Effects of nocturnal illumination on fitness-related traits of the New Zealand common triplefin (*Forsterygion lapillum)*

By

Jaye Sonoko Manaia Coriander Anemone Barclay

A thesis submitted to Victoria University of Wellington in partial fulfilment of the requirements for the degree of Master of Science in Marine Biology

2023

Abstract

Identifying determinants of variation in fitness for organisms with complex life histories has been a longstanding challenge for ecologists. Night-time conditions encompass half the lives of many organisms (Gaston et al., 2022), yet the impacts of varied nocturnal illumination on fitness-related traits across ontogeny are rarely considered. Many organisms exhibit differing patterns of development, growth, and reproduction in conjunction with natural seasonal variations in the timing, strength, and duration of light:dark periods. Interruptions to these cycles can be particularly disruptive to organisms that rely on environmental light to entrain and synchronise development or reproduction. Elucidating the unique impacts of nocturnal illumination during different stages of life history can be particularly difficult. In this thesis, I assessed the effects of nocturnal illumination on fitness-related traits of *Forsterygion lapillum* (the New Zealand common triplefin) in early (embryonic) and adult life history phases.

In Chapter 2, I conducted a laboratory experiment to appraise the impacts of the strength and timing of nocturnal light (including lunar patterns), on fitness-related traits for adults. I addressed three questions: 1) Does nocturnal illumination alter adult body condition? 2) Does growth vary in different conditions of nocturnal light? 3) How does nocturnal illumination affect reproductive behaviour? I exposed adult triplefin to four different nocturnal light treatments (regular lunar cycle, dimmed lunar cycle, 24-hr artificial light, and dark at night) over the course of three months. I then evaluated the impact of nocturnal light on the relative change in body condition for each individual during the experiment. Additionally, I extracted triplefin otoliths to reconstruct a portion of life history during the experiment and conducted growth analyses assessing if somatic growth varied between treatments. Lastly, I progressively photographed egg clutches and quantified the influence of nocturnal illumination on the timing and frequency of reproduction. Body condition was not influenced by light treatment but differed with sex and pre-experimental body condition. Female fish experienced greater reduction in body condition than males, and body condition degraded to a greater degree over the course of the experiment as pre-experimental body condition increased. While light treatment was not a determinant of body condition, the interaction of light treatment, sex, and standard length caused significant variation in growth increment width. This interaction was particularly pronounced in the 24-hr light treatment, where male growth rates increased as body size increased, but the inverse relationship was seen for females. The interaction of sex and body size varied in the other three treatments. There were no apparent lunar patterns in growth.

Reproduction was also impacted by nocturnal illumination. Fish in the 24-hr light and dark at night treatments were more likely to reproduce than those in the lunar treatments. Furthermore, fish in the 24-hr light treatment tended to lay more eggs than those in the dark at night treatment. The number of eggs laid in lunar treatments also followed semi-lunar patterns. Fish in the regular lunar treatment exhibited greater numbers of eggs laid at the first and third quarter moons, while fish in the dimmed lunar treatment had asymmetrical peaks during just after the full moon and just before the new moon. Water temperature and the number of females in each tank did not influence the likelihood of reproduction nor the number of eggs laid during reproductive events. These results suggest that nocturnal illumination has distinct and significant impacts on fitness-related traits for adult *F. lapillum* that interact with other life history traits.

In Chapter 3, I estimated the length, structure, and success of embryonic development for eggs laid during the laboratory experiment to address two questions: 1) How does nocturnal illumination influence the duration and structure of embryonic development? 2) Does exposure to nocturnal light impact hatching success of embryos? Using photographs of egg clutches taken progressively throughout the experiment, I tracked the fates of each clutch and estimated their dates of laying, eye development, hatching, and their hatching success. I used these estimates to assess the influence of nocturnal illumination on fitness-related traits for offspring. Light treatment did not impact the length or structure of development, however, eye development and overall development length followed lunar patterns. Clutches laid during the new moon had longer development periods than those laid during the full moon, and eye development was longer when it coincided with the first and third quarter moons. Warmer water temperatures at laying resulted in shorter periods of eye and total development and increased the rate of eye development relative to total development time. Conversely, clutches that had faster relative eye development also took less time to hatch. Hatching success was likewise not impacted by light treatment but followed lunar patterns. Clutches that hatched during the full moon tended to have lower hatching success than those hatched during the new moon. Larger clutches experienced much greater hatching success at higher temperatures, and water temperature did not influence hatching success for small clutches. These results emphasise the complicated interactions of environmental cues on fitness-related traits during early life history phases for common triplefin (*F. lapillum*) and highlight the need for further research into this subject.

Acknowledgements

Ko te mea tuatahi hei mihi atu ki ōku tūpuna. Nā koutou i tuku iho mai te mātauranga, te māramatanga, me te ngākaunui kia mahia te mahi nei. Ko au te tōtara e tipuria ake ana e tō koutou rā taurima. E kore au e ngaro, he kākano ahau i ruia mai i Rangiātea.

A huge and warm thank you to my supervisor, Jeff Shima, for all your support and guidance throughout my master's studies. While building my own project from scratch was a difficult (and at times frustrating) endeavour, your flexibility and encouragement empowered me to forge ahead, and my experience has been all the more rewarding for it. Thank you too to all the Shima lab group folks for the continual support in my research and fun catchups.

To all the folks down at WUCEL, thank you for the community you provided during my time with you. The memories of chasing dolphins along the Esplanade in the early days of my thesis year (with Emma, Alice, and Callum) will always sit fondly in my heart. Special thanks to the technicians; Snout and John. To Snout, thank you for your help setting up my lighting system, for your guidance in otolith extraction, and for enduring several early mornings boating with me. Thank you, John, for your enthusiasm in joining me for some of those early mornings.

A huge thank you to the besties: Emma and Tom. Your unconditional love and support throughout this experience has meant so much to me. For the excursions into the field to catch my fish, the academic advice, and the times we have spent blowing off steam together: thank you from the bottom of my heart. Meeting the two of you has been the best gift that my master's has given me. I love you both, I could not have done this without you. To the other wonderful friends that I have made since master's part 1 (Tessa, Aiden, Tory, Chelsea, Henry, Imogen, Alysha, and Laura to name a few), thank you for your companionship and camaraderie.

To my family, thank you for your endless outpouring of compassion, encouragement, and support. I would not be the person that I am today without you all, and I love you very much. In particular, thank you Mum and Dad for providing the perfect writing retreat in the final days leading up to hand-in, and for making sure I was always well-fed.

To my wonderful partner Jaime, you have been the wind beneath my wings, especially during the final count-down to the deadline. Thank you for looking after me, for the many cups of tea and back rubs, even when I have been grouchy and short-tempered. I am so grateful for your

patience and compassion, and I look forward to reciprocating that level of care if (or when) you pursue your own post-graduate studies.

Lastly, I would like to extend special thanks to Victoria University and the Morton family for funding the scholarships that supported me during my master's degree. Your support enabled me to better apply myself to my studies.

Table of contents

List of figures

List of tables

CHAPTER 1

General introduction

The influence of environmental cues on organism development, behaviour, and reproduction may vary widely over the course of an organism's lifetime (Werner and Gilliam, 1984). Yet the distinct impacts of environmental cues across ontogeny are still poorly understood. This is especially prescient for organisms with complex life histories. Complex life histories are characterised by discrete life stages associated with unique form or function, allowing an individual to occupy different ecological niches across its lifespan (Wilbur, 1980). The field of evolutionary biology is founded upon the understanding that fitness varies between individuals and changes over time, and elucidating determinants of this variation are essential to enhancing our knowledge of reproductive and developmental ecology (Cheverud, 1984, Hallgrímsson et al., 2009, Orr, 2009, Roseman, 2020).

A large body of literature aims to assess the influence of environmental cues on fitness-related traits like development and reproduction in a wide variety of organisms, such as birds (Halfwerk and Slabbekoorn, 2014), insects (Stearns et al., 1995, Wilner et al., 2019), mammals (Gaillard and Yoccoz, 2003), reptiles (Huang and Pike, 2011), amphibians (Zhang et al., 2020), and fish (Salvanes and Balino, 1998, Vindenes et al., 2016). Two important determinants of offspring development and survival (i.e., offspring fitness) during early ontogeny are parental body condition (Donelson et al., 2009, Reid et al., 2010, Farquharson et al., 2021) and local environmental conditions (Werner and Gilliam, 1984, Ridley, 2007, Burton and Metcalfe, 2014). The experiences of an individual during early life history also inform its condition in adult phases (Giménez and Anger, 2001, Shima and Swearer, 2010, Fopp-Bayat et al., 2021). The timing and success of these phases dictate the number of reproductive events an individual may have in its lifetime and have carry-over effects on the fitness of its offspring (Yamahira, 2001).

Because an individual's fitness is influenced by distinct factors over its lifetime, the specific mechanisms that determine facets of adult fitness (e.g., body condition, growth, reproductive behaviour) and offspring fitness (e.g., development and survival) are still poorly understood for many species. Within vertebrates, fish display a high degree of synchronicity in reproductive behaviours and development with seasonal environmental cues (Lowerre-Barbieri

et al., 2011). The marine environment is characterised by great fluctuation in tidal activity, current, salinity, sedimentation, temperature, and oxygen content of the water (Bleile and Rodgers, 2001, Grassle, 2013), resulting in radically variable conditions that organisms must navigate, survive, and reproduce in. For many fish, reproduction and development is innately connected to the lunar cycle (Leatherland et al., 1992, Fukunaga et al., 2020). The gravitational pull of the moon's changing position relative to the Earth across the month influences tidal activity (Takemura et al., 2004, Ikegami et al., 2014a), providing a key mechanism by which reproductive material and offspring disperse (Rowe and Epifanio, 1994, Breitburg et al., 1995, Cury and Pauly, 2000, Ando et al., 2013, Landaeta et al., 2015). Many fish rely on regular patterns of lunar light to sync reproduction, and patterns of lunar illumination modify offspring behaviour and development (Blaxter, 1968, Ricketts, 1985, Shima et al., 2020, Shima et al., 2021). Many studies investigate the influence of lunar cycles, though this is usually in the context of tidal activity.

Night-time conditions constitute half the lifespan of most animals, and key processes in fish that mediate fitness are intimately connected to patterns of nocturnal illumination in the form of lunar light (Lowerre-Barbieri et al., 2011). Yet, a comprehensive understanding of the implications of altered regimes of nocturnal light on fitness and development is lacking (Hernández-León, 2008, Park et al., 2014, Shima and Swearer, 2019, Shima et al., 2020). To achieve a holistic understanding of fitness across ontogeny it is critical to examine the influences of a wide range of environmental cues. Shifts in the strength, timing, and duration of environmental cues such as nocturnal illumination can have direct consequences for organism development and reproduction (Shaffer et al., 2020), and these consequences can differ with ontogeny (Werner and Gilliam, 1984). In assessing influences of nocturnal illumination across ontogeny, we can gain insight into how fish life histories are constructed.

1.1 Complex life histories of fish

A complex life cycle is one in which an organism may occupy different ecological niches at discrete stages of life. This type of life cycle is the product of ontogenetic adaptations to morphology, physiology, or behaviour to utilise transient resources (Wilbur, 1980). Individuals with complex life histories experience different pressures (e.g., predation, competition, resource availability) across different stages of life (Werner and Gilliam, 1984).

Metamorphosis is a key life history trait that allows movement between different habitats at certain sizes, ages, or stages of life, that maximises growth potential and minimises mortality risk (Istock, 1967, Wilbur, 1980, Willson, 1981, Werner and Gilliam, 1984).

Fish exhibit complex life histories, undergoing early phases of development before metamorphosis and later reach reproductive maturity as adults (Espinel-Velasco et al., 2018). The earliest phase: the embryonic phase, encompasses post-fertilisation development within the egg before hatching occurs. After hatching, embryos enter a prolonged phase of planktonic development in the water column called the pelagic larval duration before undergoing metamorphosis and settling as juveniles (Grosberg and Levitan, 1992). Offspring are highly sensitive and vulnerable during these early phases, and most offspring do not survive to juvenile settlement (Mcgurk, 1986, Litvak and Leggett, 1992, Parsons et al., 2014). Tolerance to environmental stressors also tends to be much narrower for embryos and larvae than adults (Septriani et al., 2021). Changes in the intensity and timing of environmental cues such as light, temperature, and pH can significantly alter the length and success of early development phases (Guma'A, 1978, El-Fiky, 2002, Martín-Robles et al., 2012). Some species circumvent this by prolonging or accelerating their development to reach maturity during favourable seasonal conditions (Johansson and Rowe, 1999). In altering developmental length to access transient resources and avoid unfavourable environmental conditions, these individuals are able to maximise their fitness (Werner and Gilliam, 1984). Adult fitness is connected to reproductive success and influences offspring fitness, and by extension, the chances that their offspring will reproduce successfully (Shaffer et al., 2020). This means it can be challenging to delineate the specific mechanisms by which environmental factors impact fitness at various stages of the complex life cycle of fish.

1.2 Nocturnal illumination as an environmental cue

There is strong evidence that seasonal (Koger et al., 1999, Nakane et al., 2013) and monthly (i.e., lunar) (Leatherland et al., 1992, Lowerre-Barbieri et al., 2011) illumination exhibits significant sway over patterns in fitness-related traits such as growth, feeding behaviour, and reproduction in fish (Kaartvedt et al., 1998, Holzman and Genin, 2003, Hanson et al., 2008, Ikegami et al., 2014a, Shima et al., 2021). In recent years, the impact of light on life history traits of fish has received increased attention (Marangoni et al., 2022). However, published

research is usually focussed on variation in nocturnal illumination on a single stage of the fish life cycle, or fails to consider both natural and artificial variation in nocturnal illumination (Tidau et al., 2021). A detailed insight into how nocturnal illumination influences fitness is therefore still lacking for many species. This is especially prescient when accounting for multiple possible sources of variation in nocturnal illumination, which may occur seasonally e.g., annual changes in photoperiod length, lunar cyclic variation in light, or can be artificially introduced (Gaston et al., 2013). For example, many fish operate on a circadian rhythm, which is entrained by changes in diurnal light throughout the year (Sweeney, 1963). The Earth's rotation provides a regular cycle of night and day, and the tilt of its axis and orbit around the sun causes seasonal variation in the timing, duration, and intensity of light:dark periods. This consistent variation is responsible for regulating the frequency and phase of the circadian endogenous clock (Menaker, 1968). In addition to the circadian rhythm, many fish also rely on lunar (monthly) or semi-lunar (fortnightly) rhythms to synchronise fitness-related processes like reproduction, and growth may also follow lunar patterns (Tanner, 1996, Takemura et al., 2006, Baker and Dekker, 2008). The circalunar rhythm is entrained by regular and predictable changes in lunar illumination across its cycle. The moon orbits the Earth in a cycle that lasts 29.53 days. Light from the sun is reflected to Earth from the moon, illuminating different portions of the lunar disc as it orbits Earth. Lunar brightness peaks when the moon is positioned at 180° to the sun from Earth's perspective (full moon) through to 270° (third quarter) (Ikegami et al., 2014b). Moonrise and moonset times likewise vary at different stages of the moon's orbit. The lunar cycle therefore presents a natural, predictable, spatially discrete window of changing duration, timing, and luminosity of lunar light (Fukunaga et al., 2020, Tidau et al., 2021). These large-scale differences in light level also vary locally due to changing weather conditions. Luminosity and light period vary by latitude, and lunar illumination is less intense in temperate latitudes (Kyba et al., 2017). In the marine context, the properties of seawater and the organisms that it contains alter the intensity, spectra, and spatiotemporal persistence of light underwater (Aksnes et al., 2009, Capuzzo et al., 2015, Tidau et al., 2021). The quality of light at the sea surface is likewise altered by atmospheric conditions including light pollution, air pollution, cloud cover, the position of the moon in the sky, and its distance from Earth (Cinzano et al., 2001). Despite these spatiotemporal differences, the light regime for any given latitude has been consistent for long periods of geographical time, providing a reliable set of environmental cues that inform ecological and evolutionary processes (Gaston et al., 2013).

Artificial light at night (ALAN), is a common technology used in human settlement and transportation (Baker and Dekker, 2008) that alters natural states of luminescence (Gaston et al., 2017). ALAN can be directly transmitted into otherwise dark environments at night, disrupting cycles of light and dark (Longcore and Rich, 2004, Navara and Nelson, 2007, Hölker et al., 2010, Perkin et al., 2011). ALAN can also be transmitted or reflected into the sky. This scattered light can have additive effects with fog or clouds to significantly increase Night Sky Brightness (NSB), especially in urban areas with high light pollution (Ribas et al., 2016). Some heavily populated areas of the planet experience "perennial moonlight" conditions, where natural patterns of luminescence are masked by heavy light pollution (Cinzano et al., 2001). While the utilisation of light at night has many beneficial outcomes for humans and allows us to extend hours of activity into the night, it also can have severe negative impacts on other organisms that we share our environment with (Gaston et al., 2022). The artificial introduction of light into an ecosystem can significantly disrupt important fitness-related processes controlled by endogenous clocks (Gaston et al., 2013). For example, saturation of nocturnal environments with artificial light can cause changes in patterns of predation, disrupt navigation, alter social and survival behaviours such as foraging and singing, inhibit diel vertical migrations of zooplankton, and cause failure in synchronized mass spawning of corals (reviewed in Gaston et al., 2017, Gaston et al., 2022, Marangoni et al., 2022), as well as reducing individual growth and likelihood of survival (Schligler et al., 2021). Over the past several decades, ALAN and its effects has become an increasing concern for ecology researchers (Depledge et al., 2010). A developing body of literature aims to assess the impacts of artificial light on organism fitness, but knowledge of the consequences of ALAN across ontogeny is limited for many species. Furthermore, little research has attempted to assess the impacts of disruptions to nocturnal light regimes on fish species endemic to New Zealand.

1.2.1 Adult fitness

Reproduction is an energetically expensive process, often involving trade-offs between maximising parental fitness versus offspring fitness (Roff and Fairbairn, 2007) or between investing energy into reproduction versus parental growth or survival (Brosset et al., 2016). These trade-offs can cause individuals to neglect growth and survival in favour of boosting fecundity (Abrahams and Dill, 1989, Donelan and Trussell, 2020) or else sacrifice fecundity to

maintain body condition or growth (e.g., Ghalambor and Martin, 2001). Fecundity is also influenced by body size and condition (Barneche et al., 2018, Mu et al., 2021), and individuals that accumulate greater energetic reserves prior to reproduction tend to experience greater reproductive success (Clark et al., 1994, Johnston et al., 2007). Larger individuals are often better equipped to meet the energetic requirements of reproduction (Clark et al., 1994). Increased access to resources translates to periods of greater growth and therefore increased body size, and conversely, periods of reduced food availability can translate to reduced growth (Jørgensen et al., 2014), resulting in decreased physical condition in preparation for reproduction. The timing of reproduction determines not only parental condition and fitness, but also the number of reproductive opportunities an individual will have in its lifetime (Yamahira, 2001). Reproductive timing, too, sets the starting conditions that an offspring will inhabit, and is therefore an important determinant of offspring fitness (Yamahira, 2001). Unique qualities of marine and freshwater environments such as water current and viscosity exert strong selection on fish reproductive life-history traits, and many fish therefore share similar reproductive traits (Cury and Pauly, 2000, Lowerre-Barbieri et al., 2009). Many fish spawn following seasonal or monthly changes in light (Koger et al., 1999, Yamahira, 2001, Lowerre-Barbieri et al., 2011). The high prevalence of periodic spawning in fish at different timescales associated with annual and seasonal variations in light suggests that there are advantages associated with breeding at a specific time (Rowe and Ludwig, 1991). This implies that spawning in tandem confers some advantage to either parents or offspring. The persistence of synchronized spawning despite increased risk of predator-based larval mortality derived from greater larval patchiness (Mcgurk, 1986) also supports this. Alternatively, aggregated spawning behaviour may enhance reproductive success and decrease predation risk by swamping predators with larvae, increasing the average chance of survival for individual larva (Johannes, 1978). Chronological factors that promote breeding within a window of time can also maximise the likelihood of gamete fertilisation alongside offspring survival (Juntti and Fernald, 2016).

1.2.2 Offspring fitness

Patterns of luminescence can influence growth, feeding behaviour, and recruitment of fish during early life phases (Hernández-León, 2008, Shima and Swearer, 2019, Shima et al., 2021).

Most larval fish, and their predators, rely on nocturnal illumination to visually locate their prey (Blaxter, 1968), so being active at certain times of the lunar month may yield multiple benefits or costs to larval growth and survival. For example, the recruitment and hatching of *Trachurus trachurus* (the Atlantic horse mackerel) has been found to increase during the new moon (Klein et al., 2018). It was speculated that this was because low levels of luminescence during the new moon provided a temporal refuge for larvae from their predators. The relationship of larval behaviour and growth to lunar phases is also mediated by lunar-induced changes in behaviour of predators and prey. Exposure of larvae to light can increase the efficiency of prey capture, feeding, and larval swimming behaviours (Blaxter, 1968, Batty, 1987). The timing and intensity of lunar light can therefore alter patterns of larval feeding across the lunar month (Gehrke, 1992), which in turn affects patterns of larval development and growth. Diel vertical migrations (DMVs) are the migration of meso-planktonic organisms up and down in the water column every night (Hernández-León, 2008, Benoit-Bird et al., 2009, Drazen et al., 2011, Jørgensen et al., 2014). Moonlight regulates this migration of organisms to surface waters, constituting the largest animal migration on Earth occurring on a nightly basis (Benoit-Bird et al., 2009, Drazen et al., 2011). Diel vertical migrants not only include planktonic food sources for larval fish, but also their predators (Drazen and Sutton, 2017). The encroachment of both prey items and predators into shallow waters is inhibited in conditions of high luminescence, so upward migration is restricted during the full moon. DMVs are therefore seen in their shallowest extent in dim conditions at the new moon (Benoit-Bird et al., 2009, Last et al., 2016, Bandara et al., 2021). These patterns of movement are also influenced by the timing of moonrise and set across the month. The lunar illumination hypothesis asserts that light at the sea surface promotes increased feeding by adult fish and lowered rates of mortality in their early planktonic phases through its control of DMVs (Hernández-León, 2008). DMVs, by virtue of influencing the presence of food and predators, therefore alter patterns of larval growth, mortality, and recruitment (Hernández-León, 2008, Shima and Swearer, 2019, Shima et al., 2020, Shima et al., 2021). The literature investigating the impact of nocturnal illumination on larval stages is well-developed, but the impact of this environmental cue is less explored in the context of embryonic development – especially lunar illumination.

1.3 Study species

This thesis investigates the influence of nocturnal illumination on facets of adult and offspring fitness for the New Zealand common triplefin, *Forsterygion lapillum* (Hardy, 1989). *F. lapillum* belongs to the family Tripterygiidae, which is characterised by a high degree of endemism (Stewart et al., 2015), representing 26 species over 14 genera (Hickey et al., 2009). The phenotype and ecology of this group of fishes is strongly influenced by environmental conditions (Syms, 1995, Hilton et al., 2008, Caiger et al., 2021). In particular, water depth and exposure have been identified as the most significant determinants of habitat differentiation for the triplefin taxon (Wellenreuther and Clements, 2007).

F. lapillum is a small-bodied demersal species of this family (Hickey and Clements, 2003, Feary et al., 2009) that is highly abundant in shallow water (0-5m; Jawad, 2008) coastal reef environments throughout New Zealand (Hickey and Clements, 2003, Feary and Clements, 2006, Wellenreuther and Clements, 2007, Wellenreuther and Clements, 2008, Smith et al., 2013). *F. lapillum* is a highly generalist triplefin species, inhabiting a wide variety of habitats (Wellenreuther et al., 2009) and displays significant phenotypic plasticity correlating with environmental conditions (Caiger et al., 2021). Common triplefin feed on tiny invertebrates such as isopods, amphipods, and polychaetes, as well as algae (Allen and Robertson, 1994, Fricke, 1994). *F. lapillum* are abundant in the Wellington region, found in densities of 1-6 fish per metre^2 for both adults and juveniles depending on time of the year (Mensink and Shima, 2014). Despite the close phenotypic association of *F. lapillum* with environmental conditions, the impact of nocturnal illumination on fitness for this species is currently unexplored. Common triplefin are also easy to maintain in a laboratory environment (Wellenreuther and Clements, 2007), making them an excellent study species for manipulative experiments.

1.4 Aims and thesis structure.

This thesis aims to evaluate the importance of nocturnal illumination on components of fitness at distinct stages of the complex life cycle of the temperate reef fish *Forsterygion lapillum*.

In Chapter 2, I assess the impact of nocturnal illumination on fitness-related traits of adult *F. lapillum*. I collected adults in the field and conducted a laboratory experiment using four

nocturnal light treatments (regular lunar cycle, dimmed lunar cycle, 24-hr artificial light, and darkness at night) to evaluate how nocturnal illumination impacted several components of adult fitness: body condition, growth, and reproductive behaviour. I measured body condition before and after the experiment to assess whether exposure to nocturnal illumination impacted individual wellbeing. I then extracted and processed otoliths post-experiment to assesses the influence of nocturnal light (treatment and lunar effects) on growth rates during the final month of the experiment. Lastly, I used photographs of egg clutches taken progressively throughout the experiment to investigate the effect of nocturnal illumination (light treatment and lunar effects) on reproductive activity (timing and output).

In Chapter 3, I examine the impact of nocturnal illumination on components of offspring fitness: development length, structure, and success. I used progressive photos of each clutch taken throughout the laboratory experiment to gather estimates of development milestones and structure (eye development, total development, and the portion of development taken to synthesise eyes) and used these estimates to evaluate if embryonic development was shaped by exposure to nocturnal illumination (light treatment and lunar effects). I also tracked the number of eggs lost from each clutch until hatching occurred to estimate the survival of embryos to hatching – i.e., clutch "hatching success" – and examined whether hatching success differed with nocturnal illumination (light treatment and lunar effects).

CHAPTER 2

The impact of nocturnal illumination on adult fitness-related traits of *Forsterygion lapillum*.

2.1 Introduction

Night-time represents half the lives of organisms, yet we rarely consider effects of nocturnal illumination on fitness. Light is a significant factor that informs the organisation of the biological world (Ragni, 2004, Bradshaw and Holzapfel, 2010). The timing, intensity, duration, direction, and spectra characteristics of natural light provide information to organisms about the time of day, month, and year, their location, and other characteristics of their environment (Neff et al., 2000, Ragni, 2004). Variation in light intensity, wavelength, polarization, and diurnal or seasonal photoperiod influence several facets of fish behaviour (reviewed in Puvanendran and Brown, 2002), growth, and reproduction (Head and Malison, 2000, Takemura et al., 2004). Nocturnal illumination, in addition to photoperiod, is therefore an important source of environmental variation that is likely to affect adult fitness. Changes in nocturnal illumination can occur naturally monthly (i.e., the lunar cycle) or seasonally (i.e., changes in photoperiod throughout the year). Lunar light moves in a predictable window over the course of a single lunar month spanning 29.53 days, with brightness peaking during the full moon and third quarter moon phases (Ikegami et al., 2014a, Fukunaga et al., 2020, Tidau et al., 2021). In addition to natural sources of variation, patterns of nocturnal illumination may be artificially altered due to human influence (e.g., Artificial light at night – ALAN) (Gaston et al., 2013).

Many fish share similar fitness-related life history traits such as egg size and the presence of a larval phase but display great variation in the timing of reproduction between species (Lowerre-Barbieri et al., 2009). Some fish spawn on a seasonal or daily (circadian) rhythm, using changes in light:dark ratios and temperature to initiate development to sexual maturity and reproduction (Koger et al., 1999). Both seasonal and lunar cycles may influence reproductive timing concurrently (Bushnell et al., 2010, Graham et al., 2021). Some fish spawn year-round in

regions where environmental conditions remain relatively consistent but still rely upon environmental cues to trigger reproduction at optimal times (Taylor, 1984). Many fish (Leatherland et al., 1992) and many other organisms across different taxa reproduce in conjunction with the lunar cycle (e.g., Clifton, 1997, Connell et al., 1997, Claydon et al., 2014, Andreatta et al., 2020, Luz et al., 2020, Shima et al., 2020) using lunar light to induce gonadal maturation, gamete production, and synchronise other reproductive behaviours such as courtship and spawning (Bhattacharya, 1992, Leatherland et al., 1992, Takemura et al., 2006, Baker and Dekker, 2008).

Some species rely on darkness as a resource to regulate growth and reproduction (Gerrish et al., 2009). For these species, consistent patterns in periods of light and darkness are essential to entraining the endogenous clocks that regulate these processes (Menaker, 1968). Furthermore, considerable energetic costs may be borne to maintain the metabolic pathways and sensory organs required to maintain endogenous rhythms (Niven and Laughlin, 2008). Artificial light - particularly in the form of artificial light at night (ALAN) - may provide misleading cues or mask cycles of light and dark, disrupting processes that are entrained by natural shifts in patterns of light (reviewed in Marangoni et al., 2022). This is of particular concern for organisms occupying highly light-saturated environments. Constant exposure to low levels of ALAN can reduce metabolic performance, causing fatigue, reduced individual health, and degradation of body condition (Beck and Gobatto, 2016, Kupprat et al., 2021). Growth can also be hindered by continued exposure to artificial light, and these impacts vary depending on ALAN wavelength colour (Head and Malison, 2000). The wavelength of artificial light can be especially disruptive to certain organisms due to differences in the spectral sensitivity of receptor organs between species (Gaston et al., 2013). Changing trends in artificial light wavelength and spectrum towards a broader spectrum of “white” light alter the colour of sky glow, potentially exacerbating biological impacts in areas of dense human settlement (Kyba et al., 2012). ALAN can be especially disruptive to organisms with complex life histories, inhibiting metabolic processes associated with growth, development, and metamorphosis, causing greater mortality risk and reduced fitness in later phases of life (Geffen and Nash, 2012, Gaston et al., 2017, Schligler et al., 2021). In species where reproduction is closely connected with seasonal and environmental cues, disruptions to the strength or timing of light may interfere with reproductive output and timing, likewise influencing offspring fitness (Shaffer et al., 2020).

Understanding the drivers of variation in fitness-related traits is essential to develop insight into the evolutionary biology and ecology of a particular species within its community. Individuals must often trade-off energy investment between maintenance of body condition, somatic growth, or reproduction (Brosset et al., 2016, Zhiegelbecker and Sefc, 2021). Individual investment choices between these factors can determine the timing, mode, and outcome of reproduction (Mitterwallner and Shima, 2022). The outcome of the energetic trade-off contributes to the timing and success of reproduction, which dictates facets of offspring fitness, and in turn affects patterns of community recruitment, population size, structure, composition, and genetic variation (Cargnelli and Gross, 1996). Achieving a holistic understanding of the impact of nocturnal illumination on fitness-related traits is therefore of paramount importance to explicate factors that shape adult fitness in species with complex life histories. Despite most fish displaying strong synchronicity in fitness-related processes and reproductive behaviours, our understanding of how many environmental cues like nocturnal illumination influence these traits is limited (Lowerre-Barbieri et al., 2011). This is especially true of lunar illumination, partially due to the general lack of emphasis in research on nocturnal processes (Park, 1940).

In this study I investigated how nocturnal illumination influences adult fitness-related traits for a species that exhibits close phenotypic association with environmental cues: the New Zealand common triplefin (*Forsterygion lapillum*). I conducted a laboratory experiment manipulating nocturnal illumination for *F. lapillum* to examine its effects on adult condition, growth, and reproduction over 3 months. I measured individual body condition before and after the experiment to assess whether exposure to nocturnal light altered adult body condition over the experiment. Additionally, I humanely euthanised fish post-experiment and extracted their otoliths to assess the impact of nocturnal illumination on somatic growth rates (light treatment and lunar effects). Lastly, I progressively photographed egg clutches laid in experimental tanks to evaluate the influence of nocturnal illumination on the timing and frequency of reproduction (light treatment and lunar effects). I explored three questions through this research: 1) Does nocturnal illumination alter adult body condition? 2) Does growth vary in different conditions of nocturnal light? 3) How does nocturnal illumination affect reproductive behaviour?

2.2 Methods

2.2.1 Study species and system

Forsterygion lapillum (the common triplefin) is a small-bodied (Hickey and Clements, 2003, Feary et al., 2009) temperate fish that is found commonly in rocky reef environments throughout New Zealand (Wellenreuther and Clements, 2007, Wellenreuther et al., 2008). *F. lapillum* are found naturally in densities of 1-6 fish per metre^2 in cobblestone environments in the Wellington region (Mensink and Shima, 2014) and feed largely on small invertebrates (Allen and Robertson, 1994, Fricke, 1994). Common triplefin exhibit close phenotypic association with environmental factors (Caiger et al., 2021) resulting in differed timing of breeding seasons across New Zealand. For example, the breeding season in Auckland ranges from June-January (Francis, 2001, Mcdermott and Shima, 2006) but they may spawn year-round except for the coldest winter months in the Wellington region (Moginie and Shima, 2018). Common triplefin are thought to be asynchronous daily spawners (Warren, 1990). During the breeding season, reproductively active males assume a black nuptial colouration and defend a small cobble territory (~1m^2) that may contain several suitable nesting sites (Wellenreuther and Clements, 2007, Mensink et al., 2014). Males provide a substrate for nesting that protects eggs from physical disturbance and predation (Thompson, 1979, Francis, 2012) and maximises the ability to attract females (Thompson and Jones, 1983). Common triplefin display resource-defensive polygyny, where males defend spawning sites and females travel to forage and find potential mates (Jones, 2013). Females anchor eggs with sticky threads to the underside of a nesting stone within the successful male's territory (Thresher, 1984, Feary and Clements, 2006). Male triplefin then assume sole parental care over offspring until eggs hatch, usually around 2-3 weeks after laying (Moginie and Shima, 2018). Males court females using a set of ritualised courtship displays and may guard clutches laid by several different females at once (Mensink et al., 2014). Males that defend higher quality spawning sites may display disproportionately high reproductive success, whereas others may not spawn at all during a breeding season (Jones, 1981). The use of relatively open nesting sites may improve the ability of males to attract greater numbers of females, and therefore increase the number of eggs laid in their nests and potential number of offspring (Feary and Clements, 2006). *F. lapillum* therefore exhibit strong preference for nesting sites (Thompson and Jones, 1983).

Other factors such as nest site quality, body size (Tornquist, 2020), and population density can significantly influence reproductive success for both sexes (Barnett and Pankhurst, 1996). Female triplefin retain the same colouration year-round, which is indistinguishable from that of non-reproductively active males (Wellenreuther et al., 2008). Gray and brown are common colours for female and non-spawning male blennies (Thresher, 1984, Wheeler, 1985, Böhlke and Chaplin, 1994, Nelson, 1994, Springer, 1998, Myrberg and Fuiman, 2002). "Streaking" occurs in some species of triplefin, wherein younger cryptically coloured males rush in while the nest-guarding male is spawning and release their own sperm to attempt to fertilize some of the eggs (Thresher, 1984). It is possible that this occurs in common triplefin, though this has not been substantiated. Visual differentiation between non-breeding males and females via non-lethal means is difficult owing to small size and relatively undifferentiated external genitalia. It is currently unknown whether reproduction of the common triplefin follows lunar patterns, or how nocturnal illumination impacts fitness-related traits for adults of this species.

2.2.2 Laboratory experiment

Tank setup and experimental conditions

I used 68 litre food-safe plastic storage containers as tanks for the experiment (645mm length, 413mm width, 397mm height: AP15 Enviro Stacka Nesta 68L - 1223RG; Stowers Containment Solutions 2010 Ltd). Each tank was fitted with a water in-tube (12mm diameter) and a PVC downpipe (16mm diameter). Tanks were supplied with continuous flow-through of filtered seawater. I provided two artificial nesting sites to each tank made from halved unglazed terracotta flowerpots (Northcote Pottery 12cm diameter, 147.03cm^3 internal surface area) and 1.5 litres of fine river stone (Garden Highlights, NZ) to emulate a cobble substrate (Fig 2.1A). Black lids were affixed to tanks, and once these were in place, tanks were entirely opaque to the passage of light. Black silicone sealant was used to block passage of light around any fittings. All tanks were threaded with one length of PVC piping (16mm diameter) that was cut and re-fitted around a length of clear vinyl (12mm diameter). The vinyl housed an LED strip attached to a bamboo stake. The PVC pipe had nine holes drilled into it at roughly equal intervals to allow light to shine into the tank (Fig 2.1B). I made additional modifications to tanks depending on which experimental light treatment they were assigned to; details are described in the following section.

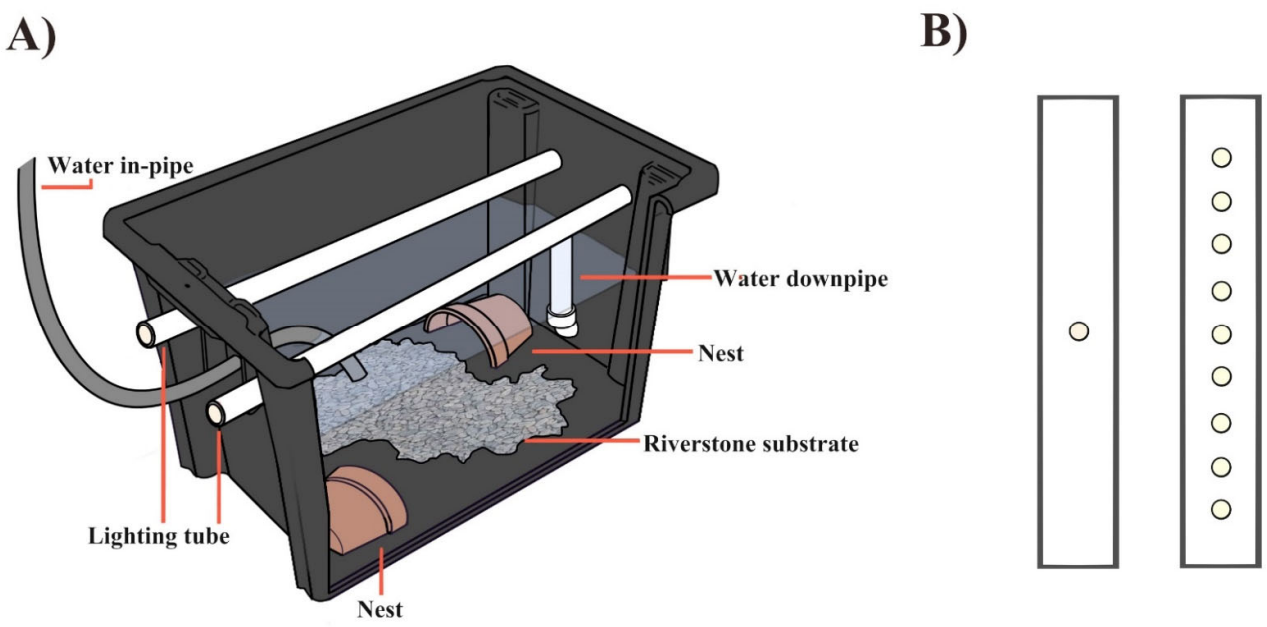

Figure 2.1: A) Cross-section of a tank with its lid removed. A water in-pipe was threaded through a hole in one side of the tank with a downpipe situated opposite allowing a continuous flow-through of seawater. Two artificial nesting sites sat at opposite ends of the tank, and fine river stone was scattered haphazardly along the tank floor. Two lighting tubes containing vinyl-housed LED strips ran lengthways through lunar treatments – one for lunar lighting and the other for diurnal lighting. Non-lunar treatments contained only a single diurnal lighting tube. **B)** Compares the number of holes drilled into a lighting tube housing lunar LEDs (left) versus a lighting tube housing diurnal light (right).

Experimental manipulation of light

I assigned tanks to one of four experimental light treatments (n = 5 per treatment, total n tanks = 20): 1) a simulated lunar cycle with luminescence approximating moonlight in clear cloudless sky conditions (regular lunar treatment); 2) a simulated lunar cycle with luminescence intended to approximate moonlight under heavy cloud cover (dimmed lunar treatment); 3) no nocturnal light (dark at night treatment); and 4) constant 24-hour artificial light (24-hr light treatment).

Tanks in the two lunar treatments and the dark at night treatment received the same diurnal light (24volt LED strip, 10watt/metre, 5000k colour temperature, approx. 75.3lux), which were set to a 12 hour on/off cycle (7:30am-7:30pm, 12L:12D). For the 24-hr artificial light treatment, these lights remained on overnight (24L:0D). Due to a change in the LED manufacturing process during experimental set-up, the lights for this treatment had slightly different specifications to those of the other three treatments (15watt/metre rather than 10watts/metre). The increased power input requirement for this strip resulted in brighter ambient light conditions for this treatment (approx. 150.7lux). Because diurnal light should be even between treatments (Tidau et al., 2021), I assigned the different set of lights to the 24-hr light treatment so that the remaining three treatments would receive equal diurnal light.

The two lunar treatments were also threaded with an additional PVC pipe (described in the previous section) containing LED lights for the lunar lighting system. The specifications for these LEDs matched those of the diurnal lights, except the colour temperature. The refraction of sunlight from the moon tends further towards the red end of the visible light spectrum than sunlight (400-700nm) (Ciocca and Wang, 2013), so I selected LEDs with 4000k colour temperature to appropriately emulate the colour of moonlight (Forsythe, 1923). Additionally, only a single hole was drilled into the PVC for lunar lighting tubes, allowing less light to penetrate the tank to mimic the perceived difference in magnitude of brightness between solar and lunar light (Fig 2.1B). To dim the lunar light in the dimmed lunar treatment, I affixed a segment of black duct tape over the hole in the PVC, limiting the light that penetrated the tank.

Tanks in each of the four treatments shared a single strip of LEDs for diurnal or artificial light (and another single strip for simulated lunar light for lunar treatments). As a result, I was not able to randomly assign tanks to treatments, so tanks in each treatment were located adjacent to one-another in a line.

The two lunar light programmes were implemented using an APEX lunar simulator module (A2 APEX base unit AOS 5.10 8F22, APEX lunar simulator LSM: Neptune Systems, Morgan Hill USA). The simulator was programmed to vary intensity, duration, and timing of luminescence like that of a natural lunar cycle across the month (Fig 2.2A). The moon rises and sets during the day at certain phases of the lunar cycle, so during periods of the experiment lunar treatments received lunar illumination during the daytime as well as at night. Simulated lunar light was not able to be detected using a HOBOware light logger (Onset, Bourne USA) (measured as 0lux), so it is unlikely that simulated lunar light had an additive impact on diurnal light (measured approx. 75.3lux) for the lunar treatments. I measured light readings for calibration purposes to compare the nocturnal brightness across the lunar cycle for the regular and dimmed lunar treatments using a Unihedron sky quality meter-LU-DL (Unihedron, Ontario Canada). Measurements were taken in magnitude per square arcsecond (MPSAS), an astronomical quantification of surface brightness. MPSAS deals with logarithmic difference in surface brightness which is constant with distance, whereas lux is calculated using distance and area. Lux therefore varies significantly depending on proximity to the object that is emitting light, and so cannot be converted to MPSAS or vice versa. Lower readings constitute greater object brightness. A decrease of a single MPSAS corresponds to a 2.5x increase in sky brightness, so 5 magnitudes lower is equal to a 100x increase in brightness. The MPSAS of the sun averages around -26.7, while the full moon is -12.6, constituting a 408,276.3x difference in brightness. MPSAS readings of both lunar and diurnal lights provided a reasonably accurate approximation of the magnitude of difference in sky brightness between day and night-time, and the regular lunar cycle provided a reasonable approximation of natural variations in lunar brightness (Fig 2.2B).

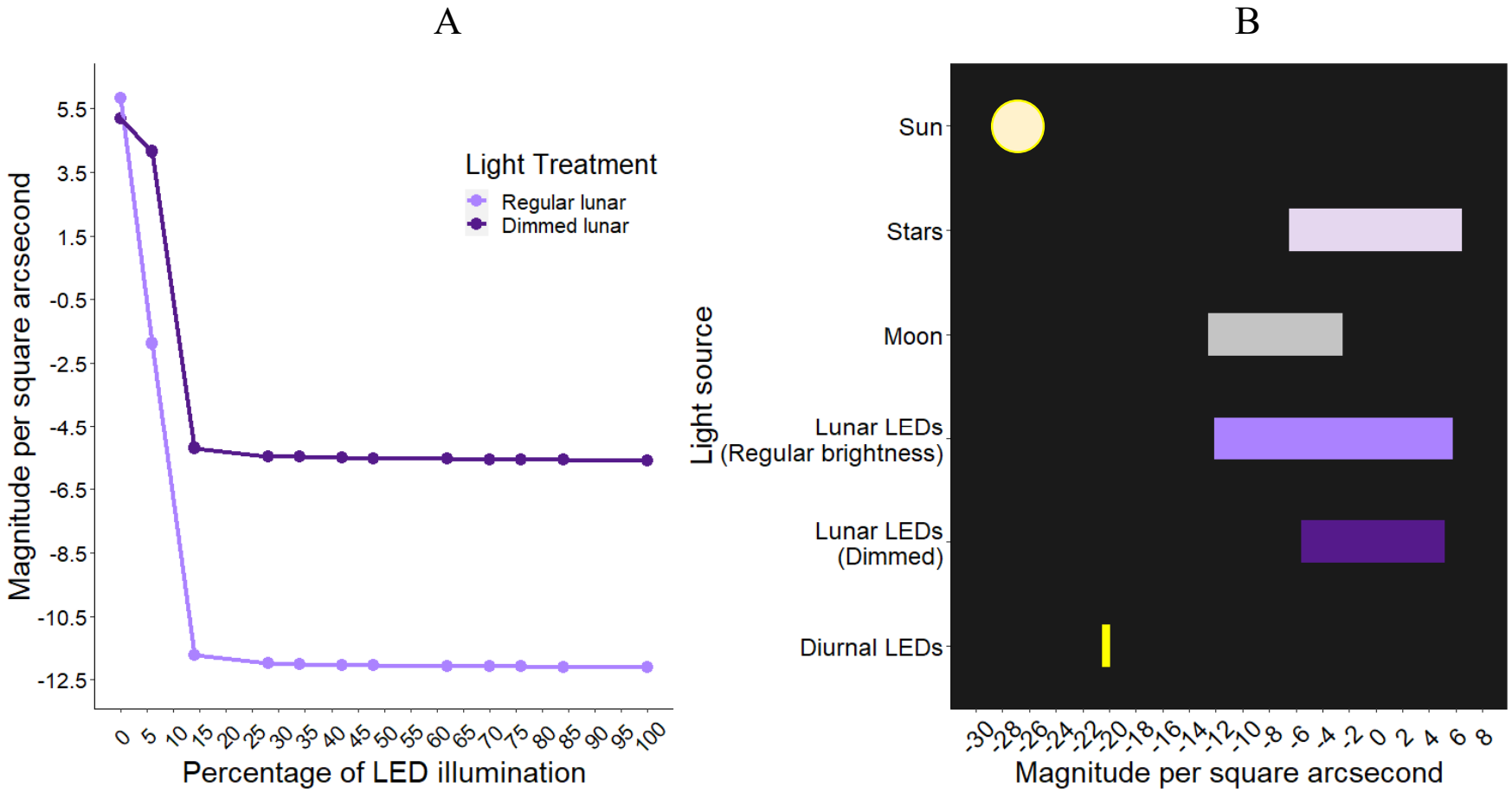

Figure 2.2 A) Magnitude per square arcsecond (MPSAS) by percentage illumination of LED lights in the regular lunar treatment (light purple) and the dimmed lunar treatment (dark purple). **B)** Magnitude per square arcsecond (MPSAS) of celestial bodies (Sun, stars, moon) (measurements obtained from Unihedron (2022) and simbad.u-strasbg.fr. (n.d.)) and lights used in the experiment (Lunar LEDs for the regular and dimmed lunar treatments, diurnal LED lights).

Fish collection and maintenance

I collected adult triplefin from Tarakena Bay (41°20'41.24"S, 174°49'14.35"E) on the south coast of Wellington between December 2021 and April 2022. I aimed to collect 40 males and 120 females. I identified males by the presence of black nuptial colouration and territorial displays. Triplefin without black colouration were initially assumed to be female, though (because this is an imperfect indication of sex) I assessed this further upon experiment completion (described below). I made collections with the aid of snorkel using hand nets. I then transported fish to the Wellington University Coastal Ecology Lab (WUCEL) alive, segregated by assumed sex in seawater-filled buckets. Upon arrival at WUCEL, I randomly assigned 2 confirmed males and 6 putative females to each of the 20 experimental tanks. I conducted the laboratory experiment between May 6th and August 3rd, 2022. I maintained fish on an *ad-libitum* diet (consisting of a single whole *Mytilus edulis*) added to each tank once per week. I assumed that this frequency of feeding was sufficient as I routinely found *M. edulis* tissue remaining in the tanks by the end of the 7-day period in the weeks prior to experiment commencement. I acclimated fish to tanks under ambient light conditions in the laboratory for at least one week prior to experiment commencement. Upon commencement of the experiment, I manipulated patterns of nocturnal light for 3 months, and quantified the effects of these treatments on the body condition, growth, and reproductive behaviour of adult triplefin. After the experiment, I euthanised the fish using clove oil in accordance with approved VUW Animal Ethics procedures (AEC 29677) and stored them in 99% ethanol.

Towards the end of the experiment 2 fish had to be euthanised due to an unknown infection (1 from the 24-hr light treatment, 1 from the dark treatment), and a further 8 died suddenly (5 from the 24-hr light treatment, 3 from the dark treatment). Common triplefin typically have a maximum lifespan of 1-1.5 years (Moginie, 2016). All fish had already reached sexual maturity prior to the experiment, and I collected many of them in December 2021. Because the experiment began in May 2022, it is likely that these fish were reaching the end of their natural lifespan. As fish were euthanised, I replaced them in the tank with members of the same putative sex that had been acclimated in laboratory conditions for at least one week. This was to ensure even breeding densities between tanks. I did not replace fish in the final 2 weeks of the experiment, assuming that exposure to nocturnal light would require time to become entrained and that this was not likely to be represented if I added fish in the final days of the experiment. Four fish were also missing by the end of the experiment from the 24-hr light

treatment. It is possible that these fish somehow escaped experimental tanks, though this is unlikely as none were found in the sea table surrounding the tanks. The sea table downpipe was also screened off, and I did not find any fish on the area of floor surrounding the sea tables. It is possible that these fish reached the end of their natural lifespan, died of natural causes, and were eaten by their tankmates, but I never found any remains. This is unlikely but not impossible given that I checked tanks once every 1-3 days so as to minimise disruption to experimental conditions.

2.2.3 Data collection and analysis

Sexing

As small-bodied fish triplefin are difficult to sex via inspection of external genitalia. Posthumously, all triplefin changed to a dark black colouration like that of the nuptial colouration displayed by males. This necessitated extra measures to confirm the number of fish of different sexes in the experiment. I sexed triplefin using a combination of sighting the urogenital opening under a dissecting microscope and specimen dissection to confirm suspected sex internally. After performing 20 dissections I was able to distinguish the appearance of male and female genital papillae from one another. The female urogenital opening tended to be larger with a spongy texture and an indistinct opening, whereas the male urogenital opening was more distinct with a puckered appearance (Fig. 2.3). I was then able to correctly predict sex in 9 out of 10 attempts based solely on inspection of the genital papilla. In cases where the genital papilla was indistinct, or I was otherwise uncertain about sex based on the appearance of the genital papilla alone, I performed dissections to confirm sex. Four missing fish from the 24-hr light treatment were not able to be sexed.

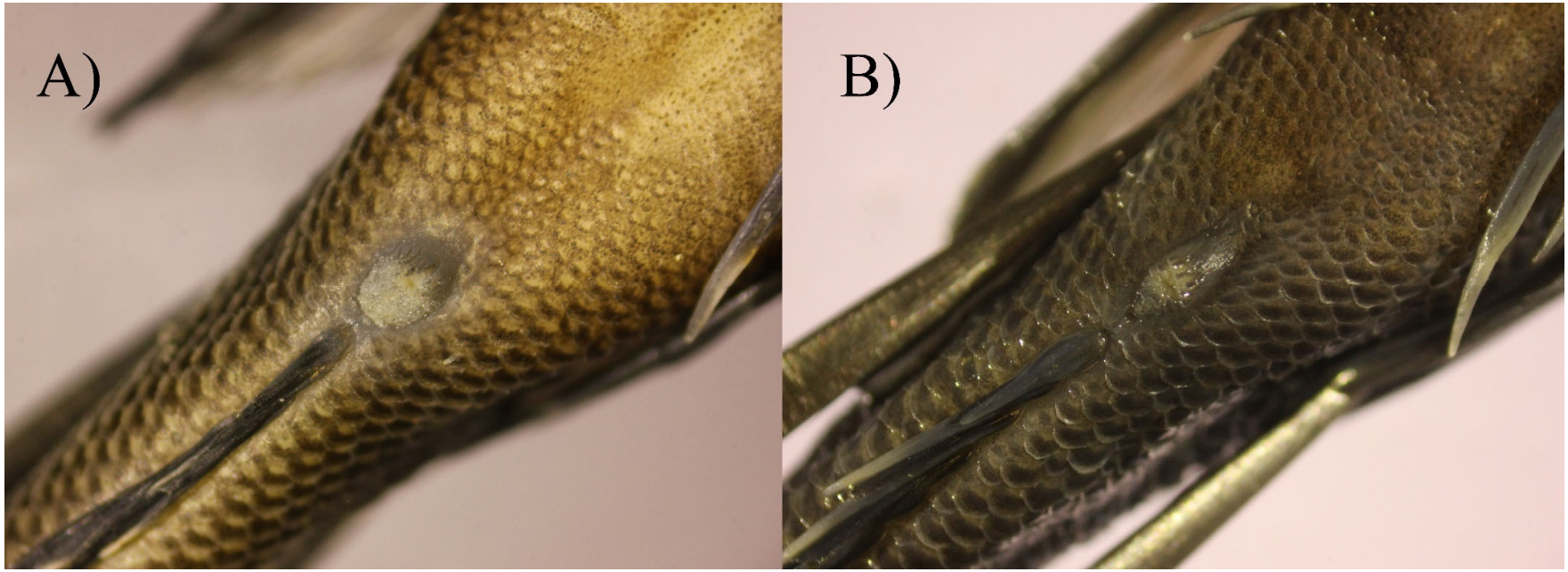

Figure 2.3: *F. lapillum* urogenital openings. **A)** Shows the spongy female urogenital opening located at the base of the anal rays. **B)** Exhibits the puckered male urogenital opening also located at the base of the anal rays.

Assessing changes in body condition

Fulton's body condition index (K) (Fulton, 1904) is a non-lethal morphometric index used to estimate body condition using weight and length measurements of each fish. It assumes that heavier fish of a certain length are in better condition, with K factors closer to 1 indicating "normal" condition (Sutton et al., 2000). Prior to experiment commencement, I measured the wet weight of each fish to the nearest 0.0001g, the standard length (from the base of the caudal fin to the tip of the snout – SL) and total length (from the tip of the anal rays to the tip of the snout) to the nearest 0.01mm using callipers. I used measurements of standard length and weight to calculate Fulton's K for each individual as:

$$K = \frac{\text{Weight(g)}}{100 * \text{length(cm)}^3}$$

In early phases of experimental design development, I attempted to tag some triplefin (that were not used in the later experiment) using visual implant elastomer (VIE) (Northwest Marine Technology, Washington, USA). Tagging results were mixed, and the dark colouration taken on by both male and female fish to camouflage with the bottom of the tank made identification of marks extremely difficult without further invasive procedures. To avoid causing unnecessary stress to the fish and to apply a consistent approach in identification, I assigned each of the eight fish in each tank a rank (1-8) based on their standard length (i.e., the largest fish in a tank was ranked 1, the smallest was ranked 8). Following the experiment, I measured the standard length (SL), total length (TL), and wet weight (WWT) of each fish again posthumously. I assumed that any variation in growth rates among fish within a given tank would be unlikely to influence rank order in their standard lengths. Given this assumption, I used the same system of ranking from the pre-experimental measurements to estimate the identity of each individual. I then used post-experimental wet weight and standard length to calculate post-experimental Fulton's K for each fish.

I estimated change in body condition by calculating the relative percentage change between pre-experimental and post-experimental Fulton's K for each fish (ΔK).

To assess the influence of nocturnal illumination on adult body condition, I built a linear mixed effects model using the percent change in Fulton's body condition across the experiment (ΔK) as the response variable and light treatment as a fixed effect. This analysis tested the null hypothesis that the relative change in body condition after 3 months of experimental rearing did not vary with light treatment. Fish may experience changes in body condition differently depending on sex (Schoenebeck et al., 2014, Angel et al., 2015) and pre-existing body condition (Schoenebeck et al., 2014, Bright Ross et al., 2021), so I included sex and pre-experimental body condition (Pre-K) as fixed effects that may additionally influence ΔK in the model. I included tank as a random effect to account for possible variation between tanks in the same light treatment. I tested all possible combinations of the model terms—main effects and their interactions—and used AICc (Akaike information criterion) to select the best fitting model (*AICcmodavg* package, Mazerolle, 2020). The best fitting model included interaction terms between all three of the fixed effects. I have included the AIC output in appendix A (Table A1) to illustrate the model selection process that I use in model selection for all analyses described in this chapter, and in chapter 3.

I analysed this model using a Wald chi-square analysis of deviance test (R Core Team, 2022) (type III error) to test the significance of main and interaction terms. The interaction terms were not indicated to influence the response variable, so I re-analysed the model using type II error due to its superior statistical power in main effects analysis. I then conducted a linear mixed effects regression to test the prediction power of linear effects (*lme4* package, Bates et al., 2015) and calculated the marginal R^2 (R^2m) and conditional R^2 (R^2c) to assess the variance in the model explained by the fixed effects and the entire model (including both fixed and random effects) respectively (*MuMIn* package, Bartoń, 2022). For categorical fixed effects of statistical significance, I conducted post-hoc pairwise testing (*emmeans* package, Lenth, 2022) to test for differences in body condition between fixed effect groups.

Estimating growth rates

Otoliths ('ear stones') preserve a record of daily growth that can be analysed by measuring the width of each daily growth increment (Shima and Swearer, 2009, Shima and Swearer, 2016).

I evaluated otolith microstructure to assess the influence of nocturnal light regime on growth rates. First, I extracted two (left and right) sagittal otoliths from each fish by cutting a wedge of skull and cranial tissue from the top of the head, extracting the brain, and removing each otolith using fine forceps. I cleaned remaining tissue off the otoliths and stored them dry in centrifuge containers (1ml). I prepared otoliths for microstructure analysis by randomly selecting one otolith per specimen and mounting it on a lapping disc using a two-part epoxy resin (EpoThin^{TM}2 Epoxy resin 20-3440-032, EpoThin^{TM}2 Epoxy hardener 20-3442-016: Buehler, Lake Bluff, IL, USA) sulcus side up. Once set, I used 9μm lapping film to remove otolith material by hand until the otolith edge was thin enough to read. I then applied a small amount of type F immersion liquid (Leica Microsystems CMS) and photographed the rostrum of the processed otoliths using a camera (Leica DMC4500, Danaher, Washington D.C.) mounted to a dissecting microscope (Leica DM2500 LED, Danaher, Washington D.C) at the 20x/0.5 objective. Temporal patterns in growth often require time to become entrained. These patterns may not have been entrained within the first two months of the experiment, so I used the Otolith M app in Image-Pro Premier v9.3 (Media Cybernetics, Bethesda, Maryland, USA) to conduct otolith analysis and measure the width (μm) of each of the outer 30 growth rings from the otolith edge – corresponding to growth occurring during the final month of the experiment. I corroborated each increment width to the date it was formed, counting back from the otolith edge, and assigned it the equivalent day of the lunar month (0-29). To ensure that all otolith measurements were taken from fish during the same window of time, I did not take growth measurements from fish that were not in the experiment for its entire duration (female n = 4, male n = 5). I successfully recorded 30 days of growth measurements for 140 fish (female n = 55, male n = 85). I assigned each otolith a rating from 1-5 based on my confidence in the accuracy of measurements, with 1 indicating low confidence and 5 indicating high confidence.

Lunar effects.

In the two lunar treatments, I also investigated whether growth varied over the lunar month by comparing increment width to lunar day. I assigned each day of the lunar month a number representing its place in the month (0 = new moon, 15 = full moon, 29 = end of the last quarter). I then allocated an angular equivalent theta (θ) to each lunar day by dividing lunar days into 360^{o} (2π radians). I applied sine and cosine transformations of θ to express the circular-periodic quality of the lunar cycle following the methods of deBruyn and Meeuwigg (2001). Cosine describes a phase shift close to 0^{o} (new moon) or 180^{o} (full moon), while the sine term describes a phase shift around 90^{o} or 270^{o} (first and last quarter respectively). A positive cosθ coefficient

would indicate a peak in growth at the new moon, while a negative value indicates a peak at the full moon. A positive sinθ coefficient represents a peak in growth during the first quarter, while a negative coefficient indicates a peak during the third quarter. I included sin2θ and cos2θ transformations of the data to investigate whether two peaks growth could be occurring per lunar month.

Does growth vary in different conditions of nocturnal light?

Light treatment.

I investigated the impact of nocturnal illumination on patterns of adult growth by building a linear mixed effect model, fitting growth increment width (μm) as the response variable with light treatment as a fixed effect. The analyses based on this model tested the null hypothesis that the growth increment width over 30 days in the experiment did not vary by light treatment. Growth may vary between individuals based on sex (Parker, 1992) or body size of the fish (Day and Taylor, 1997). To account for this possible variation in the model, I included sex and standard length (SL) as fixed effects. I also included tank as a random effect to account for random variation in growth between tanks. My confidence in the accuracy of increment width measurements also varied, so I assigned the confidence rating of each otolith as an additional random effect in the model. I then tested all possible combinations of main effects and their interactions and used AICc scores to select the best fitting model for the data (*AICcmodavg* package, Mazerolle, 2020). The best fitting model for this analysis included the interaction terms between all three of the fixed effects (standard length, sex, and treatment). I then conducted analysis of the model using a Wald chi-square analysis of deviance test (R Core Team, 2022) (type III) to test the significance of main and interaction terms. I analysed the data using a linear mixed effects regression (*glmmTMB* package, Brooks et al., 2017) to elucidate the prediction power of linear fixed effects. I calculated the marginal R^2 (R^2m) and conditional R^2 (R^2c) to understand the degree of variance that was explained by the fixed effects and the entire model (including fixed and random effects) respectively (*MuMIn* package, Bartoń, 2022). Data did not conform to assumptions of normality (based on the distribution of the Q-Q residuals plot). I performed a log transformation of the data, which improved normality of the residuals. I therefore report analyses in the results section using the log transformation of the data.

Lunar effects.

To elucidate whether adult growth changed across the course of the lunar month, I built a linear mixed effects model using growth increment width (μm) as the response variable and the lunar terms; sinθ and cosθ as fixed effects. Analysis of this model tested the null hypothesis that growth increment width (μm) in lunar treatments did not change across the final lunar month of the experiment. Following the procedure described in the previous analysis, I included tank as a random effect to account for possible random variation in a given tank. I also included the confidence rating for each otolith as a random effect to account for differing confidences in measurement. I tested every possible combination of main effects of sinθ and cosθ, with sin2θ and cos2θ added (representing dual peaks in growth), and lunar treatment, with interaction terms between lunar treatment and the lunar prediction terms and used AICc to select the best fitting model (*AICcmodavg* package, Mazerolle, 2020). The best fitting model used only sinθ and cosθ as fixed effects. I then conducted a periodic mixed effects regression (linear-circular) to analyse this lunar model (*glmmTMB* package, Brooks et al., 2017). I calculated the marginal R^2 (R^2m) and conditional R^2 (R^2c) for the data to explain the degree of variance that was explained by the fixed effects and the entire model (including fixed and random effects) respectively (*MuMIn* package, Bartoń, 2022). Residuals were not normally distributed (as illustrated by the Q-Q residuals plot), so I performed a log transformation of the data. Transformation improved normality of the residuals, so I report the results of this analysis using the log transformation of the data.

Quantifying timing and frequency of reproduction

Triplefin were able to engage in regular breeding and feeding behaviours during the experiment for 3 lunar months. I assessed reproductive activity three times per week (n sampling dates = 37) by removing nesting sites from tanks and photographing any egg clutches that were present alongside a ruler for scale. I counted eggs by hand, overlaying a small, coloured dot overtop each egg in a clutch using Paint.net version 4.3.11 (Fig 2.4). In new clutches, I changed dot colour for every 50 eggs to acquire an accurate count of clutch size (Fig 2.4A). Once a clutch was counted, I assigned a colour code to it to distinguish it from any new clutches in the same nesting site (Fig 2.4B) and tracked the fate of each clutch across the experiment by comparing

progressive photos and counting the number of eggs lost over time (Fig 2.4C) to maintain accurate counts as new clutches were continually laid.

Because triplefin spawn seasonally in Wellington in warmer temperatures, I recorded the water temperature in degrees Celsius (nearest 0.1 degree) using a thermometer each time I conducted photo sampling. The influence of other environmental cues on reproductive activity was possible but unlikely given that aquaria were self-contained and external light was blocked from the tank.

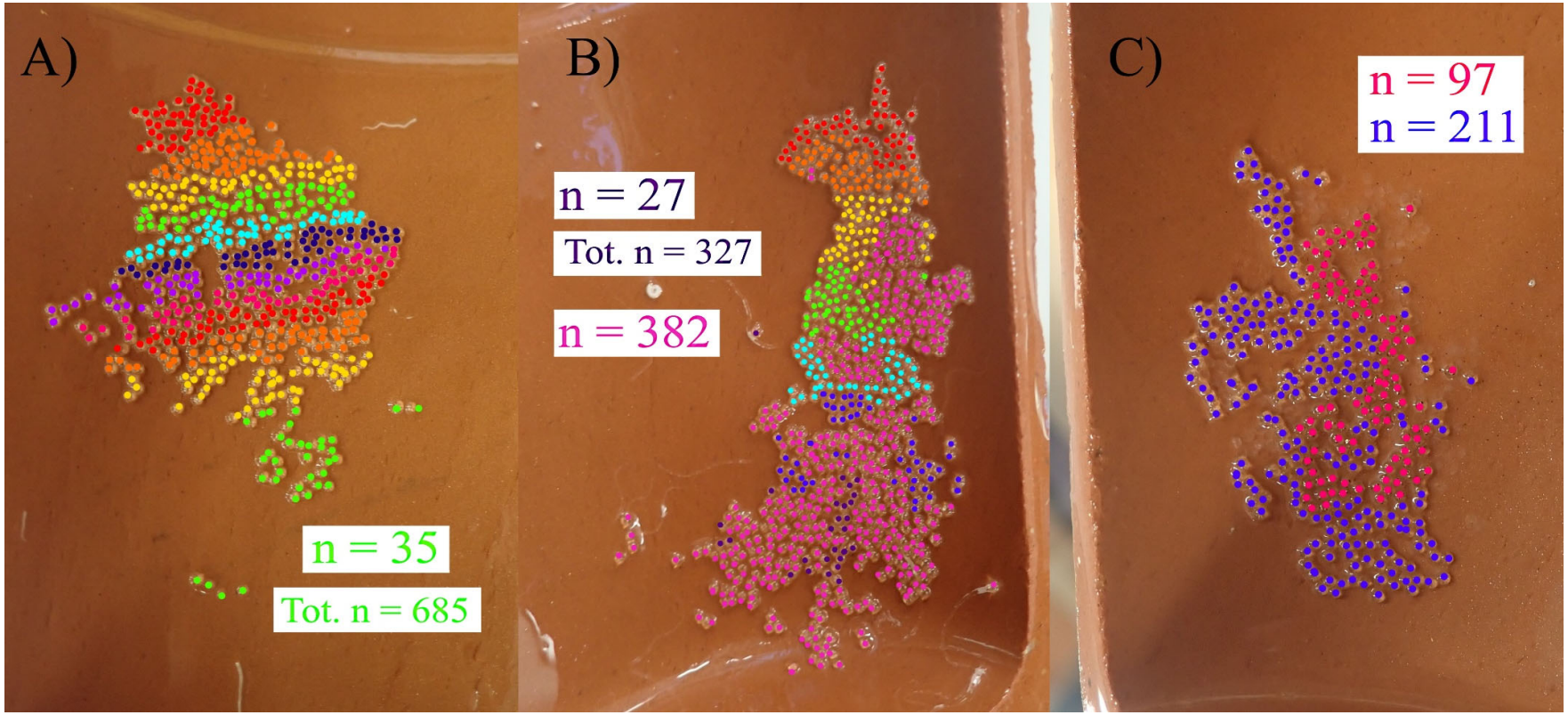

Figure 2.4: Diagrams depicting my methods for counting common triplefin eggs. **A)** Shows a freshly laid clutch. Each coloured band represents a count of 50 eggs, the remaining green band at the bottom contains 35 eggs for an overall clutch egg n = 685. **B)** Shows a freshly laid clutch (indicated in rainbow bands, purple band n = 27, total clutch egg n = 327) alongside an older clutch (indicated in pink, n = 382). **C)** Shows two older clutches, the fates of which are being tracked alongside one another (indicated in pink and blue, n = 97 and n = 211 respectively).

Because triplefin may spawn multiple times in a single day (Warren, 1990), I was unable to estimate the number of discrete spawning events that occurred. There were also differing numbers of females in each tank and the number of days between photograph sampling varied (2 or 3 days). I therefore estimated reproductive activity of fish in each tank post-experiment by calculating the average clutch size as:

$$\text{Average clutch size} = \frac{\text{Total clutch size}}{n_{Females} * \text{Days between photographs}}$$

Lunar effects.

In the two lunar treatments, I assessed whether reproductive output followed lunar patterns by comparing the number of eggs laid in each reproductive event to lunar day. Following the methods of deBruyn and Meeuwigg (2001), I assigned the estimated date the clutch was laid a lunar day corresponding to the day of the simulated lunar month (0 = new moon, 15 = full moon, 29 = end of the last quarter). I then transformed the data by allocating each lunar day an angular equivalent; theta (θ), dividing lunar days into 360^{o} (2π radians). I applied sine and cosine transformations of θ to express the circular nature of the lunar cycle. The cosine term describes phase shifts close to the new moon (0^{o}) or full moon (180^{o}). The sine term describes a phase shift close to the first quarter (90^{o}) or third quarter (270^{o}). A positive cosθ signifies a peak in the number of eggs laid at the new moon, whereas a negative coefficient indicates a peak in the number of eggs laid at the full moon. Conversely, a positive sinθ coefficient signifies a peak in the number of eggs laid during the first quarter, while a negative coefficient corresponds to a peak in the number of eggs laid during the third quarter. I also performed sin2θ and cos2θ transformations of the data to assess whether the number of eggs laid across the lunar month exhibited dual peaks.

How does nocturnal illumination affect reproductive behaviour?

Light treatment.

To evaluate the impact of nocturnal light on reproductive behaviours, I built a linear mixed-effects model fitting the average number of eggs per female per day as the response variable and light treatment as a fixed effect. Analyses of this model tested the null hypothesis that nocturnal illumination did not influence clutch size across the 3-month experiment. Triplefin

are seasonal spawners (Francis, 2001, Mcdermott and Shima, 2006, Moginie and Shima, 2018), so I included water temperature as a fixed effect to account for possible seasonal variation in spawning activity. I included tank as a random effect to consider random variation in reproductive behaviours within a given tank.

Spawning data contained a disproportionately high number of zeros. As a result, the assumption of normality was not met (as evidenced by the residual Q-Q plot). I therefore selected a hurdle model containing a zero-inflated component and a conditional component (negative binomial distribution) to analyse the data. The zero-inflated component tested whether the fixed effects influenced if the response variable was 0 or not, i.e., whether reproductive activity occurred or not. The conditional component of the model used a negative-binomial distribution and tested if the fixed effects influenced the distribution of non-zero values of the response variable (i.e., if eggs were laid, how many?). The conditional component used log transformations of the data, improving the normality of residuals (seen in the Q-Q plot of the model given by the *DHARMa* package; Hartig, 2022). Because the negative binomial distribution requires that the response variable be an integer, I reverted to total clutch size as an estimate of reproductive activity for this analysis. However, the raw count data did not account for differences in the number of females in each tank or the number of days between photographs. I therefore considered the number of females in each tank and the days between photographs as possible fixed effects, using AICc scores to select the most appropriate model (*AICcmodavg* package, Mazerolle, 2020). The model with the lowest AICc score included the number of females as a fixed effect but excluded days between photographs. I then tested every combination of these main effects and their interaction terms using the same method previously described. The best-fitting model included only the interaction term of water temperature and light treatment. It is possible to specify main effects and interaction terms for both components of the zero-inflated model. To refine model fit, I tested further combinations of inclusion of the interaction term of water temperature:light treatment in a single component of the model (zero-inflated *or* conditional) or in both. The best-fitting model included the interaction term of temperature and light treatment only in the zero-inflated portion of the model.

I excluded two tanks from the dimmed lunar treatment and 24-hr light treatment from analyses. The tank in the dimmed lunar cycle contained only males (and so did not exhibit any reproductive activity). 3 fish went missing by the end of the experiment in the tank in the 24-hr light treatment (see methods for possible explanations for this). The missing fish from this tank meant that I could not be sure of the number of females across the experiment, so I

excluded it from the model. Another tank in the same treatment was also missing a single fish at experiment conclusion. I conducted separate analyses of the data assuming that this individual was male in one and female in the other. Changing the sex of this individual did not significantly alter results or model fit. I conducted analyses assuming that this individual was male so as not to underestimate the possible impact of the number of females in each tank.

I analysed the model by conducting a Wald chi-square Analysis of deviance (type II error) to assess the significance of fixed effects. This test was only applied to the conditional portion of the model. I also conducted a generalised linear mixed effects regression (*glmmTMB* package, Brooks et al., 2017) to ascertain the influence of the fixed effects on both the zero-inflated and conditional portions of the model. For categorical fixed effects of statistical significance, I conducted post-hoc pairwise testing (*emmeans* package, Lenth, 2022). Likewise, this testing applied only to the conditional portion of the model (using the model's logarithmic scale).

Lunar effects.

To investigate the influence of patterns of lunar illumination on the reproductive output of common triplefin, I built a linear mixed effects model using clutch size (eggs per female per day) as the response variable. Analysis of this model tested the null hypothesis that the number of eggs laid during reproductive events did not vary over the course of the lunar month in the experiment. To account for possible random variation in reproduction between tanks, I selected tank as a random effect. I fit the two lunar prediction terms; $\sin\theta$ and $\cos\theta$ as fixed effects to evaluate the influence of lunar patterns. I used AICc scores to compare variations of this model that also included lunar treatment (regular versus dimmed), the lunar prediction terms accounting for two possible peaks in the number of eggs laid ($\sin2\theta$ and $\cos2\theta$), and interaction terms between lunar treatment and lunar prediction terms (indicating that the effect of lunar predictors differ between treatments) as possible fixed effects (*AICcmodavg* package, Mazerolle, 2020). The best-fitting model included $\sin2\theta$ and $\cos2\theta$ and the interaction terms of lunar treatment with lunar predictors ($\sin\theta$, $\cos\theta$, $\sin2\theta$, $\cos2\theta$). I analysed this model using a linear mixed effects periodic (circular-linear) regression (*lme4* package, Bates et al., 2015). I then calculated the marginal and conditional R^2 (R^2m, R^2c) to ascertain the degree of variance explained by the fixed effects and the overall model (including both fixed and random effects) accordingly (*MuMIn* package, Bartoń, 2022). I performed all statistical analyses in this chapter using R Statistical Software 4.2.1 (R Core Team, 2022).

2.3 Results

2.3.1 Nocturnal illumination and body condition

I subjected 163 fish to experimental conditions over the course of 3 months including 96 males (24-hr light $n_{/tank}$ = 3, 3, 4, 4, 5; dark $n_{/tank}$ = 3, 4, 5, 5, 7; regular lunar $n_{/tank}$ = 4, 5, 6, 6, 8; dimmed lunar $n_{/tank}$ = 4, 4, 5, 5, 6), 61 females (24hr light $n_{/tank}$ = 2, 3, 4, 4, 6; dark $n_{/tank}$ = 1, 3, 3, 4, 6; regular lunar $n_{/tank}$ = 0, 2, 2, 2, 4; dimmed lunar $n_{/tank}$ = 2, 2, 2, 3, 4), and 6 individuals of unknown sex (24 hr light $n_{/tank}$ = 0, 0, 0, 1, 3; dark $n_{/tank}$ = 0, 0, 0, 0, 1; regular lunar $n_{/tank}$ =0; dimmed lunar $n_{/tank}$ = 0). All tanks contained at least one female, save a single tank in the regular lunar treatment that was excluded from analyses.

The percentage change in relative body condition (ΔK) ranged from -33.63% to +27.15%. Body condition decreased on average by 6.8295% (±SE 0.8120). The change in body condition did not vary significantly between light treatments (Table 2.1, Fig. 2.5A). Female body condition decreased to a much greater degree than male body condition (Female mean ± SE; -10.29% ± 1.20, male mean ± SE; -4.49% ± 1.04) (Fig. 2.5 B), constituting a mean reduction in body condition twice as great as male fish (*emmeans* pairwise comparison; t_{133} = 4.970, p <0.0001).

Fulton's K of fish prior to the experiment (Pre-K) indicated good average body condition (Mean ± SE, 1.8480 ± 0.01328), ranging between 1.3919K and 2.6144K. As pre-experimental body condition increased, the relative percentage change in body condition tended to decrease (Fig 2.5C). The mixed effects model regression estimate indicated that an increase in pre-experimental Fulton's K by one unit (1K) resulted in a reduction in body condition of 47.771%. Conversely, individuals in relatively worse condition showed increases in Fulton's K over the experiment.

The interaction terms of Treatment:Sex, Treatment:Pre-K, Sex:Pre-K, and Treatment:Sex:Pre-K were not of statistical significance (Figure included in Appendix B; Fig. B1).

This model explained approximately 59.8% of the variation in the percentage change in body condition overall (R^2c = 0.5984431). A high proportion of this variation was attributed to the fixed effects (R^2m = 0.4821463).

Table 2.1: Analysis of Deviance table (Wald Chi-square, type II error) for the model investigating the impact of nocturnal illumination on adult body condition. The response variable (percentage change in relative body condition; ΔK) is analysed versus the main effects of pre-experimental Fulton's K index for body condition (Pre-K), sex, light treatment, and their interaction terms (Pre-K:Sex, Pre-K:Light treatment, Sex:Light treatment, and Pre-K:Sex:Light treatment). The table contains model parameters, chi-square test statistics (X^2), degrees of freedom (df), and p values. Statistically significant results ($p < 0.05$) are indicated in bold text.

Parameters	X^2	df	p value
Pre-K	**131.5246**	**1**	**2.2^{-16}**
Sex	**24.8153**	**1**	**6.309^{-07}**
Light treatment	0.7537	3	0.8605
Pre-K:Sex	0.3704	1	0.5428
Pre-K:Treatment	0.8806	3	0.8301
Sex:Treatment	2.9070	3	0.4062
Pre-K:Sex:Treatment	5.5259	3	0.1371

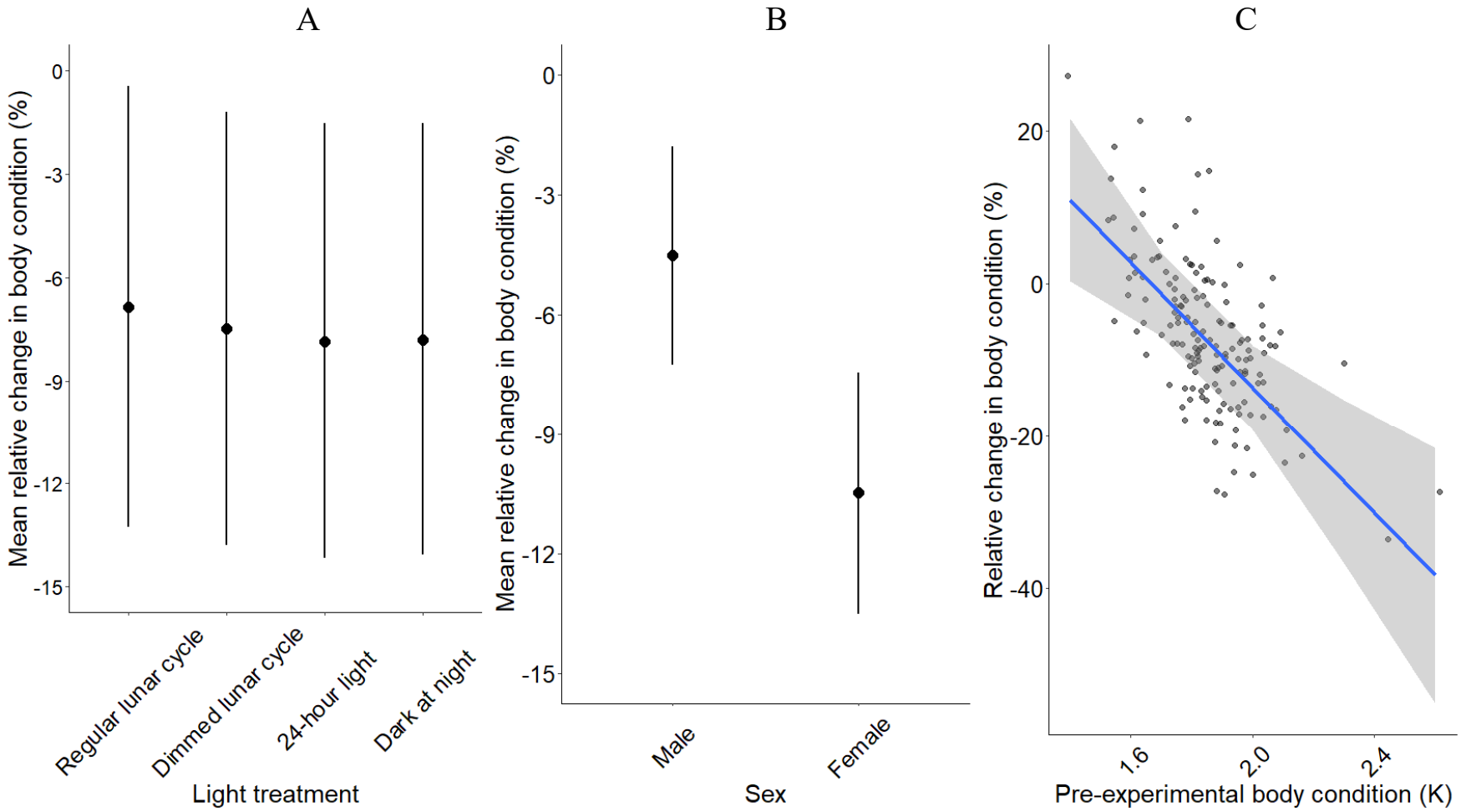

Figure 2.5: A) Mean percentage relative change in body condition (ΔK) between light treatments with 95% confidence interval. **B)** Mean percentage relative change in body condition (ΔK) between sexes with 95% confidence interval (female n = 61, male n = 96). **C)** Scatterplot of the percentage relative change in body condition (ΔK) versus pre-experimental body condition (K). The plot contains a regression line (blue) ± 95% confidence interval (grey shaded).

2.3.2 Nocturnal illumination and adult growth

Light treatment.

Daily growth increment width ranged from 0.43µm to 6.93µm, averaging at 1.4305µm (± SE 0.0097). The three main effects - standard length, sex, and light treatment - showed significant interaction (Table 2.2, Fig. 2.6), so I present results of the interaction terms over the main effects. Growth increment width did not vary significantly between sexes within the regular lunar treatment (Fig 2.6A), but increment width increased as standard length increased. This was only significantly distinct between the smallest and largest fish (for example, $female_{SL = 41mm}$ mean ± SE: 1.1306µm ± 0.0088, $female_{SL = 66mm}$ mean ± SE: 1.7630µm ± 0.1009).

Mean increment width size likewise also did not vary between sexes in the dimmed lunar treatment save for the fish with large standard lengths (Fig. 2.6B). Male growth was equivalent across body size, but female growth increment width increased as standard length increased. This resulted in females having wider growth increments than males at large body sizes (male mean ± SE: 1.2997µm ± 0.0787, female mean ± SE: 1.9237µm ± 0.1134).

Both sex and standard length interacted strongly in the 24-hr light treatment (Fig. 2.6C). Females had much wider growth increments than males at small body sizes (male mean ± SE: 1.1135µm ± 0.1234, female mean ± SE: 2.6303µm ± 0.0846). The inverse relationship was true at large body sizes (male mean ± SE: 1.6916µm ± 0.0.0848, female mean ± SE: 0.6614µm ± 0.0900). For intermediate standard lengths, growth increment width did not differ between sexes. Lastly, neither sex nor standard length caused variation in growth increment width for the dark at night treatment (2.6D).

The model explained approximately 37.7% of variation in the data ($R^2c = 0.3773465$), but only 8.48% of variation was explained by the fixed effects ($R^2m = 0.08482449$).

Lunar effects.

Growth increment width did not show any statistically significant relationships to lunar rhythms (cosθ, sinθ) (Appendix B; Table B1, Fig. B2). The model explained approximately 13.3% of variation in the data ($R^2c = 0.1330166$), and very little of this variation was attributed to the fixed effects ($R^2m = 5.448967^{-05}$).

Table 2.2: Analysis of deviance table (Wald Chi-square, type III error) for the mixed effects model with the log width of growth increments (μm) as the response variable and sex, standard length (mm), light treatment, and their interaction terms as fixed effects. The table contains chi-square test statistics (X^2), degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Parameters	X^2	df	p value
Intercept	**7.3907**	**1**	**0.006556**
Sex	0.0153	1	0.901577
Standard length	**18.5621**	**1**	**1.645^{-05}**
Light Treatment	**224.0990**	**3**	**$<2.2^{-16}$**
Sex:Standard length	0.0118	1	0.913473
Sex:Light Treatment	**103.8785**	**3**	**$<2.2^{-16}$**
Standard length:Light Treatment	**245.9179**	**3**	**$<2.2^{-16}$**
Sex:Standard length:Light Treatment	**117.5406**	**3**	**$<2.2^{-16}$**

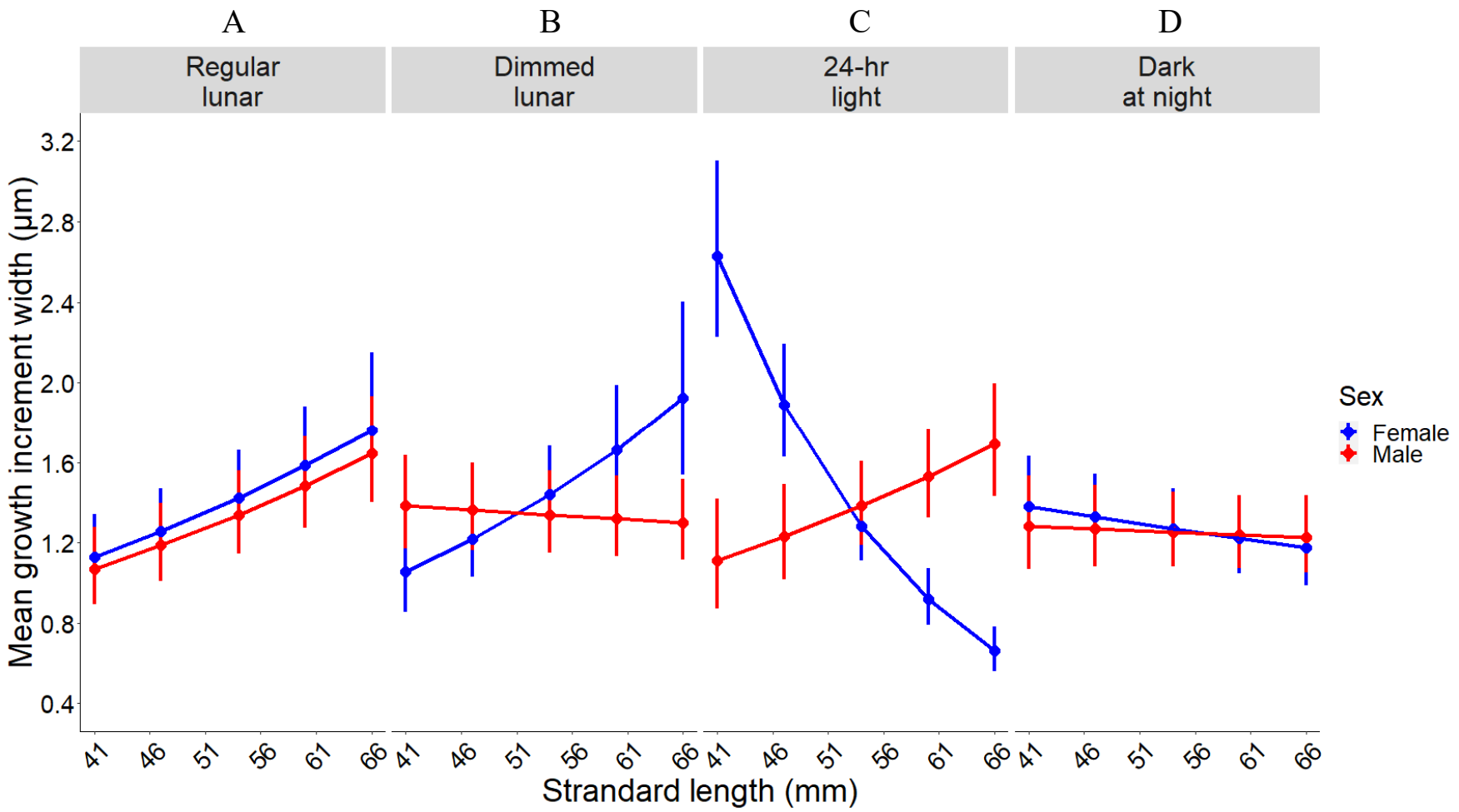

Figure 2.6: Interaction plots of the model effects (generated by the *effects* package, Fox and Weisberg, 2018) for mean growth increment width (with 95% confidence interval) by standard length (mm) for females (blue) and males (red) across light treatments (**A**: regular lunar treatment, **B**: dimmed lunar treatment, **C**: 24-hr light treatment, **D**: dark at night treatment).

2.3.3 Nocturnal illumination and reproduction

Light treatment.

162 reproductive events occurred during the experiment. 18 of these were in the regular lunar treatment, 14 occurred in the dimmed lunar treatment, 77 occurred in the 24-hr light treatment, and the remaining 53 were in the dark at night treatment. Water temperature ranged from 11.3 to 17.3°C, decreasing as the experiment progressed during the winter months (Mean ± SE; 13.59°C ± 0.052). It is not possible to extract model effects for the zero-inflated portion of the model and graphically represent them (Brooks et al., 2017). To that end I have not provided figures for the zero-inflated portion of the model and discuss the results in the context of the regression output only. The negative estimate for water temperature in the zero-inflated part of the model (Table 2.3) indicates that reproduction was more likely to occur in warmer temperatures, however this relationship was marginally not significant. Likewise, greater numbers of females in a tank increased the likelihood that reproduction would occur, but this relationship was also marginally not significant. Both the 24-hr light and dark at night light treatments had significant high likelihood that reproduction would occur. Exposure to lunar light did not affect the likelihood of reproduction occurring for fish in the lunar treatments. The only significant interaction term between water temperature and light treatment for the zero-inflated model was seen in the dark at night treatment. For this treatment, as temperature increased, the likelihood of reproduction occurring tended to decrease (estimate ± SE; 0.9900 ± 0.2420). A total of 70,490 eggs were laid by triplefin in the experiment. 6,039 eggs were laid in the regular lunar treatment, and 4,288 eggs were laid in the dimmed lunar treatment. The 24-hr light treatment had the greatest number of eggs laid at 40,959 eggs. The dark at night treatment had 19,204 eggs laid. Clutch size ranged from 6 to 1753 eggs, with a mean clutch size of 105.84 eggs (± SE 8.97). In the conditional portion of the model, light treatment significantly influenced clutch size (Wald $X^2_3 = 15.2899$, $p = 0.001585$) (Table 2.3, Fig. 2.7A). The mean clutch size for the regular lunar treatment (Mean ± SE; 335.5 ± 30.14), dimmed lunar treatment (Mean ± SE; 306.29 ± 81.56) and the dark at night treatment (Mean ± SE; 362.34 ± 27.47) did not differ. The 24-hr light treatment had the largest mean clutch size (Mean ± SE; 531.94 ± 35.73). This treatment differed significantly only from the dark at night treatment (*emmeans* pairwise comparison 24-hr – dark at night; $t_{648} = 3.384$, $p = 0.0042$). Water temperature and the number of females in each tank did not influence clutch size (Fig. 2.7B-C).

Table 2.3: Output for the zero-inflated negative binomial model assessing variation in clutch size. The table includes estimates, standard errors, z values, and p values for the zero-inflated portion (fixed effects: light treatment, water temperature, the number of females in each tank, and water temperature:light treatment) and for the conditional portion (fixed effects: Light treatment, water temperature, and the number of females in each tank). The regular lunar treatment has been used in the intercept. Significant results ($p<0.05$) are indicated in bold text.

Model	Parameter	Estimate	Standard error	z value	p value
Zero-inflated	**Intercept**	**7.5736**	**2.5539**	**2.965**	**0.00302**
	Treatment (Dimmed lunar cycle)	2.0354	3.8393	0.530	0.59601
	Treatment (24-hr light)	**-6.8485**	**3.0416**	**-2.252**	**0.02434**
	Treatment (Dark at night)	**-14.5771**	**3.3376**	**-4.368**	**1.26^{-05}**
	Water temperature	-0.3193	0.1765	-1.809	0.07047
	Number of females per tank	-0.2607	0.1357	-1.921	0.05474
	Treatment (Dimmed lunar cycle):temperature	-0.1519	0.2677	-0.568	0.57036
	Treatment (24-hr light):temperature	0.3386	0.2159	1.569	0.11673
	Treatment (Dark at night):temperature	**0.990**	**0.2420**	**4.091**	**4.29^{-05}**
Conditional	**Intercept**	**6.084919**	**0.714679**	**8.514**	**$<2^{-16}$**
	Treatment (Dimmed lunar cycle)	-0.052444	0.225513	-0.233	0.8161
	Treatment (24-hr light)	**0.390379**	**0.173947**	**2.244**	**0.0248**
	Treatment (Dark at night)	-0.001828	0.185356	-0.010	0.9921
	Water temperature	-0.027172	0.049625	-0.548	0.5840
	Number of females per tank	0.039346	0.036542	1.077	0.2816

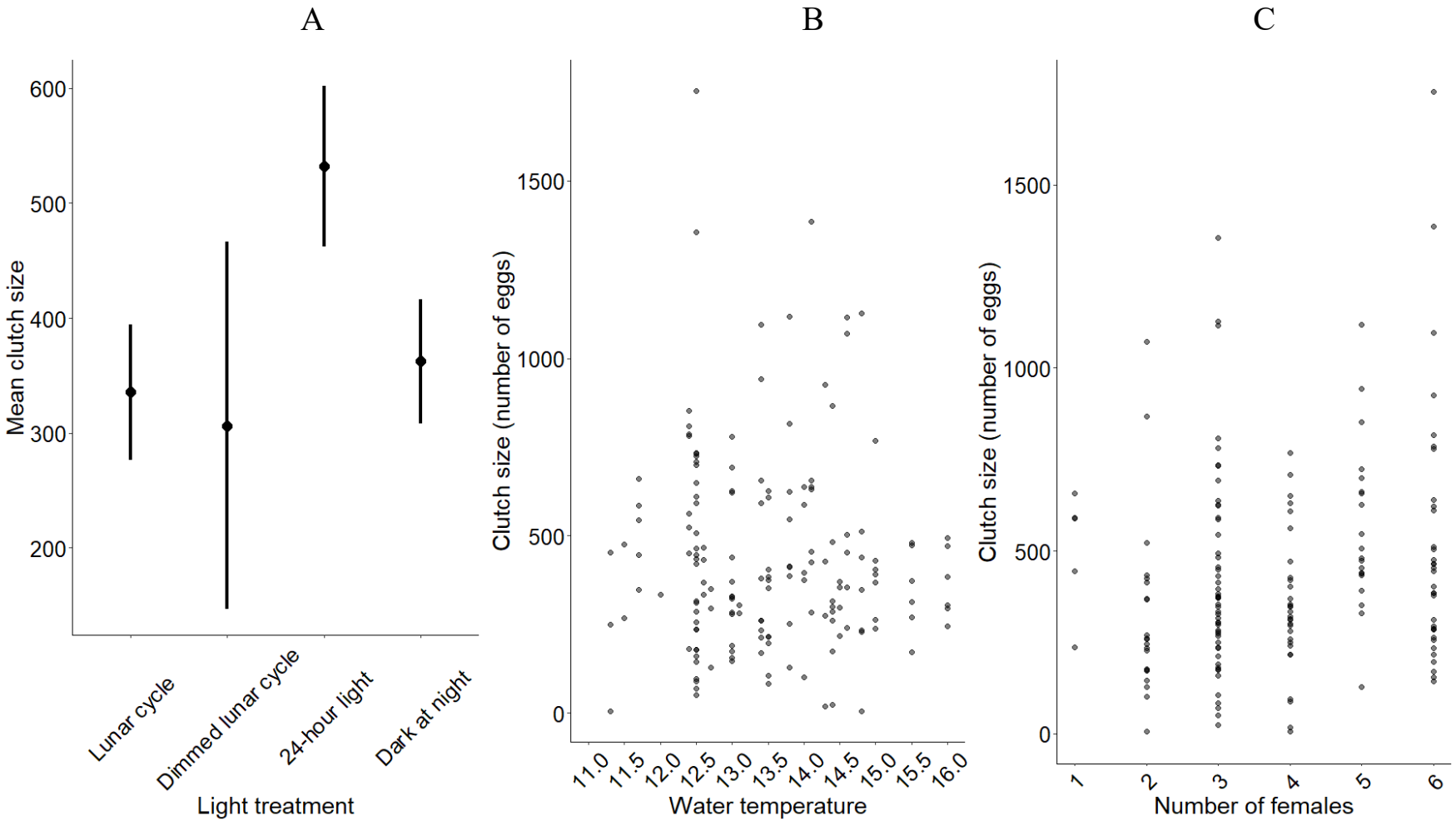

Figure 2.7: A) Mean clutch size (number of eggs) in each light treatment with 95% confidence interval. **B)** Scatterplot of clutch size (number of eggs) versus water temperature (degrees Celsius). **C)** Scatterplot of clutch size (number of eggs) versus the number of females in each tank. The relationships in B and C were not of statistical significance (linear mixed effects regression $p>0.05$), so no regression lines have been fitted.

Lunar effects.

I observed 32 reproductive events across three lunar months in the lunar treatments (regular lunar cycle n = 18, dimmed lunar cycle n = 14). Eggs per female per day in the regular lunar treatment ranged from 20.1 to 106 eggs (Mean ± SE; 53.4 ± 6.16). The range for the dimmed lunar treatment was 1.5 to 217 eggs per female per day (Mean ± SE; 60.2 ± 16.6). The average number of eggs per female per day differed between treatment groups, but this relationship was marginally not significant (Table 2.4, Fig. 2.8A, B).

The sinθ, cosθ, sin2θ, and cos2θ terms alone were not sufficient to describe patterns in reproductive activity, but the interaction terms of lunar treatment with sinθ, cosθ, sin2θ were significant. The regular lunar treatment showed significant peaks in the number of eggs per female per day during the first and third quarter moons (Fig. 2.8A). Conversely, the dimmed lunar treatment showed asymmetrical peaks in the number of eggs per female per day just after the full moon, and just before the new moon (Fig. 2.8B). Two data points in the dimmed lunar treatment were significantly larger than others, and consequentially held a great degree of sway over the results. When these data points were removed, the pattern in the number of eggs laid mimicked that of the regular lunar treatment. These reproductive events were from the same experimental tank. The model overall explained 54.66% of variation in the average number of eggs per female per day ($R^2c = 0.5466143$), and 27.18% of the variation was attributed to the fixed effects ($R^2m = 0.2718274$). Removal of these large data points eliminated the variation caused by the random effects ($R^2c = 0.2850162$, $R^2m = 0.2850162$) indicating these two points explained the large degree of variation introduced by tank as a random effect. However, I am confident that these outliers were not the result of an error in data collection, so I present the results with outliers included.

Table 2.4: Periodic mixed effects regression output of the average number of eggs per female using lunar phase terms, light treatment, and the interaction terms as fixed effects. The regular lunar treatment was used as the intercept. The table contains a list of parameters, the estimate, standard error, degrees of freedom (df), t studentized residual test statistic, and p values. Significant results ($p < 0.05$) are indicated in bold text.

Parameter	Estimate	Standard error	df	t value	p value
Intercept	40.190	28.497	13.093	1.410	0.1818
Cosθ	-12.271	26.665	17.117	-0.460	0.6512
Sinθ	-18.536	34.461	18.774	-0.538	0.5970
Cos2θ	-2.154	16.352	17.297	-0.132	0.8967
Sin2θ	-22.675	29.755	20.387	-0.762	0.4547
Light treatment (Dimmed lunar)	83.661	42.251	12.908	1.980	0.0694
Light treatment (Dimmed lunar):Cosθ	**96.654**	**43.829**	**21.970**	**2.205**	**0.0382**
Light treatment (Dimmed lunar):Sinθ	**124.762**	**47.252**	**21.694**	**2.640**	**0.0150**
Light treatment (Dimmed lunar):Cos2θ	-18.828	24.692	17.599	-0.762	0.4559
Light treatment (Dimmed lunar):Sin2θ	**125.375**	**44.594**	**21.729**	**2.811**	**0.0102**

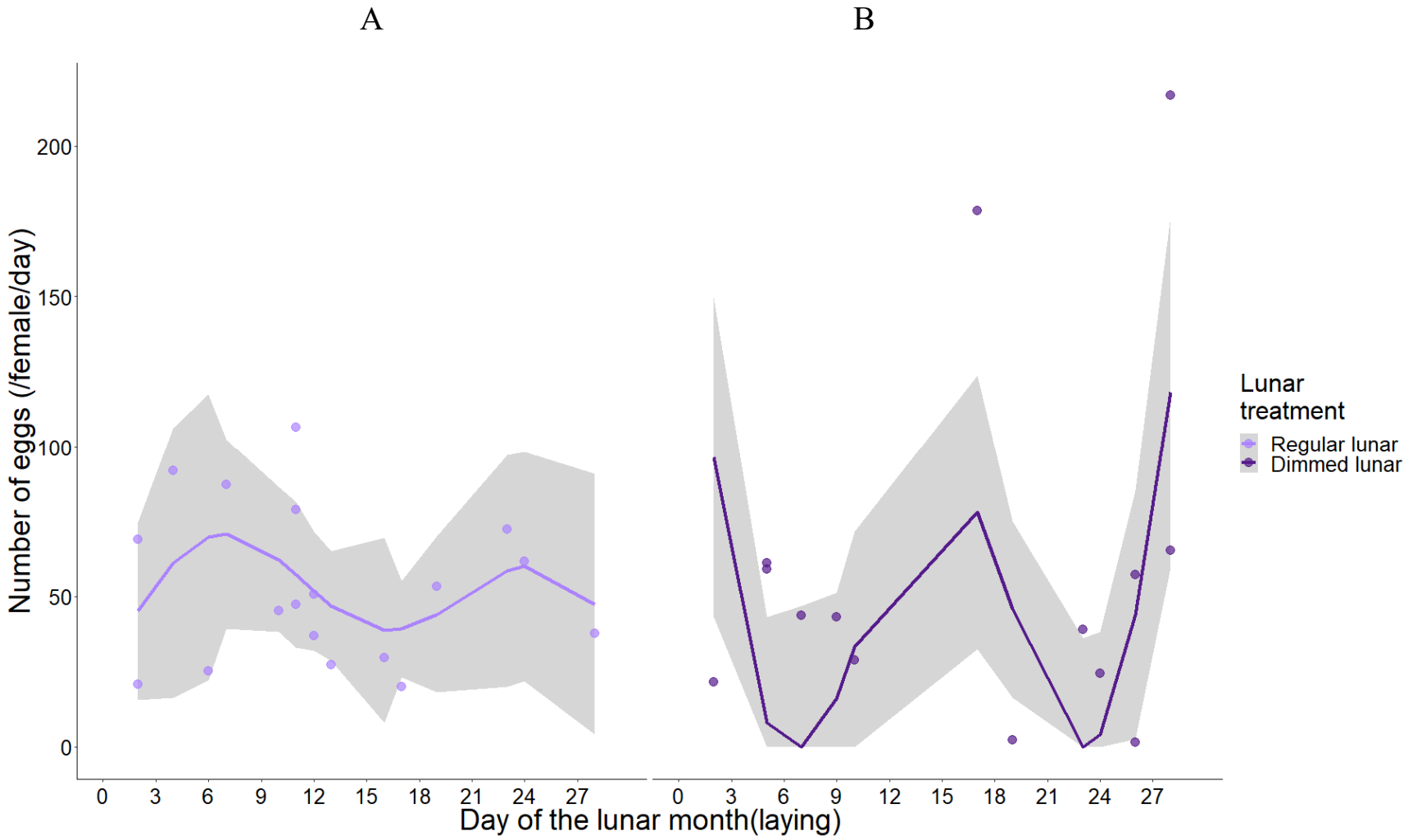

Figure 2.8: Scatterplots of the average number of eggs per female per day in **A).** the regular lunar treatment (light purple) and **B)** dimmed lunar treatment (dark purple) versus lunar day. The plots include model prediction lines generated using the *lme4* package (Bates et al., 2015) ± 95% confidence interval (grey shaded) (estimated from SE generated using *glmmTMB* package; Brooks et al., 2017).

2.4 Discussion

Does nocturnal illumination alter adult body condition?

Nocturnal illumination did not significantly impact changes in adult body condition over the course of the experiment, but females experienced a much greater relative reduction in body condition than males. The energetically expensive process of reproduction is often borne to a higher degree for females than males because eggs require more energy to produce than sperm (Trivers, 1972, Roff and Fairbairn, 2007, Hayward and Gillooly, 2011). At times in order to meet the energetic costs associated with reproduction, females must sacrifice other energetically expensive processes to maintain personal body condition (Abrahams and Dill, 1989). The greater relative decrease in the body condition of females may be reflective of serial depletion of energetic resources over the course of the reproductive season. Triplefin with better prior body condition also experienced deterioration of body condition during the experiment, while fish with worse prior body condition improved in condition. Fish reproductive energy and fecundity is directly influenced by weight and body length (Barneche et al., 2018, Mu et al., 2021), and individuals that accumulate greater energetic reserves prior to reproduction tend to experience greater reproductive success (Clark et al., 1994, Johnston et al., 2007). Triplefin with better body condition prior to experiment commencement may have been better equipped to divert resources towards reproduction, resulting in an erosion in body condition across the experiment. Conversely, fish with worse body condition may have elected to invest resources into improving body condition over reproducing.

Does growth vary in different conditions of nocturnal light?

Interactions of nocturnal illumination, sex, and body size yielded distinct differences in growth. This was most pronounced for fish in the 24-hr light treatment. Small females in the 24-hr treatment showed significantly increased growth in comparison to large females in the same treatment. Greater nocturnal brightness can increase rates of feeding and reproduction (Donelan and Trussell, 2020). Day and Taylor (1997)'s model for growth asserts that small fish preferentially allocate energy towards growth, and large fish tend to invest energy in

reproduction. The modified female growth rates across body size in this treatment could therefore be a function of shifting investment choice from growth to reproduction (Mitterwallner and Shima, 2022) as body size increased. Males in this treatment showed the opposite relationship. In some species, large body size provides an advantage in territoriality and food acquisition against conspecifics with smaller body sizes (Rhodes and Quinn, 1998, Szabo, 2002). Furthermore, common triplefin display resource-defensive polygyny (Jones, 2013), and large males tend to occupy larger, superior nesting territories, netting greater reproductive success (Tornquist, 2020). The contrasting sex effects of body size on growth for males in the 24-hr light treatment may be attributed to increased competitive ability at larger body sizes since nest size was uniform. In direct contrast to the 24-hr light treatment, fish in the dark at night treatment did not show any changes in growth across body sizes or between sexes, implying that no energetic trade-offs were occurring between reproduction and growth.

Growth rates in the regular lunar treatment did not differ between sexes but increased with body size. Following the increased growth for males in the 24-hr light treatment, this suggests that larger fish may have had an advantage in feeding over smaller-bodied conspecifics. It is possible that fish in this treatment also preferentially allocated energy towards growth over reproduction. This pattern was mirrored for females in the dimmed lunar treatment, however, males with large body sizes in this treatment exhibited less growth than females of the same size. Growth also did not follow lunar patterns for fish in the lunar treatments. Apparent lunar behaviours are the result of responses to lunar behaviours of another species in the environment (Schmoker et al., 2012, Ikegami et al., 2014b). It is possible that triplefin may have apparent lunar growth in field settings, but that the removal of triplefin from the ecosystem erased any possible lunar patterns. Supporting this, the feeding schedule of triplefin in the experiment did not conform to lunar patterns. Transferring an organism to a laboratory setting in absence of the natural lunar cycles of behaviour of predators and prey in the ecosystem may therefore remove the influence of lunar cycles on species growth.

For each model, the fixed effects explained only a small portion of variation in growth (approx. 1%< to 9%), and a much larger portion of variance was explained by the random effect of tank (approx. 13% to 30%). This indicates that while light treatment may have influenced adult growth rates, it is much more likely that factors unmeasured during the experiment influenced adult growth rates to a much greater degree than fixed effects captured in the models. For example, I did not assess possible competitive interactions during the experiment or evaluate the impact of fish density in each tank on growth. A factor that should also be considered when

interpreting these results is that I only conducted growth analyses of the last month of growth rings to account for a lag in entrainment after exposure to experimental conditions. However, including the first two months of growth and comparing growth over time would have given a clearer understanding of what long-term patterns in growth and entrainment may have occurred if the extent of my data collection had not been limited by time constraints. Likewise, I concluded that growth patterns did not follow lunar patterns, but only a single reproductive event occurred in either treatment during the final month of the experiment. It was therefore not possible for me to conclude whether growth rates varied in response to reproductive events over the lunar month. These results highlight the complexity of factors determining growth and emphasises the need for further research into this subject.

How does nocturnal illumination affect reproductive behaviour?

In the regular lunar treatment, the number of eggs laid across the lunar month exhibited semi-lunar peaks around the first and third quarter moons. Melatonin is thought to be the hormone responsible for the chemical control of lunar-entrained reproduction (Takemura et al., 2004), that also controls the sleep/wake cycle. The perception of bright lunar light supresses melatonin accumulation in the brain, resulting in reduced concentrations of melatonin in bright nocturnal conditions (Andreatta et al., 2020). This can cause increased wakefulness and vigour of some behaviours, such as feeding or swimming (Ikegami et al., 2014b, Kupprat et al., 2020). Fish in the dimmed lunar treatment followed asymmetric semi-lunar patterns in the number of eggs laid, showing peaks just after the full moon and just before the new moon. It is likely that fish with lunar synchronization perceive changes in moonlight and use this information to adjust the timing and output of reproductive behaviour (Fukunaga et al., 2020). The dimmed lunar treatment reached a maximum brightness at the full moon comparable to the first few days of the simulated new moon in the regular lunar treatment. The degree of change in luminescence for this treatment was thus more subtle than in the regular lunar treatment. This may have caused smaller-scale changes in melatonin accumulation over the lunar cycle, confusing responses in reproductive output. Furthermore, all variation introduced by tank as a random effect was attributed to two large outliers in this treatment. The data without these points appears to conform to a semi-lunar pattern exhibiting peaks at the first and third quarter moons. However, I am confident that these outliers were not due to errors in data collection, so I have

interpreted the results with these points included. Due to the influential nature of these points, my results for lunar effects in the dimmed lunar treatment should be interpreted with caution. Additionally, even within species that show strong lunar rhythmicity in reproductive timing, individual variation is still seen in the duration of reproduction, the abundance of offspring, and the frequency of reproductive events (Luz et al., 2020, Neely and Butler, 2020), so the relative strength of lunar patterns within the experiment may have been influenced by factors not accounted for in my model. This is reflected by the weak-moderate amount of variance in clutch size that was explained by lunar effects (~27%). Fish exposed to unnatural conditions of nocturnal light (i.e., 24-hr light or complete darkness) were significantly more likely to reproduce than fish in the lunar treatments. In species that utilise environmental cues to synchronise reproduction, shifts in the timing or strength of cues determine reproductive timing and output (Shaffer et al., 2020). If the reproductive output of common triplefin peaks in conditions of intermediate brightness, being kept in perpetual conditions that cause high or low melatonin accumulation could inundate the triplefin brain with confused environmental signals, inducing continual readiness to breed. This would increase propensity to reproduce in contrast to populations experiencing natural cycles of luminescence. Furthermore, fish in the 24-hr light treatment laid more eggs on average than fish in the dark at night treatment. As previously discussed, increased conditions of brightness (and lowered melatonin accumulation) can increase feeding, growth, and reproduction (Hernández-León, 2008, Kupprat et al., 2020). That average clutch size only differed between the 24-hr treatment and the dark at night treatment (but not between the two lunar treatments and the 24-hr light treatment or the dark at night treatment) suggests that conditions of increased nocturnal brightness may increase the reproductive output of common triplefin.

Reproductive activity was not significantly influenced by water temperature during the experiment. Triplefin are seasonal breeders, reproducing preferentially during the summer months in Wellington (Francis, 2001, Mcdermott and Shima, 2006, Moginie and Shima, 2018), so it was surprising that temperature did not impact the likelihood of breeding occurring, or the number of eggs laid. It is possible that seasonal windows of reproduction in wild triplefin are programmed by photoperiod rather than temperature, as triplefin in the laboratory experiment (save for the 24-hr treatment) were exposed to an unchanging cycle of 12-hr light between 7:30am and 7:30pm. The number of females in each tank also did not significantly influence the likelihood of reproduction, or the average number of eggs laid in each clutch. The reproductive success for both sexes of triplefin is significantly influenced by population density

(Barnett and Pankhurst, 1996), but triplefin also exhibit female mate selection (Feary and Clements, 2006, Mensink et al., 2014). An increased ratio of male:female triplefin may have mitigated possible impacts on reproductive behaviour by increasing the number of suitable mating candidates for females to choose from.

Conclusion

My results demonstrate the importance of assessing influences of nocturnal illumination when investigating determinants of adult fitness-related traits for species with complex life histories. Altered patterns and intensity of nocturnal light can mediate energetic investment choices. Furthermore, traits associated with fitness can have significant consequences on reproductive success and survival of adults in a breeding population. While my results indicated that nocturnal illumination impacts fitness-related traits of *F. lapillum* such as growth and reproduction, I cannot identify the mechanisms underlying these effects with certainty. Additionally, the extent to which these patterns present themselves in wild populations cannot be extrapolated from my laboratory study. These added considerations merit further study. Characteristics of the physical environment are highly dynamic and can significantly modify adult fitness. Further empirical and theoretical research is required to develop a holistic understanding of the influences and effects of nocturnal illumination on adult fitness.

CHAPTER 3

The influence of nocturnal illumination on fitness-related traits for offspring of *Forsterygion lapillum*.

3.1 Introduction

During early stages of life history and development, juveniles have a significantly narrower margin of tolerance for changes in environmental conditions than adults (Septriani et al., 2021). Embryonic development is a particularly sensitive and complex process that is influenced by factors such as temperature (Guma'A, 1978, Kilambi and Galloway, 1985, Hamel et al., 1997, Kaminski et al., 2006, Korwin-Kossakowski, 2012, George and Chapman, 2013), predator presence (Godoy et al., 2021), photoperiod (Martín-Robles et al., 2012), exposure to metabolic-enhancing acids (Francis et al., 2012), and pH (El-Fiky, 2002). Embryonic development tends to follow the same pattern for most fish, but unique differences in the timing of organ differentiation and morphology are apparent at distinct developmental stages for different species (Jafari et al., 2010). Variation in the shape, structure, and length of embryonic development can also influence the changes of embryo survival to hatching (Pearson and Warner, 2018), which consequently dictates the starting resources and conditions an individual will encounter with it enters the larval phase. Additionally, experiences in early development phases can have long-lasting "carryover" effects that shape fitness in later stages of life (Giménez and Anger, 2001, Fopp-Bayat et al., 2021). Furthermore, physiological and developmental changes that occur during embryonic to larval ontogeny (Penaz, 2001) cause variation in phases of growth and differentiation (Osse and Van den Boogaart, 1995, Wieser, 1995, Osse et al., 1997). In early phases of life history, exposure to varied intensities and wavelengths of light can cause increased energy consumption and accelerate growth (Septriani et al., 2021), modifying development length and duration of hatching (Brüning et al., 2011). Light is interpreted in the pineal gland of the fish embryo (Forsell et al., 1997). Pineal photoreceptors can differentiate before retinal cells during embryonic development (Ekstrom

and Meissl, 1997) and are specifically adapted to detect variation in luminescence (Meissl and Ekström, 1988, Kusmic et al., 1993). The pineal organ plays a key role in the entrainment of endogenous rhythms and influences metabolism, growth, and endocrine regulation during early ontogeny (Ekstrom and Meissl, 1997). Furthermore, early differentiation of pineal neurons is foundational to establishing neural patterns in the brain (Wilson and Easter, 1991) that interpret environmental stimuli and synchronise hatching activity in favourable environmental conditions (Yamagami et al., 1988, Forsell et al., 1997). Eye development and maintenance is a particularly metabolically costly process during early development, constituting an estimated 15% of resting metabolism for fish weighing less than 1 gram (Moran et al., 2015). As the pineal gland – a precursor to ocular development involved in light perception – is impacted by light exposure during embryonic development, it is conceivable that eye development is also influenced by light exposure. The rate of eye development could also theoretically alter other facets of development and hatching success. Hatching occurs once the embryo has reached a size where the energy required to maintain respiration exceeds the capacity for oxygen to diffuse through the egg envelope (Fuiman, 2002). Light can impact hatching activity uniquely between species (Korwin-Kossakowski, 2012). In some cases, exposure to light accelerates and synchronises hatching activity (Downing and Litvak, 2002), but delays development and hatching in others (Helvik and Walther, 1992, Helvik and Walther, 1993b, Helvik and Walther, 1993a), and can reduce hatching success (Arambam et al., 2020). Conversely, the embryogenesis of some species is not affected by variations in light at all (Iglesias et al., 1995). Despite the apparent and varied impacts of light on the length and success of embryonic development, the exact mechanisms underpinning these differences are still poorly understood, particularly in the context of lunar illumination. There is a developing body of literature addressing the important role that moonlight plays in regulating patterns of larval growth and development for fish (Hernández-León, 2008, Shima et al., 2018, Shima and Swearer, 2019, Shima et al., 2021), but these same considerations are conspicuously absent for the embryonic stage. The length, structure, and success of this phase influences the fitness of an individual during the larval phase, and consequently its ability to survive to adulthood and reproduce (Jonsson and Jonsson, 2014). Individual fitness of embryos consequently affects larval fitness and therefore larval recruitment and the size, genetic variation, and composition of the community (Cargnelli and Gross, 1996). Building a detailed knowledge of the impacts of nocturnal illumination on fitness-related traits for offspring is essential to understanding how development and survival are shaped during early life phases, and what implications these influences have for fitness in later stages of life.

In this study, I evaluated the influence of nocturnal illumination on fitness-related traits for embryos in the New Zealand common triplefin (*Forsterygion lapillum*). I conducted a laboratory experiment that manipulated nocturnal illumination for 3 months and examined the effects of light treatment on the length and structure of embryonic development of *F. lapillum* embryos. Furthermore, I tracked the fate of clutches laid during the experiment to assess the influence of nocturnal illumination on the survival of embryos to hatching, i.e., the "hatching success" of each clutch. I investigated two questions through this study: 1) How does nocturnal illumination influence the duration and structure of embryonic development? 2) Does exposure to nocturnal light impact hatching success of embryos?

3.2 Methods

3.2.1 Study species and system

Forsterygion lapillum (the New Zealand common triplefin) is a small-bodied temperate fish that is found commonly in rocky reef environments throughout New Zealand (Wellenreuther and Clements, 2007, Wellenreuther et al., 2008). Common triplefin are most reproductively active between August and December in the Wellington region but may spawn year-round, excluding during the coldest winter months (Francis, 2001, Mensink et al., 2014, Moginie and Shima, 2018).

Triplefin lay small, hemi-spherical eggs that are anchored to the underside of a nesting site with sticky threads by the female during spawning (Thresher, 1984). Male triplefin provide sole parental care and brood eggs inside the nest until hatching after approximately 20 days (Paulin and Roberts, 1992). Little is known about the development of common triplefin embryos in the egg, or about which factors determine the length, structure, and success of embryonic development. After hatching, larvae are planktonic (Watson, 2009), inhabiting shallow coastal waters until juvenile settlement in algal environments. Please see section 2.2.1 for more information on adult reproductive behaviours.

3.2.2 Laboratory experiment

I conducted a 3-month experiment manipulating nocturnal illumination to assess its influences on embryonic development and hatching success. Please see section 2.2.2 for detailed descriptions of tank set-up, light specifications, adult collection, and maintenance.

In brief, I collected adult *F. lapillum* from Tarakena Bay (41°20'41.24"S, 174°49'14.35"E) on the south coast of Wellington between December 2021 and April 2022 with the aid of snorkel and hand nets. I then transported the fish live to the Wellington University Coastal Ecology Lab (WUCEL) and randomly assigned them to one of 20 tanks. Tanks were assigned to one of four nocturnal light treatments for the duration of the experiment (May 6th – August 3rd, 2022). I assigned 5 tanks each to the following four light treatments: 1) a simulated lunar cycle approximating moonlight in clear and cloudless conditions (regular lunar treatment), 2) a simulated lunar cycle with illumination intensity intended to approximate nocturnal conditions of heavy cloud cover (dimmed lunar treatment), 3) no nocturnal light (dark at night treatment), and 4) constant 24-hour artificial light (24-hr light treatment).

Fish were able to participate in regular feeding and breeding behaviours over the duration of the experiment. I collected estimates of embryonic development and survival ("hatching success") from eggs laid during the experiment to evaluate the influence of nocturnal illumination on fitness-related traits for offspring of common triplefin.

3.2.3 Data collection and analysis

Quantifying duration and structure of development

To quantify length and structure of embryonic development, I took photographs of egg clutches alongside a ruler for scale three times per week throughout the course of the experiment, counted them, and tracked their fates (details of this are included in section 2.2.3). Triplefin are daily spawners and sometimes spawn several times within the same day (Warren, 1990), so it was not possible to accurately stage embryos within the same "clutch" (i.e., the cohort of eggs that had been laid between photograph sampling). I did not take photographs of clutches at a higher frequency so as not to disturb the reproductive activity of adults in the experiment.

This meant that the date that eggs were laid and hatched fell within a window of 2-3 days. I therefore estimated the date that each clutch was laid as the first day that the clutch was recorded photographically. Using this estimated date, I recorded the water temperature at laying for each clutch. I measured temperature using a thermometer to the nearest 0.1 degree Celsius. I estimated the egg diameter for each clutch using ImageJ (ver. 1.53u). I calibrated the scale for each image using the ruler photographed alongside the clutch. I then haphazardly selected 10 eggs and took two diameter measurements for each. I averaged these to acquire a single diameter measurement and calculated the egg diameter for the clutch (mm) by averaging the 10 diameter measurements for each clutch. Some clutches contained fewer than 10 eggs (n = 2). For these, I measured all present eggs and estimated egg diameter for the clutch from these measurements.

I estimated the date of eye development as the first day that eye spots became visible for any part of the clutch. Hatching activity often occurred over a period of one to several days. I estimated hatch date as the first date that hatching activity was observed (after eye development had occurred). This was usually signified by the presence of clear "empty" egg casings in place of eggs containing fully developed larvae. I estimated the length of eye development (t_{Eye}) by subtracting the estimated date of eye development from the estimated date of clutch laying. I estimated the total duration of embryonic development (t_{Hatch}) by subtracting the estimated date of hatching from the estimated date of laying. To quantify the structure of development in differing conditions, I calculated the proportion of development required for eye synthesis (P_{Eye}) as:

$$P_{Eye} = \frac{t_{Eye}}{t_{Hatch}}$$

A proportion closer to 0 indicates a very quick eye synthesis with a longer interval of development before hatching. A proportion of 1 would indicate that hatching started as soon as eye development had occurred.

Lunar effects.

In the two lunar treatments, I also investigated whether embryonic development duration varied at different times of the lunar month. Following the methods of deBruyn and Meeuwigg (2001), I assigned the corresponding day of the lunar month to the estimates for the date of laying, the date of eye development, and the date of hatching (0 = new moon, 15 = full moon, 29 = end of the last quarter). Each day was allocated an angular equivalent (θ) by dividing the lunar day by 360^{o} (2π radians). I then transformed θ by sine and cosine to express the circular quality of the lunar cycle. The cosine term relates to phase shifts corresponding to 0^{o} (new moon) or 180^{o} (full moon), and the sine term relates to phase shifts occurring near 90^{o} (first quarter) or 270^{o} (last quarter). A significant positive cosθ would correspond to a peak in the response variable around the new moon, while a negative value indicates a peak at the full moon. Conversely, a positive sinθ describes a peak in the response variable at the first quarter, while a negative value corresponds to a peak during the third quarter. I also conducted cos2θ and sin2θ transformations of the lunar date data for estimated dates of laying, eye development, and hatching to assess the possibility of dual peaks in development duration across the lunar month.

How does nocturnal illumination influence embryonic development?

Eye development.

I investigated the influence of nocturnal illumination on the duration of eye development of embryos by building a linear mixed effects model, fitting duration of eye development (t_{Eye}) as the response variable and light treatment as a fixed effect. The analysis of this model tested the null hypothesis that the length of eye development did not vary between nocturnal light treatments. I assigned tank as a random effect to account for non-independence introduced for the breeding population within a given tank. Water temperature alters embryonic development (Guma'A, 1978, Kilambi and Galloway, 1985, Kaminski et al., 2006, George and Chapman, 2013), so I included the water temperature for the estimated date of laying as a fixed effect. I compared model fit including the interaction term of water temperature and light treatment and used AICc to select the best fitting model for the data (*AICcmodavg* package, Mazerolle, 2020). The best fitting model excluded the interaction term. I then conducted analysis of the model using a Wald chi-square analysis of deviance test (R Core Team, 2022) (type II) to test the

significance of main effects and analysed the data using a linear mixed effects regression (*glmmTMB* package, Brooks et al., 2017).

I calculated the marginal R^2 (R^2m) and conditional R^2 (R^2c) to ascertain the degree of variance in eye development explained by the fixed effects and the overall model (including random effects) (*MuMIn* package, Bartoń, 2022). I conducted post-hoc testing between groups for statistically significant discrete fixed effects (*emmeans* package, Lenth, 2022).

Structure of embryonic development.

I examined the impact of nocturnal illumination on the structure of embryonic development by building a linear mixed effects model, using the proportion of embryonic development taken to synthesise eyes (P_{Eye}) as the response variable and light treatment as a fixed effect. This model tested the null hypothesis that the structure of embryonic development did not vary between light treatments. I selected tank as a random effect to consider random variation in development structure within a given tank. I included water temperature as a fixed effect to account for seasonal influences in development structure.

I assessed the fit of all possible combinations of the main effects and their interaction terms and used AICcs to select the best fitting model (*AICcmodavg* package, Mazerolle, 2020). The best-fitting model excluded interaction effects. I analysed this model using a Wald chi-square analysis of deviance test (R Core Team, 2022) (type II) to test for significant relationships to the main effects. I also conducted a linear mixed effects regression (*glmmTMB* package, Brooks et al., 2017) to gather model parameter estimates. I assessed the amount of variance in development structure that was explained by the fixed effects and the total model (including fixed effects) by calculating the R^2 (R^2m) and conditional R^2 (R^2c) (*MuMIn* package, Bartoń, 2022). For predictors of statistical significance, I conducted post-hoc pairwise comparison testing (*emmeans* package, Lenth, 2022).

Total development duration.

Lastly, to assess the impact of nocturnal illumination on overall development length I built a linear mixed effects model, fitting the total duration of embryonic development (t_{Hatch}) as the response variable and light treatment as a fixed effect. This model tested the null hypothesis

that the length of embryonic development did not vary between light treatments. I included tank as a random effect in the model to account for random variation in development length within each tank. Following prior models detailed in this section, I selected water temperature at the estimated date of laying as a fixed effect to consider possible seasonal variation in development length. I also fit the proportion of development taken to synthesise eyes (P_{Eye}) as a fixed effect to ascertain whether the structure of development influenced its duration. I compared AICc values for all possible model combinations of main effects and their interaction terms and used the lowest value to select the best-fitting model (*AICcmodavg* package, Mazerolle, 2020). The best-fitting model excluded all interaction terms. I then analysed the model using a Wald chi-square analysis of deviance test (R Core Team, 2022) (type II) to test the significance of the main effects. I conducted a linear mixed effects regression (*glmmTMB* package, Brooks et al., 2017) to acquire parameter estimates for the model. I evaluated the degree of variance in development length explained by the main effects and the total model (including random effects) by calculating the R^2 (R^2m) and conditional R^2 (R^2c) (*MuMIn* package, Bartoń, 2022). I conducted post-hoc pairwise comparison testing (*emmeans* package, Lenth, 2022) for statistically significant discrete predictors to investigate the strength and direction of relationships.

Lunar effects on embryonic development

Eye development.

To investigate the role of lunar illumination in shaping the development of eyes for embryonic triplefin, I constructed two linear mixed effects models. The first fit the duration of eye development as the response variable and the lunar predictors (sinθ, cosθ) for the estimated lunar day of laying as fixed effects. This model tested the null hypothesis that duration of eye development did not vary with the lunar time of the month at clutch laying. I fit tank as a random effect to consider random variation within a given tank. I considered dual-peak lunar predictors (sin2θ, and cos2θ) and lunar treatment (regular versus dimmed) as possible fixed effects and used AICc scores to test all combinations of main effects and their interaction terms (*AICcmodavg* package, Mazerolle, 2020). The best fitting model only used the main effects of sinθ and cosθ and excluded interaction terms.

The second model fit the duration of eye development as the fixed effect and the lunar predictors (sinθ, cosθ) for the estimated date of eye development as fixed effects. This model

tested the null hypothesis that the length of eye development did not vary with the time of the lunar month when eye development was estimated to occur. To consider random variation in eye development within a tank, I included tank as a random effect. I considered lunar predictors indicating dual peaks in eye development length (sin2θ, and cos2θ) and lunar treatment (regular versus dimmed) as possible fixed effects, then used AICc scores to test all possible combinations of main effects and their interaction terms (*AICcmodavg* package, Mazerolle, 2020). The best fitting model used sinθ, cosθ, sin2θ, and cos2θ, and excluded lunar treatment and interaction effects.

I analysed both models using Wald chi-square analysis of deviance tests (R Core Team, 2022) (type II) to assess the significance of the main effects and conducted periodic (linear-circular) mixed effects regressions (*lme4* package, Bates et al., 2015) to attain parameter estimates for each model. I also evaluated the degree of variance in eye development length explained in each model by calculating the R^2 (R^2m) and conditional R^2 (R^2c) (*MuMIn* package, Bartoń, 2022), which describe the variation explained by the main effect and total model (including random effects).

Development duration.

To assess the role of lunar illumination in determining the length of embryonic development, I constructed two linear mixed effects models. The first fit the length of embryonic development as the response variable and the lunar terms (sinθ, cosθ) for the estimated lunar day of laying as fixed effects. This model tested the null hypothesis that the length of embryonic development did not vary depending on the time of the lunar month that eggs were laid. To account for random variation in development length between tanks, I included tank as a random effect. I considered the inclusion of dual-peak lunar predictors (sin2θ, and cos2θ) and lunar treatment (regular versus dimmed) as possible fixed effects and evaluated the fit all possible model combinations of main effects and their interaction terms using AICc scores (*AICcmodavg* package, Mazerolle, 2020). The best fitting model only included sinθ and cosθ as main effects.

The second model fit the length of embryonic development as the response variable and the lunar terms (sinθ, cosθ) for the estimated lunar day of hatching as fixed effects. This model tested the null hypothesis that the length of eye development did not vary with the time of the lunar month when hatching was estimated to occur. I accounted for random variation in development length between tanks by including tank as a random effect. I also considered lunar

predictors indicating two possible peaks in development length during the month (sin2θ, and cos2θ) and lunar treatment (regular versus dimmed) as possible fixed effects and conducted model comparison of all possible main effects and their interactions using AICc scores to select the best-fitting model (*AICcmodavg* package, Mazerolle, 2020). The best fitting model used sinθ, cosθ, sin2θ, and cos2θ terms, and excluded lunar treatment and interaction effects.

I then analysed both models using Wald chi-square analysis of deviance tests (R Core Team, 2022) (type II) to test the significance of the main effects and obtained parameter model estimates using periodic (linear-circular) mixed effects regressions (*lme4* package, Bates et al., 2015). I assessed the variance explained in each model by the fixed effects and total model (including random effects) by calculating the R^2 (R^2m) and conditional R^2 (R^2c) (*MuMIn* package, Bartoń, 2022).

Assessing hatching success

I tracked the fate of eggs in each clutch across the course of the experiment by counting the number of eggs lost between each progressive photograph of the clutch until hatching occurred. I recorded the number of eggs that remained immediately prior to hatching and used this as the estimate for the number of embryos that survived to hatching. I then calculated the hatching success of each clutch as the proportion of embryos that survived from laying to hatching. I did not calculate hatching success for clutches where most embryos had not yet hatched by experiment conclusion.

Does nocturnal illumination impact hatching success?

Light treatment.

I investigated the relationship between nocturnal illumination and hatching success by fitting a linear mixed effects model using the proportion of successfully hatched larvae for each clutch as the response variable and light treatment as a fixed effect. This model tested the null hypothesis that hatching success of a clutch did not vary by light treatment. I included tank as a random effect to account for random variations in hatching success within a given tank. I considered several additional variables as mixed effects. Water temperature, egg diameter, and

clutch size are determinants of embryonic survival (Brockelman, 1975, Parker and Begon, 1986, Das et al., 2006, Johnston et al., 2007). I therefore considered these three additional variables as possible fixed effects. I conducted pairwise comparison of models containing different combinations of these five variables as fixed effects and used AICc scores to select the best-fitting combination of main effects (*AICcmodavg* package, Mazerolle, 2020). The most appropriate combination of main effects included light treatment, clutch size, and water temperature. I then used the same method to consider all combinations of these three main effects and their interactions. The best-fitting model included the interaction term of clutch size and water temperature. Hatching success data was extremely skewed towards high percentages of hatching success (Fig. 3.1), resulting in non-normal distribution of residuals (evaluated by inspecting the Q-Q residuals plot). Applying log, square root, and cube root transformations of the data did not improve distribution of the residuals. Analysing the data using a Poisson distribution also did not improve fit. The data also violated assumptions of homoscedasticity (as shown in the *DHARMa* package output for the model; Hartig, 2022), so I performed a box-cox transformation of the data (*MASS* package, Venables and Ripley, 2002). The dataset had a lambda (λ) of 5. The box-cox transformation formula is as follows:

$$\text{If } \lambda \neq 0, \qquad \frac{x^{\lambda} - 1}{\lambda}$$

$$\text{If } \lambda = 0, \qquad \log(x)$$

The transformed data conformed to assumptions of normality and homoscedasticity (*DHARMa* package, Hartig, 2022), so I present results for this analysis using the $\lambda = 5$ transformation. I conducted a Wald chi-square Analysis of Deviance (type III error) (R Core Team, 2022) test to assess the significance of the main effects. I also performed a linear mixed effects regression to obtain model parameters (*glmmTMB* package, Brooks et al., 2017). For statistically significant main effects, I conducted post-hoc pairwise comparison (*emmeans package,* Lenth, 2022) to describe the strength and direction of these relationships. I then calculated the marginal R^2 (R^2m) and conditional R^2 (R^2c) to ascertain the degree of variance in hatching success explained by the fixed effects and the overall model (including random effects) (*MuMIn* package, Bartoń, 2022).

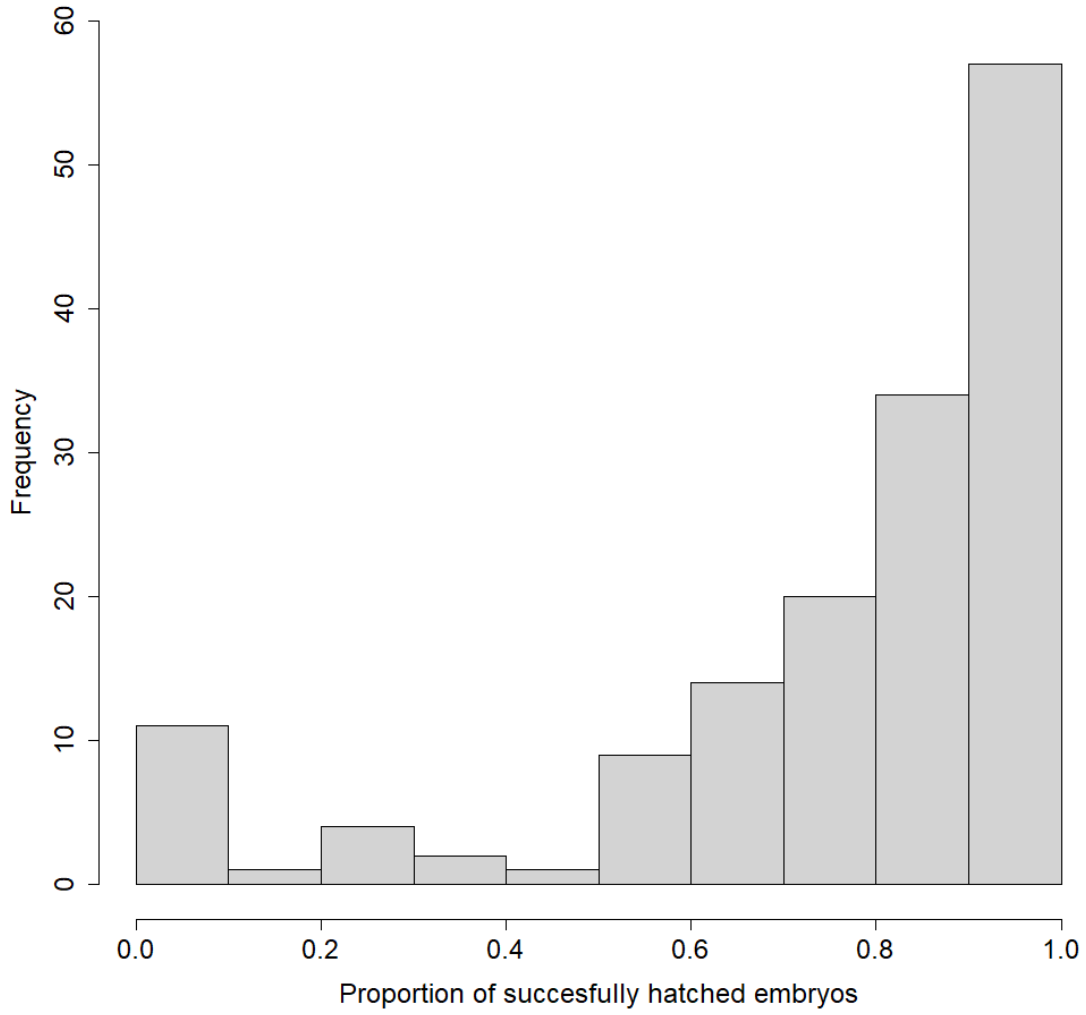

Figure 3.1: Histogram showing the distribution of hatching success data for triplefin embryos. The data shows heavy skewing towards high proportions of hatching success.

Lunar effects.

To assess whether lunar patterns influenced the hatching success of triplefin embryos, I built two linear mixed effects models. I fit tank as a random effect to both models to account for random variation in hatching success in each tank.

The first model investigated whether hatching success was influenced by the time of the lunar month at laying, fitting hatching success as the response variable, and the lunar terms for laying date (sinθ and cosθ) as fixed effects. This model tested the null hypothesis that hatching success is not influenced by lunar patterns at laying. I also considered the possibility of dual peaks of hatching success occurring in the lunar month (sin2θ and cos2θ), and of hatching success varying by lunar treatment. I tested every possible combination of main effects and interaction terms and selected the best fitting model based on AICc score (*AICcmodavg* package, Mazerolle, 2020). The best fitting model used only sinθ and cosθ as fixed effects. The second model assessed whether hatching success was influenced by the time of the lunar month at hatching, fitting hatching success as the response variable, and the lunar terms - sinθ and cosθ – for hatch date as fixed effects. This model tested the null hypothesis that hatching success is not influenced by lunar patterns at hatching. I tested models including dual-peak lunar predictors (sin2θ and cos2θ) and lunar treatment as main effects, and all possible combinations of interaction terms. I selected the most appropriate model based on AICc score (*AICcmodavg* package, Mazerolle, 2020). The best fitting model used only sinθ and cosθ as fixed effects. Like the previous section, data did not conform to assumptions of normality or homoscedasticity, so I performed a box-cox transformation (*MASS* package, Venables and Ripley, 2002). λ=3.7 for this subset of hatching success data. I transformed the data following the formula described in the previous selection. Transformed data conformed to assumptions of normality and homoscedasticity (residuals plot, *DHARMa* package, Hartig, 2022). I therefore present the results for these analyses using the transformed data.

I analysed both models using periodic (linear-circular) regression analyses (*lme4* package, Bates et al., 2015). I then calculated the delta marginal R^2 (R^2m) and conditional R^2 (R^2c) of each model to ascertain the degree of variance in hatching success explained by the fixed effects and the overall model (including random effects) (*MuMIn package,* Bartoń, 2022).

I performed all statistical analyses in this chapter using R Statistical Software 4.2.1 (R Core Team, 2022).

3.3 Results

3.3.1 Nocturnal illumination and embryonic development milestones

Eye development.

151 of the 181 clutches laid in the experiment developed eyes. 3 clutches did not develop eyes before the experiment concluded, the remaining 27 clutches died before eye development could occur. The length of eye development ranged from 7-14 days. On average, clutches took 10.25 days (±SE 0.1462) to develop eyes. Eye development did not differ significantly between light treatments (Table 3.1, Fig. 3.2A).

At warmer laying temperatures, the length of eye development decreased. (Fig. 3.2B). Parameter estimates from the regression showed that for every degree that water temperature increased, eye development tended to decrease by 0.8286 days (±SE 0.1194).

The fixed effects explained 33.36% of the total variation in eye development ($R^2m = 0.336344$). The random of tank by comparison was only responsible for 0.44175% of variation in eye development ($R^2c = 0.3380519$).

Table 3.1: Analysis of deviance table (Wald Chi-square, type II error) for the mixed effects model fitting the length of eye development (t_{Eye}) as the response variable and light treatment and water temperature as fixed effects. The table contains chi-square test statistics (X^2), degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Fixed effect	X^2	df	p value
Light treatment	6.8695	3	0.07618
Water temperature	**48.1480**	**1**	**3.952^{-12}**

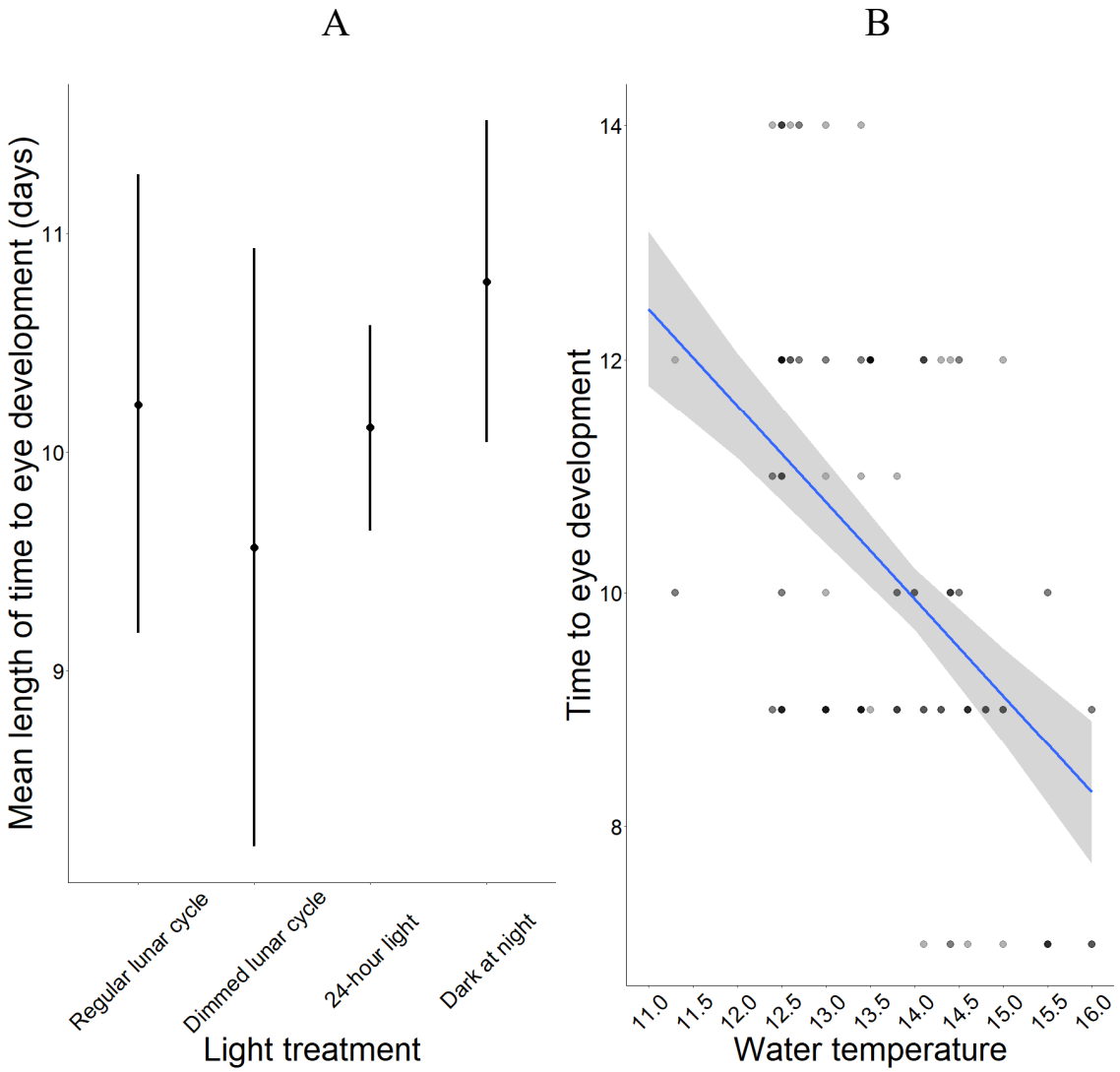

Figure 3.2: A) Mean length of eye development (with 95% confidence interval) between light treatments. **B)** Scatterplot of the length of eye development (t_{Eye}) versus water temperature at the estimated date of laying (degrees Celsius). The plot is fitted with a regression line (blue) and 95% confidence interval (grey shaded).

Development structure.

Of the 151 clutches that survived to eye development, 141 hatched. The remaining 10 clutches did not hatch before the experiment concluded. Eye synthesis lasted for at least 33.33% of development for clutches that hatched ($P_{Eye} = 0.33\dot{}$). The maximum proportion of time devoted to eye development was 1, wherein eye development and hatching occurred simultaneously (n = 9 clutches). On average, 61.26% of development time was allocated to eye synthesis (Mean ± SE: 0.6126 ± 0.0139).

The proportion of development taken to synthesise eyes did not vary between light treatments (Table 3.2, Fig. 3.3A). Warmer water temperatures at laying yielded faster eye synthesis relative to development length (Table 3.2, Fig. 3.3B). The mixed effects regression parameter estimates predicted that for every degree that water temperature increased, the proportion of development taken to synthesise eyes tended to decrease by 0.068 (±SE 0.012).

The fixed effects explained 23.53% of variation in development structure ($R^2m = 0.2353073$). Only 2.53% of additional variation was explained by the random effect of tank ($R^2c = 0.260612$).

Table 3.2: Analysis of deviance table (Wald Chi-square, type II error) for the linear mixed effects model of the proportion of development taken to synthesise eyes (P_{Eye}) versus the fixed effects of light treatment and water temperature. The table contains chi-square test statistics (X^2), degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Fixed effect	X^2	df	p value
Light treatment	2.2007	3	0.5318
Water temperature	**32.3635**	**1**	**1.279^{-08}**

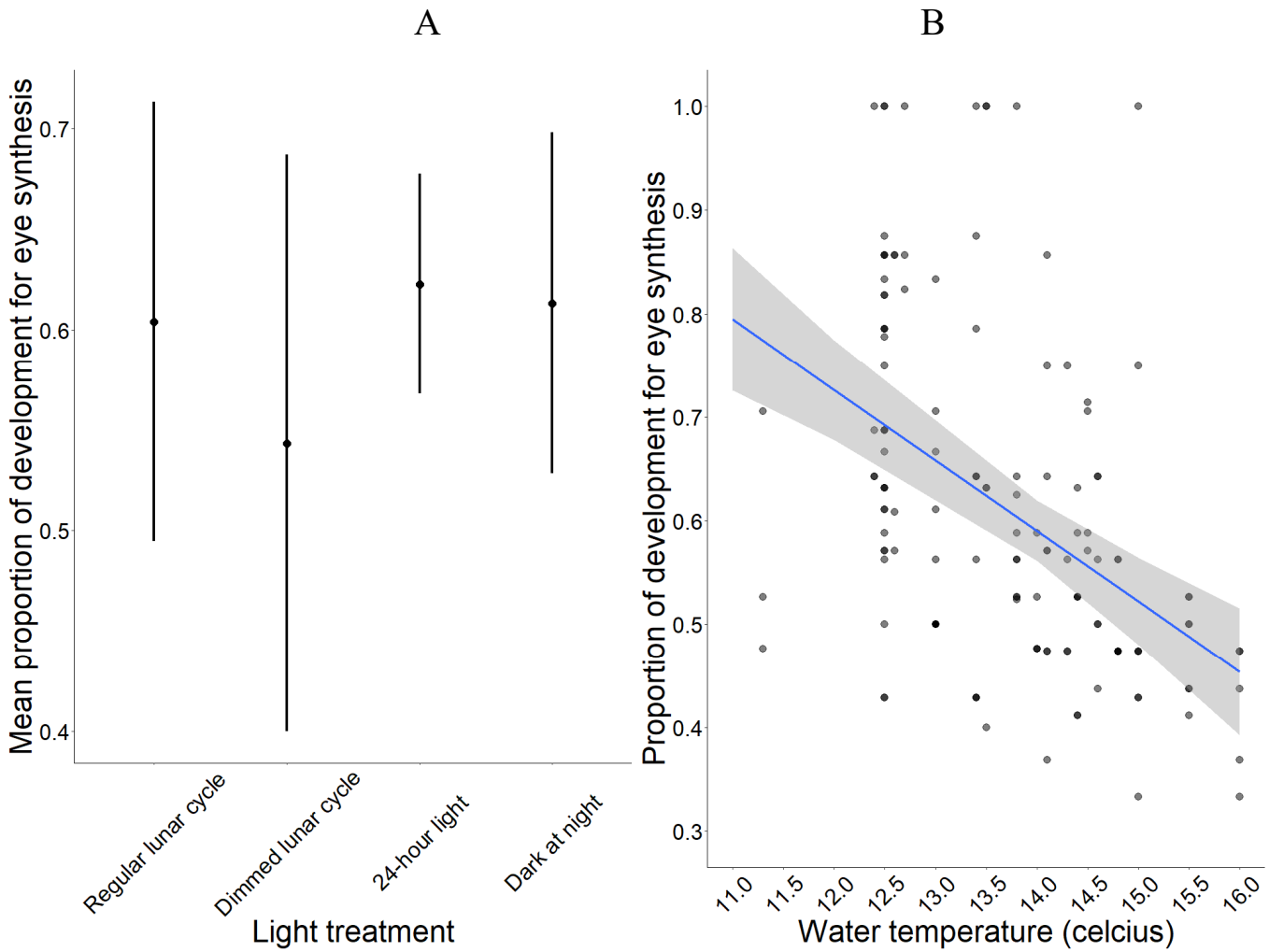

Figure 3.3: A) Mean proportion of development taken to synthesise eyes (P_{Eye}) (with 95% confidence interval) by light treatment. **B)** Scatterplot showing the proportion of development taken to synthesise eyes by water temperature at laying (degrees Celsius). The plot includes a regression line (blue) and 95% confidence interval (grey shaded).

Total duration of development.

Development length (t_{Hatch}) ranged from 9 to 30 days, with clutches taking an average of 17.16 days to hatch (±SE 2.66).

The relationship between development length and light treatment was not significant (Table 3.3, Fig. 3.4A). Warmer water temperatures at laying resulted in shorter duration of development (Fig. 3.4B). The mixed effects regression estimate predicted that for every degree increase in water temperature, development length tended to decrease by 0.45 days (±SE 0.1678).

Greater proportion of development taken to synthesise eyes resulted in shorter development length. (Fig. 3.4C). The mixed effects regression estimate indicated that an increase in the proportion of time taken to synthesise eyes by 0.1 results in a decrease in development length by 1.555 days (raw estimate = -15.5542 ± SE 1.0506).

The fixed effects of this model predicted 57.56% of variation in development length (R^2m = 0.575581), and the random effect of tank explained a further 14.24% of variation (R^2c = 0.7179618).

Table 3.3: Analysis of deviance table (Wald Chi-square, type II error) for the linear mixed effects model of development length (t_{Hatch}) versus the fixed effects of light treatment, proportion of development taken to synthesise eyes (P_{Eye}), and water temperature. The table contains chi-square test statistics (X^2), degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Fixed effect	X^2	df	p value
Light treatment	6.4132	3	0.092416
P_{Eye}	**219.1732**	**1**	**$<2.2^{-16}$**
Water temperature	**7.1966**	**1**	**0.007304**

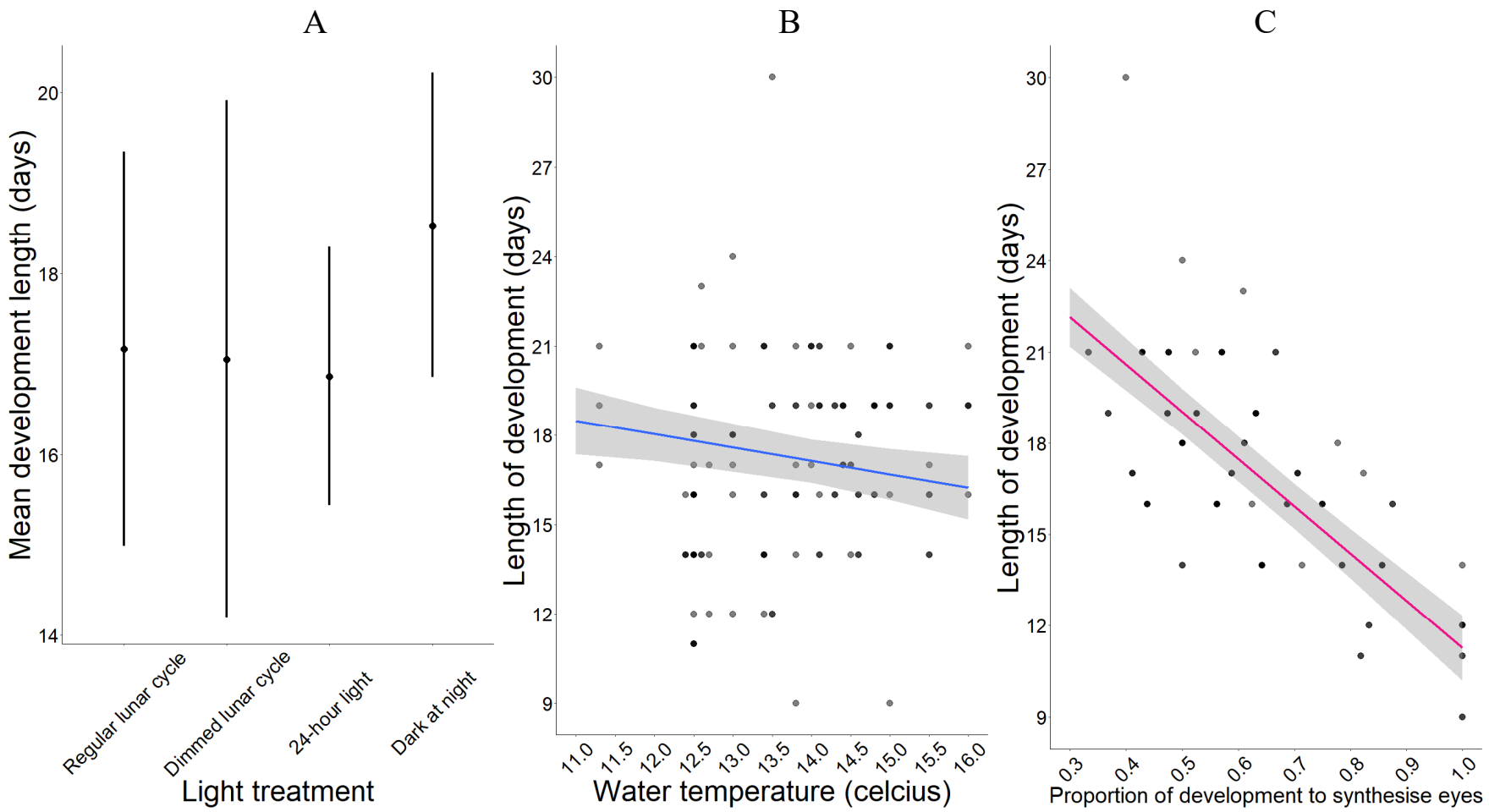

Figure 3.4: A) Mean development length (t_{Hatch}) (with 95% confidence interval) by light treatment. **B)** Scatterplot showing development length by water temperature (degrees Celsius). The plot includes a regression line (blue) and 95% confidence interval (grey shaded). **C)** Scatterplot showing development length by the proportion of development taken to synthesise eyes. The plot includes a regression line (red) and 95% confidence interval (grey shaded).

Lunar patterns of development

Eye development.

Of the 32 clutches laid in the lunar treatments during the experiment, 27 of them developed eyes and later hatched. Length of eye development (t_{Eye}) ranged from 7 to 12 days. On average, embryos developed eyes after 9.44 days (±SE 0.299). Eye development was observed throughout the lunar month, except for the days at the very start and end of the month (i.e., 0-1, 29). The lunar day of laying did not influence the length of eye development (Table 3.4, Fig. 3.5A). The fixed effects of the cosine and sine transformations of the lunar predictor θ explained 10.04% of variation in the response variable ($R^2m = 0.1003775$), while the random effect of tank explained 29.57% of variation ($R^2c = 0.3960498$). Conversely, the length of eye development exhibited dual peaks in eye development corresponding to the first and third quarters respectively (Table 3.4, Fig. 3.5 B). The longest eye development occurred during the first quarter of the month on day 6, with embryos having taken 10.714 days (±SE 0.542) to develop eyes. The length of eye development exhibited a slightly lower peak on day 23 during the third quarter of 10.486 days (±SE 0.600). Conversely, eye development for embryos that developed eyes during the full moon (day 16) showed much shorter duration at 8.197 days (±SE 0.536). The lunar prediction terms for the estimated date of eye development explained 25.13% of variation in the length of eye development ($R^2m = 0.2512858$), while the random effect of tank explained 29.25% of variation ($R^2c = 0.543763$).

Total length of development.

Embryonic development length (t_{Hatch}) in lunar treatments ranged from 11-21 days. Clutches on average took 17.81 days to hatch (±SE 0.4864). Development length was significantly predicted by cosθ transformation for the lunar day of the estimated date of laying (Table 3.5, Fig. 3.5C). The estimate for the regression output indicates that clutches laid during the new moon took longer to hatch (Mean ± SE = 19.865 days ± 1.020 at day 2) than those laid just after the full moon (Mean ± SE = 16.478 days ± 0.726 at day 19). The fixed effects for this model explained 15.79% of variation in development length ($R^2m = 0.157918$), and the random effect of tank did not explain any variation ($R^2c = 0.157918$). Contrastingly, the duration of embryonic development was not significantly related to the time of the lunar month at the estimated date of hatching (Table 3.5, Fig. 3.5D). The fixed effects of the model explained 16.18% of variation in development length ($R^2m = 0.1618101$), and the random effect of tank did not explain any variation ($R^2c = 0.1618101$).

Table 3.4: Parameter estimates of two periodic mixed effects regressions. The first model models tested for variation in the length of eye development versus lunar period of laying, using the length of time to eye development (t_{Eye}) as the response and the sine and cosine transformations of the lunar term, θ, for the time of the lunar month at laying as fixed effects. The second model models tested for variation in the length of eye development depending on the lunar period of eye development, using the length of time to eye development (t_{Eye}) as the response and the sine and cosine transformations of the lunar term, θ, for the time of the lunar month at eye development as fixed effects. These models use a student's t distribution for small sample sizes. The table contains parameter estimates, standard errors, degrees of freedom (df), t values, and p-values. Statistically significant parameters ($p<0.05$) are indicated in bold text.

Model	Parameter	Estimate	Standard error	df	t value	p value
Is eye development length related to the lunar phase of laying?	**Intercept**	**9.78095**	**0.48768**	**7.03**	**20.056**	**1.82^{07}**
	Lunar period of laying (cosθ)	-0.06192	0.45933	20.25	-0.135	0.894
	Lunar period of laying (sinθ)	0.072148	0.42262	22.35	1.707	0.102
Is eye development length related to the lunar phase of hatching?	**Intercept**	**8.0141**	**0.8701**	**21.00**	**9.211**	**8.01^{09}**
	Lunar phase of eye date (cosθ)	-1.9146	1.0065	21.04	-1.902	0.07091
	Lunar phase of eye date (sinθ)	-1.8130	0.9667	19.43	-1.872	0.07586
	Lunar phase of eye date (sin2θ)	**-2.4164**	**0.8491**	**20.52**	**-2.846**	**0.00982**
	Lunar phase of eye date (cos2θ)	-0.3375	0.3383	19.58	-0.972	0.34275

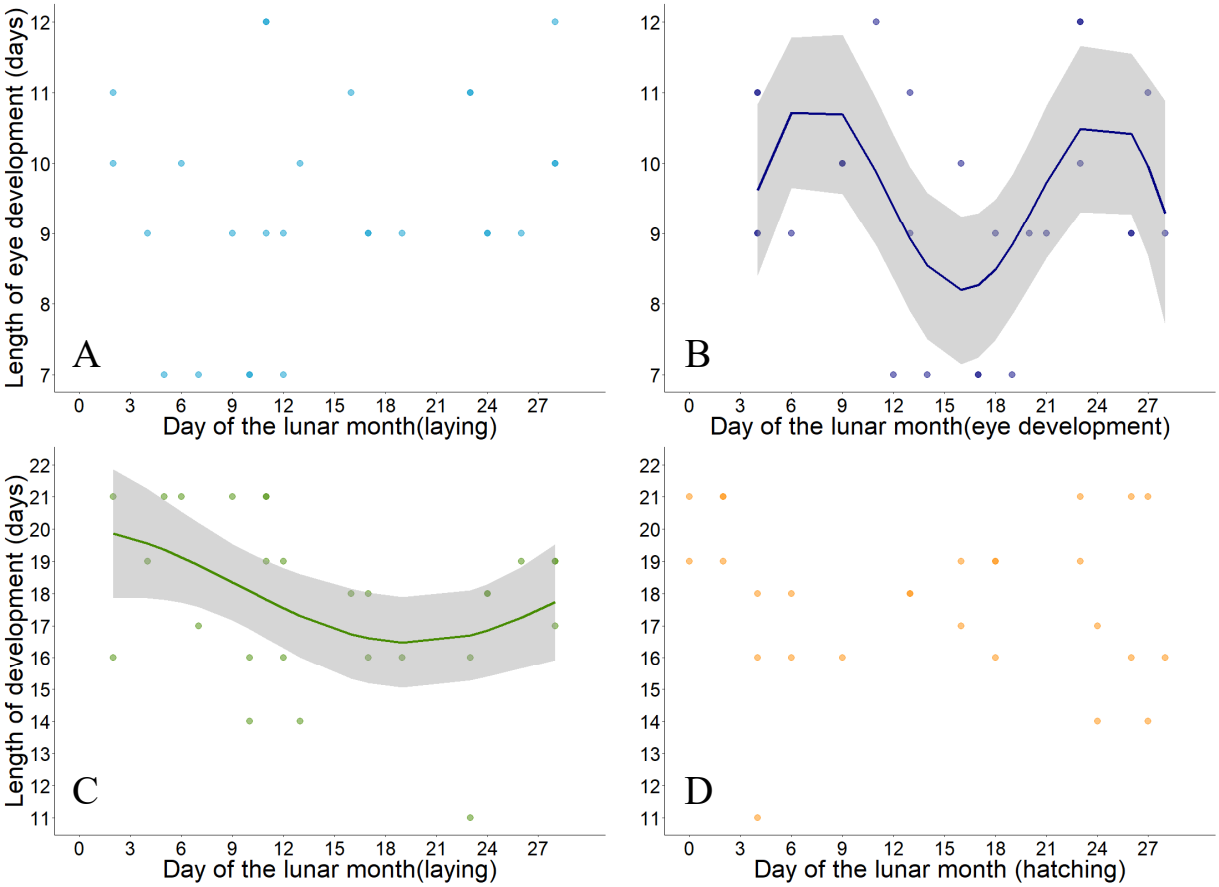

Figure 3.5: Scatterplots of **A)** length of eye development (light blue) and **B)** length of eye development (navy) by lunar day of eye development; and **C)** development length (green) and **D)** development length (orange) by the lunar day of hatching. **B** and **C** include predictor lines (generated by the *lme4* package, Bates et al., 2015) and 95% confidence interval (grey shaded) calculated from estimated SE generated using *glmmTMB* package (Brooks et al., 2017). Relationships in **A** and **D** were not of statistical significance (regression $p>0.05$), so no regression lines have been fitted.

Table 3.5: Parameter estimates of two periodic mixed effects regressions. The first model models tested for variation in the length of eye development depending on the lunar period of laying, using development length (t_{Hatch}) as the response and the sine and cosine transformations of the lunar term, θ, for the time of the lunar day at laying as fixed effects. The second model models tested for variation in the length of development depending on the lunar period of hatching, using the development length (t_{Hatch}) as the response and the sine and cosine transformations of the lunar term, θ, for the time of the lunar day at hatching as fixed effects. These models use a student's t distribution for small sample sizes. The table contains parameter estimates, standard errors, degrees of freedom (df), t values, and p-values. Statistically significant relationships predictors ($p<0.05$) are indicated in bold text.

Model	Parameter	Estimate	Standard error	df	t value	p value
Is development length related to the lunar phase of laying?	**Intercept**	**18.2555**	**0.5844**	**24.00**	**31.236**	**$<2^{e-16}$**
	Lunar phase (cosθ)	**1.7678**	**0.8367**	**24.00**	**2.113**	**0.0452**
	Lunar phase (sinθ)	0.2562	0.7475	24.00	0.343	0.7348
Is development length related to the lunar phase of hatching?	**Intercept**	**18.4535**	**0.8270**	**22.00**	**22.314**	**$<2^{e-16}$**
	Lunar phase (cosθ)	0.9650	0.9293	22.00	1.038	0.310
	Lunar phase (sinθ)	1.5869	1.2656	22.00	1.254	0.223
	Lunar phase (cos2θ)	0.8626	0.9277	22.00	0.930	0.363
	Lunar phase (sin2θ)	1.5450	1.0379	22.00	1.489	0.151

3.3.2 Nocturnal illumination and hatching success

Light treatment.

Hatching success data was calculated for 171 out of 181 clutches. The remaining 10 clutches did not hatch before experiment conclusion. The proportion of successfully hatched embryos in a clutch ranged from 0 to 1. Mean hatching success for triplefin embryos was 0.75 (± SE 0.022). Hatching success was not influenced by light treatment (Table 3.6, Appendix C, Fig. C1).

Clutch size interacted with water temperature significantly, so I therefore present the results in the context of the interaction effects (Fig. 3.6A-E). The hatching success of smaller clutches did not vary significantly across different water temperatures (Fig. 3.6 A, B). For larger clutches, hatching success was low in cooler temperatures (Fig. 3.6 C-E), with only 0-25% of embryos surviving to hatching. This increased to 75-100% in warmer temperatures. The interaction between water temperature and clutch size appears to follow a linear pattern, except in the case of very large clutches (Fig. 3.6E), which exhibited much greater disparity in hatching success between the coldest temperatures (Mean ± SE; 0 ± 0.5658495) and the warmest temperatures (Mean ± SE; 1 ± 0.1314105).

The fixed effects of the model explained only 9.11% of variation in hatching success (R^2m = 0.09105315), and the random effect of tank explained 11.76% of variation (R c = 0.208676).

Table 3.6: Analysis of deviance table (Wald Chi-square, type III error) for the linear mixed effects model of hatching success (box-cox transformed, $\lambda = 5$) versus the fixed effects of light treatment, water temperature, clutch size, and the interaction term for water temperature and clutch size. The table contains chi-square test statistics (X^2), degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Parameter	X^2	df	p value
Light treatment	2.1390	3	0.54406
Water temperature	1.5416	1	0.21438
Clutch size	**4.8680**	**1**	**0.02736**
Clutch size:water temperature	**5.5628**	**1**	**0.01835**

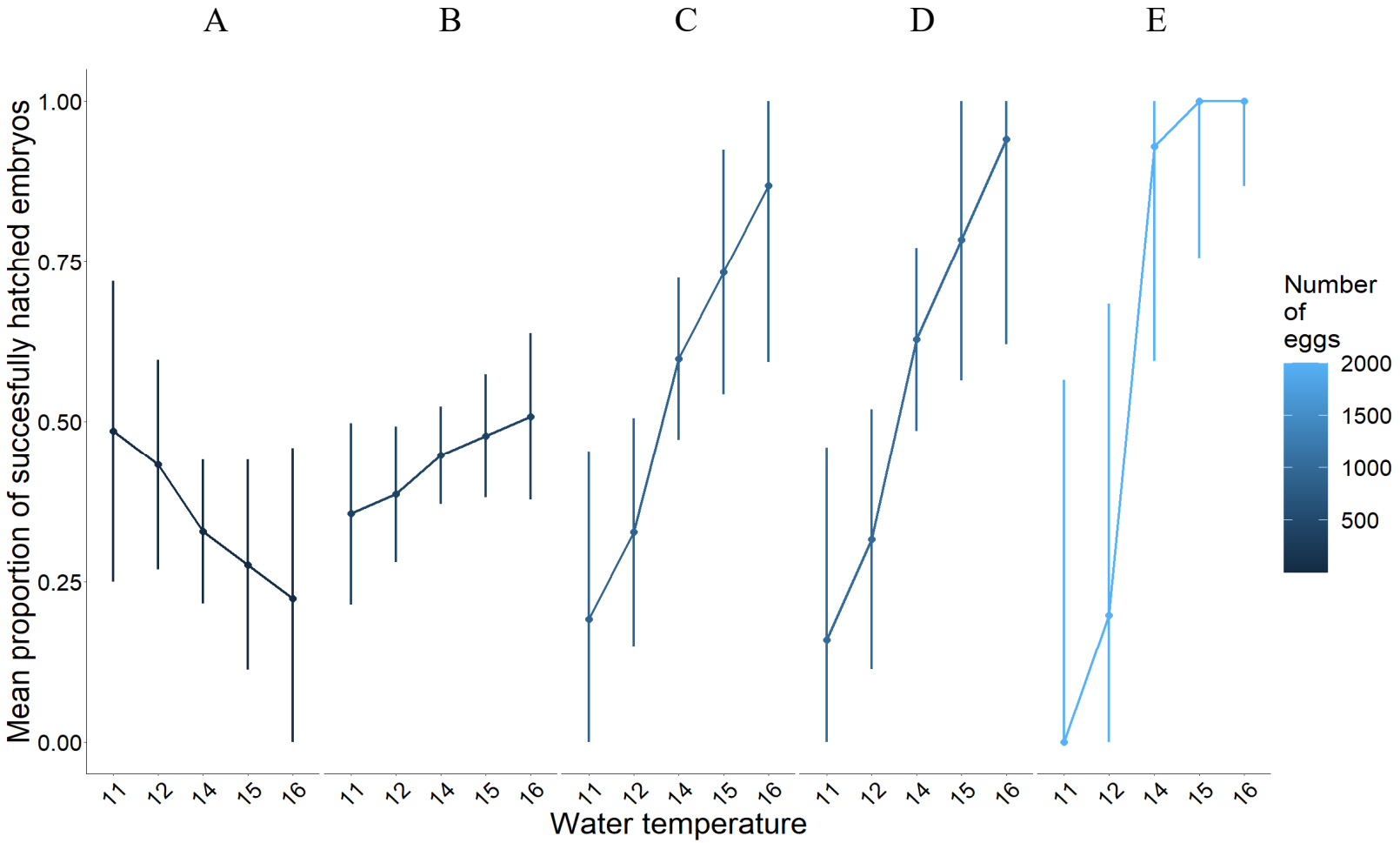

Figure 3.6: Model effects of water temperature (ºC) and clutch size derived from the *effects* package (Fox and Weisberg, 2018). Dots represent model estimates for the mean proportion of successfully hatched embryos at a given clutch size with 95% confidence intervals across water temperatures (degrees Celsius). Shades of blue indicate clutch size **(A)** *n = 6* eggs, **B)** *n = 400* eggs, **C)** *n = 900,* **D)** *n = 1000* eggs, **E)** *n = 2000* eggs).

Lunar effects.

The proportion of hatching success for the lunar treatments ranged from 0 to 1 (Mean ± SE: 0.663355 ± 0.05972657). Hatching success was not significantly related to the time of the lunar month at laying (Table 3.7, Fig. 3.7 A).

However, the regression estimates for the time of the lunar month at laying indicated significant relation of hatching success to cosθ. Clutches that hatched during the full moon tended to have lower predicted hatching success (Estimate 0.3743107 ± SE 0.2498836), than those that hatched during the new moon (Estimate 0.7517201 ± SE 0.2428795) (Fig. 3.7 B).

The fixed effects explained 14.78% of variation in hatching success ($R^2m = 0.1478318$), and the random effect of tank explained 26.35% of variation ($R^2c = 0.4113675$). This indicates that random variation between tanks influenced hatching success to a greater extent than the fixed effects of the model.

Table 3.7: Parameter estimates from periodic mixed effects regressions assessing the relationships between hatching success (box-cox transformed, $\lambda = 3.7$) versus theta transformations for the date of laying and the date of hatching. The table contains parameter estimates, standard errors, t values, degrees of freedom (df), and p-values. Statistically significant relationships ($p<0.05$) are indicated in bold text.

Model	Parameter	Estimate	Standard error	df	t value	p value
Does the time of the lunar month of clutch laying influence hatching success?	Intercept	0.04238	0.09659	7.30274	0.439	0.674
	Lunar phase (cosθ)	-0.11279	0.09609	25.37993	-1.174	0.251
	Lunar phase (sinθ)	-0.16255	0.09657	28.00631	-1.683	0.103
Does the time of the lunar month of clutch hatching influence hatching success?	**Intercept**	**0.24621**	**0.09755**	**2.89035**	**2.524**	**0.0891**
	Lunar phase (cosθ)	**0.19042**	**0.08004**	**22.32196**	**2.379**	**0.0263**
	Lunar phase (sinθ)	0.09672	0.09789	18.65685	0.988	0.3357

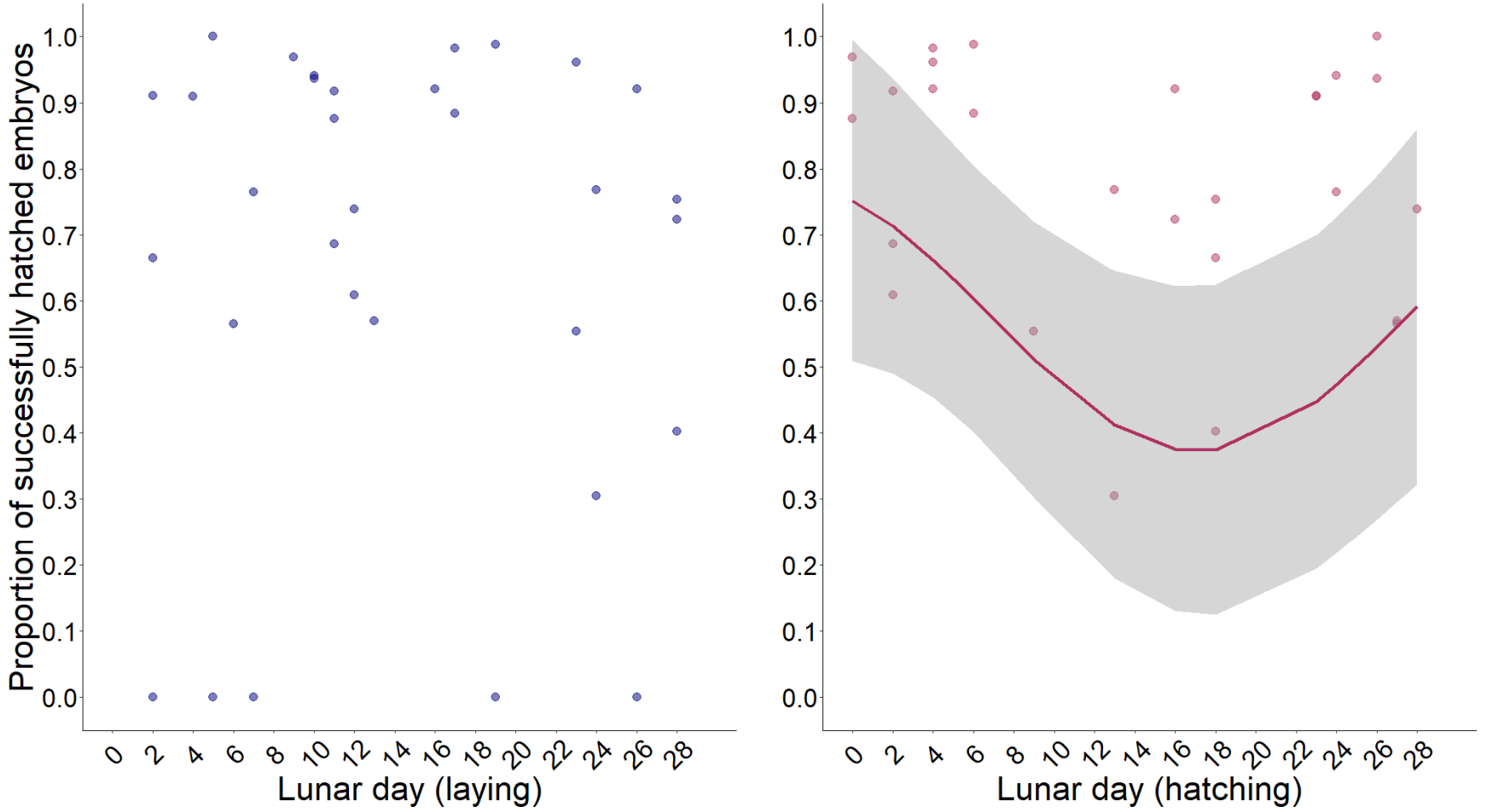

Figure 3.7: A) Scatterplot of the proportion of successfully hatched larvae versus the lunar day of laying**. B**) Scatterplot of the proportion of successfully hatched larvae versus the lunar day of hatching. The plot includes a predictor line (generated by the *lme4* package, Bates et al., 2015) (red) and 95% confidence interval (grey shaded) (calculated by estimates of the SE generated by the *glmmTMB* package; Brooks et al., 2017).

3.4 Discussion

How does nocturnal illumination influence embryonic development?

Light treatment did not impact the length or structure of embryonic development during the experiment, but lunar patterns of light modified development length. This implies that patterns of changing luminescence are more important influences on embryonic development for common triplefin than consistent exposure to light. While lunar time of the month at laying did not impact the length of eye development, eye development coinciding with the first and the third quarter moons tended to be longer than development coinciding with the full moon. Some studies have shown that exposing developing embryos to light causes accumulation of glycolytic compounds used to fuel the synthesis of cells in the outer segment of the retina (reviewed in Jaroszynska et al., 2021). It is possible that exposure to intermediate or bright lunar light during critical stages of eye development increased the rate of retinal cell synthesis, resulting in shorter developmental duration. This hypothesis is speculative as very few studies concentrate on the influence of lunar patterns in embryonic development. Furthermore, the random effect of tank explained approximately half of the model variation in the lunar effects of eye development, indicating that within-tank variation caused significant change in eye development in lunar treatments. For both models, between ~50-85% of variation in eye development and hatching length were not explained by the model, suggesting the influence of other factors I did not evaluate in the experiment. These results should accordingly be interpreted with caution.

My results suggest that the increased metabolic efficiency of development in warm temperatures existed in trade-off with modified development structure. Higher water temperatures at clutch laying yielded faster eye development, faster overall development, and smaller proportions of development needed to synthesise eyes. Warm temperatures accelerate the rate of development for poikilotherms (including fish) (Herzig and Winkler, 1986, Das et al., 2006, Lugowska and Kondera, 2018), but the specific mechanisms underpinning the acceleration of embryonic development are not well-understood (Lugowska and Kondera, 2018). It is speculated that accelerated developmental rates arise from temperature-based changes in the activity of enzymes such as chorionase (Reddy and Lam, 1991), and in gene expression (Campos et al., 2012, Papakostas et al., 2014, Martinez et al., 2016). Several key

metabolic enzymes tend to operate with greater efficiency at higher temperatures, resulting in increased rates of swimming, feeding, muscular activity, and respiration (Sumner and Doudoroff, 1938, Dabrowski, 1986). The retinal tissue of fish, like that of humans, is one of the most metabolically active tissue types in the body (Ames et al., 1992), and requires great energetic investment to develop and maintain (Jaroszynska et al., 2021). The development of vital organs such as the heart may occur prior to eye development (e.g., Yu and Guo, 2018), post-eye development (e.g., Jafari et al., 2010), or both may occur concurrently. Increased metabolic efficiency in warmer laying temperatures may have increased the rate of eye development, therefore shortening the proportion of development devoted to eye synthesis. This may also explain the shorter duration of development overall at warmer temperatures. However, a longer portion of development devoted to eye synthesis yielded shorter periods of development. This implies that a key determinant of the length of embryonic development for *F. lapillum* is the modification of development structure at different water temperatures. Despite phenotypic benefits associated with late-season developmental environments, hatching early in the season (i.e., during unfavourable conditions) confers increased survival in later life phases (Pearson and Warner, 2018). In colder temperatures, the process of eye development for embryos in the experiment may have been more metabolically expensive. Early hatching after diversion of metabolic energy towards eye development may therefore confer advantages in survival early in the hatching season. Each model evaluating non-lunar fixed effects on fitness-related traits of offspring explained a weak-moderate amount of variance in each of the response variables (~25% to 58%), while comparatively little variance was explained by tank (approx. 1%< to 14%). The remaining unexplained variance may have been a function of unassessed variables in the experiment, such as parental condition and egg quality. These results highlight the many complex factors that influence embryonic development and emphasise the need for further research to discern how these factors in turn affect fitness at later stages of life.

It should be considered that there are potentially more appropriate measurements for development milestones such as heart development, length at hatch, and yolk size. While these metrics may have provided more robust estimates of embryonic development, due to the interconnected nature of my adult and offspring studies, I was not able to collect images of high enough resolution at a high enough rate to gather data of this nature. I avoided removing eggs from nests to measure these facets of development so as not to disrupt adult reproductive activities. Likewise, as males guard and care for their eggs, I did not remove eggs for

measurement because I could not discount any possible impacts of parental absence on development. Due to the limited flexibility of my experimental set-up, I also could not collect larvae after that hatched from the tanks. My data using eye development primarily as a metric for comparisons of embryonic development should be interpreted with caution.

Does exposure to nocturnal light impact hatching success of embryos?

The regime of nocturnal illumination did not influence triplefin hatching success, but clutches that hatched during the new moon experienced greater hatching success than those that hatched during the full moon. Lunar influences in embryonic development are seldom investigated, and as such it is unclear why this pattern may have occurred during the experiment. For some species hatching success is greater during the new moon. It is speculated that hatching during the new moon could confer survival advantages to offspring due to predator avoidance (Klein et al., 2018). It is therefore possible that hatching in conjunction with extended periods of nocturnal darkness could confer an advantage to embryos at hatching. Hatching success did not vary by water temperature for small clutches but increased at higher temperatures for large clutches. Greater clutch size can reduce offspring fitness, as offspring may receive a smaller relative portion of maternal resources as the number of eggs laid increases (Charnov and Krebs, 1974, Brockelman, 1975, Parker and Begon, 1986, Godfray et al., 1991). It is possible that laying in warmer temperatures mitigated penalties to fitness from reduced maternal investment for large clutches during the experiment. In both models for hatching success (light treatment and lunar effects), fixed effects explained less than half of the model variation in hatching success for triplefin embryos. Many factors influence embryonic development, so it is possible that a complicated set of factors not captured by my data was responsible for the remaining 60-80% variance in hatching success. A potentially important example of this that I was not able to assess due to constraints in time and arising from experimental design and available equipment was quality of eggs laid by females. Both clutches laid in one tank in the regular lunar treatment did not survive to eye development and disappeared shortly after laying occurred. As triplefin males prune (eat) non-viable or poor-quality eggs, this suggests that egg quality may have influenced hatching success. Additionally, further investigation into whether the structure and duration of embryonic development determined the degree of success for clutches that hatched may yield greater insight into potential influences of nocturnal light on

hatching success. Likewise, investigating interactive effects of nocturnal illumination is required to define the determinants of hatching success for common triplefin.

Conclusion

My results highlight the complexity and sensitivity of embryonic development and demonstrate the importance of cultivating a nuanced understanding of determinants of fitness-related traits in early life history. Characteristics of the physical environment can significantly impact the length and success of development. These impacts can have carry-over effects to later stages of life, accordingly modifying development and fitness in later life phases. My results indicated that the degree of nocturnal illumination did not impact fitness-related traits for embryonic *F. lapillum*, but that lunar effects influenced the length and success of development. However, I cannot confidently corroborate the source of these patterns as existing literature exploring lunar effects on embryonic development is scant. Furthermore, I cannot account for possible contributions of parental fitness, which may explain the significant within-tank variation in lunar effects. Additional research is required to disentangle the influence of environmental cues and parental fitness on embryonic development and hatching success. Environmental cues can vary widely and may have additive interactions with other life history traits that radically alter offspring fitness. Further study is necessary to increase our understanding of the impact of nocturnal illumination on facets of offspring fitness.

CHAPTER 4

General discussion

4.1 Overview

Evolutionary theory is founded upon the notion that the ability of individuals to survive and contribute their genetic material to the next generation varies (Orr, 2009), and fitness is subject to different selective pressures with ontogeny (Werner and Gilliam, 1984). The aims of this thesis were to investigate the impact of nocturnal illumination on 1) fitness-related traits of adults, and 2) fitness-related traits of offspring during early stages of development. I concentrated on aspects of fitness involved in energetic trade-offs for adults (such as body condition, growth, and reproduction), and the length, structure, and success of embryonic development as indicators of offspring fitness. I sought to address these aims using a laboratory experiment that manipulated nocturnal illumination and evaluated its impacts on fitness-related traits of adults and offspring for a species of temperate reef fish that exhibits close association with several environmental factors, the New Zealand common triplefin (*Forsterygion lapillum*). My results have emphasised the necessity of evaluating the impact of nocturnal illumination across ontogeny, and of considering possible interactive effects of environmental cues with other aspects of life history (e.g., sex, age/size, seasonality in breeding and/or development) in studies of determinants of fitness-related traits. My study highlights the differing impacts of nocturnal illumination during early developmental and adult phases that can be used to predict fitness in distinct stages of life. This research builds on an expanding body of literature examining the consequences of variation in environmental cues and spurs further questions about the specific developmental and metabolic mechanisms that drive organism responses to these changes.

4.2 Effects of nocturnal illumination on fitness-related traits in adulthood

The results from this study highlight the complex and myriad factors that impact different facets of fitness-related traits of adult triplefin. *F. lapillum* show strong ecological and

phenotypic association with local environmental conditions (Syms, 1995, Hilton et al., 2008, Caiger et al., 2021), such as water depth and exposure, and temperature (Wellenreuther and Clements, 2007, Wellenreuther et al., 2009). It is therefore likely that fitness-related traits for adults are determined by a complex network of interacting environmental factors, yielding distinct patterns in growth, reproduction, and body condition corresponding to local environmental changes. Nocturnal illumination did not influence body condition, but showed differing impacts on growth and reproductive activity between light treatments, and in the case of reproductive behaviour, according to lunar patterns. Body condition was primarily determined by individual life history attributes. Females experienced a greater reduction in body conditions than males, likely due to depletion from serial bouts of reproduction as eggs are more costly to produce than sperm (Trivers, 1972, Roff and Fairbairn, 2007, Hayward and Gillooly, 2011). Growth likewise varied between sexes. The condition of fish with better initial body condition degraded over the experiment, while the condition of fish with worse initial body condition improved. This suggests a shifting gradient in individual energy investment from body condition maintenance to reproduction as initial body condition increased (Clark et al., 1994, Johnston et al., 2007, Barneche et al., 2018).

Reproductive activity was not significantly influenced by water temperature, suggesting that the seasonal variation of reproduction for triplefin in Wellington (Francis, 2001, Wellenreuther and Clements, 2007, Moginie and Shima, 2018) may not be solely determined by the effects of temperature on adult spawning behaviours. *F. lapillum* have been described as daily continuous spawners throughout their breeding season (Warren, 1990), and lunar influences on reproductive behaviours have not been previously assessed for this species. The timing of lunar reproduction is synchronised by a series of hormonal changes regulated by consistent variation in luminescence over time. The pineal gland is responsible for the regulation of seasonal physiological, biochemical, and behavioural processes (Kah et al., 1993). Environmental stimuli are received and processed in the hypothalamus, upregulating the expression of clock genes such as Cryptochrome (Fukushiro et al., 2011), in turn triggering a release of gonadotropin-releasing hormone (GnRH) to the pituitary gland (Bhattacharya, 1992). This causes a release of gonadotropin (GtH), beginning the development of sexual organs to reproductive maturity (Bhattacharya, 1992). During prolonged periods of darkness, the pineal gland synthesizes melatonin – a hormone associated with the sleep/wake cycle - which is then released into the blood, transferring photoperiodic information to the central nervous system and peripheral tissues (Ikegami et al., 2014a). The reception of lunar light supresses melatonin

production, so it is present in low concentrations during the full moon and accumulates at greater concentrations around the new moon (Park et al., 2006, Ikegami et al., 2014b). Melatonin is thought to be responsible in some species for triggering the process of gonadal maturation and ovulation (Kah et al., 1993) and the triggering of increased ovarian investment (Desjardins et al., 2011). Certain concentrations of neural melatonin correlate with the synthesisation of reproductive hormones like luteinizing hormone and follicle-stimulating hormone by the pituitary gland, shortly thereafter inducing reproductive activity (Falcón et al., 2010). For many species with semi-lunar coordination of spawning, melatonin accumulation on either end of the spectrum (very high or very low) triggers development to reproductive maturity (Park et al., 2006, Andreatta et al., 2020). This results in coordinated spawning events during periods of intermediate brightness (i.e., during the first or third quarter moons).

In the laboratory experiment, triplefin laid eggs in greater numbers during periods of intermediate brightness immediately following simulated conditions of nocturnal darkness (new moon) or brightness (full moon) for the regular lunar treatment. Furthermore, artificial conditions of nocturnal brightness (24-hr light) or darkness (dark at night) resulted in increased likelihood of reproduction. Given these results it is possible that, if melatonin plays a partial role in regulating lunar patterns of reproduction for *F. lapillum*, melatonin accumulation in differing conditions of nocturnal brightness may play a key role in determining temporal variation in reproductive output. In unnatural conditions such as those in the 24-hr light treatment, the dark at night treatment, and the dimmed lunar treatment, these abnormal environmental cues may disrupt the entrainment of the reproductive clock for *F. lapillum*. Artificial light at night (ALAN) has been found to disrupt reproductive behaviours and success for a range of other marine species (Gaston et al., 2017). Exposure of European perch to ALAN significantly disrupts neural release of gonadotropin (Brüning et al., 2016). Red light in particular can have more severe impacts on these processes than blue or green wavelengths of light. Artificial light exposure likewise interrupts the accumulation of ocular and neural melatonin (Bayarri et al., 2002). Some species such as clownfish appear to employ conditional lunar spawning, causing variation in the timing and number of spawning events determined by local food availability, individual fecundity, and nocturnal brightness (Seymour et al., 2018). Recent reproductive studies of the clownfish have found that continued exposure to ALAN increased the time between spawning events, decreased egg size during spawning, and increased rates of offspring hatching failure (Forbert et al., 2019, Forbert et al., 2021). ALAN can also interact with other environmental anthropogenic factors, exacerbating previously

apparent impacts on organism wellbeing (Gaston et al., 2017). The past 150 years has seen rapid increases in human utilisation of ALAN (Depledge et al., 2010). If left unmitigated, increased exposure to ALAN could have serious deleterious impacts on the breeding behaviours, timing, and success of intertidal fish species such as *F. lapillum*. These negative impacts can be reduced by avoiding and minimising the use of ALAN or changing the wavelength of light used to one that is less biologically disruptive (Gaston et al., 2022). Coastal development should therefore carefully consider the utilisation of ALAN and manage this resource to minimise possible impacts on the local ecological community as much as possible.

My results suggest that *F. lapillum* may operate on a similar conditional semi-lunar cycle, influenced by several other previously studied components of reproductive success such as body size, female choosiness, territory size, and local environmental conditions. While my study did not assess hormonal changes in conjunction with nocturnal light conditions, it is notable that somatic growth for large-bodied females in the 24hr light treatment was the lowest of any sex-treatment group. This light treatment also showed the most vigorous reproductive activity during the study. It is possible that exposure to ALAN over long periods of time forces a shift in energy away from somatic growth towards increased reproductive output for female triplefin, but until further studies assessing hormonal changes and offspring quality are conducted, this theory will remain purely speculative. If common triplefin indeed operate on a conditional semi-lunar cycle, increased light pollution (direct or indirect) may have serious impacts on the reproductive behaviour, reproductive success, and individual body condition or growth of this species. Likewise, increasingly erratic variation in weather conditions like cloud cover due to climate change may further disrupt these processes, in turn impacting larval survival, recruitment, and the overarching structure of the local biotic community. The results of this study emphasise the importance of considering the impacts of environmental cues such as nocturnal illumination from a holistic perspective and highlight the nuanced influences of environmental cues on fitness-related traits. However, the influence of lunar cycles on triplefin breeding behaviour requires further assessment through additional controlled laboratory experiments with a greater number of replicates, and increased repetition of simulated lunar cycles at varying intensities factoring in the influence of cloud cover. Further study to disentangle the mechanisms by which these fitness-related traits are regulated is also required. Are these patterns controlled hormonally? Does melatonin indeed play a role in entraining endogenous rhythms for this species? Experiments that assess triplefin hormonal chemistry,

growth, and body condition over longer periods are necessary to paint a more detailed picture of the impact of nocturnal illumination on fitness-related traits of *F. lapillum*.

4.3 Impacts of nocturnal illumination on fitness-related traits in early development

While fish are exposed to similar environmental conditions across ontogeny, the effects of abiotic selective pressures are more pronounced in early stages of development (Septriani et al., 2021). This study highlights the challenge of elucidating determinants of the trajectory and outcome of embryonic development for fish such as *F. lapillum*. In laboratory experiments assessing the impact of nocturnal illumination on embryonic development, I found that light treatment did not significantly impact any fitness-related traits, but that water temperature, lunar patterns, and other characteristics of development did.

Several studies have been conducted into the influence of illumination on embryonic development, but few discuss this in the context of lunar illumination. It is therefore assumed for *F. lapillum* embryos in this study that lunar influences in development are derived from changing patterns in intensity and timing of nocturnal light in lunar treatments that was not apparent in the 24hr light and dark at night treatments. Exposure to differing levels of nocturnal illumination has variable impacts for different species and can significantly alter key developmental processes such as lateralization (Dadda and Bisazza, 2012). In a study on haddock larvae, embryonic exposure to very bright continuous light and complete darkness produced large-bodied larvae with small yolk sacs that developed rapidly, and small-bodied larvae that had long development times respectively (Downing and Litvak, 2002). De Borsetti et al. (2011) linked long development times of zebrafish embryos kept in constant darkness to a temporary accumulation of habenular precursor cells, causing late differentiation and long-term decreases in neuronal processes. In contrast to adult fish, embryos in this study showed delayed melatonin accumulation, which in turn inhibited habenular neurogenesis and slowed development.

In my experiment, water temperature appeared to be the chief determinant of development length, structure, and hatching success in analyses using light treatment, water temperature, and various development metrics as fixed effects. However, analyses concerning only lunar

patterns of light often explained a greater proportion of variation in development and hatching success than in models grouping static light treatments (i.e., 24 hr light and dark at night treatments) with dynamic light treatments (regular and dimmed lunar treatments). During the experiment, development of embryo eyes was shorter when it coincided with the full moon, and longer when it coincided with the new moon. Embryos laid during the new moon also showed longer development than those laid during the full moon, suggesting that triplefin embryos exhibit plasticity in development according to changing patterns of lunar light. Furthermore, hatching success was greater for clutches that hatched during the new moon than those that hatched during the full moon. It is possible here that longer development for clutches laid in or around the new moon (and hatching in or around the full moon) may reduce hatching success. I did not assess this possible link with development length as it was only measured for clutches with hatching success greater than 0%, but future research that incorporates development characteristics into models of this nature could help answer this question. While results did not significantly differ between light treatments, it is possible that 24hr-light or total darkness at night may have influenced embryonic development but that comparisons of dynamic lunar light treatments with static light treatments decreased the resolution of these patterns. Indeed, despite a lack of statistical support, all developmental criteria measured were longer in duration for embryos in the dark at night treatment than for those in the 24-hr light treatment. In addition, the previously discussed developmental milestones did not differ between the dim and regular lunar treatments. This raises the possibility that the degree and patterns of nocturnal illumination may impact upon embryonic development distinctly. A thorough appraisal of the nature of these separate facets of nocturnal illumination is essential to properly understand and mitigate the possible impacts of ALAN exposure on embryonic development. For example, a recent study on surgeonfish larvae discovered that while ALAN exposure caused larvae to grow faster and heavier, it also significantly increased mortality over time (O'Connor et al., 2019). This study did not assess embryo quality prior to or post-hatching, so it is unknown to what extent similar mechanisms may have been acting upon embryos within the experiment. Most studies assessing the impact of nocturnal light on embryonic fish largely centre on Zebrafish as a model organism (e.g., Andrew et al., 2009), or aquaculture species (e.g., Novales Flamarique, 2018). Very little if any research into this topic has been conducted into *Tripterygiidae* species, or on embryos of fish species endemic to New Zealand. This study therefore highlights current gaps in the knowledge of these early and vulnerable stages of development and emphasises the importance of seeking greater insight into the impact of nocturnal illumination on fitness-related traits during the embryonic phase.

4.4 Limitations

I acknowledge that the density of fish in each experimental tank (8 per tank with a base area of 0.266 m^2, equivalent to approximately 30 per m^2) was significantly greater than naturally occurring densities (1-6 per m^2; Mensink and Shima, 2014). Based on the assumption that greater densities of triplefin would result in greater reproductive success (Barnett and Pankhurst, 1996), I assigned 8 fish to each tank, but my results should be interpreted with this in mind.

I also acknowledge that the sections of my study regarding lunar patterns in reproduction in Chapter 2 and in development and hatching success in Chapter 3 were limited by small sample sizes. This reduced the power of my statistical analyses and reduced the prediction power of the models. Repetition of the experiment in warmer conditions may have helped this. Furthermore, the simulated lunar cycles as programmed by the APEX did not have the option of ramping the lights up or down in a gradient at times of moonrise or moonset. This meant that when the simulated moon "rose" the lights would abruptly turn on, and when the moon "set" the lights would abruptly turn off. This is obviously not reflective of how the moon rises and sets in nature, but I assume that this did not influence my data significantly as the timing and intensity of light still followed a reasonable approximation of natural lunar cycles. The dimmed lunar cycle was intended to approximate a lunar cycle in cloudy conditions. Nocturnal sky brightness in overcast conditions approximately is 10x dimmer than the night sky in clear conditions (in Gaston et al., 2013), however, this magnitude difference in brightness would only apply to pristine environments with no light pollution. Cloud cover can magnify ALAN, resulting in increased sky glow in areas of dense human settlement (Kyba et al., 2011). While the difference in magnitude might approximate pristine conditions, they were unlikely to approximate conditions at the collection site located close to Wellington city, where skyglow is likely to be brighter in conditions of heavy cloud in comparison to remote locations.

Due to time restrictions and experimental design constraints, data collection of egg counts and embryonic development in Chapter 2 and Chapter 3 only occurred three times per week. I elected not to sample at a higher frequency to minimise disturbance to the reproductive activity of adults in the experiment, but this meant that I was only able to photograph egg clutches once every few days. This in turn meant that I was not able to accurately stage embryos as they developed, which necessitated the use of estimates for the dates of laying, eye development,

and hatching. While the time between recording observations (2/3 days) did not seem to impact my results, the data I recorded was of a lower resolution than it may have been if I had collected data daily. Future studies should aim to either conduct separate experiments on embryonic development, or else conduct sampling at a higher frequency for this portion of the experiment. Likewise, I was unable to ascertain which fish in each tank were participating in reproductive activity, meaning that I could not conduct analyses specifically comparing parental and offspring fitness over time. Future studies should work with single breeding pairs rather than aggregated groups to further refine an understanding of nocturnal illumination on parental and offspring fitness.

Lastly, the determinants of both adult and offspring fitness are diverse and varied. This thesis only concerns the impact of nocturnal illumination on fitness-related traits and does not account for other seasonal variation in environmental cues such as photoperiod length or tidal activity. My results, therefore, should be interpreted with caution as fitness-related traits may be influenced by a plethora of alternative factors not discussed in my research, and can have additive impacts for individuals depending on other life history traits.

4.5 Conclusion

In summation, this thesis highlights the importance of evaluating the influence of environmental cues such as nocturnal illumination across ontogeny, and how these effects may have distinct or additive effects between adults and their offspring. Many similar studies often focus only on a single phase of the complex life cycle, failing to account for changing impacts of nocturnal illumination on different facets of fitness across ontogeny. This study aimed to examine how nocturnal illumination influences fitness-related traits of adults and offspring for the New Zealand common triplefin (*Forsterygion lapillum*). My results affirm the complex and interconnected nature of fitness across ontogeny, emphasizing the need for future research to consider the impact of nocturnal illumination and its interactions with other environmental cues and life history traits when studying organisms with complex life histories. Overall, my results have contributed towards achieving a greater insight of the factors that impact fitness-related traits, and how these traits in turn influence individual fitness.

Appendix A: Example Akaike information criterion (AIC) table used for model selection.

Table A1: Akaike information criterion output assessing the relative fit of several linear models for the influence of light treatment on relative change (%) in Fulton's body condition (ΔK) across the experiment. Possible fixed effects include light treatment (treatment), sex, pre-experimental body condition (Pre-K), and combinations of interaction effects for these three main effects. The table includes the number of parameters included in the model (k), the Akaike information criterion information score (AICc), the delta AICc score, the AIC score weight, the cumulative AIC weight of the model, and the residual log likelihood of the model. Models are listed in order of goodness of fit from best to worst.

Model parameters	K	AICc	Delta AICc	AICc weight	Cumulative weight	Residual log likelihood
Treatment:Sex:Pre-K	**18**	**1001.40**	**0.00**	**1**	**1**	**-480.20**
Treatment:Pre-K + Sex	11	1034.34	32.94	0	1	-505.25
Treatment:Sex + Pre-K	11	1039.22	37.82	0	1	-507.69
Treatment + Pre-K:Sex	9	1043.18	41.78	0	1	-511.97
Treatment + Sex + Pre-K	8	1047.36	45.96	0	1	-515.19

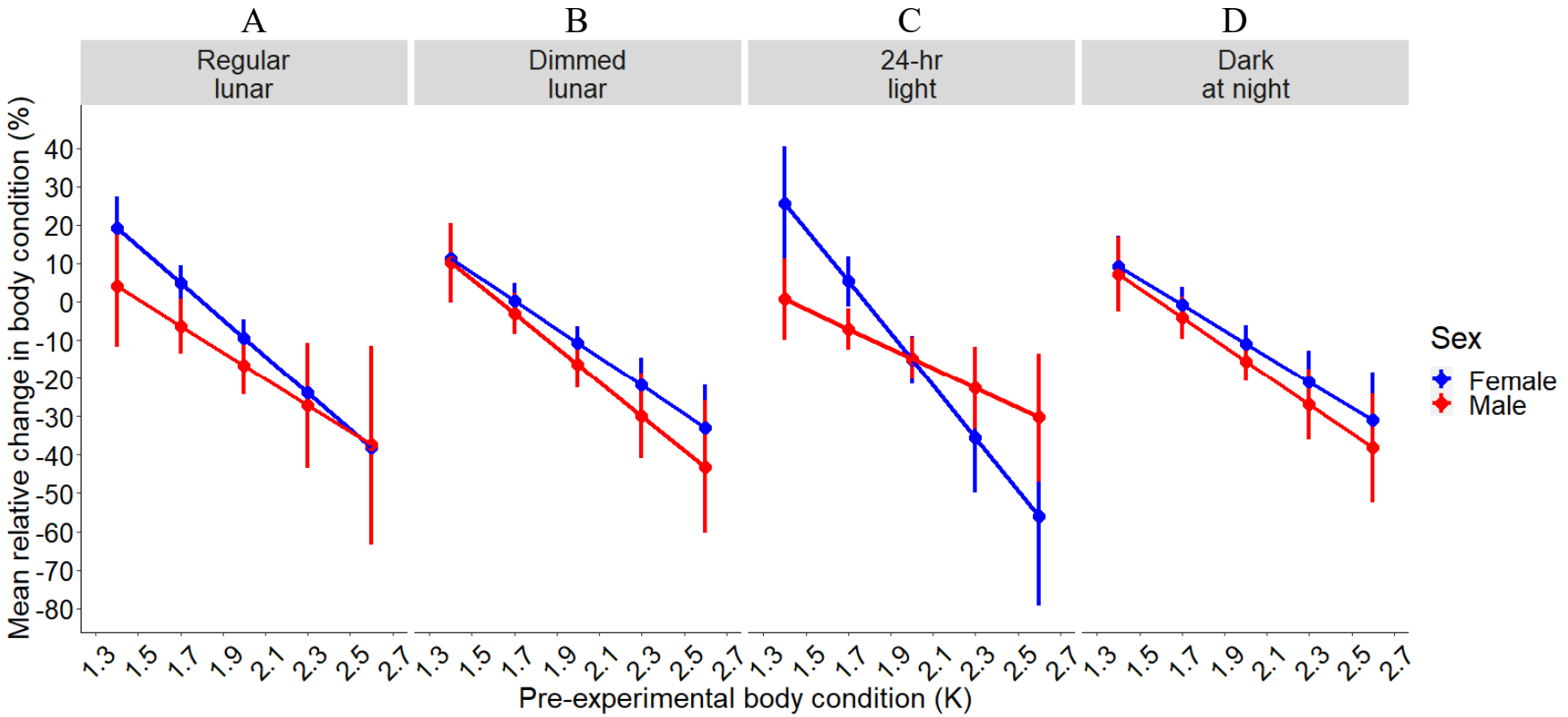

Figure B1: Interaction plots of the model effects (generated by the *effects* package, Fox and Weisberg, 2018) for the mean relative change in body condition for females (blue) and males (red) versus pre-experimental body condition (K) by light treatment (**A**: regular lunar treatment, **B**: dimmed lunar treatment, **C**: 24-hr light treatment, **D**: dark at night treatment). Each mean is bounded by a 95% confidence interval.

Table B1: Linear mixed effects regression output of log growth increment length (μm) using lunar phase terms as fixed effects and tank as a random effect. Cosθ represents a peak in activity at the new or full moon, while sinθ indicates a peak at the first or third quarter. Significant results are indicated in bold text.

Parameter	Estimate	Standard error	Z value	p value
Intercept	**0.284478**	**0.04550**	**6.252**	**4.05^{-10}**
Cosθ	0.001843	0.008821	0.209	0.835
Sinθ	-0.002725	0.008845	-0.308	0.758

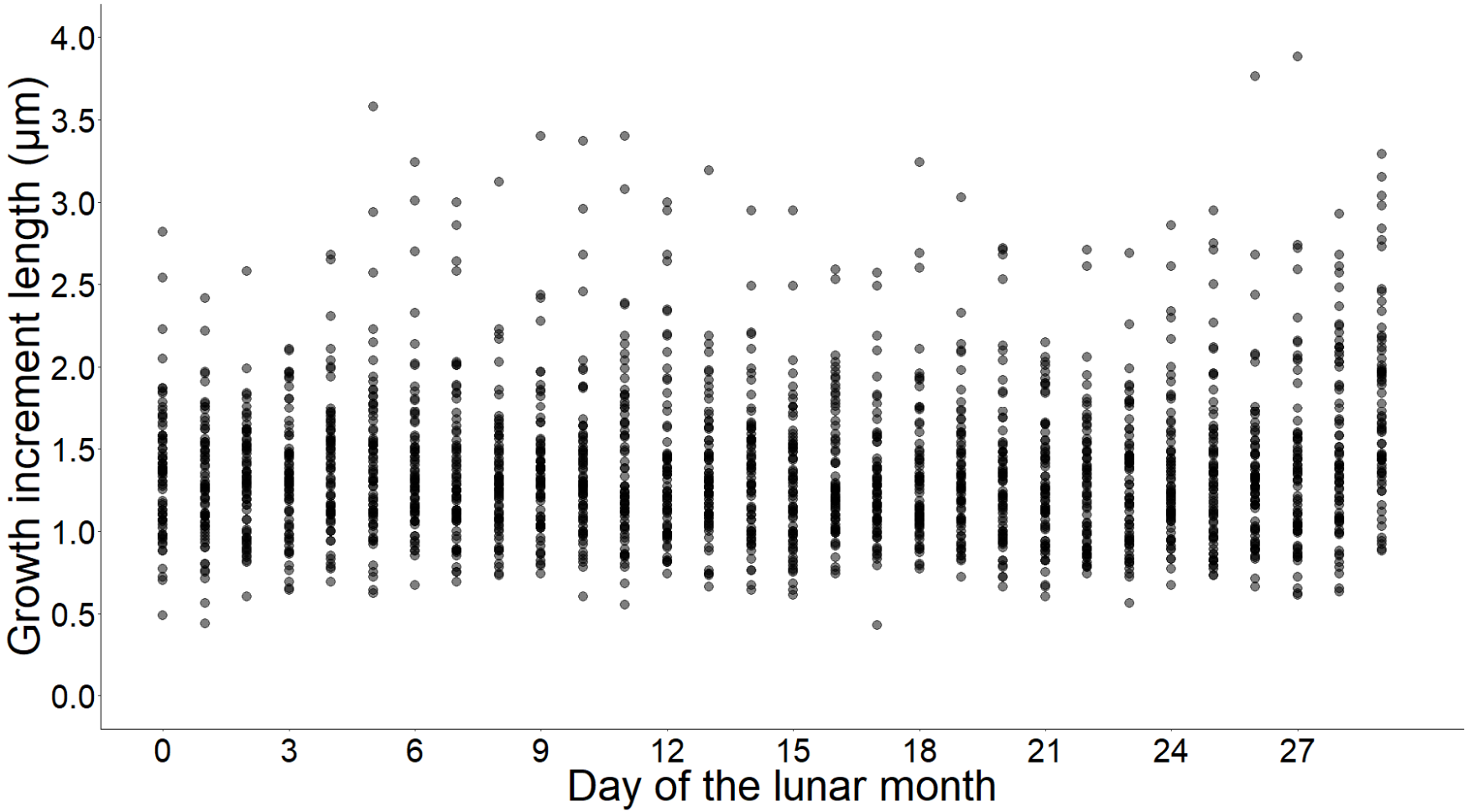

Figure B2: Scatterplot of growth increment length (µm) across the lunar month. Growth increment widths did not show lunar patterns in variation, so no regression line has been fitted.

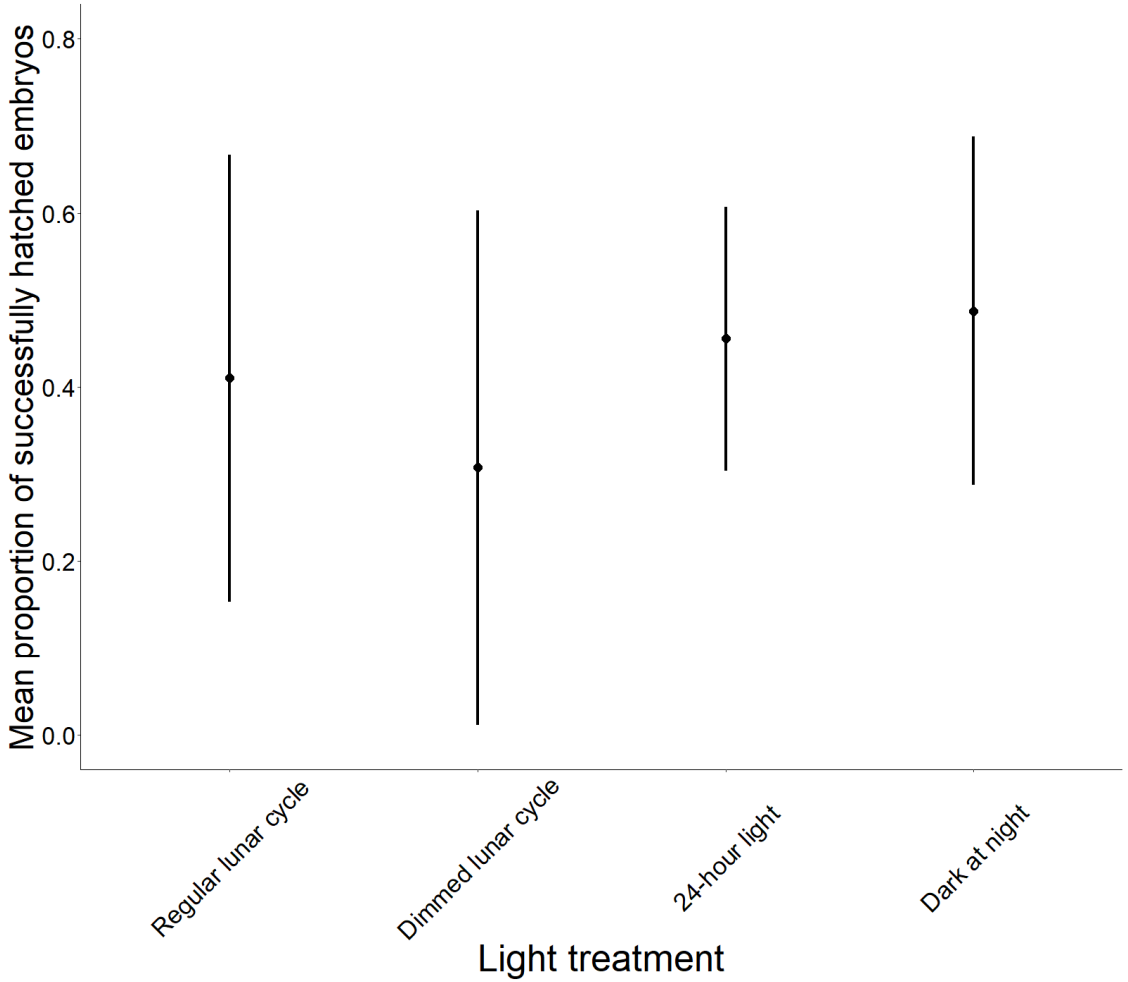

Figure C1: Mean proportion of successfully hatched embryos (with 95% confidence interval) by light treatment.

References

Abrahams, M. V. & Dill, L. M. 1989. A Determination of the Energetic Equivalence of the Risk of Predation. *Ecology* **70**: 999-1007.

Aksnes, D., Dupont, N., Staby, A., Fiksen, Ø., Kaartvedt, S. & Aure, J. 2009. Coastal Water Darkening and Implications for Mesopelagic Regime Shifts in Norwegian Fjords. *Marine Ecology Progress Series* **387**: 39-49.

Allen, G. & Robertson, D. 1994. *Fishes of the Tropical Eastern Pacific,* Honolulu, HI: University of Hawaii Press.

Ames, A., Li, Y., Heher, E. & Kimble, C. 1992. Energy Metabolism of Rabbit Retina as Related to Function: High Cost of Na^+ Transport. *The Journal of Neuroscience* **12**: 840-853.

Ando, H., Shahjahan, M. & Hattori, A. 2013. Molecular Neuroendocrine Basis of Lunar-Related Spawning in Grass Puffer. *General and Comparative Endocrinology* **181**: 211-214.

Andreatta, G., Broyart, C., Borghgraef, C., Vadiwala, K., Kozin, V., Polo, A., Bileck, A., Beets, I., Schoofs, L., Gerner, C. & Raible, F. 2020. Corazonin Signaling Integrates Energy Homeostasis and Lunar Phase to Regulate Aspects of Growth and Sexual Maturation in Platynereis. *Proceedings of the National Academy of Sciences* **117**: 1097-1106.

Andrew, R. J., Osorio, D. & Budaev, S. 2009. Light During Embryonic Development Modulates Patterns of Lateralization Strongly and Similarly in Both Zebrafish and Chick. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**: 983-989.

Angel, L. P., Wells, M. R., Rodríguez-Malagón, M. A., Tew, E., Speakman, J. R. & Arnould, J. P. Y. 2015. Sexual Size Dimorphism and Body Condition in the Australasian Gannet. *PLoS ONE* **10**: e0142653.

Arambam, K., Singh, S. K., Biswas, P., Patel, A. B., Jena, A. K. & Pandey, P. K. 2020. Influence of Light Intensity and Photoperiod on Embryonic Development, Survival and Growth of Threatened Catfish *Ompok Bimaculatus* Early Larvae. *Journal of Fish Biology* **97**: 740-752.

Baker, G. C. & Dekker, R. W. R. J. 2008. Lunar Synchrony in the Reproduction of the Moluccan Megapode *Megapodius Wallacei*. *Ibis* **142**: 382-388.

Bandara, K., Varpe, Ø., Wijewardene, L., Tverberg, V. & Eiane, K. 2021. Two Hundred Years of Zooplankton Vertical Migration Research. *Biological Reviews* **96**: 1547-1589.

Barneche, D. R., Robertson, D. R., White, C. R. & Marshall, D. J. 2018. Fish Reproductive-Energy Output Increases Disproportionately with Body Size. *Science* **360**: 642-645.

Barnett, C. W. & Pankhurst, N. W. 1996. Effect of Density on the Reproductive Behaviour of the Territorial Male Demoiselle *Chromis Dispilus* (Pisces: Pomacentridae). *Environmental Biology of Fishes* **46**: 343-349.

Bartoń, K. 2022. MuMIn: Multi-Model Inference. *R package version 1.47.1*: https://CRAN.R-project.org/package=MuMIn.

Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**: 1-48.

Batty, R. S. 1987. Effect of Light Intensity on Activity and Food-Searching of Larval Herring, *Clupea Harengus*: A Laboratory Study. *Marine Biology* **94**: 323-327.

Bayarri, M. J., Madrid, J. A. & Sánchez-Vázquez, F. J. 2002. Influence of Light Intensity, Spectrum and Orientation on Sea Bass Plasma and Ocular Melatonin. *Journal of Pineal Research* **32**: 34-40.

Beck, W. & Gobatto, C. 2016. Effect of High Wavelengths Low Intensity Light During Dark Period on Physical Exercise Performance, Biochemical and Haematological Parameters of Swimming Rats. *Acta Physiologica Hungarica* **103**: 112-120.

Benoit-Bird, K. J., Au, W. W. L. & Wisdoma, D. W. 2009. Nocturnal Light and Lunar Cycle Effects on Diel Migration of Micronekton. *Limnology and Oceanography* **54**: 1789-1800.

Bhattacharya, S. 1992. Endocrine Control of Fish Reproduction. *Current Science* **63**: 135-139.

Blaxter, J. H. S. 1968. Visual Thresholds and Spectral Sensitivity of Herring Larvae. *Journal of Experimental Biology* **48**: 39-53.

Bleile, H. & Rodgers, S. D. 2001. Marine Coatings. *In:* Buschow, K. H. J., Cahn, R. W., Flemings, M. C., Ilschner, B., Kramer, E. J., Mahajan, S. & Veyssière, P. (eds.) *Encyclopedia of Materials: Science and Technology.* Oxford: Elsevier.

Böhlke, J. & Chaplin, C. 1994. *Fishes of the Bahamas and Adjacent Tropical Waters.,* Wynnewood, PA: Published for the Academy of Natural Sciences of Philadelphia by Livingston.

Bradshaw, W. E. & Holzapfel, C. M. 2010. Light, Time, and the Physiology of Biotic Response to Rapid Climate Change in Animals. *Annual Review of Physiology* **72**: 147-166.

Breitburg, D., Palmer, M. & Loher, T. 1995. Larval Distributions and the Spatial Patterns of Settlement of an Oyster Reef Fish: Responses to Flow and Structure. *Marine Ecology Progress Series* **125**: 45-60.

Bright Ross, J. G., Newman, C., Buesching, C. D., Connolly, E., Nakagawa, S. & Macdonald, D. W. 2021. A Fat Chance of Survival: Body Condition Provides Life-History Dependent Buffering of Environmental Change in a Wild Mammal Population. *Climate Change Ecology* **2**: 100022.

Brockelman, W. Y. 1975. Competition, the Fitness of Offspring, and Optimal Clutch Size. *The American Naturalist* **109**: 677-699.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M. & Bolker, B. M. 2017. glmmTMB Balances Speed and Flexibility among Packages for Zero-Inflated Generalized Linear Mixed Modeling. *The R Journal* **9**: 378-400.

Brosset, P., Lloret, J., Muñoz, M., Fauvel, C., Van Beveren, E., Marques, V., Fromentin, J.-M., Ménard, F. & Saraux, C. 2016. Body Reserves Mediate Trade-Offs between Life-History Traits: New Insights from Small Pelagic Fish Reproduction. *Royal Society Open Science* **3**: 160202.

Brüning, A., Hölker, F., Franke, S., Kleiner, W. & Kloas, W. 2016. Impact of Different Colours of Artificial Light at Night on Melatonin Rhythm and Gene Expression of Gonadotropins in European Perch. *Science of the Total Environment* **543**: 214-222.

Brüning, A., Hölker, F. & Wolter, C. 2011. Artificial Light at Night: Implications for Early Life Stages Development in Four Temperate Freshwater Fish Species. *Aquatic Sciences* **73**: 143-152.

Burton, T. & Metcalfe, N. B. 2014. Can Environmental Conditions Experienced in Early Life Influence Future Generations? *Proceedings of the Royal Society B: Biological Sciences* **281**: 20140311.

Bushnell, M. E., Claisse, J. T. & Laidley, C. W. 2010. Lunar and Seasonal Patterns in Fecundity of an Indeterminate, Multiple-Spawning Surgeonfish, the Yellow Tang *Zebrasoma Flavescens*. *Journal of Fish Biology* **76**: 1343-1361.

Caiger, P. E., Croq, C. & Clements, K. D. 2021. Environmentally Induced Morphological Variation in the Temperate Reef Fish, *Forsterygion Lapillum* (F. Tripterygiidae). *Marine Biology* **168**.

Campos, C., Valente, L. M. P. & Fernandes, J. M. O. 2012. Molecular Evolution of Zebrafish Dnmt3 Genes and Thermal Plasticity of Their Expression During Embryonic Development. *Gene* **500**: 93-100.

Capuzzo, E., Stephens, D., Silva, T., Barry, J. & Forster, R. M. 2015. Decrease in Water Clarity of the Southern and Central North Sea During the 20th Century. *Global Change Biology* **21**: 2206-2214.

Cargnelli, L. M. & Gross, M. R. 1996. The Temporal Dimension in Fish Recruitment: Birth Date, Body Size, and Size-Dependent Survival in a Sunfish (Bluegill: *Lepomis Macrochirus*). *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 360-367.

Charnov, E. L. & Krebs, J. R. 1974. On Clutch-Size and Fitness. *Ibis* **116**: 217-219.

Cheverud, J. M. 1984. Quantitative Genetics and Developmental Constraints on Evolution by Selection. *Journal of Theoretical Biology* **110**: 155-171.

Cinzano, P., Falchi, F. & Elvidge, C. D. 2001. Moonlight without the Moon. *Earth Moon and Planets* **85-6**: 517-522.

Ciocca, M. & Wang, J. 2013. By the Light of the Silvery Moon: Fact and Fiction. *Physics Education* **48**: 360.

Clark, M. R., Fincham, D. J. & Tracey, D. M. 1994. Fecundity of Orange Roughy (*Hoplostethus Atlanticus*) in New Zealand Waters. *New Zealand Journal of Marine and Freshwater Research* **28**: 193-200.

Claydon, J. a. B., Mccormick, M. I. & Jones, G. P. 2014. Multispecies Spawning Sites for Fishes on a Low-Latitude Coral Reef: Spatial and Temporal Patterns. *Journal of Fish Biology* **84**: 1136-1163.

Clifton, K. E. 1997. Mass Spawning by Green Algae on Coral Reefs. *Science* **275**: 1116-1118.

Cone, R. S. 1989. The Need to Reconsider the Use of Condition Indexes in Fishery Science. *Transactions of the American Fisheries Society* **118**: 510-514.

Connell, J. H., Hughes, T. P. & Wallace, C. C. 1997. A 30-Year Study of Coral Abundance, Recruitment, and Disturbance at Several Scales in Space and Time. *Ecological Monographs* **67**: 461-488.

Cury, P. & Pauly, D. 2000. Patterns and Propensities in Reproduction and Growth of Marine Fishes. *Ecological Research* **15**: 101-106.

Dabrowski, K. R. 1986. Active Metabolism in Larval and Juvenile Fish: Ontogenetic Changes, Effect of Water Temperature and Fasting. *Fish Physiology and Biochemistry* **1**: 125-144.

Dadda, M. & Bisazza, A. 2012. Prenatal Light Exposure Affects Development of Behavioural Lateralization in a Livebearing Fish. *Behavioural Processes* **91**: 115-118.

Das, T., Pal, A. K., Chakraborty, S. K., Manush, S. M., Dalvi, R. S., Sarma, K. & Mukherjee, S. C. 2006. Thermal Dependence of Embryonic Development and Hatching Rate in *Labeo Rohita* (Hamilton, 1822). *Aquaculture* **255**: 536-541.

Davies, T. W., Bennie, J., Inger, R., Ibarra, N. H. & Gaston, K. J. 2013. Artificial Light Pollution: Are Shifting Spectral Signatures Changing the Balance of Species Interactions? *Global Change Biology* **19**: 1417-1423.

Davies, T. W., Duffy, J. P., Bennie, J. & Gaston, K. J. 2014. The Nature, Extent, and Ecological Implications of Marine Light Pollution. *Frontiers in Ecology and the Environment* **12**: 347-355.

Day, T. & Taylor, P. D. 1997. Von Bertalanffy's Growth Equation Should Not Be Used to Model Age and Size at Maturity. *The American Naturalist* **149**: 381-393.

De Borsetti, N. H., Dean, B. J., Bain, E. J., Clanton, J. A., Taylor, R. W. & Gamse, J. T. 2011. Light and Melatonin Schedule Neuronal Differentiation in the Habenular Nuclei. *Developmental Biology* **358**: 251-261.

DeBruyn, A. & Meeuwigg, J. 2001. Detecting Lunar Cycles in Marine Ecology: Periodic Regression Versus Categorical ANOVA. *Marine Ecology Progress Series* **214**: 307-310.

Depledge, M. H., Godard-Codding, C. a. J. & Bowen, R. E. 2010. Light Pollution in the Sea. *Marine Pollution Bulletin,* **60:** 1383-1385.

Desjardins, J. K., Fitzpatrick, J. L., Stiver, K. A., Van Der Kraak, G. J. & Balshine, S. 2011. Lunar and Diurnal Cycles in Reproductive Physiology and Behavior in a Natural Population of Cooperatively Breeding Fish. *Journal of Zoology* **285**: 66-73.

Donelan, S. C. & Trussell, G. C. 2020. Sex‐Specific Differences in the Response of Prey to Predation Risk. *Functional Ecology* **34**: 1235-1243.

Donelson, J. M., Munday, P. L. & Mccormick, M. I. 2009. Parental Effects on Offspring Life Histories: When Are They Important? *Biology Letters* **5**: 262-265.

Downing, G. & Litvak, M. K. 2002. Effects of Light Intensity, Spectral Composition and Photoperiod on Development and Hatching of Haddock (*Melanogrammus Aeglefinus*) Embryos. *Aquaculture* **213**: 265-278.

Drazen, J. C., De Forest, L. G. & Domokos, R. 2011. Micronekton Abundance and Biomass in Hawaiian Waters as Influenced by Seamounts, Eddies, and the Moon. *Deep-Sea Research Part I-Oceanographic Research Papers* **58**: 557-566.

Drazen, J. C. & Sutton, T. T. 2017. Dining in the Deep: The Feeding Ecology of Deep-Sea Fishes. *Annual Review of Marine Science* **9**: 337-366.

Ekstrom, P. & Meissl, H. 1997. The Pineal Organ of Teleost Fishes. *Reviews in Fish Biology and Fisheries* **7**: 199-284.

El-Fiky, N. 2002. The Influence of Water pH on the Embryonic Development of Grass Carp, *Ctenopharyngodon Idella*. *Egyptian Journal of Aquatic Biology and Fisheries* **6**: 233-261.

Espinel-Velasco, N., Hoffmann, L., Aguera, A., Byrne, M., Dupont, S., Uthicke, S., Webster, N. S. & Lamare, M. 2018. Effects of Ocean Acidification on the Settlement and Metamorphosis of Marine Invertebrate and Fish Larvae: A Review. *Marine Ecology Progress Series* **606**: 237-257.

Falcón, J., Migaud, H., Muñoz-Cueto, J. A. & Carrillo, M. 2010. Current Knowledge on the Melatonin System in Teleost Fish. *General and Comparative Endocrinology* **165**: 469-482.

Farquharson, K. A., Hogg, C. J. & Grueber, C. E. 2021. Offspring Survival Changes over Generations of Captive Breeding. *Nature Communications* **12**.

Feary, D. A. & Clements, K. D. 2006. Habitat Use by Triplefin Species (*Tripterygiidae*) on Rocky Reefs in New Zealand. *Journal of Fish Biology* **69**: 1031-1046.

Feary, D. A., Wellenreuther, M. & Clements, K. D. 2009. Trophic Ecology of New Zealand Triplefin Fishes (Family *Tripterygiidae*). *Marine Biology* **156**: 1703-1714.

Fobert, E. K., Burke Da Silva, K. & Swearer, S. E. 2019. Artificial Light at Night Causes Reproductive Failure in Clownfish. *Biology Letters* **15**: 20190272.

Fobert, E. K., Schubert, K. P. & Da Silva, K. B. 2021. The Influence of Spectral Composition of Artificial Light at Night on Clownfish Reproductive Success. *Journal of Experimental Marine Biology and Ecology* **540**: 7.

Fopp-Bayat, D., Nitkiewicz, A. & Chandra, G. 2021. First Report on Embryonic and Larval Development of 2n/3n Mosaic Sterlet. *Animal* **15**: 100317.

Forsell, J., Holmqvist, B., Helvik, J. V. & Ekström, P. 1997. Role of the Pineal Organ in the Photoregulated Hatching of the Atlantic Halibut. *International Journal of Developmental Biology* **41**: 591-595.

Forsythe, W. E. 1923. Colour Temperature and Brightness of Moonlight. *Nature* **111**: 533.

Fox, J. & Weisberg, S. 2018. Visualizing Fit and Lack of Fit in Complex Regression Models with Predictor Effect Plots and Partial Residuals. *Journal of Statistical Software* **87**: 1-27.

Francis, M. 2001. *Coastal Fishes of New Zealand: An Identification Guide, 3rd Edn.,* Auckland, NZ: Reed Books Ltd.

Francis, M. 2012. *Coastal Fishes of New Zealand,* Nelson, NZ: Craig Potton Publishing.

Francis, S., Delgoda, R. & Young, R. 2012. Effects of Embryonic Exposure to α-Lipoic Acid or Ascorbic Acid on Hatching Rate and Development of Zebrafish (*Danio Rerio*). *Aquaculture Research* **43**: 777-788.

Fricke, R. 1994. *Tripterygiid Fishes of Australia, New Zealand and the Southwest Pacific Ocean (Teleostei),* Koengistein: Koeltz Scientific Books.

Fuiman, L. A. 2002. Special Considerations of Fish Eggs and Larvae. *In:* Fuiman, L. A. & Werner, R. G. (eds.) *Fishery Science: The Unique Contributions of Early Life Stages.*: Wiley-Blackwell Publishing.

Fukunaga, K., Yamashina, F., Takeuchi, Y., Yamauchi, C. & Takemura, A. 2020. Moonlight is a Key Entrainer of Lunar Clock in the Brain of the Tropical Grouper with Full Moon Preference. *BMC Zoology* **5**.

Fukushiro, M., Takeuchi, T., Takeuchi, Y., Hur, S.-P., Sugama, N., Takemura, A., Kubo, Y., Okano, K. & Okano, T. 2011. Lunar Phase-Dependent Expression of Cryptochrome and a Photoperiodic Mechanism for Lunar Phase-Recognition in a Reef Fish, Goldlined Spinefoot. *PLoS ONE* **6**: e28643.

Fulton, T. W. 1904. The Rate of Growth of Fishes. *Twenty-second annual report*: 141-241.

Gaillard, J. M. & Yoccoz, N. G. 2003. Temporal Variation in Survival of Mammals: A Case of Environmental Canalization? *Ecology* **84**: 3294-3306.

Gaston, K. J., Bennie, J., Davies, T. W. & Hopkins, J. 2013. The Ecological Impacts of Nighttime Light Pollution: A Mechanistic Appraisal. *Biological Reviews* **88**: 912-927.

Gaston, K. J., Davies, T. W., Nedelec, S. L. & Holt, L. A. 2017. Impacts of Artificial Light at Night on Biological Timings. *Annual Review of Ecology, Evolution, and Systematics* **48**: 49-68.

Gaston, K. J. & Sánchez De Miguel, A. 2022. Environmental Impacts of Artificial Light at Night. *Annual Review of Environment and Resources* **47**: 373-398.

Geffen, A. J. & Nash, R. D. M. 2012. Egg Development Rates for Use in Egg Production Methods (Epms) and Beyond. *Fisheries Research* **117-118**: 48-62.

Gehrke, P. C. 1992. Diel Abundance, Migration and Feeding of Fish Larvae (*Eleotridae*) in a Floodplain Billabong. *Journal of Fish Biology* **40**: 695-707.

George, A. E. & Chapman, D. C. 2013. Aspects of Embryonic and Larval Development in Bighead Carp *Hypophthalmichthys Nobilis* and Silver Carp *Hypophthalmichthys Molitrix*. *PLoS ONE* **8**: e73829.

Gerrish, G. A., Morin, J. G., Rivers, T. J. & Patrawala, Z. 2009. Darkness as an Ecological Resource: The Role of Light in Partitioning the Nocturnal Niche. *Oecologia* **160**: 525-536.

Ghalambor, C. K. & Martin, T. E. 2001. Fecundity-Survival Trade-Offs and Parental Risk-Taking in Birds. *Science* **292**: 494-497.

Giménez, L. & Anger, K. 2001. Relationships among Salinity, Egg Size, Embryonic Development, and Larval Biomass in the Estuarine Crab *Chasmagnathus Granulata* Dana, 1851. *Journal of Experimental Marine Biology and Ecology* **260**: 241-257.

Godfray, H. C. J., Partridge, L. & Harvey, P. H. 1991. Clutch Size. *Annual Review of Ecology and Systematics* **22**: 409-429.

Godoy, R. S., Weber, V., Lanés, L. E. K., Reichard, M., Gemelli, T., Hohendorff, R. V. & Maltchik, L. 2021. Recognizing the Enemy: Do Predator Cues Influence Hatching in Neotropical Annual Killifish? *Journal of Fish Biology* **99**: 1476-1484.

Graham, P. M., Franks, J. S., Anderson, E. J., Leaf, R. T. & Tilley, J. D. 2021. Age and Growth of Early Life Stage Atlantic Tarpon *Megalops Atlanticus* from the Northcentral Gulf of Mexico. *Journal of Fish Biology* **99**: 1190-1200.

Grassle, J. F. 2013. Marine Ecosystems. *In:* Levin, S. A. (ed.) *Encyclopedia of Biodiversity.* 2nd ed. Waltham: Academic Press.

Grosberg, R. K. & Levitan, D. R. 1992. For Adults Only? Supply-Side Ecology and the History of Larval Biology. *Trends in Ecology & Evolution* **7**: 130-133.

Guma'a, S. A. 1978. The Effects of Temperature on the Development and Mortality of Eggs of Perch, *Perca Fluviatilis*. *Freshwater Biology* **8**: 221-227.

Halfwerk, W. & Slabbekoorn, H. 2014. The Impact of Anthropogenic Noise on Avian Communication and Fitness. *Avian Urban Ecology: Behavioural and Physiological Adaptations*: 84-97.

Hallgrímsson, B., Jamniczky, H., Young, N. M., Rolian, C., Parsons, T. E., Boughner, J. C. & Marcucio, R. S. 2009. Deciphering the Palimpsest: Studying the Relationship between Morphological Integration and Phenotypic Covariation. *Evolutionary Biology* **36**: 355-376.

Hamel, P., Magnan, P., Lapointe, M. & East, P. 1997. Timing of Spawning and Assessment of a Degree-Day Model to Predict the in Situ Embryonic Developmental Rate of White Sucker, *Catostomus Commersoni*. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 2040-2048.

Hanson, K. C., Arrosa, S., Hasler, C. T., Suski, C. D., Philipp, D. P., Niezgoda, G. & Cooke, S. J. 2008. Effects of Lunar Cycles on the Activity Patterns and Depth Use of a Temperate Sport Fish, the Largemouth Bass, *Micropterus Salmoides*. *Fisheries Management and Ecology* **15**: 357-364.

Hardy, G. S. 1989. The Genus *Forsterygion* Whitley & Phillipps, 1939 (Pisces: Tripterygiidae) in New Zealand and Australia, with Descriptions of Two New Species. *Journal of Natural History* **23**: 491-512.

Hartig, F. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. *R package version 0.4.6*: https://CRAN.R-project.org/package=DHARMa.

Hayward, A. & Gillooly, J. F. 2011. The Cost of Sex: Quantifying Energetic Investment in Gamete Production by Males and Females. *PLoS ONE* **6**: e16557.

Head, A. B. & Malison, J. A. 2000. Effects of Lighting Spectrum and Disturbance Level on the Growth and Stress Responses of Yellow Perch Perca Flavescens. *Journal of the World Aquaculture Society* **31**: 73-80.

Helvik, J. V. & Walther, B. T. 1992. Photo‐Regulation of the Hatching Process of Halibut (*Hippoglossus Hippoglossus*) Eggs. *Journal of Experimental Zoology* **263**: 204-209.

Helvik, J. V. & Walther, B. T. 1993a. Development of Hatchability in Halibut (*Hippoglossus Hippoglossus*) Embryos. *International Journal of Developmental Biology* **37**: 487-490.

Helvik, J. V. & Walther, B. T. 1993b. Environmental Parameters Affecting Induction of Hatching in Halibut (*Hippoglossus Hippoglossus*) Embryos. *Marine Biology* **116**: 39-45.

Hernández-León, S. 2008. Natural Variability of Fisheries and Lunar Illumination: A Hypothesis. *Fish and Fisheries* **9**: 138-154.

Herzig, A. & Winkler, H. 1986. The Influence of Temperature on the Embryonic Development of Three Cyprinid Fishes, *Abramis Brama*, *Chalcalburnus Chalcoides Mento* and *Vimba Vimba*. *Journal of Fish Biology* **28**: 171-181.

Hickey, A. J. R. & Clements, K. D. 2003. Key Metabolic Enzymes and Muscle Structure in Triplefin Fishes (*Tripterygiidae*): A Phylogenetic Comparison. *Journal of Comparative Physiology B* **173**: 113-123.

Hickey, A. J. R., Lavery, S. D., Hannan, D. A., Baker, C. S. & Clements, K. D. 2009. New Zealand Triplefin Fishes (Family *Tripterygiidae*): Contrasting Population Structure and Mtdna Diversity within a Marine Species Flock. *Molecular Ecology* **18**: 680-696.

Hilton, Z., Wellenreuther, M. & Clements, K. D. 2008. Physiology Underpins Habitat Partitioning in a Sympatric Sister-Species Pair of Intertidal Fishes. *Functional Ecology* **22**: 1108-1117.

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., Hänel, A., Kappeler, P. M., Völker, S., Schwope, A., Franke, S., Uhrlandt, D., Fischer, J., Klenke, R., Wolter, C. & Tockner, K. 2010. The Dark Side of Light: A Transdisciplinary Research Agenda for Light Pollution Policy. *Ecology and Society* **15**.

Holzman, R. & Genin, A. 2003. Zooplanktivory by a Nocturnal Coral-Reef Fish: Effects of Light, Flow, and Prey Density. *Limnology and Oceanography* **48**: 1367-1375.

Huang, W.-S. & Pike, D. A. 2011. Climate Change Impacts on Fitness Depend on Nesting Habitat in Lizards. *Functional Ecology* **25**: 1125-1136.

Iglesias, J., Rodriguez-Ojea, G. & Peleteiro, J. B. 1995. Effect of Light and Temperature on the Development of Turbot Eggs (*Scophthalmus Maximus L.*). *ICES Journal of Marine Science* **201**: 40-44.

Ikegami, T., Takeuchi, Y., Hur, S. P. & Takemura, A. 2014a. Impacts of Moonlight on Fish Reproduction. *Marine Genomics* **14**: 59-66.

Ikegami, T., Takeuchi, Y. & Takemura, A. 2014b. Lunar Clock in Fish Reproduction. *In: Annual, Lunar, and Tidal Clocks.* Springer Japan.

Istock, C. A. 1967. The Evolution of Complex Life Cycle Phenomena: An Ecological Perspective. *Evolution* **21**: 592.

Jafari, M., Salleh Kamarudin, M., Saad, C. R., Arshad, A., Oryan, S. & Guilani, M. H. T. 2010. Embryonic Development of Caspian Kutum, *Rutilus Frisii Kutum*. *Journal of the World Aquaculture Society* **41**: 378-390.

Jaroszynska, N., Harding, P. & Moosajee, M. 2021. Metabolism in the Zebrafish Retina. *Journal of Developmental Biology* **9**: 10.

Jawad, L. A. 2008. Second Revision of the New Zealand Triplefin Genus Forsterygion Whitley and Phillips, 1939 (Pisces: Tripterygiidae). *Journal of Natural History* **42**: 2943-2989.

Johannes, R. E. 1978. Reproductive Strategies of Coastal Marine Fishes in the Tropics. *Environmental Biology of Fishes* **3**: 65-84.

Johansson, F. & Rowe, L. 1999. Life History and Behavioral Responses to Time Constraints in a Damselfly. *Ecology* **80**: 1242-1252.

Johnston, T. A., Wiegand, M. D., Leggett, W. C., Pronyk, R. J., Dyal, S. D., Watchorn, K. E., Kollar, S. & Casselman, J. M. 2007. Hatching Success of Walleye Embryos in Relation to Maternal and Ova Characteristics. *Ecology of Freshwater Fish* **16**: 295-306.

Jones, G. 2013. Ecology of Rocky Reef Fish of Northeastern New Zealand: 50 Years On. *New Zealand Journal of Marine and Freshwater Research* **47**: 334-359.

Jones, G. P. 1981. Spawning-Site Choice by Female *Pseudolabrus Celidotus* (Pisces, Labridae) and its Influence on the Mating System. *Behavioural Ecology and Sociobiology* **8**: 129-142.

Jonsson, B. & Jonsson, N. 2014. Early Environment Influences Later Performance in Fishes. *Journal of Fish Biology* **85**: 151-188.

Jørgensen, C., Opdal, A. F. & Fiksen, Ø. 2014. Can Behavioural Ecology Unite Hypotheses for Fish Recruitment? *ICES Journal of Marine Science* **71**: 909-917.

Juntti, S. A. & Fernald, R. D. 2016. Timing Reproduction in Teleost Fish: Cues and Mechanisms. *Current Opinion in Neurobiology* **38**: 57-62.

Kaartvedt, S., Knutsen, T. & Holst, J. 1998. Schooling of the Vertically Migrating Mesopelagic Fish *Maurolicus Muelleri* in Light Summer Nights. *Marine Ecology Progress Series* **170**: 287-290.

Kah, O., Anglade, I., Leprêtre, E., Dubourg, P. & De Monbrison, D. 1993. The Reproductive Brain in Fish. *Fish Physiology and Biochemistry* **11**: 85-98.

Kaminski, R., Kamler, E., Korwin-Kossakowski, M., Myszkowski, L. & Wolnicki, J. 2006. Effects of Different Incubation Temperatures on the Yolk-Feeding Stage of *Eupallasella Percnurus* (Pallas). *Journal of Fish Biology* **68**: 1077-1090.

Kazemi, R., Yarmohammadi, M., Hallajian, A. & J, J. 2020. Influence of the Photoperiod and Light Intensity on Growth Performance, Melatonin and Insulin-Like Growth Factors Gene Expression on *Acipenser Persicus* During the Embryonic Stage. *Iranian Journal of Fisheries Sciences* **19**: 1175-1192.

Khan, J. R. & Herbert, N. A. 2012. The Behavioural Thermal Preference of the Common Triplefin (*Forsterygion Lapillum*) Tracks Aerobic Scope Optima at the Upper Thermal Limit of its Distribution. *Journal of Thermal Biology* **37**: 118-124.

Khan, J. R., Iftikar, F. I., Herbert, N. A., Gnaiger, E. & Hickey, A. J. R. 2014. Thermal Plasticity of Skeletal Muscle Mitochondrial Activity and Whole Animal Respiration in a Common Intertidal Triplefin Fish, *Forsterygion Lapillum* (Family: *Tripterygiidae*). *Journal of Comparative Physiology B* **184**: 991-1001.

Kilambi, R. V. & Galloway, M. L. 1985. Temperature Preference and Tolerance of Hybrid Carp (Female Grass Carp, *Ctenopharyngodon Idella* x Male Bighead, *Aristichthys Nobilis*). *Environmental Biology of Fishes* **12**: 309-314.

Klein, M., Beveren, E. V., Rodrigues, D., Serrão, E. A., Caselle, J. E., Gonçalves, E. J. & Borges, R. 2018. Small Scale Temporal Patterns of Recruitment and Hatching of Atlantic Horse Mackerel (L.) at a Nearshore Reef Area. *Fisheries Oceanography* **27**: 505-516.

Koger, C. S., Teh, S. J. & Hinton, D. E. 1999. Variations of Light and Temperature Regimes and Resulting Effects on Reproductive Parameters in Medaka (*Oryzias Latipes*). *Biology of Reproduction* **61**: 1287-1293.

Komyakova, V., Jaffres, J. B. D., Strain, E. M. A., Cullen-Knox, C., Fudge, M., Langhamer, O., Bender, A., Yaakub, S. M., Wilson, E., Allan, B. J. M., Sella, I. & Haward, M. 2022. Conceptualisation of Multiple Impacts Interacting in the Marine Environment Using Marine Infrastructure as an Example. *Science of the Total Environment* **830**: 18.

Korwin-Kossakowski, M. 2012. Fish Hatching Strategies: A Review. *Reviews in Fish Biology and Fisheries* **22**: 225-240.

Kraak, S. B. M. & Bakker, T. C. M. 1998. Mutual Mate Choice in Sticklebacks: Attractive Males Choose Big Females, Which Lay Big Eggs. *Animal Behaviour* **56**: 859-866.

Kupprat, F., Hölker, F. & Kloas, W. 2020. Can Skyglow Reduce Nocturnal Melatonin Concentrations in Eurasian Perch? *Environmental Pollution* **262**: 114324.

Kupprat, F., Hölker, F., Knopf, K., Preuer, T. & Kloas, W. 2021. Innate Immunity, Oxidative Stress and Body Indices of Eurasian Perch *Perca Fluviatilis* after Two Weeks of Exposure to Artificial Light at Night. *Journal of Fish Biology* **99**: 118-130.

Kusmic, C., Barsanti, L., Passarelli, V. & Gualtieri, P. 1993. Photoreceptor Morphology and Visual Pigment Content in the Pineal Organ and in the Retina of Juvenile and Adult Trout, *Salmo Irideus*. *Micron* **24**: 279-286.

Kyba, C. C. M., Mohar, A. & Posch, T. 2017. How Bright is Moonlight? *Astronomy & Geophysics* **58**: 1.31-1.32.

Kyba, C. C. M., Ruhtz, T., Fischer, J. & Hölker, F. 2011. Cloud Coverage Acts as an Amplifier for Ecological Light Pollution in Urban Ecosystems. *PLoS ONE* **6**: e17307.

Kyba, C. C. M., Ruhtz, T., Fischer, J. & Hölker, F. 2012. Red is the New Black: How the Colour of Urban Skyglow Varies with Cloud Cover. *Monthly Notices of the Royal Astronomical Society* **425**: 701-708.

Lambert, Y. & Dutil, J. D. 2000. Energetic Consequences of Reproduction in Atlantic Cod (*Gadus Morhua*) in Relation to Spawning Level of Somatic Energy Reserves. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 815-825.

Landaeta, M. F., Zavala-Muñoz, F., Palacios-Fuentes, P., Bustos, C. A., Alvarado-Niño, M., Letelier, J., Cáceres, M. A. & Muñoz, G. 2015. Spatial and Temporal Variations of Coastal Fish Larvae, Ectoparasites and Oceanographic Conditions Off Central Chile. *Revista de biología marina y oceanografía* **50**: 563-574.

Last, S., Kim, Hobbs, L., Berge, J., Brierley, S., Andrew & Cottier, F. 2016. Moonlight Drives Ocean-Scale Mass Vertical Migration of Zooplankton During the Arctic Winter. *Current Biology* **26**: 244-251.

Leatherland, J. F., Farbridge, K. J. & Boujard, T. 1992. Lunar and Semi-Lunar Rhythms in Fishes. *In:* Ali, M. A. (ed.) *Rhythms in Fishes.* Boston, MA: Springer.

Lenth, R. V. 2022. emmeans: Estimated Marginal Means, Aka Least-Squares Means. *R package version 1.8.2*: https://CRAN.R-project.org/package=emmeans.

Litvak, M. & Leggett, W. 1992. Age and Size-Selective Predation on Larval Fishes: The Bigger-is-Better Hypothesis Revisited. *Marine Ecology Progress Series* **81**: 13-24.

Longcore, T. & Rich, C. 2004. Ecological Light Pollution. *Frontiers in Ecology and the Environment* **2**: 191-198.

Lowerre-Barbieri, S., Henderson, N., Llopiz, J., Walters, S., Bickford, J. & Muller, R. 2009. Defining a Spawning Population (Spotted Seatrout *Cynoscion Nebulosus*) over Temporal, Spatial, and Demographic Scales. *Marine Ecology Progress Series* **394**: 231-245.

Lowerre-Barbieri, S. K., Ganias, K., Saborido-Rey, F., Murua, H. & Hunter, J. R. 2011. Reproductive Timing in Marine Fishes: Variability, Temporal Scales, and Methods. *Marine and Coastal Fisheries* **3**: 71-91.

Lugowska, K. & Kondera, E. 2018. Early Development of Vimba (*Vimba Vimba*) at Different Temperatures and Temperature-Related Anomalies. *Aquaculture Research* **49**: 2336-2344.

Luz, B. L. P., Di Domenico, M., Migotto, A. E. & Kitahara, M. V. 2020. Life‐History Traits of *Tubastraea Coccinea*: Reproduction, Development, and Larval Competence. *Ecology and Evolution* **10**: 6223-6238.

Marangoni, L. F. B., Davies, T., Smyth, T., Rodríguez, A., Hamann, M., Duarte, C., Pendoley, K., Berge, J., Maggi, E. & Levy, O. 2022. Impacts of Artificial Light at Night in Marine Ecosystems - a Review. *Global Change Biology* **28**: 5346-5367.

Martín-Robles, Á. J., Aliaga-Guerrero, M., Whitmore, David, Pendón, C. & Muñoz-Cueto, J. A. 2012. The Circadian Clock Machinery During Early Development of Senegalese Sole (*Solea Senegalensis*): Effects of Constant Light and Dark Conditions. *Chronobiology International* **29**: 1195-1205.

Martinez, E., Porreca, A. P., Colombo, R. E. & Menze, M. A. 2016. Tradeoffs of Warm Adaptation in Aquatic Ectotherms: Live Fast, Die Young? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **191**: 209-215.

Mazerolle, M. J. 2020. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(C). *R package version 2.3-1*: https://cran.r-project.org/package=AICcmodavg.

Mcdermott, C. & Shima, J. 2006. Ontogenetic Shifts in Microhabitat Preference of the Temperate Reef Fish *Forsterygion Lapillum*: Implications for Population Limitation. *Marine Ecology Progress Series* **320**: 259-266.

Mcgurk, M. 1986. Natural Mortality of Marine Pelagic Fish Eggs and Larvae: Role of Spatial Patchiness. *Marine Ecology Progress Series* **34**: 227-242.

Meissl, H. & Ekström, P. 1988. Photoreceptor Responses to Light in the Isolated Pineal Organ of the Trout, *Salmo Gairdneri*. *Neuroscience* **25**: 1071-1076.

Menaker, M. 1968. Extraretinal Light Perception in the Sparrow. I. Entrainment of the Biological Clock. *Proceedings of the National Academy of Sciences* **59**: 414-421.

Mensink, P. J., Geange, S. W. & Shima, J. S. 2014. Reproductive Success of Parasitized Males in a Marine Reef Fish. *Marine Biology* **161**: 2689-2696.

Mensink, P. J. & Shima, J. S. 2014. Patterns of Co-Occurrence and Interactions between Age Classes of the Common Triplefin, *Forsterygion Lapillum*. *Marine Biology* **161**: 1285-1298.

Mitterwallner, P. & Shima, J. S. 2022. Influence of the Lunar Cycle and Spatial Gradients on Size-Dependent Male and Female Reproductive Investment Decisions of a Protogynous Reef Fish. *Marine Biology* **169**.

Moginie, B. & Shima, J. 2018. Hatch Date and Growth Rate Drives Reproductive Success in Nest-Guarding Males of a Temperate Reef Fish. *Marine Ecology Progress Series* **592**: 197-206.

Moginie, B. M. 2016. *Going All the Way: The Implications of Life History and Phenotype on Reproductive Success of the Common Triplefin Forsterygion Lapillum.* MSc thesis, Victoria University of Wellington.

Moran, D., Softley, R. & Warrant, E. J. 2015. The Energetic Cost of Vision and the Evolution of Eyeless Mexican Cavefish. *Science Advances* **1**: 9.

Mu, X., Zhang, C., Xu, B., Ji, Y., Xue, Y. & Ren, Y. 2021. Accounting for the Fish Condition in Assessing the Reproductivity of a Marine Eel to Achieve Fishery Sustainability. *Ecological Indicators* **130**: 108116.

Myrberg, A. & Fuiman, L. A. 2002. The Sensory World of Coral Reef Fishes. *In:* Sale, P. (ed.) *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem.* San Diego, CA: Academic Press.

Nager, R. G., Monaghan, P. & Houston, D. C. 2001. The Cost of Egg Production: Increased Egg Production Reduces Future Fitness in Gulls. *Journal of Avian Biology* **32**: 159-166.

Nakane, Y., Ikegami, K., Iigo, M., Ono, H., Takeda, K., Takahashi, D., Uesaka, M., Kimijima, M., Hashimoto, R., Arai, N., Suga, T., Kosuge, K., Abe, T., Maeda, R., Senga, T., Amiya, N., Azuma, T., Amano, M., Abe, H., Yamamoto, N. & Yoshimura, T. 2013. The Saccus Vasculosus of Fish is a Sensor of Seasonal Changes in Day Length. *Nature Communications* **4**: e2108.

Navara, K. J. & Nelson, R. J. 2007. The Dark Side of Light at Night: Physiological, Epidemiological, and Ecological Consequences. *Journal of Pineal Research* **43**: 215-224.

Neely, K. L. & Butler, C. B. 2020. Seasonal, Lunar, and Diel Patterns in Spawning by the Giant Barrel Sponge, *Xestospongia Muta*. *Coral Reefs* **39**: 1511-1515.

Neff, M. M., Fankhauser, C. & Chory, J. 2000. Light: An Indicator of Time and Place. *Genes & Development* **14**: 257-271.

Nelson, J. 1994. *Fishes of the World,* Upper Saddle River, NJ: Prentice-Hall.

Niven, J. E. & Laughlin, S. B. 2008. Energy Limitation as a Selective Pressure on the Evolution of Sensory Systems. *Journal of Experimental Biology* **211**: 1792-1804.

Novales Flamarique, I. 2018. Light Exposure During Embryonic and Yolk‐Sac Alevin Development of Chinook Salmon *Oncorhynchus Tshawytscha* Does Not Alter the Spectral Phenotype of Photoreceptors. *Journal of Fish Biology* **95**: 214-221.

O'Connor, J. J., Fobert, E. K., Besson, M., Jacob, H. & Lecchini, D. 2019. Live Fast, Die Young: Behavioural and Physiological Impacts of Light Pollution on a Marine Fish During Larval Recruitment. *Marine Pollution Bulletin* **146**: 908-914.

Olsen, P. D. & Cockburn, A. 1993. Do Large Females Lay Small Eggs? Sexual Dimorphism and the Allometry of Egg and Clutch Volume. *Oikos* **66**: 447-453.

Orr, H. A. 2009. Fitness and its Role in Evolutionary Genetics. *Nature Reviews Genetics* **10**: 531-539.

Osse, J. & Van Den Boogaart, J. 1995. Fish Larvae, Development, Allometric Growth, and the Aquatic Environment. *ICES Marine Science Symposia* **201**: 21-34.

Osse, J. W. M., Vandenboogaart, J. G. M., Vansnik, G. M. J. & Vandersluys, L. 1997. Priorities During Early Growth of Fish Larvae. *Aquaculture* **155**: 249-258.

Papakostas, S., Vøllestad, L. A., Bruneaux, M., Aykanat, T., Vanoverbeke, J., Ning, M., Primmer, C. R. & Leder, E. H. 2014. Gene Pleiotropy Constrains Gene Expression Changes in Fish Adapted to Different Thermal Conditions. *Nature Communications* **5**: e4071.

Park, Y.-J., Park, J.-G., Kim, S.-J., Lee, Y.-D., Saydur Rahman, M. & Takemura, A. 2006. Melatonin Receptor of a Reef Fish with Lunar-Related Rhythmicity: Cloning and Daily Variations. *Journal of Pineal Research* **41**: 166-174.

Park, Y.-J., Park, J.-G., Takeuchi, Y., Hur, S.-P., Lee, Y.-D., Kim, S.-J. & Takemura, A. 2014. Influence of Moonlight on Mrna Expression Patterns of Melatonin Receptor Subtypes in the Pineal Organ of a Tropical Fish. *Marine Genomics* **14**: 67-70.

Parker, G. A. 1992. The Evolution of Sexual Size Dimorphism in Fish*. *Journal of Fish Biology* **41**: 1-20.

Parker, G. A. & Begon, M. 1986. Optimal Egg Size and Clutch Size: Effects of Environment and Maternal Phenotype. *The American Naturalist* **128**: 573-592.

Parsons, D. M., Middleton, C., Smith, M. D. & Cole, R. G. 2014. The Influence of Habitat Availability on Juvenile Fish Abundance in a Northeastern New Zealand Estuary. *New Zealand Journal of Marine and Freshwater Research* **48**: 216-228.

Paulin, C. & Roberts, C. 1992. The Rockpool Fishes of New Zealand (Te Ika Aaria O Aotearoa). Museum of New Zealand (Te Papa Tongarewa).

Pearson, P. R. & Warner, D. A. 2018. Early Hatching Enhances Survival Despite Beneficial Phenotypic Effects of Late-Season Developmental Environments. *Proceedings of the Royal Society B: Biological Sciences* **285**: 20180256.

Penaz, M. 2001. A General Framework of Fish Ontogeny: A Review of the Ongoing Debate. *Folia Zoologica* **50**: 241-256.

Perkin, E. K., Hölker, F., Richardson, J. S., Sadler, J. P., Wolter, C. & Tockner, K. 2011. The Influence of Artificial Light on Stream and Riparian Ecosystems: Questions, Challenges, and Perspectives. *Ecosphere* **2**: 1-16.

Puvanendran, V. & Brown, J. A. 2002. Foraging, Growth and Survival of Atlantic Cod Larvae Reared in Different Light Intensities and Photoperiods. *Aquaculture* **214**: 131-151.

Ragni, M. 2004. Light as an Information Carrier Underwater. *Journal of Plankton Research* **26**: 433-443.

Reddy, P. K. & Lam, T. J. 1991. Effect of Thyroid Hormones on Hatching in the Tilapia, *Oreochromis Mossambicus*. *General and Comparative Endocrinology* **81**: 484-491.

Reid, J. M., Bignal, E. M., Bignal, S., Mccracken, D. I., Bogdanova, M. I. & Monaghan, P. 2010. Parent Age, Lifespan and Offspring Survival: Structured Variation in Life History in a Wild Population. *Journal of Animal Ecology* **74**: 851-862.

Rhodes, J. S. & Quinn, T. P. 1998. Factors Affecting the Outcome of Territorial Contests between Hatchery and Naturally Reared Coho Salmon Parr in the Laboratory. *Journal of Fish Biology* **53**: 1220-1230.

Ribas, S. J., Torra, J., Paricio, S. & Canal-Domingo, R. 2016. How Clouds Are Amplifying (or Not) the Effects of ALAN. *International Journal of Sustainable Lighting* **18**: 32-39.

Ricketts, E. F., Calvin, J., Hedgpeth, J. W., Phillips, D. W. 1985. *Between Pacific Tides,* Standford, CA: Standford University Press.

Ridley, A. R. 2007. Factors Affecting Offspring Survival and Development in a Cooperative Bird: Social, Maternal and Environmental Effects. *Journal of Animal Ecology* **76**: 750-760.

Roff, D. A. & Fairbairn, D. J. 2007. The Evolution of Trade-Offs: Where Are We? *Journal of Evolutionary Biology* **20**: 433-447.

Roseman, C. C. 2020. Exerting an Influence on Evolution. *eLife* **9**: e55952.

Rowe, L. & Ludwig, D. 1991. Size and Timing of Metamorphosis in Complex Life Cycles: Time Constraints and Variation. *Ecology* **72**: 413-427.

Rowe, P. & Epifanio, C. 1994. Flux and Transport of Larval Weakfish in Delaware Bay, USA. *Marine Ecology Progress Series* **110**: 115-120.

Salvanes, A. G. V. & Balino, B. M. 1998. Productivity and Fitness in a Fjord Cod Population: An Ecological and Evolutionary Approach. *Fisheries Research* **37**: 143-161.

Seymour, J. R., Barbasch, T. A. & Buston, P. M. 2018. Lunar Cycles of Reproduction in the Clown Anemonefish Amphiprion Percula: Individual-Level Strategies and Population-Level Patterns. *Marine Ecology Progress Series* **594**: 193-201.

Schligler, J., Cortese, D., Beldade, R., Swearer, S. E. & Mills, S. C. 2021. Long-Term Exposure to Artificial Light at Night in the Wild Decreases Survival and Growth of a Coral Reef Fish. *Proceedings of the Royal Society B: Biological Sciences* **288**: 20210454.

Schmoker, C., Arístegui, J. & Hernández-León, S. 2012. Planktonic Biomass Variability During a Late Winter Bloom in the Subtropical Waters Off the Canary Islands. *Journal of Marine Systems* **95**: 24-31.

Schoenebeck, C. W., Turco, M., Fahrlander, R. M., Darveau, K. M. & Freeman, T. L. 2014. Sex and Age Group Specific Changes in Body Condition of Red-Tailed Hawks in Central Nebraska. *The Wilson Journal of Ornithology* **126**: 575-580.

Septriani, S., Kiddane, A. T., Kim, G. D. & Brown, C. L. 2021. Effects of Different Wavelength from Light Emitting Diodes (Leds) on Growth and Development in Zebrafish (*Danio Rerio*) Embryos and Larvae. *E3S Web of Conferences* **322**: 01033.

Shaffer, M. R., Davy, S. K., Maldonado, M. & Bell, J. J. 2020. Seasonally Driven Sexual and Asexual Reproduction in Temperate Tethya Species. *The Biological Bulletin* **238**: 89-105.

Shima, J. & Swearer, S. 2009. Spatially Variable Larval Histories May Shape Recruitment Rates of a Temperate Reef Fish. *Marine Ecology Progress Series* **394**: 223-229.

Shima, J. S., Noonburg, E. G., Swearer, S. E., Alonzo, S. H. & Osenberg, C. W. 2018. Born at the Right Time? A Conceptual Framework Linking Reproduction, Development, and Settlement in Reef Fish. *Ecology* **99**: 116-126.

Shima, J. S., Osenberg, C. W., Alonzo, S. H., Noonburg, E. G., Mitterwallner, P. & Swearer, S. E. 2020. Reproductive Phenology across the Lunar Cycle: Parental Decisions, Offspring Responses, and Consequences for Reef Fish. *Ecology* **101**.

Shima, J. S., Osenberg, C. W., Noonburg, E. G., Alonzo, S. H. & Swearer, S. E. 2021. Lunar Rhythms in Growth of Larval Fish. *Proceedings of the Royal Society B: Biological Sciences* **288**: 20202609.

Shima, J. S. & Swearer, S. E. 2010. The Legacy of Dispersal: Larval Experience Shapes Persistence Later in the Life of a Reef Fish. *Journal of Animal Ecology* **79**: 1308-1314.

Shima, J. S. & Swearer, S. E. 2016. Evidence and Population Consequences of Shared Larval Dispersal Histories in a Marine Fish. *Ecology* **97**: 25-31.

Shima, J. S. & Swearer, S. E. 2019. Moonlight Enhances Growth in Larval Fish. *Ecology* **100**: e02563.

Simbad.U-Strasbg.Fr. n.d. *Simbad Query Result.* [Online]. Available: http://simbad.u-strasbg.fr/simbad/sim-sam?Criteria=Vmag%3C6.5 [Accessed 7 Jan. 2023].

Smith, A. N., Duffy, C. a. J. & Leathwick, J. R. 2013. *Predicting the Distribution and Relative Abundance of Fishes on Shallow Subtidal Reefs around New Zealand*: Publishing R Core Team, Department of Conservation Wellington, New Zealand.

Smyth, T. J., Wright, A. E., Edwards-Jones, A., Mckee, D., Queiros, A., Rendon, O., Tidau, S. & Davies, T. W. 2022. Disruption of Marine Habitats by Artificial Light at Night from Global Coastal Megacities. *Elementa-Science of the Anthropocene* **10**: 17.

Springer, V. 1998. Blennies. *In:* Eschmeyer, W. & Paxton, J. (eds.) *Encyclopedia of Fishes.* 2nd ed. San Diego, CA: Academic Press.

Stearns, S. C., Kaiser, M. & Kawecki, T. J. 1995. The Differential Genetic and Environmental Canalization of Fitness Components in *Drosophila Melanogaster*. *Journal of Evolutionary Biology* **8**: 539-557.

Stewart, A., Clements, K., Stewart, A. & Struthers, C. 2015. Family *Tripterygiidae*. *The fishes of New Zealand* **4**: 1491-1523.

Sumner, F. B. & Doudoroff, P. 1938. Some Experiments Upon Temperature Acclimatization and Respiratory Metabolism in Fishes. *The Biological Bulletin* **74**: 403-429.

Sutton, S. G., Bult, T. P. & Haedrich, R. L. 2000. Relationships among Fat Weight, Body Weight, Water Weight, and Condition Factors in Wild Atlantic Salmon Parr. *Transactions of the American Fisheries Society* **129**: 527-538.

Sweeney, B. M. 1963. Biological Clocks in Plants. *Annual Review of Plant Physiology* **14**: 411-440.

Syms, C. 1995. Multi-Scale Analysis of Habitat Association in a Guild of Blennioid Fishes. *Marine Ecology Progress Series* **125**: 31-43.

Szabo, A. 2002. Experimental Test of Intercohort Competition for Food and Cover in the Tidepool Sculpin (*Oligocottus Maculosus* Girard). *Canadian Journal of Zoology* **80**: 137-144.

Takemura, A., Rahman, M. S., Nakamura, S., Park, Y. J. & Takano, K. 2004. Lunar Cycles and Reproductive Activity in Reef Fishes with Particular Attention to Rabbitfishes. *Fish and Fisheries* **5**: 317-328.

Takemura, A., Ueda, S., Hiyakawa, N. & Nikaido, Y. 2006. A Direct Influence of Moonlight Intensity on Changes in Melatonin Production by Cultured Pineal Glands of the Golden Rabbitfish, *Siganus Guttatus*. *Journal of Pineal Research* **40**: 236-241.

Tanner, J. E. 1996. Seasonality and Lunar Periodicity in the Reproduction of Pocilloporid Corals. *Coral Reefs* **15**: 59-66.

Taylor, M. H. 1984. Lunar Synchronization of Fish Reproduction. *Transactions of the American Fisheries Society* **113**: 484-493.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. https://www.R-project.org/.

Thompson, S. M. 1979. *Ecological and Behavioural Factors Influencing the Distributionand Abundance Patterns of Tripterygiid Fishes with Particular Reference to Tripterygion Varium.* MSc Thesis, University of Auckland.

Thompson, S. M. & Jones, G. P. 1983. Interspecific Territoriality and Competition Forfood between the Reef Fishes *Forsterygion Varium* and *Pseudolabrus Celidotus*. *Marine Biology* **76**: 95-104.

Thresher, R. 1984. *Reproduction in Reef Fishes,* Neptune City, NJ: T.F.H. Publications.

Tidau, S., Smyth, T., Mckee, D., Wiedenmann, J., D'angelo, C., Wilcockson, D., Ellison, A., Grimmer, A. J., Jenkins, S. R., Widdicombe, S., Queirós, A. M., Talbot, E., Wright, A. & Davies, T. W. 2021. Marine Artificial Light at Night: An Empirical and Technical Guide. *Methods in Ecology and Evolution* **12**: 1588-1601.

Tornquist, M. G. 2020. *Disentangling the Determinants of Reproductive Success in a Temperate Reef Fish, Forsterygion Lapillum.* MSc thesis, Victoria University of Wellington.

Trivers, R. 1972. Parental Investment and Sexual Selection. *In:* Campbell, B. (ed.) *Sexual Selection and the Descent of Man, 1871-1971.* Chicago, IL: Aldine.

Unihedron. 2022. SQM-LU-DL: Operator's Manual. Ontario, Canada: Author.

Venables, W. N. & Ripley, B. D. 2002. *Modern Applied Statistics with S,* New York, NY: Springer.

Villamizar, N., Blanco-Vives, B., Oliveira, C., Dinis, M. T., Di Rosa, V., Negrini, P., Bertolucci, C. & Sánchez-Vázquez, F. J. 2013. Circadian Rhythms of Embryonic Development and Hatching in Fish: A Comparative Study of Zebrafish (Diurnal), Senegalese Sole (Nocturnal), and Somalian Cavefish (Blind). *Chronobiology International* **30**: 889-900.

Vindenes, Y., Langangen, Ø., Winfield, I. J. & Vøllestad, L. A. 2016. Fitness Consequences of Early Life Conditions and Maternal Size Effects in a Freshwater Top Predator. *Journal of Animal Ecology* **85**: 692-704.

Warren, E. J. 1990. *Spawning Patterns within the Breeding Seasons of Favonigobius Lateralis (Family: Gobiidae) and Forsterygion Lapillum (Family: Tripterygiidae).* MSc thesis, University of Auckland.

Watson, W. 2009. Larval Development in Blennies. *In: The Biology of Blennies.* 1st ed. Boca Raton, FL: CRC Press.

Wellenreuther, M. & Clements, K. D. 2007. Reproductive Isolation in Temperate Reef Fishes. *Marine Biology* **152**: 619-630.

Wellenreuther, M. & Clements, K. D. 2008. Determinants of Habitat Association in a Sympatric Clade of Marine Fishes. *Marine Biology* **154**: 393-402.

Wellenreuther, M., Syms, C. & Clements, K. D. 2008. Body Size and Ecological Diversification in a Sister Species Pair of Triplefin Fishes. *Evolutionary Ecology* **22**: 575-592.

Wellenreuther, M., Barrett, P. T. & Clements, K. D. 2009. The Evolution of Habitat Specialisation in a Group of Marine Triplefin Fishes. *Evolutionary Ecology* **23**: 557-568.

Werner, E. E. & Gilliam, J. F. 1984. The Ontogenetic Niche and Species Interactions in Size-Structured Populations. *Annual Review of Ecology and Systematics* **15**: 393-425.

Wheeler, A. 1985. *The World Encyclopedia of Fishes,* London, UK: Macdonald.

Wieser, W. 1995. Energetics of Fish Larvae, the Smallest Vertebrates. *Acta Physiologica Scandinavica* **154**: 279-290.

Wilbur, H. M. 1980. Complex Life Cycles. *Annual Review of Ecology and Systematics* **11**: 67-93.

Willson, M. F. 1981. On the Evolution of Complex Life Cycles in Plants: A Review and an Ecological Perspective. *Annals of the Missouri Botanical Garden* **68**: 275.

Wilner, D., Greenway, E. V., Cirino, L. A. & Miller, C. W. 2019. Environmental Effects on Behavior and Fitness: The Impact of Temporary Nutritional Deprivation on Future Reproduction in the Leaf-Footed Cactus Bug (*Narnia Femorata*). *Integrative and Comparative Biology* **59**: E249-E249.

Wilson, S. W. & Easter, S. S. 1991. A Pioneering Growth Cone in the Embryonic Zebrafish Brain. *Proceedings of the National Academy of Sciences* **88**: 2293-2296.

Yamagami, K., Hoar, W. S. & Randall, D. J. 1988. Mechanisms of Hatching in Fish, . *Fish Physiology* **XI**: 447-499.

Yamahira, K. 2001. Experimental Examination of Interpopulation Variation in Reproductive Timing of a Fish. *Oecologia* **128**: 389-399.

Yu, T. & Guo, Y. 2018. Early Normal Development of the Paradise Fish *Macropodus Opercularis*. *Russian Journal of Developmental Biology* **49**: 240-244.

Zhang, W., Zhi, H. & Sun, H. 2020. Effects of Heavy Metal Pollution on Fitness and Swimming Performance of *Bufo Raddei* Tadpole. *Bulletin of Environmental Contamination and Toxicology* **105**: 387-392.

Ziegelbecker, A. & Sefc, K. M. 2021. Growth, Body Condition and Contest Performance after Early‐Life Food Restriction in a Long‐Lived Tropical Fish. *Ecology and Evolution* **11**: 10904-10916.