higher elevations, where it is generally established that there is an increment between 5.5 ºC and 6.5 ºC for each 1,000 m of ascent (Anslow & Shawn, 2002), but also at lower lowlands where thermal regime are affected by differing land use intensification.

Lipids play an essential role in maintaining insect biological functions; this in turn influences an individual's development, survival and fecundity (Casas *et al.*, 2005). In insects, lipids are comprised of phospholipids and triglycerides, the latter representing more than 90% of the overall total lipids (Beenakkers *et al.,* 1985). As the most abundant form of energy storage, triglycerides represent the primary component for the insect biological membrane (Zera, 2005). They are also a major component in neutral lipids, along with smaller fractions of monoglyceride, diglyceride and fatty acid which are stored in the insect fat body to sustain energy-demanding activities (Foray *et al.,* 2012). Higher amounts of triglycerides in insect tissue are associated with longer life-span and active flight behaviour (Zera & Harshman, 2001); they are also a crucial component for egg production in insects (Beenakkers *et al.,* 1985; Grapes *et al.,* 1989).

Carbohydrate also serves as an important indicator for insect reproductive output and life-span (Maklakov *et al.*, 2008). Many studies manipulate carbohydrate for insect nutritional intake; lack of carbohydrates leads to insect nutrient deficiency that influences survival and reproduction (Olson *et al.,* 2000; Nestel *et al.,* 2003; Fanson & Taylor, 2012). In this present study, higher nutrient allocation in reproductive (carbohydrates) and somatic (neutral lipids) tissues for upstream *H. siltalai* may represent higher reproductive investment upon emergence. Nutrient allocation in reproductive and somatic components in upstream *H. siltalai* may also reflect reproductive allocation to current reproduction. Insects exhibit increased investment to current reproduction under favourable conditions; for example during periods of high resource availability (Billman *et al.,* 2014) or optimal environmental conditions (Le Lann *et al.,* 2014). Exposure to physicochemical constraints in downstream *H. siltalai* may exhibit reproductive restraint as physiological adaptations require more resource investment to somatic maintenance (Congdon *et al.*, 2001).

Caddisfly populations living downstream experience different environmental conditions, but both *H. siltalai* and *R. dorsalis* showed physiological adaptation to ensure survival. Upstream *H. siltalai* represent greater nutrient content (%) in reproductive tissue. Changes in water physicochemical changes along altitudinal gradient indicate a potential adaptive response with downstream *H. siltalai* showing greater nutrient allocation into somatic tissue. *Rhyacophila dorsalis*, however, showed different response with downstream individuals showing greater nutrient content (%) in reproductive and somatic tissue compared to upstream individuals. Flatt *et al.* (2013) explained that under favourable conditions; organisms may invest more resources into reproduction at the cost of somatic maintenance, while under stressful conditions, they may reallocate resource for improved somatic maintenance, thereby optimizing fitness. An insect's ability to adjust life history response (plasticity) under different environmental conditions has been established in both flies and bees, and is known as an *adaptive life history switch* (Finch 1990; Finch & Rose 1995; Tatar & Yin 2001; Flatt *et al.*, 2013). Caddisfly resource allocation (present study) showed that, when comparing upstream and downstream individuals, *H. siltalai* and *R. dorsalis* represent different physiological responses towards changing environmental conditions. This implies that different caddisfly species may vary considerably in their sensitivity towards physicochemical constraints. Higher allocation of triglycerides to *H. siltalai* somatic tissue indicates that, for upstream individuals, upon emergence, more energy is required for an active flight prior to oviposition. In addition, higher triglycerides content in reproductive tissue for upstream *H. siltalai* indicates higher egg mass upon emergence. Similar observation were reported by Moiroux *et al.* (2010) for the drosophilid parasitoid, *Leoptopilina boulardi*. In this latter study, female parasitoids from colder climates invested more into early reproduction than females from warmer climates; this resulted in them having a higher lipid content during the first 24 h of adult life. In another study by Pearson and Connolly (2000) on the caddisfly shredder species, *Anisocentropus kirramus,* adult insects accumulated large stores of triglycerides and free fatty acids for successful reproduction.

5.5.2 Physicochemical variables effect on caddisfly nutrient content

Water physicochemical variables may influence invertebrate species composition, along with their individual life history functions (Vaughan & Ormerod, 2012; Al-Shami *et al.*, 2013; Storey & Quinn, 2013). The current study predicted that caddisfly communities would reflect their response towards different physicochemical variables by altering resource allocation pattern to optimize fitness (Congdon *et al.*, 2001). Friberg and Jacobsen (1999) showed that leaf-shredding caddisfly, *Sericostoma personatum* growth performances are more restricted to habitat constraints (e.g. physicochemical conditions) than available resources (e.g. leaf litter quality), even when macroinvertebrates may respond differently to physicochemical contents in plants (Suren & Lake, 1989). This is because shredders are capable of satisfying their nutritional requirements for growth and metabolism by increasing their consumption of litter mass even on poor quality leaf litter (Fugère *et al.*, 2012). When associated with stressful environments, caddisflies are exposed to multiple stressors that may potentially limit available resources for growth and metabolism (Gessner *et al.*, 2012).

Caddisfly responses to temperature are illustrated in nutrient allocation between reproductive and somatic tissue. Newly-emerged *H. siltalai* allocate higher lipid and lower protein to somatic tissue under conditions of high temperature. Meanwhile, newly emerged *R. dorsalis* showed lower glycogen in somatic tissue and lower carbohydrate and neutral lipid in reproductive tissue. Higher resource investment for somatic maintenance is part of an insect’s adaptive response to a warming environment (Clarke, 2003). Insects are capable of adjusting lipid composition in response to changes in surrounding temperature to maintain optimal function of membrane-associated proteins (Van Dooremalen *et al.,* 2013). The results from the current study suggest that *H. siltalai* may shift resource from protein into lipid as an adaptive measure to cope with higher temperature. Lower nutrient allocation to reproductive tissue in *R. dorsalis* may also suggest reduced reproductive output at higher temperature. Alp *et al.,* (2013) suggested that stream degradation (hydromorphological variables), coupled with increasing temperature, may constrain reproduction of freshwater species.

Increases in conductivity and anion (nitrate, chloride and phosphate) concentrations between upstream and downstream sites may be suggestive of an eutrophication gradient (Hrovat *et al.*, 2014). Throughout this study, period of measurement during and after the summer period may influence the result; summer low flows, for example, increase the risk of eutrophication (Jarvie *et al.,* 2005). Several other studies, however, have suggested that conductivity and nitrate can serve as apparent variables indicating an eutrophication gradient (Hrovat *et al.,* 2009; Floury *et al.,* 2012), as in this present study (**refer to Figure 5.5**). Results from the present study do suggest that land-use intensification along the stream gradient may have enhanced nutrient input due to urban waste and agricultural intensification. Increases in nitrate concentrations contributed to the positive relationship with protein in *H. siltalai*, and lipid in *R. dorsalis* somatic and reproductive tissue (**see Table 5.3**). It is expected that increased nitrate concentration may lead to abundant chironomid populations in downstream sites as their communities are positively associated with eutrophication (Rosenberg *et al.,* 1986). Their presence will provide higher foraging options for *R. dorsalis* larva and may contribute to higher nutrient content at increasing nitrate concentrations. Similarly, *H. siltalai* benefits from increased nitrate by higher resource acquisition due to the abundance of phytoplankton and zooplankton under higher nitrate conditions. This influences the volume of seston particles captured by *Hydropsyche* sp. larvae (Alexander & Smock, 2005). Increasing populations of *Hydropsyche* sp. in downstream locations has previously been reported previously by Valett and Stanford (1987). The Stress-gradient Hypothesis (Bertness & Callaway, 1994) proposes that a switch from negative to positive interactions can occur as the magnitude of environmental stress becomes stronger; in this present study, increased nitrate at downstream sites was expected to increase resource acquisitions for *H. siltalai* and *R. dorsalis* during larval stage, contributing to a positive effect on resource acquisition. In addition, increase lipid consumption for *R. dorsalis* may contribute to somatic maintenance which explained why there were no difference in nutrient allocation among populations along the altitudinal gradient..

In summary, resource allocation pattern into major life history traits may be driven by changes in physicochemical variables, as shown in *H. siltalai*, where upstream individuals invest more resources into early reproduction compared to those downstream. This, however, differs between species as different taxa employ different life history strategies; in *R. dorsalis*, for example, overall nutrient content did not differ between upstream and downstream individuals. Our understanding of resource allocation-based life history (Congdon *et al.,* 2001) explains how individuals may express different suites of resource allocation favouring specific environmental conditions. These include the allocation of assimilated resources such as nutrients into competing life history functions (e.g. growth, development, maintenance and reproduction).

In the context of the present study, investment into early reproduction in upstream *H. siltalai* showed that physicochemical variables may shape insect reproductive strategy. Upstream *H. siltalai* may employ an early reproductive strategy, with higher nutrient allocation into reproductive tissue upon emergence. The costs of early reproduction are, however, high as investment into reproductive traits requires high levels of resource (Eller & Jervis, 2004). As physicochemical conditions change along a stream gradient, *H. siltalai* shift available resources for somatic maintenance reducing resource investment in reproduction. In contrast, increased lipid consumption in *R. dorsalis* may promote species tolerance with physicochemical constraints. This may explain the similar resource investment pattern observed in both upstream and downstream individuals for *R. dorsalis*. Under natural environmental conditions, insects will invest more resource into reproduction to maintain their life history success (Rosenheim, 2011). As environmental conditions change, species may adapt to constraint by employing different resource allocation strategies, as illustrated in *H. siltalai*. The interrelationship between species traits and environmental conditions are, however, more complex and context-dependent as they depend on the adaptive value of individual phenotypes (Verberk *et al.,* 2008). More research is needed to explain species adaptation under changing environment; understanding life history strategy is clearly a phenomenon that must be taken into account as different species can employ different strategies to maximise overall fitness.

6. A Translocation Study to assess the Potential Influence of Climate Change on Resource Allocation in Stream Caddis Larvae

6.1 Abstract

1. Insect life history choices and in particular the trade-off between nutrient allocation to life-span versus reproductive performance, are sensitive to environmental conditions, and hence environmental change. There have, however, been relatively few investigations of potential global change effects on resource allocation to life history traits, particularly in freshwater environments.
2. In this study, unique translocation experiments over a thermal gradient of 1.5 - 4.5 °C upstream and downstream of a cave system were conducted to mimic climate change effects on the net-spinning caddis, *Hydropsyche siltalai.* Protein, carbohydrate, glycogen and lipid allocations to somatic and reproductive tissues were assessed.
3. Simulated warming resulted in lower adult somatic and reproductive mass. Warming also increased protein, carbohydrate, glycogen and total and neutral lipid allocation to somatic tissue, and increased lipid allocation to reproductive tissue. Surprisingly, colder environment lead to increased nutrient allocation in female somatic and reproductive tissue. The findings were, however, influenced by heavily skewed sex ratios within the translocated organisms. The findings from the study were also limited by the small number of individuals used; this was a consequence of the short time period available for the translocation studies.
4. These results do, however, demonstrate how exposure to increased temperature in late caddisfly instars can affect adult fitness by reducing body size and diverting most nutrients from reproduction into survival. The one exception in this study, that of greater lipid investment into reproduction, is nevertheless consistent with advanced reproductive timing in warmer conditions.

6.2 Introduction

Climate change is recognised increasingly as a major threat to global biodiversity and ecosystem processes (Walther *et al.,* 2002; Stenseth, *et al.,* 2002; Dawson *et al.,* 2011; Woods *et al.,* 2014; Pacifici *et al.,* 2017). Between 2003 and 2012, global surface temperatures rose, on average, by 0.78 [range: 0.72 - 0.85] ºC (IPCC, 2014; Rummukainen, 2012; Stocker *et al.,* 2013), continuing a longer-term trend. As well as increasing average temperature, events of extreme high temperature have increased in frequency during the past decade (Barriopedro *et al.,* 2011; Perkins *et al.,* 2012), consistent with predicted future climate scenarios (IPCC, 2007). A 'heatwave' is defined as a period lasting at least five consecutive days with maximum temperatures at least 5ºC higher than 'typical' (Frich *et al.,* 2002; Tebaldi *et al.,* 2006). The past decade has seen heatwave duration and intensity increasing in some areas with recorded temperatures up to 12 ºC higher than average (reviewed by Perkins, 2015).

As ectotherms, insects are highly sensitive to changing temperature, and from an ecological perspective a range of responses to such changes have been observed, including pest outbreaks in the cabbage butterfly, *Pieris rapae* (see Kingsolver *et al.,* 2007), European pine sawfly, *Neodiprion sertifer* (see Kollberg *et al.,* 2013), European spruce bark beetle, *Ips typographus* (see Marini *et al.,* 2012) and the potato tuber feeding moths, *Tecia solanivora*, *Symmetrischema tangolias* and *Phthorimaea operculella* (see Dangles *et al.,* 2013). Species interactions, for example, insect resistance to parasites and pathogens, between parasitoids and their hosts, and between insects and host plants, are also affected under a warming climate (Relyea & Hoverman, 2006; Rahel & Olden, 2008; Schowalter, 2011). Individual adaptation may, however, differ between species depending on their flexibility to adapt. To understand fully how insects respond to climate change, more detailed ecophysiological information is required.

Indications that insects are physiologically sensitive to both average temperature and to episodes of high temperature are illustrated by their phenology and metabolic activity (Stott *et al.,* 2010; Mawdsley, 2011). As well as insect locomotion, immune function, sensory input, foraging ability, copulation and feeding (Angilletta *et al.,* 2002; Harper & Peckarsky, 2006), temperature primarily affects growth, development and reproduction which can result in substantial impact on morphology and behaviour (Bale & Hayward, 2010; Bannerman *et al.*, 2011). In the case of life history, warming effects have often been recorded in terms of thermodynamic influences on physiology (Musolin, 2007), morphology and dispersal behaviour (Arribas *et al.*, 2012). Increased temperature and more frequent heatwaves could, however, alter the balance of resource allocation between reproduction and survival, mainly due to increased somatic functions (Angiletta, 2009). Even under ambient environmental conditions, most insects have limited energy reserves, usually as a consequence of environmental constraints restricting the possibilities of resource acquisition and allocation (Flatt & Heyland, 2011). As a result, insect survival and reproduction cannot be maximised simultaneously (Roff, 1992; Ellers & Van Alphen, 1997; Saeki *et al.*, 2014), and insects employ different strategies for trading-off resource demands (Roff *et al.*, 2002). A general pattern is that increased temperatures result in increased metabolic rates which, in turn, have led to shorter developmental times and greater early reproductive efforts albeit with lower lifetime fecundity (Frazier *et al.*, 2006; Cannon, 1998; Newman, 2006).

As with terrestrial systems, aquatic ecosystems are sensitive to climate change through effects on discharge, thermal regime and important solutes such as oxygen (Durance & Ormerod 2007). Increasing water temperature affects aquatic insects by changing their abundance and seasonal activity (Gillooly & Dodson, 2000; Durance & Ormerod, 2007; IPCC, 2014), as well as through individual respiratory (Buchwalter *et al.*, 2003; Rotvit & Jacobsen, 2013) and basal metabolic rates (Brown *et al.*, 2004) rates. Together, these have the potential to contribute to significant changes in growth rate, body size and the onset of insect emergence (Sweeney *et al.*, 1991; Chadwick & Feminella, 2001; Rahel & Olden, 2008; Woodward *et al.*, 2010). These faster maturation effects are seen as aquatic insect adaptation to warmer temperatures, albeit to a smaller body size (Hering *et al.*, 2009; Li *et al.*, 2011 Such a range of physiological responses to brief or prolonged exposure to warm waters make aquatic insects excellent subjects for the investigation of climatic effects on life history trade-offs, but few such studies have ever been carried out.

Among aquatic insects, the Trichoptera represent a sizeable proportion of running-water communities, both in species richness and population abundance (Malm *et al.*, 2013); they also exhibit a high degree of endemism, making them extremely vulnerable to any changes in environmental conditions (Brand & Miserendino, 2012). While there is longstanding evidence that temperature affects the distribution of some trichopteran families (Hildrew & Edington 1979) there is a dearth of information on the impacts of elevated temperature on these species. Nevertheless, as discussed in Chapter 5 higher temperatures increases resource investment towards somatic functions that affect overall fitness.

This chapter describes an experiment using field mesocosms that were reciprocally translocated over a thermal range intended to mimic the scale of climate change effects on a net-spinning trichopteran, *Hydropsyche siltalai*. The study species is abundant and ubiquitous in most running water bodies in England, Wales and Scotland, and intermediate altitudes between cooler and warmer-water downstream (Hildrew & Edington 1979). The species exhibits negligible, if any, adult feeding. Caddisfly dispersal is energetically costly as nutrient reserves are allocated mainly to reproduction (Stevens *et al.,* 2000; Masters *et al.*, 2007). Consequently, any changes in environmental conditions during the larval stage may influence resource investment between survival and reproduction in newly-emerged adult insects. The study explores whether altered temperature during immature development can influence resource allocation between reproduction and survival in subsequent adult caddisflies, specifically testing the hypothesis that changes in thermal regime during the penultimate instar stage will affect the balance in resource use and the rates of physiological processes. Physiological stress during the immature stage is known to have a delayed impact on adult caddisfly body mass (Stevens *et al.,* 1999; Jannot *et al.*, 2007; Mondy *et al.,* 2011).

6.3 Materials and Methods

6.3.1 Site description

The study was conducted between June and August 2015 in the Afon Mellte, Wales, in the upper Neath Valley (51°45'25.9"N 3°35'14.9"W). A large section of the River Mellte is designated as a Site of Special Scientific Interest (SSSI) and a Special Area of Conservation (SAC) for its assemblages of bryophyte and lichen, supported by complex mosaics of sessile oak (*Quercus petraea)* and ash (*Fraxinus excelsior)* woodlands (JNCC, 2015). The River Mellte provided an excellent experimental study system as the river flows into an underground cave (**Figure 6.1**) that provided natural cooling to moderate the lower downstream temperature throughout the summer period. Specifically, the river flows through a 500 m underground passage, entering at Porth yr Ogof and resurging at Pwll Glas. Sites upstream (51°48'32.8"N 3°33'02.8"W) and downstream (51°47'47.5"N 3°33'30.1"W) of the cave were therefore in an ambient and cooled river environment, respectively, but are otherwise similar in water quality (Environment Agency, 2009). Water temperatures were recorded using a data-logger (HOBO Pendant® Data Logger Model 8K-UA-002-64, Measurement System Limited, Berkshire, UK) from 16 June to 22 September 2015. Temperature data were recorded every 15 min at each stream site then collected and analysed weekly.

6.3.2 Study organism

Net-spinning caddisflies of the Family Hydropsychidae are important macroinvertebrates that regulate nutrient cycling and food webs in lotic ecosystems (Albertson & Daniels, 2016). Hydropsychid larvae spin a fine silk capture net that is anchored to the substrate and is used to collect suspended matter from flowing water (Edington & Hildrew, 1995). When congeneric *Hydropsyche* species' populations are abundant, their silk nets have been shown to provide benefits to other macroinvertebrates by generating more refuge space during high-discharge events (Cardinale *et al.,* 2004; Nakano *et al.,* 2005) and also by stabilizing the pattern of flow currents that, in turn, promotes sediment stability (Cardinale *et al.,* 2002). Hence, Hydropsychidae are identified as some of the most important suspension feeders inhabiting streams (Brown *et al.,* 2005). They are restricted to flowing waters and are commonly encountered during late spring to early summer in river margins and stream-beds. Because of their ability to withstand poor water quality (Vuori, 1993, 1995), they are also considered an important study organism for studies exploring species adaptation. In the present study, larvae of *Hydropsyche siltalai,* identified by their distinct morphological characteristics (**Figure 6.2),** were collected and fifth instars, determined by head-width measurements (see Edington and Hildrew, 2005), separated out in the field. Fifth instar larvae were collected from each stream sites, together with their plant fragment cases, using forceps to handle them and placing them individually in 50 ml centrifuge tubes filled with stream water. Centrifuge tubes were then stored in a cooler with ice before being transferred to the mesocosms (**Figure 6.3**).

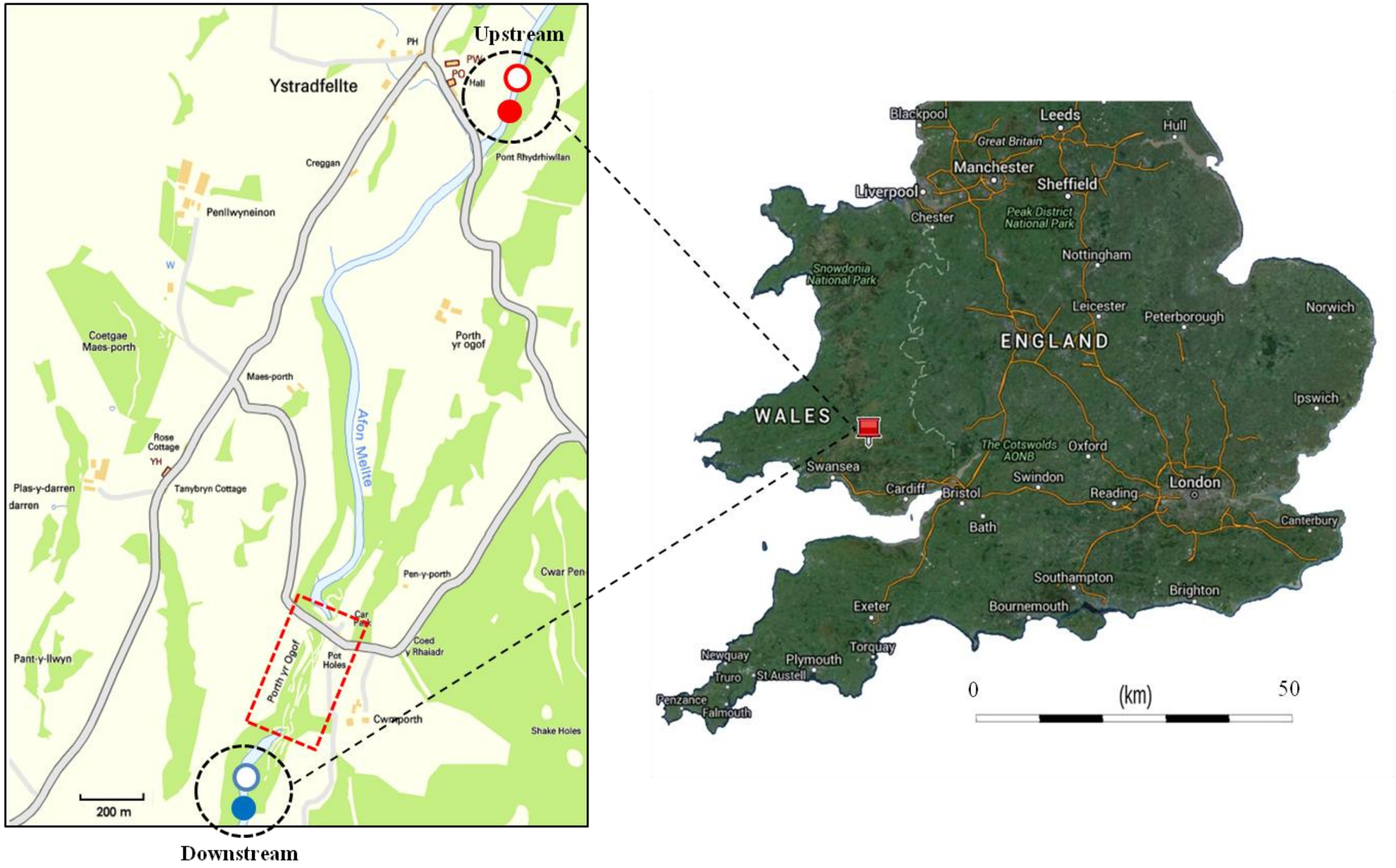


Figure 6.1: Location of River Mellte, Wales. The underground cave is located within the red broken line. Mesocosms were located in both upstream and downstream sites (blue circles represent downstream stations - solid (control), open (treatment); red circles represent upstream stations – solid (control), open (treatment)**.**

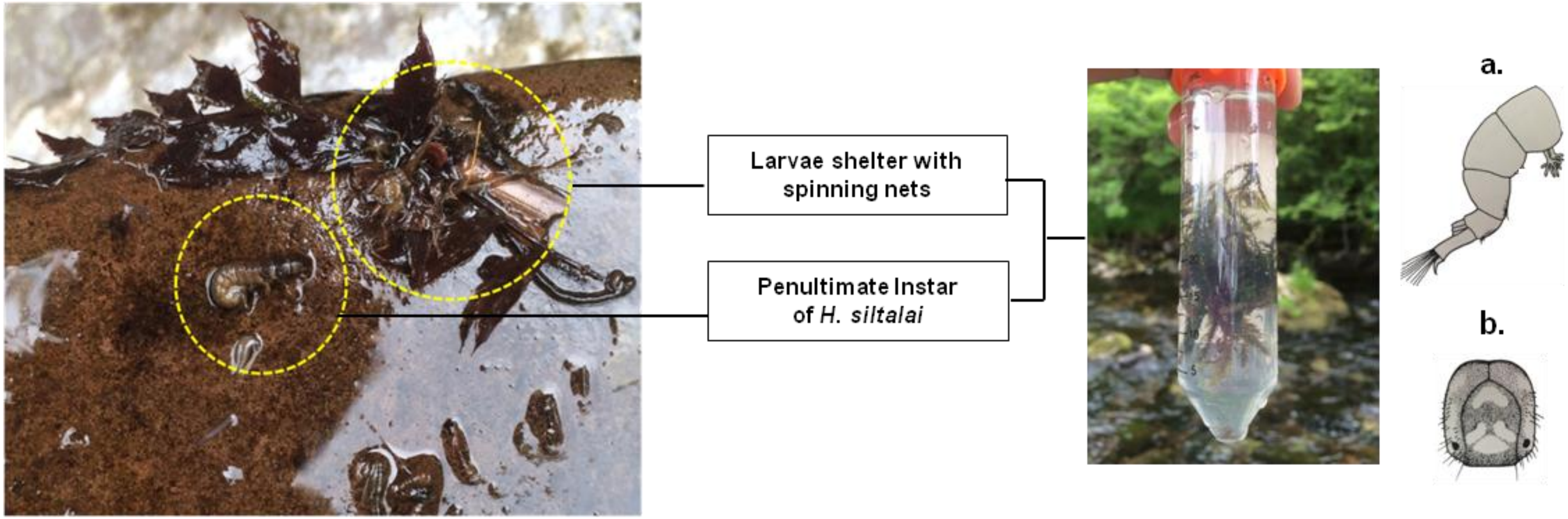


Figure 6.2: Fifth instar *Hydropsyche siltalai* were collected, together with their net-spinning tubes and transferred using centrifuge tubes into the mesocosms. Identification of *H. siltalai* larvae was based on their morphological characteristics: (a) gills absent on 7th abdominal segment, and (b) front oclypeus U or V shaped in outline of pronotum.

6.3.3 Mesocosm design

Each mesocosm consisted of an experimental channel constructed from housing thermoplastic channel (length: 1 m; width: 0.2 m) modified with a V-shape aluminium plate at the inlet to increase water flow (**Figure 6.3**). Altogether, four mesocosms were used (two for 'control' studies, two for 'treatment' studies (see below)). Each mesocosm was anchored to the stream bed using bricks and steel rods. A coarse mesh (1.2 cm) covering the inlet, outlet and channel lid prevented leaf litter clogging while still allowing suspended materials to enter and pass along the channels. The course filter also kept the experimental larvae within the mesocosms and prevented any predators from entering. Each mesocosm was filled with gravel from either the upstream or downstream site, as appropriate. The filters were scraped clean weekly, using a smooth brush, to prevent algal build-up.

Individual mesocosms were labelled (with plastic tags) as either 'control' or 'treatment' (**Figure 6.1**). Temperature regimes were recorded by data provided by HOBO data-loggers positioned within each mesocosm.

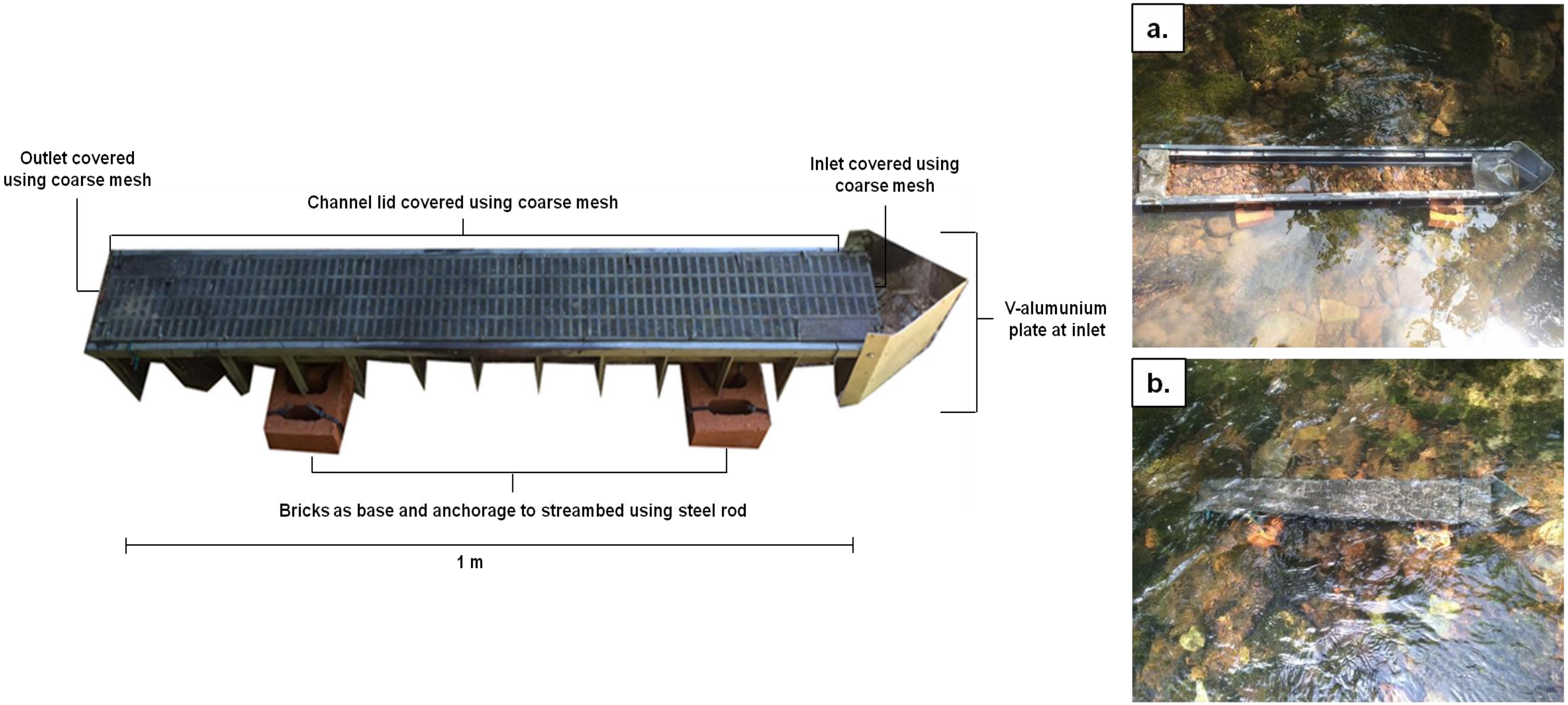


Figure 6.3: Mesocosms used to house *Hydropsyche siltalai* larvae. Each mesocosm was filled with stream gravel. Temperature loggers were attached under the channel lid for precise temperature measurements throughout the study period. Mesocosms were inspected by moving to shallow waters (a) and then being transferred back into deeper water (b).

6.3.4 Caddisfly exposure

The difference in temperature regime between the upstream and downstream sites during the experiment (varied between 1.5 and 4.5 ºC) was in line with the range of changing climate outlined by the IPCC (2007). Individual larvae, originating from either upstream and downstream sites, housed in mesocosms located at their home-stations comprised the ‘control’ studies (**Figure 6.1**). ‘Treated’ studies consisted of individual larvae collected from upstream or downstream but translocated to mesocosms at the non-home-station. Thus two mesocosms contained ‘native’ larvae ('upstream larvae' remaining upstream, and 'downstream larvae' remaining downstream), while the other two mesocosms contained translocated larvae ('upstream larvae' to downstream, and 'downstream larvae' to upstream). Larvae that were translocated from downstream to upstream sites were thus exposed to higher water temperatures than those remaining downstream, while larvae translocated from upstream to downstream were exposed to lower water temperatures than those remaining upstream (**Figure 6.1**). All fifth instar larvae remained in the mesocosms for between 1-2 weeks until they developed into pupae. Mesocosms were inspected every 2-3 days and fully developed pupae collected. The individual pupae that were eventually used for the translocation study were those collected between June and July 2015 when temperature variation was at its highest (**Figure 6.4**). Once collected, fully developed pupae were transferred into a controlled environment chamber (**r**efer to Chapter 4) rearing facility at Cardiff University. They were kept in an aerated tank (length x width x depth = 18 x 11 x 12.5 cm) containing stream water.

6.3.5 Insect nutrient assay

A total of 80 fifth instar *H. siltalai* larvae were transferred into mesocosms during the experiment. Of these, the 48 that emerged as adults (**male = 26, female = 22; Table 6.1**) were used to determine differences in mass and nutrient allocation between control and treated individuals. Using a nutrient assay protocol developed from that of Foray *et al.* (2012; see Chapter 3), the macronutrient content of both somatic and reproductive tissues in newly-emerged (less than 12 h upon emergence) caddisflies was determined. The newly-eclosed insects were weighed and deep-frozen, before dissection on cooled Petri dishes to separate out those body parts linked to somatic (head, thorax and appendages attached to the gaster) and reproductive (ovaries/testis follicles and fat bodies) functions (refer Chapter 3).

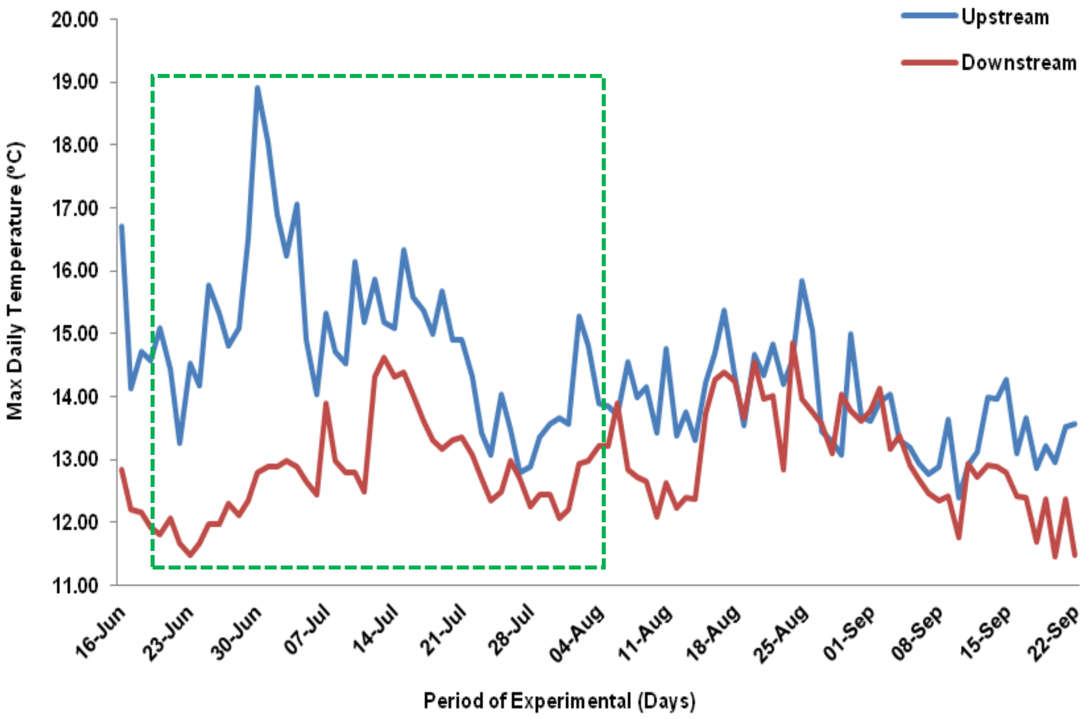


Figure 6.4: Maximum daily temperature for upstream and downstream sites throughout the study period. The translocation experiment took place from June to July 2015 when thermal variation was at its highest (green box). Reduced temperature difference between upstream and downstream sites were shown in mid-August.

6.3.6 Data Analysis

Differences in somatic and reproductive tissue mass between control and treated groups of larvae during the emergence period (number of days to adult emergence during laboratory rearing), were analysed using Analysis of Variance (ANOVA). Two-way ANOVA was used to test the main and interactive effects of translocation and sex on each nutrient (protein, carbohydrate and glycogen, and total and neutral lipid) content in both somatic and reproductive tissue. Model residuals were checked for normality and homoscedasticity assumptions. Where these assumptions were violated, alpha was set to 0.001 to reduce the risk of finding false positives. Robust post-hoc tests (Tukey HSD) were used, adjusting p-values for multiple pairwise comparisons between treatment levels (Herberich *et al.*, 2010). In addition, nutrient (protein, carbohydrate, glycogen, and total and neutral lipid) contents in somatic and reproductive tissue were combined using Principle Component Analysis (PCA), and overall nutrient scores were compared between control and treated individuals using Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) and pairwise comparisons. These latter tests were carried out using the Adonis function, based on 4,999 permutations (Oksanen *et al.,* 2012). All data analysis was carried-out using the 'R' statistical software (R Development Core Team, 2011).

6.4 Results

Throughout the experiment, maximum daily temperatures (mean (± SE)) ranged from 12.40 (± 0.03) to 18.90 (± 0.17) ºC at the upstream site, and 11.46 (± 0.01) to 14.85 (± 0.04) ºC downstream. The mean (±SE) maximum temperatures during the experimental period for upstream and downstream sites were 14.38 (± 0.12) °C and 12.92 (± 0.08) °C, respectively **(Figure 6.4)**.

6.4.1 Effects of warming on mass allocation between somatic and reproductive tissue

Warmer temperature reduced resource allocations to both types of tissue in newly-emerged adults [somatic (ANOVA; $F_{3,44} = 9.72$, $P < 0.001$) and reproductive ($F_{3,44} = 3.34$, $P = 0.028$) mass] (**Figure 6.5**). Newly-emerged *Hydropsyche siltalai* differed in somatic mass between cool control and warming treatment, as well as between warm control and warming treatment (Tukey's HSD; $P < 0.05$). Somatic mass in cooling treatment individuals did not differ from either warm or cool control (Tukey's HSD, $P > 0.05$).

Downstream individuals translocated to the warmer, upstream site (= warming treatment) had lower somatic and reproductive mass than cooling treatment and control groups (**Table 6.1**). Reproductive mass did not, however, differ between groups (Tukey's HSD, $P > 0.05$). Nor did time period to emergence of adults did not differ significantly between groups ($F_{3,44} = 1.58$, $P=0.21$) (**Table 6.1**), although times to emergence were significantly longer for females than for males ($F_{1,43} = 27.64$, $P<0.001$; females 10.27 ± 0.54 days; males 6.84 ± 0.37 days).

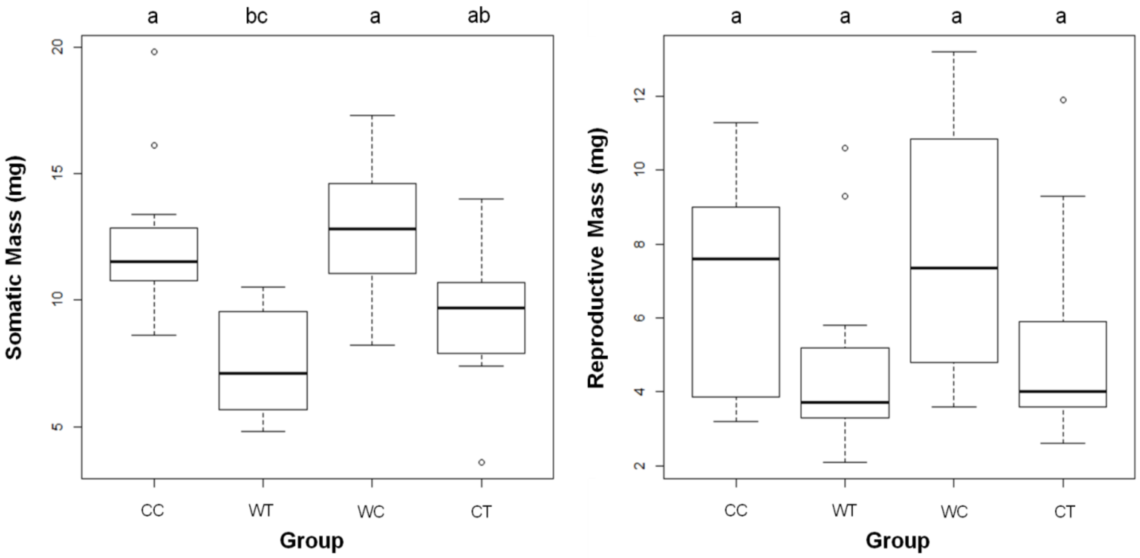


Figure 6.5: Boxplot showing mass allocation in newly-emerged somatic and reproductive tissue between control and treated individuals in the translocation experiment (12 individuals per group) (WC = warm control; CC = cool control; CT = cooling treatment; WT = warming treatment). Different letters above boxplots denote significant differences between groups (ANOVA followed by a Tukey HSD multiple comparisons test, P<0.05). Boxplots: horizontal bold line, median; box, interquartile range; whiskers, the range of the values between the interquartile that is no more than 1.5 times the interquartile range.

6.4.2 Nutrient allocation into somatic and reproductive functions

Newly-emerged *H. siltalai*, following exposure to warmer (= warming treatment) and cooler (= cooling treatment) waters had altered nutrient allocation between somatic and reproductive tissue. Translocation studies affected nutrient allocation in somatic [protein ($F_{3,44} = 11.26$, $P < 0.001$), carbohydrate ($F_{3,44} = 6.24$, $P = 0.0014$), glycogen ($F_{3,44} = 2.88$, $P = 0.047$), and total ($F_{3,44} = 9.36$, $P < 0.001$) and neutral ($F_{3,44} = 8.98$, $P < 0.001$) lipid] and reproductive [protein ($F_{3,44} = 7.44$, $P < 0.001$), carbohydrate ($F_{3,44} = 8.55$, $P < 0.001$), glycogen ($F_{3,44} = 2.48$, $P = 0.075$), and total ($F_{3,44} = 26.98$, $P < 0.001$) and neutral ($F_{3,44} = 60.68$, $P < 0.001$) lipid] tissues (**Table 6.2**). Comparison between somatic tissues between 'treatment' and 'control' group revealed significant (Tukey's HSD, $P < 0.05$) difference for protein, carbohydrate, glycogen and total and neutral lipid content. In reproductive tissue, 'treatment' and 'control' groups differed only with respect to total lipid (Tukey’s HSD, $P < 0.05$), with protein and neutral lipid only showing a significant difference between warm 'control' and downstream 'treated' individuals (**Figure 6.6**).

Specifically, male caddisfly exposed to warming treatment showed greater nutrient allocation in somatic (protein = 36.65 ug mg^{-1}, glycogen = 2.02 ug mg^{-1}, total lipid = 11.69 ug mg^{-1} and neutral lipid = 6.18 ug mg^{-1}) and reproductive (protein = 59.61 ug mg^{-1}, total lipid = 14.65 ug mg^{-1} and neutral lipid = 11.32 ug mg^{-1}) tissue (**Figure 6.7**). Meanwhile, for female caddisfly exposure to warming treatment showed greater nutrient allocation only in reproductive tissue (lipid = 11.34 ug mg^{-1}) (**Figure 6.7**). In contrast, cooling treatment showed greater nutrient allocation in female somatic (protein = 27.38 ug mg^{-1}, carbohydrate = 1.61 ug mg^{-1}, glycogen = 1.83 ug mg^{-1}, total lipid = 12.88 ug mg^{-1} and neutral lipid = 5.27 ug mg^{-1}) and reproductive (protein = 22.79 ug mg^{-1}, carbohydrate = 1.29 ug mg^{-1}, glycogen = 3.06 ug mg^{-1} and neutral lipid = 5.67 ug mg^{-1}) tissue (**Figure 6.7**). Cooling treatment showed greater nutrient allocation in male only in carbohydrate for both somatic (carbohydrate = 1.70 ug mg^{-1}) and reproductive (carbohydrate = 2.84 ug mg^{-1}) tissue (**Figure 6.7**).

Insect sex influenced nutrient content (ug per insect) in somatic and reproductive tissue. Overall, male *H. siltalai* represent greater proportional nutrient content in somatic (carbohydrate = 60%, glycogen = 50% and total lipid = 61%) and reproductive (protein = 45%) when compared to female. Female *H. siltalai* showed greater nutrient content in somatic (protein = 60%) and reproductive (carbohydrate = 44%, glycogen = 52% and total lipid 43%) tissues than males (**Figure 6.8**). Female and male caddisfly showed similar neutral lipid content in both somatic (neutral lipid = 52%) and reproductive (neutral lipid = 48%) tissues.

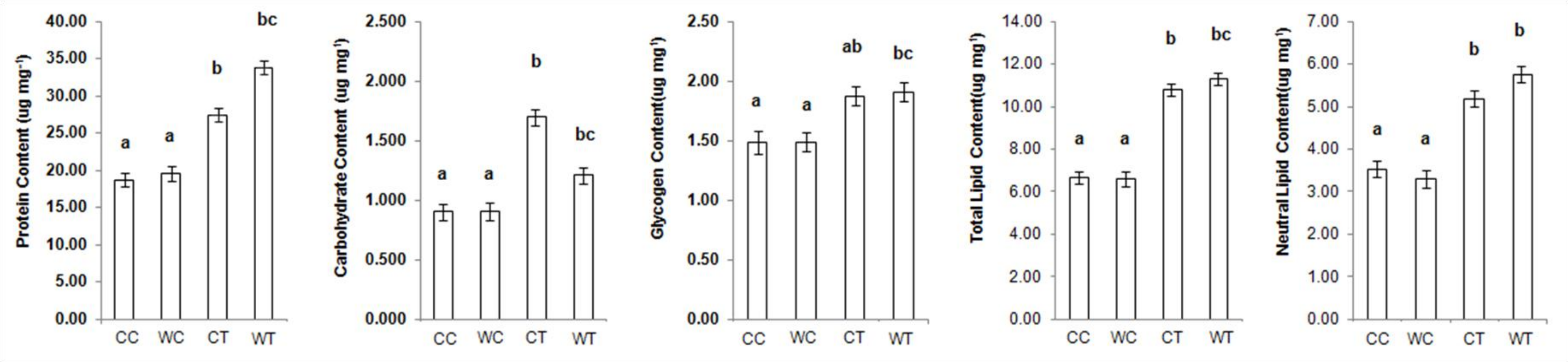
Table 6.1: Mean (± SE) values for mass allocation and nutrient content in somatic and reproductive tissue in *Hydropsyche siltalai* for control and treated individuals. Sexual difference between groups are represented by letters (F=Female; M=Male).

Stream Sites	**Caddisfly, *H. siltalai***	**Emergence Period (days)**	**Life History Traits**	**Mass Allocation (mg)**	**Protein (ug mg^{-1})**	**Carbohydrate (ug mg^{-1})**	**Glycogen (ug mg^{-1})**	**Total Lipid (ug mg^{-1})**	**Neutral Lipid (ug mg^{-1})**
Upstream	Warm Control	8.83±1.01	Somatic	12.74±0.83	19.54±0.99	0.90±0.07	1.49±0.08	6.61±0.35	3.29±0.20
	(F=8, M=4)		Reproductive	7.91±0.97	25.45±3.39	1.34±0.17	2.86±0.54	7.39±0.67	5.66±0.69
	Cooling Treatment	7.00±0.62	Somatic	9.40±0.77	27.48±2.74	1.70±0.26	1.87±0.22	10.79±1.27	5.18±0.61
	(F=2, M=10)		Reproductive	5.38±0.82	37.41±4.65	2.28±0.30	3.40±0.37	12.80±0.85	8.71±0.95
Downstream	Cool Control	9.33±0.70	Somatic	12.26±0.88	18.69±0.91	0.90±0.07	1.49±0.10	6.66±0.30	3.54±0.18
	(F=7,M=5)		Reproductive	6.94±0.78	29.25±4.30	1.47±0.21	2.74±0.44	8.13±0.77	6.45±0.78
	Warming Treatment	8.50±0.77	Somatic	7.70±0.63	33.76±3.06	1.22±0.07	1.91±0.12	11.30±0.99	5.75±0.49
	(F=5, M=7)		Reproductive	4.75±0.76	52.31±5.47	1.92±0.23	3.93±0.45	14.20±0.67	10.07±1.04

Table 6.2: Summary of two-way ANOVAs comparing *H. siltalai* nutrient content in somatic and reproductive tissue exposed to cool and warming treatment. Significant values (P<0.05) are in bold.

Macronutrients measured	**Effect**	**d.f.**	***F-statistic***	***P-value***
Somatic Tissue				
Protein	Treatment	3	11.26	**< 0.001**
	Sex	1	8.59	0.139
	Treatment × Sex	3	0.84	0.459
	Error	40		
Carbohydrate	Treatment	3	6.24	**0.0014**
	Sex	1	4.27	**0.045**
	Treatment × Sex	3	0.21	0.89
	Error	40		
Glycogen	Treatment	3	2.88	**0.047**
	Sex	1	4.92	**0.03**
	Treatment × Sex	3	0.16	0.92
	Error	40		
Total lipid	Treatment	3	9.36	**< 0.001**
	Sex	1	0.21	0.65
	Treatment × Sex	3	0.76	0.52
	Error	40		
Neutral Lipid	Treatment	3	8.98	**< 0.001**
	Sex	1	1.79	0.19
	Treatment × Sex	3	0.47	0.70
	Error	40		
Reproductive Tissue				
Protein	Treatment	3	7.44	**< 0.001**
	Sex	1	71.67	**< 0.001**
	Treatment × Sex	3	0.49	**0.049**
	Error	40		
Carbohydrate	Treatment	3	8.55	**< 0.001**
	Sex	1	70.54	**< 0.001**
	Treatment × Sex	3	0.78	0.51
	Error	40		
Glycogen	Treatment	3	2.48	**0.075**
	Sex	1	17.44	**< 0.001**
	Treatment × Sex	3	1.16	0.33
	Error	40		
Total lipid	Treatment	3	26.98	**< 0.001**
	Sex	1	13.44	**< 0.001**
	Treatment × Sex	3	1.07	0.37
	Error	40		
Neutral Lipid	Treatment	3	12.48	**< 0.001**
	Sex	1	60.68	**< 0.001**
	Treatment × Sex	3	1.00	0.40
	Error	40		

Somatic Tissue



Reproductive Tissue

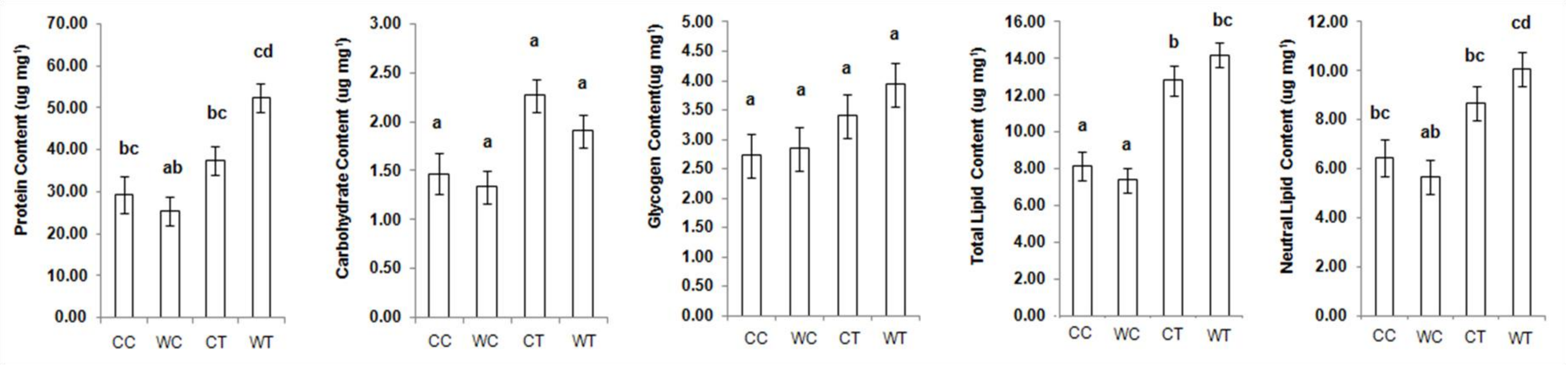


Figure 6.6: Bar-plots of mean (± SE) proportion of nutrient content in somatic and reproductive tissue of newly-emerged caddisfly between control and treated individuals under translocation experiment (n=48) (WC = warm control; CC = cool control; CT = cooling treatment; WT = warming treatment). Different letters above bars denote significant differences between groups (ANOVA followed by a Tukey HSD multiple comparisons test, $P<0.05$).

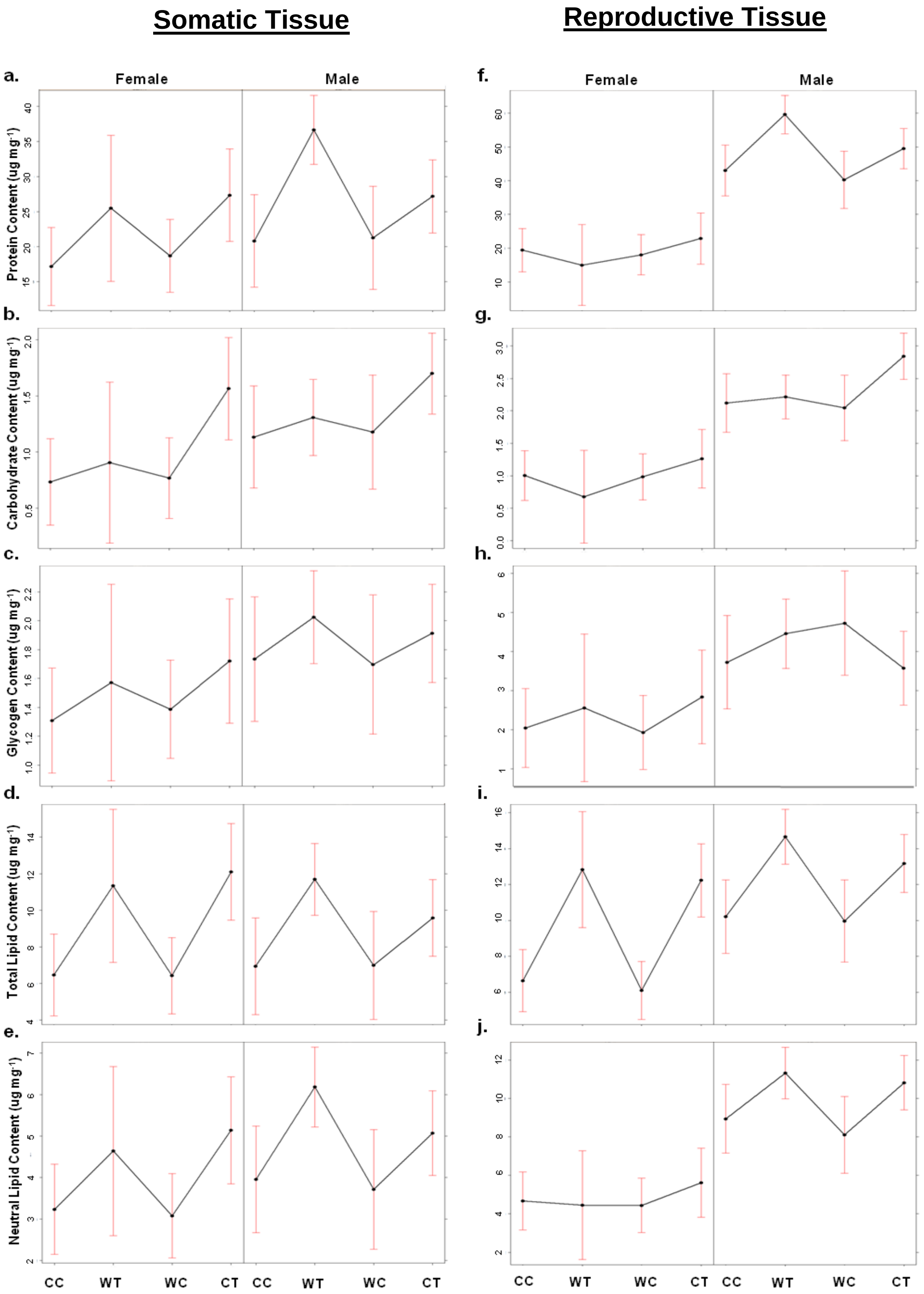


Figure 6.7: Mean (± SD) of nutrient allocation (ug mg^{-1}) in somatic (a. - e.) and reproductive (f. - j.) tissues of newly-emerged caddisfly between control and treated groups in the translocation experiment (n=48) (CC = cool control; WT = warming treatment; WC = warm control; CT = cooling treatment).

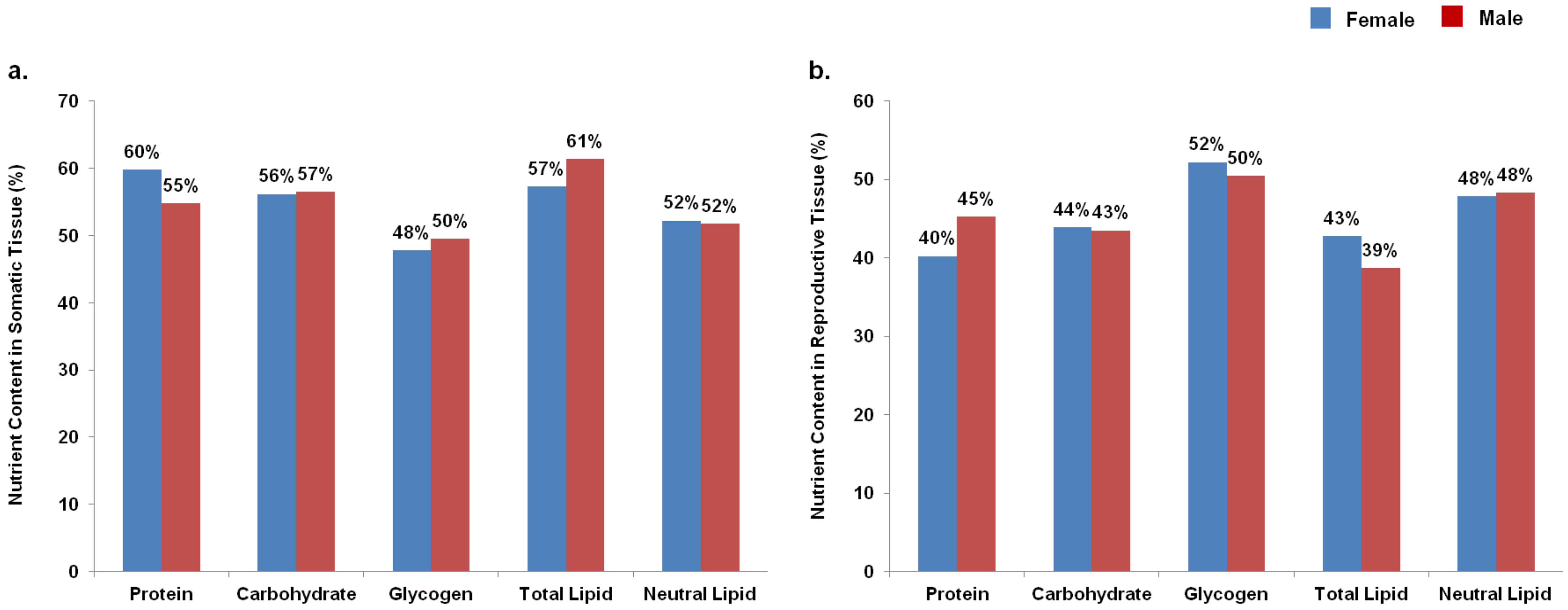


Figure 6.8: Comparisons between macronutrient content (per insect) expressed as percentage for female and male *Hydropsyche siltalai* in somatic (a.) and reproductive (b.) tissue.

6.4.3 Overall nutrient content between control and treated individual caddisfly

Macronutrient (protein, carbohydrate, glycogen, and total and neutral lipid) content measured in both reproductive and somatic tissues for control and treated individuals were combined and the overall nutrient content compared using PERMANOVA (**Figure 6.9**). Two principal components (PCs) in the Principle Components Analysis for reproductive tissue represented over 75% of the variation among individuals in nutrient allocation between groups. Protein, carbohydrate, glycogen and both lipid measurements increased along PC1, while PC2 represented increasing glycogen but decreasing protein, carbohydrate and both lipid measurements (**Figure 6.9a**). For somatic tissue, the two principle components explained 84% of nutrients with increasing protein, carbohydrate, glycogen, and both lipid measurements along PC1, similar to reproductive tissue, while PC2 represented declining carbohydrate and glycogen, with increasing protein and both lipid measurements (**Figure 6.9b**). There was a significant difference (using PERMANOVA) in overall nutrient content for reproductive ($F_{3,44}$ = 6.09, P = 0.003) and somatic ($F_{3,44}$ = 13.47, P = 0.001) tissues for all treatments (**Figure 6.9c and 6.9d**). Pairwise comparison between PERMANOVA show significant difference between control and treatment groups (**see Table 6.3**).

Table 6.3: Pairwise comparison of PERMANOVA used to evaluate differences in overall nutrient allocation between control and treatment groups (WC = warm control; CC = cool control; CT = cooling treatment; WT = warming treatment).

Groups Compared	**MS**	$F_{1,23}$	***P***
WC - CC	0.001	0.21	0.743
WC - CT	0.155	11.25	0.002
WC - WT	0.346	26.49	<0.001
CC - CT	0.172	12.67	<0.001
CC - WT	0.369	28.82	<0.001
CT - WT	0.048	2.51	0.132

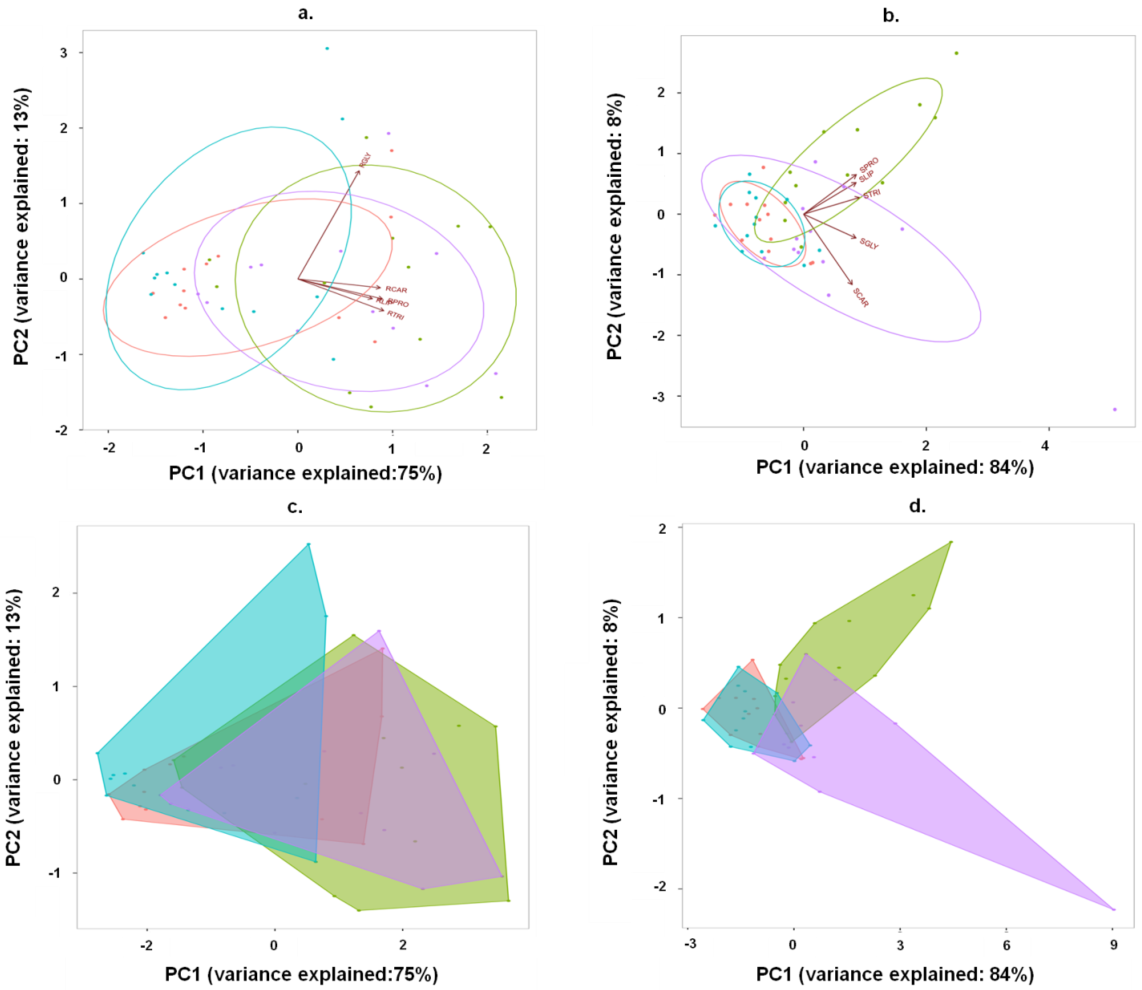


Figure 6.9: Principle Component Analysis (PCA) for different nutrient content in newly-emerged *Hydropsyche siltalai* reproductive (a.) and somatic (b.) tissues. Permutational Multivariate Analysis of Variance (PERMANOVA) was used to compare overall nutrient content for *Hydropsyche siltalai* rreproductive (c.) and somatic (d.) tissues. (Blue - warm control individuals; Red - cool control individuals; Purple - cooling treatment individuals; Green - warming treatment individuals).

6.5 Discussion

Relatively little is known of how climate change and climatic events affect resource allocation in insects. Allocation theory implies that trade-offs between survival and reproduction can be linked to allocation of mass and resources to body parts in newly-emerged adult holometabolous insects (e.g. thoracic and abdominal mass, wing dimorphism, mature and immature oocytes) (Jervis *et al.,* 2005; Stjernholm & Karlsson, 2006; Boggs, 2009). Results from this study support the prediction that *Hydropsyche siltalai,* when exposed to warmer waters during its immature stages, increases, on attaining adulthood, resource allocation towards survival. Exposure to warmer temperature resulted in lower somatic and reproductive mass with higher nutrient allocation into somatic components. Reduced body mass in newly-emerged *H. siltalai* can be explained by the Temperature-Size Rule (Atkinson, 1994). This rule states that ectotherm development is relatively faster at higher temperature and, as a result of early maturation into adulthood, leads to a reduced final weight. Unexpectedly, *H. siltalai* transferred from upstream to cooler downstream waters showed the same response, resulting in individuals with lower somatic and reproductive mass compared to control individuals. Surprisingly, male and female showed different response under translocation studies with male showing greater nutrient allocation under warmer environment and female under colder environment to somatic tissue. These patterns, however, were influenced by sex effect that revealed an experimental design weakness because of heavily skewed sex ratios within the transplanted caddisfly due to low survival rates (60%) during transition into adult stage. It is also important to highlight that the findings were limited to a small number of caddisfly population. Short-time duration (2 weeks) for translocation studies due to loss of original mesocosms during preliminary study also influenced the results.

Temperature variation and fluctuation can affect an insect’s physiological condition in many ways. In general, physiological stress caused by increased temperature can affect ectotherm body size as a result of allometric physiological limitations (Lonsdale & Levinton, 1985; Hogue & Hawkins, 1991). At higher temperature, the metabolic rate of an ectotherm may increase and developmental time shorten; this results in earlier maturation of the adult but at a smaller body size (Atkinson, 1994). Many studies have illustrated temporal trait changes in freshwater invertebrates under warming environmental conditions; for example, adult body size decreased in the mayfly *Baetis* *bicaudatus* (see Harper & Peckarsky, 2006), caddisfly *Limnephilus indivisus* (see Jannot, 2009) and damselfly *Ischnura elegans* (see Stoks *et al.*, 2012). In addition, Van Doorslaer *et al.* (2007) showed that under warmer conditions, the daphnid, *Simocephalus vetulus*, exhibited a similar response to temperature but with an increase in offspring number. These responses imply that species adaptation to a changing environment may alter the characteristic traits of an individual's life history in a trade-off between survival and reproduction. In the present study, short-term exposure to warmer waters during the immature stage had a subsequent impact on adult resource allocation patterns. Newly-emerged *H. siltalai* increased resource allocation for survival purposes; this was true for all macronutrients (protein, carbohydrate, glycogen, and total and neutral lipid) measured (**Figure 6.6**). On the other hand, *H. siltalai* allocated the highest total lipid content to reproductive tissue (**Figure 6.6**); this may suggest that the species employs an earlier reproduction strategy on emergence. Higher resource allocation into both survival and reproductive components can be compared to the response in pro-ovigenic parasitoids (i.e., parasitoids that mature all of their eggs before emergence) under similar warmer environmental conditions (Denis *et al.,* 2012). These latter authors showed that parasitoids, on emergence at higher temperature, favoured a small initial egg-load and a larger amount of lipids for maintenance.

The association of larger insects with colder environment implies that resource investment between reproductive and somatic functions respond differentially with temperature (Hogue & Hawkins, 1991; Bochdanovits & de Jong, 2003). In the study, female *H. siltalai* allocate greater nutrient into somatic and reproductive tissue with colder water temperature which were similar with male nutrient allocation under warmer water temperature. Translocation of caddisfly into warmer and colder environment may indicate a stress response that may have potential to reduce reproductive success due to greater nutrient allocation to somatic tissue. In general, insect adaptation to colder environment reveal greater survival due to longer lifespan but delayed in reproduction (Moiroux *et al.,* 2010). Angiletta *et al.* (2003) explained that thermal adaptation of enzymes can decrease the concentrations that are necessary for anabolic and catabolic processes, thus leads to more energy reserves for somatic growth under colder environment.

Previous studies highlight the use of offspring number and longevity as measures for assessing reproduction and survival in an attempt to estimate trade-offs between major traits (Boivin & Martel, 2012; Klepsatel *et al.,* 2013). In the present study, nutrient allocation between reproduction and survival yielded similar results. Resource allocation into two or more competing traits, when resources are derived from a finite pool, means that an increase in allocation to one trait necessitates a decrease in another. This leads to resource allocation trade-offs (De Jong & Van Noordwijk, 1992; Stearns, 1992). Such trade-offs may exemplify individual decisions for resource allocation between different life history functions. In *H. siltalai,* higher nutrient allocation into somatic tissue at higher thermal regimes may signal individual physiological adaptation to a warming environment. Higher nutrient allocation into survival is associated with active flight activity and enhanced somatic repair at cellular level (Judd *et al.,* 2010; Amat *et al.,* 2012; Flatt *et al.,* 2013). In a warming environment, the aquatic invertebrate community is predicted, by the Disposable Soma Hypothesis (Kirkwood, 1987), to allocate more resources into survival (Daufresne *et al.,* 2009). The adaptive response due to increased somatic investment may, however, vary under different circumstances for different insect species. Fruit flies, *Drosophila melanogaster,* for example, extend their life-span under dietary restriction (Min *et al.,* 2006). In contrast, the firebug, *Pyrrhocoris apterus,* when exposed to limited food supply, re-utilized proteins derived from flight muscles for female ovary and male accessory gland development (Socha & Sula, 2008). The present study showed that *H.siltalai,* under similar conditions, increased nutrient (protein, carbohydrate, glycogen and total and neutral lipid) allocation to somatic components while retaining nutrient (lipid) for reproduction.

Insect body size represents the interaction between resource acquisition and allocation in terms of physiological traits (Chown & Gaston, 2010). Female insects benefit from smaller body size by investing more resources into early reproduction; having more mature eggs upon emergence but at the cost of a shorter life-span (Thorne *et al.,* 2006). Larger females, on the other hand, invest more resources into future reproduction, with higher initial oocyte numbers and a fat body component that prolong adult life-span. Differences in reproductive allocation, reported between gregarious (*Aphaereta pallipes*) and solitary (*A. genevensis*) parasitoid species, are considered consequences of different species investment in clutch sizes (Pexton & Mayhew, 2002). This is shown in female of the gregarious *A. pallipes* where resources are allocated more into reproduction leading to lower fat bodies and reduced longevity (Godfray, 1994; Mayhew; 1998). In contrast, female of the solitary *A. genevensis* allocated resources toward greater fat reserves, resulting in enhanced longevity. Thus, solitary parasitoid species that lay one egg per clutch has more fat and fewer eggs than a gregarious parasitoid species that lay more eggs per clutch (Mayhew & Van Alphen, 1999). Physiological studies of insects indicate higher lipid allocation for reproduction, as seen in this study for warming treatment in *H. siltalai*. In addition, insects that are exposed to warmer environmental conditions tend to reproduce early. This has been documented for several insect species: butterflies (Berger *et al.,* 2008), parasitoids (Denis *et al.,* 2011) and caddisfly shredders (Mas-Marti *et al.*, 2015). The explanation for such behaviour is that the female insects require a sufficient temperature to convert allocated resources into eggs. Warmer temperatures provide the benefits for early egg maturation. Investment into early reproduction, by having an initial egg load upon maturation, is, however, costly and can demand higher allocations of nutrient resources such as lipids. As well as being an essential nutrient component for insect egg maturation (Casas *et al.,* 2005), lipids also represent an important nutrient content for embryonic development and insect vitellogenesis (Sayah *et al.,* 1997).

Male and female insects differ in how they maximise reproductive success (Sagarra *et al.*, 2007). Male insects with higher mating rates are expected to have relatively larger testes in order to fertilise more eggs (Blackenhorn *et al.*, 2004). As for females, just a few matings are sufficient for adequate egg maturation (Kant *et al.,* 2016). Male investment into gamete production is influenced by testes size; larger testes contribute to higher sperm production (Saglam *et al.,* 2008). In the present study, male *H. siltalai,* upon emergence, showed higher allocation than females, for all nutrients measured, into reproductive tissue. For this species, therefore, it would appear that male investment into sperm production requires higher nutrient allocation compared to a female's investment into oocyte production. This may imply that the cost of reproduction for males requires higher resource allocation into reproduction to allow earlier mating strategies. Male *H. siltalai,* upon emergence, allocate higher carbohydrate and glycogen resources to somatic tissue than females. Several studies have shown that carbohydrate and glycogen are important energy reserves to sustain insect flights as for example, in the parasitoids *Venturia canescens* (see Amat *et al.,* 2012) and *Cotesia glomerata* (see Wanner *et al.*, 2006), and in orchid bees, *Euglossa* sp. (Suarez *et al.*, 2005). Carbohydrate is an important means of energy storage that can be transformed into glycogen; this can then be used to produce glucose in the haemolymph and flight muscles (Van Der Horst, 2003; Pelosse *et al.,* 2010). The higher energetic demands of male active flight capability upon emergence are mainly associated with courtship flights (Dufour *et al.*, 2012). The different body mass between male and female insects may also contribute to the different abilities of the two sexes to withstand environmental change, with females showing higher survival under warmer environments due to higher overall body tissue (e.g. fat body, ovaries and embryonic tissues). This was observed very clearly in the aphid parasitoid, *Aphidius avenae* (see Roux *et al.*, 2010).

Investigation of insect adaptation under changing environment may provide evidence that freshwater invertebrates are capable of a rapid micro-evolutionary response to support a population's persistence (De Jong, 2005). Fitness consequences for *H. siltalai* under warmer conditions may indicate phenotypic plasticity leading to life history trade-offs for maintaining overall fitness (Moczek, 2010). Previous studies have shown that trade-offs were caused by limited food supply or various environmental factors that can affect development (Fischer & Fiedler, 2002; Jervis *et al.*, 2007). In holometabolous insects, adaptive phenotypic plasticity is related to fitness-cost related traits; adults are fully dependent on resources acquired during their larval stages for various life history functions. The degree of plastic response is, therefore, dependent on immature life experience. Trade-off signatures, using resource allocation patterns between somatic and reproduction tissues, may represent a novel approach in understanding how these allocation choices are made. Nutrient content in somatic tissue showed significant differences in all nutrient parameter comparisons between treated and control *H. siltalai* individuals **(Figure 6.6)**. Previous studies have shown that an insect undergoes changes in phenotypic characteristics, such as flight muscles and ovarian mass, as a trade-off between survival and reproduction (Mole & Zera, 1993). Warming might alter adult resource allocation patterns and this may determine whether an early or future reproductive strategy is followed. Changes in resource allocation pattern do not, however, represent changes in phenotypic characteristics in *H. siltalai* as somatic and reproductive mass showed no difference between treated and control groups. Similar observations have also been made in the damselfly, *Coenagrion scitulum* (see Therry *et al.*, 2015). In this latter study, adult *C. scitulum* from warmer environment had longer relative thorax length for better flight performance but were also able to maintain high fecundity. In another study, it was found that higher thoracic investment can indicate an evolutionary change in flight morphology, as seen in colonizing individuals of speckled wood butterfly, *Pararge aegeria* (see Hill *et al.*, 1999).

Experimental field studies provide relatively reliable information when assessing an organism's response to a warming climate. Principle Component Analyses for overall nutrient content showed significant differences in somatic and reproductive components between treated and control groups (**Figure 6.8**); resource allocations differed in translocated individuals and thus provided an understanding of biological outcomes in *H. siltalai* under warmer conditions. Increased metabolic activity from higher nutrient allocation, arising from warming of the range used in this study, can influence voltinism, the number of generations per year. Smaller body size upon emergence in newly emerged *H. siltalai* may indicate faster larval development; as faster immature development is related to increased aquatic insect voltinism with more generations per year (Shama *et al.,* 2011). Furthermore, some *Hydropsyche* spp. have been reported to exhibit both univoltine and bivoltine life histories depending on the environmental temperature and food abundance as this influences larval growth rates (Cudney & Wallace, 1980; Freeman & Wallace, 1984). Rapid population growth under a higher temperature has been observed in the dragonfly, *Gomphus vulgatissimus* (see Braune *et al.,* 2008); the voltinism of the species decreased in a population from a warmer to a colder environment. The plastic response of having a smaller body size due to rapid population may, however, in the long-term, be costly (Chown *et al.,* 2010). Rapid population growth in insects, under a warming environment, is dependent on habitat conditions; other stream ecological factors, for example, pH or dissolved oxygen content, may interact with temperature and affect insect biological functions affecting survival.

In conclusion, adaptation of the caddisfly, *H. siltalai,* in warmer conditions should lead to a generally higher nutrient allocation into somatic components, with higher total lipid allocation into reproduction. In a warming environment, and resulting from smaller body sizes, insects may employ an early reproductive strategy. Differences between overall nutrient content in somatic and reproductive components lead to higher metabolic activity with rapid growth under warmer conditions. More studies are needed to assess the impact of increased water temperature on insect life cycles in their totality; there may very well be consequences on the embryonic stage, immature physiological conditions, and adult oviposition and foraging. Coupled with other potential ecological factors, global warming could become more challenging for the survival of some insects by causing major changes in resource allocation.

7. General Discussion

7.1 Discussion

7.1.1 Revisiting the context

Insects employ different resource allocation strategies to maximise fitness and survival. How they do this depends on resource uptake, on how resources are divided between different life history functions, and also on environmental characteristics. Numerous studies have provided insight on how insects respond physiologically to environmental factors including food availability and physical conditions (Boggs, 1993; Casas *et al.,* 2003; Pelosse, 2007). Under favourable conditions, insects have the opportunity to invest more resources into reproduction at the cost of somatic functions. Conversely, harsh environmental conditions often cause a shift of resources into somatic maintenance for increased survival (Kirkwood, 1977; Flatt, 2011). While these responses, generally referred to as 'non-adaptive plasticity', provide a mechanism for insects to adjust their life histories in response to environmental variations (Ghalambor, *et al.,* 2007), they come with a physiological cost. Differential allocation of resources between somatic and reproductive traits leads to a trade-off between these two life history components (Roff & Fairbairn, 2012).

Adult growth and development in holometabolous insects are typically dependent on a single resource pool derived from larval nutrient acquisition (Boggs, 1992). Frequently, the allocation of this resource pool is influenced by the environmental conditions experienced by juvenile life-stages. For example, physicochemical constraints such as temperature and contaminants may increase larval metabolic responses and shorten developmental times, reducing nutrient acquisition and allocation (Congdon *et al.,* 2001; Jannot *et al.,* 2007; Wajnberg *et al.,* 2012). This study has focused particularly on how insect physiological traits and environmental factors influence the allocation of nutrients within reproductive and somatic tissues in order to optimize fitness. Assessments were completed considering life history theory in relation to the 'Cost of Reproduction' (Williams, 1966) (**Chapter 4**), 'Disposable Soma' (Kirkwood, 1977) (**Chapter 5**) and the 'Temperature-size Rule' (Atkinson, 1994) (**Chapter 6**).

7.1.2 Overall summary

Chapter 4, showed how body size variation in *Plodia interpunctella* affected female reproductive strategies. Smaller females employed 'current' reproduction strategies, while larger females invested more resource into 'future' reproduction. More specifically, there was clear evidence of reproduction-longevity trade-offs, in which larger female *P. interpunctella* had greater higher potential lifetime fecundity through a prolonged life-span, while smaller females maintained greater initial egg loads and a shorter life-span. Assessment of nutrient resource allocation in reproductive and somatic tissues also revealed that current reproduction required greater nutrient allocation in somatic and reproductive tissue, in line with the Cost of Reproduction Hypothesis (William, 1966). Early reproduction may require higher energy reserves for egg maturation and flight during both mating and oviposition.

Chapters 5 and **6** took the study into real-world environments, and respectively used correlative and experimental methods to evaluate how stream environments affected body size and nutrient allocation pattern in larval Trichoptera. In **Chapter 5**, resource allocation by two caddis species along an altitudinal gradient revealed how response to physicochemical constraints were species-specific. Newly-emerged *Hydropsyche siltalai* from cooler, nutrient-poor locations had higher levels of carbohydrates as well as total and neutral lipids within reproductive and somatic tissues, respectively, in comparison to warmer, more nutrient rich, downstream locations (**Chapter 5**). There was no such effect in *Rhyacophila dorsalis*. This greater allocation of nutrients to both tissue types in upstream *H. siltalai* suggest an early reproductive strategy. In contrast, *H. siltalai* in the warmer and eutrophic environments downstream shifted resource investment from reproduction into somatic maintenance, as predicted by the Disposable Soma Hypothesis (Kirkwood, 1977). This paradigm argues that a reduction in resource allocation to reproduction will lead to a shift to somatic maintenance associated with increased survival. Water physicochemistry could affect the distribution of macronutrients in caddisfly reproductive and somatic tissues in various ways. The increased lipid composition of somatic tissue in *H. siltalai* at higher temperature, for example, was similar to patterns seen in parasitoid and collembola species exposed to sub-lethal temperature events (Denis *et al.*, 2012; Van Dooremalen *et al.*, 2013). Interestingly, site-to-site variations showed that both *H. siltalai* and *R. dorsalis* responded in a similar way to nitrate levels: eutrophic environments could offer more optimal feeding conditions, and therefore nutrient acquisition. Increased lipid consumption at higher nitrate levels may, however, at least in *R. dorsalis,* promote species tolerance to physicochemical constraints in downstream sites. The results from **Chapter 5** contribute to growing evidence that trade-offs between reproduction and survival occur commonly under field conditions, thereby bringing realism to previous laboratory investigations (Roff, 1992; Stearns, 1992). The contrasts among the two species studies also extend previous knowledge by showing that there may be different adaptive responses to environmental conditions.

The translocation study using stream mesocosms explored the consequences of variable thermal exposure in fifth instar *H. siltalai* to subsequent adult fitness (**Chapter 6)**. While patterns observed in **Chapter 5** were inevitably were confounded by simultaneous chemical and thermal variations among sites, the translocation experiment between upstream and downstream of a cave system allowed focus on thermal effects alone. Exposure to warmer and colder waters during the fifth instar *H. siltalai* reduced adult reproductive and somatic mass, while increased allocation of nutrient resources into somatic tissue was also consistent with negative impacts on reproduction. Male response to warmer conditions are in line with the Temperature-size Rule (Atkinson, 1994), with specific evidence showing that higher temperature during an insect's penultimate instar stage reduces adult body size. Greater nutrient allocation into female somatic tissue at colder temperature may represent a potential negative impact in reproduction. Greater lipid investment into reproduction, however, is consistent with advanced reproductive timing when exposed to stress. The results, however, were confined to small sample size with short duration of translocation exposure. There was, throughout this experiment, evidence of a clear phenotypic response under climate change scenarios with insects becoming smaller and shifting more resource into somatic maintenance.

7.1.3 Synthesis

Together, the results from this thesis suggest nutrient allocation to different life history features in semelparous insects can provide useful metrics through which to explore plasticity in reproductive strategies and the consequences of global change. While supporting the Cost of Reproduction Theory, the findings from this study expand understanding by suggesting that early reproduction requires greater nutrient allocation to both somatic and reproductive tissue (**Chapter 4**). The study also has added realism as it incorporates from field environment data to what, previously, have been laboratory-dominated studies. **Chapter 5** illustrates how greater nutrient allocation, such as lipid in reproductive and somatic tissue, can support early reproductive efforts in insect populations under their optimal conditions – in this case *H. siltalai* within its ideal temperature range. In contrast, under warming aquatic environments, *H. siltalai* exhibited similar allocation in both tissue components (**Chapter 6**). These findings confirm that smaller insects utilising early reproductive strategies require greater lipid allocation into somatic and reproductive tissue. Early reproduction in smaller insects, however, is costly; leading to shorter adult life-span in semelparous species as they depend on resource derived from larval feeding for resource allocation. Findings herein also support the results of previous physiological studies suggesting lipid reserves as the key nutrient component for early reproduction (Zhao & Zera, 2002; Zera, 2005).

Nutrient allocation trade-off may vary for different insect species. In this thesis, emphasis has been given to highlighting how reproduction-longevity trade-off affect nutrient allocation patterns in somatic and reproductive tissue. Lipid allocation, for example, does not illustrate trade-offs between somatic and reproductive components. Such results are expected to arise from the fact that lipid are important nutrients for both these major body components. Total and neutral lipid are utilized by insects to fuel active flight (mating, oviposition) in somatic tissue and to provide eggs in reproductive tissue (**Chapters 4 & 5**). Studies on a wing-dimorphic insect, however, represent a clear dispersal-fecundity trade-off between flight-capable and flightless morph (Zera & Zhao, 2006; Socha & Sula, 2008). Flight-capable morph of the cricket, *Gryllus firmus* utilized neutral lipid in somatic components to fuel flight activity while the flightless morph used protein for egg production (Zera, 2005) – an example where specific nutrient reserves were used to support different somatic and reproductive functions. The contrast from this study is in the fact that nutrient allocation trade-offs arising from phenotypic plasticity in wing-dimorphic insects may be absent in phenotypic plasticity induced by environmental conditions. In addition, different trade-off components between reproduction-longevity and dispersal-fecundity may also differ in the way insect utilize energy reserves. In the study, insect adaptive response to environmental change may not illustrate nutrient allocation trade-offs, but still represent changes in physiological traits (**Chapter 6**). Even though nutrient allocation trade-offs were absent, nutrient allocation between somatic and reproductive tissue appears to provide a reliable indicator for fitness components under changing environment (**Chapter 5**).

Some of the best example that explained cost of reproduction in other taxa are shown in aspic vipers, *Vipera aspis* (Bonnet *et al.*, 2002), lizards, *Carlia rubrigularis* (Goodman, 2006) and mountain goats, *Oreamnos americanus* (Hamel *et al.*, 2009). Similarly, the cost of reproduction involves measuring reproductive output (i.e., clutch sizes), reproductive output upon maturation and physiological traits such as body size. These traits, however, are difficult to measure in mobile and long-lived animals as lifetime reproductive success or lifetime potential fecundity can be mediated by several factors such as survival and energy cost among habitat. In the present study, current and future reproduction is determined by the timing and number of mature eggs carried by female insects upon maturation. In addition, nutrient content between reproductive and somatic traits are used as indicator for resource investment between different life history traits. For longer-lived animals, however, differences in body conditions such as metabolism and locomotor speeds are used to measure lifetime reproductive success to estimate survival and breeding probabilities (Sinervo *et al.*, 1991). Studies also demonstrate that for longer-lived animals, cost of reproduction may only be evident at low resource availability as some females may acquire energy for reproduction due to their genetic or physiological quality (Hamel *et al.*, 2009).

The resilience and persistence of aquatic insects in response to rapid changes in environmental conditions – particularly those caused by anthropogenic change including climate - is determined in part by their ability to maintain nutrient balance for reproduction and survival (Mas-Marti *et al.*, 2014). The present study shows that *H. siltalai* emerging at smaller body size can allocate more nutrient in somatic components supporting their successful adaptation under warming environment (**Chapter 6**). This has relevance for the observation that size-spectra among freshwater communities may become smaller under warming climates (Woodward *et al.*, 2016). Smaller body size has the advantage for advance maturation and lower energetic demands (Kingsolver & Huey, 2008). In addition, a study by Radchuk *et al.* (2013) indicates a reduction in egg, larval and pupa development time with warming; potentially contributing to increased insect voltinism (generations per year). Assessments of warming effects, however, should consider resource acquisition in response to the shortened larval life-stage duration. Insects may depend on important nutrients that are derived from the larval stage to manufacture eggs as seen in hawkmoth, *Amphion floridensis* (see O'Brien *et al.,* 2002). Smaller insect body size under warming environment also represent lower potential lifetime fecundity with reduced life-span (Honek, 1993). Moreover, aquatic insects are associated with limited dispersal capability under changing environment; potentially affecting both mating opportunity and ovipositional behaviour negatively (Lancaster *et al.,* 2010). Overall, insects may show different nutrient allocation pattern as a consequence of their sensitivity to environmental changes (**Chapters 5 and 6**).

Ecological studies on caddisfly species such as *H. siltalai* have important ecological applications. As an 'ecosystem engineer', *H. siltalai* can play a major role in invertebrate communities through the generation consolidation of the substrate and the formation of refugia during high-discharge events (Cardinale *et al.,* 2004; Nakano *et al.,* 2005). *H. siltalai,* as an important suspension feeder in streams also stabilize flow currents, further promoting sediment stability (Cardinale *et al.,* 2002). Thus, ecophysiological studies regarding their adaptation to changing environment provide an important early conservative effort to assess their sensitivity within aquatic ecosystem.

7.1.4 Study limitations

As with all investigations, there were limitations in the design of this study. They include confounds in field investigations, the risk of artefacts in experiments, and the difficulties encountered in determining exact causes of nutrient allocation patterns. The present study, and specifically the work reported in Chapters 5 and 6, is confined to a small sample size of caddisfly species due to high mortality during transition from pupae into adult. Short time duration for translocation studies due to the loss of the original mesocosm (removed by river warden) also contributed to heavily skewed sex ratio that influenced results.

Explanations of nutrient anomalies during insect life history has been attributed to stochasticity in nutritional diet (Attisano *et al.,* 2012), insufficient carry-over of some egg production nutrients missing from adult diets (Fanson & Taylor, 2012) and the occurrence of adult diapause (Hahn & Delinger, 2011). In addition, while the insect nutrient assay protocol employed throughout this current study provided precise measurement of the relationship between nutrient contents of reproductive and somatic tissue, there were limitations. While the assay would have included the musculature component of the soma, the samples used would have been unlikely to contain either: (i) chitin degraded into shorter chain molecules, or (ii) protein and lipid released from the cuticle. Thus, resource allocation to the true exoskeleton component of soma may not have been fully assessed.

It is also important to note that the relationship between early reproduction and life-span involves two functional (physiological resource) trade-offs, corresponding to the 'Y' model (Roff & Fairbairn, 2007). These trade-offs occur, at one level, between reproductive and somatic components and, at a second level, between initial eggs and initial reserves, or fat bodies (Stearns, 1992; Jervis *et al.,* 2005). This latter aspect was not considered in this study. As these reserves are used both to manufacture future eggs (fuelling development of the 'currently' immature oocytes) and to support somatic functions (maintenance, repair, locomotion), they are linked - both indirectly and directly - to insect survival. Future work should attempt to incorporate trade-offs at both levels and provides a more comprehensive test for a trade-off between reproduction and survival. Despite these limitations, the data can act as a valuable basis for future investigations (Jervis *et al.,* 2005, 2007).

7.1.5 Implications

Much ecological research has addressed the importance of using different approach to assess individual and populations persistence across different environment. The methods used in this study contribute to insect evolutionary life history research through the inclusion of what is thought to be among the first comparative test of a 'Y' model concerned with allocation of major nutrient resources between reproductive and somatic tissue in insects (King *et al.,* 2011; Fanson *et al.,* 2012). Previous work on trade-offs have approximated the real allocations of carbon, nitrogen or dry mass of tissue to somatic and reproduction by comparing the allocation to head / wings / thorax (with legs) to that allocated to the abdomen (Boggs, 1981; Karlsson & Wickman, 1989; Stevens *et al.,* 1999, 2000; Min *et al.,* 2006). In this study, the allocation patterns of different nutrients components in somatic and reproductive tissue provide a realistic approach to predict insect physiological conditions. Moreover, insect species-specific response to environmental conditions can be represented when measuring fitness from reproductive and somatic components.

Field studies on insect ecophysiology provide the opportunities to assess the interactions between insects and their environment. So far, studies have highlighted insect adaptations in terrestrial landscape focusing on Lepidoptera and Hymenoptera (Casas *et al.,* 2003; Hodkinson, 2005; Karl *et al.,* 2008; Berger *et al.,* 2012; Abram *et al.*, 2016). Recent studies focusing on aquatic insect adaptation, are, however are growing (Larsen & Ormerod, 2014; Stoks, *et al.,* 2014; Woodward *et al.,* 2016) and the findings from this study might be an important contribution. In this study, caddisfly show successful adaptation to warming climate in an aquatic environment. This, however resulted in smaller body size and a potential decrease in fitness components for future reproduction. Early reproduction and shorter developmental time under warming environment may potentially increase viltinisms. This can lead to higher numbers of generations per year and increases population abundance within environment. Higher species abundance for insects such as caddisfly can be beneficial in an aquatic ecosystems as they are part of an important food resource for salmonid fishes and riverine birds. In addition, *H. siltalai* is an important suspension feeder in stream that promotes stability in benthic habitat for other macroinvertebrates. It is, however, it is also important to highlight that shorter development represent lower resource carried over from larva into adult stage. This may lead to population declines or species loss when exposed to severe environmental stress. Previous studies have shown that the establishment of population and community structure within aquatic environments provides the mechanism to understand how inter- and intra-specific relationships influence species survival and reproduction (Barnes *et al.*, 2013). Warmer climate may represent potential extinction for larger freshwater insect species. Maintaining higher metabolic rate in larger individuals are costly due to their higher *per capita* metabolic rate that may lead to decline in populations (Brown *et al.*, 2004). As smaller individuals benefits from warmer climate, this could also mean that trophic interactions within food web may shift towards a smaller aquatic species community (Woodward *et al.,* 2016).

Understanding insect ecology requires a mechanistic and quantitative approach that characterize their evolutionary response across different environments. The findings from the study show that non-adaptive plasticity may differ between insect species and indicate a species-specific response towards different physicochemical constraints. In this study, estimating individual fitness by looking at nutrient allocation between somatic and reproductive components enables an early assessment of species adaptation. Insect species may represent different tolerance to environmental variables.

Thus, the approach used provides important tools to predict individual physiological conditions and contribute towards an effective conservation effort.

7.1.6 Avenues for further research

Results for the combined nutrients were not consistently reflected in the individual nutrient results. This indicates that tissue (e.g. somatic *versus* reproductive) measures used to generate empirical support for the 'Y' model (Karlsson & Wickman, 1989; Stevens *et al.*, 1999, 2000), provide at best a crude approximation to the real pattern of resource investment in survival and reproduction. It is clear that a multi-nutrient approach should be employed in future studies, and that this should be assessed at as fine an anatomical scale as practicable. With improved micro-dissection of insects possible, ideally allocation to gut and nerve tissues, abdominal/gastral musculature, oviduct tissue and accessory glands could be quantified. In general, this would be easier to achieve with holometabolous insects (Lepidoptera), due to their larger body size and individual tissues. In addition, allocation to exoskeleton should also be properly quantified; this would require the use of additional assay method (Andersen *et al.*, 1995). There is also an obvious need to explain some of the nutrient anomalies; one way forward would be to combine the methods utilised within this study with other specialized methodologies such as stable isotope (O'Brien *et al.*, 2000, 2002). Adding the analysis of insect gut contents would also provide an opportunity to relate how different feeding strategies affects resource consumption and subsequent changes in nutrient allocation.

Empirical evidence also suggests that findings regarding trade-offs in field and laboratory studies may differ quite substantially. In this present study, nutrient allocation represent different pattern in somatic and reproductive tissue between laboratory and field studies. In laboratory studies, artificial selection for insects using nutritional diet used may influence the imaginal disk growth during larval stage (Chown & Gaston, 2010), and trade-offs pattern may reflect diet instead of adaptation. Thus, future studies should consider experimental trade-off under field experiments as it represents insect adaptations within their environment. In addition, laboratory studies need to refine insect nutrient assay methodology that reflect food resource in wild populations.

By using Indian mealmoth and caddisfly as the study species, represent protein and lipid have been used as the major nutrient reserves in reproductive and somatic tissue. These major nutrient reserves should, however, be given to the possibility that, during early adult insect life, a degree of resource re-allocation might occur. For example, Zera and Zhao (2006) showed that the flight-capable morph of polymorphic cricket, *Gryllus firmus*, was able to utilize lipids by converting more proteins into flight fuel, while the flightless morph was able to utilize proteins for use in egg manufacture. For some insects, such as parasitoid wasp species that are capable of synthesizing lipids, it is conceivable some of their carried-over protein could be converted, via intermediary metabolism, to lipid early in their life-span. Such scenarios are predicted to be one consequence of egg resorption and used by the parasitoid as a strategy for fuelling somatic maintenance (Rosenheim *et al.*, 2000; Hegazi *et al.*, 2013). Thus, future studies may consider looking at major nutrient reserves such as lipid and protein as the information is adequate to estimate insect fitness components.

Pysiological studies have indicated that diverting resource away from somatic functions and the 'cost of reproduction' cannot be considered as a physiological trade-off. Instead, a molecular signalling mechanism regulates a set of life history traits including fecundity and longevity (Leroi, 2001; Barnes & Partridge, 2003). In this case, it is not egg manufacture *per se* that is costly to survival-related traits, but the insulin signalling process that contributes to the enabling of ovigenesis (Barnes *et al.*, 2006). Reciprocal changes in allocation among physiological resources are, therefore, not a necessary outcome. While this might explain why the results on nutrient allocation do not conform to the hierarchical 'Y' model, it would require the molecular signalling mechanism to be explored for any definitive conclusion (Shingleton, 2007). The molecular signalling mechanism has so far not been widely demonstrated, and the limited information available suggest a different result (*Drosophila* flies; Leroi, 2001). Thus, phylogenetic confounding effects need to be controlled for future research.

7.1.7 Conclusion

This study has provided evidence for the occurrence of allocation trade-offs in a functionally diverse array of nutrients linked to reproduction and survival in both terrestrial, *P. interpunctella,* and aquatic, *H. siltalai* and *R. dorsalis,* insect species. In the context of current and future reproduction in *P. interpunctella*, the direction of trade-offs is consistent with the predictions of the 'Y' model. Trade-offs between different nutrient components have, however, been difficult to distinguish as the cost of reproduction requires major resource investment into survival and reproductive tissue. For the caddisfly species, nutrient allocation may differ with species-specific responses to a variety of physicochemical conditions. Changes in nutrient allocation patterns may represent species sensitivity to environmental conditions and provide important information to assess species adaptation. Although associated with caveats, the utilisation of the 'Y' model to provide an explanation for nutrient allocation patterns should not be dismissed at this stage. The approach used in this study thus provides an important ecological tool with two potentially major spin-offs. First, the approach applied throughout this thesis allows observations on how insects maximise their fitness under different environmental conditions. Second, the approach provides a potential means of measuring and understanding evolution under changing global conditions. With global change now becoming increasingly rapid, the development of such methods can provide important insights for conservation and environmental management.

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