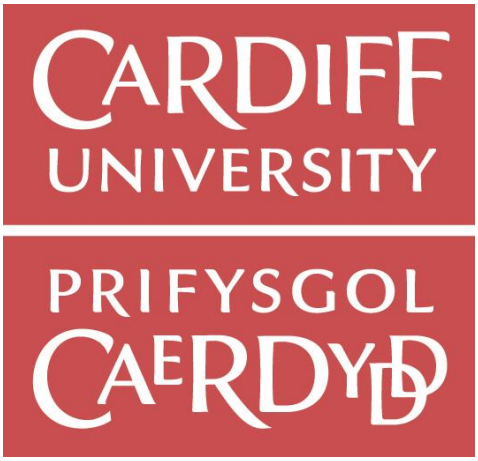
Artificial Light at Night and the Predator-Prey Dynamics of Juvenile Atlantic Salmon (*Salmo salar L.)* in Freshwater.

Rhian C. Newman



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Organisms and Environment, School of Biosciences, Cardiff University

This thesis is submitted to Cardiff University in candidature for the degree of Doctor of Philosophy

This thesis is dedicated to the memory of my late Mother, Diana M. Wilson (*1952 - 2014*).

Always my biggest supporter and my idol, the reason for all that I am and everything I have achieved.

….. I did it Mum!

Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed ..... (candidate) Date .....

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of .....(insert MCh, MD, MPhil, PhD etc, as appropriate)

Signed ..... (candidate) Date .....

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own.

Signed ..... (candidate) Date .....

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loan, and for the title and summary to be made available to outside organisations.

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Acknowledgements

I remember first seeing the advert for this PhD. I was on a decrepit computer in a hostel in Buenos Aires, killing time whilst waiting for a taxi to take me to the airport and fly home. Coming to the end of my 'gap year', I was feeling apprehensive about what was coming next and what I was going to do with myself. I couldn't wait to get home and e-mail the prospective supervisor so sent an e-mail there an then, on a keyboard with several broken letters, a stubborn space bar and an apology for the numerous typos. Dr Siân Griffiths quickly replied and the rest is history. I've always felt this PhD was perfect for my research interests and really, just meant to be (despite my dislike of working nights, Bill!).

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Summary

Artificial Light at Night (ALAN) is among the fastest growing anthropogenic influences on the natural environment. ALAN has been suggested to affect the behaviour and physiology of nearly all vertebrates and invertebrates by reducing the distinction between day and night, and by altering the cues that activate nocturnal behaviours. Information is particularly scarce for freshwater ecosystems, many of which are close to sources of ALAN. This thesis examines the behavioural and physiological impact of broad spectrum ALAN on Atlantic salmon (*Salmo salar L.*) and their invertebrate prey. After reviewing available literature of the effects of ALAN on freshwaters (Chapter 1), a series of empirical field and laboratory experiments examined the impact of ALAN on i) invertebrate drift in an experimentally artificially lit stream (Chapter 2) to determine the influence of ALAN on the primary food source of Atlantic salmon; ii) the dispersal behaviour (Chapter 3) and cortisol stress response in dispersing Atlantic salmon fry (Chapter 4); and iii) the diel pattern of foraging and refuging in Atlantic salmon parr (Chapter 5). ALAN impacted the drifting behaviour of invertebrates from contrasting taxa with a divergent effect of ALAN between taxa and functional feeding groups (FFGs), with some increasing and others decreasing under part-lighting. In dispersing Atlantic salmon fry, ALAN disrupted the timing and periodicity of nocturnal dispersal behaviour, at all experimental light intensities (1 – 8 lux). However, this behavioural change was not the result of a cortisol stress response. Finally, ALAN affected activity levels of Atlantic salmon parr through disrupting the amount and timing of refuging behaviour, with fish housed under high intensity ALAN found to refuge 28 % more than those in the control treatment. These results highlight the complex nature of the response of both Atlantic salmon and their invertebrate prey to ALAN, whereby the influence of ALAN can be difficult to generalise between taxa and species' life stages. Moreover, this thesis provides evidence to inform proposed mitigation strategies and advocates an increase in natural unlit areas.

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Publications

Papers arising from this thesis

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Chapter 1: Review of the current literature.

1.1 Overview

Anthropogenic impacts and modifications of the natural environment are likely the most pervasive and destructive threats to species and ecosystems, and along with an ever-increasing population, human activities are increasingly disrupting and outcompeting natural processes within the environment (Crutzen 2006; Lewis and Maslin 2015). This has led to the suggestion that the current geological time period, or epoch, should be re-termed the Anthropocene (Crutzen 2006; Steffen *et al.* 2007; Lewis and Maslin 2015). This term accounts for the prolific changes to the environment driven by human activity, which threatens ecosystem health and species persistence (Steffen 2007). Today, anthropogenic impacts on the environment have increased in number and diversified greatly to include a number of chemical, biological and physical factors (Fair and Becker 2000).

When considering the effects of the Anthropocene, which if nothing else has great heuristic value in drawing attention to the impact of humanity on the natural world that surrounds it, one such physical anthropogenic impact that must be recognized is light. Light is inextricably associated with human activity and urbanisation of the natural environment and as such, artificial light at night (ALAN) is now one of the fastest growing anthropogenic pressures on the environment (Falchi *et al.* 2011). Humans have long sought to artificially light the nocturnal environment (Longcore and Rich 2004), and the historically recent, rapid development of technology has meant that this form of environmental modification is now commonplace globally (Hölker *et al.* 2010b; Cinzano *et al*. 2001). Since the Industrial Revolution, and especially over the last 60 years, the number of outdoor lights has increased rapidly (Hölker *et al.* 2010b). The number of lights that line motorways and illuminate residential areas currently stands at over 7.5 million in the UK (HTMA 2011), a number thought to increase 3% annually (RCEP 2009) and on a global scale such lighting is increasing by 6% annually (Hölker *et al.* 2010b). Further, nocturnal illumination stems from advertising signs, motor vehicles and homes (Gaston *et al.* 2012). The extent of ALAN today is reflected by the fact that only 11% of those living

in England are able to see a true night sky (Riley *et al.* 2012), and 99% of those living in the USA and Europe live under light polluted night skies (Cinzano *et al.* 2001). This total modification of the natural, nocturnal environment ties in to the description of the anthropocene, whereby this anthropogenic modification of the nocturnal environment is outcompeting the natural process of light and dark (Crutzen 2006). This is perhaps the biggest modification of the natural environment, for millennia day and night were guaranteed; yet now in many urbanised areas, and even rural areas, true nighttime is not seen (Hotz 2008). Further, it is suggested that in approximately 40% of America it does not become dark enough for a human's retinas to switch to nocturnal vision (Cinzano *et al.* 2001). Thus, the lighting of our nocturnal environment is so ubiquitous that illuminated patches of the earth's surface can be seen from space (Longcore and Rich 2004) and to these ends, it is now impossible to imagine the developed world without artificial light.

Figure 1.1 Artificial light at night (ALAN) as seen from space. Image credit: (NASA Earth Observatory image by Robert Simmon, using Suomi NPP VIIRS data provided courtesy of Chris Elvidge (NOAA National Geophysical Data Center). Suomi NPP is the result of a partnership between NASA, NOAA, and the Department of Defense. Caption by Mike Carlowicz).

Despite this widespread proliferation of artificial light at night (ALAN) the recognition that it can be an ecological problem has arisen only recently (Longcore and Rich 2004; Perkin *et al.* 2011). The

current lack of scientific evidence concerning the ecological effects of ALAN means that its' impacts are so far largely unknown. Light determines the daily, seasonal and major events in the lives of many animals, both nocturnal and diurnal (Boeuf and le Bail 1999; Longcore 2004). Thus, it seems intuitive that altering the natural pattern of light and dark will not only have consequences for individuals, both behaviourally and physiologically, but also the way in which they interact with their external environment. Organisms will need to adapt their behaviour to the changing environment or risk extirpation (Sih *et al.* 2011). Research to date has demonstrated shifts in behaviour in a broad range of species, both vertebrate and invertebrate, terrertrial and aquatic (see Section 1.3.1 and Table 1.2). These behavioural changes will result in changes to both inter- and intra-species interactions and could ultimately result in ecosystem level changes (Kurvers and Hölker 2014). As such, understanding the role of ALAN in influencing the behaviour and physiology of species and as such, possibly, the survival of populations is of the upmost importance (Rich and Longcore 2006; Gaston *et al.* 2014a; Gaston and Bennie 2014).

1.2 What is ALAN?

1.2.1 Streetlights

ALAN is primarily, but not entirely, as a result of streetlights in public areas, along roads and highways (Gaston and Bennie 2014). The energy associated with light is spread across a visual spectrum that is produced by plotting the wavelength against intensity of light (RCEP 2009). Intensity is the brightness of the light and is usually expressed in lux, a unit that quantifies the brightness of light within the visual range of the human eye (Longcore and Rich 2004). Lux is perennially useful in studies for ease of comparison it provides; however, this measurement does present two central problems. Firstly, using lux carries the assumption that the visual capacity of any given species is the same as that of humans (Chaston 1969), when a more accurate measure of light for ecologists is photons/m^2/sec (Longcore and Rich 2004). Secondly, lux is intended to represent the intensity of white light and it is for this reason it is suggested that W. m^{-2}, a measure of irradiance, is a more comparable

measure of lights of differing wavelengths (Boeuf and le Bail 1999). The visual range of humans spans from violet at 380nm to red at 750nm in daylight (RCEP 2009). Many species, however, may be able to detect and respond to changes in light levels that are too subtle for human visual systems to detect, rendering lux somewhat less valuable in analysis concerning other species (Elvidge *et al.* 2001).

New technologies have resulted in a number of different types of streetlights available that vary according to the wavelength and intensity of emitted light (Boeuf and le Bail 1999; Elvidge *et al.* 2010; Gaston *et al.* 2012). The majority of the 7.5 million streetlights found across the UK are low-pressure sodium lamps (LPS) producing a single, very narrow peak emission of light at 590nm, in the yellow region of the spectrum (RCEP 2009; Bruce-White and Shardlow 2011; Gaston *et al.* 2014a,b). In addition LPS streetlights are often extremely large in size, which not only makes their installation problematic in many lamps, but this also means that they are difficult to shield, from emitting light away from the ground, thus resulting in increased sky glow (Bruce-White and Shardlow 2011). Additionally, the light produced from LPS lamps is considered to be unfavourable for human colour vision, due to the narrow waveband monochromatic light produced allowing only very poor colour discrimination (RCEP 2009; Bruce-White and Shardlow 2011; Table 1.1). Colour discrimination is measured via the Colour Rendering Index (CRI), which examines the colour perception of objects under the light in question compared with a test light (US D.O.E 2008), whereby higher values signify better colour discrimination. As such, CRI is significantly better under the new, white light lamps (e.g. LEDs) than under the LPS lamps of the past (Table 1). Increasingly, these streetlights are being replaced with a variety of new technologies (Gaston *et al.* 2012; Table 1.1).

The newer technology streetlights that are increasingly used across the UK are: high-pressure sodium (HPS), fluorescent, light-emitting diodes (LED) and metal halide (Table 1.1). Increasingly, HPS are now used in place of LPS, largely as they are more favourable for colour discrimination due to having a greater number of peaks across the colour spectrum (RCEP 2009). Further, HPS lamps are also more compact than LPS so they can be more effectively shielded to reduce escape of unwanted light (Bruce-

White and Shardlow 2011). Finally, they have a greater lifespan than LPS lamps and are therefore more cost effective for local councils (Bruce-White and Shardlow 2011).

Table 1.1 The technical properties and use of streetlights currently used or piloted across the UK (Adapted from RCEP (2010) with Elvidge and Keith (2009); Elvidge *et al.* (2010); Gaston *et al.* (2013)).

Type of Light	**Colour of Light**	**Colour Rendering Index (CRI)**	**Efficiency (Lumens per Watt)**	**Emission peak/range**	**Current Usage on UK Roads (% of lamps)** [1]
Low-pressure Sodium	Yellow/orange	0	80-200	Single peak at 590nm	44
High-pressure Sodium	Pinkish/amber -white	7-32 (up to 85 for white light lamps)	90-130 (30-45 for white light lamps)	Main peak at 819nm, secondary peaks 569-616nm.	41
Metal Halide	Bluish-white/white	64-100	60-120	Main Peaks at 819 & 617nm, secondary peaks at 474-578nm.	0
Compact Fluorescent	Warm white	5-82	67-87	Main peaks at 544 and 611nm, secondary peaks 436-574nm.	15
LED	Blue/ White	65-100	50	Primary emission 450-460nm.	NA

Fluorescent lamps are increasingly used due to their energy efficiency compared with LPS and HPS lamps (Bruce-White and Shardlow 2011) and metal halide lamps are also extremely energy efficient

[1] Data on percentage of road usage across the UK is from 2010 and is the most recent estimate of usage currently available.

and have a long lifespan (Bruce-White and Shardlow 2011). Finally, LED lights emit light with a peak wavelength in the blue part of the spectrum, at around 450nm (Bruce-White and Shardlow 2011).

These LED lights are currently undergoing trials across the UK, though they are currently, comparatively, very expensive (RCEP 2009). Despite the greater cost, they do offer numerous benefits; LED lamps have the potential to be powered by solar and wind energy (Mills 2005), while possessing a comparatively long life span and low energy consumption (Schubert and Kim 2005; Gaston *et al.* 2012).

1.2.2 ALAN as a global pollutant

Pollution can be defined as, "The introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenity, or interference with legitimate uses of the environment" (Holgate 1979). Under this definition, ALAN can be incorporated as 'energy liable to cause hazards'. In the UK chemical and noise pollution are well regulated under the Environmental Protection Act 1990, whilst light pollution has been incorporated in to the Clean Neighbourhoods and Environment Act (2005) as a statutory nuisance. Many authors have defined light pollution (e.g. Cinzano *et al.* 2000; RCEP 2009; Martin and Orlando 2009); however, a useful working definition is provided by Morgan-Taylor and Hughes (2005) "Every form of artificial light which shines outside the areas it serves to illuminate, including light which is directed above the horizontal into the night time sky, or which creates glare, or other nuisance".

Despite ALAN representing one of the fastest growing anthropogenic impacts on the environment (Hotz 2008; Hölker *et al.* 2010b; Falchi *et al.* 2011), it is only recently gaining recognition as a pollution issue in its own right (Morgan-Taylor and Hughes 2005; Perkin *et al.* 2011). The light pollution research that does exist can be split into two broad areas of interest and impact, namely ecological light pollution and astronomical light pollution. Astronomical light pollution is concerned with the way in which ALAN obscures the view of the night sky and prevents the observation of outer space (Longcore and Rich 2004). It is estimated that two thirds of the world's population no longer see

a true night sky due to the effects of light pollution (Hotz 2008). The research in this thesis deals with ecological light pollution, defined as the disruption caused by ALAN on natural light cycles within ecosystems (Longcore and Rich 2004).

The amount of light that is transmitted in the environment is determined by a number of factors. When considering light transmission in air, weather is suggested to be a decisive factor in the light quality in the atmosphere (Boeuf and le Bail 1999). For example, a study by Kyba *et al.* (2011) suggests that cloud cover can intensify the level of light pollution in the environment. This is as a result of the reflective properties of clouds, mainly composed of aerosols which reflect light back towards the ground, resulting in increased light pollution (Kyba *et al.* 2011). The way light is transmitted in air differs from the way light travels in water; the spectral properties of light is altered once it enters water, as water does not absorb all wavelengths of light, and light is scattered and filtered by particles (sediment) suspended in the water (McFarland 1986; Kusmic and Gualtieri, 1999). Water can be considered a monochromator (Tyler 1959; Lythgoe 1979; Kusmic and Gualtieri 1999) – it selectively transmits some, but not all, wavelengths of light, influencing both the colour and visual potential of light transmitted through water. Light at the red end of the spectrum does not penetrate far into water, due to the greater absorption of longer (red) wavelengths relative to the absorption of shorter (blue) wavelengths (Helfman *et al.* 2009). Turbidity reduces the amount of light that reaches the riverbed and increased turbidity also shifts the transmission of light towards the red end of the spectrum (Knowles and Dartnell 1977). In addition, the spectral distribution and intensity of light transmitted within a body of water is further influenced by depth, geographic location (Robinson *et al.* 2011) and season (Beatty 1969).

The degree to which a given light is transmitted in the environment is heavily influenced by the spectral properties of the types of lights themselves (Gaston *et al.* 2014b, Aube 2015) and thus far, little research has attempted to elucidate the effect of differing lighting types (Gaston *et al.* 2015). Falchi *et al.* (2011) examined the spectral properties of streetlights using human eye photoreceptors as a model. The lights were assigned a value of 'pollution' that is relative to the level of spectral response

seen in the photoreceptors of human eyes and it was found that the LPS lamps were the least 'polluting', whilst the LED lamps were the most 'polluting' due to their blue spectral emissions lying within the peak visual sensitivity of humans and many other organisms. Further, blue light contributes more to light pollution due to more scattering within the atmosphere (Falchi *et al.* 2011). In addition, the intensity of LED lights are such, that a move from LPS/HPS lamps to LED lamps could increase ALAN brightness by 2.5- 5 times the current levels (Falchi *et al.* 2011). Such results suggest that the move from LPS and HPS lamps to the new technology LED lamps will increase the environmental impact of these artificial lights (Falchi *et al.* 2011).

In the absence of ALAN, the nocturnal illumination intensity is solely dependent on the moon phase and cloud cover (Rich and Longcore 2006; Gaston *et al.* 2012). While nocturnal illuminance is highly variable, bright moonlight is typically considered to range from 0.1 to 0.3 lux (Austin *et al.* 1976; Brewin and Ormerod 1994; Rich and Longcore 2006; Martin 2010), with natural nocturnal intensities ranging from 0.0001 to 0.3 lux (Rich and Longcore 2006). Under ALAN the level of nocturnal illumination, and degree to which the natural environment is impacted, is dependent on a number of factors including the spectral properties and intensity of the light used (Gaston *et al.* 2012). Recorded measurements of artificially lit environments range from 0.04 to 60 lux (Perkin *et al.* 2014b; Gaston *et al.* 2012). In freshwater, Riley *et al.* (2013) measured ALAN at significant urban Atlantic salmon spawning sites in chalk streams across southern England. Measurements taken at river level were found to range from 22.7 lux on the River Frome at Dorchester and 20.0 lux on the River Itchen at Bishopstoke to 6.1 lux on the River Test at Romsey, Whilst in Berlin light measurements ranging from 0.04 to 1.4 lux were taken at a depth of 50 cm in rivers and streams (Perkin *et al.* 2014b).

1.2.3 Management strategies

To manage ALAN successfully, there needs to be a solid understanding of its ecological impacts in order to inform policy and allow for the development of appropriate management strategies. Currently, there is a paucity of information, and that which is currently available is often conflicting (Kronfield-Schor *et al.* 2013). Further, this lack of evidence is confounded by the increasing use of new lighting

types, the environmental impact of which has not yet been widely tested. The available literature suggests a number of strategies to reduce the environmental impact of ALAN (RCEP 2009; Gaston *et al.* 2014b). The first is by reducing the amount of light escape (RCEP 2009; Falchi *et al.* 2011; Day *et al.*, 2015). In order to achieve this, the way in which the streetlights are fitted should promote the maximum benefits of ALAN whilst ensuring the least amount of light pollution is produced (RCEP 2009). Streetlights should always point downwards and be shielded to prevent any horizontal or upwards escape of light, ensuring that they do not contribute to sky glow (Falchi *et al.* 2011).

In the UK, many street lamps are nearing the end of their recommended 30 year lifespan and are due to be replaced over the next few years (RCEP 2009)**.** This could provide an opportunity for lighting managers and ecologists to work together to minimize the impact of ALAN by replacing the old lamps with new technologies that are carefully selected to minimise the ecological impacts. It is important to take advantage of these advances in technology, which promise both economic and environmental benefits, though not at the expense of ecosystem health (RCEP 2009). The move from the monochromatic LPS lamps to newer, broad-spectrum lighting types (e.g. LED, metal halide) is likely to increase the potential for ALAN to impact species and ecosystems (Kyba *et al.* 2012; Gaston *et al.* 2014b). A recent study found that across much of Europe and globally, ALAN is getting brighter (Bennie *et al.* 2014a; Navara and Nelson 2007). This increasing potential for the negative impacts of ALAN will be further compounded by the aforementioned global growth of nocturnal illumination (Navara and Nelson 2007). To date, the impact of ALAN, and particularly the use of the new types of streetlights on organisms and ecosystem health, has not been largely considered outside of academia. There have been several publications outlining the need for a clearer understanding of the ecological impacts of ALAN (RCEP 2009; Hölker *et al.* 2010a; Perkin *et al.* 2011; Bennie *et al.* 2015) and investigations into the ecological effects of changes to lighting type (Gaston *et al.* 2014b).

Given the aforementioned 3% annual rise in ALAN in the UK, any attempt to stem this increase would be beneficial in order to maintain natural, unlit areas (Gaston *et al.* 2012). This increase in ALAN has recently prompted DEFRA to commit to protecting dark skies in the UK (DEFRA 2013).

Yet Hölker *et al.* (2010b) suggest that any attempt to reduce lighting levels in urban areas is a difficult task because the 'supposed' benefits of lighting are entrenched in our society (Jakle 2001). In addition, simply reducing the rate at which ALAN proliferates would not alleviate the problem in the vast areas across the UK where it is already polluting the nocturnal environment. That said, a number of small-scale light removal schemes have been successful in reducing the negative ecological impacts of ALAN (Black 2005; Tuxbury and Salmon 2005), and as such this technique could be employed in areas of high ecological importance (Bruce-White and Shardlow 2011). Recently the Highways Agency has turned off 62 miles of motorway lights (DEFRA 2013). Whilst this move to reduce ALAN is driven by a desire to reduce costs (Gaston 2013), through employing the techniques necessary to reduce energy consumption and money, it is hoped the ecosystem impacts can also be ameliorated.

When considering a management strategy, there must obviously be a balance between human safety and the environmental impacts (Falchi *et al.* 2011). In many areas, eliminating streetlights may not be achievable (Gaston *et al.* 2012). As such, a number of schemes have been piloted across the UK to reduce the amount of ALAN. Here local authorities across England and Wales have taken part in projects to determine public response to measures, outlined above, which aim to reduce ALAN (DEFRA 2011). One such measure that is now employed across many counties in the UK is 'trimming' or part-night lighting, which is simply a reduction in the duration of artificial lighting each night (Gaston *et al.* 2012; Bennie *et al.* 2014; Day *et al.* 2015). There are variations on the exact timings of illumination across local authorities but, typically lights will come on at dusk, turn off at midnight and come on for an hour or two before dawn (dependent on season). Trimming has also been applied by the Highways Agency to reduce the amount of ALAN along motorways; 59 miles of motorway lights are now turned off between 00.00 and 05.00 each night (DEFRA 2013). These timings are based on human peak activity and, if the trimming regime was designed to address ecological concerns it could be more beneficial to have lights off during dawn and dusk - often the period of peak activity and foraging for both nocturnal and crepuscular species (Moser *et al.* 2004). Whether reducing the duration of ALAN has any ecological benefits is, as yet, unknown, as currently no studies have tested its efficacy (Gaston *et al.* 2012; see Chapter 4).

Reducing the intensity of currently installed streetlights, also known as dimming, is the final proposed management strategy. In trials, dimming has been seen to reduce energy consumption by up to 40% (RCEP 2009) and decrease running costs of streetlights in counties across the UK. In Gloucestershire, for example, dimming saved the local council an estimated £172,000 per annum (DEFRA 2011). Studies have found that residents would not accept a complete turn off of road lights at a certain time; however, many found dimming an acceptable compromise (DEFRA 2011; RCEP 2009). It is suggested that dimming may also be preferable to alternative methods employed across parts of Europe such as motion-activated lighting due to the sudden flashes of light caused by the lights switching on and off being more disruptive to nocturnal organisms than continuous lighting (RCEP 2009; Inger *et al.* 2014). Light pollution has, however, been found to impact species physiology and behaviour across a wide range of light intensities, and crucially down to very low light intensities (see Chapter 3; Cos *et al.* 2006; Miller 2006; Riley *et al.* 2015). As such, the value in reducing the intensity of ALAN may be lost, however, any reduction in light intensity at source will reduce the area affected by that light source.

1.3 Ecological Impacts of ALAN

1.3.1 ALAN and ecology

Despite growing concerns (Rich and Longcore 2006; RCEP 2009; International Dark Skies Association 2010), little systematic data that demonstrates the ecological effects of ALAN is presently available (Hotz 2008; Perkin *et al.* 2011; Kronfield-Schor *et al.* 2013; Gaston *et al.* 2014a; Kyba *et al.* 2015), particularly in freshwater ecosystems. Considering the rise in global night lighting, it is important to determine the impacts of such lighting on the environment and how this can be effectively managed. Anthropogenic effects on the environment should often be considered as stressors to the species they impact upon. A stressor is defined as a factor that interferes with homeostasis; the maintenance of the body's internal milieu in an optimal state (Levy *et al.* 2004).

There has been a recent upsurge in interest in determining whether light may be having a detrimental impact on the health and functioning of organisms (Rich and Longcore 2006; Perkin *et al.* 2011; Gaston *et al.* 2014a,b; Table 1.2), and thus acting as a stressor.

Chemical and noise pollution are well defined as anthropogenic impacts; the affect that they have on the physiology and behaviour of a range of species has been extensively studied (Fowler *et al.* 1995; Sonne *et al.* 2006; Wysocki *et al.* 2006; Guillette and Edwards 2008; Barber *et al.* 2011). In contrast, while ALAN is increasingly thought to alter the behaviour and/or physiology of a broad range of species, both vertebrates and invertebrates (Rich and Longcore 2006; Wise 2007; Hölker *et al.* 2010a; Bruce-White and Shardlow 2011; Table 1.2), much current evidence is limited to a few charismatic species such as sea turtles and birds (Lorne and Salmon 2007; Kamrowski *et al.* 2012; Dominoni *et al.* 2013). Research has extensively focussed on the detrimental impact of the attraction of these species to light (Evans-Ogden 1996; Lorne and Salmon 2007; Poot *et al.* 2008), however the undetected, and likely much broader, effect of ALAN in repelling species and restricting behaviour is comparatively unstudied (Gaston *et al.* 2014b). In addition, much of our knowledge is garnered from observational studies and to date little experimental evidence has been forthcoming (Perkin *et al.* 2011; Gaston *et al.* 2014a,b).

Life on earth has evolved in response to, what are in natural circumstances, stable and predictable light regimes known as photoperiods (Longcore 2004; Bruning *et al.* 2011). The circadian clock coordinates an organism's response to a particular light regime (Bruning *et al.* 2011) and functions very strongly in the organism's metabolism, growth, endocrinology and behaviour (Dunlap 1999; Hölker *et al.* 2010a)**.** Despite the widespread understanding of the role of ALAN in influencing the daily and seasonal rhythms of organisms, relatively little research as attempted to discern the role of ALAN as a stressor or modifier of behaviours (Perkin *et al.* 2011). Since light is considered to be one of the largest influences on an organism's behaviour, it is to be expected that any changes to this, once predictable, regime under ALAN will result in large-scale behavioural changes (McConnell *et al.* 2010; Kyba *et al.* 2011).

Broadly speaking, species are either nocturnal or diurnal in their daily patterns of activity (Gaston *et al.* 2013; Bennie *et al.* 2014b) and will be specially adapted to suit their light determined niche. ALAN can be over a million times brighter than natural nocturnal illumination (Perry *et al.* 2008) and thus, creates a less obvious distinction between day and night. For this reason the cues that activate nocturnal behaviour may be lost (Bruning *et al.* 2011). Considering that 60% of invertebrate species and 30% of vertebrate species are nocturnal (Hölker *et al.* 2010a), the change to the nocturnal environment and nocturnal species behaviour has potentially far reaching consequences, and is suggested to be a major threat to species biodiversity (Hölker *et al.* 2010a; Table 1.2). ALAN is proposed to be a key contributor to declines in species that are light sensitive - nocturnal and crepuscular species (Hölker *et al.* 2010 a,b) - as through the elimination of dark skies, their temporal activity niche is being substantially modified. Nocturnal species may not be able to adapt behaviourally to the addition of light to their environment and if so, it is likely that we will face species and genotype extinctions if the effects of ALAN are not mitigated (Kyba *et al.* 2011). This issue becomes more pertinent when considering the impact that ALAN will have on species that are already a conservation concern (Mora *et al.* 2007)**.** It has been suggested, however, that evolutionary processes may allow certain species, likely those with short generation times, to overcome the issue through adaptation to the new environmental conditions (Hölker *et al.* 2010; Kyba *et al.* 2011; Sih *et al.* 2011), as has been observed with a number of other anthropogenic stressors (Hendry *et al.* 2008).

Table 1.2 Table summarising selected key publications, outlining the range of species that have been studied under ALAN and the experimental findings.

Taxon	Species	Authors and publication date	Summary of findings
Mammals	Bats *(Rhinolophus hipposideros; Eptesicus bottae; Pipistrellus kuhlii; Carollia sowelli).*	Stone *et al.* (2009); Polak *et al.* (2011); Lewanzik and Voigt (2014).	Reduction in activity; delay in onset of commuting behaviour; increased flying speed; decreased flying altitude; changes to foraging behaviour.
	Sugar glider (*Petaurus breviceps*).	Barber-Meyer (2007).	Changes to foraging behaviour and activity levels.
	Mouse lemur (*Microcebus murinus*).	Le Tallec *et al.* (2013).	Modification of daily rhythms of locomotor activity and core temperatures, along with changes to nocturnal activity and patterns of feeding behaviour.
Fish	Atlantic salmon (*Salmo salar*).	Riley *et al.* (2012; 2013; 2015)	Disruption in nocturnal behaviour during fry dispersal; delayed nocturnal smolt migration.
	European perch (*Perca fluviatilis*).	Brüning *et al.* (2015).	Inhibition of melatonin rhythms and potential for disruption of biological rhythms.
Amphibians	Frog (*Rana clamitans melanota*).	Baker *et al.* (2006).	Disruption to breeding behaviour of male frogs.
Reptiles	Sea turtles (*Caretta caretta; Chelonia mydas; Eretmochelys imbricate; Lepidochelys olivacea; Natator depressus; Dermochelys coriacea).*	Kamrowski *et al.* (2012); Zheleva *et al.* (2012); Rivas *et al.* (2015).	Attraction of hatchlings to light source, causing misorientation and resulting in increased mortality.
Birds	European blackbird (*Turdus merula*).	Dominoni *et al.* (2013); Nordt and Klenke (2013).	Irregular molt progression; undeveloped reproductive system; temporal shift in dawn song.
	American robin (*Turdus migratorius).*	Miller (2006).	Temporal shift in dawn song.
	Redshank (*Tringa totanus*)	Dwyer *et al.* (2013).	Impact the timing and distribution of foraging opportunities.
Invertebrates	Moths (various; *Mamestra brassicae*).	van Langevelde *et al.* (2011); van Geffin *et al.* (2014).	Attraction to light sources, particularly those emitting light with smaller wavelengths; Male only effects on pupal mass, pupation time and caterpillar mass.
	Freshwater Invertebrates (Various; *Gammarus roeseli*).	Meyer *et al.* (2013); Perkin *et al.* (2014a).	Decreased family richness of invertebrate drift and body size; decreased activity.
	Terrestrial Invertebrates.	Davies *et al.* (2012).	Change in the number of individuals from predator and scavenging functional feeding groups (FFG) caught in pitfall traps.
Microorganisms	Freshwater cyanobacteria (*Mycrocystis aeruginosa*).	Poulin *et al.* (2014).	Changes to photophysiology.

The behavioural response to a given light can take one of three forms: attraction (positive phototaxis); avoidance (negative phototaxis), or neutral behaviour (Rich and Longcore 2006). Neutral behaviour to light has been observed in a handful of species (Marchesan 2005)**.** This behavioural response of the individual organism can then be further characterized as neutral, beneficial or detrimental (Kyba *et al.* 2011). The way behaviour is modified by the addition of ALAN is thought to be species-specific (McConnell *et al.* 2010; Brunning *et al.* 2011) and will also vary between different life-stages of a given species (McConnell *et al.* 2010). For example, some species of bats are found actively to avoid ALAN during nocturnal activity (Stone *et al.* 2009, whilst others exploit the effect that streetlights have on attracting insects by foraging around the light (Jung and Kalko 2010). ALAN can impact a species physiology and behaviour by altering three important features of the natural environment: the level of ambient illumination, the photoperiod length and the spectral properties of light (Perry *et al.* 2008).

1.3.2 ALAN and species interactions

The impact of ALAN on the behaviour of individuals, populations and even species will have consequences for their predators, prey and competitors, and will likely alter community composition and ecosystem functioning (Smith *et al.* 2009). Species interactions have evolved in response to natural lighting regimes, thus any behavioural changes as a result of the addition of ALAN is like to affect these interactions (Kyba *et al.* 2011). Predator-prey interactions are also often determined by light, with many prey species having evolved to be active at night to avoid diurnal predators or using synchronised nocturnal movements to reduce their predation risk (Fraser *et al.* 1994; Riley *et al.* 2013). Temporal niche partitioning (diurnal/crepuscular/nocturnal) is the specialisation of species along a continuous light gradient and how ALAN affects this behavioural partitioning is currently unknown (Gaston *et al.* 2013). ALAN may increase the foraging duration of diurnal species and if these diurnal species extend their feeding into night, thus exploiting the ‘night-light niche’, then it is expected that they will prosper, (Rich and Longcore 2006; Gaston and Bennie 2014).

The impact of moonlight intensity on the predator-prey dynamics has long been studied (Clarke 1983; Kronfield-Schor *et al.* 2013; Gaston *et al.* 2013; Gaston and Bennie 2014), whereby changes in the lunar cycle influence the nocturnal activity levels of prey species (Daly *et al.* 1992; Kramer and Birney 2001). Moonlight intensity has been shown to impact the success of predators; for example, short-eared owls (*Asio flammeus*) were found to be more efficient at searching and capturing deermice (*Peromyscus maniculatus*) at higher moonlight intensities (Clarke 1983). The deermice showed an adaptive behaviour in response to the changes in light intensity and reduced their activity at higher light intensities. Thus, ALAN may similarly disrupt predator-prey dynamics and species interactions, both inter- and intra-specific. The time at which individuals forage is based on a trade-off between growth rate and mortality risk, and for those nocturnal species that are both predators and prey, the decision to forage is complicated further under ALAN (Kronfield-Schor *et al.* 2013). For a given species, if the increased feeding success under ALAN outweighs the risk of predation then nocturnal activity can be expected, and vice versa (Kronfield-Schor *et al.* 2013). Moreover, day length is known to influence the trade-off between refuging and foraging (Clarke 1983) and as ALAN, dependent on its intensity, effectively extends the day and shortens the night, it can be supposed it will strengthen these effects (Gaston *et al.* 2013).

Yurk and Trites (2000) found that the addition of ALAN on a bridge resulted in increased predation upon migrating Pacific salmon smolts (*Oncorhynchus spp.*) by harbour seals (*Phoca vitulina*). In the absence of ALAN, fewer seals fed around the bridge, suggesting that the seals were exploiting the 'night light niche' created by ALAN. Harbour seals have also been seen feeding in other artificially lit areas, including a stadium and a sawmill; ostensibly they are actively seeking lit areas to allow them to feed more efficiently (Yurk and Trites 2000). Yet when exploiting the additional light to forage, those species exploiting ALAN are then themselves at an increased risk of predation and as such the benefits must be balanced by this additional risk (Gotthard 2000). Tabor *et al.* (2004) examined the way in which the addition of ALAN impacted on the predation of juvenile sockeye salmon (*Oncorhynchus nerka*) by cottids (Family *Cottidae*), a natural predator. It was found that the cottids predated most effectively on juvenile salmon in the absence of light, presumably as a means of avoiding predation

themselves. Nocturnally active, visually foraging shorebirds were found to improve their prey intake rate under ALAN (Santos *et al.* 2010) and it is proposed visual foragers will benefit more than tactile foragers from increased foraging opportunities under ALAN (Gaston *et al.* 2013). It has been suggested, however, that the foraging success of some nocturnal predators may be impaired by the negative effect of ALAN on their vision (Buchanan 1993). Salmon have been observed to alter their predation technique when under different light intensities (Ali 1959)**.** Further, it is important to consider both the response of the predator and prey species when examining the impact of ALAN on predator-prey dynamics, as light can also play a crucial role in determining the effectiveness of predator strategies and success (Clarke 1983; Bramm *et al.* 2009; Santos *et al.* 2010).

ALAN has also been shown to alter the community composition of terrestrial invertebrates; under streetlights the numbers of predators and scavengers caught within pitfall traps increased (Davies *et al.* 2012). Beyond these species interactions, this result is suggested to lead to changes in ecosystem functioning (Davies *et al.* 2012). ALAN, then, has the potential to restructure whole ecosystems and the interactions within it (Hölker *et al.* 2010a).

1.4 Fish Research

1.4.1 Vision in fish

Light perception by teleosts is mediated via the pineal gland, a light sensitive organ located in the brain (Boeuf and le Bail 1999; Kusmic and Gualtieri, 1999; Porter *et al.* 2001), and photoreceptors in the eye (Boeuf and le Bail 1999). The pineal gland in fish is able to detect the ambient light levels even after the removal of the eyes (Kusmic and Gualtieri 1999) and facilitates the response to light. The pineal gland is referred to as an extra-retinal receptor (Kusmic and Gualtieri, 1999) and produces the hormone melatonin, which circulates throughout the organism (Migaud *et al.* 2007)**.** The production of melatonin is solely regulated by light (Falcon *et al.* 2003). Recent research has suggested that the pineal gland is also involved with navigation in teleost fish such as Tuna (Willis *et*

al. 2009). It has been shown through a number of studies that the cyclic fluctuation of this circulating hormone reflects the photoperiod the organism is experiencing, with melatonin levels seen to decrease with current light levels (Randall *et al.* 1995; Boeuf and le Bail 1999; Porter *et al.* 1999, 2001; Amano *et al.* 2006). As such, melatonin is used to demonstrate light perception in many species, including teleost fish (Porter *et al.* 2001; Falcon *et al.* 2010; Brüning *et al.* 2015). The role of the pineal gland and melatonin outlined above, however, extends beyond timekeeping to regulate the rhythm, both daily and annual, of a number of behaviours and physiological processes (Falcon *et al.* 2007). Research suggests that the pineal gland and melatonin are responsible for regulation of daily patterns of behaviour such as locomotor activity and shoaling, whilst annual, physiological, processes such as smoltificiation, growth and reproduction are controlled through perception of photoperiod through the pineal gland (Falcon *et al.* 2007). Any disruption to normal photoperiod will impact the melatonin production of the pineal gland and disrupting the function hypothalamo-pituitary axis and thus cause changes to the physiological processes outlined above (Taylor *et al.* 2006; Falcon *et al.* 2007).

The visual range of teleost fish is 250-700nm (Douglas and McGuigan 1989), with the specific visual range of salmonids being 346-690nm (Ali 1961). Since the visual sensitivity ranges of teleosts and humans are similar (see Section 1.2.1), the use of lux is also considered appropriate for studies concerning teleost fish**.** The morphology, and thus sensitivity, of the visual system in teleost fish appears to be determined by the ecology of a given species, rather than its' taxonomy (Kusmic and Gualtieri, 1999). Likely as an adaptation to their environments, marine fishes are most sensitive to wavelengths of light at around 450-550nm in the blue-green end of the spectrum, whilst freshwater fishes are more sensitive to light at the red end of the spectrum at wavelengths of around 650nm (Lythgoe 1979, 1980), though studies suggest these values are likely to vary between species and life stages (Robinson *et al.* 2011). Widder *et al.* (2005) found that red light was less disruptive to the feeding behaviour of the deep-water sablefish (*Anoplopoma fimbria*) than white light, likely as it lies outside it's visual range. It is only marine species that have been observed to exhibit positive phototaxis to light in the blue and green part of the spectrum (Loukashkin and Grant 1965). The visual sensitivity of snapper (*Pagrus auratus*), a diadromous species, was found to differ between life stages,

younger fish were more attracted to light from the red end of the spectrum whilst older fish were more responsive to the blue end of the spectrum (Robinson *et al.* 2011). Whilst the visual systems of fish are thought to be adapted to the light of the environment in which they are found, there is also thought to be a degree of plasticity, allowing for this change in sensitivity (Robinson *et al.* 2011).

In addition to environment differences, season and temperature are also thought to play a role in the visual sensitivity within a given species (Allen *et al.* 1973; Fraser and Metcalfe 1997). Juvenile salmonids in freshwater undergo seasonal changes in their behaviour and it is suggested that they undergo physiological adaptions that parallel these behavioural changes (Fraser and Metcalfe 1997). Vision is one such physiological change that occurs concomitantly with seasonal behavioural changes and in winter, when the fish are more nocturnally active, their visual sensitivity increases (Allen *et al.* 1973). This increase in visual sensitivity is mediated by a change in the amount of two visual pigments within the retina, with the number of porphyropsins and rhodopsins increasing (Allen *et al.* 1982). Such an increase in visual sensitivity is required to forage efficiently in the dark, as nocturnal foraging is an inefficient strategy for growth in salmonids (Fraser and Metcalfe 1997). Since salmon do not have any special adaptations for nocturnal foraging like most freshwater fish, they solely rely on vision for foraging (Fraser *et al.* 1993; Fraser and Metcalfe 1997).

1.4.2 Physiological impact of light

Much of the research investigating the effect of ALAN on fish has focused on aquaculture systems (Bruning *et al.* 2011; Kronfield-Schor *et al.* 2013), examining the influence of additional light on fish growth and reproductive capacity (Boeuf and le Bail 1999). Exposure to constant ALAN causes decreased maturation and increased growth in Atlantic salmon (*Salmo salar*) parr (Villareal *et al.* 1988; Stefansson *et al.* 1993; Oppedal 1997, 1999; Porter *et al.* 1999; Taranger *et al.* 1999; Migaud *et al.* 2007). In an aquaculture system, such growth enhancement is desirable as the fish are farmed for food and increased growth results in a maximum economic return (Bruning *et al.* 2011). In the wild, however, such alterations in the physiology of salmon are not desirable and may pose a threat to salmon conservation (Bruning *et al.* 2011). There is little available information regarding the effects

of ALAN on the physiology of wild fish, particularly freshwater species (Riley *et al.* 2012). Franke *et al.* (2013) found constant ALAN at 100 lx significantly reduced the expression of melatonin in European perch (*Perca fluviatilis*) during a laboratory experiment. In the freshwater stage of their lifecycle, salmonids are extremely sensitive to light and it both initiates and controls the development of smolts (Stefansson *et al.* 2007; Riley *et al.* 2012, 2015). In particular, it is the length of the photoperiod that initiates the parr-smolt transformation; fish raised in constant light fail to successfully make the transformations necessary to survive exposure to seawater (Stefansson *et al.* 2007).

The physiological effect of light on teleosts is mediated by the intensity and wavelength of the light in question (McFarland 1986; Porter *et al.* 2001; Leclerq *et al.* 2010). In Atlantic salmon, it was found that the greater the intensity of the light they were exposed to, the greater the level of maturation suppression, and thus increased growth (Oppedal *et al.* 1997; Porter *et al.* 1999; Taranger *et al.* 1999; Leclerq *et al.* 2010). The majority of the studies that are carried out into the role of artificial light and suppression of maturation look at the effects of light intensity but not of different types (spectral composition) of lights (Leclerq *et al.* 2010). The pineal gland in teleost fish is able to detect the properties of the light and it is thought that the range of wavelengths of light will impact fish in different ways. A recent study by Leclerq *et al.* (2010) examined the way in which different lighting types, each with different wavelengths, impact the suppression of sexual maturation in Atlantic salmon. The study demonstrated that lights from the both the green and red ends of the spectrum were the most effective at suppressing maturation, with both more effective than the white light that is currently used in aquaculture systems. Other studies have suggested that, in Atlantic salmon, blue light (450nm) is more effective at suppressing melatonin than red light (650nm) (Migaud *et al.* 2010; Vera *et al.* 2010). This was also shown to be the case in European sea bass (*Dicentrarchus labrax*) (Bayarri *et al.* 2002; Vera *et al.* 2010).

A number of studies, however, show little effect of light on the development of fish (Stefansson and Hansen 1989; Stefansson *et al.* 1990, 1993; Collett *et al.* 2008). As such, it may not be the characteristics of the light the fish are exposed to when under normal photoperiods that impacts

development, instead it may be the extra light and thus, the disruption of photoperiods that is the key. As a result of these changes, experimentally increasing the photoperiod the fish are exposed to leads to increased growth (Boeuf and le Bail 1999).

1.4.3 Behavioural impact of light

In fish, as well as in many other organisms, light has been described as being the 'Zeitgeber' (environmental cue) for determining the timing of major events in the organism's lifecycle (McCormick *et al.* 1998; Boeuf and le Bail 1999; Longcore 2004; Stefansson *et al.* 2007; Lecelerq *et al.* 2010). Whilst the physiological effects of artificial lights used in aquaculture systems are well known and often intended, it is thought ALAN will impact upon the physiology and behaviour of wild fish species (McConnell *et al.* 2010; Kronfield-Schor *et al.* 2013). Only a small number of studies, however, have examined aspects of fish behaviour and light, both natural and ALAN, in the field or lab studies (Moore and Scott 1988; Riley and Moore 2000; Riley *et al.* 2012, 2013, 2015). As with physiology, many of the recent studies carried out into the affect of artificial light on fish behaviour have been carried out in aquaculture systems (Huse and Holm 1993; Ferno *et al.* 1995; Oppedal *et al.* 2001, 2007; Juell *et al.* 2003; Migaud *et al.* 2007). In such systems, the impact of light on the schooling and vertical swimming distribution on Atlantic salmon is well understood (Oppedal 2011); however, wild behavioural studies are lacking. It has previously been suggested that, due to the lack of opportunities for specialisation in freshwater ecosystems compared with terrestrial ecosystems, freshwater fish species have broad tolerance of external environments (Chapman 1966) and, as such, may be able to adapt to changes in nocturnal illumination.

In fish, light is said to be a directive factor, as natural light regimes will influence their daily pattern of behaviour (Fry, 1927). Due to the importance of natural photoperiods in regulating behavioural patterns, the behaviour of fish can also be modified in many ways in response to light. McConnell *et al.* (2010) examined the impact of artificial light on the abundance of wild fish around an area of aquaculture farming in Canada. They saw an increase in the abundance of all life-stages of Pacific herring (*Clupea pallasii*) and threespine stickleback (*Gasterosteus aculeatus*) on the nights when the

light was on, suggesting that these fish exhibited positive phototaxis. This attraction to light is widely utilised in commercial fishing as an attractant, to improve catch (Dragesund 1958). For example, Becker *et al.* (2013) found the abundance of both large predatory fish and small shoaling fish increased alongside an experimentally lit jetty. Conversely, it was found that the downstream migration of the European eel (*Anguilla anguilla*) was impaired by bright moonlight, suggesting that this species exhibits a negative phototactic response (Lowe 1952)**.**

Such studies suggest that the behavioural response of fish to light is species-specific (Marchesan *et al.* 2005; McConnell *et al.* 2010; Brunning *et al.* 2011) as, between species, conflicting responses to light are reported within the literature. The response of a given fish species to ALAN may, however, be further complicated by life stage and the specificities of light. An example of this is provided by two studies on Pacific herring (*Clupea pallasii*) conducted in consecutive years by Krefft and Schubert (1950) and Schuler and Krefft (1951). These studies presented quite oppositional results: in the first study, herring exhibited positive phototaxis, whilst in the following year the herring exhibited negative phototaxis. From this divergence it could be supposed that factors such as life stage play a role in the response of the species to light (McConnell *et al.* 2010). In contrast, Kawamoto and Takeda (1951) found that the "gathering rate" of pelagic fish around a light was dependent on the spectral properties of the light and concluded that the wavelength of light used was more important in determining the attraction of fish to the light than the "energy" (i.e. intensity) of the light. Further, the behavioural change of a fish in response to ALAN may not be as simple as attraction or avoidance behaviour. Light intensity has been seen to influence the aggressive and territorial behaviour of fish, with increased light intensity resulting in increased aggression (Valdimarsson and Metcalfe 2001; Castro and Caballero 2004; Carvalho *et al.* 2013). Light intensity has also been seen to influence schooling behaviour and swimming speed (Batty *et al.* 1990; Miyazaki 2000; Oppedal *et al.* 2011; Riley *et al.* 2014).

Light is also important in foraging behaviour as many freshwater fish are reliant upon vision to detect their prey (Marchesan *et al.* 2005). When considering feeding behaviour, the majority of studies to

date have focused on the minimum light levels needed in order to permit feeding by planktivorous fish and not the light levels that would inhibit the feeding behaviour or alter the way in which the fish feed (Puvanendran and Brown 1998). Again, the impact of light on foraging behaviour is thought to be species-specific. A study by Robinson and Tash (1999) compared the feeding ability of Arizona trout (*Oncorhynchus apache*) and Brown trout (*Salmo trutta*) at different light intensities and found that the two species differed in their response to and feeding ability under the different levels of light.

1.5 Study System

1.5.1 Freshwater ecosystems

In the UK, freshwater ecosystems are some of the most productive and bio-diverse ecosystems and are a key habitat resource (UK NEA 2011). They provide a home for both freshwater specialists and species that migrate between freshwater and marine ecosystems as part of their lifecycle, such as the Atlantic salmon (UK NEA 2011). Due to their productivity, freshwater ecosystems feature heavily in conservation policy, including the EU habitats directive, EU water framework directive and many UK specific environmental legislations (UK NEA 2011). In Wales for example, 75% of nationally recognised conservation sites include freshwater habitats (UK NEA 2011).

Despite their importance biologically, freshwater ecosystems are likely to be the most heavily impacted ecosystem on earth (Revenga *et al.* 2005). Humans have relied on freshwater for millennia and settlements have historically grown up around sources of fresh water. Yet, demand for freshwater has increased sharply over the last century (Strayer and Dudgeon 2010) and as a result, freshwater ecosystems now face a great deal of anthropogenic stressors (Dudgeon *et al.* 2006; Darwall *et al.* 2008; Ormerod 2010). These multiple stressors, and their interactions, are thought to be the drivers behind the effect of urbanisation on freshwater ecosystems, often termed ‘urban stream syndrome’ (Walsh *et al.* 2005). This is true in the UK where freshwater habitats are the most heavily depleted ecosystem and, to this end, there are no intact freshwater ecosystems remaining (UK NEA 2011).

Pressures on freshwater ecosystems are diverse but are mostly anthropogenic in nature. These pressures include habitat alteration such as drainage, the addition of pollutants and changes in land cover (UK NEA 2011; Revenga *et al.* 2005). Yet, it is due of the aforementioned human reliance on freshwater that the impact of ALAN is not limited to urban streams. Human settlements have historically formed around sites of freshwater and thus, even in peri-urban and rural areas freshwater ecosystems are increasingly exposed to ALAN (Hölker *et al.* 2010a; Perkin *et al.* 2011).

Pollutants are a key and increasing pressure for freshwater ecosystems (Revenga *et al.* 2005; UK NEA 2011). A recent review highlighted the need for research into the impact of ALAN on freshwater ecosystems (Perkin *et al.* 2011), and this article could be considered a 'call to arms' for freshwater ecologists. The scale of the problem, concerning the impact of ALAN on freshwater ecosystems is not fully understood; however, given its' prevalence in developed societies, it is highly likely that in urban areas ALAN is invading and impacting freshwater ecosystems. Further, given the aforementioned increase in ALAN, natural and semi-natural areas are increasingly impacted (Bennie *et al.* 2015).

1.5.2 Atlantic salmon

Atlantic salmon (*Salmo salar*) are found across much of the North-Western Hemisphere in temperate and subartic regions surrounding the Atlantic Ocean (Shelley 2004; Aas *et al.* 2011). Atlantic salmon is a diadromous species, a trait it shares with a number of other salmonid species, meaning that they are able to live in both freshwater and marine environments. Specifically Atlantic salmon is an anadromous species, migrating from marine environments to freshwater to spawn (Aas *et al.* 2011).

In their freshwater phase, they require pristine water to survive and are not tolerant to pollutants or anthropogenic exploitation of the freshwater environment (Youngson and Hay 1996) It is for this reason that they are a valuable bio-indicator of ecosystem health (NACSO 2009). The anadromous lifecycle of Atlantic salmon includes a juvenile period spent in freshwater, extensive growth then takes place at sea, before spawning sees a migration back to freshwater (Aas *et al.* 2011; Fig. 1.2). There are

also landlocked populations of Atlantic salmon, for which the entirety of their lifecycle takes place in freshwater (Aas *et al.* 2011).

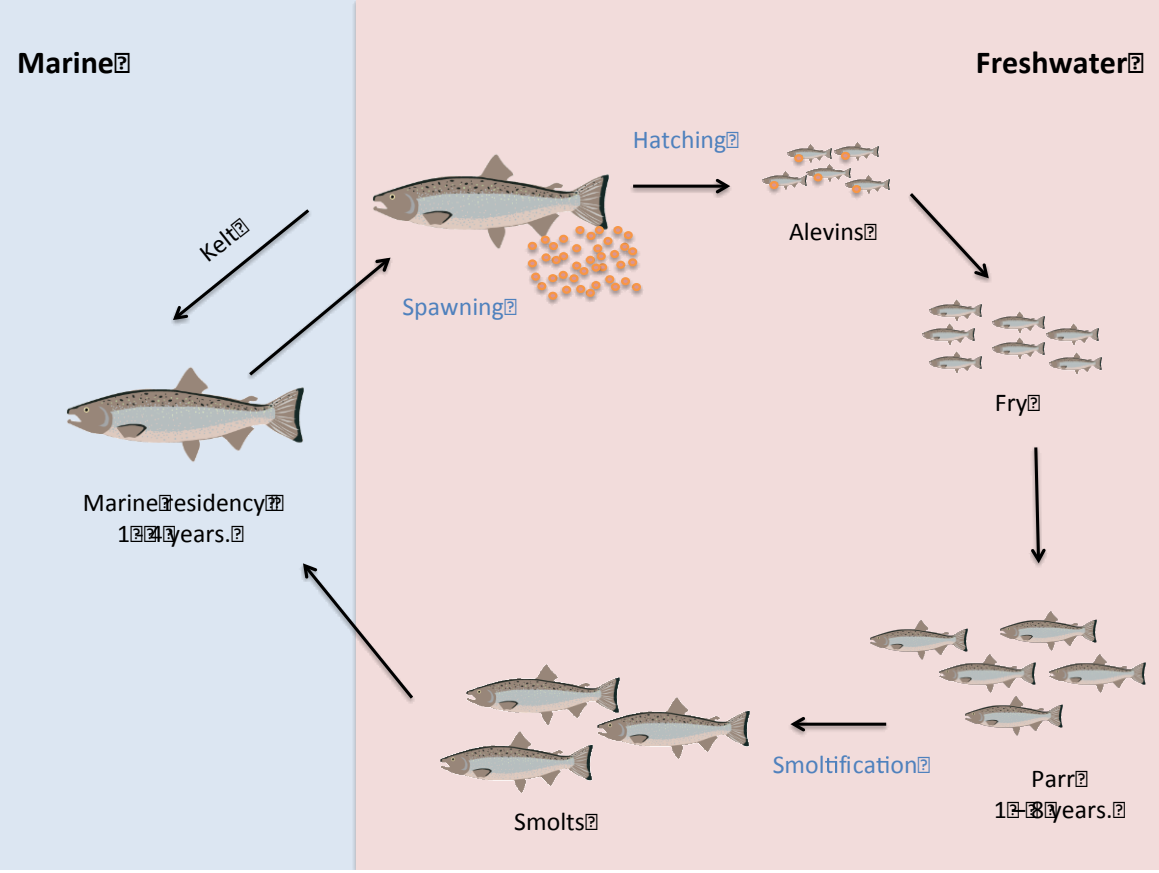


Figure 1.2 Schematic representation of the complex life cycle of Atlantic salmon across both freshwater and marine ecosystems (Adapted from Mills 1991 and Aas *et al.* 2011).

Spawning typically occurs during November and December, whereby a female digs out a large depression in the gravel in order to lay a clutch of eggs in a nest. Eventually several of these are created and together will form a "redd" (Aas *et al.* 2011). Eggs are fertilised by males and develop within the redd, hatching in the early spring. Once hatched, the juvenile fish - termed alevins - remain in the gravel, feeding only on their maternally provided yolk sac and growing for 3-8 weeks (Aas et el., 2011). Once their yolk sac is nearing depletion, the fish - now termed fry - will emerge from the gravel and disperse downstream in search of a feeding territory (Godin 1982).

The dispersal period and establishment of a feeding territory is one of high mortality and competition for a suitable territory is fierce (Armstrong *et al.* 2003; Nislow *et al.* 2004). Here they will remain

residents in freshwater as juveniles (parr) for anywhere between one and eight years, though more typically two years in UK, feeding on drifting invertebrates. During their freshwater residency the parr grow rapidly, before undergoing a physiological metamorphosis, known as smoltification, and becoming smolts (Aas *et al.* 2011). It is the smolts that undertake the seaward migration during spring, a synchronised downstream movement (Riley *et al.* 2012), towards rich feeding areas in the North Atlantic Ocean. This is also known to be a period of high mortality in the lifecycle of Atlantic salmon, where the fish are learning to adapt to a new environment with a new predator-prey dynamic (Aas *et al.* 2011; Klemetson *et al.* 2003). The fish will remain at sea for typically one to two years, before returning to their natal river and migrating back upstream to their natal spawning ground to spawn themselves.

1.5.3 Why study Atlantic salmon?

In order to determine fully the behavioural and physiological effects of ALAN, good model species are required for investigations (Gaston and Bennie 2014). Atlantic salmon are an extremely well studied species, and because much of their behaviour, ecology and physiology is so well understood they are a popular vertebrate model species for studying behaviour and response to environmental change (Aas *et al.* 2011; Finstad *et al.* 2011). As an anadromous fish, the life cycle of Atlantic salmon is complex, including two different habitats and two major developmental processes; smolting and maturation, both of which are known to be controlled by light cues (Fig. 1.1; McCormick *et al.* 1998; Boeuf and le Bail 1999; Longcore 2004; Stefansson *et al.* 2007; Lecelerq *et al.* 2010). It is for these reasons that the Atlantic salmon is an extremely interesting species to study (Shelton 2009), particularly in response to changes in nocturnal illumination levels.

Whilst a counter argument could be made that currently, too much research is focused on a few economically significant species, such as Atlantic salmon, it is important to appreciate that the present study is somewhat preliminary and explorative. Thus the study species used needs to have a wide research base from which to draw baseline conclusions of behaviour. While it would be valid then to suggest the need to study a greater range of species so that we can preserve them, it is hoped that the

results of this study can be used to inform subsequent studies on additional species of conservation concern. Through building on the foundations of this work and the application of the methodological techniques utilised in this study, the impact of ALAN can be explored for other freshwater fish species that are somewhat neglected in the current ecological literature.

Atlantic salmon have both high economic and cultural significance in the UK, making their study important for reasons other than their use as a model species in ecological research. As such, the funding afforded to this study specified that the study species used must be of high economical value. In Scotland in 1995, the revenue from salmon fishing was estimated at between £270 and £430 million (Davies *et al.* 2004). This revenue is of particular significance for the economies of communities in rural areas of Scotland (Davies *et al.* 2004). Salmonids are also of huge value to the Welsh economy, it is estimated that money from anglers fishing licenses in the UK is worth at £350 million annually, with around £74 million of this found in Wales (Mawie and Peirson 2009; UK NEA 2011). In West Wales in one river alone, the River Teifi, salmonid fishing contributes £1.1 million annually to the Welsh economy (Environment Agency 2000).

Despite their high economic and cultural status, Atlantic salmon stocks in the UK are declining (Parrish *et al.* 1998; JNCC 2010; Clews *et al.* 2010), and much effort and money has been spent on their restoration (Fraser *et al.* 2007; Ward *et al.* 2008). The main factor that is known to impact the distribution of Atlantic salmon is the presence of suitable habitat, as salmonids are extremely sensitive to habitat changes (Hendry and Cragg-Hine 2003; Armstrong *et al.* 2003; Clews *et al.* 2010). The precise habitat requirements differ between the life stages of Atlantic salmon but all stages have strict habitat requirements and demand a high level of water quality to survive (Reiser and Bjornn 1979; Bjornn and Reiser, 1991; Youngson and Hay 1996; Armstrong *et al.* 2003; Hendry and Cragg-Hine 2003). Debasement of freshwater habitats in the last century resulted in a local extinction of Atlantic salmon, particularly in Wales (JNCC 2007), however, declines in Atlantic salmon numbers was seen across the extent of their range (WWF 2001). In some river catchments in the UK, the number of juvenile salmon and trout present has reduced by around 60% (Clews *et al.* 2010; UK NEA 2011).

Much of the declines in Atlantic salmon populations have been attributed to human impacts (WWF 2001; Webb *et al.* 2007).

When considering the state of Atlantic salmon populations it is advisable to consider the ten-year mean population number, as the year on year numbers are so extremely variable (JNCC 2007). The most recent population estimates are taken from 1997-2006, with approximately 723,000 returning adults and 556,500 spawning adults across the UK as a whole (JNCC 2007). In Wales, the Atlantic salmon population is low due to the environmental scars of past industrial activity. In industrialised areas such as South Wales, particularly in the rivers that run through the Welsh Valleys, heavy mining activity for most of the last century left the rivers polluted and uninhabitable to salmon (JNCC 2007; UK NEA 2011). According to habitat surveys by the Environment Agency (2006), only one river in Wales is considered to be achieving its set target with regards to spawning levels. Much work has, however, been invested in to cleaning up the rivers and restoring them to full ecosystem function as a result of the EU Habitats Directive (JNCC 2007). Atlantic salmon are considered a priority species under the UKBAP and managing their populations for both economic and cultural importance is an ongoing issue (JNCC 2007). Yet, a lack of scientific information is a major impediment to conservation efforts (Reynolds *et al.* 2009), and for this reason it is important to fully understand the way in which ALAN impacts Atlantic salmon.

1.5.4 Salmonids and light

The scientific literature contains conflicting accounts of the behavioural attraction or avoidance responses of Atlantic salmon to artificial light (Table 1.3). A number of studies have suggested that salmon are positively phototactic (Carss 1990; Juell *et al.* 2003; Juell and Fosseidengen 2004; Oppedal *et al.* 2007; Table 3), whilst it has been observed in a number of other studies that they exhibit a negative phototactic response (Migaud *et al.* 2007; Riley *et al.* 2012, 2015; Table 1.3)**.** Despite a lack of consensus on the directional response to light, all such studies indicate a clear response of the fish to the artificial light (positive or negative) and thus behavioural disruption. Yet, it is important,

however, to note the potential for publication bias when considering these studies and as such, caution should be taken in generalising these results.

An additional study by Carss *et al.* (1990) examined the aggregations of Atlantic salmon around aquaculture farms off the coast of Scotland and found the fish to be attracted to the lit areas. It is important to note, however, that this study was observational, with no control, and as such alternative explanations for this attraction of the fish such exist, such as attraction to the food or the farmed salmon.

Table 1.3 The phototactic response of Atlantic salmon to artificial light, whereby positive and negative responses signify attraction and avoidance, respectively.

Study	Lighting Type	Study Setting	Direction of response
Juell *et al.* (2003)	1000W Metal Halogen	Aquaculture	Positive
Juell & Fosseidengen (2004)	400W/1000W Metal Halogen	Aquaculture	Positive
Oppedal *et al.* (2007)	400W	Aquaculture	Positive
Migaud *et al.* (2007)	Blue and white LED	Aquaculture	Negative
Riley *et al.* (2012)	45W Metal halide	Field	Negative
Riley *et al.* (2013)	45 W Metal halide	Lab	Negative
Riley *et al.* (2015)	45W Metal halide	Lab	Negative

Riley and Moore (2000) found a significantly negative relationship between the level of (natural) nocturnal illumination and the number of Atlantic salmon fry emerging from redds. Under natural conditions fry emerge from redds synchronously at night, with this behaviour likely evolving as a means of avoiding predation pressure (Gustafson-Marjanen and Dowse 1983; Fraser *et al.* 1993; Riley and Moore 2000) and can also be seen in sea trout (*Salmo trutta*) (Moore and Scott 1988). Further, Riley *et al.* (2013; 2015; see Chapter 2) found that ALAN, at differing light intensities (1 - 12 lux), influenced the timing and diel periodicity of Atlantic salmon fry dispersal from artificial redds in laboratory studies.

Natural migratory behaviour of Atlantic salmon smolts predominantly occurs at night, also as a mechanism to reduce predation pressure (Solomon 1982; Roberts *et al.* 2009) and, as such, ALAN will likely impact on smolt migration. Smolts have, however, been observed to migrate during the day later in the season (Solomon 1978; Thorpe and Morgan 1978), a change that may be mediated by temperature or day length/light intensity (Fraser *et al.* 1993; McCormick *et al.* 1998). Riley *et al.* (2012) examined the behaviour and timing of migration in Atlantic salmon smolts leaving their home stream using Passive Integrative Transponders (PIT) antennae under both ALAN and control conditions. It was found that under ALAN conditions, smolt migration was delayed until the following morning. This delayed migration may reduce the fitness of smolts through increasing their predation risk or by impacting their reproductive success (Einum and Flemming 2000; Riley *et al.* 2002; Riley *et al.* 2012). Further, in laboratory studies the addition of artificial light was found to increase the behavioural avoidance of high velocity gradients in Chinook salmon (*Oncorhynchus tshawytscha*) and brown trout (*Salmo trutta*) in experimental flumes (Vowles *et al.* 2014; Vowles and Kemp 2012). In contrast, the presence of artificial light in an experimental flume aided the downstream migration of Pacific salmon smolts (Genus: *Oncorhynchus*); under artificial light the fish were better able to pass a submerged weir (Kemp and Williams 2009). The contrasting results suggest a species and/or context specific response to light within salmonids.

1.5.5 Gaps in current knowledge

When considering the impact of ALAN on Atlantic salmon, there is a dearth of information regarding the way their behaviour is impacted by light. Whilst there is some understanding of the way ALAN influences the dispersal behaviour of fry, the impact of reducing the intensity of light on the level of disruption seen in fish behaviour and, further at what intensity light causes behavioural disruption in dispersing fry is yet to be determined (see Chapter 3). In addition, we do not have any understanding of the physiological mechanism behind the previously reported behavioural disruptions (see Chapter 4). When considering the impact of ALAN on individuals post-dispersal, we have an understanding of the influence of ALAN on the migration behaviour of smolts, yet we do not know how ALAN influences their behaviour during freshwater residency, between dispersal and smolt migration (see

Chapter 5). Furthermore, whilst there have been management strategies proposed to mitigate the unwanted ecological effects of light, these have not been tested in proximity to freshwater and as such, we have little to no understanding of their efficacy (see Chapters 3 and 5). Each chapter, then, provides an new addition to the literature which taken as a cohesive whole offers fresh insight to further our understanding of ALAN on aquatic ecosystems and provides important insights into how these impacts can be mitigated in the field.

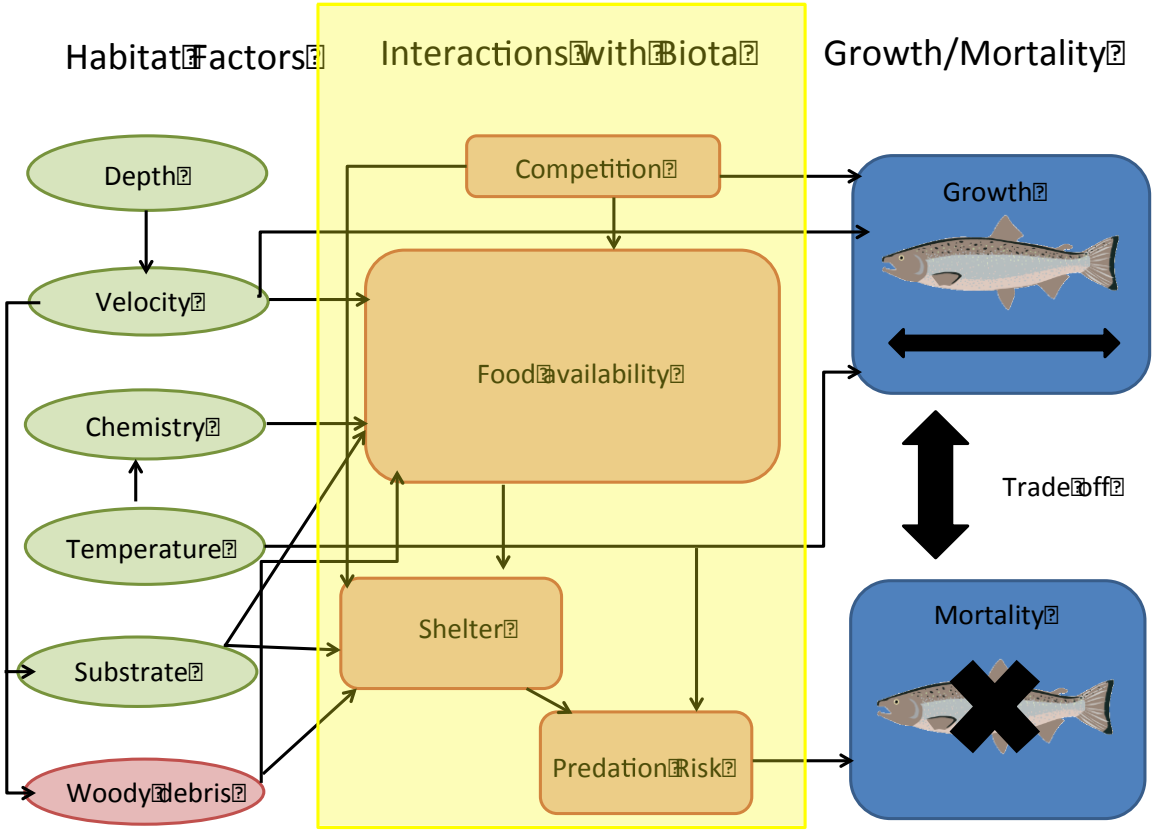


Figure 1.3 How abiotic habitat factors of freshwater ecosystems influence the biotic interactions of Atlantic salmon and subsequently impact their growth and mortality. The highlighted section suggests the biotic interactions that could be influenced by the addition of ALAN and that will be investigated as part of this thesis (Adpated from Aas *et al.* 2011).

In particular, this thesis specifically sets-out to determine how ALAN influences the predator-prey dynamics of Atlantic salmon in freshwater, As outlined above, ALAN has the potential to affect the behaviour and physiology of Atlantic salmon, and thus will influence the interaction of individual fish with their environment, predators, conspecifics and competitors (Fig. 1.3). In turn, the effect of ALAN

on these interactions will have consequences for individuals' growth and mortality and scale up to population level effects. Previous studies have focussed on the impact of ALAN at the individual level (Kurvers and Hölker 2015) and therefore there are major gaps in current knowledge concerning the influence of ALAN on species interactions (predator-prey, inter- and intra-specific competition and social interactions).

1.6 Aims and Hypotheses

The overall aim of this thesis is to examine the influence of artificial light at night (ALAN) on the predator-prey dynamics of the Atlantic salmon in freshwater. There are two component parts to realising this aim in practice:

1) Identify the gaps in knowledge around the impact of ALAN on Atlantic salmon behaviour and physiology;
2) Assemble a solid evidence base of the effects of ALAN on the juvenile stage of Atlantic salmon in freshwater, to contribute towards filling the existing knowledge gaps.

By combining these two elements, the aim of this thesis will operate in order to contribute towards the protection of Atlantic salmon, and other freshwater fish, against the unwanted and currently unmitigated impacts of this environmental pollutant. In pursuing this aim, the following chapters use a range of experimental methodologies to examine how ALAN impacts the behaviour and physiology of Atlantic salmon during the freshwater stage of its' development, and that of its invertebrate prey.

Given that the scope of the thesis encompasses a wide range of scientific disciplines and as such, this first chapter sought to place the thesis in context and summarise the necessary literature to provide a clear and concise background to this current research. Each of the individual chapters will undertake

three objectives that contribute towards meeting the overall aim of the thesis through a range of different approaches, outlined below.

Chapter 2

Chapter 2 takes a quantitative approach to reviewing the current literature to highlight research trends and identify gaps in the current knowledge concerning the ecological effects of ALAN. This is conducted with a view to highlighting the importance of this thesis in building our knowledge of the impact of ALAN on fish.

The three objectives for Chapter 2 are to:

- Identify publications trends and data pertaining to the ecological impacts of ALAN;
- Synthesize the literature to draw out important themes, scope and impact of existing research, and;
- Analyse the results to highlight the key gaps in the literature that require addressing.

Chapter 3

Chapter 3 seeks to examine the effect of ALAN on the nocturnal activity of freshwater invertebrates. This is primarily conducted with a view to determining how ALAN might affect the food source of drift feeding fish, like Atlantic salmon and also to examine how ALAN may impact ecosystem health. The chapter hypothesises that: (1) ALAN will reduce the family level richness of the drift when compared with control nights; (2) ALAN will reduce the total number drift abundance when compared with control nights; (3) Periodicity of the drift will differ between lighting regimes of each treatment; (4) ALAN will differentially affect families within the drift, and; (5) Part-night lighting (trimming) will reduce the impact of ALAN compared to whole-night lighting.

The three objectives for Chapter 3 are:

- Determine the impact of light on invertebrate drift;
- Assess the efficacy of a proposed management strategy (part-lighting), and;
- Deduce how predator-prey dynamics and ecosystem health may be influenced.

Chapter 4

Chapter 4 aims to determine the impact of ALAN on the nocturnal dispersal behaviour of Atlantic salmon fry in an empirical laboratory study. The study looks to examine the effect of reducing the intensity of broader wavelength street lamps on fry dispersal, and aims to determine whether this will significantly reduce the impact of ALAN on Atlantic salmon fry dispersal. Though Chapter 4 also has its own hypothesis and follows a distinct set of objectives to ensure it contributes towards meeting the overall thesis aim, these are not set out in the chapter itself as the chapter reflects a published manuscript and thus the content must be kept as per the final journal paper. The hypotheses tested in this experiment are that: (1) ALAN will cause delay and disruption to fry dispersal behaviour; (2) the higher the intensity ALAN the greater the level of disruption; and (3) fry dispersing under ALAN will be lower in weight due to delayed dispersal.

The three objectives for Chapter 4 are:

- Examine the impact of ALAN on fry dispersal behaviour;
- Identify patterns of disruption to dispersal related to ALAN at different intensities, and;
- Determine how ALAN can impact the fitness of the dispersing fry.

Chapter 5

Following on from the effects on dispersal described in Chapter 4, the aim of Chapter 5 was to investigate whether the disruption to the nocturnal synchronous dispersal of Atlantic salmon fry was mediated via a cortisol stress response in the dispersing juveniles. The hypotheses tested as part of the experiment are: (1) ALAN will induce a cortisol stress response in those fry dispersing from experimentally lit incubators; (2) the magnitude of the stress response will increase with light intensity; and (3) fry dispersing under ALAN will be lower in weight due to delayed dispersal.

The three objectives for Chapter 5 are:

- Assess the impact of ALAN on the cortisol stress response of individual dispersing fry;
- Identify the population level cortisol response to ALAN, and;
- Evaluate possible alternative physiological mechanisms behind the observed behavioural disruption under ALAN.

Chapter 6

Chapter 6 examines the influence of ALAN on the diel pattern of refuging and foraging behaviour in 1+ Atlantic salmon parr using continuous PIT monitoring of the fishes' behaviour around the 24-hour clock. The hypotheses tested as part of the experiment are: that fish under ALAN will (1) spend more time refuging than their control counterparts and (2) spend less time foraging than their control counterparts; (3) ALAN will disrupt the diel timings and pattern of foraging and refuging, and; (4) the higher the intensity of ALAN the greater the disruption of natural diel behaviours.

The three objectives for Chapter 6 are:

- Assess the impact of ALAN on the diel patterns of refuging and foraging behaviour;

- Determine how altering the intensity of light influences the effect of ALAN, and;
- Infer the consequences for predator-prey dynamics.

Chapter 7

To draw these different studies together, Chapter 7 summarises the results of the empirical data chapters, looking to provide clear recommendations for future research and to inform management strategies. It draws together the research findings to highlight the overall contribution made to the literature and identifies key issues for further investigation, including research questions that need to be addressed in order to increase both the breadth and the depth of knowledge.

Chapter 2: Quantitative Review of the literature concerning the ecological impact of artificial light at night (ALAN).

2.1 Introduction

As outlined previously (see Chapter 1), despite the number and proliferation of artificial light at night (ALAN), the recognition that it can be a problem has come only recently (Longcore and Rich 2004). Yet, from the limited existing studies, it appears that ALAN has the potential to disrupt the behaviour and physiology of many taxa and even to disrupt ecosystem processes (Navara and Nelson 2007; Stone *et al.* 2009; Riley *et al.* 2012; Perkin *et al.* 2012) and is proposed to have far-reaching consequences. As such, it is important to identify gaps in our current knowledge in order to guide future research and provide evidence for successful mitigation.

This brief investigation sought to quantify the currently available literature regarding the influence of ALAN on species and ecology, to identify research gaps, trends in publications and to help answer the overarching question:

What is the currently available evidence for the impact of ALAN on organisms and environments?

In order to answer the above question, more discrete, structured questions were asked of the data:

- *(Q1) When do the publications stem from? Are there peak years for publication of studies?* - This examines the rate at which interest in researching ALAN is increasing.

- *(Q2) What is the focus of the current literature? Is research equitably distributed across taxa?* - This allows for taxa that are being neglected by current research to be identified.

- *(Q3) Are most papers experimental or theoretical?* - This determines the type and amount of new evidence being reported for the ecological impacts of ALAN.

- *(Q4) Which journals feature strongly in the literature? Are these high impact journals?* - This allows for the determination of the breadth of readership and dissemination of the research articles.

- *(Q5) Which papers are the most heavily cited?* - This outlines the papers that are having the biggest impact on research within the broader academic community.

These questions sought to assess the scope and impact of the current literature, in order to guide the focus of this thesis and address one of the overall thesis aims, to identify the gaps in knowledge around the impact of ALAN on Atlantic salmon behaviour and physiology. As such, the specific objectives for this chapter are to: Identify publications trends and data pertaining to the ecological impacts of ALAN; synthesise the literature to draw out important themes, scope and impact of existing research, and; analyse the results to highlight the key gaps in the literature that require addressing.

2.2 Methodology

2.2.1 Literature searches

Literature searches were performed using Google scholar. The articles included in this review were selected based on their relevance and contribution to the field of ecological impacts of ALAN research. An initial search was performed (August 2014) for the search term "Ecological Light Pollution". This initial search term was selected in order to produce clearer results than simply searching for "Light Pollution" or "Artificial Light" by increasing the level of specificity. This search was intended to retrieve papers that would form the main structure of the dataset, as such a specific search term was chosen. The initial search retrieved 352 articles, and a total of 94 articles were included as part of this systematic literature review after meeting the inclusion criteria, outlined below.

A second search was performed (October 2014) using the search term “artificial light at night”. This search returned 689 results, of which 8 new articles were added to the existing dataset. A further search was performed (October 2014) as a title only search containing the broad phrase “light pollution”. This search returned 504 results, of which a further 4 articles were added to the dataset along with the previously collated papers. This search was performed in order to ensure inclusion of papers that were published prior to 2004, which did not use the specific phrase “ecological light pollution”. That the two later searches only returned a total of 12 articles that were not covered by the initial search, indicating that the phrase “Light Pollution and Ecology” had returned the bulk of the available literature.

Two final searches were attempted (October 2014) for “environmental light pollution” “light pollution and ecology”; however, these did not reveal any further relevant results. It was at this point that the dataset was deemed complete, as further search terms had not identified any additional articles.

2.2.2 Selection criteria

Article titles and abstracts were read and reviewed based on a set of predefined inclusion criteria, whereby the title and abstract of all returned search results were examined for relevance. Firstly it was decided that the studies should be published in an international peer-reviewed journal. Non-peer reviewed PDFs or information sheets produced by environmental charities were not included in this evaluation. This was decided due to such reports recovered during the search being secondary review papers citing articles already included as part of the study. In addition, searches only included articles, the option for patents and citations were removed.

Secondly, there must be mention of ALAN, light pollution or artificial light in relation to an ecosystem, species or higher order of biological classification mentioned within the title or abstract. The impact of light could relate to numerous factors of environmental or organismal well being, including but not limited to, functioning, health, population or behaviour. Articles that briefly

mentioned light pollution as part of a list of environmental or organismal stressors were, however, not included as the aim was to find articles that centre on the influence of light and contribute to the field of study. The decision was made to exclude the impact of lighting regimes on laboratory rodents. These studies typically focus on the influence of light on disease and morbidity for use in human health research, whereas the aim of this review and the research that follows is to identify the influence of ALAN, typically street lighting, in ecosystems and on wild organisms in their natural environment.

Finally, articles that examined the influence of natural light/dark regimes were not included as part of this study, nor were studies that investigated the impact of constant daylight, strobe lighting or any other experimental lighting regimes that are not representative of ALAN from public lighting.

2.2.3 Analysis

The articles that were selected to form part of this study were input to a database to allow for easy manipulation and analysis. From each of the selected papers information was collected regarding: authorship, journal of publication, year of publication, number of citations, focus of the article and whether they were experimental or theoretical in nature. Modelling, field observation and mapping studies were considered as being experimental papers, whilst literature reviews and opinion pieces were categorised as being theoretical.

2.3 Results and Discussion

This section presents the research articles included as part of this investigation and seeks to answer the outlined research questions. Whilst the analysis is not statistical in nature, the analysis looks to provide an overall quantitative picture of the research area as it stands and identify gaps in current knowledge.

Q1. When do the publications stem from? Are there peak years for publication of studies?

The literature searches identified 105 studies published between 2004 and 2014 that identify, experientially manipulate or discuss the ecological impact of ALAN (Fig. 2.1). From 2008 a clear upward trend in the number of publications can be seen. This trend steepens from 2010 with 75 of the 105 studies located being published in the last four years. The earliest publication that formed part of the dataset was from 2004, however, it was not until 2006 that the number of publications steadily began to increase. There can then be seen to be a sharp increase in the number of papers published annually between 2009 and 2010, with the number more than doubling from six to eleven papers. Between 2012 and 2013 the number of papers published also rose sharply from 13 to 21. The peak year for publications was 2014; the final year for which studies were included.

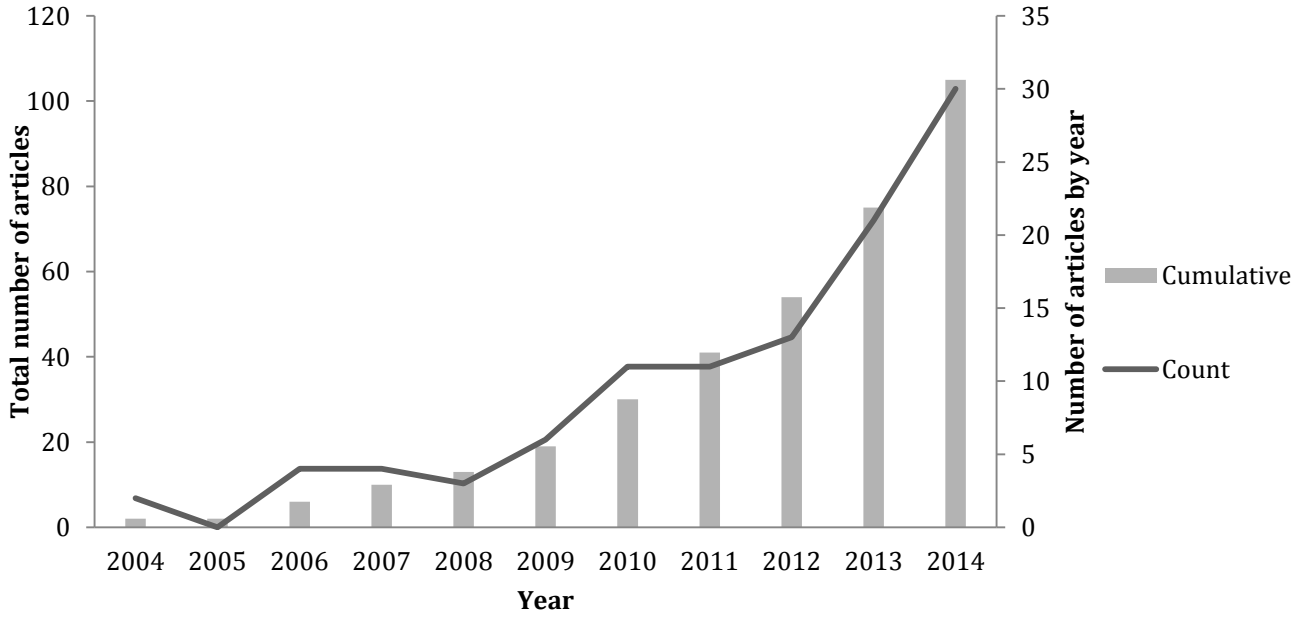


Figure 2.1 Number of publications by year (line graph) and cumulative number of studies (bar graph) from 2004-2014 retrieved from the literature search.

The publication trend shows that ALAN - or ecological light pollution (a term coined by the pioneering paper by Longcore and Rich 2004) - is becoming a hot topic in the ecological literature. Interest and awareness of ALAN as a stressor, which has the potential to modify the behaviour and

physiology of all species, appears to be growing, with researchers looking to tackle the lack of knowledge through an investment in research.

Q2. What is the focus of the current literature? What groups of organisms does the existing literature focus on?

An examination of the articles revealed that most, 74.4%, focus on the impact of light on different species, genera or families. The remaining 24.4% of papers look at ecosystem and ecological impacts, whilst only a small proportion (1.16%) looks at the physiological impacts of ALAN (Fig. 2.2).

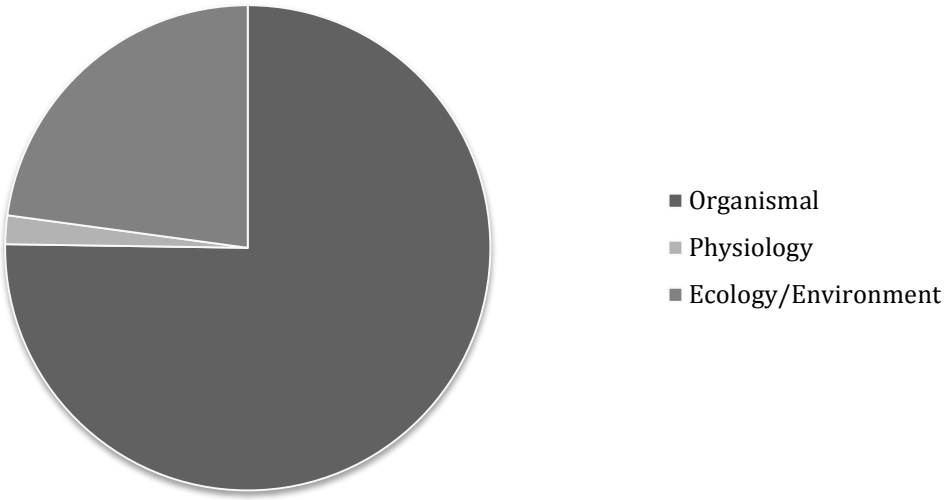


Figure 2.2 The distribution of papers in the dataset presented separately for organismal, physiological and ecological/environmental studies.

The majority of the organismal papers consider birds and invertebrates, with 29.5% and 19% of papers, respectively, investigating the impact of ALAN on these organisms (Fig. 2.3). Mammals, mostly bats, feature comparatively heavily in the literature also, comprising 10.5% of studies (Fig. 2.3).

Fish are comparatively under-studied, with only 7.6% of the articles looking at the impact of ALAN on fish (Fig. 2.3) and when this is broken down further by species, only 2.4% of studies examine the study species of this thesis, Atlantic salmon (*Salmo salar* L.). Furthermore, reptiles and amphibians are the most poorly represented classes in the current literature, with only 5.7% and 1% respectively (Fig. 2.3).

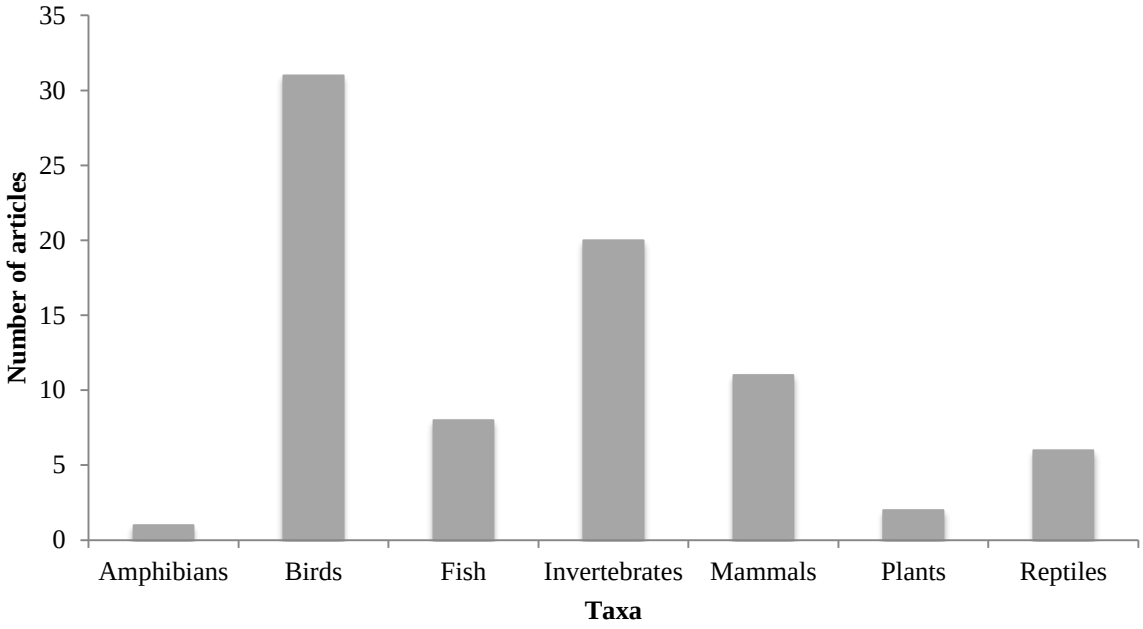


Figure 2.3 The distribution of the number of papers in the dataset presented separately for the higher order animal groups.

All currently published articles acknowledge that there are major gaps in our knowledge about the impact of light on most species, yet the above figures are the first to quantify this. These figures also serve to identify some of the taxa that are currently being overlooked and require further research investment. Taxonomic bias is an established problem in the conservation and ecology literature (Bonnet *et al.* 2002; Fazey *et al.* 2005; Martin-Lopez *et al.* 2009). Studies in these fields were found to be biased towards vertebrates (Fazey *et al.* 2005), in particular birds and mammals (Bonnet *et al.* 2002), and single species in place of ecosystems or community composition (Fazey *et al.* 2005). When looking at the articles in this review, broken down by specific study species or higher taxonomic group, a bias towards birds, mammals and invertebrates can clearly be seen (Fig. 2.3). Birds were the focus of most species-focussed articles included in this review and this appears to be a common trend in the ecological literature (Bonnet *et al.* 2002). Whilst 79% of all species known to science are invertebrates, only 26% of the organismal articles included in this review examine the influence of light on invertebrates. By comparison, vertebrates that comprise 3% of known species are the focus of 74% of the organismal papers.

Fish are historically comparatively under-studied (Bonnet *et al.* 2002; Clark and May 2002; Fazey *et al.* 2005). When comparing the number of papers published in the ecological literature on individual vertebrate classes with the number of known species, Bonnet *et al.* (2002) found fish to be particularly under-represented given that they are known to represent 48% of ectothermic species yet were only studied in 14% of the 1171 papers included in their analysis. This is not a bias unique to studies of ALAN; when Clark and May (2002) analysed all back issues of two internationally recognised conservation journals *Conservation Biology* and *Biological Conservation* between 1987 and 2001 they found only 8% of the conservation literature, on any topic, within these two journals reported on fish. Likewise, Fazey *et al.* (2005) also found fish to be poorly represented in their analysis of papers published in three conservation specific journals (*Conservation Biology, Biological Conservation and Biodiversity and Conservation*) in the year 2001. Fazey *et al.* (2005) also found that only 7% of papers included their aforementioned analysis examined conservation in an ecosystem setting, compared with 54% of papers focussed on individual species or populations. This can also be seen in the proportion of articles in this review, the number of articles that focus on ecosystem or community effects (23%) was considerably less than the number of studies that focussed on single species or on species assemblages (75%) (Fig. 2.2). Therefore the species-focused bias seen in current research needs to be addressed to fill the large gaps in our current knowledge of the species-interactions and community-level effects of ALAN. Such studies are crucial if successful mitigation against the detrimental impacts of light on ecosystems is to be developed.

Q3. Are most papers experimental or theoretical?

The articles included in this analysis were assigned to either theoretical or experimental categories, with the greater proportion (77.2%) of papers found to be experimental and only a small proportion were theoretical (22.8%).

Of the 30 papers published in 2014 so far, 73% of these were experimental papers. This surge of interest in experimental investigations into the ecological impact of ALAN will prove invaluable in informing management policies and future research. Given the lack of knowledge concerning the

ecological impact of ALAN, empirical experimental papers are extremely useful in furthering knowledge in this area. They further serve in identifying species or ecosystems at risk of being negatively impacted by ALAN. That the use of ALAN is now considered entrenched in our society and the numbers of lights are growing annually (RCEP 2009; Hölker *et al.* 2010b), it is of the upmost importance that empirical evidence is provided to support mitigation strategies.

It is only through a clear understanding of the impact of ALAN on species and ecosystems that informed mitigation strategies can be developed and it is assumed that scientific studies will provide clear evidence to inform mitigation, however this is not always the case. In the field of invasive species biology, a phenomenon termed the 'knowing-doing gap' (Esler *et al.* 2010, Matzek *et al.* 2014b) describes the mismatch between scientific knowledge and on-the-ground action and conservation (Matzek *et al.* 2014a, McNie 2007). This mismatch is often attributed to either failings with the research itself or lack of action from those responsible for developing conservation strategies (Matzek *et al.* 2014b). For this reason, it is important not only that studies are performed which provide results that are both rigorous and directly applicable for conservation management, but also, that research is published and widely disseminated, in order raise awareness of the ecological impacts of ALAN.

Q4. Which journals feature strongly in the literature? Are these high impact journals?

Papers were found from a total of 80 journals, highlighting the varied nature of the currently available literature concerning the ecological impacts of ALAN (Table 2.1).

Articles concerning ALAN are published in a wide variety of journals from broad scope journals such as PLoS One and Current Biology to focussed niche journals such as *Freshwater Biology, Journal of Mammalogy* and *Journal of Insect Conservation.* Also included in this review are well-known, high impact, international journals such as *Nature* (IF 42.351) to the relatively unknown and low impact journals such as *Transcations of Tanjin University* (IF 0.00).

Table 2.1 The journals publishing papers on light pollution and ecology. TP= total publications, TP%= the percentage each journal represents from the total dataset, CI= total number of citations, MCI= mean number of citations and IF= impact factor of the journal. (Impact factors taken from www.citefactor.org- accessed October 2014).

Journal	TP	TP%	CI	MCI	IF
PloS One	5	4.76	66	13.2	3.534
Journal of Applied Ecology	4	3.81	34	8.5	4.754
Frontiers in Ecology and the Environment	3	2.86	354	118	8.412
Current Biology	3	2.86	165	55	9.916
Biological Conservation	3	2.86	41	13.7	4.036
Ecosphere	2	2.33	21	10.5	2.595
Environmental Reviews	2	2.33	0	0	2.359
European Journal of Wildlife Research	2	2.33	8	4	1.208
Freshwater Biology	2	2.33	0	0	2.905
Global Change Biology	2	2.33	4	2	8.224
Journal of Animal Ecology	2	2.33	14	7	4.726
Journal of Mammology	2	2.33	35	17.5	2.225
Journal of Insect Conservation	2	2.33	0	0	1.789
Oecologia	2	2.33	15	7.5	3.011
The Condor	2	2.33	54	27	1.347
Ecological Applications	2	2.33	1	0.5	4.126
Sum	40	42.78	812	284.4	N/A

Of the 80 journals included in this review, five published more than two articles on ALAN and ecology; the remaining 75 journals published one or two articles on the topic. Of these top five journals, one is the open access journal *PloS One* and has, to date, published the most articles for a single journal with 5 published articles included in this review. Whilst *PLoS One* doesn't have the highest impact factor of journals featured in this review, its open access nature makes it a popular choice for submissions and ensures a broad readership for the articles, thus increasing the number of people that are aware of the growing concerns surrounding light pollution and ecology.

Amongst the journals with the most number of articles published, several are high-ranking journals with high Impact Factors (IF) including; *Current Biology* (IF 9.916), *Frontiers in Ecology and the*

Environment (IF 8.412) and Global Change Biology (IF 8.224). With the exception of *Global Change Biology*, these papers can also be seen to have the highest mean number of citations (Table 2.1).

The publication of articles investigating/discussing the ecological impacts of ALAN in high impact and open access journals will result in greater dissemination of the results and increased awareness of ALAN as an ecological issue. Whilst impact factors are not always a true indicator of readership, a higher impact factor journal is likely to be more mainstream and thus will disseminate the results of a published study to a wider audience. Within a given niche specialism, journals may have a low impact factor but be read by everyone conducting research within that specialism. When considering a research topic such as the ecological impacts of ALAN, that is beginning to attract interest, it is important to consider the influence of publishing in some of the journals with higher impact factors will have on stimulating new research.

Q5. Which papers are the most heavily cited? What are these papers focussed on?

The most heavily cited article is the inaugural paper by Longcore and Rich (2004) with 306 citations, followed by Navara and Nelson (2007) with 193 (Table 2.2).

Table 2.2 The five papers with the greatest number of citations, in order, found within the dataset and the mean citations per year based on publication date.

Article	Citations	Mean citations per year
Longcore and Rich (2004)	306	31
Navara and Nelson (2007)	193	28
Stone *et al.* (2009)	93	19
Johnsen *et al.* (2006)	73	9
Neil and Wu (2006)	71	9

Both of these articles are theoretical in nature, making them a good source of information to be cited themselves in a broad range of articles, both experimental and theoretical. Longcore and Rich (2004) is the seminal paper, beginning the current interest in to ALAN now seen in the ecological literature, as such it is the most cited article to date and has received the highest number of mean citations per

year. This is likely to be because most articles, regardless of their specific focus, cite Longcore and Rich (2004) as the main reference for the ecological impacts of ALAN. Navara and Nelson (2007) is the only paper to speculate on the physiological impacts of ALAN in animals, thus accounting for the high number of citations it has received.

Neil and Wu (2006) is another theoretical article that is highly cited, likely due to it being one of only two articles that examine the influence of light pollution on the plant kingdom. As the only other plant focussed article included in this data set (Poulin *et al.* 2013) is an experimental article and published considerably later than Neil and Wu (2006), it is likely that Neil and Wu (2006) will be used as a reference for all plant papers published when discussing ALAN. The two remaining articles, Stone *et al.* (2009) and Johnsen *et al.* (2006), are experimental papers. Stone *et al.* (2009) examine the influence of ALAN on bats and Johnsen *et al.* (2006) studied moths. Moths and bats are amongst the most studied taxa in relation to ALAN so it is unsurprising that these are amongst the most cited articles in this research area.

1.10 Conclusions

The search for scientific articles concerning ALAN and ecology prior to the year 2004 returns no relevant search results. Post 2004, however, and the publication of Longcore and Rich's 2004 review paper "Ecological light pollution" in *Frontiers in Ecology and the Environment* and their subsequent 2006 book "Ecological Consequences of Artificial Night Lighting", research has steadily increased in to this topic. However, there has recently been an upsurge in interest in this ecological issue and the majority of articles included in this review have been published within the past four years. Further, 2014 had the most publications per year to date. The trend for increasing research is promising and suggests that the ecological impacts of ALAN on a wide variety of species will increasingly be identified. However, given that the impacts of ALAN appear to be species and even life stage specific (McConnell *et al.* 2010), a great investment of research is required to elucidate the problem fully.

Despite this surge in interest in determining the ecological impact of ALAN, it is important to note the future uncertainties concerning the nocturnal environment. Firstly, lighting types in the UK are changing to be more energy and cost efficient, a move that could prove them to have more impact on organisms (Falchi *et al.* 2011; Kyba *et al.* 2012; Gaston *et al.* 2013). Secondly, species are becoming subject to global climate change, a process that, to date, has not been accounted for in ecological studies of ALAN. The role of multiple stressors has yet to be determined, though it is thought that species declines are more rapid when subjected to multiple stressors (Mora *et al.* 2007; Darling and Cote 2008). Given that these changes are already occurring, it becomes all the more pertinent that we begin to engage fully with the subject of ALAN.

This quantitative review of the literature highlights that there are still enormous gaps in our knowledge. Importantly, fish, birds and reptiles are comparatively under-studied and current articles focus strongly on individual species effects rather than community and ecosystem level impacts. Research into the species-level effects of ALAN needs to be equitably distributed amongst all taxa, not only to ensure effective management of ALAN for all species but also due to the possibly community and ecosystem level effects that may result if a given taxon is impacted by ALAN. These gaps in our knowledge can only be filled through investment of research into the ecological impacts of ALAN from artificial lights both, old and new and on species big and small.

Chapter 3: Artificial light at night (ALAN) and the nocturnal drifting behaviour of freshwater invertebrates

3.1 Abstract

Despite growing concerns about the ecological impacts of artificial light at night (ALAN), there have been few assessments of the potential consequences for invertebrate drift in streams. Drift is primarily a nocturnal, active dispersal behaviour triggered by darkness and inhibited by light. This study assessed the behavioural impacts of ALAN regime (partly-lit, fully-lit and unlit) on the drifting behaviour of invertebrates in an experimentally-lit temperate lowland stream. A high-pressure sodium (HPS) broad-spectrum streetlight was introduced to an ALAN-naïve stream and invertebrate drift was investigated at night, under the ALAN regimes at three distances from the streetlight (13.5, 7 and 2.5 lux). The number and diversity of freshwater invertebrates obtained at three sampling times (23.30, 01.30 and 03.30 BST) were compared with an upstream, unlit control net (0.01 lux) sampled at the same times. The results suggest a taxon-specific response to ALAN, with the two most prevalent taxa, Gammaridae and Baetidae, displaying differing drifting behavioural responses to ALAN; Gammaridae were found to be significantly reduced by ALAN, whilst there was no effect on Baetidae. Further, these results reveal the varying impacts of ALAN across the different functional feeding groups (FFGs) comprising an invertebrate community - the first study to do so in a freshwater ecosystem. ALAN most strongly influenced the behaviour of shredders and grazers, through increased drifting behaviour under part-night lighting. Given the importance of invertebrates in aquatic ecosystem functioning, these results suggest that ALAN may have effects on wider ecosystem processes and may influence the food availability of economically important drift-feeding fishes such as salmonids.

3.2 Introduction

Despite growing concerns (Rich and Longcore 2006; RCEP 2009, International Dark Skies Association 2010), systematic data appraising the ecological effects of artificial light at night (ALAN) is still lacking (Hotz 2008; Perkin *et al.* 2011). Globally there is currently a 6% annual rise in artificial night lighting (Hölker *et al.* 2010b), thus it is important to consider how light is impacting the environment and how these impacts can be managed effectively. Due to the increase in street lighting over the past century, many freshwater ecosystems in the UK are subject to ALAN, yet the physiological and behavioural impacts on freshwater species are still largely unknown (Riley *et al.* 2013). Indeed, despite their importance biologically, freshwater ecosystems are likely the most heavily impacted ecosystem on earth as a result of anthropogenic pressures (Revenga *et al.* 2005). In the UK, freshwater habitats are the most heavily degraded ecosystem and, as such, there are no pristine freshwater ecosystems remaining according to the latest national ecosystem assessment (UK NEA 2011).

A recent review highlighted the need for research into the impact of ALAN on freshwater ecosystems (Perkin *et al.* 2011), suggesting that any disruption to freshwater invertebrate behaviour, in particular, could have far reaching ecosystem consequences. Thus, it is important to understand the influence of ALAN on the behaviour of freshwater invertebrates in order to mitigate against the unwanted environmental impacts of urban development.

Invertebrate drift is the downstream, primarily nocturnal, movement of aquatic invertebrates within the water column of flowing water systems (Johansen *et al.* 2000) and is considered a defining characteristic of this habitat (Leung *et al.* 2009). Drift behaviour differs between taxa; each with its own daily periodicity or distance of movement, and this can be further divided by the life-cycle stage (Brittain and Eikeland 1988). This drifting behaviour of freshwater invertebrates allows for both habitat selection (Muller 1974) and avoidance of predators (Flecker 1992) and can be divided into passive and active drift (Brittain and Eikeland 1988; Johansen *et al.* 2000). Passively drifting animals

are carried by the current after being dislodged from the benthos (Bishop 1969), whilst active drifters choose when to drift and for how long. Since active drift must be induced behaviourally, it occurs in response to one or more stimuli that can include food abundance, the presence of predators, changes in water quantity or quality and, importantly, light quantity or quality (Loose and Davidewicz 1994; Hieber *et al.* 2003; Blachowiak-Samolyk *et al.* 2006). These cues are all likely to interact, to produce the observed behavioural patterns of drift (Hieber *et al.* 2003; Blachowiak-Samolyk *et al.* 2006).

Evasion of visually foraging predators may be an explanation for the nocturnal drifting behaviour in invertebrates (Lampert 1993; Loose and Davidewicz 1994). Through the strategic use of this plastic behavioural mechanism, actively drifting invertebrates attempt to maximise their foraging opportunity with the least risk of predation (Ringelberg 1991). It is light intensity, however, that appears to be the critical factor controlling the activity patterns of invertebrate species (Ringelberg 1987; Haney 1993) as the overall activity of invertebrates peaks after the onset of darkness (Holt and Waters 1967; Chaston 1969; Bishop 1969; Allan 1995; Gal *et al.* 1999; Moore *et al.* 2000). Much work has focussed on determining the level of light that triggers the onset of nocturnal active drift (Chaston 1969; Brittain and Eikeland; 1988, Allan 1995). At least in streams where invertebrates are at risk from drift-feeding predators, drift is initiated as light intensity decreases from daylight levels to approximately 0.01 lux, equivalent to bright moonlight (Bishop 1969; Brewin and Ormerod 1994; Martin 2010), and ceases once light reaches 5 lux, equivalent to late twilight (Muller 1965; Martin 2010). It appears, however, there is considerable variation in the level of light that can be seen to suppress drift and previous studies have put this value between 1 and 5 lux (Muller 1965; Chaston 1969; Holt and Waters 1967; Brittain and Eikeland 1988; Allan 1995). Intensity has been suggested to be the light property that controls drifting behaviour, whereas differences in wavelength are not thought to do so (Bishop, 1969; Chaston 1969). It is therefore expected that the modification of their natural environment with the addition of ALAN will alter their normal drifting behaviour (Bruning *et al.* 2011).

A number of studies have found that the active drifting behaviour of nocturnal aquatic invertebrates is suppressed by the addition of ALAN (Meyer *et al.* 2013; Perkin *et al.* 2014a,b; Henn *et al.* 2014). Here, the natural the environmental cue that ordinarily triggers drifting behaviour is being masked by the addition of ALAN (Moore *et al.* 2000). A suppression of drift in invertebrates, to near daytime levels, has been demonstrated under a continuous ALAN regime both in field and lab experiments (Waters 1966). Further, the role of ALAN has been investigated in a number of freshwater stream systems; in laboratory (Chaston 1969; Perkin *et al.* 2014a) and field contexts (Holt and Waters 1966; Moore *et al.* 2000; Perkin *et al.* 2014b,c; Henn *et al.* 2014), and in both temperate (Perkin *et al.* 2014b,c) and arid (Henn *et al.* 2014) climes. ALAN has been found to reduce the number of drifting invertebrates between paired reaches of control and experimentally lit streams (Perkin *et al.* 2014b; Henn *et al.* 2014). Conversely a study by Perkin *et al.* (2014a) found there to be no impact of ALAN on the nocturnal drifting behaviour of *Gammaridae.* The impact of ALAN on drift in different taxa remains to be formally tested; however, the aforementioned studies are indicative of a family-specific response to light.

Despite the apparent suppressive effects of ALAN on drift, some studies have reported positive phototatic responses to ALAN (Bishop 1969; Hansson *et al.* 2007). In both marine (McConnell *et al.* 2010) and freshwater (Mamcarz 1995) environments, ALAN has been shown to increase the local abundance of aquatic invertebrates. Further, in regions north of the Arctic Circle, summertime day length is near continuous (Johanssen *et al.* 2000). Here, under continuous daylight, the number of drifting invertebrates does not differ between day and night (Johanssen *et al.* 2000). This pattern is suggestive of continuous light interfering with the diel periodicity of the invertebrates' behaviour, though it may also represent a local adaptation influenced by biotic factors such as predation risk (Hays 2003; Hansson *et al.* 2007).

The impact of ALAN on the behaviour of invertebrate species will have consequences for their predators, prey and competitors, and will likely alter the community composition of the streambed (Smith *et al.* 2009). Firstly, the effect of ALAN on predator-prey dynamics warrants investigation. If

zooplankton were to decrease their drifting behaviour as a result of ALAN, this could have a negative effect for the drift-feeding fish that rely upon them as a food source (Leung *et al.* 2009; Brunning *et al.* 2011). Thus, the nature and timing of changes in ALAN may be influential in determining the availability of this food source to the fish (Bramm *et al.* 2008). Further, street lighting has recently been shown to alter the community composition of terrestrial invertebrates - a result that the authors suggest will lead to changes in ecosystem functioning (Davies *et al.* 2012). The suppression of freshwater invertebrate drift and changes to invertebrate diversity/community composition could also have far-reaching community and ecosystem impacts, which as yet have remained unstudied. Invertebrates are commonly used in stream-wide health assessments (Wallace and Webster 1996, Merrit *et al.* 2002), as they are good indicators of localised conditions (Barbour *et al.* 1999). Invertebrates are particularly good indicators of ecosystem decline due to their intermediate position within the food chain, meaning they are affected by both bottom-up and top-down changes (Wallace and Webster 1996).

As a means to assess the potential for ALAN to disrupt community composition this study will use functional feeding groups (FFG), a grouping that assigns families to guilds based on their feeding behaviour rather than their taxonomy. If ALAN affects the community composition and diversity of species across FFGs then this could impact upon the functioning of freshwater ecosystems and species interactions. Thus, when considering the implication of ALAN on the freshwater phase of the Atlantic salmon lifecycle, and in all fisheries management it could be argued, it is important to consider the implications that habitat modifications, such as ALAN, will have for ecosystem health and function, and FFG are a widely accepted proxy (Wallace and Webster 1996).

This study is the first to assess the efficacy of one of the proposed management techniques for mitigating ALAN - part lighting or “trimming” (RCEP 2009; Gaston *et al.* 2014a,b; see Chapter 1) through experimental investigations. A recently published study modeled the peak activity of a bat species (*Rhinolophus ferrumequinum*) in a streetlit area and predicted, due to peak timings of activity, part lighting would not be a suitable management technique to minimise the influence of ALAN in

bats (Day *et al.* 2015). Given the 3% annual rise in ALAN in the UK, any attempt to stem this increase would be beneficial in order to maintain natural, unlit areas (Gaston *et al.* 2012), however, when implementing a management strategy, there must be consideration of both human safety and the environmental impacts (Falchi *et al.* 2011). This technique was selected as being one of the most viable options, since attempts to reduce the whole night ALAN is often met with resistance from people who use the artificially lit areas. ALAN is associated with a sense of security and wellbeing and as such, their use in urban, suburban and even periurban areas is entrenched in our society (Hölker *et al.* 2010a; Perkin *et al.* 2011).

3.3 Aims and Hypotheses

The aim of this study is to examine the effect of ALAN on the nocturnal activity of freshwater invertebrates. This is primarily conducted with a view to determining how ALAN might affect the food source of drift feeding fish, such as Atlantic salmon and also to examine how ALAN may impact ecosystem health. This study will contribute towards the overall aim of the thesis by meeting the following objectives: (1) determine the impact of light on invertebrate drift; (2) assess the efficacy of a proposed management strategy (part-lighting), and; (3) deduce how predator-prey dynamics and ecosystem health may be influenced. The chapter hypothesises that: (1) ALAN will reduce the family level richness of the drift when compared with control nights; (2) ALAN will reduce the total number drift abundance when compared with control nights; (3) Periodicity of the drift will differ between lighting regimes of each treatment; (4) ALAN will differentially affect families within the drift, and; (5) Part-night lighting (trimming) will reduce the impact of ALAN compared to whole-night lighting.

3.4 Materials and Methods

3.4.1 Field study design

All experimental work was conducted at a light naïve (permanently unlit at night) section of the Llanmaes Brook at Llanmaes, Vale of Glamorgan, UK (29°80'31"E, 16°94'44"N) over a nine-day period in June 2013. A high-pressure sodium lamp (Phillips SON-T Pro 70w), as used widely in the local area for street lighting, was mounted on a steel tripod and positioned centrally across the width of the river, 1 m above the streambed. The light was powered using mains electricity (240 volts). Neutral-density filters (E-Colour+ #298 0.15, transmission = 69.3%; Rosco Laboratories Inc., Stamford, Connecticut, USA) were attached to the lamp to reduce the intensity of the light without altering its frequency. This was to ensure that the experimental light intensity corresponded with light levels measured in existing street-lit stream settings (Riley *et al.* 2013).

Invertebrate drift was measured on nine consecutive nights. One of three differing lighting treatments was applied to the stream at night as follows: (1) unlit (i.e. natural moonlight only), acting as the control; (2) fully-lit for the whole duration of the sampling period (21.30-03.30 BST the following day), and; (3) partly-lit (21.30-00.30) (Fig. 3.1).

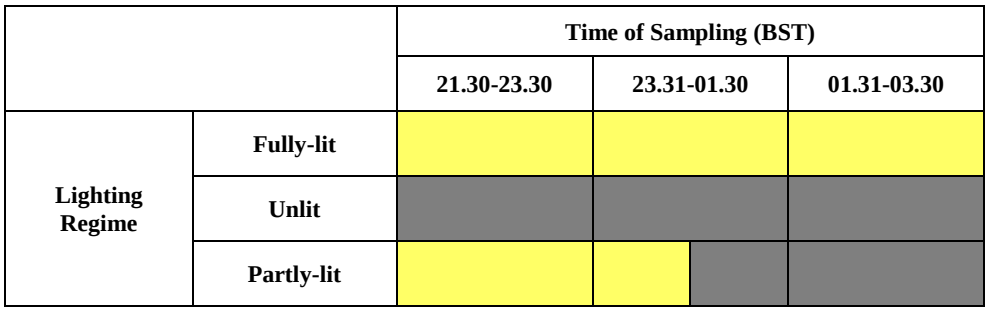


Figure 3.1 Schematic representation of the lighting regime during the three experimental sampling periods for each of the three lighting regimes: grey represents control conditions (<0.1lux) and yellow represents experimentally lit conditions.

The durations of the ALAN regimes were based on the lighting regime used by council-operated streetlights in the surrounding area for the time of year when the study was conducted. All treatments

were replicated three times, to quantify and control for variation due to environmental or lunar factors. The treatments were conducted on successive nights and the order of each treatment was randomised in order to minimise any longitudinal sampling effect.

3.4.2 Invertebrate sampling

Four drift nets (500µm mesh size, EFE and GB Nets, Cornwall) were stationed either upstream or downstream of the HPS light (Fig. 3.2). Three experimental nets were placed at 2 m, 2.25 m and 2.5 m downstream of the streetlight. One net was stationed 2 m upstream of the streetlight, in an unlit reach; this was intended to act as a reference (always unlit) net to take into account the background level of drift when comparing drift sampled by the experimental nets in each of the lighting treatments. The three downstream nets were positioned in a staggered arrangement across the stream, to ensure (1) that the nets did not intercept drift that could enter another net, and (2) so that the area in front of all nets was illuminated by the streetlight without any net casting shadows on the stream bed upstream of a neighbouring net. It was also important that all nets could be accessed from behind, downstream of the light, so that they could be emptied without disturbing the streambed in front of the nets.

Drift nets were emptied every 2 hours during the nocturnal sampling period at 23.30, 01.30 and 03.30 BST (Fig. 3.1), to enable shifts in patterns of nocturnal drifting behaviour to be identified. The sampling period commenced at 21.30 BST at sunset (sunset 21.29-21.34 BST) when the local streetlights turned on, and concluded at 03.30 BST (sunrise 04.56-04.57 BST). The final sample was taken at 03.30 as the timing of sunrise meant a full two-hour nocturnal sampling period could not be achieved.

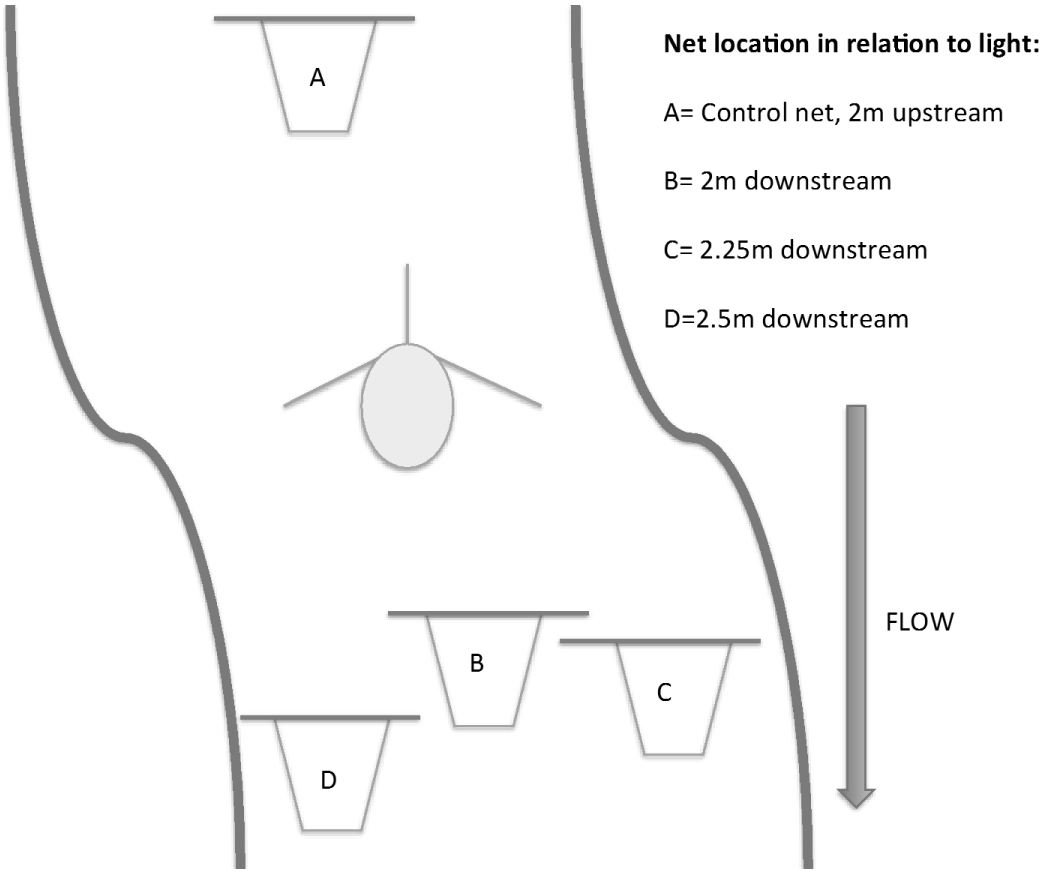


Figure 3.2 Schematic representation of the experimental field set-up, outlining the position of nets A-D in relation to the light source.

Light measurements were taken with a lux light meter (HANNA instruments HI 97500, accuracy ±6%, to 0.01 lux) at the 1.30am sampling point. This was chosen, as it was the middle of the sampling period and the naturally darkest point of the night. Mean light intensities across the four nets for 1 m upstream of the net and directly in front of the mouth of the net are given below for both experimental treatments, fully-lit and partly-lit, and the control unlit treatment (Table 3.1). Due to constraints in the field set-up, light intensities 1 m upstream of drift nets B-D were higher than would commonly be experienced in a street-lit setting. The light intensities at the mouth of the nets, however, were representative of natural field settings; thus, in this experiment sampling occurs across a steep light gradient (1m – mouth of the net). This is an exploratory study and thus if no effect of ALAN is seen across this high light gradient, then it would be unlikely to have an effect at lower intensities.

Light intensity measurements were taken 1m upstream of the drift nets, as under low flow conditions invertebrates are thought to drift less than 2m on average (Townsend and Hildrew 1976). The

minimum light intensity that could be measured on the lux meter was [<] 1 lux and as such this is the lowest resolution possible for the control light intensities. Due to the low flow conditions of the stream, flow measurements were not readily measurable using a flow meter (Tamar Digital Stream Meter/EFE and GB Nets, Cornwall), and so statistical modelling controlled for variations in the background (un-manipulated) level of drift, using Net A as an upstream (unlit) control.

Table 3.1 Light intensities for all nets, measurements taken at both the mouth of the net and 1m upstream. Net A is always a control net with no exposure to ALAN regardless of experimental treatment and nets B-D are experimental nets that will differ in lighting regime dependent on the treatment night. The location of nets B-D was fixed, thus they were always placed at 2, 2.25 and 2.5 from the light respectively.

	Unlit (lux)		**Partly- and Fully -lit (lux)**	
Net	**1m upstream of net**	**Mouth of net**	**1m upstream of net**	**Mouth of net**
A	<1	<1	<1	<1
B	<1	<1	170	13.5
C	<1	<1	76.5	7
D	<1	<1	54	2.5

3.4.3 Analysis of samples

Drift samples were transferred to lidded pots and preserved in 70% ethanol on site. Each sample was sifted and the total number of drifting invertebrates in each sample was counted. Identification was conducted under a dissecting microscope at 6x magnification. The invertebrates in all samples were identified to family level to allow for analysis of family-level richness under each experimental treatment.

Finally, families were grouped into functional feeding groups (FFG) (Appendix 1). Species were assigned to one of six functional groups; Shredders, Filterers, Predators, Parasites, Grazers and Gatherers (Moog 1995). Based on the classifications of Moog (1995), families that displayed tendencies of multiple FFG were handled in one of two ways: (i) those that displayed a clear preference for a given FFG were assigned to the group in line with their highest affinity and (ii) for those families whose tendencies were split evenly between two or more FFG, the invertebrate

abundance was split equally between the groups. Where a clear affinity was present the first method was employed in order to simplify the family-level allocations and allow for any treatment effect to be readily identified. Since the use of joint-assignments has been said to blunt the sensitivity of the FFG approach (MacNeil *et al.* 1997; King *et al.* 1988), this joint-assignment approach was only used when no clear affinity was present. This allowed the impact of ALAN on community composition and prevalence of species with different feeding strategies to be determined.

3.4.4 Statistical analysis

All statistical analysis was conducted in R (Version 2.13.2, R Development Core Team 2012). Factors influencing the number of invertebrates and the species richness in each drift sample were evaluated using a Generalised Linear Model (GLM) based on a Poisson error distribution where computationally possible, and otherwise a Gaussian error distribution. Separate models were used to model the abundances of drifting invertebrates in each family and FFG (dependent variable). Independent variables were: Date, time of night, location of the drift net, and treatment and each two-way interaction between independent variables was also included in the initial model. The models were refined using the "dredge" function (available in the "MuMin" R package), which selects the best (statistically most plausible) final model from a "saturated" starting model containing all independent variables and biologically plausible interactions, using Akaike information criteria (AIC). The models used the drift sampled by Net A as an offsetting variable, to control statistically for variation in the background level of drift between and within nights, to ensure that any apparent differences in flow in the downstream experimental nets (B-D) were due to the different lighting treatments and not due to variations in the background (unmanipulated) drift that could be caused by factors such as variation in stream flow.

3.5 Results

3.5.1 Species richness and drift abundance

A total of 6,670 drifting individuals were caught over the duration of the experiment, representing 51 families (Appendix 2). The most common invertebrate families found in the samples taken were Baetidae and Gammaridae, composing 36% and 25% of the overall count, respectively. Other well-represented families in the samples were Helophoridae (11%), Chironomidae (8%) and Simuliidae (6%). The most commonly sampled invertebrates and their mean numbers across each net and treatment are summarised below (Table 3.2). These were found to make up >96% of the total drift abundance in all samples. The remaining invertebrates were made up of several much less common families (Appendix 2).

Table 3.2 Total numbers of drifting invertebrates, in each lighting treatment (Fully lit, Unlit and Partly-lit; n=3 replicate nights in each case). Data are presented for the ten most abundant invertebrate families and are given separately for each light treatment and net (Control, 2m, 2.25m and 2.5m). For clarity, control conditions are shaded. Total drift abundance, Shannon diversity index and Family level species richness are given for the total sample (Appendix 2).

	Control			B			C			D		
Species	**Fully lit**	**Unlit**	**Partly-lit**	**Fully lit**	**Unlit**	**Partly-lit**	**Fully lit**	**Unlit**	**Partly-lit**	**Fully lit**	**Unlit**	**Partly-lit**
Mesovilidae	4	4	13	6	1	4	3	1	7	0	2	5
Flatworm	8	5	13	0	1	2	2	5	6	2	2	6
Psychodidae	1	0	5	1	0	7	2	2	14	1	0	10
Hydrachnidae	16	15	14	19	6	7	16	26	22	12	13	19
Elmidae	6	14	114	13	11	47	12	8	47	8	10	39
Simuliidae	7	11	30	44	35	65	23	20	48	17	47	51
Chironomidae	29	5	47	59	42	94	37	60	88	29	42	75
Helophoridae	27	11	357	6	6	42	29	9	90	4	11	123
Gammaridae	45	42	61	98	99	180	130	274	426	53	109	164
Baetidae	90	69	57	375	316	188	231	320	228	98	234	166
Total	**243**	**178**	**734**	**631**	**522**	**650**	**498**	**729**	**996**	**235**	**479**	**668**
% accounted for by most abundant families	96.8	97.3	98	99.8	99.6	98.8	97.6	99	97.6	98.3	98.6	98.1
Total Drift Abundance	251	183	749	632	524	658	510	736	1021	239	486	681
Shannon Diversity Index	2.055794	1.917232	1.867811	1.395168	1.283297	1.924239	1.714492	1.40726	1.878792	1.815559	1.607492	2.02763
Family Level Species Richness	19	15	28	16	13	24	20	19	31	19	21	22

General Linear Modelling (GLM) revealed there to be no significant impact of light treatment on family level richness, with the control treatment not being significantly different from the experimentally lit treatments (Fig 3.3a; Table 3.3). H1 predicted that ALAN would reduce the taxonomic diversity of the drift, however this does not appear to be the case: species richness was not significantly changed in either the partly-lit treatment or the fully lit treatment when compared with the unlit treatment. Total drift abundance was also not significantly different between treatments, although treatment was retained as a variable in the most plausible model (Fig 3.3b; Table 3.3). H2 predicted that ALAN would reduce the total number of drifting invertebrates; however, as with the family level richness/diversity effects, there was no significant difference between the control and the experimentally lit treatments.

Table 3.3 Results of the Generalised Linear Models (GLM) used to evaluate the effects of treatment, time and net on the drift abundance and family-level richness found within the drift samples. Terms included in the final GLMs are shown in the table. Significant P values (P <0.05) are emboldened.

Category	Term	Df	LRT	P	Explained deviance
Drift Abundance	Day	1	6.6053	**0.01210**	0.132139
	Treatment	2	2.5593	0.08392	
Family-level Richness	Day	1	4.3040	**0.038023**	0.233065
	Time	2	13.2806	**0.001307**	
	Net	2	5.1107	0.077664	

Both the total drift abundance and family level richness were found to vary by day (Table 3.3) and family level richness was also seen to vary across the sampling period and net (Table 3.3). The family level richnesses of the drift at 23.30 and 3.30 were significantly different from the drift at 01.30 across all nets.

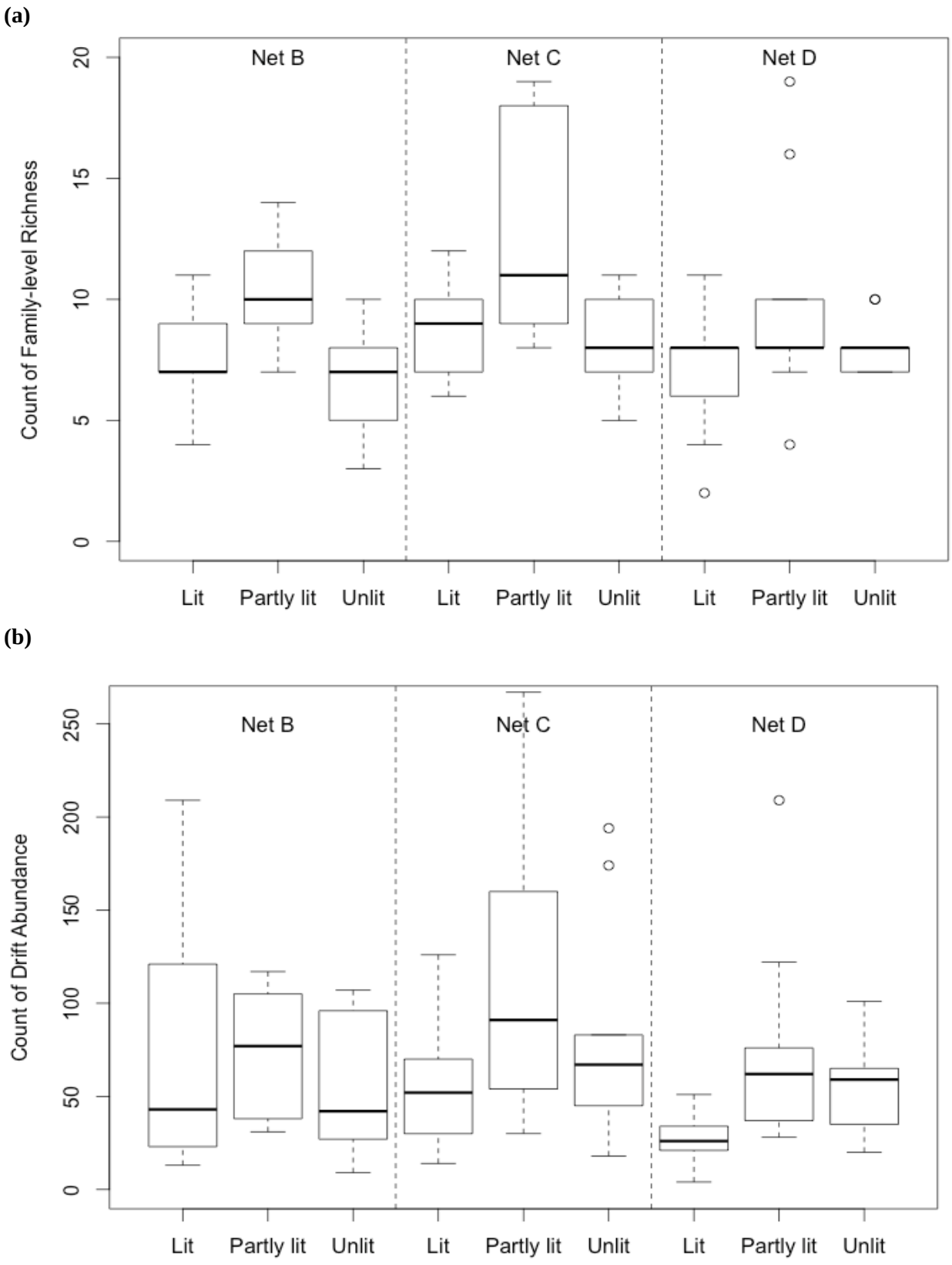


Figure 3.3 The mean counts of (a) family-level richness and (b) drift abundance across the total number of invertebrates caught, by treatment and net across the three replicate nights.

3.5.2 Family-level responses

The total numbers of the most prevalent invertebrate family in the samples, Baetidae, were not significantly affected by the treatment, however, Baetidae were significantly affected by time of night, day and net (Table 3.4).

Table 3.4 Results of Generalised Linear Models (GLM) used to evaluate the effects of treatment, time and net on the number of invertebrates from each taxa found within the drift samples. Terms included in the final GLMs are shown in the table. Significant P values (P <0.05) are emboldened.

Taxa	Term	Df	LRT	P	Explained deviance
Gammaridae	Treatment:Day	2	2.836	0.657	0.554
	Treatment:Time	4	3.851	**0.007**	
	Net	3	11.254	**<0.001**	
Baetidae	Net	2	2.840	0.065	0.373
	Time	2	6.901	**<0.001**	
	Day	1	25.131	**0.002**	
Chironomidae	Treatment	2	4.118	**0.021**	0.149
	Time	2	2.552	0.085	
Simuliidae	Time	2	2.744	0.071	0.066
Helophoridae	Treatment	2	6.923	**0.002**	0.195
	Time	2	2.267	0.111	

Whilst variation in abundance between nets was found to be significant in the GLM for Gammaridae, there were significantly more gammarids in Net C when compared with Nets B and D (Table 3.4).

Unlike Baetidae, however, Gammaridae were significantly affected by the lighting treatment, with the significance of the effect dependent on time (Table 3.4; Fig 3.4). The numbers of drifting Gammaridae across the sampling period differed between the treatments at the different sampling points (Fig. 3.4a). This is supportive of H3, which predicted that periodicty of the drift would differ between the lighting treatments.

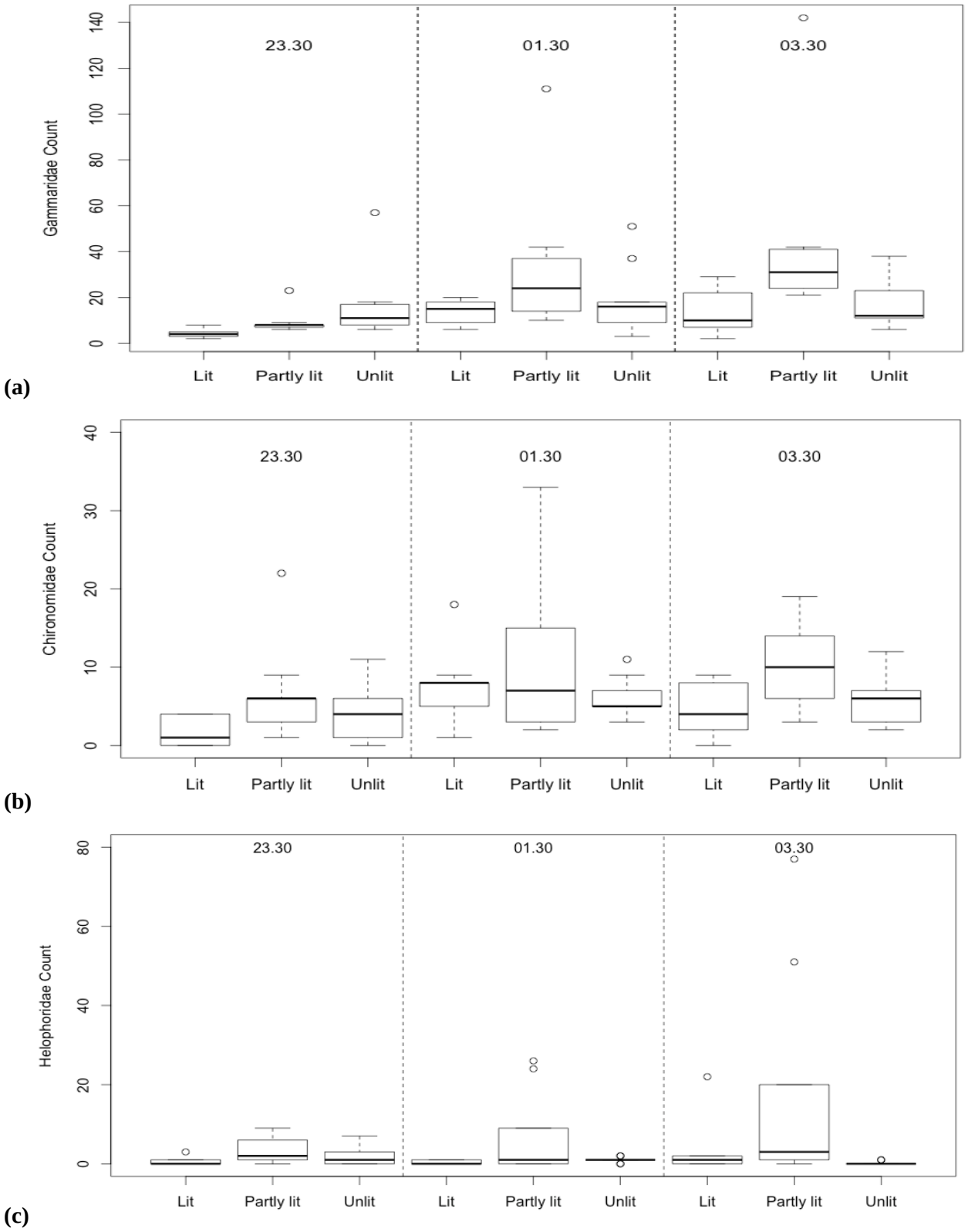


Figure 3.4 Abundance of (a) Gammaridae, (b) Chironomidae and (c) Helophoridae, by lighting treatment and time, mean value across the three replicate nights and experimental nets B-D.

This is also the case for the next two most abundant families within the drift; both Chironomidae and Helophoridae were significantly affected by treatment and can be seen to increase in the partly-lit treatment (Table 3.4; Fig. 3.4b, 3.4c). Both families were also further significantly affected by time (Table 3.4). Finally, Simuliidae were not significantly affected by treatment, however, the number of drifting simulids were significantly different across the different sampling times (Table 3.4). The difference between the taxa in whether their drifting behaviours are affected by the experimental light regimes is indicative of family-specific responses to light.

3.5.3 Functional feeding groups (FFGs)

In order to test H4, which predicted ALAN would alter community composition of the drifting biota, when compared with control nights, the proportion of invertebrates in each of the functional feeding groups (FFG) was examined between treatments. The greatest numbers of families were assigned to the gatherer and predator groups, whilst the fewest numbers of families assigned as parasites (Appendix 1). The numbers of invertebrates present in the drift samples assigned to each of the FFG were seen to vary between treatments and nets (Fig. 3.5). Gatherers were the most abundant group across all experimental treatments and nets, with parasites being the least abundant (Fig. 3.5). Despite predators being the second most family rich FFG found within the drift (Appendix 1), they are one of the least abundant groups (Fig. 3.5).

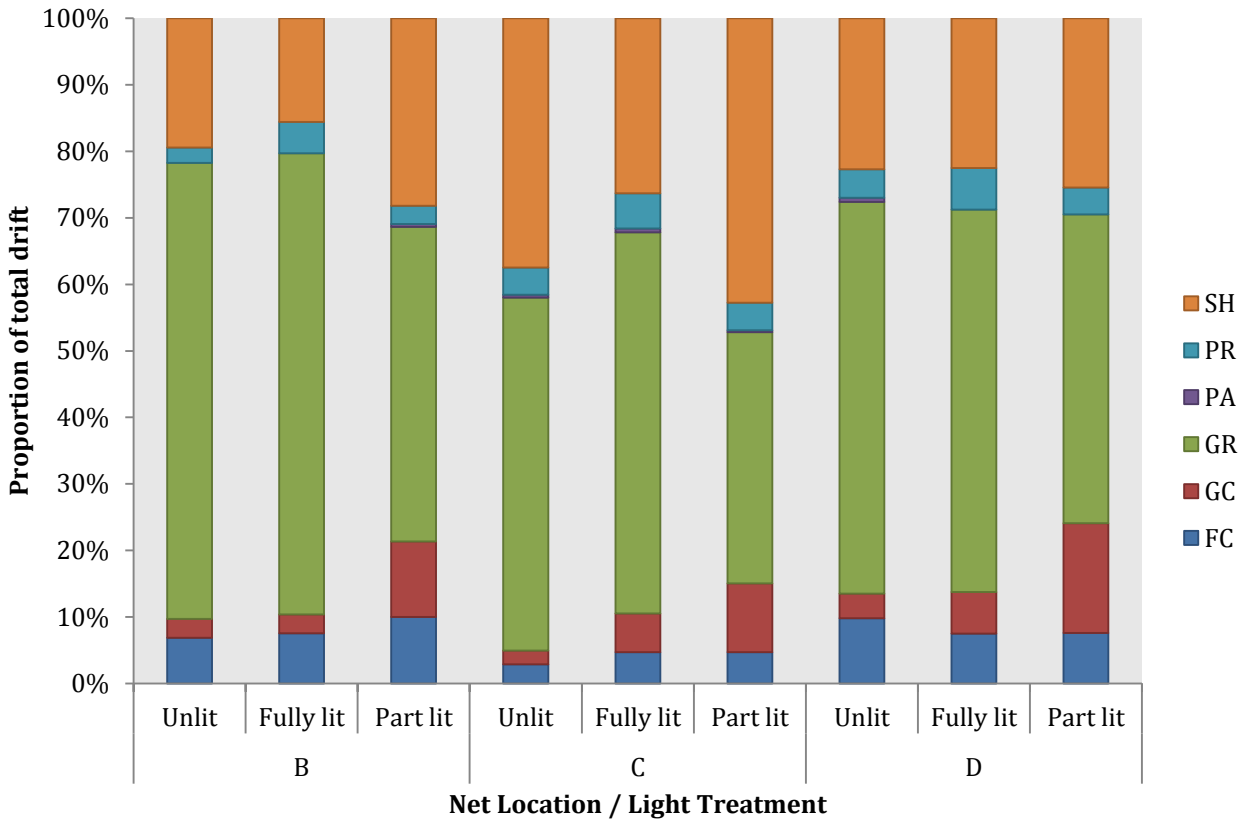


Figure 3.5 Percentage of each functional feeding group (FFG) for each treatment, by net, mean number across the three replicate nights. SH=Shredders, PR=Predators, PA=Parasites, GR= Gatherers, GC=Grazers, FC=Filterers.

The counts of invertebrates assigned to each of the FFG sampled from the drift varied by treatment (Fig. 3.6 a-e). General Linear Modelling (GLM), including two-way interaction terms (Table 3.5), determined that the influence of ALAN on the drifting behaviour of the different FFG was complex. Experimental light treatment had a significant effect on the drifting behaviour of grazers (Table 3.5; Fig. 3.6b), parasites (Table 3.5; Fig. 3.6c) and shredders (Table 3.5; Fig. 3.6f), but did not impact the numbers of the remaining FFG sampled from the drift. Grazer numbers were significantly increased in the partly-lit treatment when compared with the unlit treatment, whilst there was no significant difference seen between the unlit and the fully lit treatments (Fig. 3.6b). For shredders, there was no significant difference between the treatments; however, the overall treatment effect was significant (Fig. 3.6f). In the case of predators the treatment effects are being masked by variations in the time term, as revealed by the 2-way interactions in the GLM (Table 3.5; Fig. 3.6d)

Table 3.5 Results of Generalised Linear Models (GLM) used to evaluate the effects of treatment, time and net on the number of invertebrates from each FFG found within the drift samples. Significant p values ($p<0.05$) are in bold.

Functional group	Term	Df	LRT	P	Explained deviance
Filterers	Time	2	2.5548	0.0842	0.0614796
Grazers	Treatment	2	7.3864	**0.001154**	0.159237
Parasites	Treatment	2	8.0207	**0.0006911**	0.3192058
	Time	2	0.6658	0.5168126	
	Treatment:Time	4	3.5203	**0.0111**	
Predators	Treatment	2	1.5811	0.21243	0.2962232
	Time	2	3.2046	**0.04612**	
	Treatment:Time	4	4.7155	**0.001951**	
Gatherers	Treatment	2	3.0573	0.05268	0.07269472
Shredders	Treatment	2	4.8363	**0.0105417**	0.3922014
	Time	2	5.9960	**0.003949**	
	Net	2	7.7472	**0.0008668**	
	Net:Treatment	4	1.1318	0.348658	

The number of filterers, grazers, predators and shredders present in the drift was found to differ significantly by time of night in all cases and the number of shredders was also found to differ between nets (Table 3.5). Differences between the nets (B-D) are indicative of a local effect of light intensity, as the closer to the light the net is (Fig. 3.2) the greater the lighting intensity when the light is on. This suggests that shredders respond differently to the lighting treatment depending on the intensity of the light itself.

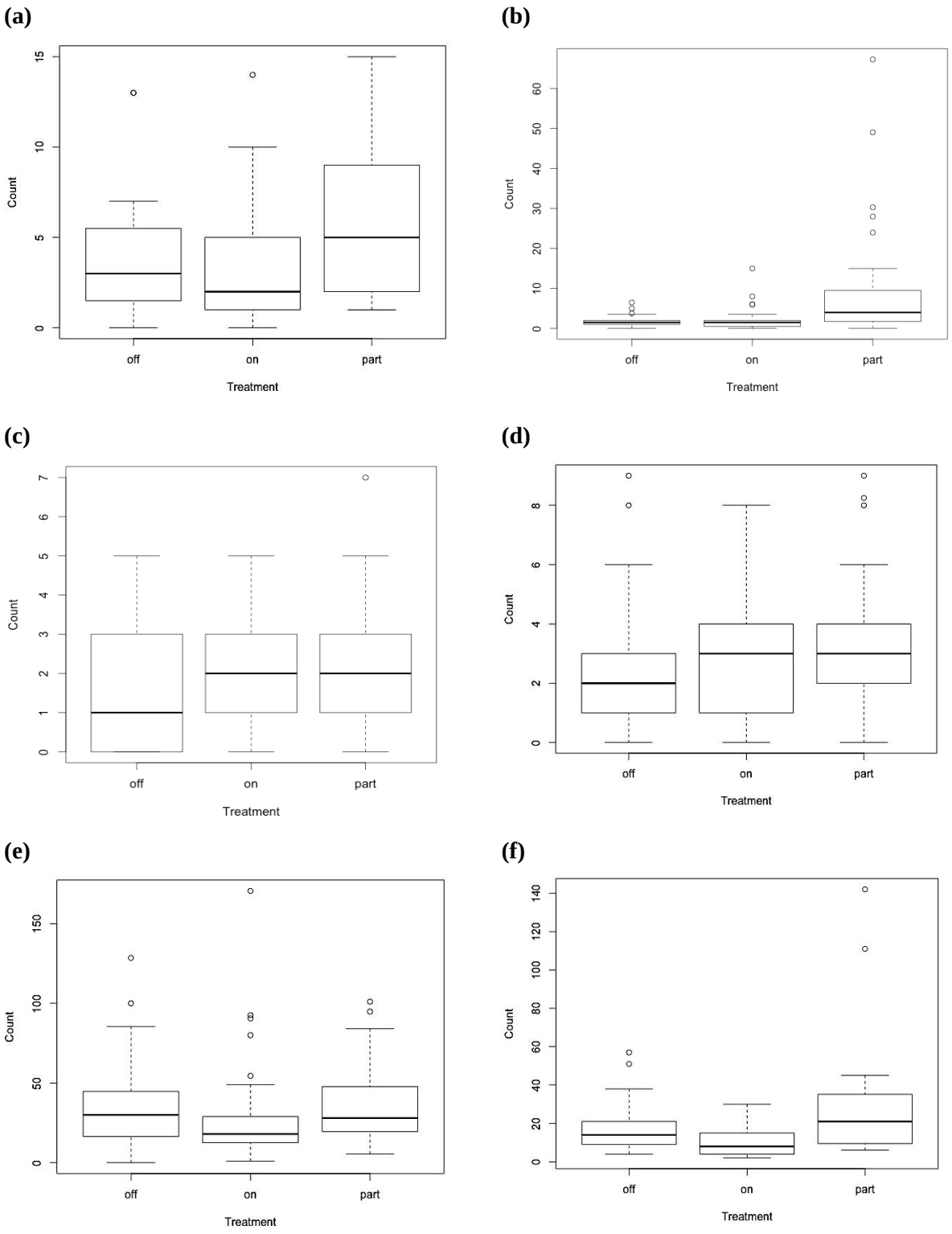


Figure 3.6 Total number of invertebrates sampled from each of the functional feeding groups (FFG) within the drift by treatment **(**a) Filterers, (b) Grazers, (c) Parasites, (d) Predators, (e) Gatherers and (f) Shredders. Boxplots show median number across the experimental nets and replicate nights for each lighting regime.

When simply looking at the treatment effect, the positive response to part lighting of both grazers and shredders, and the non-significant result of the remaining groups, is indicative of light influencing the behaviour of the FFGs differently. This therefore suggests that ALAN will affect the community composition of the drift, through changing the drifting behaviour of invertebrates from different FFG within the streambed community.

3.6 Discussion

The drifting behaviour of freshwater invertebrates appears to have a complex response to ALAN. On the one hand ALAN was not found to decrease either the family level richness of the drift (H1), nor the total number of drifting invertebrates (H2) and the periodicity of the drift did not differ between lighting treatments (H3). On the other hand ALAN significantly and differentially affected different families (H4). The final hypothesis, that part-night lighting reduces the impact of ALAN compared to whole-night lighting (H5) was falsified as, contrary to expectations, part-night lighting had a greater effect than whole night lighting for the affected taxa. The data from the current investigation have identified the presence not only of a family specific response to light, but also of differing responses to ALAN between FFGs. These findings support previous studies in suggesting that the response to ALAN is taxon-specific (Meyer *et al.* 2013; Henn *et al.* 2014; Perkin *et al.* 2014 a,b).

Unlike the present study, ALAN has previously been found to reduce the drift abundance of invertebrates in reaches of artificially lit streams (Perkin *et al.* 2014b), where the number of invertebrates drifting in the lit reaches was approximately half that in the control reaches. When examining the response of individual taxa, a taxon-specific response is seen to light, with a difference in response between the two most abundant families Baetidae and Gammaridae. ALAN significantly affected Gammaridae drifting

behaviour in an interaction with time, whilst in Baetidae drifting behaviour was not significantly altered by ALAN. Interestingly, the number of Gammaridae in the drift was reduced at 23.30 under both the fully lit and partly-lit treatments, as hypothesised, but increased under the partly-lit treatment at 01.30 and 03.30 whilst they were unaffected by the fully lit treatment at these times. The same can also be seen in Chironomidae, whilst Helophoridae increase under part lighting at all three sampling times. Henn *et al.* (2014) found that the addition of ALAN significantly reduced the number of drifting invertebrates in large streams. Most notably, the numbers of drifting Simuliidae and Baetidae were reduced by 58% and 51%, respectively. However, as with this study, light was not found to influence the taxonomic diversity of the drift (Henn *et al.* 2014). These results contrast with the present study, where ALAN did not affect the numbers of both Baetidae and Simuliidae present in the drift samples. Henn *et al.* (2014) conducted their study in an arid region of North America, thus individual species and/or climatic differences may account for the differences seen between that study and the present one.

Further, Perkin *et al.* (2014a) examined the influence of ALAN on the drifting behaviour of *Gammarus* spp. under ALAN in experimental flumes and found no impact of ALAN on the nocturnal drifting behaviour of this taxon across the light gradient. In this present study, light was found to have a significant effect on the numbers of drifting Gammaridae in an interaction with time. Perkin *et al.* (2014a) sampled at four points in a 24-hour period, with two sampling points during "night" in their experimental set-up taken between 16.00-08.00. For this reason, it is possible that any effect of treatment across time may have been lost over such a large sampling interval, and had they sampled at more regular intervals an effect of treatment in an interaction with time may have been seen for the *Gammarus* spp.

The number of drifting Gammaridae were also found to differ by net, and thus in relation to light intensity. The effect of increasing light intensity, however, was not shown simply to increase or decrease the number of drifting invertebrates, and is more likely a result of local differences in gammarid abundance. This is suggestive that light has an effect, which is not increased with further increasing ALAN intensity.

This has previously been seen in response to ALAN in dispersing Atlantic salmon (*Salmo salar*) fry (Riley *et al.* 2015). The nocturnal dispersal behaviour of fry was found to be disrupted by ALAN at an intensity of between 0.1 and 1 lux, with little additive impact as light intensity increased to 8 lux. It is, however, important to note that there are likely differences between the number and composition of drifting invertebrates between the drift nets placed at different locations across the stream. That said, the experimental design and statistical analysis was designed to determine differences in invertebrate drift abundance and composition between light treatments and not between the different drift nets.

The results here also present the first experimental evidence of the impact of the proposed management technique of part-night lighting on the drifting behaviour of the aquatic freshwater invertebrate assemblage. The investigation demonstrates that part lighting does not act to reduce the impact of ALAN, as intended. In fact, part lighting has a greater impact on the drifting freshwater invertebrates than full night lighting. This surprising finding is important, as ALAN is increasing in the UK at a rate of 3% annually (RCEP 2009) and there is a growing realisation that nocturnal environments must be protected from unwanted, potentially damaging effects of ALAN. Whilst part lighting is intended to reduce the negative ecological impacts of ALAN (Gaston *et al.* 2014a,b), the results presented in this study suggest that part lighting of our nocturnal environment has a greater impact on the behaviour of certain freshwater invertebrates than full night lighting. In nocturnal invertebrate species there are two main types of drifting behaviour, "bigeminus" and "alterans" (Brittain and Eikeland, 1988). Both represent two peaks in nocturnal activity; a bigeminus pattern is a larger peak after sunset and a smaller peak before sunrise and vice versa for an alterans pattern. From these drifting patterns, it can be seen that the exogenous stimulus for the initiation of this active drifting behaviour is light (Holt and Waters 1967; Bishop 1969; Chaston 1969). That part lighting has a greater effect on certain taxa than full lighting is possibly due to the period of lighting corresponding with the two peaks in invertebrate activity, after sunset and before sunrise (Brittain and Eikeland 1988). Whilst the impact of part lighting is greater, its effect is, however, opposite to the effect originally predicted - although the cause for this is unclear.

The current investigation has identified the presence not only of a family specific response to light, but also differing responses to ALAN between functional groups. Lighting treatment was found to alter the drifting behaviour of species from FFGs. When considering the main effect of lighting treatment, the most apparent impact can be seen in shredders and grazers. Of the two artificial-lighting treatments, part lighting can be seen to have the greatest impact on these two FFGs, as counts of invertebrates assigned to these two categories are significantly higher under this lighting regime. Of the remaining FFG, parasites have a treatment effect, predators have a treatment effect that is dependent on time of sampling, and gatherers and filterers are not significantly affected by light treatment.

This is believed to be the first study to demonstrate how ALAN differentially affects the behaviour FFG of invertebrates in a freshwater environment. However, ALAN has previously been shown to influence the composition of terrestrial invertebrates caught in pitfall traps (Davies *et al.* 2012) and the results of the present study are consistent with this investigation; whereby light impacts the nocturnal behaviour of certain FFGs. In the terrestrial study of Davies *et al.* (2012), the number of predators and scavengers in a terrestrial ecosystem are significantly impacted by ALAN whilst the remaining FFGs are not. The change in the number of drifting invertebrates in different FFGs, seen in this current investigation, is suggestive of light having the potential to disrupt ecosystem services and trophic interactions. This effect is shown to be dependent on time of night in predators and parasites and independent of both time and net in grazers and shredders, which suggests that any change in nocturnal lighting regime will differentially influence the drifting behaviour of the FFGs; with some increasing and others decreasing, the effect further dependent on the specifics of the ALAN regime. This will lead to changes in the composition of the drift as well as potentially in the streambed community composition.

The composition of drifting invertebrates from the different FFGs in freshwater can be used to assess the role of anthropogenic influences on ecosystem health (Masese *et al.* 2014). Any FFG- or taxon-specific behavioural changes may lead to community-level disruption (James *et al.* 2009). For example, Moore *et*

al. (2000) suggest that urban ALAN may have knock-on effects for water quality in urban areas, as invertebrate grazing is responsible for managing algal levels in riverine systems and if the abundance or behaviour of invertebrates are affected by ALAN, then algal blooms may result. It is important to exercise caution in the use of the FFG concept, due to the classifications being somewhat superficial and an oversimplification of trophic variability (MacNeil *et al.* 1997; see Chapter 7). In this study, however, FFG classifications were used as means of assessing how broad invertebrate grouping may differentially respond to ALAN and a whether this environmental change could potentially result in ecosystem-level changes, not as a rigorous assessment of ecosystem dynamics. Given that, in this investigation, light has been shown to affect the proportion of invertebrates drifting from different FFGs, this may be an appropriate tool to determine the longer-term ecosystem effects of ALAN in a freshwater ecosystem. Determining how ALAN influences the behaviour of different FFGs will allow an understanding of how light influences trophic relationships, organic-matter processing and energy flow, thus enabling the most effective mitigation to be implemented (Masese *et al.* 2014).

Given the wide variety of roles played by freshwater invertebrates (Wallace and Webster 1996) it is important for ecosystem function that biological diversity is maintained. If freshwater invertebrate communities are impacted by light, with individual taxa and FFG affected in different ways, this could have consequences for whole ecosystem functioning (Wallace and Webster 1996). Individual species are also used as bio-indicators of habitat degradation, particularly as a result of chemical pollutants. *Daphnia magna* (Tomasik and Warren 1996) and more recently *Gammarus spp*. (Gerhardt 2011) have been used in studies examining the effect of anthropogenic pollutants on freshwater ecosystems. The use of pollution-sensitive invertebrate species allows for a quick assessment of the health of a stream, as their sampling is relatively quick and low cost. Given that drifting behaviour of Gammaridae appears to be affected by light in the short-term, monitoring of the impact of ALAN could utilise this taxon/genus as an indicator species to determine the long-term impacts ALAN on freshwater ecosystems. Finally, FFGs are commonly used as surrogates for measuring ecosystem attributes (Merritt *et al.* 2002) and can be used to evaluate

ecosystem health (Merritt *et al.* 2002; Cummins *et al.* 2005). Given that, in this investigation, light has been shown to affect the proportion of invertebrates drifting from different FFGs, this may be an appropriate tool to determine the longer-term ecosystem effects of ALAN in a freshwater ecosystem. Determining how ALAN influences the behaviour of different FFGs will allow an understanding of how light influences trophic relationships, organic-matter processing and energy flow, thus enabling the most effective mitigation to be implemented (Masese *et al.* 2014).

The results of this short-term study allow us to infer how light influences the drifting behaviour of different invertebrate taxa and FFG; however, in order to determine the influence of light on the community composition of the streambed, long-term manipulation experiments would be required (see Chapter 7). Any disruption to the community composition and the volume and composition of invertebrate drift will have implications for the wider predator-prey dynamics in the freshwater ecosystem. Drifting invertebrates are the main food source of many salmonid fish and provide the link in the nutritional cycle between primary producers and higher trophic levels (Wallace and Webster 1996; Rader 1997; Leung *et al.* 2009).

In line with foraging theory, it has been shown that fish biomass can be correlated with drift abundance (Wilzbach *et al.* 1986; Shannon *et al.* 1996; Vehanen 2003). Rader (1997) developed a classification system for invertebrates based on two factors; their drifting behaviour and their significance in salmonid diets. As drift availability will have fitness implications for drift feeding fish (Leung *et al.* 2009), the abundance of drifting invertebrates can be used as a biological indicator of habitat quality for the fish (Nislow *et al.* 1998, 1999). Atlantic salmon parr (the freshwater residency phase in the salmon lifecycle), feed upon drifting invertebrates and feed primarily at dawn and dusk, likely in response to the abundance of prey since this is when invertebrate drift peaks (Valdimarsson and Metcalfe 1999, Amundsen 1999) but, also, as a predator avoidance strategy (Bédard *et al.* 2005)**.** The increase in the drifting behaviour of certain taxa and FFG under part-lighting may increase the opportunity for drift feeding in these fish,

depending on their dietary preference. Post young of the year (PYOY) Atlantic salmon are known to feed on Ephemeroptera, an order of insects containing baetids (Mookerji *et al.* 2004) and also simulids (Jonsson and Jonsson 2011), thus they may not be too adversely impacted by any changes to drift induced by ALAN since in this study baetids and simulids did not show any response to differing ALAN regimes. Young of the year (YOY) Atlantic salmon, however, are thought to feed on Chironomidae larvae and pupae preferentially (Jonsson and Jonsson 2011), which in this study were shown to be significantly impacted by ALAN, the extent to which is dependent on the lighting regime. Gammarids were also significantly impacted by ALAN, however *Gammarus* spp. are not a preferred prey species for wild juvenile Atlantic salmon in freshwater (Reiriz *et al.* 1998). Further, the foraging behaviour of salmonids may itself be affected as a result of the ALAN as a number of studies have shown an impact of ALAN on the nocturnal behaviours of Atlantic salmon (Riley *et al.* 2012, 2013, 2015).

The impact of ALAN on the drifting behaviour of freshwater invertebrates described in the present study, suggests that it has the potential to influence predator-prey dynamics and the functioning of the freshwater environment. Future research directions should attempt to elucidate the impacts of ALAN outside of immediate behavioural responses. Long-term studies are needed to examine the broader influence of ALAN on stream communities' recruitment, population size, community composition and functioning as a whole (RCEP 2009; Gaston *et al.* 2014a,b). Studies that have been conducted to date are limited in that they only examine one aspect of the influence of ALAN, in this case invertebrate behaviour. Moreover, interactions between predator and prey, competitors or conspecifics may all be affected if ALAN causes disruption to individual invertebrate taxon drifting behaviour. It is therefore imperative that we understand how ALAN may influence the decline of freshwater ecosystems. The way in which light influences long-term community composition, leaf litter decomposition and salmonid diet are all areas of research that warrant investigation.

Chapter 4: A laboratory experiment to determine the dispersal response of Atlantic salmon (*Salmo salar*) fry to street light intensity

*This chapter is based on a manuscript that has been published as - Riley, W.D., Davison, P.I., Maxwell, D.L., **Newman, R.C.** and Ives, M.J. (2015). A laboratory experiment to determine the dispersal response of Atlantic salmon (Salmo salar) fry to street light intensity. Freshwater Biology **60**: 1016-1028.*

***Declaration-** This chapter was conducted in collaboration with W.D.R and team at Cefas, Lowestoft; the experiment was designed by W.D.R. R.C.N jointly performed the experimental set up and experimental sampling with W.D.R, M.J.I and P.I.D. Statistical analysis was conducted by D.L.M and P.I.D. R.C.N contributed to the preparation of the manuscript and was responsible for authoring sections of the introduction and the entirety of the discussion.*

4.1 Abstract

1. The effect of a range of ecologically relevant broader spectrum street light intensities on the dispersal timing of Atlantic salmon (*Salmo salar*) fry was investigated to assess the efficacy of a proposed management tool, the dimming of lamp brightness, for reducing the ecological consequences of artificial light at night (ALAN) on aquatic ecosystems. Dispersal timing under ALAN at intensities of 8, 4, 2 and 1 lux was compared to that under a control night light intensity of 0.1 lux, representative of approximately half that experienced from a full moon.

2. Dispersal timing of the fry was significantly delayed (by 1.4 to 2.2 days), and the diel pattern was significantly disrupted under ALAN. The dose–response for both delay and disruption effects, however, was not linear, with a strong effect apparent at 1 lux, and little or no additional impact seen when the light

intensity was increased further. Under control conditions, the mean time of dispersal was 3:58 h after dusk, with very few fry (<4%) dispersing during daylight hours. For the ALAN treatments the mean time of dispersal of fry was significantly later (5:31 h after dusk at 1 lux) at night, and a much wider distribution of fry dispersal times was apparent with many more fry (19% at 1 lux) dispersing during daylight hours.

3. Survival to dispersal in aquarium conditions was high (≥97.8%) and comparable in the control and ALAN treatments. In the wild, however, the period between fry dispersal and the establishment of feeding territories is considered to be of critical importance in the dynamics of salmonid populations and any disruption may significantly increase predation and reduce fitness.

4. The findings of this aquarium-based investigation suggest that the dimming of lamp brightness has little potential as a successful management strategy to reduce the disruptive impact of ALAN surrounding freshwater ecosystems. We therefore recommend that the best course of action is to maintain and increase natural unlit areas.

4.2 Introduction

Artificial light at night (ALAN) has increased dramatically during the last century raising concern regarding the potential impact on populations and ecosystems throughout the biosphere (Longcore and Rich 2004; Rich and Longcore 2006; Sutherland *et al.* 2006; RCEP 2009; Hölker *et al.* 2010b). Globally, the use of ALAN is continuing to increase (estimated at 6% per annum; Hölker *et al.* 2010b) both in previously unlit regions of the developing world and in heavily developed countries (estimated at 3% per annum in the U.K.; RCEP 2009). Different types of streetlights have varying spectral compositions. The most common type of streetlight in the U.K. (low-pressure sodium vapour lamps) emits light that is narrowly concentrated in the longer wavelengths of the visible spectrum, appearing yellow or orange to the human eye. Replacement lights, for example metal halide, compact fluorescent light (CFL) and light-emitting diode (LED) lamps, emit considerably more light across the visible spectrum especially at shorter wavelengths, providing superior colour rendering for human vision (RCEP 2009). These more naturalistic whiter lights, however, could lead to significant changes in the impact of ALAN on natural systems (Rich and Longcore 2006; RCEP 2009), particularly in aquatic ecosystems where penetration through water will increase (Becker *et al.* 2013). Moreover, there is growing concern regarding how anthropogenic freshwater stressors might interact with each other (Ormerod *et al.* 2010) and that the effects of ALAN may be confounded with other urban stressors making it difficult to determine the role it has played in declines in freshwater biodiversity and ecosystem functioning (Perkin *et al.* 2011). Freshwater ecosystems are often the most significantly impacted (Revenga *et al.* 2005), and those tasked with their preservation are becoming increasingly concerned with the way ALAN is altering these ecosystems (Perkin *et al.* 2011). This change to the nocturnal environment and the behaviour of nocturnal species has potentially far reaching consequences and is a major threat to species biodiversity (Hölker *et al.* 2010b; Perkin *et al.* 2011). The issue becomes more pertinent when considering the impact that ALAN will have on species that are already a conservation concern (Mora *et al.* 2007), such as Atlantic salmon (*Salmo salar*) (Riley *et al.* 2013).

For fish, light is a directive factor, as natural light patterns will influence their behaviour (Fry 1971) and recent evidence suggests that the diel behaviour of fish can be modified in response to ALAN (see review in Nightingale, Longcore and Simenstad 2006). In salmonids, the emergence and dispersal of fry from spawning redds occurs principally at night (see review in Riley *et al.* 2013). The period between fry emergence and the establishment of feeding territories is a time when mortality can be very high and appears to be of critical importance in the dynamics of salmonid populations (Armstrong *et al.* 2003). Synchronous nocturnal salmonid fry emergence and dispersal is a predator avoidance tactic (Peterman and Gatto 1978; Godin 1982; Fraser, Huntingford and Thorpe 1994; Riley and Moore 2000; Tabor, Brown and Luiting, 2004). A recent investigation, however, demonstrated that the dispersal of Atlantic salmon fry is both delayed and disrupted by broader wavelength street lamps at a light intensity level of 12 lux (Riley *et al.* 2013), suggesting that recruitment under such conditions may be reduced. Riley *et al.* (2013) used a light intensity of 12 lux to reproduce the maximum artificial night light intensities they measured at river level at significant urban Atlantic salmon spawning sites in chalk streams across southern England: River Frome at Dorchester, up to 22.7 lux; River Avon at Salisbury, up to 11.3 lux; River Test at Romsey, up to 6.1 lux; River Itchen at Bishopstoke and Winchester, up to 20.0 and 12.9 lux, respectively (Riley *et al.* 2013). Although 12 lux is ecologically relevant, and below some national guidelines for minimum horizontal illuminance values for street lighting levels (a mean of 15 lux in Great Britain; British Standards Institute, 2003, 2008: and a mean of 20 lux in North America; Illuminating Engineering Society of North America, 2000), it is towards the upper end of night light intensities likely to be encountered at river level at urban salmon spawning locations. More typical levels of urban street lighting will fall between the intensities measured from direct street lighting by Riley *et al.* (2013) and indirect urban sky glow that can be as high as 0.5 lux (Kurtze 1974). Indeed, a recent study reported that common moderate to high ambient intensities of ALAN in urban stream reaches were between 0.6 and 4.0 lux (Meyer and Sullivan 2013).

Recent studies have reviewed management options and developments for reducing the ecological consequences of ALAN (RCEP 2009; Gaston *et al.* 2012, 2014b). These have included: (i) preventing areas from being artificially lit, (ii) reducing the trespass of lighting, (iii) changing the spectrum of lighting, (iv) limiting the duration of lighting and (v) changing the intensity of lighting (from Gaston *et al.* 2012, 2014b). Along riparian corridors, the first three of these are likely to conflict with other social, economic or ecological objectives, and will therefore be very difficult to achieve because: (i) riparian corridors are often preferred sites for human habitation/activities so the complete or partial removal of ALAN may be neither practical nor desirable; (ii) luminaire developments are generally aimed at directing ALAN downwards (i.e. to illuminate either the road surface or objects below the light source) thereby reducing the ecological impact of trespass in an upward or horizontal direction; and (iii) there is a drive towards more energy efficient whiter light sources providing superior colour rendering for human vision, often perceived to improve public safety through crime and road accident reduction (RCEP 2009; Gaston *et al.* 2012, 2014a). The fourth proposed management option is likely to be ineffective at alleviating many impacts on nocturnal or crepuscular animals, including salmonid fry dispersal and smolt migratory behaviour, because peak demand for ALAN (the hours immediately after dusk and before dawn) often coincides with peak activity (Riley and Moore, 2000; Gaston *et al.* 2012; Riley *et al.* 2012, 2013). Therefore, this study targeted the remaining management option: (v) reducing the intensity of broader wavelength street lamps, and aimed to determine whether this will significantly reduce the impact on Atlantic salmon fry dispersal.

The aim of this study was to examine the dispersal timing of fry from artificial redds housed in an aquarium under a range of ecologically relevant ALAN intensities. The study looks to determine the effect of reducing the intensity of broader wavelength street lamps on fry dispersal, and aims to determine whether this will significantly reduce the impact of ALAN on Atlantic salmon fry dispersal. The three objectives for Chapter 4 are: (1) Examine the impact of ALAN on fry dispersal behaviour; (2) identify patterns of disruption to dispersal related to ALAN at different intensities, and; (3) determine how ALAN

can impact the fitness of the dispersing fry. The hypotheses tested in this experiment are that: (1) ALAN will cause delay and disruption to fry dispersal behaviour; (2) the higher the intensity ALAN the greater the level of disruption; and (3) fry dispersing under ALAN will be lower in weight due to delayed dispersal. The results will provide evidence-based information that can be used as a management tool to identify sites where potential impacts may currently exist and help guide mitigation strategies along riparian corridors, aiding the protection of freshwater fish species in urban environments.

4.3 Materials and Methods

Experiments were conducted in 10 75-L black plastic, deep substratum incubators (Edmonds, Riley and Maxwell, 2011; Riley *et al.* 2013) at the Cefas Laboratory aquarium, Lowestoft, U.K. (52°270330N, 1°440220E). The incubators were positioned in the aquarium to produce pairs of replicates (in mirror image away from the artificial night light source) exposed to artificial night light intensities at c. 8, 4, 2, 1 and 0.1 lux, while maintaining all 10 incubators at similar light intensities during the day. The 0.1 lux artificial night light intensity is representative of approximately half that experienced from a full moon (0.2 lux, Austin *et al.* 1976; 0.1 to 0.3 lux, Rich and Longcore, 2006) and as such is considered to be the control.

Artificial night lighting was provided using a lamp (Philips Master Cosmo White; CPO-T White 45W/ 628PGZ12) (Royal Philips Electronics Inc., Amsterdam, the Netherlands) fitted in a luminaire (Philips 'iridium series' opti-C street lighting unit) installed at a height of 1.7 m. This equipment is typical of the energy-efficient ceramic metal halide street lamps currently used to illuminate city centres, residential areas, minor roads and pathways, and is typically installed at a height of 4 to 6 m. To compensate for a lower installation height in the aquarium, 10 neutral density filter sheets (E-Colour+ #298 0.15, transmission = 69.3%; Rosco Laboratories Inc., Stamford, Connecticut, U.S.A.) were fitted over the

luminaire (plus an additional filter over only one half of the luminaire) to reduce the lights intensity without changing its spectral distribution. Daytime lighting in the aquarium was provided by eight, daylight mimicking, low-pressure mercury discharge fluorescent lamps (Philips Master TL-D 58W/865; 1.5 m in length) fitted into four luminaires (Philips Pacific 29 TL-D 58W 220–240 V HFR). Each lamp was dimmable through a 0–10 V signal on the electronic ballast fitted in each luminaire. A mechanical timer switch (Grasslin, St. Georgen, Germany) was used to trigger a bespoke electronic timer (EFI Ltd, Lowestoft, U.K.) that both switched the daytime and night lighting on or off at the correct time and also increased or decreased the 0–10 V signal to the electronic ballast within each daylight luminaire to provide a 5-min mimicked dawn and dusk period each day. Day length was calculated for the study location using the method of Hohenkerk and Yallop (2004), and adjusted accordingly each week. Once hourly sampling commenced, however, day length was maintained at a constant duration of 14 h.

Light intensity readings (measured using a digital light meter, Tenmars TM-201, minimum resolution = 0.1 lux, accuracy 8%; Tenmars Electronic Co Ltd, Taipei, Taiwan) were taken (at the start and end of the experiment) at the water surface in the centre of each incubator during daytime lighting, and at night once the street lamp had fully warmed up. In addition, minimum and maximum day and night light intensity readings were determined by scanning the entire water surface of each incubator (Table 4.1). For daytime lighting, the range of light intensities overlapped across all incubators. However, there was some evidence of the 1.0 lux incubators having slightly lower intensities than the 0.1 lux incubators, relative to the variation between replicates (centre: 1.0 lux coefficient = 155.5, $P = 0.02$; maximum: 1.0 lux coefficient = 129.0, $P = 0.03$; minimum: 1.0 lux coefficient = 141.5, $P = 0.05$; each based on an ANOVA with d.f. = 4,5). For night lighting, the intensity at the centre was the same for each replicate pair of incubators. The maximum levels were clearly separated ($P < 0.001$) between treatments, as were the minimum levels ($P < 0.001$), although at ≥1 lux there was some overlap between the maximum at one intensity and the minimum at the next intensity level.

Table 4.1 Light intensity readings (lux) measured at the water surface of each incubator during daytime and artificial night lighting (light meter minimum resolution = 0.1 lux).

Incubator	Light intensity (lux)					
	Daytime lighting			Night lighting		
	Centre	Max	Min	Centre	Max	Min
0.1 A	1062	1120	849	0.1	0.1	0.1
0.1 B	1072	1157	893	0.1	0.1	0.1
1.0 A	902	1004	731	1.0	1.5	0.7
1.0 B	921	1015	728	1.0	1.3	0.7
2.0 A	1045	1117	813	2.0	3.0	1.1
2.0 B	927	1001	667	2.0	2.8	1.2
4.0 A	1012	1083	854	4.0	5.9	2.9
4.0 B	1089	1102	904	4.0	5.5	2.6
8.0 A	1109	1135	921	8.0	11.4	4.0
8.0 B	1169	1177	864	8.0	12.1	4.1

De-chlorinated mains water inflow (ambient temperature: mean 9.7°C; max 11.3°C; min 7.7°C) was gravity-fed (mean 252.8 L h1 ; max 300 L h1 ; min 220 L h1) to each incubator from a large outdoor header tank through a perforated pressure plate, lined with 20 to 30 mm of pea gravel, fitted into each incubator base. Outflow was via standing overflow pipes discharging into perforated stainless steel counting boxes placed within fry troughs. The mirror image replicates for each artificial night light intensity treatment discharged into different fry troughs. Water temperature measurements were collected from within these troughs using Tinytags (Gemini Data Loggers UK Ltd.). These programmable data loggers underwent a three-point calibration (at 0, 15 and 30°C) by Gemini before deployment and were programmed to record temperature once every hour. Subsequent analysis of the temperature data showed no significant differences (Student's t-test; n = 1438, d.f = 1437, P = 0.49) between the mean temperatures (both 9.7°C) recorded in each trough.

On 22 February 2012, 500 eyed Atlantic salmon eggs (development c. 260 degree-days) were randomly assigned to each incubator. To replicate reported burial depths (Crisp and Carling 1989; Bardonnet and Bagliniere 2000; Armstrong *et al.* 2003) and the substratum exploited by spawning salmonids in the chalk

streams of southern England (Crisp and Carling 1989; W. D. Riley unpublished data), the eggs were buried 150 to 180 mm deep within washed 15- to 40-mm gravel. The water depth between the gravel surface and the outflow was measured at 100 mm in all incubators.

The perforated stainless steel counting boxes into which the water from each incubator discharged were checked each day for any dispersing fry. As soon as one fry had dispersed, the number and total wet mass (nearest 0.01 g) of fry dispersing from each incubator was recorded at dawn and dusk each day.

Hourly sampling commenced when developmental degree-days suggested that initial dispersal was imminent (Edwards 1978). Prior to hourly sampling a total of six fry were recorded (across all incubators), and these fish were included in the analysis of overall survival, but excluded from all other analysis. Hourly sampling continued post-peak dispersal until <20 fry had dispersed (across all incubators) over a 24-h period. The substratum from each incubator was then carefully removed and the number of fry that had not dispersed was recorded; a total of 35 fry were removed (across all incubators) during this process; again these were included in the analysis of overall survival but excluded from all other analysis.

To transform the hourly sampling periods into a numerical variable, a decimal 'sampling day' was assigned to each fry dispersal as sampling day + sampling hour/24. Hourly sampling commenced following dusk on 31 March (Day 1) and continued until dusk on 21 April (Day 22). The mean time of fry dispersal was calculated for each incubator. For these mean values, analysis of variance with Tukey's honest significant difference (HSD) method for pairwise comparison (with 5% family-wise error rate) was used to compare the dispersal times for the different treatments, with each other, and with the controls. Analysis was carried out in R v3.0.1 (R Development Core Team, 2013).

The mean mass of dispersing fry per incubator per hour was calculated from the hourly totals for each incubator. An exploratory analysis was carried out on trends in mean mass with days since the start of hourly sampling by fitting a generalised additive model (Wood 2006) to the data from each incubator by

day and night separately. These models used a thin plate regression spline with shrinkage and basis dimension (k) of 10. For further analysis, a mixed effects model (Pinheiro and Bates 2000) was fitted to mean mass with fixed effects being factors for day or night and night light level (0.1, 1, 2, 4 or 8 lux), a linear trend for time since the start of hourly sampling, and all interactions of the three variables, and random effects being the mean and linear trend for each incubator. This allowed any additional variation between incubators to be included in analysing the effect of light level. All the statistical models used the number of fry that each mean mass was derived from as statistical weight; this was to account for the fact that the mean mass calculated when more fry dispersed will be more precise. Model selection was then carried out on the fixed effects, using likelihood ratio tests and a 5% significance level, to produce a simplified final model, which was refitted using restricted maximum likelihood.

Hourly patterns of dispersal were investigated using circular statistics (Batschelet 1981). For each night light intensity, the mean vector (l) (mean time of dispersal ±95th percentile confidence limits) and the mean vector length (r, expressed as a value between 0 and 1, with higher values indicating that observations are clustered more closely around the mean) were calculated. Rayleigh's uniformity tests were performed for each treatment to calculate the probability (P) of the observations under the null hypothesis that dispersal was uniformly distributed throughout the diel cycle. Differences between the treatments were investigated by a circular version of the chi-square test and by comparing the data from the incubators exposed to the street-lit conditions with the mean vector of the control data (V-test). All circular statistics were carried out in Oriana (www.kov-comp.com/oriana).

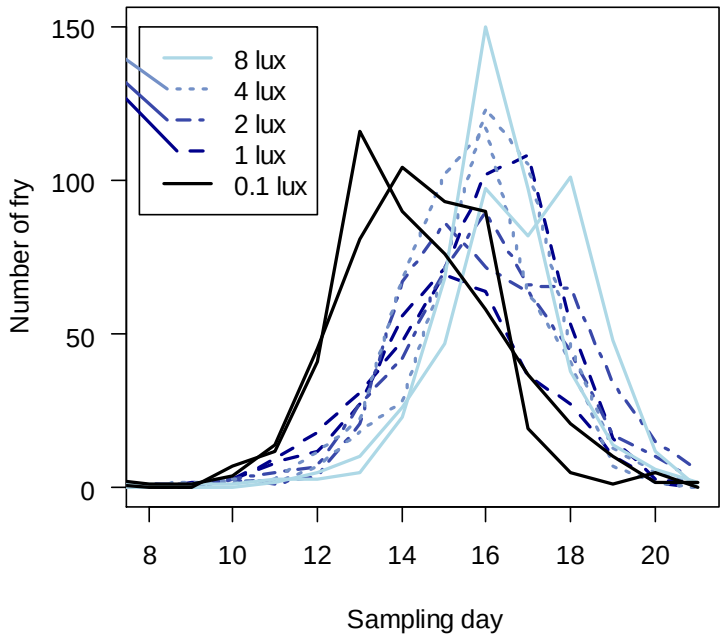
The percentage of fry surviving during the whole experimental period was calculated for each incubator, and survival under street-lit (1 to 8 lux) and control (0.1 lux) conditions were compared using a randomisation test based on all 45 possible combinations of the 10 incubators to the two groups (Manly, 2001).

4.4 Results

4.4.1 Dispersal day

The number of fry dispersing from each of two incubators by sampling day at the five levels of night light intensity was recorded during both, the night (Fig. 4.1a) and daylight hours (Fig. 4.1b). The dispersal data (Table 4.2, Fig. 4.2a) show a wide range of dispersal dates in all incubators. Between the control (0.1 lux) and the treatment artificial night light intensities, however, there was an increase in dispersal day for the central section of the distribution and the differences in mean dispersal day were statistically significant relative to the variation between incubators (ANOVA, d.f. = 4,5, F = 18.7, P = 0.003).

(a)



(b)

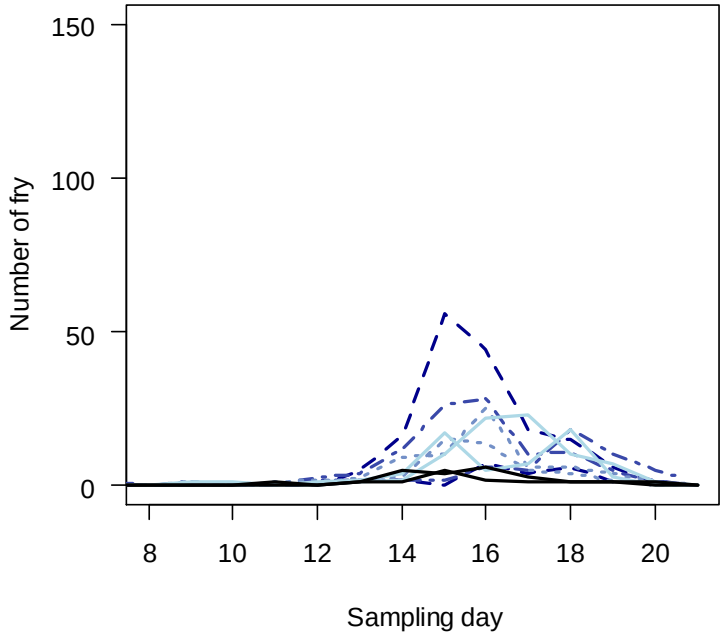


Figure 4.1 Number of fry dispersing from each of two incubators by sampling day at five levels of night light intensity: (a) during night hours; (b) during day hours. X-axis truncated at sampling day 8; during night hours a total of 19 fry emerged earlier than day 8, during day hours a total of 3 fry emerged earlier.

Table 4.2 Summary of survival and dispersal for each incubator. All incubators had 500 eggs at the start of the experiment. Hourly sampling commenced following dusk on 31 March (day 1) and continued until dusk on 21 April (day 22).

Incubator	Total no. surviving	Total % surviving	No. fry emerging during 24 hr sampling	Dispersal day mean	Dispersal day sd
0.1 A	489	97.8	484	14.4	2.14
0.1 B	500	100.0	498	14.6	2.14
1.0 A	492	98.4	483	15.7	1.99
1.0 B	489	97.8	487	16.0	1.94
2.0 A	497	99.4	492	16.1	1.84
2.0 B	492	98.4	488	16.5	2.22
4.0 A	490	98.0	489	16.3	1.71
4.0 B	493	98.6	491	15.8	1.69
8.0 A	494	98.8	489	16.5	1.55
8.0 B	496	99.2	490	16.9	1.90

Mean dispersal day was between 1.4 and 2.2 days later under the treatment artificial night light intensities (Table 4.3, Fig. 4.2b), and all pairwise comparisons against the control were statistically significant (Tukey's HSD, adjusted $P < 0.05$). The latest mean dispersal corresponded to the highest artificial night light level (8.0 lux), but the 95% confidence intervals for the different treatment intensities of street lighting all overlapped (Table 4.3, Fig. 4.2b) and all pairwise comparisons between treatment artificial night light intensities were non-significant (Tukey's HSD, adjusted $P > 0.05$). Including daytime light intensities did not improve the model fit after accounting for differences in night light intensities (d.f. = 1,4; $F = 0.84$; $P = 0.41$).

Table 4.3 Analysis of variance coefficients for effect of light intensity on mean dispersal day, intercept is estimate at 0.1 lux and other coefficients show difference from intercept, s.e. is standard error, CI is 95% confidence interval.

	Coefficient	s.e.	95% CI	t value	p
Intercept	14.48	0.194	(14.0, 15.0)	74.57	< 0.001
1 lux	1.36	0.275	(0.7, 2.1)	4.95	0.004
2 lux	1.80	0.275	(1.1, 2.5)	6.54	0.001
4 lux	1.54	0.275	(0.8, 2.2)	5.62	0.002
8 lux	2.23	0.275	(1.5, 2.9)	8.12	< 0.001

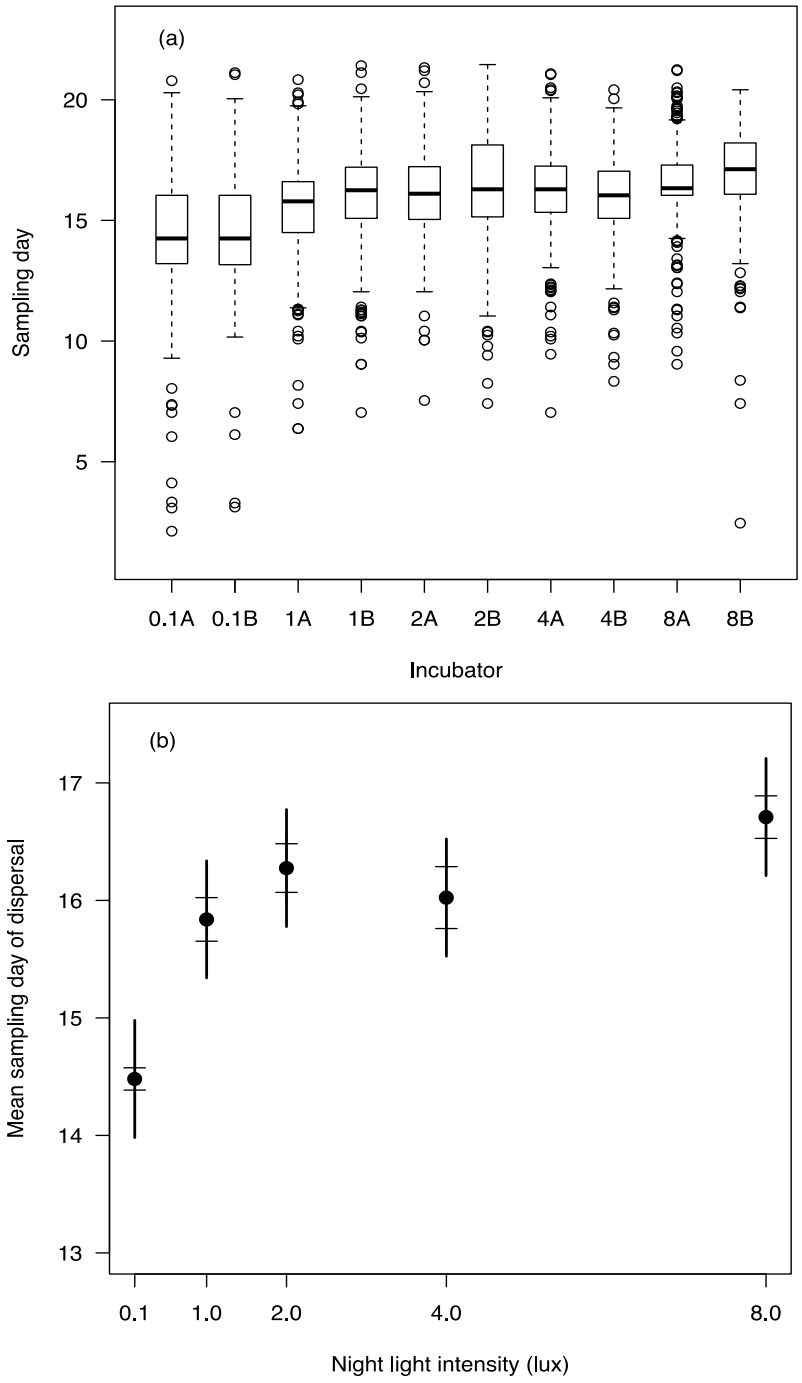


Figure 4.2 (a) Boxplot showing the distribution of dispersal day for fry from each of two incubators by sampling day at five levels of night light intensity. Horizontal lines show the medians, the boxes indicate the central 50% of the values, dashed vertical lines extend to cover the majority of the data and circles show outlying values. **(b)** Mean dispersal day (filled circles) for fry at each night light intensity with 95% confidence intervals for mean (vertical lines) from analysis of variance and observed means by incubator (horizontal lines). Night light intensity 0.1 lux is considered to be the control. Note that the y-axis does not start at Day 1.

4.4.2 Diel dispersal pattern

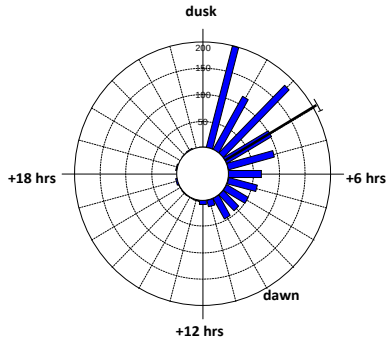
For all night light intensities, the null hypothesis was rejected in favour of directedness (Rayleigh test, $P < 0.001$); thus, dispersal times were directed around particular periods of time following the onset of dusk. Under control conditions, the mean time of dispersal was 3:58 h after dusk ($r = 0.74$), with very few fry ($P < 0.001$) later in the night: mean times of 5:31, 4:47, 5:45 and 5:31 h after dusk, respectively (Table 4.4).

Table 4.4 Summary of circular statistics and dispersal data by night light intensity during the period of hourly sampling: *n* = number of fry that dispersed, μ = the mean time after dusk (hrs) and associated confidence limits (CL), *r* = mean vector length, significance (*p*) of the Rayleigh test for randomness, significance (*p*) of the Chi-squared (χ^2) test, significance (*p*) of the V-test for directedness, and the number of fry dispersing during daylight hours (percentage in parenthesis).

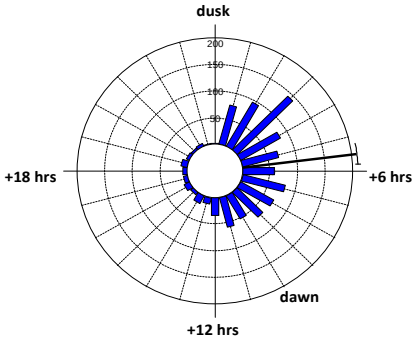
	Night light intensity (lux)				
	0.1	1	2	4	8
n	982	970	980	980	979
μ	03:58	05:31	04:47	05:45	05:31
95% CL (±)	00:11	00:17	00:16	00:14	00:15
r	0.736	0.541	0.559	0.639	0.605
p (Rayleigh test)	<0.001	<0.001	<0.001	<0.001	<0.001
p (χ^2 test) *		<0.001	<0.001	<0.001	<0.001
p(V-test)**		<0.001	<0.001	<0.001	<0.001
Daylight *n* (%)	35 (4)	184 (19)	155 (16)	113 (12)	136 (14)

Compared to the controls, significantly more fry (Student's t test; d.f. = 8; $P < 0.003$) dispersed during the hours of daylight in the four treatment groups (12–19%), with the most in the 1.0 lux artificial light treatment (Table 4.4; Fig. 4.1b). Circular plots of the data revealed that the diel patterns of dispersal between the control conditions and the street-lit treatments were very different (Fig. 4.3). This is due to both the later, but also much wider distribution of fry dispersal times under street-lit treatments. These differences were found to be significant ($P < 0.001$) when comparing the mean vectors (95% CLs) by performing a chi-square test on the sample distributions and when comparing the dispersal times of the fry exposed to street-lit conditions against the mean vector of the control group (V-test for combined data) (Table 4.4).

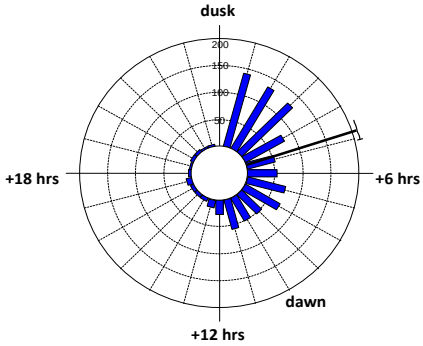
0.1 lux (control)



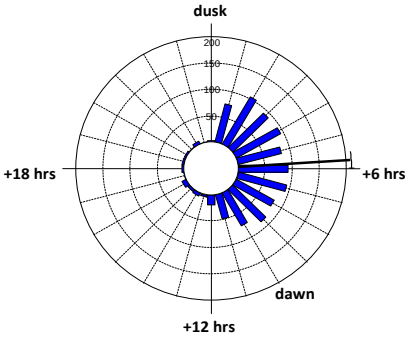
1.0 lux



2.0 lux



4.0 lux



8.0 lux

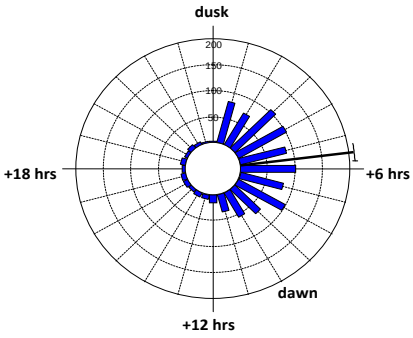


Figure 4.3 Circular plots of the diel patterns of fry dispersal in relation to hours following the onset of dusk. Data are presented for the control conditions (0.1 lux) and each artificial night light intensity treatment. Also indicated are the mean dispersal times (95% CLs) for each treatment and the onset of dawn.

4.4.3 Fry mass

Examining the mean mass of fry dispersing from each incubator each hour (Fig. 4.4) and fitting GAM curves (not shown) to each incubator day/night combination showed a mixture of constant trends, linear trends and fluctuations in mean mass across time, for example for 0.1 lux at night. Overall, there were no consistent nonlinear trends so linear trends were used in further modelling. The selected linear mixed effects (lme) model (Table 4.5, Fig. 4.4) indicated there was a marginally larger mean mass at day relative to night after accounting for sampling day and hour (+0.004 g, P = 0.0007), and different slopes by light level (P = 0.014), with an almost constant trend for the control incubators at 0.1 lux (0.00006 g d1) and small decreases in mean fry mass with time for the treatment street-lit incubators (between 0.0011 and 0.0023 g d1). The variation between incubators (SD = 0.010) was small in relation to the variation between individual fry (within-group SE = 0.025) indicating that mean masses were not strongly correlated within incubators or between treatments. There was relatively large amount of individual variation around the fitted means (Fig. 4.4). Data and model checking showed outlying masses in the Day 1 lux and Night 8 lux data and one fry dispersing early (Day 2.5) in the Day 8 lux data (see Fig. 4.4). These values were checked and confirmed as valid. Trials removing them and then refitting the selected model did not change the conclusions drawn.

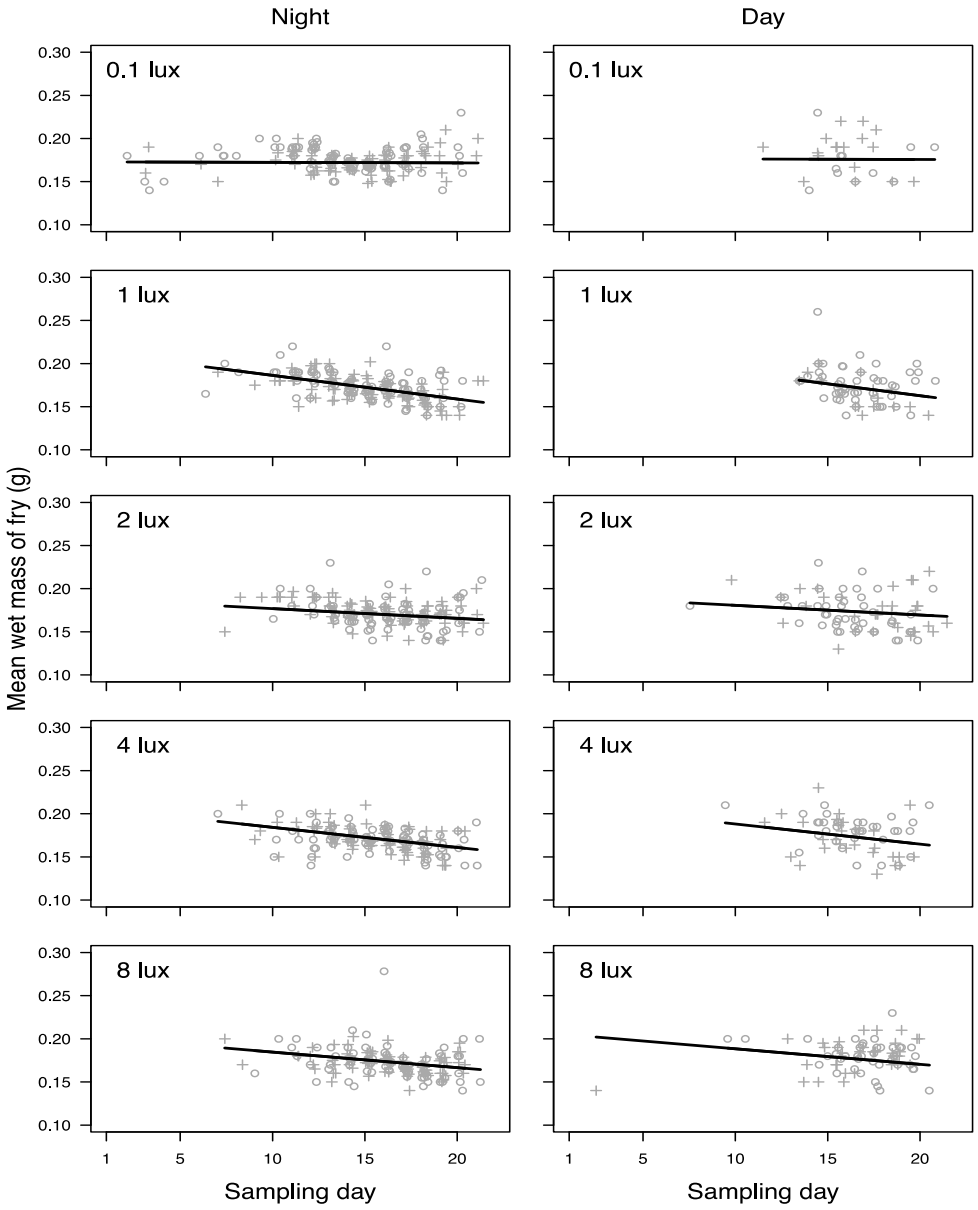


Figure 4.4 Mean wet mass of dispersing fry per hour per incubator against sampling day; cross and circle symbols denote the two incubators at each light level, solid line is the model fit from a weighted lme model (see text for details and Table 5 for model coefficients).

3.4.4 Survival

Survival was high (≥97.8%) in all incubators (Table 4.2) and comparable in the control and street-lit treatments (control mean 98.9%, street-lit mean 98.6%; randomisation test P = 0.71).

Table 4.5 Linear mixed effects model for mean dispersal mass. Dayhr is sampling day and hour (as decimal days), lux is night light intensity, night defined as the 10 hours after dusk and nfry is the number of fry in the hourly dispersal data for each mean mass.

Fixed effects	numDF	denDF	F-value	p-value
(Intercept)	1	1120	42661.84	< .0001
dayhr	1	1120	28.19	< .0001
lux	4	5	0.68	0.637
day/night	1	1120	11.51	0.0007
dayhr:lux	4	1120	3.12	0.0144

		Approx 95% CI	
Fixed effects	Estimate	Lower	Upper
Intercept	0.173	0.155	0.191
dayhr	-0.00006	-0.00120	0.00109
1 lux	0.041	0.007	0.075
2 lux	0.015	-0.019	0.049
4 lux	0.035	-0.001	0.070
8 lux	0.030	-0.006	0.065
day/night = day	0.00387	0.00167	0.00606
dayhr:1 lux	-0.00269	-0.00434	-0.00103
dayhr:2 lux	-0.00107	-0.00271	0.00057
dayhr:4 lux	-0.00227	-0.00398	-0.00056
dayhr:8 lux	-0.00174	-0.00345	-0.00003

Random Effects: Formula: ~dayhr | Incubator			
	Estimate	Lower	Upper
sd(Intercept) i.e. Incubator	0.01011	0.00328	0.03121
sd(dayhr)	0.00063	0.00020	0.00200
cor(Intercept,dayhr)	-0.973	-0.998	-0.643
within-group se	0.0250	0.0240	0.0261

Variance function:	Power of variance covariate
Formula: ~nfry	power -0.5

4.5 Discussion

The dispersal of Atlantic salmon fry in an aquarium was significantly delayed and disrupted by broader spectrum ALAN intensity levels of 1 to 8 lux. The dose response for both delay and disruption effect is

not linear, with a strong effect apparent at 1 lux and little additional impact seen when the light intensity is increased further to 2, 4 and 8 lux. The current investigation has therefore identified the intensity at which ALAN has a disruptive impact on natural Atlantic salmon fry dispersal behaviour, somewhere between natural full moonlit conditions of 0.2 lux (Austin *et al.* 1976) and ALAN conditions of 1.0 lux, with little additive effect of changing behaviour with increasing light intensity once this low threshold intensity was breached.

The period between fry dispersal and the establishment of defended feeding territories is a critical period in the life cycle of the Atlantic salmon, and populations experience high mortality during this time (Armstrong *et al.* 2003). Any disruption to the timing of important life history events will have fitness consequences for organisms (Bradshaw and Holzapfel 2010). In the wild, the delay and disruption to fry dispersal reported in this investigation would most likely impact fitness initially by diminishing the protectoral role afforded by natural synchronous nocturnal fry dispersal as a predator avoidance tactic (Peterman and Gatto 1978; Godin 1982; Fraser *et al.* 1994; Riley and Moore 2000; Tabor *et al.* 2004). In addition, the small decrease in mean fry mass, with time, observed in those dispersing under the ALAN treatments (despite the relatively crude method used to weigh each of the batches of dispersing fry) is suggestive that these fry, having delayed their dispersal, have reduced their available energy reserves. Fry that delay dispersal are likely to be physically weaker and may find it harder to compete for a feeding territory. The energy reserves with which fry would ordinarily leave the natal redd give them a degree of flexibility in the timing of their first feeding (Miller *et al.* 1988) in order to allow them to compete with other fry for a prime feeding territory. As such, those fry dispersing without this are at a competitive disadvantage. This reduction in fitness at the individual level can have population level consequences, through increased mortality rates (Armstrong *et al.* 2003; Milner *et al.* 2003), although in the controlled aquarium environment used in this investigation, survival to dispersal was unaffected by the ALAN treatments.

The light intensity at which Atlantic salmon fry dispersal is shown to be impacted in this investigation is much lower than that previously reported (Riley *et al.* 2013). This finding is of particular importance, as ALAN is often associated with urban sky glow and light escape from large urban conurbations, yet a light intensity of between 0.2 lux and 1 lux will commonly be found in suburban, periurban and rural areas (Perkin *et al.* 2011). Although the majority of adult Atlantic salmon spawn in upland reaches of river systems (Riley *et al.* 2013), away from urban areas subjected to the effects of ALAN, the results of this investigation suggest that even a few streetlights used to light villages and footpaths could have an impact on the dispersal behaviour of wild salmon fry in these areas. In addition, with clear small streams more susceptible to the penetration of light (Perkin *et al.* 2014 a, b), there is an increased potential for an impact of ALAN in these headwater reaches.

Given the rate at which the level of ALAN is increasing, both in the U.K. and globally, there is likely to be much discord between the need to protect our freshwater ecosystems from this stressor and the trend of lighting our nocturnal environment. With this in mind, this investigation sought to determine the threshold intensity of ALAN at which broader spectrum street lamps no longer impacted the dispersal behaviour of Atlantic salmon fry. This was conducted to assess the efficacy of a proposed management tool, the dimming of lamp brightness, selected as being the most universally applicable management strategy for mitigating the negative impacts of ALAN around freshwater ecosystems, as previously outlined in the introduction. The results of the investigation, however, suggest that this method has little potential as a successful management strategy, due to the triggering threshold of light disrupted behaviour being somewhere between 0.2 lux and 1 lux. Given that a reduction in ALAN below 1 lux is unlikely to be accepted, we recommend that the best course of action is to maintain and increase natural unlit areas as the most effective measure in reducing the disruptive impact of ALAN. Any reduction in light intensity at a purposefully lit source, however, will ultimately reduce the area affected by that light source. Either of these approaches will have social and economic conflicts, as many people associate the presence of nocturnal street lighting with feeling safe and secure in their environment, thus are resistant to any

perceived reduction in lighting numbers, intensity or duration (Hölker., *et al.* 2010a). Nevertheless, a number of schemes have been piloted across the U.K. to reduce the amount of ALAN, with counties across England and Wales taking part in projects to determine public response to measures reducing ALAN (Lockwood *et al.* 2011). It was found that residents would not accept a complete turn off of streetlights at a certain time; however, many found dimming an acceptable compromise (RCEP 2009; Lockwood *et al.* 2011).

An alternative solution that may provide the required compromise is the use of flexible control systems; including on-demand street lighting along riparian footpaths. These may be useful tools for mitigating impacts by providing street lighting when required for human use, but also ensuring there are sufficient dark periods for normal nocturnal behaviour. There is a current drive towards ensuring street lighting is more energy efficient, whether necessitated by environmental concern or the need to adhere to budget cuts; this movement is replacing yellow low-pressure sodium vapour lamps with white light metal halide or LED lamps. While this shift in lighting colour has the potential to increase the amount of light penetrating water (Becker *et al.* 2013), these new lamps, unlike their sodium vapour predecessors, are better able to operate using flexible on-demand lighting systems (RCEP 2009). In addition, the use of red lights, which have limited penetration through water, along riparian corridors could also be considered (Becker *et al.* 2013). Neither one these measures, however, have been tested in a field setting to ascertain their environmental impact, and as such warrant further investigation. It has also been suggested that the unpredictable periods of light produced by on-demand lighting will be more disruptive and disorientating to nocturnal animals than the constant light of full night illumination (RCEP 2009).

A final area that warrants further investigation is the role of multiple stressors and the interaction between them in impacting species behaviour and population viability. In the U.K., no pristine freshwater ecosystems remain; almost all have been impacted by diverse anthropogenic activity (including habitat alteration, addition of pollutants and changes in land use and drainage) (Revenga *et al.* 2005) and most are

now managed to a lesser or greater extent (UK NEA 2011). There are concerns that multiple interacting stressors in freshwater ecosystems (Ormerod *et al.* 2010) may confound the role of ALAN in urban systems (Perkin *et al.* 2011) and form part of the complex drivers behind the widely observed effect of urbanisation on freshwater ecosystems, often termed 'urban stream syndrome' (Walsh *et al.* 2005). Atlantic salmon are known to have some key spawning areas in rivers around towns and villages (Riley *et al.* 2013) where there are likely to be multiple anthropogenic stressors acting, such as water pollution and increased sedimentation from urban run-off. In addition, climate change is a further stressor that is of great concern for freshwater ecosystems. Rivers are most sensitive to climate change as they are directly affected by both changes in temperature and changes in rainfall (Ormerod 2009). It has been suggested that population declines seen in Atlantic salmon are a result of warmer, dryer summers (Clews *et al.* 2010).

The way in which climate change will impact freshwater species over the next decade is difficult to predict (JNCC 2007); however, some studies suggest that a rise in temperature may have already impacted Atlantic salmon (Clews *et al.* 2010). It is thought that some animal populations will be unable to cope with multiple stressors that occur simultaneously (Novacek and Cleland 2001; Folke *et al.* 2004; Mora *et al.* 2007). Multiple disturbances can act together and strengthen the impact of the others, resulting in a much quicker rate of biodiversity losses (Mora *et al.* 2007; Solomon *et al.* 2007; Darling and Cote 2008). For example, declines in rotifer populations have been reported to be up to 50 times faster, when multiple stressors are acting, compared to control populations at constant temperatures (Mora *et al.* 2007). It is therefore imperative that we attempt to understand the way ALAN interacts with other anthropogenic stressors on endangered species, populations and ecosystems; it is only with a clear and comprehensive understanding of these issues that we can successfully develop effective management strategies (Didham *et al.* 2007; Mora *et al.* 2007; Perkin *et al.* 2014 a, b).

Chapter 5: Non-invasive sampling methods to assess the cortisol stress response in dispersing Atlantic salmon (*Salmo salar* L.) fry exposed to artificial light at night (ALAN).

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5.1 Abstract

Atlantic salmon (*Salmo salar*) is a species of conservation and economic importance, and it is a model for examining the responses of fish (in freshwater) to anthropogenic stressors, with its ecology and behaviour well documented. A recent laboratory experiment demonstrated that the dispersal behaviour of salmon fry from redds (egg nests) is disrupted by artificial light at night (ALAN), although it is not yet known whether physiology is also affected. In this study, dispersing salmon (swim-up fry) exposed to different ALAN intensities were examined for an endocrine (cortisol) response. Here two physiological methodologies were applied to this ecological concern; firstly, deployable passive samplers were used to determine the population level stress response and secondly, individual measures of stress were assessed using static water samples. Dispersing fry exposed to experimental confinement showed elevated cortisol levels, indicating the capacity to mount a stress response at this early stage in development. Only one of the two methods for sampling cortisol supported the hypothesis that ALAN affected the cortisol levels of dispersing salmon fry, the population measure of cortisol with a positive relationship between light and stress response, but a cortisol-mediated response to light was not strongly supported.

5.2 Introduction

Artificial light at night (ALAN) has increased rapidly since the Industrial Revolution, and particularly over the last 60 years. The current rate of increase in the number of artificial lights is 6% per annum globally (Hölker *et al.* 2010b), and the impact of light pollution on wildlife is of concern (RCEP 2009). ALAN can be over a million times brighter than natural nocturnal illumination and changes to the, once predictable, lighting regime may result in large-scale behavioural changes (Perry *et al.* 2008). ALAN can alter behaviour, physiology and ecology in a broad range of species and taxa (Rich and Longcore 2006). For example, ALAN has been documented to disrupt the daily rhythms of nocturnal primates (LeTallec *et al.* 2013), bird singing behaviour (Miller 2006) and the community composition of terrestrial invertebrates (Davies *et al.* 2012). There is a notable paucity of information on the impacts of ALAN on aquatic systems (Perkin *et al.* 2011; Kronfield-Schor *et al.* 2013). For successful conservation and mitigation, the extent of the impact of ALAN needs to be fully understood for individual species and ecosystems (Rich and Longcore 2006; Gaston *et al.* 2014b).

As yet there are little systematic data demonstrating impacts of ALAN on wildlife, although evidence is accumulating that it may be having a detrimental impact on the health and functioning of organisms (Rich and Longcore 2006; Gaston *et al.* 2014b). Fish use diurnal and seasonal changes in light as cues for behavioural and physiological changes, and artificial lighting is used in aquaculture systems to manipulate physiology (Boeuf and LeBail 1999; Migaud *et al.* 2007). Further, there is evidence that ALAN can cause physiological stress (increased plasma cortisol and glucose) in farmed Atlantic salmon (*Salmo salar* L.) (Migaud *et al.* 2007). ALAN may therefore impact upon the physiology of wild fish species (McConnell *et al.* 2010).

A stressor is defined as a factor that interferes with homeostasis; the maintenance of the body's internal milieu in an optimal state (Bonga 1997; Levy *et al.* 2005). For the most part, maintenance of homeostasis

is controlled by the endocrine system via hormones that act by signalling and targeting specific cells. When a given species is exposed to a stressor, this interference with homeostasis results in a hormone cascade whereby the body attempts to minimise the impact (Iwama *et al.* 1999; Barton 2002). This hormonal response to a stressor is accompanied by behavioural changes – adaptive measures that serve to help the organism cope with the change it is experiencing and maintain homeostasis (Bonga 1997; Barton 2002). A species stress response is not intrinsically harmful (Barton 2002) it is a period threatened homeostasis that is counteracted by a range of intricate hormonal responses (Chrousos 1998). It is possible, however, that the organism is unable to restore their internal environment to its optimal homeostatic state (Barton 2002) and, in such cases, the hormonal responses, particularly cortisol, become maladaptive and can cause a myriad of problems, including immune and reproductive interference (Barton *et al.* 1987; Pickering and Pottinger 1989; Bonga 1997; Barton 2002).

The initial hormone changes seen in a newly stressed organism are classed as the primary stress response (Mazeaud *et al.* 1977) and controlled by the neuroendocrine system. This primary response is characterised by the release of two classes of hormones: catecholamines from the chromaffin tissue in the brain; and corticosteroids, such as cortisol, from the hypothalamic-pituitary-interrenal (HPI) axis (Barton and Iwama 1991; Bonga 1997; Schreck *et al.* 2000). The chromaffin tissue release of catecholmines occurs almost instantaneously upon the organism experiencing the stressor, whilst the release of cortisol is slightly delayed due to the feedback loop associated with production (Bonga 1997). The time taken for the cortisol response is species-specific (Schreck *et al.* 2000; Barton 2002). In fish, several other hormones have been proposed as indicators of primary stress levels, namely thyroxine, prolactin, melatonin and somatolactin. Using such hormones as indicators of primary stress in fish has, however, not been fully validated to date (Bonga 1997; Barton 2002; Larson *et al.* 2004) and thus cortisol is the most frequently used physiological measure (Ellis *et al.* 2004).

Previous research suggests strong species-specificity regarding the ontogeny, timing, magnitude and duration of the cortisol response (Feist and Schreck 2002; Fanouraki *et al.* 2011). Specifically, the developmental stage at which fish able to mount a stress response to stressors is dependent upon both species and environment (De Jesus and Hirano 1992; Barry *et al.* 1995; Stephens *et al.* 1997; Stouthart *et al.* 1998; Jentoft *et al.* 2002; Feist and Schreck 2002; Auperin and Geslin 2008). Whilst a number of species appear able to synthesise cortisol at the time of hatching, the development of a cortisol response to stressors appears later in development (Jentoft *et al.* 2002). Necheav *et al.* (2006) found that Atlantic salmon were not capable of mounting a stress response until 72 days post hatching at 6°C (432 degree days). Although previous studies all examine salmonid species (*Oncorhynchus mykiss* Barry *et al.* 1995, Aupern and Geslin 2008; *Oncorhynchus tshawytscha* Feist and Schreck 2002), there appear to be differences between them in the ontogeny of a cortisol stress response.

It has been shown that ALAN has a behavioural impact on freshwater stages in the lifecycle of anadromous Atlantic salmon, delaying dispersal and dispersal of fry from spawning redds, and migration of smolts (Riley *et al.* 2012, 2013, 2015). These synchronous nocturnal movements are thought to occur to avoid visual predators (Riley and Moore 2000), so any disruption to timing of dispersal may increase predation risk. Emergence is the time when young salmon fry establish their feeding territories, there is high competition for feeding territories and, as such, delaying emergence may result in starvation, placing strong selection pressure on the fry to emerge early and secure an optimal feeding territory.

Whilst these studies have demonstrated effects of ALAN on the behaviour of dispersing salmon fry, it is not yet known whether there are also physiological effects, such as a cortisol stress response, mediating the observed behavioural disruption. If ALAN represents an increased predation risk to the migrating fish, it will result in an elevation of cortisol (Bell and *Sih* 2007). Previous studies have demonstrated a stress response in teleost fish in response to differential aquarium lighting regimes, and the magnitude of the response appears to differ with lighting type, colour and intensity (Richards *et al.* 2007; Migaud *et al.*

2007; Heydernejad *et al.* 2011). Variations in lighting have also been seen to alter the size and direction of the stress response mounted by fish when confronted with a known stressor (Volpato and Barreto 2001; Karakatsouli *et al.* 2008). A recent study, however, found no effect of ALAN on the cortisol response over a 24-hour period of European perch (*Perca fluviatilis*) after being housed for ten days at intensities of 1, 10 and 100 lux (Brüning *et al.* 2015). As such, work is required to determine the physiological mechanism behind the previously reported behavioural disruption during fry dispersal, as a result of ALAN (see Chapter 3; Riley *et al.* 2013, 2015).

5.3 Aims and Hypotheses

Following on from the effects on dispersal described previously (see Chapter 4), the aim of this was to investigate whether the disruption to the nocturnal synchronous dispersal of Atlantic salmon fry was mediated via a cortisol stress response in the dispersing juveniles. In this study, novel physiological methodology is applied to test an ecological hypothesis; that the behavioural delay in dispersing Atlantic salmon fry under ALAN is mediated via a cortisol stress response. Elevated cortisol levels are associated with changes in various fish behaviours, including activity (see Ellis *et al.* 2012). It is also examined whether dispersing salmon fry, at this early ontogenetic stage, demonstrated a cortisol stress response to an external stimulus. The three objectives for this chapter are: (1) Assess the impact of ALAN on the cortisol stress response of individual dispersing fry; (2) Identify the population level cortisol response to ALAN, and; (3) Evaluate possible alternative physiological mechanisms behind the observed behavioural disruption under ALAN.

The following hypotheses tested as part of this experiment are: (1) ALAN will induce a cortisol stress response in those fry dispersing from experimentally lit incubators; (2) the magnitude of the stress

response will increase with light intensity; and (3) fry dispersing under ALAN will be lower in weight due to delayed dispersal.

5.4 Materials and Methods

5.4.1 Experimental set up

Experimental work was conducted at Centre for Environment, Fisheries and Aquaculture Science (Cefas) Laboratory aquarium, Lowestoft, UK (52°27'33"N, 1°44'22"E). The diurnal lighting levels in the aquarium were manipulated to be representative of natural daylight (1177-728 lux, 14 hL : 10 hD) using daylight mimicking, low-pressure mercury discharge fluorescent lamps (Philips Master TL-D 58W/865; 1.5 m in length). A Metal Halide streetlight (Philips Master Cosmo White; CPO-T White 45W/628PGZ12) was mounted in a luminaire (Philips 'iridium series' opti-C unit) 1.7 m above the incubators to enable to manipulation of nocturnal light levels across them. The alternation in the lighting system was controlled via a bespoke electronic timer (EFI Ltd, Lowestoft, UK) that triggered the switch from day to night time lighting levels, and also activated an electronic ballast in the daytime luminaires that resulted in a five minute warming up/cooling down period each day to mimic dawn and dusk.

Neutral density filters (E-Colour+ #298 0.15, transmission = 69.3 %; Rosco Laboratories Inc., Stamford, Connecticut, USA) were attached to the lamp in order to reduce the intensity of the light produced without altering the spectrum, thus corresponding with light levels measured in field settings (Riley *et al.* 2013). The light levels used in the experiment were 8, 4, 2, 1 and 0.1-lux, with two incubators at each light level treatment (Table 4.1). The 0.1 lux level is representative of a moonlit night (Longcore and Rich 2006; Riley *et al.* 2013, 2015) and was considered a control treatment. The light intensities chosen were selected based on the previously reported effect of ALAN at 12 lux and field measurements of ALAN across

streams in southern England (Riley *et al.* 2013). Further, the intensities were biased towards the lower end intensities as this study sought to identify a threshold light intensity at which light does not impact the emergent fry. The incubators were arranged in the aquarium in relation to this light level, with light intensity readings (measured using a digital lux meter, RS 180-7133, accuracy ± 4%+2 Digits; RS Components Ltd, Corby, UK) measured on the surface of the water in each incubator (Table 5.1).

Table 5.1 Light intensity (lux) measured on the surface of each incubator, experimental and control (shaded), during daytime and artificially lit night (resolution = 0.1 lux).

Incubator	Light Intensity (lux)					
	Daytime Lighting			Night Lighting		
	Centre	Max	Min	Centre	Max	Min
0.1A	1062	1120	849	0.1	0.1	0.1
0.1B	1072	1157	893	0.1	0.1	0.1
1A	902	1004	731	1	1.5	0.7
1B	921	1015	728	1	1.3	0.7
2A	1045	1117	813	2	3	1.1
2B	927	1001	667	2	2.8	1.2
4A	1012	1083	854	4	5.9	2.9
4B	1089	1102	904	4	5.5	2.6
8A	1109	1135	921	8	11.4	4
8B	1169	1177	864	8	12.1	4.1

On 22 February 2012, 500 Atlantic salmon fertilized eggs (development ~260 degree days) sourced from wild caught broodstock (Kielder Hatchery, Northumberland) were implanted into to each of ten 75L black plastic deep substrate incubators (Edmonds *et al.* 2011). The eggs were evenly distributed and buried using washed 15-40 mm gravel at a depth of 150-180 mm in order to replicate the previous study conducted by Riley *et al.* (2013) and reported burial conditions of Atlantic salmon redds (DeVries *et al.* 1997). In all incubators the water depth was approximately 100 mm above the surface of the gravel. De-chlorinated ambient temperature (mean 9.7 °C, min 7.268 °C max 11.261 °C) mains water was supplied to each incubator, mean 252.54 L h^{-1}, min 200 L h^{-1}, max 300L h^{-1}, via a perforated pressure plate at the base. The incubators were lined with 20 to 30 mm of pea gravel to ensure an even distribution of inflow water.

The outflow from each incubator was through standing overflow pipes directed in to a mesh, collecting box within the outflow trough. The inflow was recorded manually twice daily for each inflow tap and the temperature was recorded hourly using Tinytags (Gemini Data Loggers UK Ltd.). For further details of the aquarium set up see Riley *et al.* (2015; see Chapter 4).

5.4.2 Sampling procedure

Eggs were allowed to develop in the gravel under the different lighting intensities until their natural dispersal whereby the fish swam up in to the water column to migrate downstream, as they would in the wild. 24-hour monitoring of the fry dispersal began on 31 March 2012; however, fish did not begin emerging from all incubators until 9 April 2012 (see Chapter 4). Upon dispersal, individual fish swam downstream and out through the outflow of the incubators where they were retained in mesh collecting boxes. All fish used as part of this study were sampled immediately from the outflow of the incubators upon dispersal during a three-hour period between 21.00 and 00.00 (GMT), to rule out any effects of diel rhythms in cortisol production.

5.4.3 Population water cortisol sampling from the incubators

Due to the low expected concentrations of cortisol in the water of the incubator, direct point sampling was not attempted; a novel method was used in which cortisol is absorbed by a passive sampler to provide an integrated hormonal history of a fish population over time (Scott and Ellis 2007). Although it is assumed that passive samplers absorb steroids at a rate dependent upon their concentration in the water, their use for cortisol has not yet been validated. It must also be recognised that tank-specific factors such as water flow (mean 252.54 L h^{-1}, min 200 L h^{-1}, max 300L h^{-1}) and biofilm growth on samplers could affect uptake rate.

Polar Organic Chemical Integrated Samplers (POCIS) were prepared in a standard method (see Alvarez *et al.* 2004) and one was deployed in each incubator, in similar positions on the surface of the gravel. The

POCIS were deployed when it was calculated that the embryos had absorbed the majority of their yolk-sac and were close to dispersal (based on predicted development using degree-days) to limit uptake of any residual maternal cortisol and reflect any cortisol response to treatment. The POCIS were deposited once the fry had absorbed the majority of their yolk sac and were close to dispersal, based on their degree-days development time. This was in order to limit, as much as possible, the amount of maternal cortisol that was absorbed by the membranes. The POCIS were placed in all incubators on 25 March 2012, removed on 21 April 2012 and stored at -20 °C. Cortisol was extracted from the sorbent by methanol elution, followed by solid phase extraction (see Alvarez *et al.* 2004) with ethyl-acetate as the final eluate. Extracts were stored at -20°C until evaporation (under nitrogen) and reconstitution in 1 ml of radio-immunoassay (RIA) buffer for assay (Ellis *et al.* 2004). The use of RIA has been validated for Atlantic salmon and other species (Ellis *et al.* 2004; Fanouraki *et al.* 2008) and is becoming an increasingly popular technique for non-invasive hormone sampling (Scott and Ellis 2007).

5.4.4 Individual water cortisol sampling using separate static containers

Individual level sampling began on 10 April 2012 and continued for nine consecutive nights. In this method, individual fish are removed from the mesh collecting boxes and placed in a small container of clean water for a standard time. Static sampling, a non-invasive sampling technique, utilizes the fact that fish excrete free steroids from the bloodstream into the water over the gills (Ellis *et al.* 2004; Adams *et al.* 2004; Fanouraki *et al.* 2011). It has been used to assess release of a variety of steroids in a diverse range of fish species (Ellis *et al.* 2013), however, it has not previously been applied to fish <0.5 g in weight, at an early ontogenetic stage. It suffers from the potential disadvantage that the procedure itself (handling and confinement) may affect the amount of cortisol released into the water; this impact can be limited by restricting the duration of the collection period.

Immediately after entry into the mesh collecting box, individual fry were netted and placed in a beaker containing 10 ml of clean inflow water. After 30 min, the fry were removed and weighed. Water samples

were placed temporarily on ice (maximum 2h) before storage at -20°C. Thawed water samples were passed through solid phase extraction cartridges, the cortisol retrieved by ethyl-acetate elution, and this eluate was evaporated under nitrogen before reconstitution in 0.5 ml of RIA buffer for assay (Ellis *et al.* 2004). Additional quality control samples (blank samples of clean inflow water; cortisol spiked inflow water samples) were prepared and processed contemporaneously.

As this method has not previously been applied to small fish at an early ontogenetic stage, three individual fry were placed in beakers of clean inflow water for 1 h (ascribed as positive control, PC). This was to determine whether salmon fry at the dispersal stage mount a cortisol response to a putative stressor (handling and confinement). A second positive control, 10ml of clean water spiked with 50µl of cortisol, was used to determine the recovery rate of the assay. Finally, a negative control was produced using a frozen sample of the clean water used in the study. All samples were stored on ice after collection and frozen within 2 hours of at -20°C.

5.4.5 Statistical analysis

All statistical analysis was conducted in R (Version 2.13.2, R Development Core Team 2012). For statistical comparisons, individual water cortisol sample values were converted to a release rate (pg g^{-1} h^{-1}) while population water cortisol values (from POCIS samples) were not converted. Factors (light treatment, weight, day and incubator) influencing the cortisol release rate of the emergent fry were evaluated using a generalised linear modelling (GLM). Two-way interactions between independent variables were evaluated. GLMs were also used to look for differences in cortisol levels between control and pooled experimental group means/positive controls.

5.5 Results

A total of 297 fish were individually sampled over a dispersal period of nine days. Initial analysis was conducted on a representative subset of 51 samples from across the sampling period and experimental incubators. This subset comprised three samples from the positive controls and 48 across all each experimental incubator (and thus each light treatment) over the nine sampling days to test the effect of light on the stress response of emergent fry. Due to the non-significant treatment effect demonstrated by the preliminary analysis, the remaining samples were not analysed and so all data presented are from the preliminary sub-set of the total data.

5.5.1 Population water cortisol sampling from the incubators

Cortisol was readily measurable in all 10 POCIS samples, falling in the middle of the RIA standard curve (Table 5.2).

Table 5.2 Results of the population level sampling, cortisol extraction values (pg) in 100 uL of assayed solution, for the deployed POCIS for each of the eight experimental incubators and the two control incubators (shaded). Extractions conducted on 7/12/12.

Incubator	RIA result - Cortisol (pg)	Cortisol in extract (pg)
0.1A	26.4	264
0.1B	25.6	256
1A	23.2	232
1B	19.3	193
2A	23.9	239
2B	34.8	348
4A	29.1	291
4B	33.6	336
8A	24.9	249
8B	56.2	562

General lindear modeling revealed light intensity had a marginally significant influence on the cortisol content of the POCIS ($p = 0.0415$), with the cortisol content of the POCIS membrane increasing with increasing light intensity (Fig. 5.1).

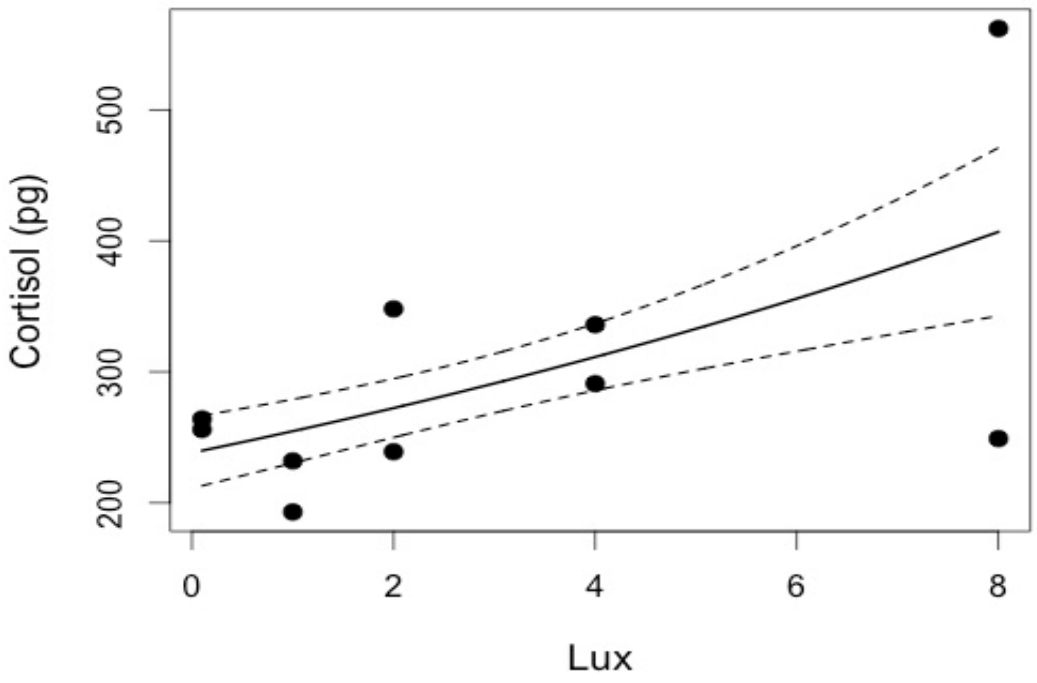


Figure 5.1 Amounts of cortisol (pg) retrieved from the POCIS samples at each of the experimental light intensities (lux) with a line of best fit (+/- 1SE) generated from a GLM model with a Gamma error family and a log-link function ($F_{1,8}$ =5.979, P = 0.0415).

Effect size was calculated for the mean cortisol content of the POCIS between the control (0.1 lux) and highest experimentally-lit treatment (8 lux), and there was found to be a large effect of the light on the cortisol level between these two treatments (d = 1.314).

5.5.2 Individual water cortisol sampling using separate static containers

A representative subset of 48 treatment samples from across the sampling period and experimental incubators was initially assayed, plus the three PC and six quality control samples (Appendix 3). Cortisol was readily measurable in the positive control samples (confined for 1h), with samples falling in the middle of the RIA standard curve. The amounts of cortisol in treatment samples (confined for 30 min)

were lower, falling within the upper third of the RIA standard curve. The three quality control blank samples returned zero or negligible cortisol values, and the three spiked quality control samples provided highly variable recoveries of 125% to 350%.

5.5.2.1 Weight

Although the total cortisol within the water extract was not significantly influenced by fish weight, subsequent analysis was conducted on cortisol release rate data (i.e. adjusted for duration), as this is a more widely used measure of stress in the literature and eliminates any potential influence arising from differences in weight across days.

No significant differences were found in the weights of sampled fish between days, individual experimental incubators or light intensities.

Table 5.3 Results of a Generalised Linear Model (GLM) used to evaluate the effects of experimental light treatment and day on the Cortisol release rate (pg/g/h) of the individually sampled dispersing fry. Significant P values ($P < 0.05$) are in bold. Adjusted R-squared = 0.3630.

Value	Term	Df	F	P
Cortisol Release Rate (pg/g/h)	Light treatment	4, 36	2.006	0.114
	Day	1, 36	9.793	**0.003**

5.5.2.2 Light

Cortisol release rates (i.e. adjusted for duration) were greater in the positive control fry than treatment fry, indicating that dispersing fry mounted a cortisol response to handling and/or confinement (Table 5.3; Fig 5.2). Within the light treatment groups, there was no significant difference in cortisol release rate between the control (0.1 lux) and experimental (1, 2, 4 and 8 lux) groups; however the cortisol release rate of fry dispersing at 2 lux was significantly lower than that from the other light intensities (Table 5.3; Fig. 5.2).

There was no significant difference in cortisol release rates between the control group (0.1 lux) and experimental groups (1, 2, 4 and 8 lux) of fish (Fig. 5.2). There was, however, a statistically significant difference between the control group of fish (0.1lux) and the positive control actively stressed fish (t = 4.8847, df = 36, P = 2.136e-05, Fig. 5.2).

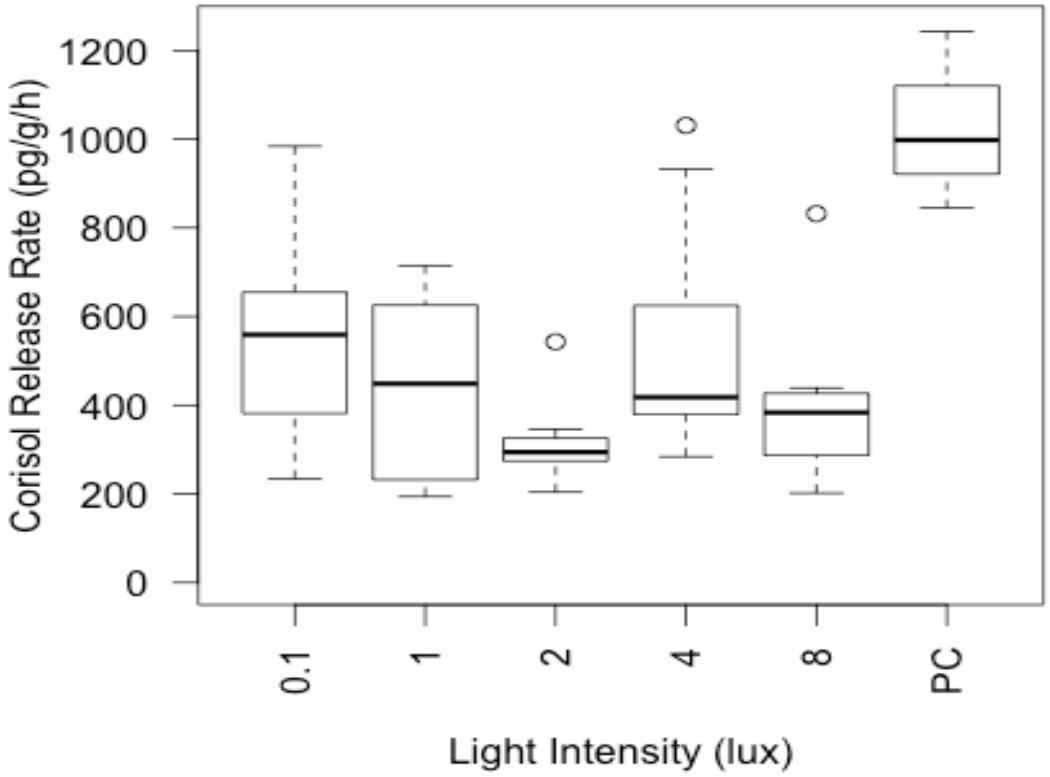


Figure 5.2 Boxplot depicting cortisol release rate against nocturnal light level (0.1-8 lux) for each of the experimental light treatments (n = 48; no. of samples per treatment: 0.1 lux n = 9, 1 lux n = 8, 2 lux= 8, 4 lux n = 9, 8 lux n =10) and the positive control (PC, n = 3).

5.5.2.3 Sampling Day

There was a significant effect of sampling day on cortisol release rates (Table 5.3, Fig. 5.3) and a negative trend can be seen in the data (Fig. 5.3), suggesting that cortisol release rates were lower among fish sampled later in the study period.

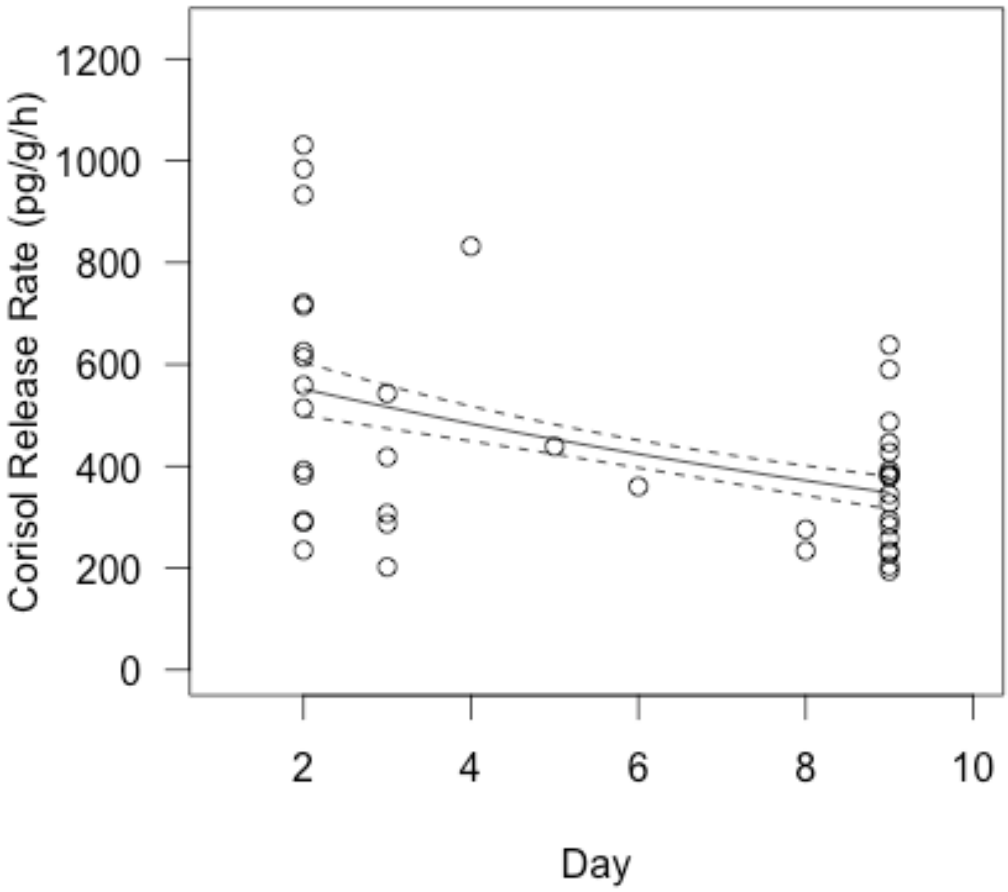


Figure 5.3 Cortisol release rate declined significantly across the nine sampling days. The line of best fit (+/- 1SE) was generated from the GLM model with a Gamma error family and a log-link function ($F_{1,36} = 9.793$, $p = 0.003$).

5.6 Discussion

No significant effect of ALAN, at varying intensities, was observed on the cortisol stress response of individually sampled fry. As such, the results do not support the hypothesis that the previously observed delay in dispersal behaviour caused by ALAN (Riley *et al.* 2013, 2015) is associated with a cortisol stress response. This lack of impact is in contrast with the aforementioned behavioural delay reported in the literature (Riley *et al.* 2013, 2015). The results show that ALAN did not significantly affect the levels of cortisol sampled in individual fish, although there was a marginally significant effect in the population

data as sampled using the POCIS method. The relatively small sample size limits the power of these tests, and further samples would clarify the result.

The work described here demonstrates two important points concerning the ontogeny of the cortisol response in juvenile salmonids and appropriate sampling methods. Firstly, this is the first study to reveal a cortisol stress response to an external stimulus in Atlantic salmon at this early stage of development. Though Necheav *et al.* (2006) induced a stress response in developing fry via an injection of adrenaline, the results in this chapter are the first evidence of Atlantic salmon producing a cortisol stress response to an external stimulus at this ontological stage. Here we demonstrate that, in line with other salmonid species (*Oncorhynchus mykiss* Barry *et al.* 1995; *Oncorhynchus tshawytscha* Feist and Schreck 2002), dispersing Atlantic salmon fry can mount a cortisol response to an external stimulus. Previous research suggests strong species-specificity regarding the ontogeny, timing, magnitude and duration of the cortisol response (Feist and Schreck 2002; Fanouraki *et al.* 2011). Studies have demonstrated that the point at which fish are able to mount a stress response to stressors post hatching is dependent upon both species and environment (De Jesus and Hirano 1992; Barry *et al.* 1995; Stephens *et al.* 1997; Stouthart *et al.* 1998; Jentoft *et al.* 2002; Feist and Schreck 2002; Auperin and Geslin 2008). Whilst a number of species appear able to synthesise cortisol at the time of hatching, the development of a cortisol response to perceived stressors does not seem to appear until later in development (Jentoft *et al.* 2002).

Secondly, we have demonstrated that non-invasive sampling of cortisol is possible at this early stage in the development of Atlantic salmon. Previous studies examining the ontogeny or presence of a cortisol response have measured total body cortisol or plasma cortisol levels. Passive sampling and separate static container sampling proved to be viable non-invasive techniques for investigating the cortisol status of fry at the dispersal stage, although additional refinement and validation is required (see Ellis *et al.* 2013; see Chapter 6). The physiological stress response of vertebrates, characterised by elevated cortisol levels, is an adaptive process designed to help individuals cope with exposure to a stressor (Bonga 1997; Schreck *et al.*

2000; Barton 2002; Romero 2004), and is increasingly used by ecologists as a means of understanding species' responses to environmental stressors (Nakano *et al.* 2004; Frisch and Anderson 2005; Zuccarelli *et al.* 2008).

What is apparent from this study is that whilst behavioural delay has been reported for fry in response to ALAN during dispersal (Riley *et al.* 2013, 2015), it is not clear that this delay is mediated via a cortisol stress response. As such, it is possible that other hormones, such as melatonin, are responsible for controlling the behaviour of fish in response to the ALAN. Melatonin has been shown to be a powerful regulator of diel behavioural rhythms in response to light dark cycles in teleost fish (Ekstrzm and Meissl 1997; Okimoto and Stetson 1999; Larson *et al.* 2004), and melatonin levels have even been shown to act as a bioindicator of social stress (Larson *et al.* 2004). Due to the low concentration of melatonin obtained from this non-invasive technique it was not possible for us to measure it in this instance. It represents a viable next step (Jones *et al.* 2015), however, in attempting to elucidate the physiological mechanism behind the observed behavioural disruption to dispersal (Riley *et al.* 2013, 2015).

Dispersal is a critical period in the Atlantic salmon lifecycle (Armstrong *et al.* 2003; Riley *et al.* 2015), as the fry need to secure an optimal feeding territory and are at risk of both starvation and predation (Brannas 1995; Riley and Moore 2013; Riley *et al.* 2013). Mortality during emergence and the establishment of feeding territories is known to be very high with previous experiments suggesting this figure to be approximately 67.5% in the first few weeks, increasing to 83.5% when fish are monitored for four months post emergence (Einum and Fleming 2000). It is, therefore, intuitive that disruption to the timing of important life history events will have fitness consequences for organisms (Bradshaw and Holzapfel 2010) as early dispersal confers a competitive advantage in establishing a feeding territory through prior residency (Cutts *et al.* 1999; Einum and Fleming 2000). The fitness cost of late dispersal is all the more pertinent when the fish's fitness may be impaired due to the effects of chronic stress (Bonga 1997), as physiological condition has been suggested to be important in establishing dominance in competition

feeding interactions (Metcalfe *et al.* 1995). In addition, that the mean fry weight can be seen to decline across the experiment (see Chapter 3; Riley *et al.* 2015). That a difference in weight was not seen in this experiment, yet was clearly seen in the associated behavioural experiment (see Chapter 3) is likely as an artifact of sampling – with the fish used in this experiment representing a small subset of the overall experimental population. This decline of weight, however, is suggestive of the fry having depleted their energy reserves (yolk sac). This will further reduce their competitive ability in obtaining a feeding territory (Riley *et al.* 2015), as the fish are facing starvation and thus have little flexibility in establishing a feeding territory in order to feed (Miller *et al.* 1988). Any reduction in the survival rate of individual fry will have population level implications (Armstrong *et al.* 2003; Riley *et al.* 2013). For this reason it is of the upmost importance to fully understand the influence of any anthropogenic impacts on juvenile Atlantic salmon.

As outlined above, there is strong selection pressure during dispersal and this has been suggested to be a factor in the degree to which behavioural coping mechanisms are expressed (Vaz-Serano *et al.* 2011). Coping mechanisms, used to explain individual differences in behaviour in animals (Koolhaas *et al.* 1999, 2008; Overli *et al.* 2007), may explain why we are unable to detect individual responses to ALAN in our study. Coping mechanisms divide populations into reactive and proactive individuals (Koolhas *et al.* 1999; Ruiz-Gomez *et al.* 2011). Reactive individuals exhibit more flexible behaviour when confronted with a stressor (Ruiz-Gomez *et al.* 2008) and, as such, may delay their dispersal. Proactive individuals are bolder and follow set routines (Vaz-Serano *et al.* 2011) so may be undeterred by the light, simply performing their normal dispersal behavioural instincts. Further, proactive fish are characterized by lower cortisol responses (Ruiz-Gomez *et al.* 2008; Ruiz-Gomez *et al.* 2011) and so it is possible that by sampling only during the first few hours of darkness we did not obtain a representative sample of the population. Sampling those fish first to disperse under ALAN, it is possible that we were sampling the bolder, proactive fish and had we sampled throughout the night we may have seen a difference in the cortisol release rate indicating that the reactive fish were dispersing later in the dark period. This behavioural

mechanism could explain the observed results in this study; the behavioural coping mechanism of individually sampled fish masked any effect of light whilst the population level effect was represented in the (non-significant) positive trend seen in the POCIS data. Further investigations would be required to confirm the presence of this behavioural mechanism (see Chapter 7).

A final point to consider is the significant decline in cortisol release rate as the experiment progressed. It is possible that this is an artifact of the sampling, with the results representing a subset of the total fish sampled during the 10-day period. An alternative hypothesis, however, is that the apparent decline in cortisol release rates represents acclimation to the perceived stressor. Whilst any further comment is speculative, given that there was not seen to be any change in the behavioural pattern of dispersal that suggested acclimation to the light (Riley *et al.* 2015; see Chapter 3) it is perhaps more likely to be the sub-samples selected driving the trend.

It is important to consider that due to the exploratory nature of the methodologies and further work is required to validate the methodologies used in this chapter (see Chapter 7). Further work is required to elucidate whether the physiological condition of dispersing fry is influenced by ALAN and to determine the physiological mechanism behind the previously reported behavioural disruption (Riley *et al.* 2013, 2015), as the impact of light will likely have both physiological and conservation implications. Melatonin has been suggested as being the possible mechanism behind the behavioural disruption under ALAN (Jones *et al.* 2015). Further work could also investigate the possibility of the presence of two distinct coping styles in Atlantic salmon fry in response to ALAN (see Chapter 7).

Chapter 6: The effect of artificial light at night (ALAN) on diel patterns of refuging and foraging in Atlantic salmon (*Salmo salar*).

6.1 Abstract

Recent concern over the rise of artificial light at night (ALAN) has led to an increase in research seeking to determine the ecological and behavioural impact on organisms. The diel pattern of light and dark is one of the largest influences on organisms' behaviour, it is proposed to have widespread implications, yet no previous studies have examined the impact of ALAN on the behavioural patterns of individual organisms around the 24-hour clock. In this study, post young of the year (PYOY) Atlantic salmon (*Salmo salar* L.) parr were individually housed in experimental tanks under differing, but ecologically relevant, lighting regimes (two experimentally lit treatments, high – 7 lux and medium – 4 lux, and a control) to determine the impact of ALAN on the daily patterning of behaviour of the fish, and the effect on both their refuging and feeding behaviour. Parr were PIT tagged to allow for continuous monitoring of their behaviour around the 24-hour clock and to determine any behavioural shifts under ALAN. Fish housed under the highest intensity ALAN refuged a mean 28% more than those housed under control conditions, and the periodicity of their refuging behaviour significantly affected. The medium intensity ALAN treatment, however, was not found to influence either the amount or periodicity of refuging behaviour. Further, ALAN at either experimental intensity did not significantly impact the amount or periodicity feeding behaviour, fish mass was found to be a greater determinant of propensity to feed than lighting treatment. The data presented in this chapter are supportive of ALAN disrupting the diel pattern of refuging behaviour in Atlantic salmon parr.

6.2 Introduction

Chronobiology is the study of species adaptation to the earth's diurnal and seasonal rhythms (Kyba *et al.* 2011) and how their circadian clock coordinates an organism's response to lighting regimes (Bruning *et al.* 2011). The internal circadian clock plays an important role in regulating the organism's metabolism, growth, endocrinology and behaviour (Dunlap 1999; Hölker *et al.* 2010a)**.** Organisms have evolved in response to stable and predictable patterns of alternation between light and dark, known as photoperiods (Longcore and Rich 2004; Bruning *et al.* 2011). Artificial light at night (ALAN) is increasing globally (Hölker *et al.* 2010b), yet the physiological and behavioural impacts of this on freshwater species are still largely unknown. Given this increase in ALAN, there is a less obvious distinction between day and night, and for this reason the natural cues that would previously have activated nocturnal behaviour may be lost (Bruning *et al.* 2011). Light is considered to be one of the largest influences on an organism's behaviour and thus it is to be expected that any changes to this once predictable regime will result in large-scale changes (McConnell *et al.* 2010; Kyba *et al.* 2011).

When considering the timing of organisms' daily events; such as when they are active, when they feed and when they shelter, broadly speaking they are either nocturnal or diurnal in their patterns of activity and have evolved to suit their light-determined niche. Time-activity budget studies examine the diel patterning and extent of behaviours in a given organism and provide valuable insight into the habitat use of organisms (Michot *et al.* 2006; Crook *et al.* 2009). Time-activity budgets are fundamental aspects of the behavioural ecology of animals, and will have consequences for their fitness and survival, as they determine the timing and frequency of interactions the animals will have with both their prey and predators (Sih *et al.* 1998). Further, the distribution of time spent between hiding from predators and actively feeding will not only influence the evolutionary fitness of the individual but will also affect the population as a whole (Walters and Juannes 1993).

An individual organism's behavioural choices concerning refuging (hiding or sheltering from predators) and feeding are intrinsically plastic, based on their perception of their predation risk and abundance of prey at any given time (Lima and Dill 1990; Stephens and Krebs 1986; Sih *et al.* 1998; Vehanen 2003). Individuals behave in a way that balances their mortality risk (μ) and feeding rate (f) (Fraser *et al.* 1993; Clark and Levy 1988), and attempting to minimise μ/f (Sih *et al.* 1998). Since most organisms cannot feed whilst refuging and must therefore choose between growth and survival the outcome of this trade off can be considered as animal decision-making (Dill 1987; Sih *et al.* 1988; Metcalfe *et al.* 1999; Bradford and Higgins 2001) and will influence predator-prey interactions. Whilst peak-feeding generally occurs during the day, this is also the period with greatest risk in terms of predation (Sih *et al.* 1998; Metcalfe *et al.* 1998; Johnston *et al.* 2004). Switching activity from diurnal to nocturnal is thought to reduce the predation risk of organisms across all taxa, with nocturnal feeding being safer per unit of food obtained than diurnal feeding despite the reduced feeding efficiency due to reduced visibility at night (Sih *et al.* 1998; Metcalfe *et al.* 1998, 1999).

In fish, during times of increased predation risk, refuging behaviour is a higher priority than feeding and thus feeding behaviour will be inhibited (Hart 1986). As such, nocturnal activity is considered to be safer for juvenile freshwater fish due to the reduced numbers of visual predators, namely birds and larger predatory fish that are diurnally active. The availability and detectability of prey is another extrinsic factor that influences the motivation of fish to feed (Hart 1986; Sih *et al.* 1998). Food abundance has been found to influence the amount of time spent both in feeding and in refuging in juvenile Atlantic salmon; hungrier fish are more likely to take risks by foraging in the presence of predators (Vehanen 2003). Foraging behaviour increases the movement and decreases the vigilance of the fish, thereby increasing their risk of predation (Milinksi 1986). Suppression of feeding due to perceived predation risk or decreased food abundance will have knock-on effects for individual growth and fitness, with potentially fatal consequences, as although fish have low metabolic rates, they are still are sensitive to starvation (Hart 1986).

The diel activity patterns of Atlantic salmon (*Salmo salar* L.) parr have been well studied (Gries *et al.* 1997; Gries and Juanes 1998; Metcalfe *et al.* 1998, 1999; Johnston *et al.* 2004). During residence in freshwater, Atlantic salmon parr were generally thought to be exclusively nocturnal during the winter and primarily diurnal during the summer months (Gries *et al.* 1997; Valdimarsson *et al.* 1997), though evidence does exist to suggest that parr are nocturnal in summer also (Gries *et al.* 1997; Johnston *et al.* 2004; Orpwood *et al.* 2006). When not feeding, juvenile salmon seek refuge in the streambed in order to minimise their predation risk, during which time they are not able to feed (Valdimarsson *et al.* 1997; Metcalfe *et al.* 1998). As such, fish should adjust the diel patterning of their feeding activity to minimise their predation risk whilst ensuring they obtain the food required for survival and growth (Bull *et al.* 1996). Whilst the abundance of drifting invertebrates is higher during the night (Waters 1962; Elliot 1973), the feeding efficiency of Atlantic salmon parr declines with light intensity and thus feeding during the day is more profitable (Fraser and Metcalfe 1997; Imre and Boisclair 2005).

The switch between nocturnal and diurnal behaviour has previously been proposed to be mediated by temperature (Fraser *et al.* 1993, 1995; Valdimarsson *et al.* 1997; Johnston *et al.* 2004); however, Atlantic salmon parr have been observed to be nocturnally active during mild summer nights (Gries *et al.* 1997; LeDrew *et al.* 1996; Valdimarsson *et al.* 1997; Aumundsen *et al.* 1999, 2000) and refuging during the daytime (Fraser *et al.* 1995; Gries and Juanes 1998). Johnston *et al.* (2004) suggests that the duality in activity patterns can be explained by size, with young of the year (YOY) and post young of the year (PYOY) behaving differently according to the trade-off between growth and predation risk. Here, the bigger PYOY are acting to minimise their predation risk according to the Asset Protection Principle (Clark 1994). The Asset Protection Principle has been shown to be a biologically sound model for juvenile salmonids (Reinhardt 2002); the principle suggests that larger individuals should preferentially act to minimise predation risk, whilst smaller individuals should prioritise growth (Clark 1994). This and other studies (Metcalfe *et al.* 1998) are suggestive of PYOY Atlantic salmon parr being primarily nocturnally active, regardless of temperature and season, due to avoidance of predation being the more important

factor in the predation-growth trade-off, where nocturnal foraging offers the lowest ratio of predation risk to food obtained (Metcalfe *et al.* 1998).

The unwanted ecological effects of ALAN are increasingly examined in terms of the individual species impacts (Stone *et al.* 2009; Riley *et al.* 2012, 2013; Dominioni *et al.* 2013) and ecosystem level effects (Longcore and Rich 2004; Davies *et al.* 2013; Perkin *et al.* 2011). It has been suggested that ALAN will affect the diel behavioural patterns of individual organisms, as the cue for the activation of these behaviours are related to light intensity (Kurvers and Hölker 2015). This is particularly true of the trade-off between feeding and refuging (Kronfeld-Schor *et al.* 2013). ALAN has been shown to affect the feeding behaviour of a broad range of species, from beach-mice (*Peromyscus polionotus leucocephalus*) (Bird *et al.* 2004) to wading birds (Santos *et al.* 2010) to bats (Blake *et al.* 1994; Polak *et al.* 2011), and can be seen to both increase (Blake *et al.* 1994; Santos *et al.* 2010; Polak *et al.* 2011) and decrease (Bird *et al.* 2004; Polak *et al.* 2011) foraging. When examining the impact of ALAN on the diel behavioural patterning in any given species, however, it is important to remember that no organism exists in isolation and any individual level effects will influence not only that particular species, but also their predators and their prey. ALAN has been seen to disrupt normal nocturnal patterns of behaviour in Atlantic salmon (Riley *et al.* 2012, 2013, 2015) and it is thought that this is due to a perceived predation risk. Given that avoidance of predation is thought to be the primary objective of PYOY parr, and their activity is increasingly thought to occur under the cover of darkness (Gries *et al.* 1997; Johnston *et al.* 2004; Orpwood *et al.* 2006) it is important to understand the impact of ALAN and illumination the nocturnal environment on this behaviour.

ALAN has been suggested to increase the nocturnal activity of diurnally active organisms and to decrease the nocturnal behaviour of those that are normally nocturnal through increasing the diurnal period (Kronfield-Schor *et al.* 2013). High nocturnal illumination, due to a full moon or ALAN, decreases the nocturnal activity of juvenile rainbow trout (*Oncorynchus mykiss*; Contor and Griffith 1995), whilst in

Atlantic salmon no correlation was found between moon phase and the numbers of nocturnally active fish (Imre and Boisclair 2005). For the PYOY parr, it could be suggested that ALAN will represent an increased predation risk, as is the case for daylight and as such, will impact on the timing and frequency of nocturnal foraging behaviour. In contrast since the feeding efficiency of Atlantic salmon is much higher on bright nights (Imre and Boisclair 2005), it is also possible that the parr may take advantage of the increased feeding potential. Given the plasticity of the nocturnal/diurnal foraging behaviour of Atlantic salmon, they represent an interesting species to study the effects of ALAN on their nocturnal behaviour. As such, allowing for the behavioural plasticity of Atlantic salmon parr, the way in which ALAN affects their diel pattern of activity may depend upon other aspects of their state (e.g. body size) or environment (e.g. water temperature) as outlined above.

Despite the increasing interest in determining the effect of ALAN on organisms, the impact on the diel periodicity of behaviour is relatively unstudied. Whilst no previous studies have investigated how ALAN affects the behavioural decisions of individual fish in a full 24-hour period, studies have examined the impact of ALAN on the dispersal behaviour of individual Atlantic salmon fry (Riley *et al.* 2013, 2015; see Chapter 3) and smolts (Riley *et al.* 2011) around the 24-hour clock. A complete picture of how ALAN influences the behavioural trade-offs in individuals, across both night and day, is required in order to elucidate how diel patterns of refuging and feeding are affected. Due to the importance of the freshwater phase in the Atlantic salmon life cycle for population persistence (Imre and Boisclair 2005), an understanding of how ALAN may impact the growth and survival of PYOY parr is crucial to prevent further population declines.

6.3 Aims and Hypotheses

This study seeks to determine the influence of ALAN on the diel pattern of refuging and feeding behaviour in post young of the year (PYOY) Atlantic salmon parr using continuous PIT monitoring of the

fishes' behaviour around the 24-hour clock. The three objectives for Chapter 6 are to: (1) Assess the impact of ALAN on the diel patterns of refuging and foraging behaviour; (2) Determine how altering the intensity of light influences the effect of ALAN, and; (3) Infer the consequences for predator-prey dynamics. The hypotheses tested as part of the experiment are: that fish under ALAN will (1) spend more time refuging than their control counterparts and (2) spend less time foraging than their control counterparts; (3) ALAN will disrupt the diel timings and pattern of foraging and refuging, and; (4) the higher the intensity of ALAN the greater the disruption of natural diel behaviours.

6.4 Materials and Methods

6.4.1 Acquisition of fish

Thirty-one Atlantic salmon parr (PYOY fish) (weight mean 11.2g, min 7.4 g, max 14.6g; fork length mean 10 cm, min 8.8 cm, max 11 cm) were obtained from Maerdy Hatchery, Corwen, Wales and were transported to Cynrig Hatchery, Brecon, also in Wales. Here they were housed at low density, to minimise stereotypical hatchery behaviour, and were introduced to a live diet, to reduce acclimation time, prior to the start of the experiment. The parr were naïve to predators and were not exposed to predation risk prior to the start of the experiment. At Cynrig, all fish were anaesthetised (0.4 mL L-1 2-phenoxy-ethanol), PIT tagged, measured for fork length (nearest mm) and weighed (nearest 0.1g). They were allowed to recover *in situ* before being transported to Lowestoft, SE England, where they were given a further week to recover before the experimental trials. At all locations, fish were maintained on a natural photoperiod and not exposed to artificial light. Prior to the start of each trial, fish were weighed and fork lengths measured again.

6.4.2 Experimental set up

Experimental work was conducted at the Cefas Laboratory aquarium, Lowestoft, UK (52°27'33"N, 1°44'22"E) during 4 – 23 of July 2013. The experiment was conducted using a Passive Integrated Transponder (PIT) Multi-Point Decoder (MPD) antennae system (Wyre Micro Design Ltd, Lancashire, UK; Riley *et al.* 2003) to allow for uninterrupted monitoring of fish behaviour. The antennae system was composed of an MPD/antenna multiplexer unit (16-channel), two 12V gel lead-acid batteries and 16 circular panel antennae (22mm deep and 300mm in diameter including a 20mm surrounding flange). Each antenna was read every 3.6 seconds and the MPD unit was connected to a logger that stored the detection data on a removable 32GB flash memory card. The PIT system was chosen for use in this study due to the readily available and continuous data the system provides.

Eight experimental tanks (190x50 cm) were installed at the Cefas Laboratory aquarium, with two PIT tag antennae mounted on the exterior of each of the tanks. This positioning of the antennae ensured that the antennae were not used as refuges by the fish. The antennae were supported under the tanks and positioned so that they were 12 cm from the far edge and centered in all cases. The maximum detection range of the antennae was found to lie between the edge of the antennae and the edge of the flange. This meant that the fish would not be detected as they swam around the outside of the tank, only when they moved in to the middle of the tank to feed or refuge.

De-chlorinated ambient temperature (mean 17.2°C, min 15.4°C, max 19.1°C) mains water was supplied to each tank (100L h^{-1}/2.78 $m^3.s^{-1}$), the outflow from each tank was through standing overflow pipes. The temperature was recorded hourly using Tinytags (Gemini Data Loggers UK Ltd.). The water depth used in the first experimental run was 7 cm, however, due to the loss of a fish that jumped out of a tank, the water depth was lowered to 6.5 cm for the second run. During the second run, however, 3 fish jumped out of their tanks. Three experimental runs were conducted; however, due to high temperatures the third run was

terminated early so that the health of the fish was not compromised. Therefore, the data from this third run do not form part of this chapter.

6.4.3 Experimental conditions

The diurnal lighting levels in the aquarium were manipulated to be representative of natural daylight using daylight mimicking, low-pressure mercury discharge fluorescent lamps (Philips Master TL-D 58W/865; 1.5m in length). A streetlight (Philips Master Cosmo White; CPO-T White 45W/628PGZ12) was mounted in a luminaire (Philips 'iridium series' opti-C street lighting unit) 1.7 m above the tanks to enable the manipulation of nocturnal light levels across them. At the time of the study, the lamp was the most commonly installed streetlight across the UK. The diel periodicity of the lighting was controlled by an electronic timer that triggers the switch from day to night time lighting levels and activates an electronic ballast (EFI Ltd, Lowestoft, UK) in the daytime luminaires that result in an 8 minute dawn and dusk period. The simulated day length during the course of the experiment was representative of natural seasonal conditions and was fixed for the duration (Daytime lights off 20.33 GMT, Streetlight on 20.41 GMT, Streetlight off 03.25 GMT, Daytime lights on 03.33 GMT).

Neutral density filters were attached to the lamp to reduce the intensity of the light produced to correspond with levels measured in street-lit field settings (Riley *et al.* 2013), without altering the frequency. The different light intensity treatments were achieved by manipulation of the number and location of the neutral density filters on the street lamp. Light intensity readings were taken (measured using a digital lux meter, RS 180-7133, accuracy ± 4%+2 Digits; RS Components Ltd, Corby, UK) on the surface of the water in each tank at several points and care was taken to ensure that each paired replicate had matching light intensities. Three night-time light intensities were used during the experiment; control (0.1 lux), medium and high (Table 6.1). Due to the shadow created by the sides of the tank, the medium light intensity tanks had a strip of shadow along the length of the tank measuring 14 cm in each. The 0.1 lux

level is representative of a bright moonlit night (Longcore and Rich 2006) and as such is the control intensity for the lit tanks.

Table 6.1 Light intensities across experimentally lit (Medium and High, 1-4) and control tanks (5-8). Minimum and maximum light intensities are given, along with the intensities at the two PIT detectors situated at the refuge and feeding station.

		Intensity (lux)			
Tank	**Treatment**	**Feeding station**	**Mouth of refuge**	**Min**	**Max**
1	Medium	4.4	3.6	0.2	6.4
2	High	7.3	5.3	4.6	8.3
3	High	7.7	5.0	4.0	8.4
4	Medium	4.7	3.1	0.3	6.1
5, 6, 7, 8	Control	0.1	0.1	0.1	0.1

6.4.4 Experimental procedure

Fish were held in a stock tank under natural photoperiod and fed frozen bloodworm (*Chironomidae tetans*) (Gamma TMC, Hertfordshire) scattered on the surface of the water once daily. At the start of each experimental run, eight fish were randomly selected, removed from the stock tank and placed in the experimental tanks, however, due to the loss of fish the actual sample sizes were 7 for the first run and 5 for the second run. Fish were housed individually in the experimental tanks and their behaviour was continuously monitored by the PIT antennae system. Each experimental run was monitored for five consecutive 24-hour periods, the fish were then removed and placed back in the stock tank. The MPD logger data were downloaded and the food parcels were changed at a set time each day, (11.00 GMT) in the middle of the light period to ensure minimal disruption to the fish. The fish were otherwise undisturbed for the duration of each experimental run.

Each tank was provided with an identical refuge, created using half a terracotta plant pot, and a feeding station, all orienting in the same direction, with the distance between the two being 108 cm in each tank.

In all tanks, the food parcel and refuge were each placed above the centre of one of the two the PIT antennae, 25 cm from the end of the trough, so that the fish's presence in either location could be separately recorded. Each food parcel was made using two squares of netting wrapped around a frozen bloodworm cube (Gamma TMC, Hertfordshire), weighted with a stone and wrapped with an elastic band to form a parcel. The feeding stations were positioned downstream of the refuge in order to lessen the possibility of food being carried towards the refuge, ensuring that the fish had to leave the refuge in order to feed. Due to the design of the food parcel, with small mesh squares, and the low flow conditions within the tanks, bloodworm was not readily distributed around the tank. Thus, the parr needed to actively feed from the food parcel and ensuring that feeding attempts were captured by the PIT system. Whilst parr typically drift feed, to quantify the amount of feeding taking place under the differing ALAN regimes it was necessary to provide a single food source that would not readily spread throughout the experimental trough. It is for this reason that the fish were transitioned on to bloodworm prior to commencing the experiment. Further, since the parr used as part of the experiment were hatchery fish, they were not habituated to drift feeding. As such, the feeding behaviour in the experiment is representative of the parr's propensity to feed rather than attempting to mimic drift-feeding behaviour under natural conditions. In the first experimental, run only one block of frozen bloodworm was offered in each of the feeding station; however, due to many of the parcels being empty upon changing (once daily), this was increased to two bloodworm blocks in each parcel for the subsequent runs.

6.4.5 Statistical analysis

Analysis of the PIT detection data was conducted in R v2.13.2 (R Development Core Team, 2011). General Linear Mixed Modelling (package lme4) was used to determine the influence of independent variables (day, lighting treatment and their interaction) on the amount of time that parr spent refuging and feeding (number of detections at each PIT antennae) using fish ID as a random factor in the models.

Patterns in the timing of daily refuging and feeding behaviours for each fish and lighting treatment were investigated using circular statistics (Batschelet 1981); all circular statistical analysis was carried out in R (using the package "circular"). Through the creation of a circular vector from the raw time of day data, circular descriptive statistics were calculated from the data. These included information regarding the timing and pattern of activity; the mean and median time of activity and Rho, as well as measures of variation within the data; angular variance, angular deviation and circular standard deviation. Rho is a measure of the uniformity of behaviour around the 24-hour clock; a value of 1 indicates a single sharp peak in behaviour, whilst a value of 0 represents uniform behaviour around the clock.

Watson's test for Circular Uniformity was applied to the median timing of activity, for each fish on each day, for the two behaviours (refuging and feeding) under the three different lighting treatments, to test in each case for deviation from a constant (uniform) level of activity around the clock. A significant P value signifies that the pattern of behaviour is significantly different from uniform.

The effects of light level on the timing of feeding and refuging behaviour were examined using a circular linear model of the timing of feeding and a second model of the timing of refuging, with light intensity (lux) as a continuous linear independent variable in each case. The repeated measures nature of the data was represented in the model by including Day as a continuous linear independent co-variate. Lux values (mean value of measurements taken across the experimental tanks) were used in place of treatment level (High, Medium, Control) because there is currently no circular GLM-type model structure available, by which categorical (Treatment) and continuous (Day) variables could be combined as independent variables in the same model (Pewsey *et al.* 2013). Daily median times of behaviour were (one median per fish per day) were used as the dependent data in these models rather than mean timing, because the median values are less strongly influenced by outliers.

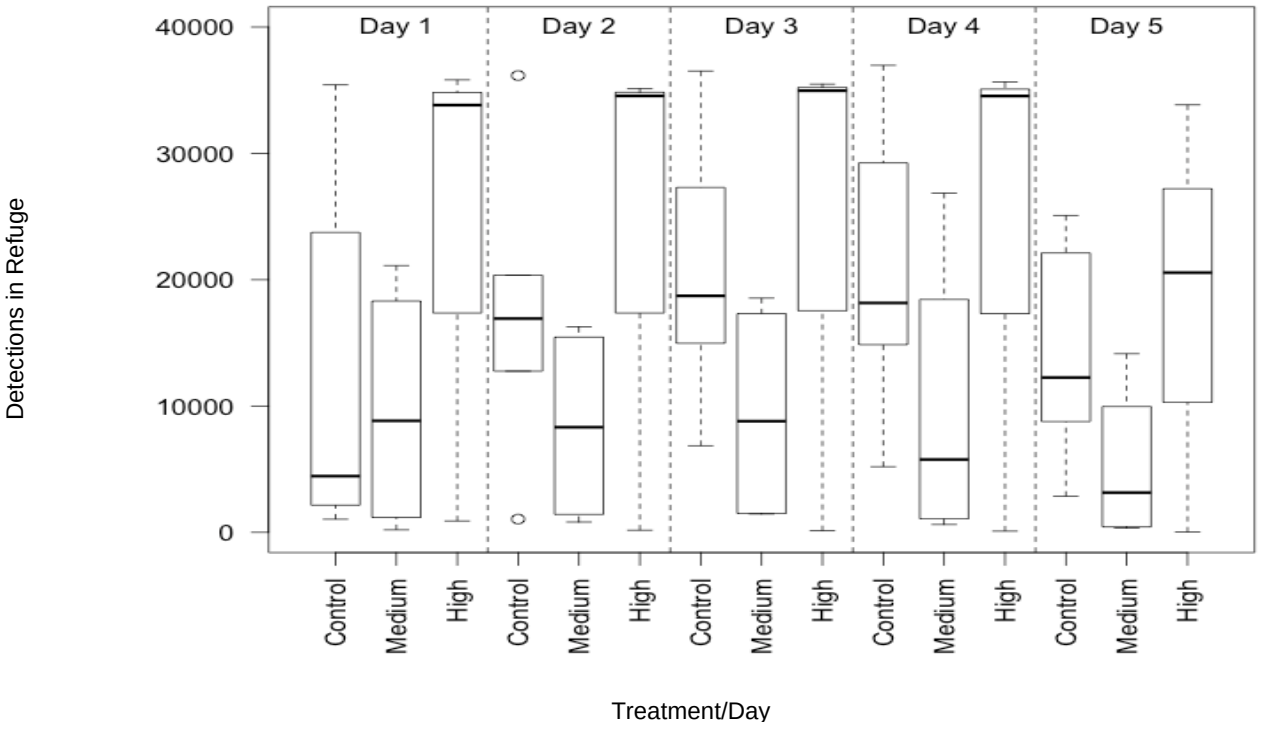
6.5 Results

6.5.1 Diel activity levels

The descriptive statistics for the detection data of the PIT antennae at both the feeding station and refuge provide an overview of the time spent in each location of the fish in each treatment over the duration of the experimental runs (Appendix 4). The amount of time spent refuging, determined by the number of detections in the refuge during the 24-hour period, was seen to differ between the lighting treatments (Fig. 6.1a). The greater the number the detections of the fish in the refuge, the more time fish spent in the refuges. General Linear Mixed Modelling (GLMM) revealed a significant difference in the time spent in each location between treatment levels (binomial GLMM: $Chi^2 = 10.302$, d.f = 2, P = 0.006), with the fish at the higher light intensity being detected in the refuge significantly more than those in the medium treatment (t = 3.165, d.f. = 1, P = 0.0032; Fig. 6.1a), whilst the number of detections in the refuge for the fish in the control treatment were not significantly different from the medium treatment (t = 1.589, d.f = 1, P = 0.164).

Conversely, the amount of feeding, determined by the number of detections at the feeding station during the 24-hour period, was not seen to differ from the control treatment in both the high (t = 0.012, d.f = 2, P = 0.986; Fig. 6.1b) and medium (t = - 0.065, d.f = 2, P = 0.948; Fig. 5.1b) experimentally lit treatments. There appears to be little discernable effect of lighting treatment on the number of detections of the parr at the feeding station each day. General Linear Mixed Modelling (GLMM) revealed that the number of detections at the feeding station varied significantly by day in the control treatment (t = -2.249, d.f = 8, P = 0.0245; Fig. 6.1b), and a marginally non-significant effect for the high ALAN treatment (t = -1.952, d.f = 8, P = 0.0509; Fig. 6.1b) with an apparent downwards trend over the duration of the experimental runs, indicating a reduction in feeding activity over the timescale of the experimental run.

(a)



(b)

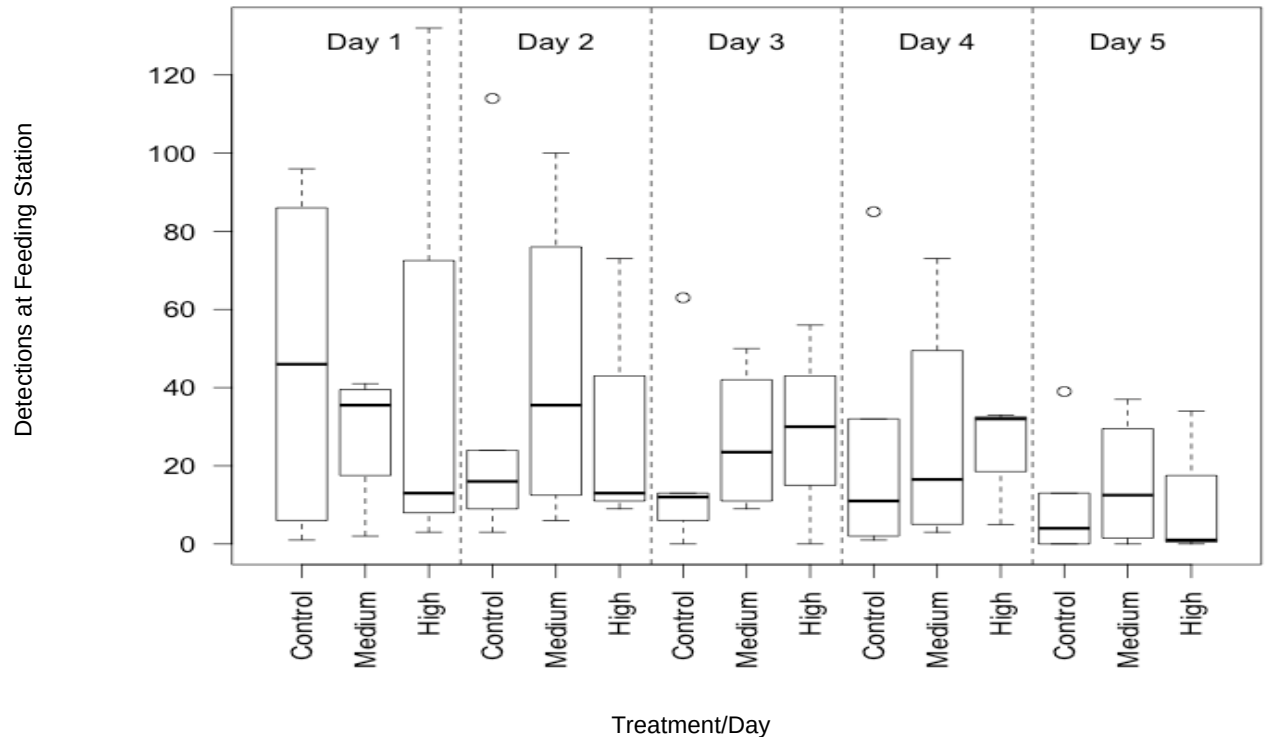


Figure 6.1 Boxplots showing (a) the number of detections in the refuge for each of the lighting treatment, by day, across the two experimental runs and (b) the number of visits to the feeding station for each of the three lighting treatments, by day, across the two experimental runs.

General Linear modelling revealed fish mass had a significant positive association with the number of detections at the feeding station (t = 2.529, d.f = 1, P = 0.0142; Fig. 6.2) with larger fish feeding more, regardless of light treatment. Mass did not significantly influence the number of detections of the fish within in the refuge (t = 0.159, d.f = 1, P = 0.874).

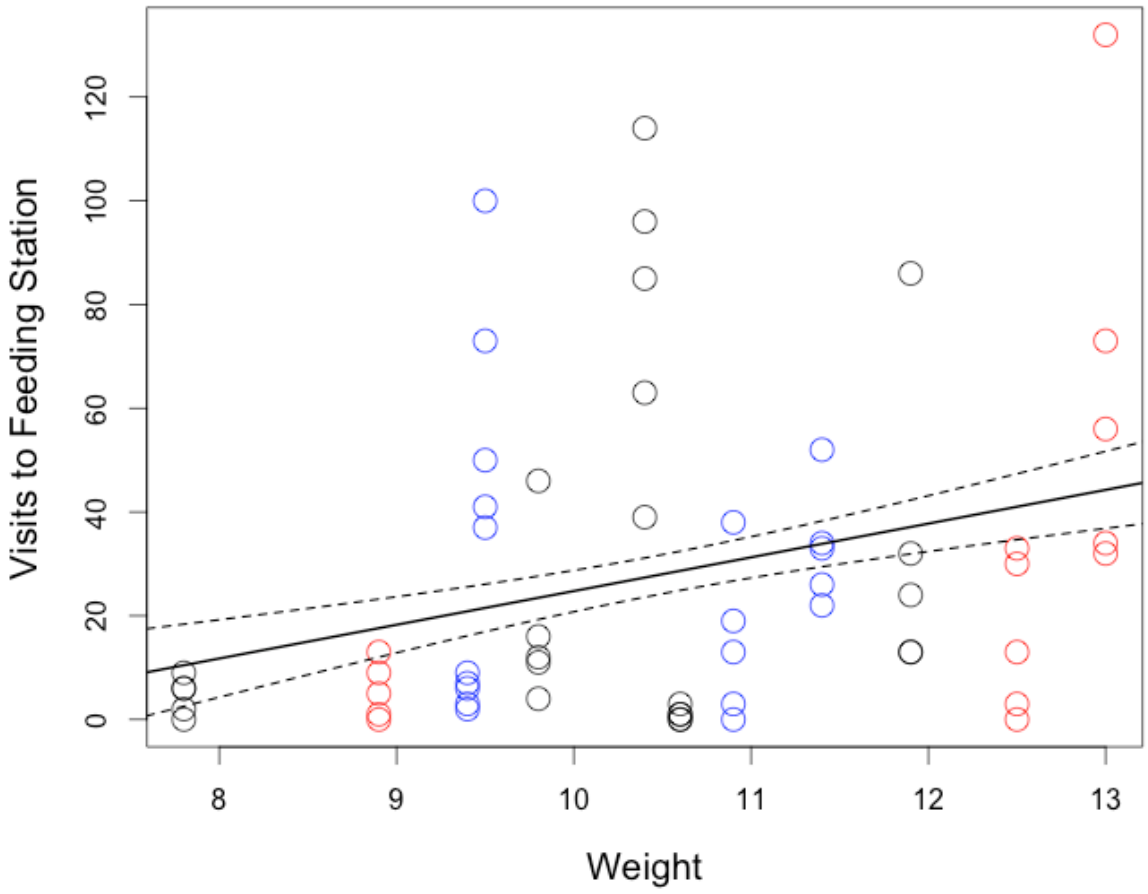


Figure 6.2 The number of visits to the feeding station by the individual fish of differing mass (g), for the three lighting treatments (Control = black; Medium = blue; High = red) with a line of best fit (+/- 1 SE) generated from a GLM model with a Gaussian error family and an identity-link function ($F_{2, 57} = 13.810$, P = 0.0188).

6.5.2 Diel behavioural patterns

The effect of lighting treatment on the timing of both feeding and refuging were calculated using circular statistics (Fig. 6.4, Fig. 6.5; Table 6.2; Table 6.4; Appendix 4).

Table 6.2 Summary statistics mean, median and Rho, for the timing of both behaviours, across all fish and grouped by light treatment – full table of data in Appendix 3.

Behaviour	Treatment	Mean	Median	Rho
Refuging	Control	13.49	11.71	0.166
Refuging	Medium	05.21	05.59	0.319
Refuging	High	14.29	14.10	0.683
Feeding	Control	14.93	13.37	0.170
Feeding	Medium	01.61	02.06	0.257
Feeding	High	12.01	13.11	0.254

Rho, the measure of the concentration of behaviour around the 24-hour clock, was examined for each behaviour type (Refuging or Feeding) under the three different lighting treatments (Table 6.3; Fig. 6.3) using General Linear Mixed Modelling (GLMM). Light treatment had no significant effect on the Rho value for the refuging behaviour of the parr (binomial GLMM: $Chi^2 = 0.6879$, d.f = 2, P = 0.709); however, Rho was significantly higher in the high intensity ALAN treatment (t = -2.076, d.f = 2, P = 0.0378) than the control and medium light intensity treatment.

There was a significant interaction between the high light treatment and day in the model of Rho data for feeding behaviour (t = -2.386, d.f = 8, P = 0.017), indicating that the effect of light on the concentration of feeding activity changed over the days of the experimental run. Specifically, Rho can be seen to decrease over the course of the five experimental days, meaning under high levels of ALAN, behaviour is becoming less concentrated and more evenly spread around the 24-hour clock (Fig. 6.3).

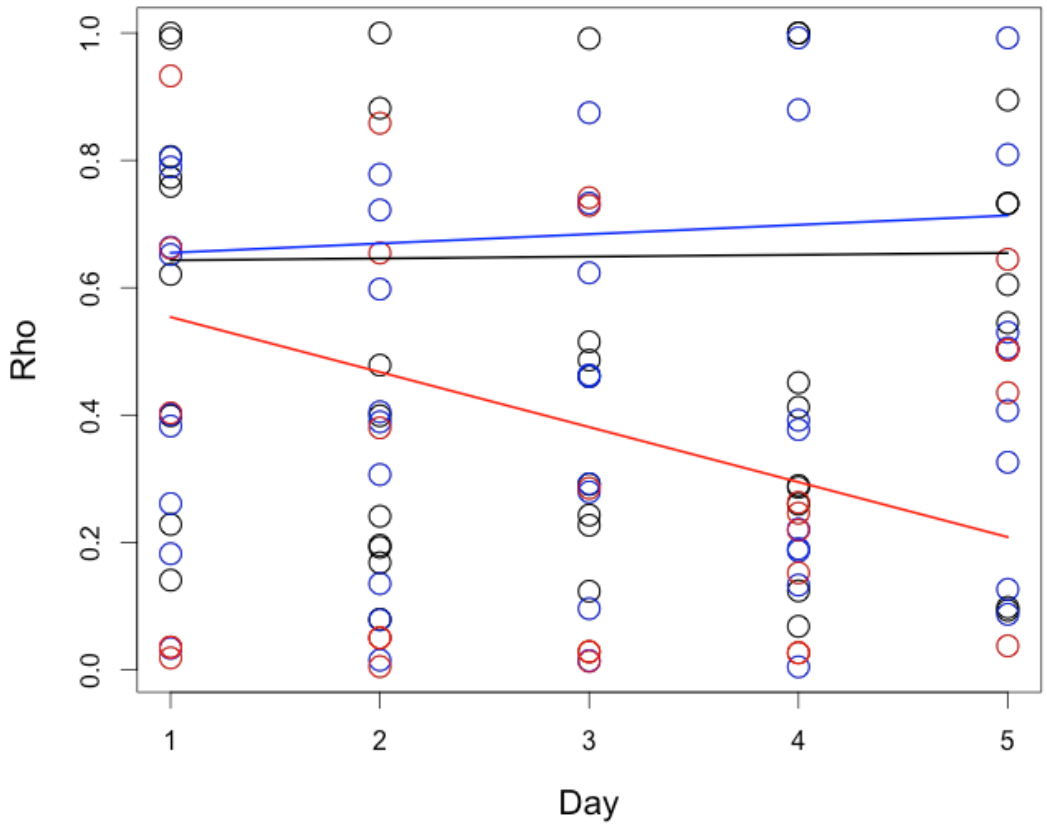


Figure 6.3 Scatterplot of Rho plotted against day, under each of the three lighting treatments (Control = black; Medium = blue; High = red with model prediction lines for each lighting treatment, in corresponding colours, plotted using a GLMM model (see text for details).

A Watson's test for circular uniformity was conducted on the median time of refuging and feeding behaviour (Table 5.4), to test for deviation from a uniform pattern in the circular distribution of the data (Fig. 5.4; Fig. 5.5). There was a significant deviation from uniformity for the median timing of refuging behaviour under the high ALAN treatment (Table 6.4; Fig. 6.4a); however, the median timing of the refuging behaviours under the medium (Table 6.4; Fig. 6.4b) and control (Table 6.4; Fig. 6.4c) treatments did not significantly deviate from uniform. The median timings of foraging behaviour under all three lighting treatments (Table 6.4; Fig. 6.5a-c) were not significantly different from uniform.

Table 6.3 Summary of the Watson test for uniformity, conducted on median timing of both behaviours, under the three lighting treatments, from data for individual fish on each sampling day (Table 6.2; Appendix 4).

Behaviour	Treatment	Test statistic	P value
Refuging	Control	0.058	> 0.1
Refuging	Medium	0.179	> 0.05
Refuging	High	0.466	**< 0.001**
Feeding	Control	0.056	> 0.1
Feeding	Medium	0.112	> 0.1
Feeding	High	0.048	> 0.1

The median timing of both refuging and feeding behaviours were also modelled in relation to light levels and day (Table 6.3). A circular linear model (Pewsey *et al.* 2013) to explain the median timing of refuging revealed a significant effect of light intensity (lux: parameter estimate = + 0.733, t = 2.480, d.f = 1, P = 0.007; Fig. 6.4) while controlling for a significant change in refuging over the five-day sampling period (parameter estimate = - 0.733, t = 2.427, d.f = 1, P =0.008).

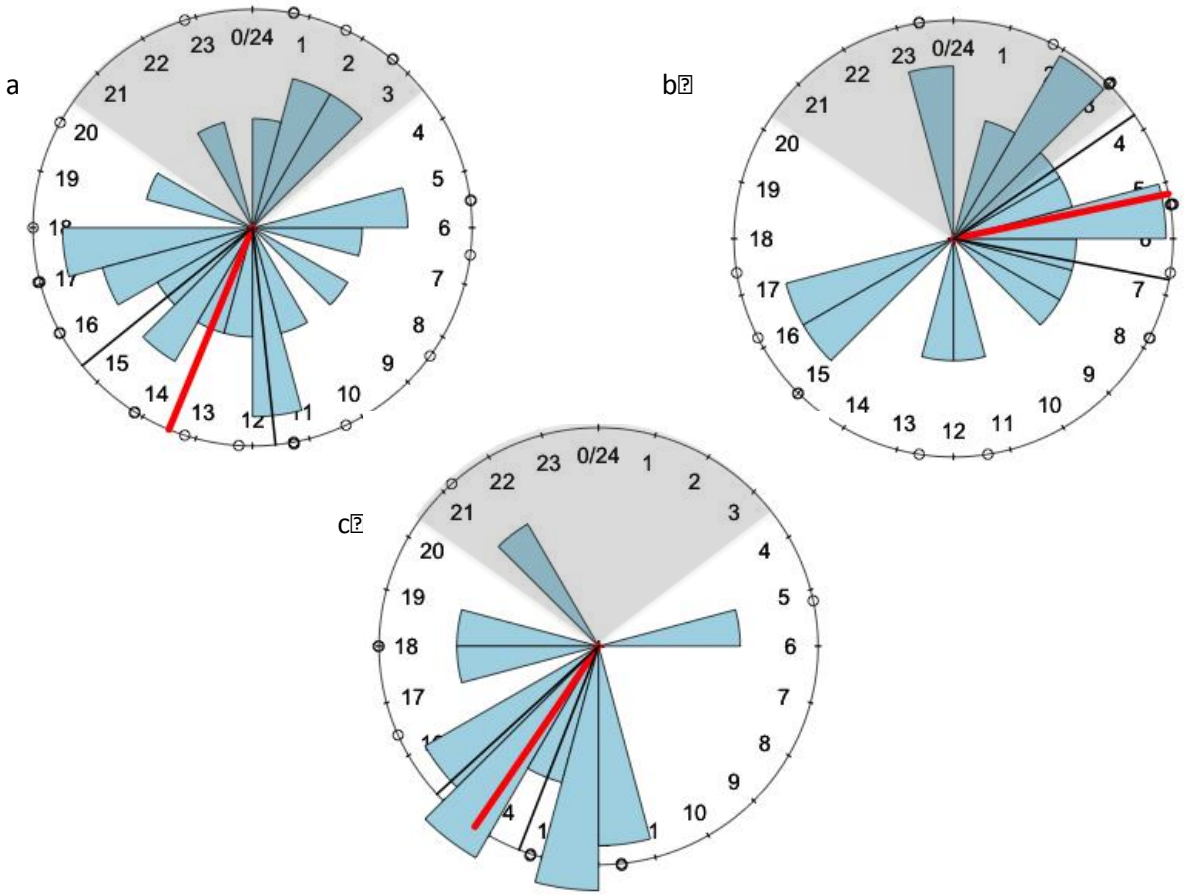


Figure 6.4 Summary circular graphs representing the mean time of refuging for each fish across the five experimental days (n = 60), under the three lighting regimes - a) control (lux = 0.1), b) medium ALAN (lux = 3.6/3.7) and c) high ALAN (lux = 6.3/6.4), Mean time of foraging (red line), +/- 1 SE (black lines). Shaded area represents the nocturnal period, between dawn and dusk, where the streetlight was on (20.33 - 3.25 GMT).

Similarly for feeding, a circular linear model to explain the median timing of this behaviour revealed a marginally non-significant effect of light intensity (lux: parameter estimate = 0.336, t = 1.632, d.f = 1, P = 0.0514; Fig. 6.5) while controlling statistically for a significant change in foraging over the five sampling days period (parameter estimate = - 0.579, t = 1.768, d.f = 1, P =0.0385).

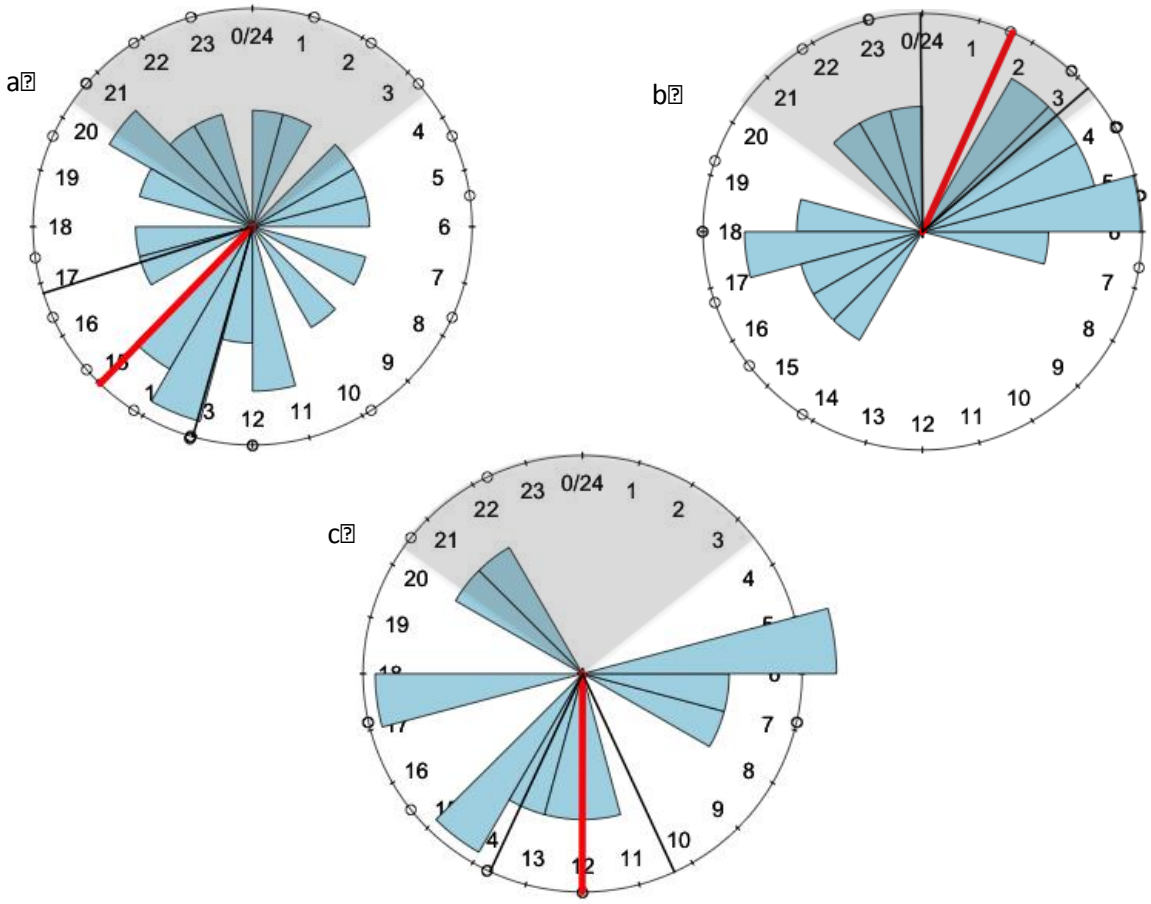


Figure 6.5 Circular graphs representing the mean time of foraging for each fish across the five experimental days, under the three lighting regimes - a) control (lux = 0.1), b) medium ALAN (lux = 3.6/3.7) and c) high ALAN (lux = 6.3/6.4), Mean time of foraging (red line), +/- 1 SE (black lines). Shaded area represents the nocturnal period, between dawn and dusk, where the streetlight was on (20.33 - 3.25 GMT).

Fish mass had a significant impact on the timing of refuging behaviour (parameter estimate = - 0.1638, t = 2.472, d.f = 1, P =0.007) but had a marginally non-significant effect on the timing of foraging behaviour (parameter estimate = - 0.2466, t = 1.513, d.f = 1, P =0.0651).

6.6 Discussion

There was a significant effect of ALAN on the amount of refuging behaviour of fish, with fish housed under higher intensity ALAN refuging 28% more than those in the control treatment. This supports H1; that fish under ALAN will spend more time refuging than their control counterparts. Conversely, there was found to be no effect of ALAN on the amount of feeding activity (H2), but the amount of feeding changed over the duration of the experimental run, regardless of treatment. In addition to fish housed under the highest intensity ALAN treatment refuging significantly more, there was also an effect of ALAN on the timing of refuging behaviour (H3) with the highest intensity ALAN found to disrupt the pattern of refuging behaviour seen in the control tanks (H4). As such, the results clearly support the hypothesis that ALAN disrupts the diel timings and pattern of refuging of the Atlantic salmon parr; yet feeding behaviour was not disrupted by ALAN - with both the amount and timing of foraging being not significantly affected by lighting treatment (H3 and H4). The higher intensity ALAN treatment, however, significantly affected the uniformity of the distribution of feeding behaviour throughout the 24-hour sampling period, in an interaction with sampling day. Further, the number of visits to the feeding station varied significantly across the sampling days in the control treatment. The results presented here clearly demonstrate that ALAN significantly affects the amount of refuging in PYOY Atlantic salmon parr, as well as the timing of refuging behaviour and at a high intensity, has a disruptive impact on the uniformity of feeding behaviour.

First, is is important to note that this is believed to be the first study to examine the impact of ALAN on the full diel periodicity of individual behaviour in fish, and provides valuable information concerning the way that ALAN affects the diel pattern of an individuals' refuging and feeding behaviour. Previous studies have looked at the behaviour of individual fish under ALAN (Riley *et al.* 2011, 2013, 2015), but no studies have examined the impact on full daily behavioural patterns around the 24-hour clock. Here individual fish were only sampled once (upon dispersal) and thus sampling points were individual fry as

they moved from the surface of the gravel in to the water column to disperse downstream. Riley *et al.* (2013, 2015) demonstrated that ALAN, at experimental light intensities between 1 and 12 lux, disrupted the periodicity and synchronicity of fry dispersal. Further, Riley *et al.* (2011) found ALAN to disrupt the nocturnal migration behaviour of Atlantic salmon smolts.

ALAN is shown in this study not only to affect the diel distribution of the timing of behaviour in Atlantic salmon, but also the total amount that fish will refuge over each day-night cycle. Given that the timing and frequency of an individual's refuging and feeding behaviour are intrinsically plastic, based on both their perception of their predation risk and abundance of prey at any given time (Sih *et al.* 1988; Lima & Dill 1990; Stephens and Krebs 1986; Vehanen 2003), it is perhaps no surprise that ALAN has been found by this investigation to disrupt the normal pattern of behaviour in PYOY parr. As predicted, fish refuged for a greater length of time under brightly lit conditions at night, as individuals seek to balance their mortality risk and feeding rate (Fraser *et al.* 1993; Clark and Levy 1988). Whilst the way ALAN is perceived by fish is unknown, given that fish housed under the high intensity ALAN treatment sheltered significantly more than those housed in the control treatment, is suggestive that ALAN at certain intensities represents a perceived increase in predation risk to Atlantic salmon parr. That the fish can be seen to use the provided refuge extensively throughout the experiment, despite being unable to forage, is suggestive of the fish choosing to conceal themselves (Valdimarsson and Metcalfe 1998). Thus here the fish are choosing to maximise their survival under the increased perceived predation risk by refuging.

This investigation is able to provide further detail as to the light intensity (lux) that appears to cause increased refuging under ALAN; given that fish housed in the medium intensity ALAN treatment did not show increased refuging behaviour. Thus the light intensity at which ALAN disrupts the normal periodicity of behaviour and increases the refuging behaviour of Atlantic salmon parr appears to lie between 3.6 and 5 lux. The intensities of ALAN on the surface of the water of streams known to be inhabited by Atlantic salmon, in urban areas across southern England has been measured beteween 6.1 and

22.7 lux (Riley *et al.* 2013). As such, there is the potential for widespread disruption for the behaviour of Atlantic salmon parr in freshwater ecosystems in urban and peri-urban areas (Hölker *et al.* 2010a; Perkin *et al.* 2011).

The aforementioned behavioural trade-off between refuging (predator avoidance) and feeding (growth) is not fixed; it is somewhat dependent on the physiological condition and life history strategy of individual fish (Metcalfe *et al.* 1998). Fish mass was found to have an effect on the overall amount of time spent at the feeding station, with heavier fish spending more time feeding. This is in contrast to lighting treatment, which did not impact the number or timing of visits to the feeding station. Further, fish mass did not affect the periodicity of foraging behaviour. The freshwater phase is the juvenile stage and is extremely important in the lifecycle of the Atlantic salmon. During this time, the ability of the fish to obtain a suitable feeding territory and gain weight determines when they will undergo the parr-smolt transformation, termed smoltification (Jonsson and Jonsson 2011). The duration of the freshwater phase of their lifecycle and thus the timing of this transformation is flexible. The main determinant of whether Atlantic salmon parr will undergo smoltification is thought to be the growth of the fish (Solomon and Lightfoot 2008) and is determined by growth that occurs approximately nine months in advance of the transformation (Thorpe 1989).

The daily activity patterns of individual fish are a complex trade-off between maximising growth whilst simultaneously minimising predation (Sih *et al.* 1998). The addition of ALAN is likely to complicate the behavioural decisions faced by individuals further still when considering how ALAN may affect the availability and periodicity of certain taxa within the drift/drift abundance (see Chapter 2; Henn *et al.* 2014). Previous studies have demonstrated that the nocturnal drifting behaviour of freshwater invertebrates is reduced under ALAN (Perkin *et al.* 2014b; Henn *et al.* 2014). Perkin *et al.* (2014b) found the number of invertebrates drifting in the reaches that were artificially-lit was approximately half that in the control reaches. Further, the results presented in this thesis found that ALAN reduced the number of

drifting gammarids and chironomids. Thus, depending on the local community composition, the availability of food for drift feeding salmonids may be reduced.

An important point to note about this investigation is a potential caveat due to the high ambient water temperatures during the duration of the experimental period. There were three groups ("runs") of experimental replicates conducted as part of the investigation; however, due to concerns over fish health it was necessary to terminate the third experimental run early. Likely as a result of stress induced by the high temperatures, the fish began suffering from a fungal infection (Bruno 1989) that meant for ethical reasons the experiment could not continue. Due to the higher than normal temperatures, however, the fish may not have been feeding normally throughout the experiment as Atlantic salmon have strict thermal limits, with high temperatures known to impact their feeding behaviour (Solomon and Lightfoot 2008; see Chapter 6); often stopping feeding once water temperatures near their upper critical temperature limit for feeding of 22 °C (Elliott and Elliott 2010). Thus any effect of temperature on the feeding behaviour of the fish may have masked an effect of the lighting regime if the fish had decreased feeding propensity. As such, the results presented here could be considered a provisional estimate of the likely impact of ALAN for fish at more typical temperatures.

Further work is required to determine the long-term impact of ALAN on the behaviour of Atlantic salmon around the 24-hour clock and in controlled field conditions. In the present experiment, each experimental replicate lasted five days and as such, it would be interesting and useful for management to extend this study over a longer period, to determine how the effect of ALAN changes over time. Subsequent fieldwork should also be conducted in a controlled field investigation, whereby ALAN is introduced to a light naïve stream and the refuging behaviour of parr, under differing light intensities, is monitored using experimental (PIT antenna-equipped) shelters. Since the behavioural disruption, seen in this experiment, will likely disrupt predator-prey dynamics (e.g. by causing a mismatch between the timing of feeding and the availability of prey) there may be conservation implications for species that are already at risk of

extirpation. As such, understanding the way ALAN influences behavioural periodicity will be of great importance in mitigating against this ecological pollutant. In addition, exploration of the experimental light intensities at which behavioural disruption occurs further aiding management.

Ethics Declaration

The insertion of PIT tags in to parr is a licensable procedure and was conducted under Home Office project license number 30/2667 and conducted by Home Office personal license number 30/10226.

Chapter 7: General Discussion

7.1 Summary

Those concerned with the preservation and protection of species and ecosystems are fast becoming concerned about the ecological effects of ALAN; however, at the time of the conception of this investigation, empirical evidence was severely lacking. The overall aim of this thesis was to examine the influence of artificial light at night (ALAN) on the predator-prey dynamics of the Atlantic salmon in freshwater, with emphasis on the effect on predator-prey interactions. In order to realise this aim, two specific objectives were addressed: (1) Identify the gaps in knowledge around the impact of ALAN on Atlantic salmon behaviour and physiology, and; (2) Assemble a solid evidence base of the effects of ALAN on the juvenile stage of Atlantic salmon in freshwater, to contribute towards filling the existing knowledge gaps. These are systematically addressed in Chapters 2-6. It was determined that since 2010 there has been an upsurge in interest in determining the ecological effects of ALAN, yet fish remain comparatively understudied and little evidence concerning the behavioural and physiological impacts of ALAN on aquatic systems in general has been forthcoming (see Chapter 2).

Using a range of investigative methodologies, in both laboratory and field settings; this study examined the impact of ALAN on the nature and timing of behaviour in drifting invertebrates (see Chapter 3) and dispersing Atlantic salmon fry (see Chapter 4) as well as the cortisol stress response of Atlantic salmon fry (see Chapter 5) and the behaviour of Atlantic salmon parr (see Chapter 6). The findings provided firm support for the overarching hypothesis that ALAN affects the behaviour and predator-prey dynamics of Atlantic salmon, however the nature and magnitude of the effects appear to be highly complex and dependent on numerous factors including: the timing and intensity of the artificial lighting, and on the taxon and life stage of the affected organisms.

7.2 Synthesis

Chapter 1 laid the foundations for this thesis through covering extensive background literature on the properties of light and ALAN, introducing the context of the problem (and potential benefits) of ALAN as a pollutant and reviewing and synthesising the available literature on the ecological impacts. Moreover, the chapter also sought to highlight the benefits and importance studying of Atlantic salmon, and why ALAN may be of particular concern to those tasked with preserving freshwater ecosystems. Chapter 2 quantitatively examined the he ALAN literature and identified research trends and noted an increase in the number of studies being published in this research area. The three objectives for Chapter 2 were to: (1) Identify publications trends and data pertaining to the ecological impacts of ALAN; (2) Synthesise the literature to draw out important themes, scope and impact of existing research, and; (3) Analyse the results to highlight the key gaps in the literature that require addressing. The findings of this chapter highlighted the key gaps in current knowledge and determined the importance of studying fish, as they are comparatively understudied.

Chapter 3 investigated whether ALAN influenced the nocturnal drifting behaviour of freshwater invertebrates; the primary food source of drift feeding fish, such as Atlantic salmon. The three objectives for Chapter 3 were: (1) Determine the impact of light on invertebrate drift; (2) Assess the efficacy of a proposed management strategy (part-lighting), and; (3) Deduce how predator-prey dynamics and ecosystem health may be influenced. ALAN was not found to reduce the overall drift abundance or family-level richness of the drift, however there were found to be effects of ALAN when examining the individual taxa and functional feeding groups (FFGs) of those invertebrates drifting under the different lighting treatments. For example, ALAN increased the number of drifting gammarids but had no effect on baetids. The primary finding of this investigation was that the response of drifting invertebrates to ALAN is complex and is dependent on a number of variables; time of night, lighting duration (partly lit vs. fully lit treatments), taxon, and FFGs. Further, this chapter demonstrates the surprising result that a proposed

management technique, trimming the duration of ALAN each night, had a greater impact on the number and composition of drifting invertebrates than full night lighting throughout.

Chapter 4 sought to examine the behavioural impact of ALAN at differing intensities (1, 2, 4, 8 lux) on fry dispersal. The three objectives that were met in Chapter 4 are: (1) Examine the impact of ALAN on fry dispersal behaviour; (2) Identify patterns of disruption to dispersal related to ALAN at different intensities, and; (3) Determine how ALAN can impact the fitness of the dispersing fry. It was found that ALAN influenced the dispersal behaviour of Atlantic salmon fry in an empirical laboratory study. The study found that broad spectrum ALAN, between 1 and 8 lux, significantly delayed and disrupted the dispersal behaviour of Atlantic salmon fry. Further, the response to light is not linear, with a strong threshold effect at a nocturnal light intensity of 1 lux and little additional disruption seen in response to further increasing experimental intensities. This study also saw a decrease in the mass of those fry dispersing later, suggestive that the fish that delayed their dispersal were physically and competitively weaker. This chapter also demonstrates that a proposed management technique, dimming the intensity of ALAN to reduce their ecological impact will not ameliorate the disruptive effect on Atlantic salmon fry dispersal since a strong effect of ALAN is apparent even at 1 lux. This chapter has been accepted for publication in Freshwater Biology (Riley *et al.* 2015 - Appendix I).

Following directly on from the behavioural study described in Chapter 4, Chapter 5 sought to identify whether a cortisol stress response was the physiological mechanism behind the observed behavioural changes as a result of ALAN (Riley *et al.* 2013; Riley *et al.* 2015; see Chapter 4). The three objectives met in Chapter 5 were to: (1) Assess the impact of ALAN on the cortisol stress response of individual dispersing fry; (2) Identify the population level cortisol response to ALAN, and; (3) Evaluate possible alternative physiological mechanisms behind the observed behavioural disruption under ALAN. Cortisol levels were not elevated in individual fish dispersing under ALAN, and thus a cortisol stress response appears not to be the physiological mechanism mediating the behavioural changes seen as a result of

ALAN. In contrast cortisol was marginally elevated under ALAN in the population level sampling using polar organic chemical integrative samplers (POCIS). This study provided two important points of note; firstly this is the first study to demonstrate that Atlantic salmon fry, at this early stage of ontogeny, are able to mount a cortisol stress response. Secondly, this study demonstrates the first use of non-invasive cortisol sampling, both passive population level sampling using the POCIS and static sampling for individual cortisol measurements, in fish at this early stage of development. This chapter is currently in review (invited revision) as a methodological short communication manuscript with Conservation Biology, with myself as lead author.

Finally, Chapter 6 investigated the effect of ALAN on the diel behavioural pattern of refuging and foraging in Atlantic salmon parr. The three objectives met in Chapter 6 were to: (1) Assess the impact of ALAN on the diel patterns of refuging and foraging behaviour; (2) Determine how altering the intensity of light influences the effect of ALAN, and; (3) Infer the consequences for predator-prey dynamics. This is the first study to examine the impact of ALAN on the diel behavioural routines of individual fish. High intensity ALAN was found to alter the refuging behaviour of Atlantic salmon, causing increased refuging. Conversely there was no effect of ALAN on the amount of foraging in either of the two ALAN treatments (high and low intensity ALAN). When considering the periodicity of refuging and foraging behaviour, again high intensity ALAN was found to disrupt the timing of refuging behaviour, whilst the timing of foraging behaviour was non-significantly affected by light but declined significantly across the five sampling days. It is possible that this reduction in foraging behavior across the course of the experiment is as a result of satiation. Further, the results of this study provide evidence for the impact of ALAN extending beyond the nocturnal period, they show how light disrupts the behaviour of individual fish during the daytime and not simply during the artificially lit period at night. This important result demonstrates that the disruptive effects of ALAN may extend temporally beyond the nocturnal period.

When combined with the current literature, thes results presented in this thesis demonstrate how ALAN has the potential to affect the freshwater stages of the Atlantic salmon lifecycle. The results indicate a complex and variable response of freshwater species to ALAN; the strength of which appears dependent on species and even life stage. The major finding was that the two most widely used management techniques for reducing ALAN (dimming the intensity of ALAN and "trimming" the duration of ALAN) are ineffective for use around freshwater; with trimming having a greater disruptive effect than full night ALAN. The results provide empirical support for claims that ALAN is indeed a problem for freshwater ecosystems and support the call for increased research into determining the full extent of its impact and appropriate ways to mitigate these impacts.

7.3 Predator-Prey Dynamics Under ALAN

Though not explicitly empirically tested, the results of this study allow for the impact of predator-prey dynamics of the Atlantic salmon under ALAN to be inferred. The results presented in this thesis together imply that ALAN has the potential to affect the predator-prey dynamics of Atlantic salmon - through disruption in the behaviour of both the fish and their invertebrate prey. By disrupting the normal patterns of activity of individuals, ALAN will influence the interactions between predators and their prey, and between conspecifics and competitors (Kurvers and Hölker 2015). The hypothesised disruption to normal nocturnal behaviour was supported for some taxa of drifting invertebrates (see Chapter 3), dispersing Atlantic salmon fry (see Chapters 4 and 5), and Atlantic salmon parr (see Chapter 6). Moreover, the impacts of ALAN on the behaviour of organisms, as demonstrated in this thesis, is likely to have far-reaching consequences for species interactions in freshwater ecosystems (Kurvers and Hölker 2015).

The increase in refuging behaviour by Atlantic salmon parr under ALAN may lead to increased competition for refuges. Organisms typically refuge to avoid predation, and increased predation risk will

result in increased refuging behaviour (Sih *et al.* 1988). Conversely, increased competition, both inter- and intra specific, for suitable refuges may result in an increased risk of predation for those competitively weaker individuals that are not able to secure a shelter. Atlantic salmon parr, in freshwater, are thought to be nocturnally active to avoid predation (Metcalfe *et al.* 1999), however it is predicted that under ALAN diurnal species will extend their activity into the now artificially lit night (Kurvers and Hölker 2015), thus Atlantic salmon may be subject to increased predation from diurnally active visual foragers. Indeed, predatory species have previously been shown to increase their nocturnal activity under ALAN (Davies *et al.* 2012). Further, previous studies have demonstrated ALAN being exploited by visual predators (harbour seals) as a means of increasing their predation rate on migrating salmonids (Yurk and Trites 2000).

An interesting point to consider is whether ALAN is always a negative influence on species' ecology. Under ALAN, diurnal species are proposed to extend their activity and foraging (Rich and Longcore 2006; Gaston and Bennie 2014), thus ALAN can be considered to be freeing these species from visual constraints on nocturnal foraging. Those species that prosper under ALAN are said to be exploiting the 'night-light niche', as demonstrated above in harbour seals (*Phoca vitulina*) (Yurk and Trites 2000). This has also been seen in some species of bats (Order: Chiroptera), which forage around streetlights – exploiting the attraction of aerial insects to ALAN (Jung and Kalko 2010). It is important, however, to consider both the predator and prey species when examining the impact of ALAN on species interactions and increased predation as a result of nocturnal predators extending their foraging period may result in over exploitation of prey species. Further, the effect of 'non-native' predators has been suggested to be more detrimental for 'naïve prey' species, as due to a lack of evolutionary history between the new predator and this prey their antipredator behaviours may be ineffective (Sih *et al.* 2010). Predator species may also be impacted by increased competition for resources. As such, ALAN and the elimination of dark skies, is likely to represent a huge loss for crepuscular and nocturnal species (Hölker *et al.* 2010 a,b), as their temporal activity niche is being substantially modified.

In this study, and in previous studies (Meyer *et al.* 2013; Perkin *et al.* 2014b; Henn *et al.* 2014), ALAN has been shown to affect the nocturnal drifting behaviour of certain taxa of drifting invertebrates. Whilst an overall reduction in total abundance of drifting invertebrates was not demonstrated in the current study, previous studies have found a reduction in abundance (Henn *et al.* 2014), biomass and density (Meyer *et al.* 2013) under ALAN. Thus there appears to be the potential for a reduction in the primary prey of drift feeding fish, such as Atalntic salmon. When faced with a reduction in nocturnal food intake, Atlantic salmon parr have, however, been found to extend their foraging behaviour into daytime, to which they are better visually adapted, to maintain growth (Fraser *et al.* 1993).

ALAN has the potential to disrupt the predator-prey dynamics of freshwater ecosystems, and the results presented in this study provide evidence to suggest that it will. However, studies that go beyond the current approach of studying individual species' responses to light are needed to empirically investigate the influence of ALAN on species interactions (predator-prey, inter- and intra-specific competition and social interactions) and determine the extent and ecological consequences of the disruption.

7.4 Strengths of approaches used and potential caveats

The strength of this study lies in the diversity of its methodological approaches, and the breadth of its remit, from behavioural to physiological. Thus, the range of methodologies used in this thesis allowed for the determination of both the behavioural and physiological impacts of the impact of ALAN on Atlantic salmon and a behavioural assessment of their invertebrate prey. Further, the thesis sought to identify the potential impacts of ALAN on the life of Atlantic salmon in freshwater and as such, looked at multiple stages in its' lifecycle. In each chapter, caution has been incorporated in the interpretation of results; here several potential caveats are highlighted.

Firstly, when considering Chapter 3, the practicalities of the experimental field set-up resulted in light intensities 1 m in front of the drift nets being higher than would commonly be experienced in a river exposed to ALAN. The intensity of the light attenuated steeply across the 1 m in front of the net, however, so that the light measurements at the mouth of the net were representative of street-lit settings in the UK. As such, invertebrates caught in each of the drift nets will have been subject to a gradient of ALAN intensities that in parts may be brighter than commonly experienced as a result of streetlights. That said, Henn *et al.* (2014) examined drift in experimentally manipulated sections of a stream at artificially lit intensities of 1482 lux, thus the light intensities used as part of this thesis are more reflective of rivers exposed to ALAN in the UK than other authors' work. A further consideration is the use of functional feeding groups (FFG) in the analysis of the results. As previously considered, caution should be exercised in the use of the FFG concept, as the classifications have been criticised as being an oversimplification of true trophic variability (MacNeil *et al.* 1997). Infact, MacNeil *et al.* (1997) refer to FFG as 'fictional feeding groups'.

When considering the experimental laboratory set-up of both Chapters 4 and 5, and the experimental light intensities used, it is important to consider how the experiment could have been improved through the use of a lower range of light intensities. With hindsight it is possible to reflect that the results of the experiment would have been more interesting had the threshold intensity, between 0.1 and 1 lux at which light stops impacting the behaviour of the dispersing fry, been identified. Had a lower range of intensities been chosen, for example 2, 1, 0.5, 0.25 and 0.1 lux, this may have been possible. However, given the variability in natural nocturnal illumination this may not have been as informative for streetlight management purposes.

The non-invasive physiological methodologies used in Chapter 5 had previously not been applied to fish at such an early ontological stage, and it was unknown whether fish of this developmental age (<0.5 g) could mount a stress response to an external stimulus. The results found that fish of this developmental

age are able to mount a stress response and demonstrated the viability of non-invasive sampling methodologies at this early stage in Atlantic salmon development. There is the potential that the procedure itself (handling and confinement) may affect the amount of cortisol released into the water; this impact can be limited by restricting the duration of the collection period. Whilst individual fish were placed in beakers of water for 30 minutes, this duration may not be sufficient to detect the extent of the cortisol stress response in the dispersing fry or it may have been too long, in which case the stress response as a result of handling and confinement may have masked a treatment effect. Further, the static-sampling methodology was somewhat exploratory and its use had not been validated or refined. Future refinement of this methodology may lead to a more sensitive cortisol assay. Moreover, due to the cost of analysis, only a small subset of the samples was analysed and as such, the small sample size limits the power of the statistical analysis. This is also the case for the population-level sampling using the polar organic chemical integrative samplers (POCIS), where there was a marginally significant effect of light on the cortisol content of the absorbent membrane. The POCIS were deployed as three disc membranes per incubator; however, these were combined during chemical analysis and thus each incubator only had one data point for the population measure of cortisol. Thus, here the relatively small sample size limits the power of the analysis, and further samples would likely clarify the result.

Finally, Chapter 6 was methodologically strong; the continuous monitoring of the fish through the use of PIT tagging provided large data sets, which allow for statistical confidence in the patterns observed. Whilst the sample size was smaller than initially planned, the enormity of the datasets collected, when analysed using a model to control for repeated measures, allows for confidence in the behavioural patterns observed. When considering the results of the two differing experimental light treatments it is also important to note the potential for the lack of an effect of the ALAN in the medium intensity treatment to be as a result of the experimental set up. Due to the constraints of the laboratory set-up, in the medium intensity treatment, the side of the tank created a shadow measuring 14 cm on the surface of the water. It could be suggested that the fish utilised this darkened area as an additional refuge, thus accounting for the

fact that there was not an increase in refuging behaviour seen in this treatment. Further, a limitation of the PIT tag design used in this in this experiment is that the fish can only be accounted for when either in the refuge or the feeding station. Had we used infrared cameras to record the fish around the 24-hour clock, we would have a better idea of their whereabouts when not present in either of the two PIT antennae locations. Thus, it would be possible to definitively say whether the fish were utilising the shadowed area as an additional refuge and also, provide a greater understanding of the behavioural patterns of the parr in the different lighting regimes. A final limitation of the laboratory set up was related to the flow of water through the tanks, in natural conditions Atlantic salmon parr typically inhabit streams with a greater water velocity (Armstrong *et al.* 2002). The velocity of water in the laboratory was limited by both financial and technical constraints; to these ends, an experimental system that utilised the natural flow of water from a stream through a flume channel would be a better study system for future research.

A further important consideration for Chapter 6 is the potential effect of extreme high temperatures during the experimental sampling period. As outlined previously, salmonids are extremely sensitive to temperature stress (Elliott 1991; Elliott and Elliott 2010; Crossin *et al.* 2008) and Atlantic salmon have strict temperature limits, both upper and lower (Elliott and Elliott 1995, 2010). The upper limit for feeding has been suggested to be around 22 °C (Elliott and Elliott 2010), and during the course of the experiment water temperatures reached 19.1 °C. Unfortunately, there was little that could be done to combat the high temperatures as the de-chlorinated mains water at Cefas, Lowestoft is held in a header tank on the roof of the laboratory and as such, would warm throughout the day in the strong sunshine. Thus, it is possible that the results seen for the foraging data are compromised by decreased propensity to feed in the fish. That said, fish behaviour was remarkably consistent between days with different temperature conditions, and between the two experimental runs (temperatures in Run 2 were higher than in Run 1), implying that temperature did not have a major effect in this study. That said, due to the high temperatures and deteriorating fish health as a result of temperature stress, Run 3 was terminated early and the data is not included in this study. Moreover, it is important to note that ALAN impacted the refuging behaviour of

Atlantic salmon despite the potential effect of temperature, suggesting that under more typical conditions and with a greater sample size, the effect of ALAN may be even more apparent.

7.5 Management Implications

As outlined in Chapter 1, the successful management of ALAN needs to be informed by solid evidence and a clear understanding of the range of unwanted ecological impacts of the pollutant. This thesis examined the potential efficacy of two proposed management techniques, "dimming" and "trimming", that are currently used in the UK. Dimming of lamp brightness was selected to evaluate as part of this thesis as it has previously been cited as being the most universally applicable management strategy for reducing the unwanted ecological impacts of ALAN. The results of this thesis however, suggest that these strategies are not effective at ameliorating the unwanted ecological effects of ALAN – at least as is the case for Atlantic salmon and many freshwater invertebrates.

The results of this thesis suggest that the intensity at which ALAN has a behavioural effect on Atlantic salmon lies between 0.2 and 1 lux. When assessing the efficacy of dimming, this technique does not seem appropriate for use around freshwater, as it is not feasible to reduce ALAN below 1 lux while maintaining its function as illumination for human activities. Trimming or part lighting was also found to be unsuitable for reducing the unwanted ecological effects of ALAN, despite its wide use across the UK. This thesis found that trimming had a greater impact than continuous night lighting, on disrupting the nocturnal drifting behaviour of freshwater invertebrates.

An important step in managing the impact of ALAN is garnering public interest in the issue, as through education it can be hoped that people will be more receptive to a reduction of ALAN in their local area. There are currently 44 "Dark Skies" parks throughout Europe (Charlier and Bourgeois 2013), one of which has recently been designated in the Brecon Beacons National Park, South Wales. The aim of these unlit areas

is to provide areas of natural ecosystems for the benefit of astronomy and the environment, and further serve to educate the public on the negative impacts of ALAN.

As such, the best course of action in the short term would be to maintain and increase natural unlit areas, whilst developing research initiatives investigating more appropriate management strategies. Given that ALAN is growing at a rate of 3% per annum in the UK (RCEP 2009) and newer technology lamps, including broad-spectrum lights, are now replacing many of the old low-pressure sodium (LPS) streetlights across the UK, it is of the upmost importance that successful management strategies are identified. This cannot be achieved; however, without a clear understanding of the ecological impact of these broad spectrum lights and as such, much more research is required to elucidate the problem.

7.6 Future Research

This thesis sought to determine if ALAN impacted the behaviour of Atlantic salmon and ascertain how their predator-prey dynamics might be affected. This research has laid the foundation for further investigations into the ecological effects of ALAN, and as such has highlighted several areas of research that warrant more detailed future investigation. This thesis formed part of a larger programme of study, focusing on the impact of ALAN in freshwater; however, future research should look to determine the impact of ALAN on the migratory life stages of Atlantic salmon, as many salmonid populations will migrate through more urban areas and thus be subjected to greater levels of ALAN.

Firstly, when considering the effect of ALAN on the behavioural disruption of salmonids, it would be interesting to investigate the possibility of the presence of two distinct coping styles in response to ALAN. The results from chapters 4, 5 and 6 suggest that individual fish may respond to ALAN in different ways. Considerable literature exists on the presence of coping styles (proactive/reactive; see Chapter 4) in

response to known stressors in Atlantic salmon and other salmonid species (Rainbow trout, *Oncorhynchus mykiss* - Overli *et al.* 2006; Koolhaas *et al.* 1999), as such it may be that fish belonging to the different coping styles are differentially affected by ALAN and may mask the treatment effect. This behavioural mechanism is thought to be widespread throughout a range of taxa (Koolhaas *et al.* 1999) and for this reason it would be prudent to determine whether there is a divergent response to ALAN within species as a result of these distinct coping styles. An extension of the fry dispersal experiment that samples emergent fish around the 24-hour clock could investigate the presence of different stress coping styles in Atlantic salmon in response to ALAN.

Further work is required to elucidate the physiological mechanisms behind the behavioural disruptions observed in numerous species under ALAN. In this thesis, cortisol was investigated in dispersing Atlantic salmon fry and was not found to be the primary mechanism behind previously observed and concomitant delays in dispersal behaviour. As such, alternative physiological mechanisms should be investigated to allow for the cause of disrupted behaviour to be identified. In Atlantic salmon fry delaying their dispersal, it appears they are not highly stressed by dispersing under ALAN and as such the cause of the behavioural delay is not clear. Future research should examine the influence of ALAN on melatonin levels in animals exposed to ALAN as this represents a viable physiological mechanism for behavioural disruption. It was planned to examine melatonin in this thesis; however, due to the constraints of trailing new methodologies it was not possible in this instance.

The results of the Chapter 5 have also highlighted the need to validate and refine the non-invasive technique used to determine the influence of light on the cortisol stress response of dispersing salmon fry. This novel methodology was applied to dispersing Atlantic salmon, and demonstrated that the use of non-invasive techniques is possible at such an early stage of development in this species. Previous studies have used total-body cortisol as a measure of the cortisol stress response in fish at this stage of ontogeny. However, this is a destructive technique and as such, does not allow for repeated sampling. The validation

of this non-invasive methodology in young fish will allow for repeated measurements to be taken from individual fish, thus reducing the number of fish needed during experiments.

Studies that have been conducted to date are limited in nature, as most only examine one aspect of the influence of ALAN, usually focusing on short-term behavioural observations or manipulations. Further studies that examine the impact of ALAN on the full 24-hour pattern of behaviour in individual organisms are necessary to fully understand the impact of ALAN beyond nocturnal disruption. If the behaviour of organisms that are normally nocturnally active becomes significantly disrupted, this will have implications for daytime behaviour too, and well as predator-prey interactions, inter-specific competition and may have serious conservation implications. As such, it is imperative that we have a full picture of how ALAN may influence the behaviour of organisms around the 24-hour clock, and not simply during artificially lit nights. In Fish, further use of PIT tagging allows us to build a clear picture of patterns of behaviour and can be achieved in field studies through the use of experimental (PIT antenna-equipped) shelters.

Furthermore, studies that examine the longer-term impacts of ALAN are necessary. As previously discussed, the majority of studies to date have been short-term behavioural observations. No studies have been conducted that attempt to ascertain the long-term impacts of ALAN. If nocturnal behaviour is modified as a result of ALAN, does this eventually / subsequently result in local extirpation? If the composition of invertebrates caught under ALAN differs, does this lead to changes in community composition? These are questions to which the answers are currently unclear, but that are readily answerable through an investment in long-term experimental manipulations. Long-term investigations will also allow the impact of light on physical processes within ecosystems to be determined. If ALAN affects the behaviour of keystone species within the ecosystem, processes such as leaf litter decomposition and microbial synthesis could well be affected. This is suggestive of a food web approach being needed to fully elucidate the influence of ALAN on predator-prey dynamics and ecosystem functioning.

A final area that requires further investigation is the interaction between ALAN and other stressors and whether multiple stressors occurring at once exacerbate the unwanted ecological impacts of ALAN. This is a particular concern when studying the ecological effects of ALAN on freshwater ecosystems as they are the most heavily debased ecosystem globally and are subject to many stressors, both anthropogenic (pollution, habitat alteration and invasive species) and as a result of climate change. Rivers are most sensitive to climate change as they are directly affected by both changes in temperature and changes in rainfall (Ormerod 2009) and the way in which climate change will impact freshwater species over the next decade is difficult to predict (JNCC 2007). A concern when examining the impact of proliferating ALAN on species must be that, if they are already under stress as a result of climate change, they will be less able to adapt to the altered light regime, or vice versa, since it has been suggested that populations are unable to cope with multiple stressors that occur simultaneously (Folke *et al.* 2004; Novacek and Cleland 2001; Mora *et al.* 2007). Multiple disturbances can act together and strengthen the impact of the other, resulting in a much accelerated rate of biodiversity loss (Darling and Cote 2008; IPCC 2007; Mora *et al.* 2007). Experimental manipulations suggest that population declines are up to 50 times more rapid when multiple stressors are acting synergistically (Mora *et al.* 2007). Thus, it is imperative that we attempt to understand the way in which additional stressors, such as climate change, interact with ALAN to endanger species and populations further.

7.7 Conclusions

It is hoped that this study provides several points of interest to stimulate future research and a number of important points to consider when seeking to manage the ecological influence of ALAN around freshwater ecosystems and beyond. ALAN is increasing globally and a wide understanding of the ecological effects of this pollutant is required in order for successful mitigation. This study has contributed towards furthering the current knowledge base, highlighting the need to consider the differential effect of

light on individual taxa and life stage. In summary, the influence of ALAN on Atlantic salmon and their invertebrate prey in freshwater is complex, and the results given in this thesis provide a broad starting point, from which future research can work to fully elucidate the impact of ALAN on freshwater predator-prey dynamics.

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Appendices - additional Data:

Appendix 1. Details of the families sampled from the drift assigned to each of the functional feeding groups (FFG), SH=Shredders, GR= Gatherers, PR=Predators, PA=Parasites, GC=Grazers, FC=Filterers (Moog 1995).

	Functional Feeding Groups (FFG)					
	GR	**PR**	**GC**	**SH**	**FC**	**PA**
Family	Asellidae	Ceratopogonidae	Elmidae	Lymnaeidae	Bithyniidae	Nematoda
	Baetidae	Cyclopoida	Collembola	Chrysomelidae	Dixidae	
	Caenidae	Dytiscidae	Haliplidae	Dryopidae	Hydropsychidae	
	Chironomidae	Ecnomidae	Helophoridae	Gammaridae	Simuliidae	
	Ephemerellidae	Empididae	Hydraenidae	Hydrophilidae	Sphaeridae	
	Helophoridae	Hebridae	Hydrobiidae	Limnephilidae		
	Hydrobiidae	Hirudinea	Hydrophilidae	Statiomyidae		
	Hydrophilidae	Hydracarina	Lymnaeidae	Tipulidae		
	Oligochaete	Hygrobiidae	Physidae	Scatophagidae		
	Psychodidae	Mesoveliidae	Planorbidae			
	Scatophagidae	Nepidae	Psychodidae			
	Scirtidae	Noteridae	Scatophagidae			
	Statiomyidae	Pediciidae	Statiomyidae			
	Syrphidae	Scatophagidae	Lymnaeidae			
	Valvatidae	Veliidae				
	Lymnaeidae					

Appendix 2. Total numbers of drifting invertebrates, in each lighting treatment (Fully lit, Unlit and Partly-lit; n=3 replicate nights in each case) for all invertebrate families caught in the drift shown by treatment and net (Control, 2m, 2.25m and 2.5m). For clarity, control conditions are shaded. Total drift abundance, Shannon diversity index, Simpson's Index and Family level species richness are given for the total sample.

	A			B			C			D		
Species	**Fully Lit**	**Unlit**	**Partly-lit**	**Fully Lit**	**Unlit**	**Partly-lit**	**Fully Lit**	**Unlit**	**Partly-lit**	**Fully Lit**	**Unlit**	**Partly-lit**
Caenidae	1	0	2	2	0	1	2	0	7	5	4	0
Cyclopoidae	1	0	1	1	4	2	5	1	1	0	2	0
Collembola	1	0	6	0	0	3	0	1	3	1	0	1
Ephemerellidae	0	0	0	1	0	1	0	0	1	1	1	2
Oligocheate	0	0	1	2	0	1	2	2	3	1	0	2
Velidae	7	2	8	2	0	3	2	0	1	3	1	2
Limephilidae	0	0	5	0	1	3	2	0	4	0	1	3
Mesovilidae	4	4	13	6	1	4	3	1	7	0	2	5
Flatworm	8	5	13	0	1	2	2	5	6	2	2	6
Psychodidae	1	0	5	1	0	7	2	2	14	1	0	10
Elmidae	6	14	114	13	11	47	12	8	47	8	10	39
Simuliidae	7	11	30	44	35	65	23	20	48	17	47	51
Chironomidae	29	5	47	59	42	94	37	60	88	29	42	75
Helophoridae	27	11	357	6	6	42	29	9	90	4	11	123
Gammaridae	45	42	61	98	99	180	130	274	426	53	109	164
Baetidae	90	69	57	375	316	188	231	320	228	98	234	166
Asellidae	0	0	0	0	0	0	1	0	0	0	0	0
Scirtidae	0	0	1	0	0	2	1	0	3	0	0	1
Nematode	2	0	0	0	0	1	2	2	1	0	1	0
Lymnaeidae	1	0	0	0	0	0	0	0	0	0	0	2
Hydrobiid	0	1	0	0	0	1	0	0	0	0	0	0
Hebridae	0	1	0	0	0	0	0	0	0	0	0	0
Halplidae	0	0	1	0	0	0	1	0	1	0	0	1
Hirudinea	0	0	0	0	0	0	1	0	3	0	0	0
Hydrophilidae	1	1	2	0	0	0	4	0	0	0	1	0
Dryophidae	0	0	0	0	1	0	0	1	0	0	0	0

Sphaeridae	0	0	0	0	0	1	0	0	0	0	0	0
Physidade	0	0	0	0	0	0	0	1	0	1	1	0
Hydropsychidae	0	0	0	0	0	0	0	1	0	0	0	0
Coratopogenidae	0	0	1	0	0	0	0	0	3	1	2	0
Nepidae	0	0	1	0	0	0	0	0	1	0	1	0
Bithyniidae	3	1	0	1	0	0	0	0	0	0	0	0
Economidae	1	0	1	1	0	1	0	1	3	0	0	1
Dixidae	0	0	2	0	0	0	0	0	0	0	0	1
Chrysomodidae	0	0	0	0	1	0	0	0	0	0	0	4
Tipulidae	0	0	1	0	0	2	0	0	3	0	0	1
Valuvitidae	0	0	0	0	0	0	0	0	1	0	0	0
Dytiscidae	0	0	2	0	0	0	0	1	1	0	0	0
Hygrogrobidae	0	0	3	0	0	0	0	0	1	0	0	0
Syrphidae	0	0	0	0	0	0	0	0	2	0	0	0
Statiomyidae	0	0	0	0	0	0	0	0	1	0	0	0
Scatophagidae	0	0	0	0	0	0	0	0	1	0	0	0
Empididae	0	0	0	0	0	0	0	0	0	0	0	0
Noteridae	0	0	0	1	0	0	0	0	0	0	0	1
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	0	0	2	0	0	1	1	1
Hydrachnida	16	15	14	19	6	7	16	26	22	12	13	19
Total	251	183	749	632	524	658	510	736	1021	239	486	681
Simpsons Index	0.8065904	0.7831228	0.7304087	0.6087716	0.5889663	0.8038128	0.7152567	0.6633868	0.7556456	0.7561613	0.6979955	0.8272102
Shannon Diversity Index	2.055794	1.917232	1.867811	1.395168	1.283297	1.924239	1.714492	1.40726	1.878792	1.815559	1.607492	2.02763
Family Level Species Richness	19	15	28	16	13	24	20	19	31	19	21	22

Appendix 3. Cortisol extraction values (pg) and Cortisol release rate (pg/g/h) for each of the sampled fish across all eight experimental incubators and the two control incubators. Cortisol extraction values (pg) are also given for the negative controls (NC), positive controls (PC) and spiked positive controls (PCSP).

Sample ID	Day	Temperature (℃)	Light Intensity (lux)	Incubator	Fish weight (g)	Volume buffer added (uL)	Volume assayed (uL)	Cortisol release rate (pg/g/h)	Cortisol in extract (pg)
0.1A/1	2	9.995303	0.1	1	0.18	500	100	559	48
0.1A/2	2	9.995303	0.1	1	0.16	500	100	984	61
0.1A/32	8	9.858745	0.1	1	0.20	500	100	234	23
0.1A/33	8	9.858745	0.1	1	0.19	500	100	276	25
0.1B/1	2	9.995303	0.1	2	0.16	500	100	1625	126
0.1B/2	2	9.995303	0.1	2	0.14	500	100	4135	289
0.1B/3	2	9.995303	0.1	2	0.20	500	100	720	70
0.1B/33	9	9.875018	0.1	2	0.21	500	100	590	54
0.1B/34	9	9.875018	0.1	2	0.16	500	100	487	37
1A/1	2	9.995303	1	3	0.19	500	100	614	58
1A/2	2	9.995303	1	3	0.19	500	100	383	35
1A/3	2	9.995303	1	3	0.18	500	100	235	21
1A/38	9	9.875018	1	3	0.23	500	100	194	21
1A/39	9	9.875018	1	3	0.16	500	100	229	17
1B/1	2	9.995303	1	4	0.19	500	100	715	61
1B/2	2	9.995303	1	4	0.19	500	100	514	44
1B/37	9	9.875018	1	4	0.14	500	100	638	43
2A/1	2	9.995303	2	5	0.18	500	100	290	25
2A/2	3	9.995319	2	5	0.23	500	100	307	30
2A/32	9	9.875018	2	5	0.17	500	100	345	28
2A/33	9	9.875018	2	5	0.14	500	100	204	14
2B/1	2	9.995303	2	6	0.19	500	100	293	26
2B/2	3	9.995319	2	6	0.19	500	100	543	49
2B/32	9	9.875018	2	6	0.15	500	100	295	22
2B/33	9	9.875018	2	6	0.18	500	100	258	22
4A/1	2	9.995303	4	7	0.16	500	100	1031	78

4A/2	2	9.995303	4	7	0.15	500	100	933	66
4A/30	9	9.875018	4	7	0.15	500	100	379	28
4A/31	9	9.875018	4	7	0.16	500	100	284	22
4B/1	2	9.995303	4	8	0.18	500	100	625	55
4B/2	2	9.995303	4	8	0.19	500	100	392	36
4B/3	3	9.995319	4	8	0.19	500	100	418	34
4B/32	9	9.875018	4	8	0.17	500	100	446	36
4B/33	9	9.875018	4	8	0.18	500	100	327	29
8A/1	3	9.995319	8	9	0.17	500	100	287	22
8A/2	3	9.995319	8	9	0.19	500	100	202	19
8A/28	9	9.875018	8	9	0.20	500	100	392	39
8A/29	9	9.875018	8	9	0.18	500	100	384	33
8B/1	4	9.984107	8	10	0.16	500	100	832	60
8B/2	5	9.9962	8	10	0.20	500	100	439	43
8B/3	6	9.981162	8	10	0.18	500	100	360	32
8B/35	9	9.875018	8	10	0.16	500	100	427	34
8B/36	9	9.875018	8	10	0.20	500	100	233	22
8B/37	9	9.875018	8	10	0.18	500	100	383	34
PC1	NA	NA	PC	0	0.18	500	100	845	130
PC2	NA	NA	PC	0	0.18	500	100	1243	222
PC3	NA	NA	PC	0	0.18	500	100	998	169
PCSP1	NA	NA	PCSP	0	NA	500	100	NA	54
PCSP2	NA	NA	PCSP	0	NA	500	100	NA	27
PCSP3	NA	NA	PCSP	0	NA	500	100	NA	76
NC1	NA	NA	NC	0	NA	500	100	NA	0
NC2	NA	NA	NC	0	NA	500	100	NA	17
NC3	NA	NA	NC	0	NA	500	100	NA	0

Appendix 4. Complete results for the analysis of the refuging and foraging data, mean value across the five experimental days for each line of data for all lighting regimes (control (shaded), medium and high). Means are provided for mean time of refuging, Rho, SD circular, angular variance and angular deviation, whilst the median was taken of the median time of refuging and sample size is the total number of detections across the five days.

Antennae	Run	Weight	Sample size	Median time	Mean time	Rho	Angular variance	SD circular	Angular deviation	Behaviour	Treatment	Condition	Lux
10	1	10.4	397	11	-5.998	0.4506	1.0989	1.2947	1.0290	Feed	Control	Unlit	0.1
12	1	9.8	89	13	0.459	0.5169	0.9661	1.2211	0.9231	Feed	Control	Unlit	0.1
14	1	11.9	168	15	-2.877	0.5377	0.9246	1.1166	0.9491	Feed	Control	Unlit	0.1
14	2	10.6	5	4	5.758	0.9606	0.0787	0.1671	0.1620	Feed	Control	Unlit	0.1
16	2	7.8	23	-3.5	-2.5	0.9957	0.0086	0.0655	0.0654	Feed	Control	Unlit	0.1
4	1	12.5	79	6	6.256	0.6946	0.6108	0.8436	0.7116	Feed	High	Lit	6.4
6	1	8.9	28	12	-0.412	0.1286	1.7428	2.3134	1.3108	Feed	High	Lit	6.3
6	2	13	327	8	-3.244	0.3963	1.2073	1.3831	1.0887	Feed	High	Lit	6.3
2	1	9.5	301	14.5	-5.060	0.5071	0.9857	1.2150	0.9601	Feed	Medium	Lit	3.7
8	1	10.9	73	3	3.533	0.8447	0.3106	0.5338	0.5045	Feed	Medium	Lit	3.6
2	2	11.4	167	5.5	5.168	0.3907	1.2209	1.4435	1.0789	Feed	Medium	Lit	3.7
8	2	9.4	27	-2	-3.765	0.7531	0.4938	0.7026	0.6245	Feed	Medium	Lit	3.6
9	1	10.4	167190	16	-7.579	0.1146	1.7707	2.5731	1.3207	Refuge	Control	Unlit	0.1
11	1	9.8	67211	10	1.115	0.3376	1.3248	1.5120	1.1334	Refuge	Control	Unlit	0.1
13	1	11.9	78400	0	-2.008	0.2490	1.5065	1.7132	1.2203	Refuge	Control	Unlit	0.1
13	2	10.6	104150	11	9.408	0.2424	1.5152	1.83460	1.2040	Refuge	Control	Unlit	0.1
15	2	7.8	16997	3	-3.318	0.3496	1.3008	1.5025	1.1339	Refuge	Control	Unlit	0.1
3	1	12.5	1312	14	-2.926	0.5388	0.9228	1.1275	0.9437	Refuge	High	Lit	6.4
5	1	8.9	160430	12	-0.412	0.1286	1.7426	2.3134	1.3029	Refuge	High	Lit	6.3
5	2	13	173890	15	-7.257	0.0453	1.9421	2.8952	1.4015	Refuge	High	Lit	6.3
1	1	9.5	95280	8	5.354	0.4113	1.1775	1.3550	1.0784	Refuge	Medium	Lit	3.7
7	1	10.9	7661	3	0.94	0.2764	1.3811	1.6660	1.1962	Refuge	Medium	Lit	3.6
1	2	11.4	3518	11	-3.569	0.4444	1.1219	1.2890	1.0542	Refuge	Medium	Lit	3.7
7	2	9.4	63677	2	1.692	0.1453	1.7094	1.9754	1.3072	Refuge	Medium	Lit	3.6