There and Back Again: Spatial and Temporal Variation in the Recruitment Dynamics of an Amphidromous Fish

Conor Neilson

A thesis submitted to Victoria University of Wellington in partial fulfilment of the requirements f
Victoria University of Wellington

2016

Contents

Chapter 1

Preface

One planet, one experiment

- E.O. Wilson

1.1 Abstract

A primary goal of ecology is to identify the factors underlying recruitment variability, and how they may shape population dynamics. Recruitment is driven by the input of new individuals into a population. However, these individuals often show high diversity in phenotypic traits and life histories, and the consequences of this variation are poorly understood. Phenotypic variation is widespread among the early life stages of fish, and this variation may be influenced by events occurring across multiple life stages. While many studies have investigated phenotypic variation and its effect on population dynamics, comparatively few studies use an integrated approach that evaluates patterns and processes across multiple life history stages. Here I focus on a native amphidromous fish, *Galaxias maculatus*, and I explore patterns and consequences of phenotypic variation during larval stages, migratory stages, and post-settlement stages of this fish.

I explore variability in phenotypes and early life history traits of G. maculatus through both space and time. I use metrics derived from body size and otolith-based demographic reconstructions to quantify potentially important early life history traits. I found that cohorts of juvenile fish sampled later in the year were comprised of individuals that were older, smaller, and grew more slowly relative to fish sampled earlier in the year. I also found that two sampled sites (the Hutt River and the Wainuiomata River) showed different temporal trends, despite their close geographical proximity.

I then investigated whether phenotype was related to mortality. I used otolith-based traits to characterise larval 'quality' for individual fish. I then calculated the average larval quality for discrete cohorts of fish, and used catch-curve analysis to estimate mortality rates for these cohorts. I investigated the overall relationship between quality and mortality, and compared the trend between two sites. My results indicate that phenotype and mortality were not significantly correlated. However, this inference may be limited by low statistical power; the non-significant trends suggest that the relationship might be negative (i.e., larvae of higher quality tend to have lower rates of mortality). This trend is typical of systems where population expansion is limited by food rather than predators.

I then investigated whether phenotypic traits in the juvenile cohorts were correlated with traits in adult cohorts. I resampled the focal populations ~6 months after sampling the juvenile stages (i.e., targeting fish from sampled cohorts that had survived to adulthood), and I used data from otoliths to reconstruct life history traits (hatch dates and growth histories). I compared adult life history traits to the traits of discrete juvenile cohorts.

My results suggest that fish that survived to adulthood had comparatively slower growth rates (reconstructed for a period of larval/juvenile growth) relative to the sampled juvenile cohorts (where growth rate was estimated for the same period in their life history). I also found that the distributions of hatch dates varied between sites. Fish that survived to adulthood at one site hatched later in the breeding season, while adult stages from the other site had hatch dates that were distributed across the entire breeding season. Both hatch date and growth rate are likely linked to fitness, and their interaction may have influenced patterns of survival to adulthood. These results provide evidence for carry-over effects of larval phenotype on juvenile success.

Collectively my thesis emphasises the importance of phenotype and life history variability in studies of recruitment. It also highlights the importance of spatial scale, and how biological patterns may differ between geographically close systems. Some of the general inferences from my study may extend to other migratory Galaxiid species, and perhaps more generally, to many species with extensive larval dispersal. Finally, my work highlights potentially important interactions between phenotypes, life histories, and mortality, which can ultimately shape recruitment, and the dynamics of populations.

1.2 Acknowledgements

I always suspected that the acknowledgements section would end up being the longest section of this thesis. In truth, there has been a phenomenal amount of people who have contributed to this in some way, and it wouldn't feel right if I didn't thank you all.

First and foremost, I want to thank my supervisor, Jeff Shima. Jeff, thank you for everything you've done for me over the past two years. You have helped me to grow and develop as a scientist, and your input has always been appreciated. Thank you especially for reigning in the first thesis plan I submitted to you. That would have kept me working until 2020! My gratitude also goes out to the members of the Shima Lab. Thanks for listening to me rabbit on about whitebait, and for providing support and advice.

To the VUCEL community, I've really enjoyed being a part of this group of people. Cheers for the BBQs, the morning teas, and the general get-togethers. You've all made my Master's a fantastic experience. John, Dan, and Snout, thank you for all the technical assistance. Everyone would be lost without you three!

This thesis wouldn't have been possible without the small army of volunteers I had come and assist with whitebaiting. In no particular order, thank you to Kayla, Tory, Savita, Heyes, Andrew, Chris, Vinnie, Jessie, Ali, Mel, Eden, Emily, Anna, Jordan, James, and Max. I also want to thank John, Danny, Tom, Kelly, and Jim for donating samples and general advice on whitebaiting.

Chris, Jess, and Vinnie, thanks for being my partners in crime during this journey. It's been great to collaborate, share data, and tackle Galaxiid ecology as a team. Cheers for listening to my ridiculous experimental ideas, and stopping me using models that no normal human would run. Vinnie, thank you in particular for your incredible amount of help in the field. You made me keep going when I was ready to give up, and kept on pushing when everything kept going wrong.

To all of my friends, and particularly my flatmates, thank you for understanding why I neglected you. Your support has meant the world to me. Thanks also needs to be said to Alex, for getting me out of a tight spot, Ben, for some much needed advice, Lisa Woods, who knows more about statistics than anyone I've met, and Phoebe, for answering seemingly endless questions about everything.

Chris, this concludes five years of us studying together. Thank you for always being there as a source of advice, ideas, and generally helping me to feel better when everything goes wrong. I'm going to miss working alongside you.

There are three people in particular I need to mention. Snout, thank you so much for your guidance. This thesis never would have got here without you. Your knowledge of logistics, fieldwork, otoliths, and everything in between has been invaluable to me, and I cannot thank you enough for all your patience. Also, your cooking skills are second to none! Secondly, I owe a huge debt of gratitude to Mark Kaemingk. Mark, you have been like a second supervisor to me. You introduced me to whitebait, and you have totally changed the way I think about science. This thesis has been shaped by you in so many ways, and it has been a true pleasure having you as a mentor and friend.

And to my partner Elyse. Thank you for all your love and support. You may have no interest in fish population ecology, but you understood my passion, and always encouraged and supported me.

Lastly, I want to say a massive thank you to my parents, Ian and Vicki. You have always supported me in whatever path I chose to pursue, and for that I am thankful.

Chapter 2

Introduction

Understanding the patterns, causes, and consequences of recruitment variability in marine systems is one of the primary goals among marine ecologists (Hjort 1914, Fogarty et al. 1991, Pepin 1991, Caley et al. 1996, Sutherland et al. 2013, Johnson et al. 2014). Many marine organisms have stage-structured life cycles with a distinct larval and adult stage (Thorson 1950). Mortality rates are extremely high during the larval stage (McGurk 1986, Rumrill 1990, Gosselin and Qian 1997), and even small variations in these rates can drive large fluctuations in the abundance of individuals surviving to adulthood (Houde and Hoyt 1987). While many early studies have focused on how larval abundance may regulate recruitment through density-dependent processes (Hjort 1914, Roughgarden et al. 1988, Jones 1990, Murdoch 1994, Caley et al. 1996), there has been a growing appreciation for how the phenotypic composition of a population may affect population dynamics (Gaillard et al. 2000, Schoener 2011). Marine species with planktonic larval stages have the potential to undertake long distance dispersal (Thorson 1950), and encountering novel environments during this dispersal may cause phenotypic plasticity in individuals (Agrawal 2001). However, understanding how phenotype distributions can explicitly drive changes in population dynamics remains a difficult task (Saccheri and Hanski 2006). Thorough understandings of phenotype distributions in both larval and adult populations, and the fitness benefits of these phenotypes, are essential for understanding population dynamics (Johnson et al. 2014).

2.1 Drivers of recruitment

Recruitment dynamics are fundamentally driven by the supply of larvae, both in quantity and quality, which in turn depends on dispersal (Roughgarden et al. 1988, Fogarty et al. 1991, Caley et al. 1996, Cowen and Sponaugle 2009). The processes affecting dispersal can be broadly categorised into physical processes

and biological traits (Largier 2003, Pineda et al. 2007). Coastal environments can experience strong interactions between topography, water columns, tidal forces, and wind (Largier 2003), variations in which may either promote long distance dispersal or high rates of retention. Landscape features like eddies (Sponaugle et al. 2005), heterogeneous bottom topography (Largier 2003), and frontal convergences (Graham and Largier 1997) will likely restrict access to offshore currents and limit dispersal. Furthermore, larvae can disperse through active or passive means. Many invertebrates and plants are likely to be passive dispersers, whereas fish may more commonly have actively swimming larvae (Cowen 2002, Leis 2006). Regardless of mechanism, dispersal will determine which environments individuals will encounter (Cowen and Sponaugle 2009, Pfaff et al. 2015), and these environments may then affect the survival and phenotype of individuals (Jonsson 1985, Kerr and Secor 2009). Phenotypic traits are known to vary extensively among individuals (Cushing 1975, Jenkins and King 2006, Shima and Swearer 2009), and these traits may be sensitive to surrounding conditions (Houde and Zastrow 1993).

Genetics will obviously play a considerable role in the quality of individuals, as will pre-hatch factors such as parental condition (McCormick 2006), and reproductive timing (Cargnelli and Gross 1996). However, many marine species display substantial phenotypic plasticity in response to environmental factors. Current paradigms suggest that dispersal pathways may change stochastically in time and space (Siegel et al. 2003, Woodson and McManus 2007), so therefore these pathways will determine what environments will be encountered (Cowen and Sponaugle 2009). Phenotype can determine the quality of an individual, and therefore its rearing environment can have substantial impacts on success (Pepin 1991, Shima and Swearer 2009). While many phenotypic traits can be environmentally influenced, growth and size are among the most responsive and most studied (Anderson 1988, Litvak and Leggett 1992, Meekan et al. 2003, Sponaugle and Pinkard 2004, Phillips 2005, Sponaugle et al. 2006, Fiksen et al. 2007). Growth is often correlated with condition, and therefore growth has been used as a proxy to infer fish quality (Bolger and Connolly 1989, Rätz and Lloret 2003, Shima and Swearer 2009). Early work supported the 'bigger is better' hypothesis, suggesting that larger, faster growing individuals are less susceptible to size-selective mortality (Oliver et al. 1979, Post and Prankevicius 1987, Miller et al. 1988, Tsukamoto et al. 1989, Cargnelli and Gross 1996). The growth-mortality framework of Anderson (1988) provided three conceptual mechanisms for the relationship between growth and mortality. First, if mortality is a function of size, then larger individuals of equal age will experience lower rates of mortality (Leggett and Deblois 1994). Second, if mortality is inversely related to size, then faster growing individuals will have lower mortality rates as they spend less time at vulnerable sizes (Ware 1975). Third, if mortality is dependent on ontogeny, and juveniles have lower mortality rates than larvae, then individuals that develop the fastest and transition from larvae to juvenile earliest will experience the lowest mortality (Chambers and Leggett 1987). However, subsequent studies have found either a lack of, or contradictory

support for faster growth being beneficial for survival (Amara et al. 1994, Good et al. 2001, Munch et al. 2003, Holmes and McCormick 2006). Predators were also proposed to be the mechanism regulating the growth-mortality hypothesis through size selective mortality (Bailey and Houde 1989), and predation is thought to be the dominant regulating mechanism especially in freshwater systems (Werner et al. 1977, Tonn and Paszkowski 1986, Savino and Stein 1989). However, contrary to the 'bigger is better' hypothesis, predators have been shown to select larger prey due to their increased visibility (Litvak and Leggett 1992). There remains substantial evidence that growth and phenotype have significant effects on individual success, but the direction and context may be system dependent.

Dispersal typically occurs during the larval stage, and is completed when larvae metamorphose into the adult form at settlement. However, pelagic species may also disperse as juveniles or adults (Cowen and Sponaugle 2009). In particular, migratory species often disperse in their metamorphosed form, meaning they must adopt life history strategies to survive in a range of environments. Timing of migration movements can coincide with ontogenetic shifts, and evidence suggests that selective processes may change with ontogeny (Meekan et al. 2006, Gagliano et al. 2007). Studies on reef fish indicate that selective processes often favour fish that settle young and grow fast (Grorud-Colvert and Sponaugle 2011). However, selective pressures may change with settlement, ontogeny, and habitat, and high condition in one life stage may not be an indicator of success in later life stages (Johnson and Hixon 2010). Carry-over effects (i.e., effects of early life history on subsequent life stages), have been documented throughout the animal kingdom (amphibians: Smith 1987, Berven 1990, Scott 1994, insects: Taylor et al. 1998, marine invertebrates: Crean et al. 2011, birds: Norris 2005, Sorensen et al. 2009, and fish: Ward and Slaney 1988, Shima and Findlay 2002, Gagliano et al. 2007, Grorud-Colvert and Sponaugle 2011). Carry-over effects can be widespread in fish due to the prevalence of migratory species that will naturally develop in different habitats over their life cycle. In particular, species with diadromous life cycles, such as amphidromy, make excellent model systems for studying these effects, as many amphidromous fish will develop into juveniles in saltwater, and then into adults in freshwater. Amphidromy is distinct from its sister categories, anadromy and catadromy, due to the migration across biomes being trophic rather than gametic (McDowall 2007). Whereas anadromous and catadromous fish cross the marine/freshwater biome as reproductively mature adults and immediately undertake spawning (Myers 1949), amphidromous fish continue to develop into adults after migration and will spawn after undertaking further development in freshwater (McDowall 2007). Undertaking diadromous migrations is energetically costly, however the primary benefit appears to be exploiting the food rich marine environment (Gross et al. 1988, Edeline 2007). Food availability in oceans is known to vary with temperature, upwelling, and nutrient supply (Bunt 1975), and there is evidence that migration patterns appear to follow food supply (Gross et al. 1988). Food and temperature are known to be the primary determinants of growth rate (Houde and Zastrow 1993), so

fish phenotypes are likely to vary during migration as they experience different environmental factors (Schluter et al. 1991, Searcy and Sponaugle 2001, Gagliano et al. 2007, Johnson and Hixon 2010). For species with migratory life cycles, phenotypes conferring high larval fitness may become disadvantageous in the juvenile or adult stages due to new challenges posed by a novel environment.

Fish present an excellent system for studying phenotypic plasticity, carry-over effects, and recruitment dynamics, due to a daily record of their growth history being recorded in their otoliths (small calcium carbonate structures that are found in the inner ear; Campana and Neilson 1985). Otoliths form by regular accumulation of growth rings, which can be used to infer growth history, determine age (Pannella 1971), and identify major events in an individual's life history (Victor 1982). A variety of hard structures have been used for seasonal growth validation, including vertebrae (Brown and Gruber 1988), opercula (Baker and Timmons 1991), scales (Robillard and Marsden 1996), and fin rays (Cass and Beamish 1983). However, the use of otoliths is the most commonly applied method and allows accurate reconstructions of recruitment patterns (Casselman 1987, Wilson and McCormick 1997). Measuring the distance between successive rings can be used to estimate daily somatic growth (Campana and Neilson 1985). While otoliths provide a powerful analytical tool, they must be treated with caution. Abrupt and intense physiological changes may decouple the relationship between otolith growth and somatic growth (Francis et al. 1993, Hoey and McCormick 2004, Baumann et al. 2005, Baumann and Gagliano 2011). This can often occur at settlement, meaning that post-settlement otolith rings may not be a reliable indicator of growth (Hoey and McCormick 2004). Thus, interpretations of otolith growth and somatic growth must include an understanding of the life history and ecological context of the species of interest.

While the formation of rings is influenced by physical processes, a critical step in the accurate aging of fish is the validation of rings forming in a regular pattern. This has been done for a considerable number of species (Taubert and Coble 1977, Fowler and Doherty 1992, Stewart et al. 1995, Newman et al. 1996, Vigliola 1997, Cappo et al. 2000, Vilizzi and Copp 2013, Peel et al. 2016, Taylor et al. 2016), and for the focal species of this thesis, Galaxias maculatus (McDowall et al. 1994).

2.2 Study species

The geographically widespread fish Galaxias maculatus provides an excellent study species for observational evaluations of recruitment dynamics. G. maculatus is an amphidromous fish that is found throughout New Zealand, Australia, and South America (McDowall 1978, Berra et al. 1996, Cussac et al. 2004). Adult G. maculatus lay their eggs amongst submerged vegetation during high spring tides (McDowall and Charteris 2006). Eggs are exposed to the air as the tide recedes and develop in this moist environment for approximately two

weeks, before hatching with the next spring tide and dispersing into the marine environment (Benzie 1968a). Larvae will spend three to six months developing in the marine environment before migrating back to freshwater streams as metamorphosed juveniles (McDowall et al. 1994). The majority of these migrations take place from August to November (McDowall and Eldon 1980). Juvenile fish settle further up the river and develop into reproductively mature adults over the ensuing six months (Cussac et al. 1992). Mature adults move downstream to spawn in estuaries, and will typically die following spawning (Benzie 1968a). During this thesis I will be discussing recruitment at several life stages, both in the traditional sense of juvenile fish being added to the adult population (Fogarty et al. 1991), and in the sense of migrating juveniles entering the freshwater river. At migration, when juvenile fish enter a freshwater stream they can be considered 'recruiting' to the stream. Therefore, juveniles caught at the river mouth will be referred to in this thesis as 'recruits'.

G. maculatus individuals show very high phenotypic plasticity (Barriga et al. 2012). Studies have validated plastic responses to changes in temperature, food availability, and predation risk. Food rich environments promote deeper bodies with shorter caudal peduncles, and vice versa in food limited environments (Kekalainen et al. 2010). Body size can also change in response to predation risk, favouring streamlined shapes that promote efficient swimming (Milano et al. 2006). Furthermore, both the migrating juveniles and the spawning adults can be easily caught, which facilitates identification of shifts in phenotypic distributions across life stages.

2.3 Thesis research

This thesis has three primary aims: (1) to characterise the extent of phenotypic variability at recruitment in early life history traits of G. maculatus, (2) to estimate mortality rates for spatially and temporally discrete cohorts of juvenile G. maculatus, and (3) to determine the effect of early life history traits on future success. In Chapter 2, I compare phenotypes of recruiting G. maculatus, both spatially across sites and temporally within sites. In Chapter 3, I estimate mortality rates for cohorts of recruits and assess whether these mortality rates vary as a function of larval quality. In Chapter 4, I quantify the early life history traits of adult fish to determine whether specific phenotypes show higher success than others. In Chapter 5, I synthesise the results from the previous three chapters and discuss hypotheses generated from these studies. This thesis represents a longitudinal study that investigates G. maculatus recruitment at three distinct life stages, and thus it represents one of the few studies that takes a holistic view of recruitment across the entire life cycle. By considering the entire life history, I provide a more complete understanding of recruitment in an amphidromous fish; a complex and difficult dynamic rate function to understand.

I have prepared the following data chapters in the form of independent manuscripts to facilitate submission to peer-reviewed journals. Therefore, each data chapter has its own Introduction and Discussion section, and consequently, there is some repetition across chapters.

Chapter 3

Phenotypic Variation of Recruting *Galaxias Maculatus* Over Small Spatial and Temporal Scales

3.1 Introduction

Recruitment is notoriously variable among fish populations, both in marine and freshwater systems (Houde 1994, Caley et al. 1996). While most studies focus on fluctuations in the abundance of recruits and their subsequent effects on year class strength (Hjort 1914, Houde and Hoyt 1987, Fogarty et al. 1991, Bailey 1994, Bjørnstad et al. 1999, Bastrikin et al. 2014), there is also extensive variation in the phenotype and developmental histories of these recruits (Houde 1989, Hadfield and Strathmann 1996, Searcy and Sponaugle 2000, Grorud-Colvert and Sponaugle 2006, Sponaugle et al. 2006). Fish populations also experience very high mortality during their early life stages (Dahlberg 1979, Bailey and Houde 1989, Sogard 1997, Chambers and Trippel 2012). Marine larvae will often disperse during their larval stage and settle away from their natal origin (Cowen and Sponaugle 2009). During this dispersal phase, individuals may experience highly fluctuating and unpredictable environments that can shape phenotypes, alter the expression of life histories, or ultimately die if they cannot adapt (Stearns 1992). Variation in phenotypes across populations may suggest local adaptation to a larval rearing environment (Harrod et al. 2010). Therefore, phenotype may be useful to infer dispersal patterns, developmental history and successful matches to environments encountered.

Variation in phenotype can result from several different biological processes.

Natural levels of genetic variation will produce distributions of phenotypic traits, which have varying levels of representation in the population (Shapiro et al. 2004). These traits may then be further influenced during ontogeny (Losos et al. 2000, Trussell and Smith 2000, Bergenius et al. 2005). For instance, variation in fitness-linked traits may lead to certain individuals experiencing higher levels of mortality than phenotypically different conspecifics (Searcy and Sponaugle 2001), which can reduce the frequency of the more susceptible phenotype. Several studies have demonstrated this selective mortality on variable life history traits, i.e. size and growth rate (Anderson 1988, Sogard 1997), and body condition (Buijse and Houthuijzen 1992, Hoey and McCormick 2004). Alternatively, environmental influences may cause some traits to show plasticity in response to conditions experienced by individuals. Phenotypic plasticity is well documented in fish, and phenotypes have been shown to be responsive to food availability (Günther et al. 2015), temperature (Fouzai et al. 2015), predation pressure (Kekalainen et al. 2010), and water flow (Imre et al. 2002). There is evidence that these early life experiences can shape an individual's developmental trajectory and future success (i.e., carry-over effects) and therefore it is critical to understand the extent of variation in these early life histories (Shima and Findlay 2002).

I chose to examine the recruitment dynamics of the amphidromous fish, Galaxias maculatus, a geographically widespread species native to New Zealand (McDowall 1968). After spending approximately six months developing in the open ocean, G. maculatus migrate to freshwater streams as metamorphosed juveniles (McDowall et al. 1994). During this migration, they can be caught just as they enter the mouth of the river. While they are known to migrate year round, peak spawning season is from March to June, and peak recruitment season is from August to November (McDowall et al. 1994). It is generally assumed that amphidromous species (and G. maculatus specifically) do not show high levels of natal homing, and therefore adult populations are made up of individuals originating from multiple natal origins (Fitzsimons et al. 1990, Radtke and Kinzie 1996, Waters et al. 2000, McDowall 2003, Hickford and Schiel 2016). Therefore, marine returning cohorts of G. maculatus are likely comprised of individuals of different natal origin and dispersal pathways. Due to spatial variation in environmental factors such as food availability and water temperature, fish with differing dispersal pathways may have experienced different environmental conditions during ontogeny (Moody et al. 2015). These conditions can result in phenotypic changes of fish if they have spent sufficient time in said environment (Chambers 1993).

Recruitment is well known to vary over a range of spatial and temporal scales, both for G. maculatus (McDowall and Eldon 1980, McDowall 1994, Barbee et al. 2011), and in other fish species (Myers et al. 1997). However, comparatively few studies have addressed how variable G. maculatus recruitment might be over very small temporal (i.e., day to day) and spatial (i.e., <20 km) scales. The aim of this chapter was to investigate the extent of phenotypic variation among spatially and temporally discrete cohorts of recruiting juvenile G. maculatus.

Specifically, I sampled juvenile fish in the peak recruitment season across two spatially unique sites through time, and measured individual traits (e.g., growth, size) known to be responsive to environmental variation. I hypothesized that I would find differences in phenotypes over larger temporal scales (i.e., month to month), but not over smaller temporal (i.e., day to day), or spatial scales. I analyse differences in developmental characteristics over these separate scales, and conclude with a discussion of potential causes and consequences of this variation.

3.2 Methods

3.2.1 Fish Collections

I sampled juvenile Galaxias maculatus from two rivers in the Wellington region: the Hutt River and the Wainuiomata River (3.1). The two river mouths are spatially separated by approximately 20km (as the crow flies). The Hutt River empties into Wellington Harbour, which is a semi-sheltered, mixed, and productive environment (Maxwell 1956). In contrast, the Wainuiomata River empties into Cook Strait, which is more exposed, with fast flowing currents, and is less nutrient rich (Bowman et al. 1983). I collected fish on a monthly schedule between August and November 2015, fishing over a period of four consecutive days within each month (16 days total, both sites were sampled on each day). Each river was sampled simultaneously during fishing days to minimize temporal variability across sites. All fishing was conducted close to the river mouth (<500m inland for the Hutt River, <100m inland for the Wainuiomata). Standard gear used by whitebaiters generally consists of A-frame set nets (65 x 120 cm frame; 90 cm long; 2 mm mesh) or sock nets (75 x 113 cm frame; 220 cm long, 3 mm mesh). Set nets are suited for shallow rivers and correspondingly slow currents, while sock nets fish better in deep rivers with fast currents. For this reason I used two A-frame set nets in the Hutt River, placed within 1m of the riverbank, and one A-frame and one sock net in the Wainuiomata River. Nets were set approximately two hours before high tide, and fishing was conducted for approximately four hours. Local fisherman occasionally supplied samples onsite, which I used to supplement my own collections. Collected individuals were returned to the Victoria University Coastal Ecology Laboratory (VUCEL), euthanized in accordance with AEC permit 22038, and preserved in 99.9% ethanol for further analysis.

3.2.2 Evaluating Developmental Characteristics

I randomly sub-sampled daily catches for a target sample size of 30 fish per river per day for further analysis. I successfully caught fish on 15 separate days in the Hutt River, and 11 days in the Wainuiomata River. For days in which fewer

than 30 fish were available I used all collected individuals (average sample size per day = 23 fish; 20 days had a sample size > 10 fish. During November, the Wainuiomata River was closed due to gravel build up, preventing juvenile G. maculatus from entering the river. Therefore, no samples were collecting during November in the Wainuiomata River.

To estimate fish size I photographed each fish using an Olympus TG-3 camera with a reference ruler in the photo frame. Standard length measurements were obtained with ImageJ v1.49 (Schneider et al. 2012). I extracted the sagittal otoliths from each fish to measure age and growth history. I cleaned one otolith from each pair by placing it in a solution of 15% H2O2 buffered with NaOH for 16 hours. To expose daily growth rings I embedded the otoliths in resin, and polished them along the sagittal plane using a 3 m diamond lapping film. Otoliths were then photographed at either 200x or 400x magnification using a Canon EOS 70D camera connected to a Leica compound microscope. Between 2 and 5 photographs were taken of each otolith at slightly different focal planes (but with the same field of view) to expose all growth rings; photographs were then stitched together to make a composite image using GIMP v2.8.16 (GIMP Team 2016).

Composite images were analysed using the Otolith M app in Image-Pro Premier v9.1 (Media Cybernetics 2016). I counted the daily rings manually, and I measured the distance between each successive daily ring. I estimated 'age' as the number of daily rings, and average otolith growth rate as the length of the otolith radius divided by total number of daily rings.

3.2.3 Statistical Analysis

To evaluate spatio-temporal variation in G. maculatus developmental characteristics I fit three nested linear models (using standard length, average growth rate, and age as response variables in three separate models). Predictor variables included in each model were *site* (Hutt and Wainuiomata), *month* (4 months in the Hutt, 3 in the Wainuiomata), and *day* (4 days per month for each site). I included main effects of site, month, and day, and the interaction term of site x month. The day effect was nested within the interaction term as I only wanted to compare days that occurred within the same month and site. I hypothesized that all three response variables would show different patterns across months given divergent dispersal patterns and associated environmental conditions experienced. I did not expect to see any differences across days or between sites as I assumed larvae would all have experienced similar environmental conditions (or similar enough that differences would not be detectable). Therefore, I treated all terms in the model as fixed effects so I could specifically evaluate the differences between the levels of each factor. I conducted post hoc tests, using the 'lsmeans' procedure from the 'lsmeans' package (Lenth 2016), to evaluate 4 aspects of each model: Do developmental characteristics (1) vary between sites (main effect: site); and (2) vary across months (main effect: month). (3) Does

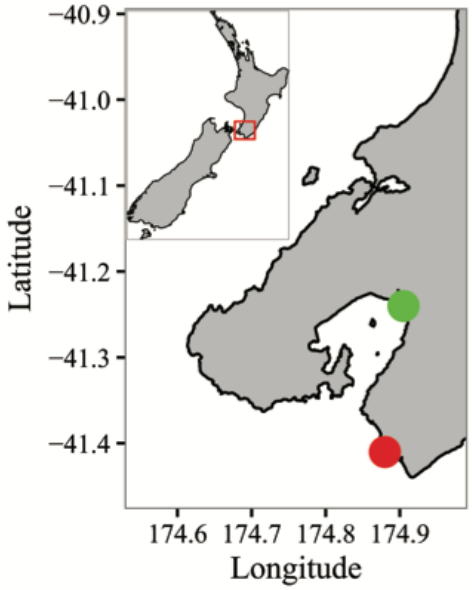

Figure 3.1: Sampling locations for juvenile G. maculatus. Green = Hutt River. Red = Wainuiomata River. River mouths are approximately 20 km apart. Data for the maps comes from the 'maps' (Becker et al. 2016) and 'mapdata' (Becker et al. 2016) R packages.

the pattern of variation between sites differ across months (interaction: month x site). (4) Using the nested term I also evaluated variation in developmental characteristics across days within sites and months (nested main effect: day). When there was a significant interaction, I ran post hoc tests to evaluate aspects (1) and (2), see above. If there was no significant interaction, post hoc tests were run on each main effect.

3.3 Results

I evaluated spatial and temporal variation in developmental characteristics with a sample of 496 fish. Standard length ranged from 33.7 to 51.2mm (mean = 45.5, SD = 2.3). Ages ranged from 105 to 233 days (mean = 175, SD = 18.5). Otolith growth rates ranged from 1.27 to 2.25 m-1day-1 (mean = 1.67, SD = 0.163).

3.3.1 Spatio-temporal variation in standard length

I found a non-significant effect of the interaction term ($F_{2, 470} = 1.95$, $p = 0.144$, 3.2) suggesting that patterns of variation in length across months were similar between sites. Therefore I evaluated main effects. Fish from the Wainuiomata River were longer than fish from the Hutt River (main effect of site variable, $F_{1, 470} = 10.74$, $p = 0.001$, 3.3).

	Standard length			**Growth rate**			**Age**		
	d.f.	F	p	d.f.	F	p	d.f.	F	p
Site	1	10.74	0.001	1	2.88	0.090	1	11.97	<0.001
Month	3	38.11	<0.001	3	6.95	<0.001	3	6.45	<0.001
Site:Month	2	1.95	0.144	2	6.49	0.002	2	7.74	<0.001
(Site:Month)/Day	19	5.21	<0.001	19	5.27	<0.001	19	5.35	<0.001
Residual	470			470			470		

Figure 3.2: Spatio-temporal variation in length, growth rate, and age of juvenile G. maculatus. "Site:Month" represents the interaction term, and "(Site:Month)/Day" represents the day term, nested within the month and site interaction term.

Length also varied across months (main effect of month variable, $F_{3, 470} = 38.11$, $p < 0.001$, 3.4). A post hoc test revealed that fish caught in August were significantly larger than fish from September ($p < 0.0001$), October ($p = 0.0026$), and November ($p < 0.0001$). Fish from September and October

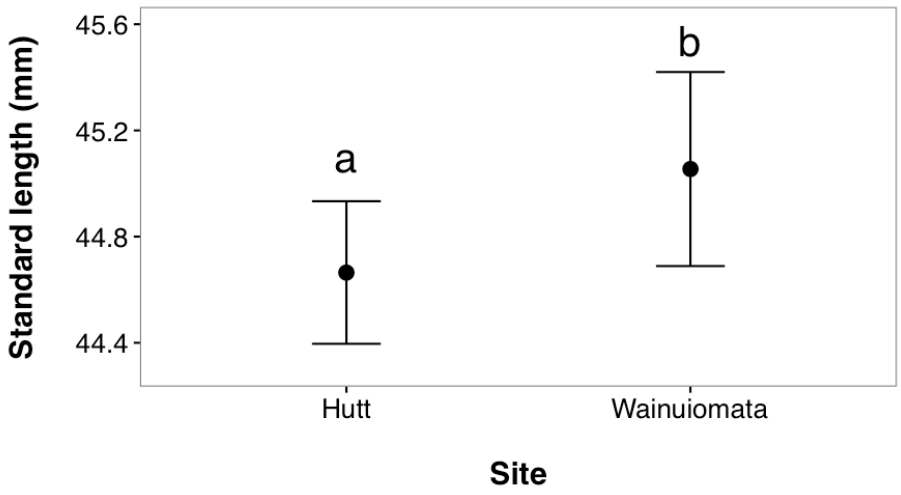

Figure 3.3: Spatial variation in standard length of juvenile G. maculatus collected from two sites (Hutt River and Wainuiomata River). Given are L-S means (i.e. corrected for other sources of variation in the statistical model, see table 2-1) ± 95% CI. Dissimilar lowercase letters indicate a significant difference based upon post hoc tests.

were both significantly larger than November fish ($p < 0.0001$ for both) but not different from one another ($p = 0.4505$).

The standard length of G. maculatus varied significantly among days nested within sites ($F_{19, 470} = 5.210$, $p < 0.0001$, 3.5). A post hoc test (3.6) indicates that a small number of pairwise comparisons appear to be driving the significance of this effect. 3.5 suggests that sizes of G. maculatus are heterogeneous across consecutive days within some months (i.e. October, November) for the Hutt River in particular.

3.3.2 Spatio-temporal Variation in Average Growth Rate

I found a significant interaction between month and site ($F_{2, 470} = 6.489$, $p = 0.0017$, 3.7), indicating that growth rate changes over time and sites (3.2). A post hoc test showed that, in the Hutt River, fish caught in August grew faster than fish caught in September ($p < 0.0001$), October ($p = 0.0265$) and November ($p = 0.0134$). September did not differ to October ($p = 0.3105$) or November ($p > 0.9999$). October and November also did not differ ($p = 0.6749$). In the Wainuiomata River, August fish did not have a significantly different growth rate to fish caught in September ($p > 0.9999$) or October ($p = 0.3072$). Fish from September and October also did not differ significantly ($p = 0.5708$).

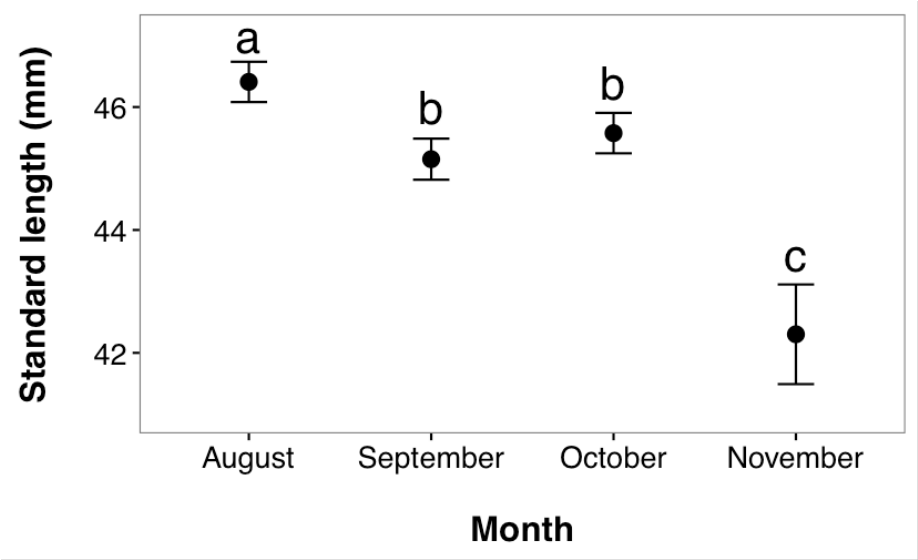

Figure 3.4: Temporal variation in standard length of juvenile G. maculatus collected from two sites. Given are LS means ± 95% CI. Dissimilar lowercase letters indicate a significant difference based upon post hoc tests.

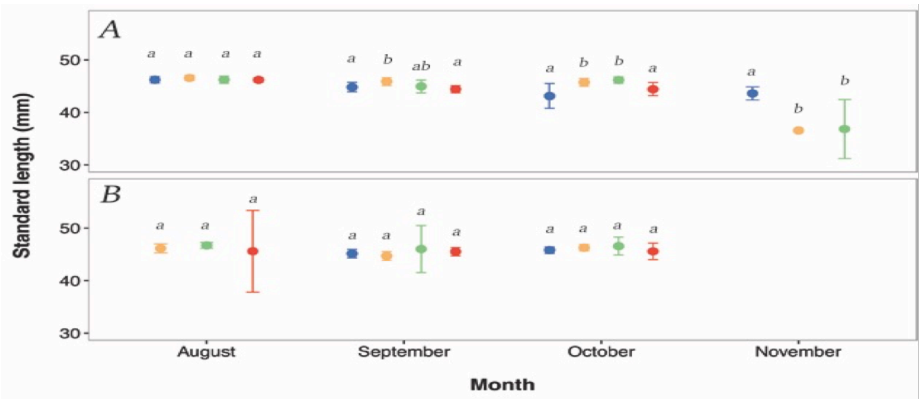

Figure 3.5: Daily (within month) temporal variation in standard length between (A) Hutt River, and (B) Wainuiomata River. Given are LS means ± 95% CI. Different colours represent the different sampling days. Blue=day 1, orange=day 2, green=day 3, red=day 4. Missing symbols indicate days were no fish were sampled. Confidence intervals are obscured by size of symbols for several observations. Dissimilar lowercase letters indicate a significant difference based upon post hoc tests; separate analyses were conducted for each site and month

Month	Day pairs	Hutt River p-values	Wainuiomata p values
August	1 and 2	0.498	NA
	1 and 3	0.994	NA
	1 and 4	0.972	NA
	2 and 3	0.503	0.296
	2 and 4	0.481	0.701
	3 and 4	0.966	0.429
September	1 and 2	0.048 *	0.470
	1 and 3	0.835	0.455
	1 and 4	0.488	0.529
	2 and 3	0.1556	0.278
	2 and 4	0.008 *	0.230
	3 and 4	0.434	0.675
October	1 and 2	<0.001 *	0.381
	1 and 3	<0.001 *	0.299
	1 and 4	0.060	0.763
	2 and 3	0.443	0.692
	2 and 4	0.021 *	0.375
	3 and 4	0.003 *	0.288
November	1 and 2	<0.001 *	NA
	1 and 3	<0.001 *	NA
	2 and 3	0.890	NA

Figure 3.6: Pairwise comparisons of standard length between days nested within months and sites. No fishing was conducted in the Wainuiomata during November due to river mouth closure. No fish were successfully caught on the 1st day in August in the Wainuiomata or the 4th day in November in the Hutt (as indicated by "NA"). Asterisks indicate a significant difference in length between day pairs.

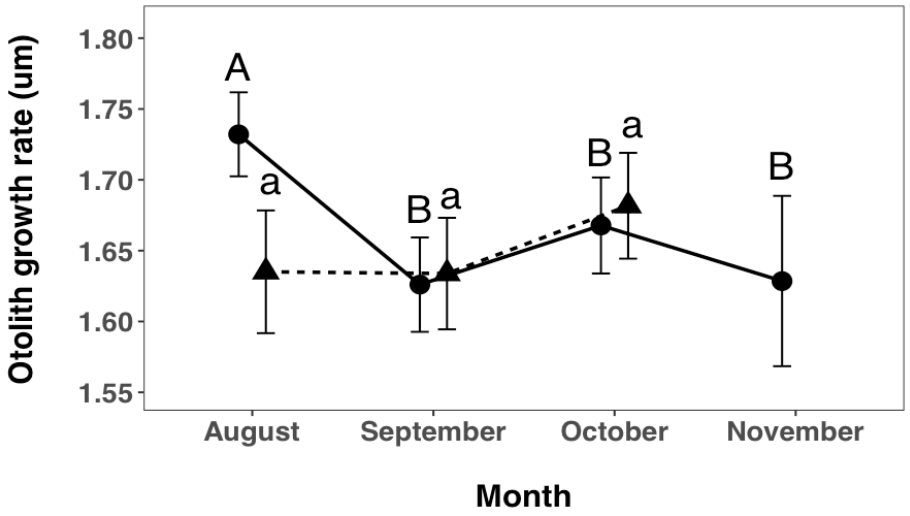

Figure 3.7: Spatial and temporal variation in otolith growth rate of juvenile G. maculatus collected from two sites (circles/uppercase letters: Hutt River, triangles/lowercase letters: Wainuiomata River). Given are LS-means (i.e. corrected for other sources of variation in the statistical model (Table 2-1) ± 95% CI. Dissimilar letters indicate a significant difference within sites, across time (e.g., no difference across months within the Wainuiomata River). Sampling did not occur in the Wainuiomata River during November due to river mouth closure.

The otolith growth rate varied significantly among days nested within months and sites (F19, 470 = 5.2703, p < 0.0001, 3.8). A post hoc test (Table 2 3) indicates that a small number of pairwise comparisons are driving the significance of this effect. 3.8 suggests that otolith growth rates of G. maculatus are heterogeneous across days within all months for the Hutt River and homogenous across days within all months for the Wainuiomata River.

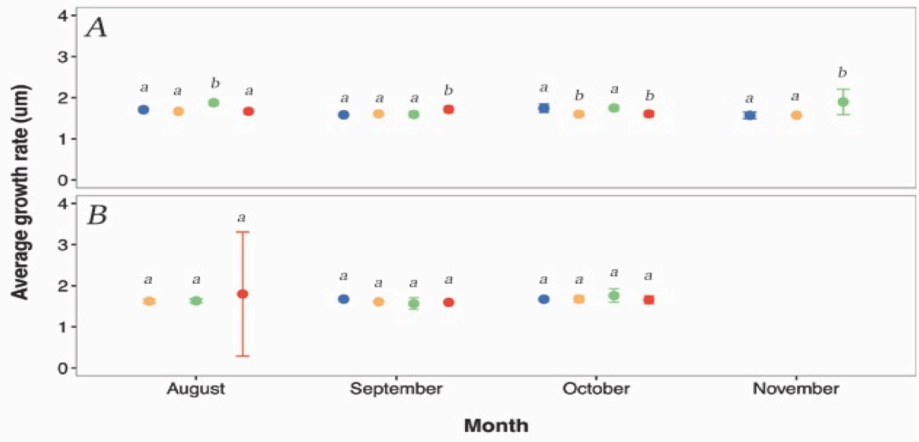

Figure 3.8: The otolith growth rate varied significantly among days nested within months and sites (F19, 470 = 5.2703, p < 0.0001, Figure 2 6). A post hoc test (Table 2 3) indicates that a small number of pairwise comparisons are driving the significance of this effect. Figure 2 6 suggests that otolith growth rates of G. maculatus are heterogeneous across days within all months for the Hutt River and homogenous across days within all months for the Wainuiomata River.

3.3.3 Spatio-temporal Variation in Ages

I found a significant interaction between month and site (F2, 470 = 7.7421, p = 0.0004, 3.10), indicating that patterns of age variation changed across time and sites. A post hoc test showed that, in the Hutt River, fish caught in August were significantly younger than fish caught in September (p < 0.0001), and October (p = 0.0029) but not November (p = 0.3783). Fish caught in September did not differ to fish from October (p = 0.4134) or November (p = 0.2774). There was also no difference in fish caught from October and November (p = 0.8869). In the Wainuiomata River, fish caught in August showed no difference in age to fish caught in September (p = 0.9934) or October (p = 0.7513). Fish caught in September also showed no difference to fish caught in October (p = 0.8709).

The ages of juvenile G. maculatus differed significantly among days nested within month and site (F19, 470 = 5.3537, p < 0.0001, 3.11). A post hoc

Month	Day pairs	Hutt River p-values	Wainuiomata River p-values
August	1 and 2	0.303	NA
	1 and 3	<0.001 *	NA
	1 and 4	0.315	NA
	2 and 3	<0.001 *	0.805
	2 and 4	0.988	0.109
	3 and 4	<0.001 *	0.129
September	1 and 2	0.589	0.186
	1 and 3	0.867	0.221
	1 and 4	0.002 *	0.081
	2 and 3	0.784	0.626
	2 and 4	0.009 *	0.790
	3 and 4	0.016 *	0.730
October	1 and 2	0.005 *	0.936
	1 and 3	0.936	0.110
	1 and 4	0.010 *	0.814
	2 and 3	<0.001 *	0.133
	2 and 4	0.890	0.775
	3 and 4	0.001 *	0.147
November	1 and 2	0.976	NA
	1 and 3	<0.001 *	NA
	2 and 3	0.044 *	NA

Figure 3.9: Pairwise comparisons of average otolith growth rate between days nested within months and sites. No fishing was conducted in the Wainuiomata during November due to river mouth closure. No fish were successfully caught on the 1st day in August in the Wainuiomata or the 4th day in November in the Hutt (as indicated by "NA"). Asterisks indicate a significant difference in length between day pairs.

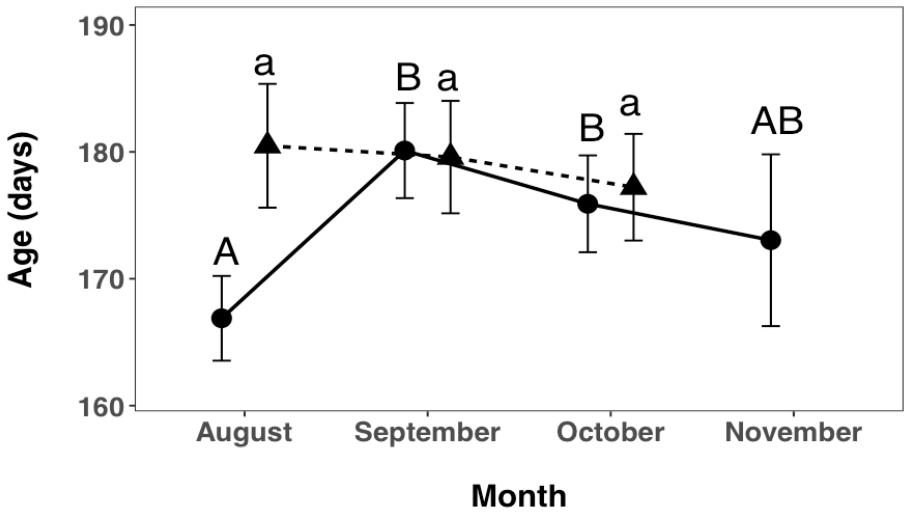

Figure 3.10: I found a significant interaction between month and site (F2, 470 = 7.7421, p = 0.0004, Figure 2 7), indicating that patterns of age variation changed across time and sites. A post hoc test showed that, in the Hutt River, fish caught in August were significantly younger than fish caught in September (p < 0.0001), and October (p = 0.0029) but not November (p = 0.3783). Fish caught in September did not differ to fish from October (p = 0.4134) or November (p = 0.2774). There was also no difference in fish caught from October and November (p = 0.8869). In the Wainuiomata River, fish caught in August showed no difference in age to fish caught in September (p = 0.9934) or October (p = 0.7513). Fish caught in September also showed no difference to fish caught in October (p = 0.8709).

test (**??**) again indicates that the significance of this effect is driven by a small number of pairwise comparisons in the Hutt River. 3.11 suggests that ages of G. maculatus are heterogeneous across days within all months for the Hutt River and homogenous across days within all months for the Wainuiomata River.

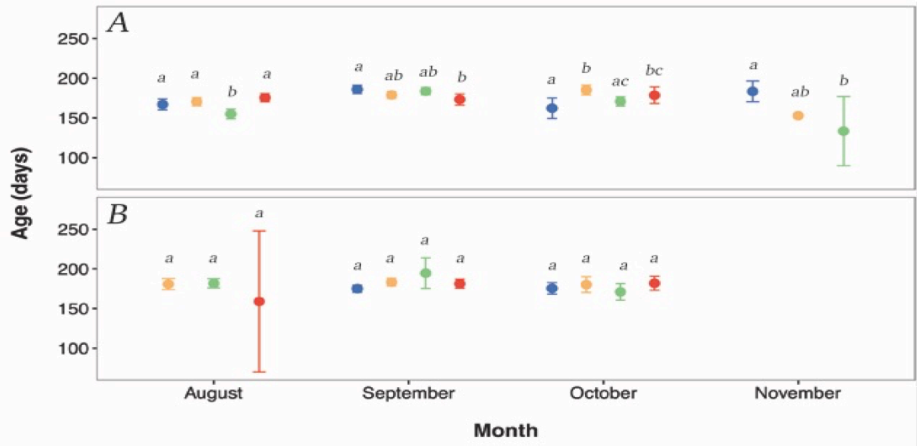

Figure 3.11: Daily (within month) temporal variation in age between (A) Hutt River, and (B) Wainuiomata River. Different colours represent the different sampling days. Blue=day 1, orange=day 2, green=day 3, red=day 4. Missing symbols indicate days were no fish were sampled. Error bars represent 95% confidence intervals. Confidence intervals are obscured by size of symbols for several observations. Dissimilar lowercase letters indicate a significant difference based upon post hoc tests; separate analyses were conducted for each site and month. Sampling did not occur in the Wainuiomata River during November due to river mouth closure.

3.4 Discussion

3.4.1 Summary of Results

I found site-specific trends in the developmental histories of G. maculatus. Juvenile G. maculatus entering the Wainuiomata River showed no difference in growth rate or age across months, although they did show a decrease in standard length across months. Fish from the Hutt River also shared this decrease in standard length, but also showed a decrease in otolith growth rate. Age showed a hump shaped curve, where the youngest recruiting fish were in August and November. Fish in the Hutt River during August, were the youngest, fastest growing, and largest, a pattern that was not reflected in the Wainuiomata. However, while fish from the Wainuiomata River did not show significant differences

in otolith growth rate and age, there did appear to be non-significant trends that matched the results from the Hutt River.

There was no day-to-day variation in any developmental characteristics of fish sampled from the Wainuiomata River. While fish from the Hutt River did show day-to-day variation, there was significant variation in the direction and magnitude of trends. Therefore, the two main points of interest become (1) why was there daily and monthly variation through time, and (2) why was there more variation in the Hutt River?

3.4.2 Spatial Differences in Developmental Histories

I propose two hypotheses that could explain my results (and these are not mutually exlusive): (1) the Hutt River may be replenished by fish from a wider variety of source populations than the Wainuiomata River, which could lead to greater variation in developmental histories among cohorts (natal source hypothesis), and/or (2) recruits from the Hutt River may have experienced greater environmental variability during their pelagic larval dispersal phase, which could lead to different phenotypic distributions through individual fish experiencing phenotypic plasticity or selective mortality (environmental experience hypothesis).

A difference in the composition of source populations entering each river is dependent on the extent of dispersal. G. maculatus have very strong swimming capabilities (Barker and Lambert 1988), and considerable research has examined the extent of population mixing and natal return (Barker and Lambert 1988, Berra et al. 1996, Waters and Burridge 1999, Waters et al. 2000) with current paradigms suggesting that G. maculatus does not show extensive natal homing (Waters et al. 2000, Hickford and Schiel 2016). However most evidence is based off a lack of genetic structure among sampled populations, and genetic structuring may be mediated by only a small number of mixing individuals (Hartl 1988). Furthermore, most studies have been concerned with broad spatial hypotheses (Barriga et al. 2007, Barbee et al. 2011, Barriga et al. 2012), rather than considering the characteristics of individual systems that may facilitate a higher level of retention than the majority of source populations. Harbour systems have been shown to have highly retentive properties due to physical and hydrodynamic processes acting on the water currents (Maxwell 1956, Bowman et al. 1983, Anderson 1988). Therefore I suggest that the hydrodynamic characteristics of the Wellington Harbour may promote higher retention of larval G. maculatus than would be expected by a coastally positioned system, thus promoting self recruitment (Jones et al. 2005, Levin 2006, McDowall 2009). However, I do not assume that the Wellington Harbour is completely isolated from other (perhaps coastally derived) G. maculatus populations, and I would expect it to still receive input from other source populations around New Zealand (McDowall et al. 1975, Caley et al. 1996, McDowall 2002, Swearer et al. 2002). The combined input of recruits from other source populations (with their own variations in phenotype), plus the resident population in the Wellington Harbour, may com-

bine to produce a more heterogeneous population of G. maculatus (Shima and Swearer 2009). Fish from the Wellington Harbour would therefore show a wider distribution in phenotypes than the Wainuiomata River, which may not have a resident population, and is only replenished by regional source populations (that shared more similar environmental conditions). These differences in the spread of potential phenotypes may be driving the lack of significant differences in the Wainuiomata, while accounting for the range of patterns documented in the Hutt River.

Marine habitats can show considerable variation in temperature, water flow, light availability, and salinity (Johnston 2006) which may vary extensively through time. Pelagic fish may experience phenotypic plasticity as a result of this environmental variability, and therefore their phenotype may correlate with conditions experienced during dispersal. If my two study sites are replenished by different combinations of source populations, with differing dispersal histories, then the environmental conditions experienced may be driving these site specific differences. During dispersal, cohorts may encounter novel environments that impose directional selection on phenotypic traits (Reznick and Ghalambor 2001, Grether 2005), which shifts the mean phenotype to a new peak (Lande and Arnold 1983). Environmental pressures may be either biotic (Handelsman et al. 2013) or abiotic (Carrera et al. 2012) but all have the potential to drive phenotypic shifts (Agrawal 2001). This hypothesis is dependent upon Wellington Harbour showing a higher degree of temporal variability in its biotic and abiotic conditions. Under the assumption that it is more variable, individuals with recent resident periods in the harbour may have experienced phenotypic plasticity, and therefore developed phenotypic characteristics representative of the conditions at the time (Agrawal 2001, Barriga et al. 2012, Chapman et al. 2015). Depending on the scale of this variability it may account for both monthly and daily differences. In contrast, if the Cook Strait shows a less temporally variable environment then that may explain the fairly consistent trends in phenotypes of recruits.

General trends in harbour systems have shown evidence of circulation currents leading to high levels of nutrients (Mackas and Harrison 1997) and zooplankton (Soetaert and Herman 1994). They have also shown that abiotic conditions can be highly variable between seasons (Muylaert and Raine 1999). Results by Maxwell (1956) indicate average water temperatures in the Wellington Harbour increase from August to November, yet there is also considerable fluctuation over shorter time scales, with changes of up to 2.5°C within a three day period. Maxwell (1956) also postulated that the causes of this high variability was due to the sheltered positioning of the harbour. In contrast, Cook Strait has very high energy, fast flowing currents (Bowman et al. 1983), and its lack of shelter may not promote high levels of abiotic variability. Cook Strait is highly dynamic with complex patterns of water circulation, but there is little evidence for its low productivity waters being temporally variable (Bowman et al 1983). While it may be a high energy environment, I argue that the consistent nature of it is not enough to drive phenotypic differences in resident cohorts of G. maculatus.

Chapter 4

Applications

Some *significant* applications are demonstrated in this chapter.

4.1 Example one

4.2 Example two

Chapter 5

Final Words

We have finished a nice book.