THE PHYSIOLOGICAL RESPONSES OF AN AMPHIDROMOUS FISH, GALAXIAS MACULATUS, TO ENVIRONMENTAL CONDITIONS EXPERIENCED DURING A TRANSITIONARY PHASE

by

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A thesis

submitted to the Victoria University of Wellington

in partial fulfilment of the requirements for the degree of

Master of Science in Marine Biology

Victoria University of Wellington

2017

ABSTRACT

Understanding how the developmental environments encountered during early life stages of fish affects the development and future success of populations is an important subject of ecological research. An amphidromous life cycle enables fish to exploit the nutrient-rich marine environment during a key developmental phase, but is associated with many risks. Morphological comparisons can be used to infer niche differences between species, as well as hypothesise the different conditions individuals within the same species have encountered over their life. Phenotypic plasticity is a process where individuals undergo morphological changes, typically better adapting them to their environment. This is especially common in fish, as they encounter a wide variety of environmental conditions over their lifetime. Another way to examine effects of environmental pressures on a population is to investigate fluctuating asymmetry (FA). While phenotypic plasticity potentially reduces stress, FA is thought to arise from stress during an organism's development and may reflect both the magnitude of stress and the organism's ability to cope with it. Analysing asymmetry in fish otoliths to measure the health and/or condition of a population is a relatively new topic that is rapidly gaining attention.

Galaxias maculatus is a widespread amphidromous fish with cultural, economic and ecological significance in New Zealand. Typically, this fish spawns in fresh water, spends 3-6 months in the ocean undergoing pelagic larval development, before migrating back to fresh water to complete its lifecycle. This species provides an ideal opportunity to investigate how environmental conditions experienced during early life stages affects survival, growth, morphology and stress.

I reared larval *G. maculatus* in 4 different water treatments (fresh + calm, fresh + turbulent, salt + calm, salt + turbulent), to simulate natural conditions they are likely to experience during their larval and transitionary phases. I compared survival between treatments and found that exposure to salt and/or turbulent environments decreases survival time. I also analysed variation in growth, condition and morphology and found that in most cases, these factors were greater in fresh than salt water. Individuals

inhabiting turbulent salt water generally had the smallest trait sizes and lowest body weight and condition. In contrast, individuals inhabiting calm fresh water generally had the largest trait sizes and higher weight and body condition. I looked for evidence of stress in response to body condition and rearing environment by comparing asymmetry of otolith size. I found that otolith area shows evidence of increasing asymmetry over time, although this was not affected by treatment. Conversely, otolith perimeter was the only trait to show evidence of FA in response to my treatments, and indicated that turbulent salt water causes increased stress.

My study shows that larval *G. maculatus* who undergo a prolonged marine duration are likely to experience high mortality and delayed development. My results also indicate that the combined effects of stressors such as turbulence and increased salinity result in high mortality, reduced growth rates and increased FA which may have negative carry-over effects. This highlights the importance of ensuring rivers remain open & accessible to migrating larval fish, as well as providing high habitat diversity so individuals can seek refuge from adverse conditions. My results emphasise the importance of including more descriptive measures, such as otolith shape, in future studies of FA. This thesis contributes to the growing understanding of the relationship between varying developmental environments and survival, growth, condition, morphology and stress in larval fish. This information can be used to better inform conservation and management decisions.

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1 INTRODUCTION

The input of offspring is vitally important to the persistence of any population (Jones, 1990; Grosberg & Levitan, 1992; Caley *et al.*, 1996; Hixon *et al.*, 2012). Both the quantity and quality of new recruits entering a local population are heavily influenced by the developmental environments and mortality experienced during early life stages (Fogarty *et al.*, 1991; Lecchini & Galzin, 2003; Siegel *et al.*, 2008; Hare, 2014). This important role of early developmental history can also shape the distribution of traits within an adult population, and the overall fitness and success of that population in the future (Fogarty *et al.*, 1991; Conover & Schultz, 1997; Schoener, 2011). Most marine organisms produce large numbers of offspring that often disperse during their larval phase and settle away from their natal origin (Cowen & Sponaugle, 2009). During this larval phase, dispersing individuals are likely to encounter variable and highly fluctuating environments (Fogarty *et al.*, 1991; Sponaugle & Pinkard, 2004; Cowen & Sponaugle, 2009) that can alter survival, shape phenotypes and ultimately affect an individual's future fitness and success (McCormick, 1994; Kerrigan, 1996; Agrawal, 2001). Species with long pelagic larval durations are particularly vulnerable to these environmental fluctuations and can therefore experience greater recruitment variability (Fogarty *et al.*, 1991). Considering that the early life experiences of fish have the potential to impose such strong selective pressures that determine survival and/or successful reproduction (Fogarty *et al.*, 1991; Conover & Schultz, 1997), it is imperative that we fully understand how the environmental conditions experienced during the early life stages affects growth, development and future success of populations.

1.1 Amphidromy

Fish have developed a wide range of life history strategies (e.g., diadromous, oceanadromous, fluvial, lacustrine, estuarine etc. Helfman *et al.,* 2009) that enable them to disperse and/or exploit their environments (Southwood, 1977; Townsend & Hildrew, 1994; Blanck & Lamouroux, 2007; Goto *et al.,* 2015; Augspurger *et al.,* 2017). Some species reside in the same locality their whole life, while other species migrate long distances between feeding and spawning grounds (Dingle & Drake, 2007; Jørgensen *et al.,* 2008). Amphidromy is a type of diadromy that includes migrations between fresh water and marine environments (in either direction), for purposes other than breeding (in contrast to anadromous and catadromous species that have migrations associated with reproduction: Myers, 1949; McDowall, 1997). For many amphidromous species, reproduction occurs in fresh water and larvae undergo pelagic larval development in the marine environment before returning to fresh water to further develop into adults (Myers, 1949; McDowall & Eldon, 1980; McDowall, 2007; Iida *et al.,* 2010). Amphidromous life histories have been described from a diverse range of fish families, including Cottidae, Galaxiidae, Gobiidae, Eleotridae, Pinguipedidae, Mugilidae and Retropinnidae, and occur across a broad geographic range throughout the world, but are disproportionately represented among fish that are endemic to small oceanic islands (Keith, 2003; McDowall, 2004, 2007; Miles *et al.,* 2009).

As amphidromy does not occur for breeding purposes, its evolutionary origin has been extensively debated (McDowall, 1997, 2004, 2007; Closs *et al.,* 2013; Hogan *et al.,* 2014; Augspurger *et al.,* 2017). Popular theories have speculated that amphidromy is an adaptation for dispersal, predator avoidance, and/or increased fecundity (McDowall, 1988, 1997, 2007; Chapman *et al.,* 2012a; Chapman *et al.,* 2012b; Closs *et al.,* 2013). McDowall (2010) favoured dispersal as the primary adaptive explanation of amphidromy. However, many have recently questioned the role of dispersal in amphidromy. Young pelagic larvae are highly susceptible to starvation and other adverse physical and chemical conditions that might be encountered prior to metamorphosis and/or settlement (Iguchi & Mizuno, 1999; Iida *et al.,* 2010; Jarvis & Closs, 2015). Therefore, it is not always favourable to disperse during the larval phase,

as larvae have a very low chance of surviving long enough during long-distance marine dispersal to settle in suitable new habitats (Sorensen & Hobson, 2005; Watanabe *et al.*, 2014; Goto *et al.*, 2015). Furthermore, amphidromy is not always obligate; many species are facultatively amphidromous, and larval development occurs entirely in fresh freshwater (Taylor *et al.*, 2000; McDowall, 2007; Hogan *et al.*, 2014), suggesting that oceanic dispersal is not essential.

Many examples of facultative amphidromy occur in the scientific literature, where one population has ocean-going larvae, while a neighbouring population of the same species is either partially or wholly land-locked in freshwater systems (McDowall, 1988; Closs *et al.*, 2003; David *et al.*, 2004; Maruyama *et al.*, 2004; Chapman *et al.*, 2012a; Chapman *et al.*, 2012b; Closs *et al.*, 2013). In such cases, both populations are generally stable, but often exhibit phenotypic differences (Humphries, 1990; Closs *et al.*, 2013). A number of galaxiid species in New Zealand and Australia exhibit evidence of this facultative amphidromy, where populations have access to the ocean, yet larvae develop in fresh water lakes (Pollard, 1971; Humphries, 1990; David *et al.*, 2004; Chapman *et al.*, 2009). Another popular theory for amphidromy is that the marine environment is more productive than fresh water systems, and developing larval fish require these abundant food resources for development, that are not available in fresh water systems (Gross *et al.*, 1988; Edeline, 2007; McDowall, 2007; Thibault *et al.*, 2007). In support of this theory, individuals that exhibit facultative amphidromy often display longer larval durations, slower growth and lower fecundity than their ocean-going counterparts, which has carryover effects to the adult life stage (Closs *et al.*, 2013; Hogan *et al.*, 2014).

Transitioning between fresh water and marine systems incurs additional hazards for larval fish. For example, extended periods in salt water result in high osmoregulatory costs for non-marine fish (McCormick *et al.*, 2003; Marshall & Grosell, 2006; Urbina *et al.*, 2013). Non-marine fish can only tolerate living in the ocean for a limited period of time before the additional metabolic demands of osmoregulation result in a disproportionately high reduction in the energy available for growth (Iida *et al.*, 2010; Iguchi & Takeshima, 2011). The benefits of amphidromous fish retaining their marine

dispersal phase and gaining access to increased food resources must therefore outweigh the risks and challenges associated with migrating between fresh water and marine systems. Marine larval development can be flexible in its duration (3-6 months for *G. maculatus* [McDowall *et al.,* 1994]; 3-5 months for the native gobiids of Hawaii [Radtke *et al.,* 1988]; 1.5-2.1 months for some Sicydiinae gobies [Taillebois *et al.,* 2012]; 2.4-4.5 months for other Sicydiinae gobies [Lord *et al.,* 2010] and 5.7-8.4 months for *Sicyopterus japonicas* [Iida *et al.,* 2008]). However, it is unknown how long this marine phase can last before the benefits no longer outweigh the costs. There are no documented cases of amphidromous fish remaining in salt water and developing into adults in the ocean, instead of in fresh water. Therefore, we can infer that larvae of amphidromous fish must eventually return to fresh water in order to continue development and complete their life cycle. It is important to know how long larval amphidromous fish can tolerate living in the marine environment before it becomes essential to return to fresh water, as well as the costs of a delayed return to fresh water. Natural and anthropogenic changes to river mouths, as well as strong offshore oceanic currents can hinder larval movement back into fresh water systems, resulting in decreased recruitment and potential population declines (McDowall & Eldon, 1980; McDowall, 1995). This is an especially important issue for short-lived semelparous species that are almost completely dependent on each annual influx of new recruits to replenish adult populations (McDowall, 1995). However, no study has investigated the effect of delayed marine duration on amphidromous fish.

1.2 Environmental impacts on fish growth and morphology

Fish often show morphological differentiation at both the inter- and intra-specific level. Morphological comparisons can be used to infer differences in niche occupation between species, as well as hypothesise the different conditions individuals within the same species have been exposed to over their life (Koehl, 1996). The relationship between form and function is well known among fish taxa (Webb, 1984a; Lauder, 1989; Blake, 2004). Fish swimming performance is affected by the interaction between fish

morphology and hydrological conditions (Nikora *et al.*, 2003; Leavy & Bonner, 2009). Performance-related variations in fitness can therefore manifest as morphological responses to extrinsic pressures (Haas *et al.*, 2015). In general, fish with fusiform, torpedo-shaped bodies with fineness ratios around 4.5 (measured as body length/body depth) are the most streamlined. These attributes are therefore more energetically efficient for species that travel long distances in open environments or inhabit high flow environments, such as the run and riffle habitats of rivers (Leavy & Bonner, 2009; Langerhans & Reznick, 2010). This body shape improves swimming efficiency, enables prolonged swimming, and reduces energy lost to drag by helping an individual to maintain a steady position in the water column (Gosline, 1971; Webb, 1975; Webb, 1984a; Weihs, 1989; Videler, 1993; Vogel, 1994; Blake, 2004). In contrast, shorter, deeper bodies with off-centre lateral fins are better for low flow environments and navigating through structurally complex habitats, such as ocean reefs and the littoral zones of lakes, as this body shape is better suited for manoeuvring than for prolonged swimming (Gosline, 1971; Webb, 1975; Webb, 1984a; Weihs, 1989; Videler, 1993; Vogel, 1994; Blake, 2004).

Other morphological measurements can be used to infer differences in life modes between fish species. For example, rapid swimming pelagic fish, such as tuna (genus Thunnus), are likely to have long, curved pectoral and pelvic fins, a forked lunate caudal fin, a narrow caudal peduncle and a high tail fin aspect ratio (measured as caudal fin height^2/caudal fin surface area) as these features maximise thrust, minimise drag and enable efficient, fast, prolonged swimming (Sambilay, 1990; Blake, 2004; Langerhans & Reznick, 2010). Whereas slower, bottom-dwelling fish, such as gobies (family Gobiidae), are likely to have rounded fins and very low tail fin aspect ratios (Weihs, 1989; Sambilay, 1990; Blake, 2004). However, while these interspecific comparisons are useful in understanding adaptations to different environmental conditions, they are clouded by differences in other aspects of the physiology, morphology and ecology of the species being studied. Morphologies can be altered in response to variable local conditions, such as predation (Langerhans *et al.,* 2004; Abrahams, 2006; Langerhans *et al.*, 2007; Abate *et al.,* 2010; Kekäläinen *et al.*, 2010), temperature (Oufiero & Whitlow, 2016), food

availability (Baltz *et al.*, 1998; Barriga *et al.*, 2012), water flow (Ferriz *et al.*, 2001; Langerhans, 2008; Moody *et al.*, 2015) and numerous other environmental conditions. Therefore, intraspecific variation in morphology can provide additional and powerful insights into the functional significance of phenotypic traits (Taylor & McPhail, 1985; Wilson, 1998; Ojanguren & Braña, 2003; Chapman *et al.*, 2015).

Morphological variation within a population can result from several different biological processes. Naturally occurring genetic variation results in a variety of phenotypic traits within a population (Via & Lande, 1985; Shapiro *et al.*, 2004). Natural selection can then result in a shift in the traits retained within a population (Lande & Arnold, 1983; Slatkin, 1987). Alternatively, phenotypic traits may undergo physical changes in response to pressures exerted by the external environment. This is referred to as 'phenotypic plasticity' (West-Eberhard, 1989; Scheiner, 1993).

Phenotypic plasticity is an important subject of ecological research (Sutherland *et al.*, 2013). Many vertebrate species exhibit intraspecific variations in morphology as a result of differences in resource use or local environmental conditions (Wimberger, 1994; Hadfield & Strathmann, 1996; Mittelbach *et al.*, 1999). These morphological differences are typically due to either local adaptation, wherein the different morphologies are the product of selection acting on heritable phenotypic characteristics; or phenotypic plasticity, wherein phenotypic changes occur in response to a particular environmental condition, but are not heritable (Wimberger, 1994; Robinson & Wilson, 1996; Smith & Skulason, 1996; Mittelbach *et al.*, 1999). Fish are especially prone to these phenotypic changes, due to the variety of environmental conditions that they experience (Hadfield & Strathmann, 1996; Oufiero & Whitlow, 2016). Many studies have found that, similar to how species are adapted to exploit different niches, individuals of the same population are often morphologically and behaviourally specialized to forage on different resources or in different habitats (Behnke, 1972; Ehlinger & Wilson, 1988; Skulason *et al.*, 1989; Ehlinger, 1990; Robinson & Wilson, 1994, 1996; Jonsson & Jonsson, 2001; Blake, 2004).

Oceans and fresh water systems tend to experience large environmental fluctuations in temperature, food availability and water dynamics, both temporally and spatially (Houde, 1989b; Friedland *et al.*, 2000; MacKenzie & Kiorboe, 2000; Shima & Swearer, 2009; Neuheimer *et al.*, 2011). This can cause differences in growth and phenotypes between cohorts of fish (Jenkins & King, 2006; Shima & Swearer, 2009). Within a habitat, fine-scale differences in environmental conditions, due to heterogeneous environments (e.g. coral reefs, or riffles and pools in a lotic system), may result in further differences in growth and phenotypes, within a cohort (Nielsen, 1992; Baltz *et al.*, 1998; Untersteggaber *et al.*, 2014; Wehrberger & Herler, 2014). For example, Law and Blake (1996) found that, within a population of a lake-dwelling sticklebacks, the limnetic-dwelling individuals were more streamlined, whereas the benthic-dwelling individuals had larger, deeper bodies.

To further complicate the study of phenotypic plasticity, phenotypes that are successful during one life stage may have reduced fitness at the next. Therefore, fish species with complex life cycles should be expected to evolve life-stage-specific adaptations to maximise survival throughout development, and through to sexual maturity (Werner & Gilliam, 1984; Williams, 1992). This is especially the case for amphidromous fish species, as they are vulnerable to stressors across fresh, brackish and salt water environments, and distinct ontogenetic changes occur with each transition into a new medium. However, the consequences of prolonged duration in suboptimal environments that were once optimal or even essential for growth and survival are still poorly understood. Field studies investigating phenotypic plasticity often measure a variety of morphological and environmental traits and attempt to find correlations (Humphries, 1990; Baltz *et al.*, 1998; Ferriz *et al.*, 2001; Hjelm & Johansson, 2003; Barriga *et al.*, 2012; Figuerola *et al.*, 2012; Haas *et al.*, 2015). However, results of studies like these are likely confounded, as an individual will have encountered a multitude of environmental conditions throughout its life, but it is exceedingly difficult to determine which environment, and at which life stage the change in phenotype was caused, and whether the conditions measured are acting alone or synergistically.

In the past, the majority of empirical studies have focused on resource competition and predation as the main drivers of phenotypic variation (Robinson & Wilson, 1994; Smith & Skulason, 1996; Hjelm & Johansson, 2003; Bolnick, 2004). Many studies have linked increased predation risk to changes in morphology that increase an individual's predator avoidance and prey escape abilities (Langerhans *et al.*, 2004; Milano *et al.*, 2006; Kekäläinen *et al.*, 2010; Barriga *et al.*, 2012), take advantage of predator gape limitations (Abate *et al.*, 2010), or increase growth and size of defensive structures (Abrahams, 2006). However, when effects of predation and resource competition are removed, other environmental variables become a driving force of morphological change. Water velocity, for example, can be a powerful driver of morphological variation among stream fish populations (Hendry *et al.*, 2006; Kerfoot & Schaefer, 2006; Zuniga-Vega *et al.*, 2007; Jacquemin *et al.*, 2013; Vanderpham *et al.*, 2013a, b). However, the direct relationship between turbulence and morphology is still little understood as it varies between species and it is difficult to link an exact flow regime to a specific morphology, especially given the vast array of flow regimes found within marine and fresh water environments (Vogel, 1996).

Another way to examine the effects of environmental pressures on a population is to investigate fluctuating asymmetry (FA) (Clarke, 1995; Palmer, 1996; Allenbach, 2011). This has been the focus of a wide range of evolutionary and ecological studies. FA is defined as small, random deviations from perfect bilateral symmetry in a morphological trait (e.g. arm length) (Clarke, 1995; Palmer, 1996; Palmer & Strobeck, 2003). This distinguishes it from directional asymmetry which is biased to one side (e.g. right-handedness in humans), or antisymmetry, which has equally large numbers of asymmetrical individuals at both ends of the spectrum, yet few in the middle, resulting in a bimodal distribution (e.g. the claws of male fiddler crabs) (Rasmuson, 2002). Phenotypic plasticity occurs to better adapt an individual to its environment, potentially reducing stress, whereas FA is thought to arise from stress during an organism's development and may reflect both the magnitude of stress and/or the ability of an organism to cope with stress (Palmer, 1996; Lens *et al.*, 2002). Individuals of low quality (e.g., poor health and body condition, reduced genetic quality etc.) (Hunt *et al.*, 2004; Neff & Pitcher, 2005; Wilson & Nussey, 2010) are developmentally less stable and unable

to develop traits that are bilaterally equal. However, the topic is controversial, as outcomes of field studies are often confounded by uncontrolled factors, and even in controlled experiments, the response of FA to stress is often trait-, species- and stress-specific (Somarakis *et al.*, 1997a; Bjorksten *et al.*, 2000; Rasmuson, 2002). Therefore, a thorough understanding of the specific stressors that cause FA, and the traits that best express FA for each species is vital.

Studies exploring FA have found that individuals exposed to high levels of stress, whether it be genetic (e.g. inbreeding, hybridization) (Markow & Ricker, 1991; Clarke, 1998; Vollestad *et al.*, 1999; Andersen *et al.*, 2002) or environmental (e.g. temperature, pollutants, food shortage) (Leary & Allendorf, 1989; Lu & Bernatchez, 1999; Eeva *et al.*, 2000; Hardersen, 2000; Rasmuson, 2002; Grønkjær & Sand, 2003), tend to show a higher degree of asymmetry. Furthermore, individuals with increased asymmetry tend to experience a reduction in growth (Møller, 1999), fecundity (Møller, 1999; Hechter *et al.*, 2000), survival (Clarke, 1995; Møller, 1999) and resistance to parasitism (Polak, 1993; Folstad *et al.*, 1996; Cuevas-Reyes *et al.*, 2011). This has led to the promotion of FA as a useful measure of the health and/or condition of fish populations (Clarke, 1995; Bjorksten *et al.*, 2000; Díaz-Gil *et al.*, 2015). Ideally FA could be used to recognise a population under stress before impacts such as high mortality and low fecundity are evident and have negatively influenced the reproductive potential of a population. However, there are a number of studies that have unsuccessfully detected FA, or failed to correlate it with the measured environmental stressors (Bergstrom & Reimchen, 2000; Bjorksten *et al.*, 2000; Rasmuson, 2002). Analysing FA can be problematic, as asymmetry may be expressed in some traits and not others (Blanckenhorn *et al.*, 1998; Woods *et al.*, 1999) and the magnitude of asymmetry can depend on the type of stress encountered (Campbell *et al.*, 1998; Roy & Stanton, 1999). Therefore, a more thorough understanding of the specific stressors that cause FA and the traits most sensitive to these stressors is necessary to improve the efficiency of future FA analyses (Bjorksten *et al.*, 2000; Lens *et al.*, 2002; Palmer & Strobeck, 2003; Díaz-Gil *et al.*, 2015).

Otoliths or 'ear stones' are bilaterally paired structures in fish that are convenient for measuring FA (Somarakis *et al.,* 1997b; Palmer *et al.,* 2010; but see Vignon & Morat, 2010). Otoliths are calcium carbonate structures in the inner ear of fish that are important for balance, sound perception and navigation (Popper & Lu, 2000; Popper *et al.*, 2005; Oxman *et al.*, 2007; Green *et al.*, 2009), meaning symmetry between left and right otoliths is necessary for their effective function. Therefore, it is believed that asymmetry in otoliths indicates reduced physiological condition and fitness (Anken *et al.*, 1998; Gagliano *et al.*, 2008). A prominent exception to this is flatfish (Pleuronectiformes) that undergo a substantial metamorphosis, including cranial deformation and the repositioning of one eye to the other side of the head (Policansky, 1982; Brewster, 1987; Okada *et al.*, 2001; Bao *et al.*, 2011). This results in skewed carbon accretion rates and generally faster growth of otoliths on the blind side, resulting in an asymmetry of otolith mass (Sogard, 1991; Fischer & Thompson, 2004; Helling *et al.*, 2005; Mille *et al.*, 2015). However, as most fish do not undergo such an extensive lopsided metamorphosis, it may be reasonable to assume that otoliths should be bilaterally symmetrical in optimal conditions (Popper & Lu, 2000; Popper *et al.*, 2005).

Fluctuating asymmetry in otoliths is typically studied in the early life stages of fish (Somarakis *et al.*, 1997b; Somarakis *et al.*, 1997a; Fey & Hare, 2008; Starrs *et al.*, 2016), as larvae and juveniles generally experience faster growth rates, lower capability to tolerate stress, and have a stronger link between growth performance and fitness (Anderson, 1988; Houde, 1989a). Increased FA in otoliths has been correlated with poor nutritional condition of juvenile fish (Grønkjær & Sand, 2003) and decreased recruitment of larval fish during poor environmental conditions (Somarakis *et al.*, 1997b; Lemberget & McCormick, 2009). Since it was discovered that growth rings in fish otoliths are deposited daily (Pannella, 1971), otolith studies have rapidly become widespread among fish biologists and ecologists. However, studies of FA in otoliths are relatively rare, and many of these have been unable to document significant correlations between asymmetry and environmental stressors (Fey & Hare, 2008; Palmer *et al.*, 2010; Díaz-Gil *et al.*, 2015). Folkvord *et al.* (2000) reported no relationship between FA in otoliths and condition. However, as stated earlier, FA may be expressed in some traits but not in others, so this lack of relationship may have been the result of only measuring otolith

radius. Furthermore, different variables within a single trait may express FA differently (e.g. otolith area vs otolith length) (Panfili *et al.*, 2005; Oliver, 2013). An approach to overcome this is to measure multiple traits (Lens *et al.*, 2002), or integrate a number of different variables describing otolith size (Panfili *et al.*, 2005; Oliver, 2013), or to measure the shape of the otolith (Palmer *et al.*, 2010; Libungan & Pálsson, 2015).

1.3 Study species

The amphidromous fish, *Galaxias maculatus* (Jenyns, 1842), commonly known as inanga, provides an excellent study species for evaluating growth variation, phenotypic plasticity and fluctuating asymmetry in controlled laboratory experiments. Mitchell (1989b) has already demonstrated that *G. maculatus* survive well in captivity. Juveniles and adults are relatively easy to obtain, and they adjust readily to conditions experienced in captivity (typically characterised by confinement in small containers with poor water quality: Mitchell, 1989a). Larvae and juveniles are not aggressive or cannibalistic, so can be kept in higher than normal densities. *G. maculatus* also survive well on a range of commercially available foods (Mitchell, 1989b). Field studies have already shown that *G. maculatus* exhibit evidence of phenotypic plasticity in response to environmental conditions such as food availability and predation risk (Barriga *et al.*, 2012). Previous studies on otolith FA are dominated by tropical reef fish (Lemberget & McCormick, 2009; Vignon & Morat, 2010), and North Atlantic Ocean species (Folkvord *et al.*, 2000; Fey & Hare, 2008; Mille *et al.*, 2015). Therefore, *G. maculatus* provides an ideal representative to investigate patterns of FA in otoliths of a Southern Hemispheric temperate fish species.

G. maculatus is a widespread diadromous fish species found naturally in the Southern Hemisphere throughout New Zealand, Australia, Lord Howe Island, Chatham Island, Chile, Argentina, and the Falkland Islands (McDowall, 1968; Berra *et al.*, 1996; McDowall, 2000; Cussac *et al.*, 2004). It is the most common galaxiid species in New Zealand,

comprising the majority of whitebait fishery catches (McDowall, 1965; McDowall & Eldon, 1980; McDowall *et al.*, 1994). In autumn, *G. maculatus* spawn at the peak of spring tides, depositing eggs in moist soil amongst riparian vegetation along the upper tidal reaches of streams and rivers (Benzie, 1968; Mitchell, 1991; Allibone, 2003; McDowall & Charteris, 2006; Hickford *et al.*, 2010). Eggs undergo approximately 3 weeks of embryonic development, then hatch during the next spring tide (Benzie, 1968; McDowall, 2000). The roughly 7mm long larvae are then swept out into the ocean where they undergo marine pelagic development for 3-6 months, before migrating back to fresh water in spring as juveniles (McDowall, 1968; McDowall & Eldon, 1980; Mitchell, 1989a; McDowall *et al.*, 1994). Following their return to fresh water, juveniles migrate upstream and settle in suitable habitat where they continue to develop into mature adults for a further 6 months. *G. maculatus* is unique in that it is semelparous and generally has a lifespan of only 1 year (but see Stevens *et al.,* 2016), whereas other New Zealand galaxiid species live for multiple years and can spawn several times throughout their life (McDowall & Eldon, 1980; McDowall *et al.*, 1994; McDowall, 2000).

It is not known exactly what triggers the return of *G. maculatus* to fresh water – early theories that migration occurs once an individual reaches a certain age or size have largely been disproven, due to the wide variety in ages and sizes of returning juveniles (Stokell, 1955; Benzie, 1968; McDowall & Eldon, 1980; McDowall *et al.*, 1994). Alternative theories have been proposed (e.g., changes in temperature or photoperiod triggers migration) but none have been unequivocally established. A credible theory is that, owing to their small size, G. maculatus larvae (like many other marine larvae) are likely at the mercy of the ocean currents for the majority of their time at sea and are therefore carried far offshore. McDowall *et al.* (1975) reports that, although most larval galaxiid fish were caught within 50km of the coast, larval galaxiids were caught as far as 700km offshore (between the Bounty and Antipodes Islands). If larval swimming ability is indeed poor, then timing of migrations back into fresh water may be dependent on ocean currents transporting individuals back inshore. Alternatively, if swimming ability strengthens greatly, timing could depend on the time it takes for larval fish to swim back inshore from wherever the ocean currents carried them (McDowall & Eldon, 1980). The latter may be more likely, as juvenile *G. maculatus* actively navigate towards river

mouths in response to the decreasing salinity gradient and exhibit positive rheotaxis as they swim upstream (McDowall & Eldon, 1980). However, juvenile *G. maculatus* swimming ability may indeed be limiting, as they cease migrating when the current becomes too strong, for example, when the tide is receding (McDowall & Eldon, 1980). It is important to know how long larval *G. maculatus* can tolerate living in the marine environment before it becomes essential to return to freshwater and what the costs are of an extended marine duration/delayed return to fresh water, yet no studies have investigated this.

G. maculatus has been extensively studied in the Southern Hemisphere, but the majority of early studies were conducted in New Zealand and focused on its life history (Stokell, 1955; McDowall, 1965; Benzie, 1968; McDowall, 1968; Woods, 1968; McDowall *et al.*, 1975; McDowall & Eldon, 1980; Mitchell, 1989b; McDowall *et al.*, 1994). Despite the importance of the marine life stage, we know very little about it, mainly due to the difficulties associated with tracking small larval fish in the ocean. However, the importance of the larval phase is amplified when we consider that *G. maculatus* typically lives for 1 year and spawns once, therefore any loss of a major proportion of the progeny will result in a correspondingly high decline of the entire population. This means that the *G. maculatus* population is very vulnerable to sudden and serious decline, and is subject to high recruitment and population variability. Therefore, catastrophic events such as droughts and floods, which are expected to increase in number and severity due to climate change (Lehner *et al.*, 2006; Lindner *et al.*, 2010; Cai *et al.*, 2014) will have pronounced effects on the population and therefore the fishery (McDowall & Eldon, 1980). We have already seen evidence of this in previous years; whitebait caught in 1971 were smaller and in poorer condition than in other years, indicating poor growth while at sea. This had a direct impact on catch, as loss in condition was sufficient enough to account for a 50% decline in catch from the previous year for the entire West Coast of the South Island (McDowall & Eldon, 1980). Seeing how reduction in fish condition has the potential to cause such huge declines in catch, it is clear that fish size and condition are of major importance in fluctuations in the whitebait fishery.

As dispersing *G. maculatus* individuals are vulnerable to adverse conditions across fresh, brackish and salt water environments, it is key that we focus research efforts across all these environments. Furthermore, *G. maculatus* experience different environmental conditions and consequent mortality across all stages of their life, which may influence the characteristics found in adult populations (Barbee *et al.*, 2011). However, we still know relatively little about the larval and juvenile phases, which are arguably the most important life stages of this semelparous species. An area particularly lacking in knowledge is how amphidromous fish, like *G. maculatus*, react to the different environmental conditions that they encounter across their lifetime. A small number of studies have found evidence that *G. maculatus* decrease in body length after migrating into fresh water (Benzie, 1968; Woods, 1968; McDowall & Eldon, 1980), however the reason for this is still uncertain. In addition to this, the diversity in inanga morphology (evidenced in Barriga *et al.,* 2012), and the range of environmental conditions they experience over their lifecycle makes this an ideal species to study environmental effects on survival, growth and morphology.

The whitebait fishery has been a lucrative as well as culturally important activity for many people in New Zealand for over 100 years (McDowall, 1965, 1968; McDowall & Eldon, 1980; Mitchell, 1989b; McDowall, 2000; McDowall, 2011). However, *G. maculatus* numbers are generally considered to be declining throughout New Zealand (McDowall & Eldon, 1980; Jowett *et al.*, 1998; Hickford & Schiel, 2013, 2014), yet there is currently very little catch regulation. Therefore, there is a growing need to improve population sizes nationwide, in order to safeguard this important fishery. A small number of projects have attempted to raise *G. maculatus* in captivity (Mitchell, 1989b; Walsby, 2012; Wylie & Lokman, 2012), but due to the complexity of their terrestrial egg-laying and amphidromous lifecycle, very few have successfully raised *G. maculatus* all the way from embryo to sexual maturity (Mitchell, 1989b; Pearl, 2014). It is vital that we learn what environmental conditions are conducive to high growth rates, optimal body condition and therefore increased fecundity in important fishery species like *G. maculatus*.

1.4 Thesis Research

In this thesis I aim to address these gaps in the literature and determine how environmental conditions such as different water types and water movement affect survival, growth, morphology and stress of *G. maculatus* in a controlled laboratory environment. I also aim to contribute towards the growing understanding of the relationship between environmental stress and FA. The more we know about FA, the easier it becomes to make informed management decisions about whether a population is under stress, and/or being over-exploited, thus resulting in better management of important fishery species. My results could also contribute to the current efforts being made to produce large, healthy laboratory populations of amphidromous fish, potentially reducing the strain on declining wild populations.

My hypotheses are: 1) survival will be lower in salt water, especially in salt + turbulent conditions, as I expect this to put more of a strain on the fish, in regards to energy usage and osmotic stress. 2) *G. maculatus* will exhibit a high level of phenotypic plasticity, and individuals will modify their morphology slightly to become better suited to the environment they are placed in. For example, in turbulent conditions fish will develop more streamlined bodies, and traits that enhance efficient swimming ability. 3) Individuals will undergo reduced growth rates and decreased body condition when exposed to stressors such as increased salinity and turbulence. 4) Otolith FA will increase over time in individuals from the treatments that cause decreased body condition.

2 METHODS

2.1 Study species

The common whitebait, *Galaxias maculatus* (inanga) is an amphidromous fish native to New Zealand. Amphidromous larvae typically hatch in fresh water during autumn months (Benzie, 1968; McDowall, 1968; Mitchell, 1991; Richardson & Taylor, 2002) then disperse out to sea where they undergo larval development for 3-6 months (McDowall & Eldon, 1980; Barbee *et al.*, 2011). After this period at sea, juveniles return to fresh water where they develop into adults (McDowall & Eldon, 1980; McDowall *et al.*, 1994; McDowall, 1995). *G. maculatus* are widespread throughout New Zealand and form the majority of the whitebait catch (McDowall, 1965; McDowall & Eldon, 1980; McDowall *et al.*, 1994). They are a culturally, economically and ecologically important species to New Zealand (McDowall, 1968; Mitchell, 1989b; McDowall, 2011; Noble *et al.*, 2016), yet numbers are generally thought to be declining due to anthropogenic activities (McDowall & Eldon, 1980; Jowett *et al.*, 1998; Hickford & Schiel, 2013, 2014).

2.2 Sample Collection

I collected juvenile *G. maculatus* from a single location at the Hutt River mouth in the Wellington Harbour (41°14'04.36"S, 174°54'02.94"E). I sampled juveniles over a 4-day period (17th – 20th November), using 2 A-frame set nets (65 x 120 cm frame; 90 cm long; 2 mm mesh) that were set ~20m apart. I checked nets for *G. maculatus* and emptied them at 30 minute intervals over a 5-hour period. *G. maculatus* were then transported to the Victoria University Coastal Ecology Laboratory (VUCEL) in buckets filled with water from the site of collection for distribution into experimental treatments.

2.3 Experimental manipulation of water type and movement

I conducted a 2-way fully crossed laboratory experiment to investigate the separate and combined effects of water movement and water type on survival, growth, development and stress of *G. maculatus*. Each factor had two levels: for water type, treatments were either 'salt water' or 'fresh water'; for water movement, treatments were either 'calm' or 'turbulent'. I manipulated water type and movement at the scale of 60 litre plastic bins, and my design included 3 replicate bins per treatment (12 bins total), with a starting density of ~92 fish per bin (N = 1,104).

2.3.1 Manipulating water movement

I manipulated movement with an Aquapro AP1050 water pump; all bins contained a pump, but only bins assigned to the 'turbulent' treatments contained working pumps, which were set to the minimum volume output of ~800 litres/hour). I chose to include pumps in the bins assigned to the calm treatment in order to control for the presence a pump (e.g., physical structure, etc.). However, I noted that this fails to control for the potential effects associated with additional heat generated by an operating pump, so I took additional steps to mitigate and measure this effect (described below). Pumps were isolated from fish to prevent mortality directly induced by the pump intake. I constructed an isolation chamber within each bin, using a 2L plastic ice cream container covered with 1mm mesh. An air bubbler was also placed in each bin and an opaque lid covered each bin, to prevent fish from escaping and to minimise disturbance from external sources.

2.3.2 Manipulating water type

G. maculatus were initially contained in an aquaria containing brackish water from the site of collection (~20ppm). After bringing *G. maculatus* into the lab, I gave them a 1-

hour acclimation period, and then I proceeded to adjust water type by increasing or decreasing salinity by ~5ppm per hour until target salinities of 0ppm (fresh water) or 35ppm (salt water) were achieved. Fish remained in acclimating tanks at the target salinity overnight, and the following day I evaluated the appearance and behaviour of fish (all appeared healthy), then I randomly and evenly assigned them to treatment bins to achieve equal starting densities.

2.3.3 Maintenance of experiment

Fish were maintained in treatments and fed a diet of Nutrafin Max small tropical fish micro granules *ad libidum,* from 21st November 2015 until 25th February 2016, for a total of 97 days. Partial water changes (80%) were made at one week intervals (salt water was sourced from the ocean directly outside VUCEL; fresh water was sourced from the Hutt River ~2.6km upstream from the site of my fish collection). During these weekly water changes, all uneaten food, faeces and other particles were removed and all surfaces, including the interior and exterior of the pumps, were wiped down to prevent a build-up of biofilms. To mitigate temperature fluctuations and potential artefacts associated with the continuous operation of pumps in turbulent treatments, all bins were immersed in a water bath of constantly flowing seawater. I monitored temperature variation using HOBO pendant temperature loggers in each bin and found that in the calm bins the temperature ranged from 14.2^{o}C - 22.2^{o}C (depending on the time of day), with a mean of 18.6^{o}C ± 0.013^{o}C, whereas in the turbulent bins the temperature ranged from 14.4^{o}C - 22.5^{o}C, with a mean of 18.8^{o}C ± 0.017^{o}C. I checked bins daily to document *G. maculatus* survival and assess trends through time. I removed any fish that had died and immediately preserved them in 90% ethanol, for subsequent measurements. At the end of the experiment, any remaining survivors were euthanized in an ice slurry and preserved in 90% ethanol.

2.4 Growth and morphological variation

I evaluated a subsample of 40 fish from each bin (120 fish per treatment, 480 fish in total) to characterise variation in morphological attributes of fish as a function of water type and movement. Each subsample comprised of individuals that had died naturally throughout the experiment, as well as the individuals that were euthanized at the conclusion of the experiment. My subsamples were selected at random and included fish that survived for variable lengths of time in treatments, but I excluded any individuals that showed evidence of partial decomposition, damaged fins etc. Fish were weighed using a Mettler Toledo AB204-S microbalance. To obtain the morphometric measurements, I photographed each fish using an Olympus TG-3 camera with a reference ruler within the frame. Pins were used to secure the fish, splay out the fins and pinpoint features for measuring. I took similar measurements to that of Assumpção *et al.* (2012) and Fisher and Hogan (2007). I measured 11 morphological features, including, standard length, body depth, body width, head length, head height, head width, pectoral fin length, caudal peduncle depth, tail fin height, tail fin area and caudal peduncle area using the program ImageJ v1.50i (see Figure 1 and 2). I calculated fineness ratio as standard length/body depth (Langerhans & Reznick, 2010). I calculated tail fin aspect ratio as tail fin height^2/tail fin area (Sambilay, 1990; Langerhans, 2008). I used Fulton's condition factor "K" to estimate body condition with the equation $100 \times$ (wet weight $\div$ standard length^3) (Froese, 2006).
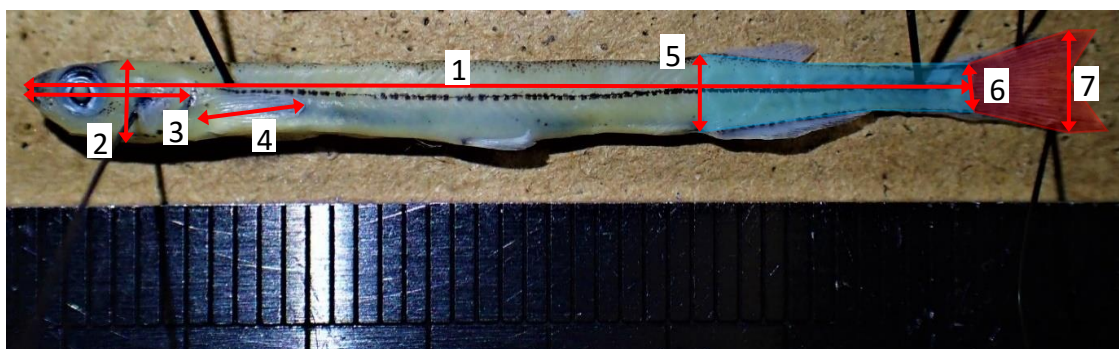
Figure 1. Lateral view of juvenile *G. maculatus* showing 9 morphometric measurements. 1=Standard length. 2=Head height. 3=Head length. 4=Pectoral fin length. 5=Body depth. 6=Caudal peduncle depth. 7=Tail fin height. Blue area=Caudal peduncle area. Red area=Tail fin area.

Figure 2. Dorsal view of juvenile *G. maculatus* showing 2 additional morphometric measurements. 8=Head width. 9=Body width

2.5 Otolith asymmetry

I explored potential evidence for developmental abnormalities related to stress by evaluating asymmetry in sizes of paired otoliths. To determine whether juvenile *G. maculatus* otoliths exhibited fluctuating asymmetry (FA), I selected a random subsample of 20 *G. maculatus* from each bin (N=60 per treatment) that survived ≥ 33 days. I assumed this to be a sufficient duration for treatments to cause asymmetry in otolith size and for the observed asymmetry to be primarily due to my treatments and not pre-treatment environmental stressors. Both left and right sagittal otoliths were extracted from 240 fish (i.e., 480 otoliths). Otoliths were photographed at 100x magnification

using a Canon EOS 70D camera connected to a Leica compound microscope. Following the methods of Libungan and Pálsson (2015), I photographed all otoliths against a black background, and I orientated each otolith so the flat side was inferiorly positioned (i.e. sulcus facing upwards) and the rostrum was pointing to the left. I used ImageJ v1.50i to measure the area, perimeter, length (major axis) and width (minor axis) of each otolith.

Following the methods used by Oliver (2013), I considered three indices for my FA analyses: the signed asymmetry index (to determine whether one otolith was consistently larger than the other), the unsigned asymmetry index (i.e., variation without consistent directionality), and the trait-size index. I calculated signed asymmetry as left minus right (L-R) for each variable. The unsigned asymmetry index was the absolute value of the signed asymmetry index (|L-R|) for each variable. I calculated the trait-size index as (L+R)/2 for each variable (this provides an overall measure of average otolith size for each fish). The magnitude of FA can be artificially inflated if it is correlated with the size of a trait (Bjorksten *et al.*, 2000; Palmer & Strobeck, 2003). Seeing as it is highly likely that magnitude of FA is correlated with otolith size (Oliver, 2013), I obtained the residuals of the linear least-squares regression between the unsigned asymmetry index and the trait-size index, and used these residuals to estimate a new signed and unsigned residual asymmetry index (Oliver, 2013). I considered only these 'size-corrected' estimates for further analysis. This method is assumed to control for variation due to otolith size, and retains variation due to true FA (Lemberget & McCormick, 2009).

2.6 Statistical analyses

2.6.1 Survival

I evaluated variation in fish survival as a function of water movement and water type using a Kaplan-Meier survival analysis. This analysis modelled survival time (the

response variable) as a function of 'treatment'. For this analysis, I considered the experiment to consist of four treatments (fresh + turbulent, salt + turbulent, fresh + calm, salt + calm). I did not evaluate these effects as an orthogonal design due to difficulties associated with the implementation of this in a Kaplan-Meier framework.

2.6.2 Growth and morphology

I evaluated variation in *G. maculatus* morphology as a function of water type and water movement using a permutation multivariate analysis of covariance (MANCOVA) using Euclidean distance matrices. I ran a permutation MANCOVA as my data had several multivariate outliers, which meant the assumptions of covariate normality and multivariate homogeneity of covariances were not met for a parametric MANCOVA. Following the methods of Reist (1985), I removed the effect of fish size by obtaining the residuals of a linear regression between standard length and each of my morphological traits, except fineness ratio and body condition because these two traits are already standardised to fish length. I then ran linear regressions between all possible pairs of standard length, fineness ratio, body condition and the residuals of the 12 other traits, to determine which traits were strongly correlated. Based on the suggestion of Tabachnick and Fidell (2013), I removed traits that had a correlation of 0.9 or higher, so that my data satisfied the assumption of absence of multicollinearity. This resulted in the removal of body condition, body depth, body width and tail fin height. I then ran a permutation MANCOVA with the remaining 11 traits as response variables, my treatments (water type and water movement as well as their interaction) as independent factors, bin as a blocking effect (to account for replicate bins within each treatment) and time as a covariate.

The MANCOVA suggested a significant overall effect of my treatments on *G. maculatus* morphology, so I performed separate univariate analyses to further identify where these differences were occurring. I further evaluated variation in fish morphology as a function of water movement and water type using a set of linear mixed-effects models (analysed separately for each morphological trait). Specifically, I evaluated models that included

'water type' and 'water movement' as fixed effects, 'time' as a covariate (because fish experienced bin environments for different survival durations) and 'bin' as a random effect nested within treatment (to control for shared environments among fish from the same bin). My models included the 3-way interaction term (water type*water movement*time) and all lower order terms. I also included 'standard length' (a measure of overall body size) as an additional covariate in some models, to evaluate trait size while controlling variation in body size. I included this additional covariate in all models except where the response variable was standard length, fineness ratio, or body condition, (because the latter two response variables are already standardised to body size).

For each response variable, I estimated the best reduced model by sequentially removing non-significant (p≥0.05) higher order interaction terms. I present these reduced models in Appendix 1 (unless the 3-way interaction term was significant, in which case I present the full model).

2.6.3 Otolith asymmetry

To evaluate the effects of water movement and water type on fluctuating asymmetry in otoliths, I used a set of linear mixed-effects models (analysed separately for each otolith trait measured). Specifically, I evaluated models that included 'water type' and 'water movement' as fixed effects, 'time' and 'body condition' as covariates, and 'bin' as a random effect. I included the 3-way interaction term (water type*water movement*time) and all lower order terms, and determined the best reduced model as described above (models are provided in Appendix 2).

All morphology and otolith data was checked to ensure it met the assumptions of normality and constant variance. Normality was checked using Shapiro-Wilk's normality test and a residuals vs quantiles of standard normal plot. Homogeneity of variance was checked with a residuals vs fitted values plot. The majority of data satisfactorily met

these assumptions, however I performed a square root transformation on the body weight data and all four measures of unsigned otolith asymmetry, and a natural log transformation on the fineness ratio data, in order for it to meet these assumptions.

I conducted the survival analysis in IBM SPSS Statistics 23. I used the 'vegan' package in the R Statistical Software Package V 3.3.3 to conduct the permutation MANCOVA and the 'nlme' package in the R Statistical Software Package V 3.3.2 to conduct the linear mixed effects analyses (for evaluating morphology & otolith asymmetry) (R Core Team, 2016).

3 RESULTS

3.1 Survival

Of the 1,104 fish distributed among bins at the start of the experiment, 10 survived to the end of the experiment. Five of these were in the salt + calm treatment, four were in the fresh + calm treatment and one was in the fresh + turbulent treatment. Employing the Generalized Wilcoxon test, survival estimates between treatments were significantly different (χ^2_3 =381.15, p<0.0001). Pairwise comparisons show that all treatments are significantly different from each other (Table 1).

Table 1. Results of Generalised Wilcoxon pairwise comparisons

Treatment	F+C		F+T		S+C		S+T	
	χ^2	p-value	χ^2	p-value	χ^2	p-value	χ^2	p-value
F+C			85.41	0.00	12.97	0.00	334.35	0.00
F+T	85.41	0.00			23.49	0.00	56.91	0.00
S+C	12.97	0.00	23.49	0.00			170.39	0.00
S+T	334.35	0.00	56.91	0.00	170.39	0.00		

As mean survival time cannot be reliably determined with censored data in which death has not occurred for some individuals (Gong & Fang, 2012; Jett & Ventre, 2015), I present median survival in Figure 3. Figure 3 shows that although median survival time is significantly different between all treatments, the calm treatments are closely grouped together, as are the turbulent treatments. Collectively, these patterns suggest that the turbulent environments decrease survival time (relative to calm conditions) and have a stronger effect than water type.
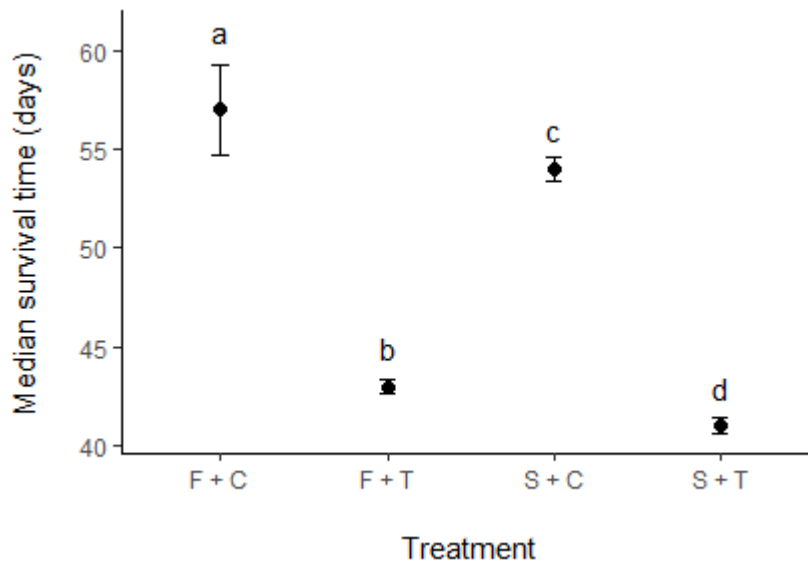
Figure 3. Median survival time for each treatment ± 95% confidence intervals. Different lowercase letters indicate a significant difference in survival time, based on pairwise tests.

The patterns of survival through time reflect divergent patterns in mortality, however. Kaplan-Meier survival curves for each treatment are shown in Figure 4. Fish numbers from both salt water treatments showed an instant steady decline, followed by a rapid decline midway through the experiment. All fish in the salt + turbulent treatment had died by Day 58, whereas fish numbers in the salt + calm treatment persisted at low numbers until the end of the experiment. Fish numbers in both fresh water treatments persisted at high levels for a longer initial period before also rapidly declining midway through the experiment. Fish numbers then persisted at low numbers in the fresh + calm treatment until the end of the experiment. Whereas, fish numbers in the fresh + turbulent treatment experienced a second levelling off and subsequent decline.
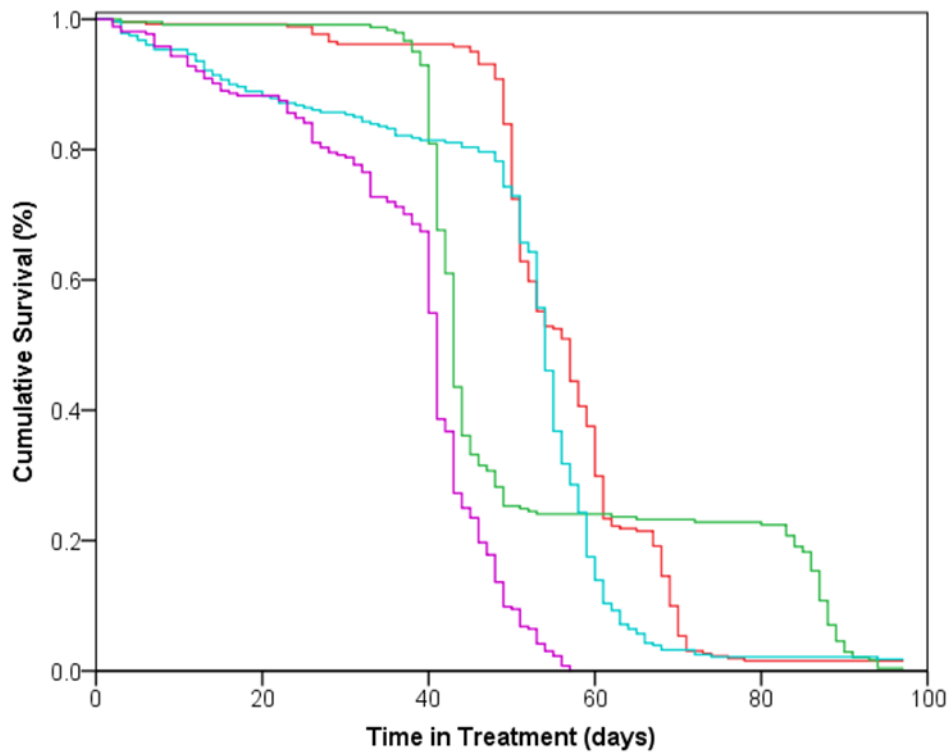
Figure 4. Kaplan-Meier survival curves showing cumulative survival vs time, by treatment. Red = Fresh + Calm. Green = Fresh + Turbulent. Blue = Salt + Calm. Purple = Salt + Turbulent.

3.2 Growth and morphological variation

The multivariate analysis of covariance shows that variation in *G. maculatus* morphology is significantly affected by water type, water movement, and their interaction (all p-values <0.05, Table 2). There is also a significant effect of time and bin (p-values <0.05, Table 2). Water type appears to be a better explanatory variable (R^2=0.20678, Table 2) than water movement (R^2=0.01457, Table 2). To further explore this relationship between treatment and *G. maculatus* growth and morphology, I present the results of a linear mixed-effects model for each morphological trait measured.

Table 2. Results of permutation MANCOVA investigating separate and joint effects of water type (salt versus fresh) and water movement (turbulent versus calm) on overall *G. maculatus* morphology, with exposure time as a covariate and bin as a block effect. P-values marked with a '*' represent significance at the α=0.05 level.

	Df	Sums of Squares	Mean Squares	F Model	R^2	Pr (>F)
Water Type	1	3158.6	3158.63	141.051	0.2068	0.001*
Water Movement	1	222.6	222.55	9.938	0.0146	0.001*
Time	1	820.9	820.91	36.658	0.0537	0.001*
Bin	2	213.1	106.56	4.758	0.0140	0.002*
Water Type*Water Movement	1	267.8	267.83	11.960	0.0175	0.001*
Residuals	473	10592.2	22.39		0.6934	
Total	479	15275.2			1.00	

3.2.1 Positive effect of treatment

The full model for *G. maculatus* standard length suggests that body length increases over time in all 4 treatments (t_{464} = 5.834797, p < 0.0001; Table 3, Appendix 1). However, the effect of water type over time differed, depending on water movement (t_{464} = 4.802323, p < 0.0001; Table 3, Appendix 1). Standard length increased fastest in salt + turbulent treatment (0.149mm per day), second fastest in the fresh + calm treatment (0.109mm per day), third fastest in the salt + calm treatment (0.056mm per day), and

slowest in the fresh + turbulent treatment (0.049mm per day). Figure 5 depicts this and shows that in fresh water, fish grow faster in calm conditions, whereas in salt water, fish grow faster in turbulent conditions. I also found that all other morphological traits increased in size as *G. maculatus* standard length increased.

Figure 5. Relationship between standard length and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 3, Appendix 1.

3.2.2 Positive effect of fresh water, negative effect of salt water

Many *G. maculatus* morphological traits increased in size over time in fresh water but decreased in salt water, regardless of water movement. For the square root of weight the effect of water type over time differs, depending on water movement (t_{463} = - 2.172542, p = 0.0303; Table 4, Appendix 1). Weight increased faster in the fresh + turbulent treatment than in the fresh + calm treatment, whereas it decreased faster in the salt + turbulent treatment than in the salt + calm treatment (see Figure 6). For body depth there is a statistically significant interaction between water type and time (t_{465} =

-3.14383, p = 0.0018; Table 5, Appendix 1). Body depth increases in the fresh treatments, but decreases in the salt treatments. There is also a statistically significant effect of water movement (t_9 = -3.24335, p = 0.0101; Table 5, Appendix 1), keeping all other factors constant, body depth is lower in the turbulent treatments than in the calm treatments (see Figure 7). For caudal peduncle depth there are statistically significant interactions between water type and time (t_{464} = -5.035958, p < 0.0001; Table 6, Appendix 1), and water movement and time (t_{464} = -2.735364, p = 0.0065; Table 6, Appendix 1). Caudal peduncle depth increased faster in the fresh + calm treatment than in the fresh + turbulent treatment, whereas it decreased faster in the salt + turbulent treatment than in the salt + calm treatment (see Figure 8). For tail fin height there are statistically significant interactions between water type and water movement (t_8 = 2.584471, p = 0.03245; Table 7, Appendix 1), and water type and time (t_{465} = -2.524576, p = 0.0119; Table 7, Appendix 1). Tail fin height increased at the same rate in the two fresh treatments, and decreased at the same rate in the two salt treatments (see Figure 9). For tail fin aspect ratio there are statistically significant interactions between water type and time (t_{464} = -2.508875, p = 0.0125; Table 8, Appendix 1), and water movement and time (t_{464} = -2.251, p = 0.0249; Table 8, Appendix 1). Tail fin aspect ratio increased faster in the fresh + calm treatment than in the fresh + turbulent treatment, whereas it decreased faster in the salt + turbulent treatment than in the salt + calm treatment (see Figure 10).
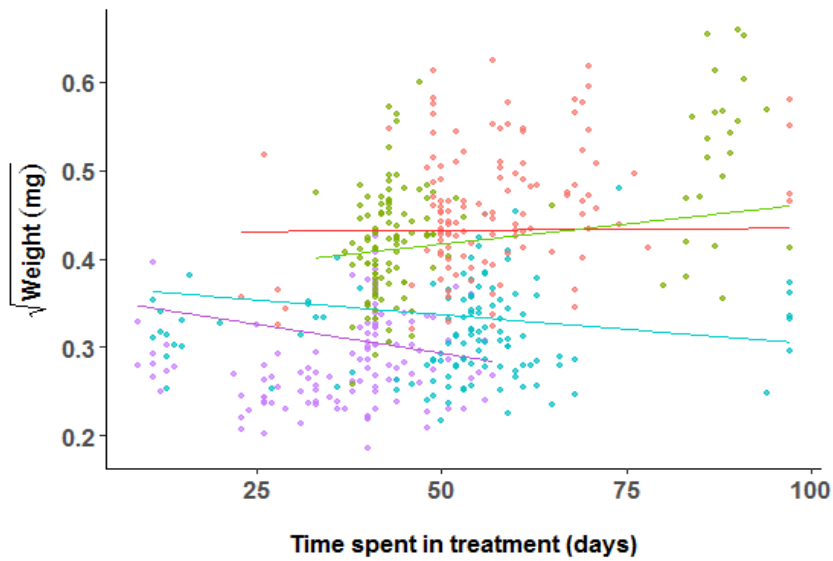
Figure 6. Relationship between square root weight and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 4, Appendix 1.

Figure 7. Relationship between body depth and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 5, Appendix 1.
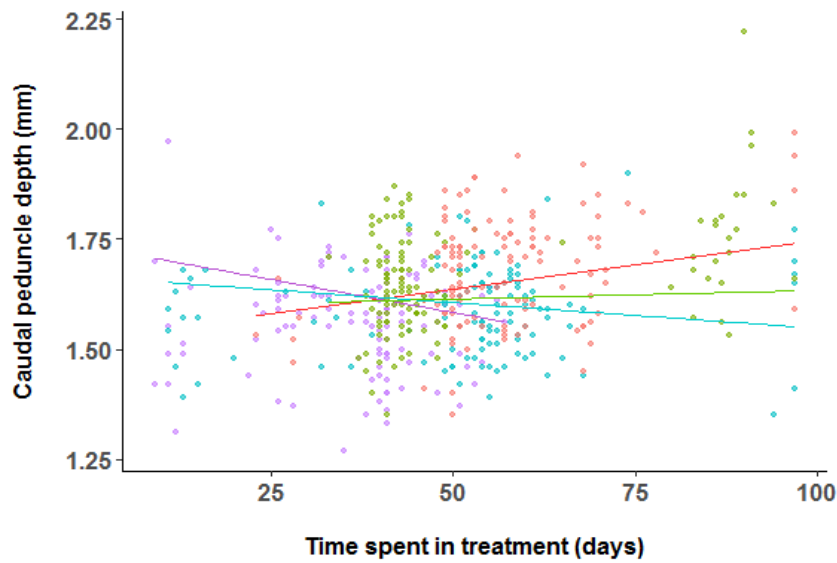
Figure 8. Relationship between caudal peduncle depth and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 6, Appendix 1.
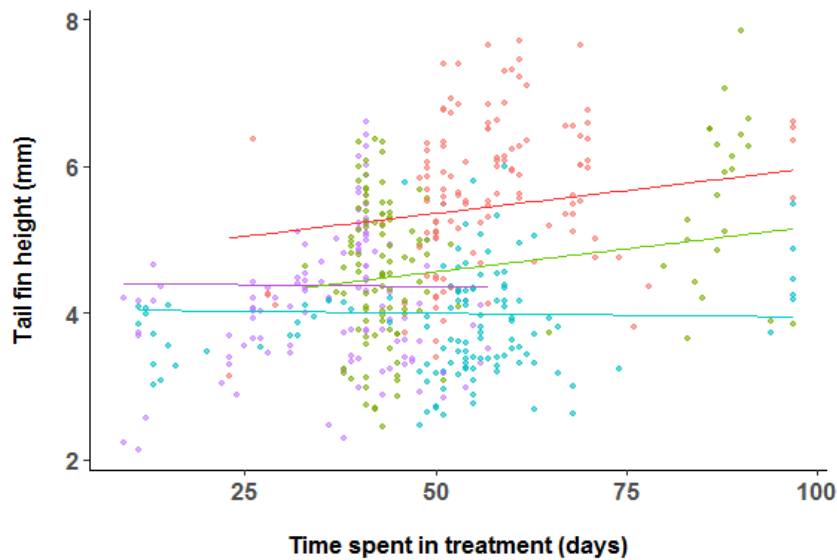
Figure 9. Relationship between tail fin height and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 7, Appendix 1.
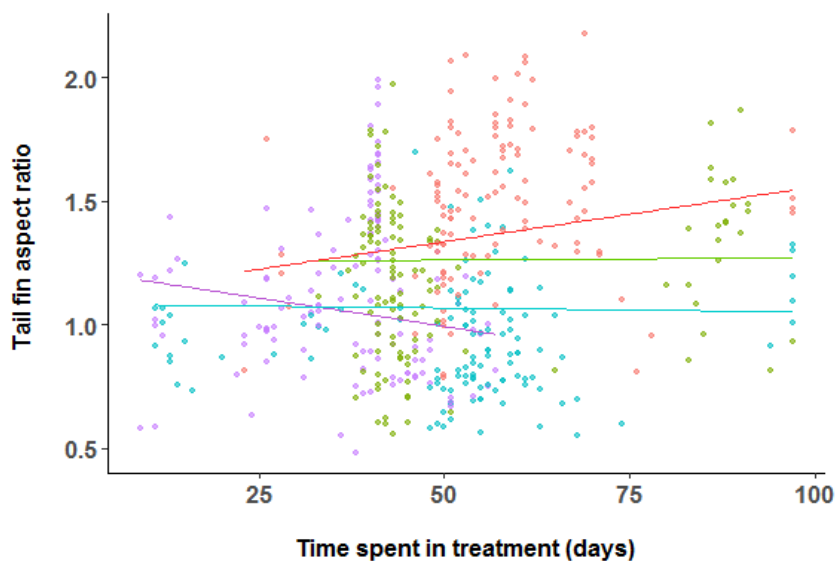
Figure 10. Relationship between tail fin aspect ratio and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 8, Appendix 1.

3.2.3 Negative effect of fresh water, positive effect of salt water

The reduced model for *G. maculatus* log fineness ratio suggests that there is a statistically significant interaction between water type and time (t_{466} = 2.92102, p = 0.0037; Table 9, Appendix 1). Log fineness ratio increases at the same rate over time in the salt treatments, but decreases at the same rate over time in the fresh treatments (see Figure 11). There is also a statistically significant effect of water movement (t_9 = 3.30739, p = 0.0091; Table 9, Appendix 1). Regardless of water type, log fineness ratio is greater in turbulent conditions than calm conditions (see Figure 11).
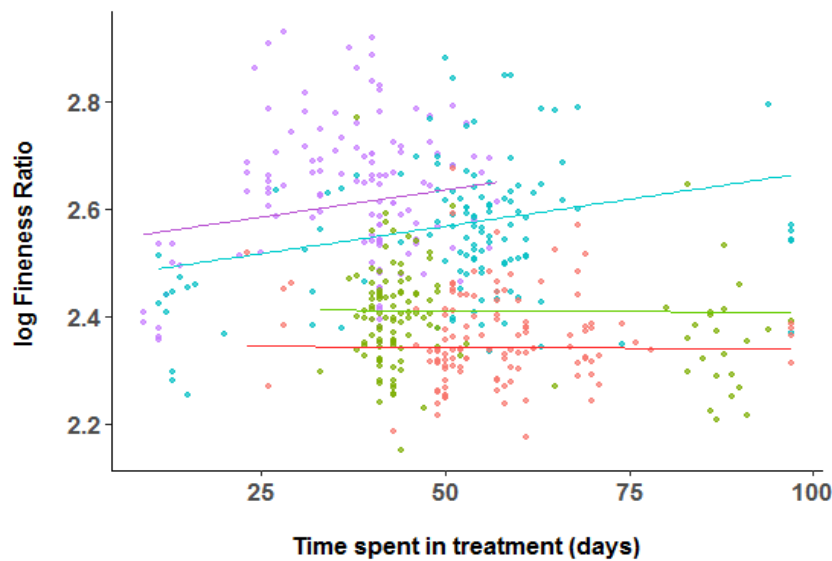
Figure 11. Relationship between log fineness ratio and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 9, Appendix 1.

3.2.4 Positive effect of fresh water and salt + calm conditions

G. maculatus head height and head width showed the same pattern, trait size decreases over time in the salt + turbulent treatment, but increases in all other treatments. For both traits, the effect of water type over time differs, depending on water movement (height: t_{463} = -3.29993, p = 0.0010; Table 10, Appendix 1; width: t_{463} = -2.252344, p = 0.00248; Table 11, Appendix 1). Head height and width increase fastest in fresh + turbulent treatment, second fastest in the fresh + calm treatment, and slowest in the salt + calm treatment, whereas they decrease in the salt + turbulent treatment (see Figure 12 and 13).
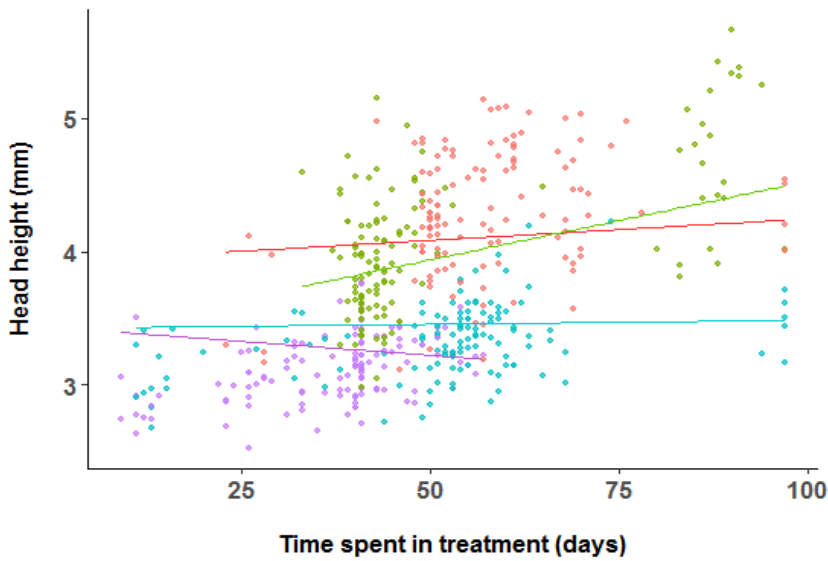
Figure 12. Relationship between head height and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 10, Appendix 1.
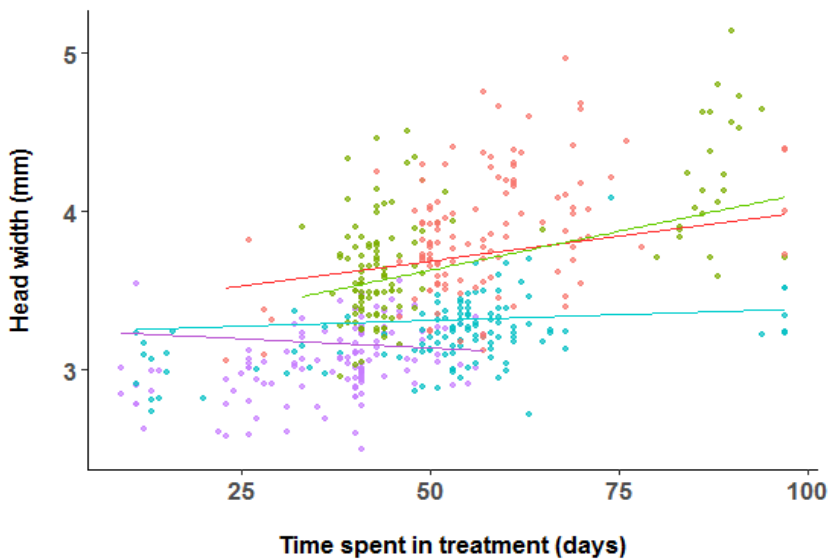
Figure 13. Relationship between head width and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 11, Appendix 1.

3.2.5 Negative effect of salt water and fresh + calm conditions

G. maculatus body condition and caudal peduncle area showed a similar pattern, trait size increases over time in the fresh + turbulent treatment, but decreases over time in all other treatments. For both traits, the effect of water type over time differs, depending on water movement (body condition: t_{464} = -2.1615, p = 0.0312; Table 12, Appendix 1; caudal peduncle area: t_{463} = -2.25272, p = 0.0247; Table 13, Appendix 1). Body condition and caudal peduncle area decrease fastest in the salt + turbulent treatment, second fastest in the salt + calm treatment, and slowest in the fresh + calm treatment, whereas they increase in the fresh + turbulent treatment (see Figure 14 and 15).
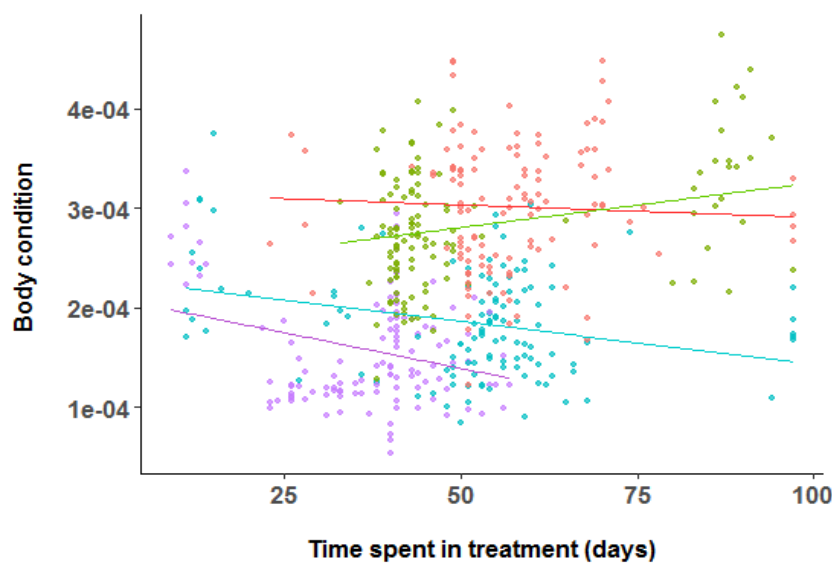
Figure 14. Relationship between body condition (Fulton's K) and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 12, Appendix 1.
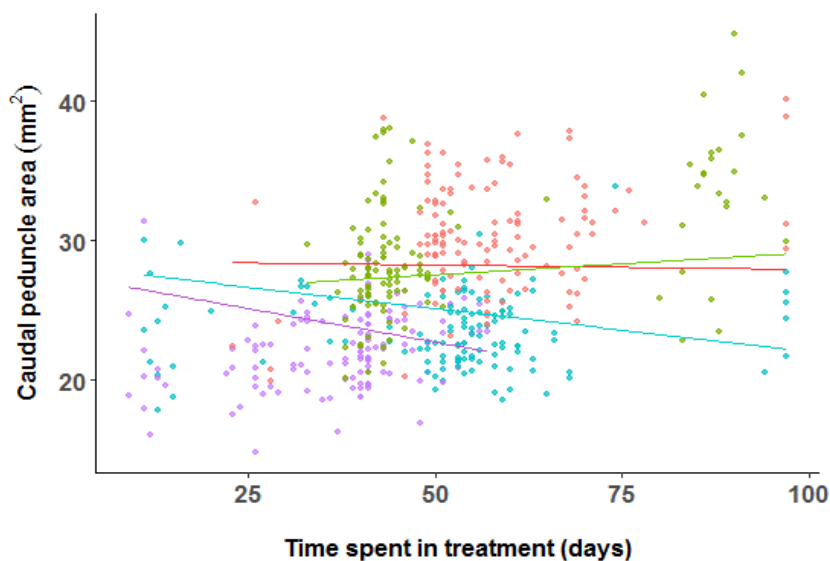
Figure 15. Relationship between caudal peduncle area and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 13, Appendix 1.

3.2.6 No effect of turbulence

Many *G. maculatus* morphological traits were not significantly affected by water movement. In head length, pectoral fin length, tail fin area and body width the effect of water movement was non-significant (p-values>0.05). For head length, pectoral fin length and tail fin area there is a statistically significant interaction between water type and time (head length: t_{465} = -3.08004, p = 0.0022; Table 14, Appendix 1; pectoral fin: t_{465} = -2.598075, p = 0.0097; Table 15, Appendix 1; tail area: t_{465} = -3.68222, p = 0.0003; Table 16, Appendix 1). Head length, pectoral fin length and tail fin area all increase faster in the fresh treatments than in the salt treatments (see Figures 16, 17 and 18). For body width there is a statistically significant interaction between water type and time (t_{465} = -3.719783, p = 0.0002; Table 17, Appendix 1). Body width increases in the fresh treatments, but decreases in the salt treatments (see Figure 19).
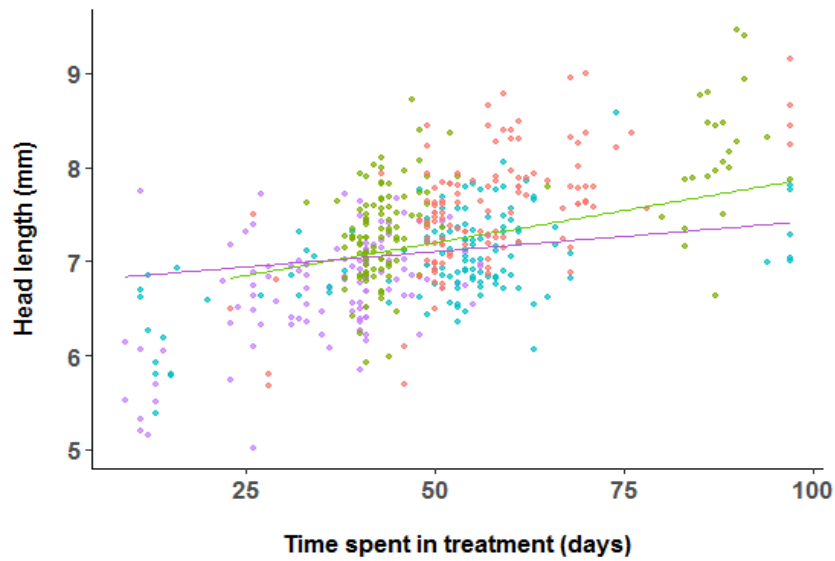
Figure 16. Relationship between head length and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Green line=Fresh. Purple line=Salt. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 14, Appendix 1 and plotted assuming turbulent conditions.

Figure 17. Relationship between pectoral fin length and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Green line=Fresh, Purple line=Salt. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 15, Appendix 1 and plotted assuming turbulent conditions.
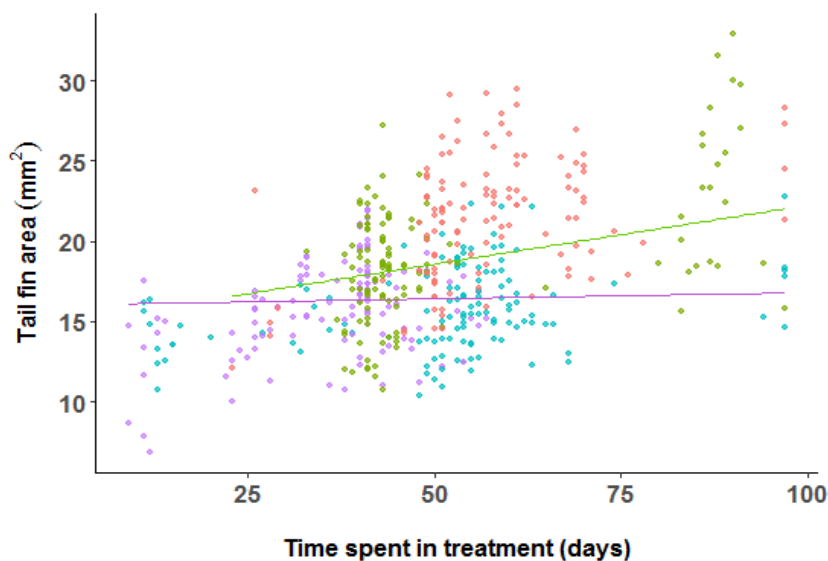
Figure 18. Relationship between tail fin area and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Red line = Fresh. Purple line = Salt. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 16, Appendix 1 and plotted assuming turbulent conditions.
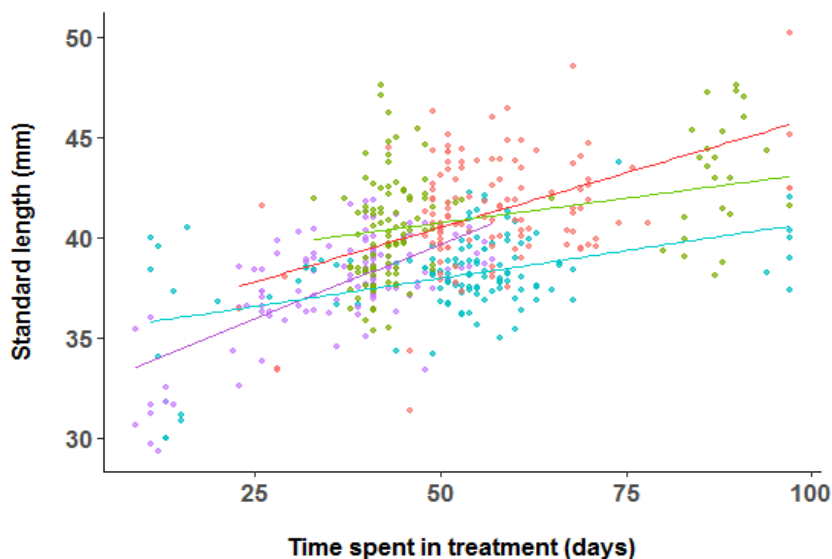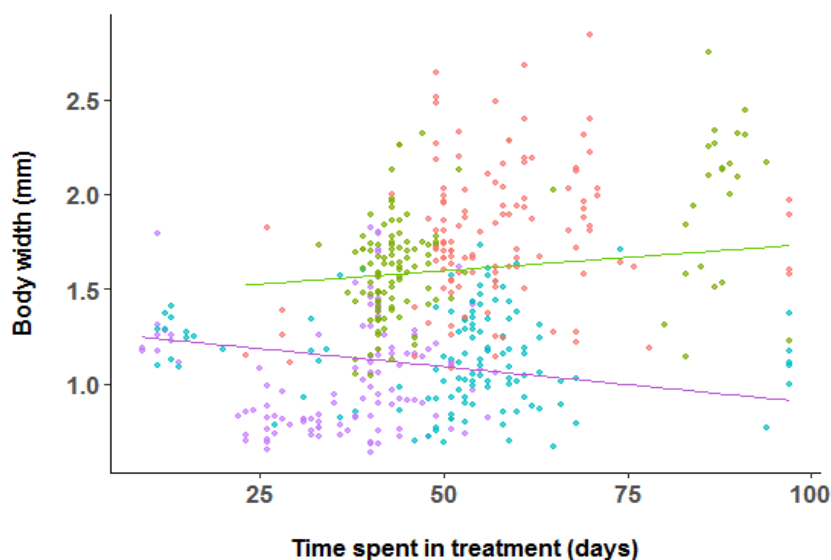

Figure 19. Relationship between body width and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Green line=Fresh. Purple line=Salt. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 17, Appendix 1 and plotted assuming turbulent conditions.

3.2.7 Summary

In summary, both water type and water movement influenced *G. maculatus* growth and morphology, but this effect was not the same for all morphological traits. Individuals in the salt + turbulent treatment generally had the lowest body weight, body condition, body depth, head height, head width, caudal peduncle area and tail fin aspect ratio out of all the treatments, regardless of time. In contrast, individuals in the fresh + calm treatment generally had the highest body depth, caudal peduncle depth, tail fin height and tail fin aspect ratio out of all the treatments. Individuals in the salt + calm and fresh + turbulent treatments generally experienced intermediate effects between these two. In almost all other aspects of morphology, trait size was greater in the fresh water treatments than in the salt water treatments, regardless of water movement and time. The notable exception of this was fineness ratio, which was highest in the salt + turbulent treatment, and lowest in the salt + calm treatment. In general, freshwater had a strong positive effect on trait size, while salt water had a strong negative effect. Water movement had a weaker and less consistent effect on *G. maculatus* morphology.

3.3 Otolith asymmetry

3.3.1 Signed asymmetry

The reduced models for otolith area, perimeter, length and width signed index suggest that neither water type, water movement, time nor body condition had a significant effect on the level of signed otolith asymmetry (all p-values>0.05, Table 18-21, Appendix 2). This means that neither the right nor the left otolith is getting progressively bigger than its counterpart over time or between treatments. This pattern can be seen in Figure 20, as it shows that the relationship between signed asymmetry in otolith area (20A), otolith perimeter (20B), otolith length (20C), otolith width (20D) and time spent in treatment is random, with no discernible pattern favouring left or right and no effect of treatment.
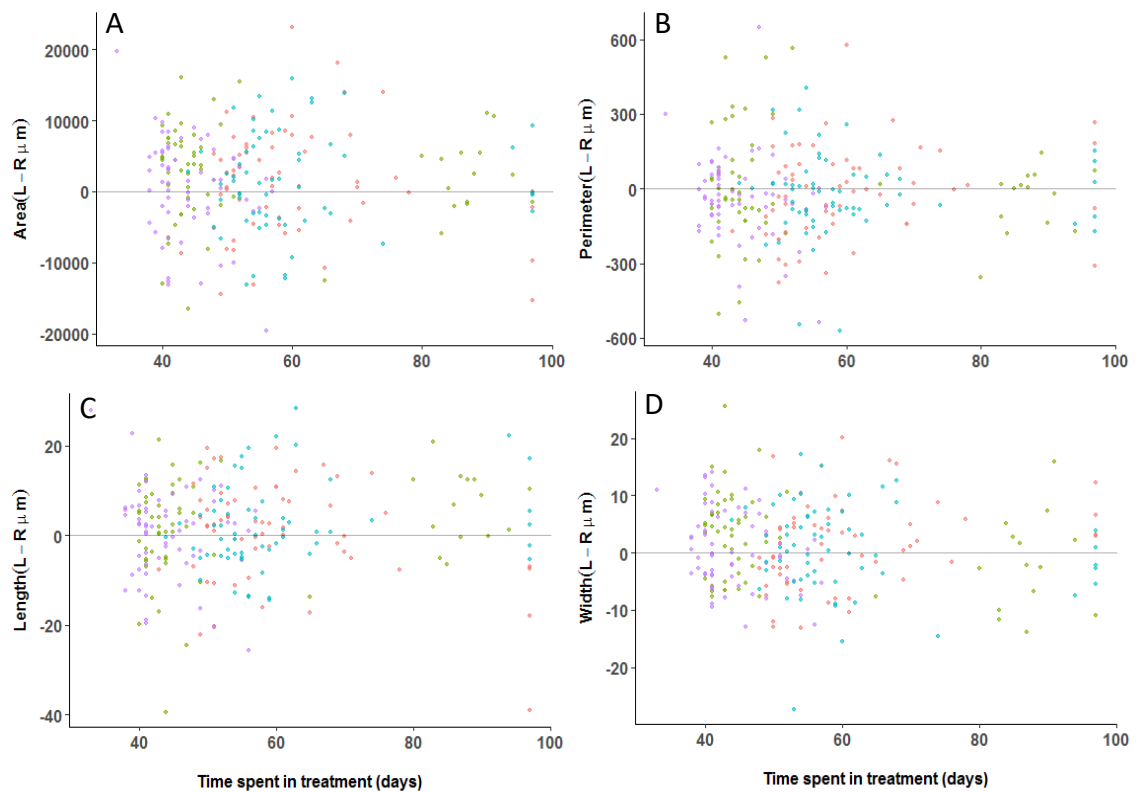
Figure 20. Relationship between time spent in treatment and otolith area signed asymmetry index (A), otolith perimeter signed asymmetry index (B), otolith length signed asymmetry index (C) and otolith width signed asymmetry index (D). Different treatments are shown by colour: Red=Fresh + Calm, Green=Fresh + Turbulent, Blue=Salt + Calm, Purple=Salt + Turbulent.

3.3.2 Absolute asymmetry

My analysis suggests that, keeping all other factors constant, the square root of absolute asymmetry of otolith area is increasing by 0.24μm per day (t_{226} = 2.2809024, p = 0.0235; Table 22, Appendix 2). This pattern can be seen in the upwards sloping line of Figure 21. I found that no other factor, apart from body condition, which was trending towards significance (p=0.0692; Table 22, Appendix 2), had a significant effect on the level of absolute asymmetry in otolith area.
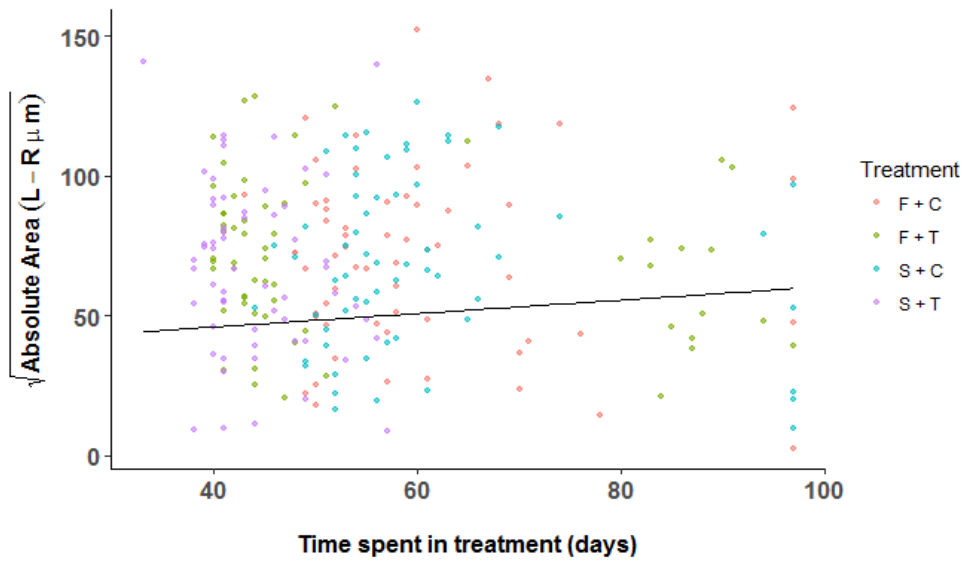
Figure 21. Relationship between otolith area unsigned asymmetry index and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Black line = effect of time, calculated based on coefficient estimate values given in Table 22, Appendix 2, averaged across all 4 treatments.

My analysis suggests that for absolute asymmetry of otolith perimeter the effect of water type over time differs, depending on water movement (t_{223} = 2.4727111, p = 0.0142; Table 23, Appendix 2). Absolute asymmetry in otolith perimeter increased faster in the salt + turbulent treatment than the fresh + calm treatment, whereas it decreased faster in the salt + calm treatment than in the fresh + turbulent treatment (see Figure 22).
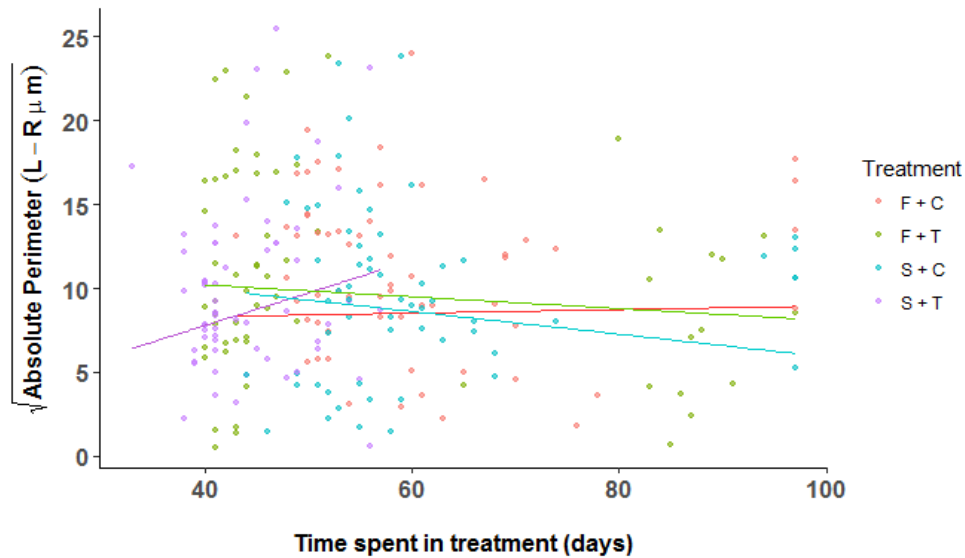
Figure 22. Relationship between otolith perimeter unsigned asymmetry index and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent. Fitted lines are calculated based on coefficient estimates from the mixed model in Table 23, Appendix 2.
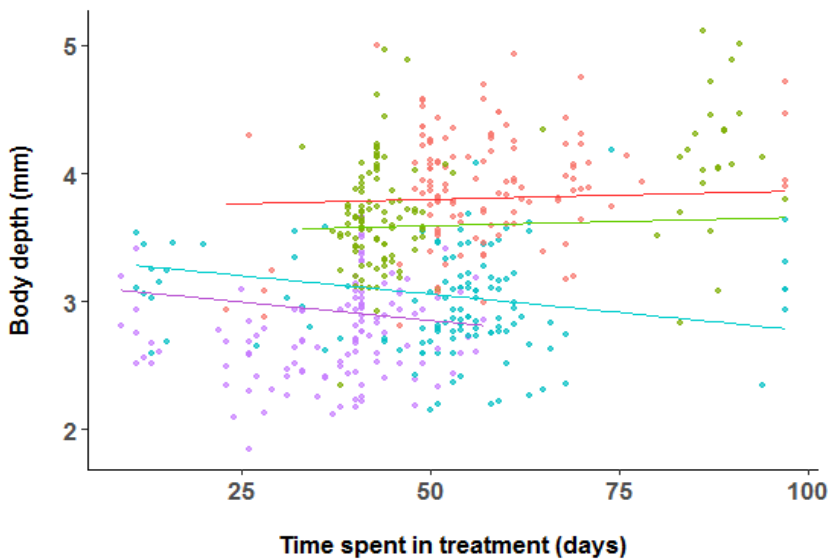
There were four vateritic otoliths in my sample, one in the fresh + turbulent treatment, one in the salt + calm treatment, and two in the salt + turbulent treatment. A vateritic otolith has a very jagged, uneven outline (see Figure 23), due to having a different crystalline structure than a regular otolith (Tomás & Geffen, 2003). Due to this, the difference in perimeter between the vateritic otolith and its smooth-edged counterpart would be much larger than usual, even if there was little difference in area, length and width. There is a possibility that these vateritic otoliths may be driving the pattern seen here, so I re-ran the analysis with vateritic otoliths removed. This removal resulted in less pronounced differences between the treatments, but the three-way interaction between water type, water movement and time remained statistically significant (t_{223} = 2.4727111, p = 0.0142; Table 24, Appendix 2), and the interaction plot showed an almost identical pattern to that shown in Figure 22. This indicates that the vateritic otoliths are not driving the pattern and it must be due to something else.
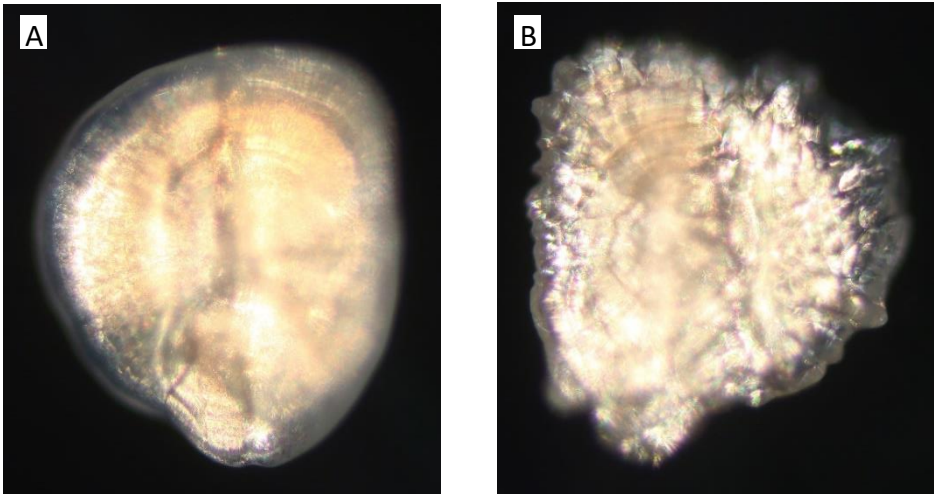
Figure 23. Dorsal view of a regular (A) and vateritic (B) otolith from the same *G. maculatus* individual.

My analysis suggests that neither water type, water movement, time or body condition had a significant effect on the level of absolute asymmetry in otolith length or width (Table 25 and 26, Appendix 2). This pattern can be seen in Figure 24 as it shows that the relationship between unsigned asymmetry in otolith length (Figure 24A) or otolith width (Figure 24B) and time spent in treatment is random, with no discernible pattern or effect of treatment.
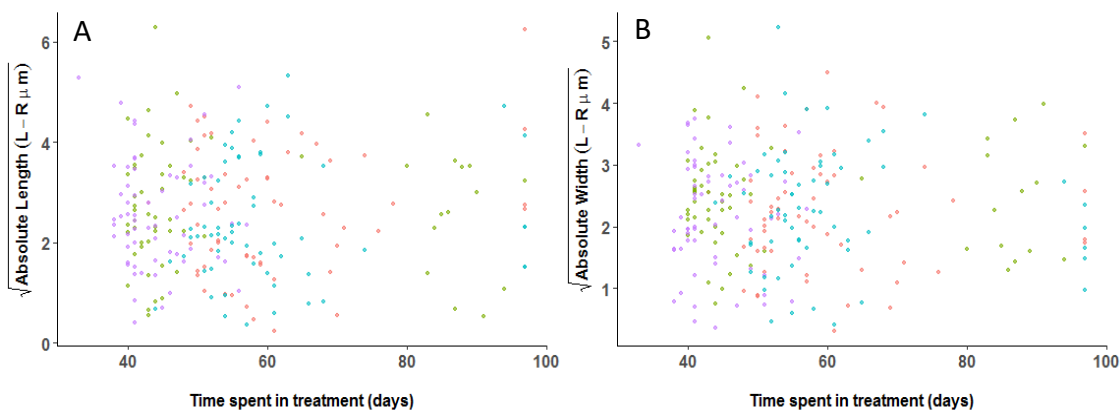
Figure 24. Relationship between otolith length (A) and otolith width (B) unsigned index and time spent in treatment. Different treatments are shown by colour: Red = Fresh + Calm, Green = Fresh + Turbulent, Blue = Salt + Calm, Purple = Salt + Turbulent.

3.3.3 Summary

I found that *G. maculatus* otoliths do not display directional asymmetry, as one side was not consistently larger than the other. Body condition did not have a significant effect on fluctuating asymmetry (FA). Otolith length and width showed no evidence of FA, whereas otolith area showed evidence of increasing absolute asymmetry over time, but this was not affected by treatment. Otolith perimeter was the only trait to show evidence of FA in response to my treatments. Absolute asymmetry increased over time in the salt + turbulent and fresh + calm treatments, but decreased in the fresh + turbulent and salt + calm treatments.

4 DISCUSSION

This experiment simulates natural conditions that *G. maculatus* are exposed to during their larval and transitionary phases from salt to fresh water. The salt + calm treatment represents environmental conditions fish would experience if they were retained in a harbour (i.e. low flow). The salt + turbulent treatment represents conditions fish would experience if they were in open coastal waters and experiencing strong ocean currents, meaning more energy would be exerted in order to return to fresh water. The fresh + calm treatment represents the environment fish would encounter within slower flowing rivers, pools or eddies, while the fresh + turbulent treatment represents conditions experienced in faster flowing rivers or riffle habitats. I investigated how these environments affected the survival, growth, morphology and stress of *G. maculatus*. I expected to find that when fish are required to exert more energy for swimming, they will experience higher mortality, as they will not be able to dedicate as large a portion of energy to growth and maintaining condition. I also expected to find that *G. maculatus* experience higher growth rates in fresh, calm water as they are not having to expend large amounts of energy on swimming and osmoregulation, whereas I anticipated slower growth rates in salt, turbulent water as they will be expending large amount of energy. I expected otolith FA to increase over time in the more stressful treatments (mainly salt + turbulent) and show an increase with decreasing body condition.

4.1 Survival

Results of my experiment suggest that *G. maculatus* may be limited in their ability to extend their larval development time in the sea. Fish died at a greater rate over their first ~40 days of development in salt water treatments relative to fish kept in fresh water

treatments. My results also suggest that the larvae are not well equipped to cope with turbulent environments for long periods of time. Mortality rates between calm and turbulent conditions were initially very similar, however populations maintained in turbulent conditions collapsed in comparison to those in calm conditions after about 30 days of chronic exposure, suggesting that fish can accommodate such conditions for periods of time, but chronic exposure exerts a physiological cost.

Iida *et al.* (2010) found that each life stage of the amphidromous goby *Sicyopterus japonicas* was adapted to the specific salinity regime inhabited during each stage of their lifecycle. For example, eggs and adults experienced higher survival in fresh water, while larval forms experienced higher survival in salt water. This suggests amphidromous fish undergo physiological changes at each life stage that enables survival in the specific salinity regime of that stage of their life (Foster, 1969). In addition, Keith *et al.* (2008) found that osmoregulatory changes to better adapt to fresh water in the larvae of the amphidromous goby *Sicyopterus lagocephalus* occur when individuals first arrive in an estuarine environment. Therefore, the increased mortality I witnessed in salt water treatments may have been the result of the larval fish I caught at the river mouth already beginning to undergo metamorphosis to adapt to living in fresh water. Therefore, retention in salt water likely resulted in high osmoregulatory stress (Morgan & Iwama, 1991; Tandler *et al.*, 1995), which may explain the reduced survival. This could imply that my salt water treatments were not a fair test of *G. maculatus* ability to prolong the marine phase. Had I caught and tested larvae before they began their transition into fresh water, the results may have been different, and lower levels of mortality may have been observed.

All treatments experienced very high mortality between days 40 and 60. This was unexpected, as prior to this fish numbers in salt water treatments had been declining, but at a slow steady rate, and fresh water treatments had been experiencing very little mortality. One theory is that the fish contracted a disease from the water collected from the Hutt River or the ocean and this was spread among treatment bins via shared cleaning equipment or tools used to remove dead individuals, although there is no way to prove this. Temperature loggers showed no sudden temperature fluctuations during

this period, and temperature stayed within the range of 15-20°C, well within *G. maculatus* tolerance limits (Boubee *et al.*, 1991; Richardson *et al.*, 1994). Therefore it is unlikely that temperature was the main cause of this increased mortality. Alternatively, mortality may have been due to a common factor among treatment bins, such as feeding regime. The food I supplied may not have catered for an ontogenetic shift in diet (or other resource) that may occur at a particular stage in the *G. maculatus* lifecycle. Ontogenetic shifts in diet have been demonstrated in other amphidromous species (Schoenfuss & Blob, 2007). For example, structures associated with feeding in the amphidromous goby (*Sicyopterus lagocephalus*) undergo changes at the end of the larval stage, which corresponds with a dietary shift from planktonic copepods to benthic algae (Keith *et al.*, 2008). A similar change may have also occurred in my test fish, resulting in nutritional deficits and thus higher rates of mortality.

4.2 Growth, condition and morphology

Very few studies have looked at the growth and development variability during the transitionary period from salt to fresh water. My results suggest that *G. maculatus* cannot remain in the marine environment indefinitely and may be constrained by physiology. If larvae are unable to return to freshwater at the end of their larval phase, and therefore remain in salt water for an extended period of time, a resulting decrease in developmental rates will occur, compared to those who successfully return to freshwater at the end of their larval phase. This is an interesting result as many studies have shown that the marine environment is highly productive and larval fish gain an adaptive advantage by spending time in this nutrient-rich environment (Gross *et al.*, 1988; Edeline, 2007; McDowall, 2007; Thibault *et al.*, 2007). Therefore, one would expect that spending an extended period of time in this high food environment would benefit larval fish, as they would develop quicker and be able to achieve greater sizes (Gross *et al.*, 1988; McDowall, 2007; Thibault *et al.*, 2007). However, I found that while standard length increased over time in all treatments, other traits, such as weight, body

condition, body depth, body width, head height and head width all decreased in the salt water treatments.

Woods (1968) characterised larval *G. maculatus* as elongate and transparent, and stated that "on entering fresh water the larvae gradually become pigmented and eventually stouter bodied". My results indicate that *G. maculatus* kept in salt water (i.e. those that experience an extended marine duration) retain their larval properties and do not gain weight or transform into the adult morph described by Woods (1968). An example of this is demonstrated in Figure 25. These fish both perished on day 65 (more than halfway through the experiment), but one was subjected to the salt water treatment (A) and one was subjected to the fresh water treatment (B). There are clear morphological differences between the individuals. The salt water representative has a slender body, with a larger head than body depth, and is both transparent and devoid of pigmentation. This is typical of larval *G. maculatus* that have not yet migrated back into fresh water. The fresh water individual has a deeper, more rotund body, with similar head and body depths and the beginnings of body colour and pigmentation, typical of juvenile or adult *G. maculatus* following settlement in fresh water (Woods, 1968). These results indicate that a delayed return to fresh water for larval *G. maculatus* causes retention of larval characteristics, regardless of age. This could indicate the presence of a signal in fresh water that triggers development from the larval form to the adult form.

Figure 25. Lateral view of two G. maculatus that perished on day 65, showing morphological differences between individuals from salt water (A) and fresh water (B).

In partial support of this theory, Iida *et al.* (2010) found that salt water was necessary for larval development of the amphidromous goby *S. japonicus*, as development of larval traits such as eye pigmentation, mouth opening and yolk absorption did not occur unless individuals were inhabiting salt water (but see Pollard, 1971; Chapman et al., 2009; Barriga et al., 2012; Augspurger et al., 2017). Many other studies have investigated the influence of environmental salinity on fish development and found that certain salinity levels (ranging from 0ppm to 35ppm) are required for each stage of early development to proceed optimally, in both marine and fresh water fish (Watanabe *et al.*, 1989; Hart & Purser, 1995; Tandler *et al.*, 1995; Peterson *et al.*, 1996; Swanson, 1996; Fielder & Bardsley, 1999; Smith *et al.*, 1999; Specker *et al.*, 1999). It is likely that a similar situation is true for larval *G. maculatus,* where they require fresh water in order to develop into adults. I should note that the marine larval phase is not essential for my study species, as some populations of *G. maculatus* are wholly or partially landlocked and complete their lifecycle entirely within freshwater. Therefore, salt water (or a salinity change) is not a requirement for *G. maculatus* (Pollard, 1971; Chapman *et al.*, 2009; Barriga *et al.*, 2012; Augspurger *et al.*, 2017).

Phenotypic variation in response to water type (i.e. salt or fresh water) was fairly consistent among morphological traits. I expected higher growth rates and larger trait sizes in fresh water, as individuals should be suffering less osmotic stress and can allocate more energy to growth. This was indeed the case for all morphological traits. Many studies have shown that fish species previously acclimated to a range of salinities experience higher growth in fresh water than in salt water, regardless of the acclimation salinity (Clarke *et al.*, 1981; McKay & Gjerde, 1985; Heyward *et al.*, 1995; Alava, 1998). Even juveniles of anadromous species such as steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*), which spend a proportion of their lives in the marine environment, show decreased growth rates and higher metabolic rates in higher salinities (Morgan & Iwama, 1991). Osmoregulation has been estimated to use as much as 50% of available energy in fish, depending on environmental salinity (Rao, 1968; Nordlie & Leffler, 1975; Nordlie, 1978; Furspan *et al.*, 1984; Nordlie *et al.*,

1991; Toepfer & Barton, 1992). Therefore, in situations of elevated osmotic stress, very little energy remains for swimming and growth. Thus, it is likely that the reduced growth exhibited in my salt water treatments was due to the high energetic cost of osmoregulation in salt water, resulting in limited energy available for growth.

I expected to find that *G. maculatus* exhibit lower growth rates in turbulent water as they need to expend larger amounts of energy on swimming and maintaining their position in the water column (Hinch & Rand, 1998; Enders *et al.*, 2003). Contrary to this, the effect of turbulence was not consistently negative. Phenotypic variation in response to water movement (i.e. calm or turbulent) was inconsistent among morphological traits and often depended on water type. For example, in both water types, body depth was consistently greater in the calm treatment, while in salt water tail fin height was greater in the turbulent treatment, whereas in fresh water it was greater in the calm treatment. In addition, head height and width experienced increased growth in all treatments except salt + turbulent, whereas body condition and caudal peduncle area experienced decreased growth in all treatments except fresh + turbulent.

A high tail fin aspect ratio, narrow caudal peduncle and fineness ratio near 4.5 represents the optimal fish design for prolonged, efficient swimming, as it maximises thrust and minimises drag (Sambilay, 1990; Blake, 2004; Fisher & Hogan, 2007), so I expected fish in turbulent treatments to display these characteristics, as it would help them maintain their position in the water column and conserve energy. Instead, I found fish in turbulent conditions often had lower tail fin aspect ratios and higher fineness ratios (further from 4.5) than those in calm conditions. There was however evidence of lower caudal peduncle depths in individuals from turbulent conditions in the later stages of the experiment. A deep caudal peduncle is found in fish described as 'accelerators'. They specialise in quick, darting movements, but have poor sustained swimming speeds (Webb, 1994; Blake, 2004). Conversely, a narrow caudal peduncle is characteristic of thunniform fish, which use an axially oscillating tail fin to generate thrust for cruising at high speed (Webb, 1984b; Webb & Weihs, 1986). This could explain why my fish, which also swim using an axially oscillating tail fin, showed lower and/or decreasing caudal peduncle depths in turbulent treatments, as opposed to calm treatments. A narrower caudal peduncle conveys an advantage when sustained swimming is necessary, as may

have been the case in the turbulent treatments. However the stronger differences in fineness ratio and tail fin aspect ratio indicate that any advantage due to a narrower caudal peduncle is likely negated.

During steady swimming in micro-turbulent flow (turbulence that varies over small scales), the pectoral fins may be inactive in species with more ventrally positioned pectoral fins and a relatively horizontal fin base, such as trout (subfamily Salmoninae) (Drucker & Lauder, 2003). Whereas fish with a more lateral fin position and relatively vertical fin base, such as perch-like fish, may swim steadily using only their pectoral fins (Gibb *et al.*, 1994; Drucker & Jensen, 1996a, b; Westneat, 1996; Walker & Westneat, 1997; Drucker & Lauder, 1999; Drucker & Lauder, 2000; Drucker & Lauder, 2003). However, in trout, the introduction of increased turbulence can prompt pectoral fin activity, which is correlated with corrective motions that aid fish in maintaining their position in the water column (Liao *et al.*, 2003). Although my results showed no significant effect of water movement on pectoral fin length, suggesting *G. maculatus* do not use their pectoral fins for steady swimming in turbulent conditions, they may be similar to trout in that pectoral fin activity is induced in more turbulent conditions (i.e. they need to utilise their pectoral fins to steady themselves). However, my results may not have been able to show this, as I only measured pectoral fin length, not fin area or aspect ratio.

Perhaps the lack of significant and consistent changes in trait size due to water movement was because the level of turbulence was too weak to cause significant differences. Another possibility is that fish in the turbulent conditions did not spend their time in the water column, swimming against the current, but instead inhabited refuges where the water was less turbulent, such as the bottom of the experimental bin, in corners, or up against the pump housing. I witnessed this on a number of occasions, and this has also been observed during a study investigating fish swimming ability, where individuals favoured lower velocity regions of the test flumes (Plew *et al.*, 2007). This meant they did not have to undergo significant morphological change to better adapt to local conditions.

It is possible that the differences in body condition, weight, head size, caudal peduncle area and tail fin aspect ratio between calm and turbulent treatments are the result of another factor, such as temperature or food availability. Many studies have shown that these are powerful drivers of phenotypic variation (Clarke *et al.*, 1981; Pepin, 1991; Baltz *et al.*, 1998; Barriga *et al.*, 2012; Oufiero & Whitlow, 2016). However, in my experiment the mean temperature was on average only 0.2°C higher in the turbulent treatments than the calm treatments and therefore, I believe, not strong enough to cause significant morphological differences. Also, the water temperatures only fluctuated between 14.2°C and 22.5°C in accordance with the natural daily temperature fluctuations. This is within the preferred temperature range of *G. maculatus*, as reported by Boubee *et al.* (1991) and Richardson *et al.* (1994). Each treatment was fed twice daily until the fish were satiated, meaning I was, in theory, meeting the energy-consumption demands of the fish in each treatment. Therefore, it is unlikely that food limitation was a driving factor of morphological change in this study.

It should be noted that although the previously discussed morphological variations occurred in response to water movement and type (therefore demonstrating phenotypic plasticity), it is likely that not all changes resulted in an adaptive advantage. We see lower body depth in turbulent conditions within each water type, and lower weight, body condition, head height, head width and caudal peduncle area in turbulent conditions in salt water. These traits are related to overall fish health and condition, so these results suggest that the morphological differences between treatments were instead due to the added stress of living in turbulent conditions or salt water, resulting in decreased fish health. In terms of energy availability, individuals in salt water would likely not have had the opportunity to change their morphology. It appears the stress placed on them by inhabiting salt water meant all available energy was expended on survival and maintaining osmoregulation, therefore the fish likely did not have extra reserves for growth and adaptation to their environment.

Fineness ratio displayed the opposite pattern to that seen in other measured morphological traits. It increased in the salt water treatments but decreased in fresh

water treatments, and was higher in salt water treatments than fresh water treatments. Higher fineness ratios result from fish with shallow, elongated bodies, while low fineness ratios result from deep, short bodies (Walker *et al.*, 2013). Typically in fish the optimum fineness ratio for minimal drag and maximal streamlining is 4.5 (which equates to log 1.5) (Aleyev, 1977; Blake, 1983; Fish, 1993). However, the optimal fineness ratio differs from this between species, and also between different life stages within a species (Bainbridge, 1960; Ahlborn *et al.*, 2009; Walker *et al.*, 2013). For example, Nikora *et al.* (2003) found that the optimal log fineness ratio for larval *G. maculatus* was on average 1.9-2.1, but for juveniles and adults it was 2.3. This would indicate that fish in my fresh water treatments were approaching an optimal fusiform shape, and were more streamlined than those in the salt water treatments. This may be because *G. maculatus* in fresh water treatments were beginning to develop into the adult morph (as discussed earlier) and this life stage naturally has a deeper body (Woods, 1968), resulting in lower fineness ratios. The larval form is thinner and more elongated, resulting in higher fineness ratios. McHenry and Lauder (2006) demonstrated a similar pattern to this, as zebrafish (*Danio rerio*) developed thicker trunk and caudal regions as they developed from larvae into adults, which contributed to improved streamlining.

Individuals were more streamlined in calm conditions than turbulent conditions within each water type. This was unexpected as I assumed streamlined individuals would have an advantage in turbulent conditions, as they would have to exert less energy to overcome the drag force of the water, and therefore would conserve more energy than their less-streamlined conspecifics (Blake, 2004; McHenry & Lauder, 2006; Langerhans, 2008; Langerhans & Reznick, 2010). If this were the case, one could expect individuals in turbulent treatments to either exhibit phenotypic plasticity, developing more streamlined phenotypes, or that less streamlined individuals would perish earlier, leaving more streamlined individuals. However, this was not the pattern I saw. The most reasonable cause is that fish were skinnier in turbulent conditions, as they had to expend more energy to maintain their position in the water column and therefore allocated less energy to growth, resulting in their higher fineness ratios.

Standard length increased over time in all treatments. This contradicts the findings of some observational studies, that *G. maculatus* decrease in body length following migration to fresh water (Benzie, 1968; Woods, 1968; McDowall & Eldon, 1980). This indicates that it is not simply the entry into fresh water causing this reduction in size. These papers hypothesise that the observed reduction is due to the exertions of swimming upstream for long distances while experiencing a period of starvation during this time. As fish in my experiments did not undergo a period of starvation, and most did not experience conditions similar to upstream migration, they were not expending such large proportions of energy swimming against the current and could instead utilise this energy for growth. Energy levels were also supplemented by regular feeding. My fresh + turbulent treatment most closely resembles the natural conditions encountered by *G. maculatus* during upstream migration and this is the treatment that showed the slowest increase in growth. This is partially consistent with the results of Benzie (1968), Woods (1968) and McDowall & Eldon (1980). The likely reason for slower growth, rather than decrease in fish length was that the fish were sufficiently fed in my treatments, whereas under natural conditions they may have undergone a period of reduced food intake at this stage.

4.3 Otolith Asymmetry

I expected to find that fluctuating asymmetry (FA) in *G. maculatus* otoliths increased with decreasing body condition and increased over time, particularly in the more stressful treatments (mainly salt + turbulent). Contrary to these expectations, I found little evidence of FA in *G. maculatus* otoliths. Neither increased salinity, increased turbulence nor decreased body condition caused a consistent change in the asymmetry of otolith traits measured. This is a similar result to other studies, which found that FA was not influenced by salinity (Panfili *et al.*, 2005) or body condition (Folkvord *et al.*, 2000; Panfili *et al.*, 2005). However my results differ to that of Grønkjær and Sand (2003) and Oliver (2013), who did demonstrate a correlation between body condition and FA. A possible reason for the lack of otolith asymmetry in my study is that poor quality

individuals (those with low health, body condition and fitness) are less able to buffer themselves against environmental perturbations (Palmer, 1996; Lens *et al.*, 2002). These fragile individuals often have more asymmetric otoliths, and were more likely to die earlier in my study. I only investigated otolith asymmetry in fish that perished after day 33, as I wanted to increase the likelihood that any FA witnessed was due to the experimental conditions, rather than pre-capture environments, therefore the most asymmetrical individuals may not have been included in this analysis. Fish that lived longer were likely the healthiest and best adapted individuals, and therefore had the least asymmetrical otoliths. Another cause that may contribute to the lack of asymmetry is the high natural mortality observed in the early life stages of fish (Bailey & Houde, 1989). I collected fish that had spent three to six months in the marine environment, a period in which they naturally experience high mortality (McDowall & Eldon, 1980). Weaker, more asymmetrical individuals may have already been removed from the population during this time (Downhower *et al.*, 1990; Gagliano *et al.*, 2008). My results highlight the importance of studying the correct temporal window in order to link otolith FA to fitness (Díaz-Gil *et al.*, 2015).

It is possible that the environmental stressors my fish were exposed to (water type and movement) were not the correct 'type' of stress to cause a corresponding increase in FA. Many studies have found increases in FA due to conditions such as temperature (Beacham, 1990; Campbell *et al.*, 1998; Campbell, 2000; Robinson & Wardrop, 2002; Turner *et al.*, 2007; Eriksen *et al.*, 2008) or predation (Moodie & Reimchen, 1976; Moodie, 1977; Bergstrom *et al.*, 2003) but few studies have found an effect of salinity on FA (for example, Panfili et al., 2005; and reviewed in Allenbach, 2011). Alternatively, otoliths may not be the most ideal structure to use in analysing effects of increased salinity or turbulence on FA. Lajus *et al.* (2003) found salinity was one of the most important factors determining FA in eelpout (*Zoarces viviparus*), but used 17 bilateral meristic characters (the number of holes in cranial bones) to analyse FA, not otoliths. From this, it seems that FA is very stress- and trait-specific. Therefore, when conducting future analyses on this subject, it would be wise to include measures of a range of environmental stressors and compare measures from a number of different traits in

addition to otoliths, to determine which stressors cause a significant FA response and which traits display the most reliable response.

I found no evidence of FA in otolith length or width, whereas otolith area showed evidence of increasing asymmetry over time, which was not influenced by treatment. This may be a factor of the time taken for otolith size to deviate (i.e. the longer an otolith is growing, the more likely it is to vary in size between left and right, and produce noticeable differences). Furthermore, otolith perimeter showed variation in asymmetry in response to my treatments. These results seem to be common in otolith FA studies. Downhower *et al.* (1990) found otolith length asymmetry in two sculpin species (*Cottus bairdi and Cottus* gobio) was not correlated with any environmental variables that they measured. Escós *et al.* (1995) found that otolith shape provided the most sensitive measurement of otolith FA in parasitized Pacific hake (*Merluccius productus*), whereas otolith length and width did not show evidence of FA. Fazio *et al.* (2005) found no relationship between parasite load and FA in otolith length and width of yellow eels (*Anguilla anguilla*). Alados *et al.* (1993) found that FA in shape and mass of adult Pacific hake otoliths were good indicators of El Niño conditions, but the otolith length and width were not. Fey and Hare (2008) found otolith area was a reliable indicator of FA in Atlantic menhaden (*Brevoortia tyrannus)*, but likewise, otolith length and width were not. In a review of FA at the time, Allenbach (2011) found that of the studies investigating FA in fish otolith traits, otolith length showed significant evidence of FA in only 30% of the studies, while otolith diameter was less successful at 20%. Yet otolith area and perimeter were more successful at capturing and displaying significant evidence of FA (57.1% and 33.3% success, respectively). Otolith length and width may not be sufficiently sensitive metrics, as they can only account for differences in one linear direction, whereas measures of shape such as perimeter and area are more comprehensive (Alados *et al.*, 1993; Fey & Hare, 2008; Allenbach, 2011). As otolith perimeter was the only trait to exhibit variation in asymmetry in response to my treatments, this may indicate that stress is better manifested in otolith shape than linear measures because there are more ways in which shape can vary than a linear measurement.

This relatively large amount of evidence indicates there is little to be gained using unidirectional otolith measurements such as otolith length, width and diameter to investigate otolith FA in fish species. While some studies have found that unidirectional otolith measurements do display FA in response to certain environmental conditions, these patterns were also represented in the measurements of shape (Somarakis *et al.*, 1997b; Grønkjær & Sand, 2003; Oliver, 2013). Thus, it seems unidirectional measurements may add additional evidence and support and give a clearer response to the tested environmental variables. However, they should be used in conjunction with other measurements of otolith shape, such as area and perimeter.

4.4 Combined implications

Presumably, the ability to remain in salt water depends on whether the added benefits of staying at sea (i.e. increased quantity and quality of food in salt water: Gross et al., 1988; Edeline, 2007; McDowall, 2007; Thibault et al., 2007) outweigh the osmotic costs. In my experiment, the combined results of reduced survival and loss in body condition and weight in the salt treatments indicates that the costs exceeded any benefits (Morgan & Iwama, 1991; Tandler *et al.*, 1995). In saying this, my feeding regime may not reflect realistic food levels in salt water, due to the standardised feeding regime between treatments. Assuming my treatments are a reasonable test of the ability of *G. maculatus* to prolong the marine phase, this result does not bode well for larval *G. maculatus* that become hindered in the marine environment, whether due to strong offshore ocean currents or because they are prevented from entering a fresh water tributary. My results suggest that should this happen, it is likely that larval *G. maculatus* will experience high mortality and delayed development.

Inaccessibility of a river mouth may not result in certain death for larval *G. maculatus*, however. It is not known exactly how whitebait shoals respond if a river mouth becomes blocked due to weather or anthropogenic influences, however some believe the fish

simply utilise adjacent rivers (McDowall & Eldon, 1980). For example, in 1972 the Waita River mouth was obstructed for long periods and catch in that river was poor. However, catch in the nearby Haast River (~12km away) was very high compared to other years (McDowall & Eldon, 1980). There are potential problems with this situation too, however. If delayed larval *G. maculatus* eventually do make it to fresh water, my results suggest they will be less developed, of poorer body condition and smaller body size than their conspecifics who did not experience extended marine durations, and thus may be at a disadvantage. This may have negative carry-over effects throughout their life (Räsänen *et al.*, 2002; Harrison *et al.*, 2011; Hettinger *et al.*, 2012), as it may also result in delayed spawning of those individuals (Benzie, 1968). It appears there are peak times to spawn and recruit for *G. maculatus* (Benzie, 1968; McDowall *et al.*, 1994; McDowall, 1995), and if individuals miss this window then it may have negative consequences as each life stage may no longer coincide with the optimal environmental conditions for survival, growth and development. In saying this, *G. maculatus* larvae are known to enter river mouths throughout the year (McDowall, 1968, 1995). This indicates that, provided a river mouth does not remain closed for the entire year, these off-peak migrations may be sufficient to sustain and/or replenish local populations, albeit at lower numbers and not during optimal migration times.

Collectively, my results suggest that turbulent conditions in salt water may be the most stressful environment for continued development of post-larval *G. maculatus*. Fish in this treatment died sooner, experienced reduced growth, and displayed increasing otolith asymmetry over time. This has implications for the long-term persistence of this species, as osmoregulatory stress and sub-optimal environments (e.g., turbulent marine conditions) encountered during early life stages likely deplete energy reserves and leave fish susceptible to other stressors, such as pollutants, contaminants or other adverse environmental conditions (Pistole *et al.*, 2008; Brooks *et al.*, 2012). This may have negative carry-over effects (Räsänen *et al.*, 2002; Harrison *et al.*, 2011; Hettinger *et al.*, 2012). G. maculatus typically live 1 year and only spawn once (Benzie, 1968; McDowall, 1968). This means they have limited recovery time before spawning occurs and may result in fish not having sufficient energy reserves to produce large quantities of

offspring, or provision them with adequate nutrients to maximise their survival. Potentially resulting in poor contribution to the future population.

It should be noted that my models assume the relationship between the response variable (e.g. standard length, weight etc.) and time is linear. Without such high mortality midway through the experiment, and if there was more overlap between the treatments (particularly between the salt + turbulent and other treatments), I may have found that *G. maculatus* growth was not linear and instead was better represented by an increasing asymptotic curve.

My feeding regime likely did not imitate the natural feeding regime of *G. maculatus*, of foraging throughout the day. Instead they were fed twice a day on store-bought fish food, so perhaps they were not provided with all the necessary nutrients, obscuring some of the treatment effects on morphology or otolith asymmetry. Furthermore, most individuals were measured after dying of natural causes within my treatments, so there is a potential bias in my sampling as I was sampling the 'unfit' individuals (i.e. the ones who could no longer meet the metabolic demands for survival).

4.5 Wider applications

The results of my study show that *G. maculatus* have limited ability to cope with turbulent conditions and therefore are likely to seek out calmer or slower-flowing sections of water. Over half of New Zealand's indigenous fish species migrate upstream at a small size (Boubée *et al.*, 1999; McDowall, 2000). Because of their small size, these species are more easily hindered or confused by fast flowing and/or turbulent water (Boubée *et al.*, 1999; Odeh *et al.*, 2002; Nikora *et al.*, 2003). This highlights the importance of ensuring the lower reaches of rivers are easily accessible to small migrating fish, and provide high habitat diversity (i.e., both riffles and pools), so fish can seek refuge and rest between bouts of upstream swimming. Anthropogenic

modifications of waterways through farming, deforestation and urbanisation have reduced beneficial habitat diversity in freshwater systems (Kauffman & Krueger, 1984; Wang *et al.*, 2001; Hickford & Schiel, 2011, 2014). In particular, culverts, weirs and any artificial funnelling of rivers can cause homogeneous high velocity sections of water, making upstream migration and settlement increasingly difficult for young fish (Boubée *et al.*, 1999; Richardson & Taylor, 2002; Leavy & Bonner, 2009). Removing these obstacles or reducing their impact by providing fish-friendly passages with slow-flowing currents (like those discussed in Agostinho *et al.* (2008) and Assumpção *et al.* (2012)) would benefit upstream migration and likely prevent excessive mortality in fresh water life stages.

The openness of a river mouth relies on the force of water flowing down and sweeping gravel and sand aside being stronger than the opposing force of ocean currents and waves that deposit gravel and sand in the river mouth (McDowall, 1995; Tanaka *et al.*, 1996). River mouth closure is a natural phenomenon, but anthropogenic manipulation of river flows through processes such as water extraction for irrigation and diversion for hydroelectricity, has led to higher rates of river mouth closure worldwide (McDowall, 1995; Schlacher & Wooldridge, 1996; Molle *et al.*, 2010). These closures have significant implications for galaxiids and all other migratory fish, in terms of both survival and productivity of the fisheries they support. In New Zealand, upstream migrations of diadromous fish peak in spring, between August and December, but overall, diadromous migrations occur throughout the year in fresh water (McDowall, 1995; McDowall, 2000). Natural events such as floods and droughts cannot be prevented, and are expected to increase in number and severity due to climate change (Lehner *et al.*, 2006; Lindner *et al.*, 2010; Cai *et al.*, 2014). In order to mitigate the impacts of these events on river flow and structure, and thus the accessibility for upstream migrations, it is important that anthropogenic factors such as irrigation and hydropower facilities are managed effectively, bearing such migrations in mind. It may become necessary to artificially open river mouths (this already occurs every spring at blockage-prone rivers such as Lakes Ellesmere and Forsyth in the Canterbury region: McDowall, 1995) and/or put measures in place to ensure rivers remain open and accessible to migrating fish for the majority of

the year, particularly during peak migrations. This is vital if we are to safeguard our culturally, economically and ecologically significant fish species.

My research contributes to the growing interest and efforts towards cultivating diadromous fish in captivity (Mitchell, 1989b; Walsby, 2012; Wylie & Lokman, 2012). I found that more optimal body conditions were achieved when fish were kept in calm water, regardless of salinity, and were not delayed from entering fresh water at the conclusion of their larval phase. I also found that an abundant food supply following entry into fresh water may be sufficient to prevent the body size decrease sometimes experienced in wild populations. To cultivate economically important fish, such as *G. maculatus*, in captivity may have the added benefit of reducing the strain on wild populations, as it may result in less fishing pressure in order to meet public demand, and captive-bred individuals could be used to restock depleted wild populations. This could allow wild populations to recover and increase in number, halting species decline and reducing the risk of extinction.

My results contribute to the growing evidence that FA is stress-dependent and is expressed in some traits but not others (Lens *et al.*, 2002; Panfili *et al.*, 2005; Allenbach, 2011). Therefore, it is important to measure asymmetry in a number of different morphological traits, rather than one single trait. There is strong evidence that it is sufficient to measure a range of linear and simple shape measurements (such as area, perimeter, length and width) from otoliths to test for FA (Somarakis *et al.*, 1997b; Grønkjær & Sand, 2003; Oliver, 2013). This methodology has the added benefits of being relatively simple to conduct and easy to analyse and interpret (Palmer & Strobeck, 2003; Oliver, 2013). In addition, there is compelling evidence that shape-describing traits (such as area and perimeter) are more desirable to use for FA analysis, as they provide the most information, are more descriptive and are more sensitive to asymmetry (Palmer *et al.*, 2010; Allenbach, 2011).

4.6 Future research

As I found that *G. maculatus* experience high mortalities and delayed development if retained in salt water indefinitely, it may be advantageous to investigate what happens if *G. maculatus* have a delayed marine phase, but eventually make it back to fresh water a month or so later. Specifically testing the sub-lethal effects of a delayed marine duration would enable researchers to determine more specifically what happens to their development. For example, individuals may undergo a sudden burst of development and catch up with fish that entered fresh water earlier, or they may remain less developed than their counterparts their entire life. The latter could have negative carry over consequences for timing of later life events, such as spawning (Benzie, 1968). Future studies could also determine the optimal length of time for larval fish to spend in the marine environment, and whether *G. maculatus* (or other amphidromous species) benefits from a prolonged marine phase. As the marine environment is typically more productive and nutrient-rich than fresh water systems (Gross *et al.*, 1988; Edeline, 2007; Thibault *et al.*, 2007), the extended period in the ocean may provide access to better food resources, therefore resulting in better body condition by the time individuals enter fresh water.

It would also be beneficial to investigate the effects of prolonged larval duration in landlocked populations of typically amphidromous fish, as they do not experience varying salinity regimes and the consequent osmotic stresses. Landlocked larvae develop in lakes, rather than in the ocean, and return to rivers to mature into adults (Pollard, 1971; Humphries, 1990; David *et al.*, 2004; Chapman *et al.*, 2009; Barriga *et al.*, 2012). Therefore, from a purely energetic or food availability viewpoint, it would be beneficial for larvae to stay in the more nutrient-rich environment of a lake instead of a river for long periods of time, maybe even the entire lifecycle, yet no study has investigated this. This would help to inform researchers on the purposes and benefits of amphidromy.

When conducting future analyses of FA, it would be wise to include measures of a range of environmental stressors and compare the asymmetry of a number of different

morphological traits (e.g., otoliths as well as pectoral fin rays). This will aid in determining which stressors cause a significant FA response and which traits display the most reliable response. Pre-existing studies of FA encompass a wide range of traits and stressors, with varying degrees of success (Bjorksten *et al.*, 2000; Rasmuson, 2002; Allenbach, 2011; Díaz-Gil *et al.*, 2015), which it makes comparisons between studies difficult. Determining which traits are more sensitive to environmental perturbation, and thus more likely to display FA, will give future studies more direction and focus. Future studies of FA would also benefit from using more computationally intensive methods to compare FA in otolith shape, such as those used by Lemberget and McCormick (2009), Palmer *et al.* (2010), Libungan and Pálsson (2015), and Harbitz (2016), as they are more comprehensive than methods using univariate measurements and have been shown to detect FA more easily. For example, Palmer *et al.* (2010) found that otolith FA clearly differed between populations of razor fish (*Xyrichthys novacula*), when analysing the shape of the otolith, but when using a univariate trait (otolith radius) from the same dataset, between-population differences in FA could no longer be detected.

4.7 Conclusion

Overall, this study suggests that an extended marine duration is not beneficial to larval *G. maculatus* as it causes increased mortality and delayed development. Turbulence is also detrimental to *G. maculatus* growth, condition and survival, but does not always result in adaptive phenotypic plasticity. Shape-depicting measurements, such as otolith area and perimeter appear to be the more superior measurements to use to detect FA in response to environmental stressors. This research contributes to the growing understanding of the relationship between varying developmental environments and survival, growth, condition, morphology and stress in larval fish. This information can be used to better inform conservation and management decisions.

APPENDIX 1

Table 3. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final standard length of G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination and time, 8.01% of the variation in length is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	35.04762	1.149994	464	30.47634	0.0000
Water Type	0.12112	1.367741	8	0.088557	0.9316
Water Movement	3.21979	1.41158	8	2.280986	0.0520
Time	0.10888	0.01866	464	5.834797	0.0000*
Water Type*Water Movement	-6.17864	1.789227	8	-3.45325	0.0087
Water Type*Time	-0.05334	0.022	464	-2.42474	0.0157*
Water Movement*Time	-0.05975	0.023017	464	-2.59593	0.0097*
Water Type*Water Movement*Time	0.15303	0.031865	464	4.802323	0.0000*

Table 4. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final weight of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 8.48% of the variation in weight is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	-0.136333	0.042097	463	-3.23856	0.0013
Water Type	-0.059356	0.029189	8	-2.03346	0.0764
Water Movement	-0.058691	0.030301	8	-1.93689	0.0888
Time	0.000052	0.000410	463	0.12770	0.8984
Standard length	0.014349	0.000976	463	14.68793	0.0000*
Water Type*Water Movement	0.047224	0.038696	8	1.22036	0.2571
Water Type*Time	-0.000717	0.000470	463	-1.52580	0.1277
Water Movement*Time	0.000863	0.000492	463	1.75108	0.0806
Water Type*Water Movement*Time	-0.001506	0.000693	463	-2.17254	0.0303*

Table 5. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final body depth of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 6.60% of the variation in body depth is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.540694	0.280805	465	1.925515	0.0548
Water Type	-0.38393	0.130633	9	-2.93902	0.0165*
Water Movement	-0.20575	0.063438	9	-3.24335	0.0101*
Time	0.00135	0.001761	465	0.767049	0.4434
Standard length	0.08087	0.007	465	11.55239	0.0000*
Water Type*Time	-0.0071	0.002258	465	-3.14383	0.0018*

Table 6. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final caudal peduncle depth of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 10.75% of the variation in caudal peduncle depth is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.6079644	0.08260378	464	7.360007	0.0000
Water Type	0.1393536	0.03980213	9	3.50116	0.0067*
Water Movement	0.0695102	0.03883705	9	1.78979	0.1071
Time	0.0022307	0.0006534	464	3.413919	0.0007*
Standard length	0.0232423	0.00193489	464	12.012191	0.0000*
Water Type*Time	-0.0033941	0.00067398	464	-5.035958	0.0000*
Water Movement*Time	-0.0018387	0.00067219	464	-2.735364	0.0065*

Table 7. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final tail fin height of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 17.16% of the variation in tail fin height is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.2746942	0.6815307	465	0.403055	0.6871
Water Type	-0.6828744	0.43363	8	-1.574786	0.1540
Water Movement	-0.7952378	0.3146557	8	-2.527327	0.0354*
Time	0.0124942	0.0041642	465	3.000354	0.0028*
Standard length	0.113013	0.0161094	465	7.015363	0.0000*
Water Type*Water Movement	1.1573325	0.4478025	8	2.584471	0.0324*
Water Type*Time	-0.0135352	0.0053614	465	-2.524576	0.0119*

Table 8. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final tail fin aspect ratio of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 30.59% of the variation in tail fin aspect ratio is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.3211743	0.24006134	464	1.337884	0.1816
Water Type	-0.0300265	0.14050222	9	-0.213708	0.8355
Water Movement	0.1388942	0.13811405	9	1.005648	0.3409
Time	0.0044446	0.00183653	464	2.420085	0.0159*
Standard length	0.0200948	0.00530995	464	3.784366	0.0002*
Water Type*Time	-0.0047637	0.00189875	464	-2.508875	0.0125*
Water Movement*Time	-0.0042606	0.00189276	464	-2.251	0.0249*

Table 9. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final fineness ratio of G. maculatus. Bin was included as a random effect in the model to

account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination and time, 7.08% of the variation in fineness ratio is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	2.3460605	0.03459729	466	67.81052	0.0000
Water Type	0.1206695	0.04106375	9	2.93859	0.0165*
Water Movement	0.0679095	0.02053268	9	3.30739	0.0091*
Time	-0.0000633	0.00053416	466	-0.11856	0.9057
Water Type*Time	0.0020838	0.00071339	466	2.92102	0.0037*

Table 10. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final head height of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 0.81% of the variation in head height is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.32116	0.250932	463	1.279885	0.2012
Water Type	-0.4974	0.157863	8	-3.15132	0.0136*
Water Movement	-0.5737	0.159099	8	-3.60622	0.0069*
Time	0.0032	0.002338	463	1.381715	0.1677
Standard length	0.09135	0.005936	463	15.39	0.0000*
Water Type*Water Movement	0.58061	0.205181	8	2.829774	0.0222*
Water Type*Time	-0.0026	0.002743	463	-0.95836	0.3384
Water Movement*Time	0.00855	0.002761	463	3.099204	0.0021*
Water Type*Water Movement*Time	-0.0133	0.004047	463	-3.29993	0.0010*

Table 11. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final head width of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are

given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 4.79% of the variation in head width is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.6895321	0.199591	463	3.45472	0.0006
Water Type	-0.1365238	0.133365	8	-1.02368	0.3359
Water Movement	-0.2373022	0.137696	8	-1.72337	0.1231
Time	0.0062413	0.001935	463	3.22519	0.0013*
Standard length	0.0681037	0.004674	463	14.57057	0.0000*
Water Type*Water Movement	0.2529579	0.175480	8	1.44151	0.1874
Water Type*Time	-0.0047744	0.002228	463	-2.14257	0.0327*
Water Movement*Time	0.0035963	0.002312	463	1.55530	0.1206
Water Type*Water Movement*Time	-0.0073966	0.003283	463	-2.25234	0.0248*

Table 12. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final body condition of G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination and time, 9.87% of the variation in body condition is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	$3.1622e^{-4}$	$2.9299e^{-5}$	464	10.7925	0.0000
Water Type	$-8.6844e^{-5}$	$3.4993e^{-5}$	8	-2.4817	0.038*
Water Movement	$-8.0386e^{-5}$	$3.6147e^{-5}$	8	-2.2238	0.0568
Time	$-2.5224e^{-7}$	$4.6935e^{-7}$	464	-0.5374	0.5912
Water Type*Water Movement	$6.2003e^{-5}$	$4.5927e^{-5}$	8	1.3500	0.214
Water Type*Time	$-6.0416e^{-7}$	$5.5247e^{-7}$	464	-1.0935	0.2747
Water Movement*Time	$1.1546e^{-6}$	$5.7972e^{-7}$	464	1.9916	0.047*
Water Type*Water Movement*Time	$-1.7292e^{-6}$	$8.0000e^{-7}$	464	-2.1615	0.0312*

Table 13. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final caudal peduncle area of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment

for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 5.53% of the variation in caudal peduncle area is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	-14.346196	1.954490	463	-7.340119	0.0000
Water Type	-0.365386	1.316603	8	-0.277522	0.7884
Water Movement	-2.593104	1.361666	8	-1.90436	0.0933
Time	-0.006347	0.018993	463	-0.334185	0.7384
Standard length	1.087926	0.04568	463	23.812063	0.0000*
Water Type*Water Movement	1.918691	1.735306	8	1.105679	0.3010
Water Type*Time	-0.055311	0.021838	463	-2.532767	0.0116*
Water Movement*Time	0.037974	0.022721	463	1.67126	0.0953
Water Type*Water Movement*Time	-0.072505	0.032185	463	-2.25272	0.0247*

Table 14. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final head length of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 7.08% of the variation in head length is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	1.104998	0.293828	465	3.760703	0.0002
Water Type	0.271056	0.137616	9	1.969656	0.0804
Water Movement	-0.05568	0.068072	9	-0.8179	0.4345
Time	0.013804	0.001844	465	7.487412	0.0000*
Standard length	0.138444	0.007313	465	18.93139	0.0000*
Water Type*Time	-0.00728	0.002364	465	-3.08004	0.0022*

Table 15. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final pectoral fin length of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment

for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 4.56% of the variation in pectoral fin length is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	1.8159909	0.3761091	465	4.828362	0.0000
Water Type	0.1389293	0.1695661	9	0.819323	0.4338
Water Movement	-0.1579622	0.0752832	9	-2.098241	0.0653
Time	0.012151	0.002344	465	5.18391	0.0000*
Standard length	0.0630499	0.0094478	465	6.673479	0.0000*
Water Type*Time	-0.007819	0.0030095	465	-2.598075	0.0097*

Table 16. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final tail fin area of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 12.11% of the variation in tail fin area is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	-7.746487	2.2293378	465	-3.474793	0.0006
Water Type	1.145623	1.1112016	9	1.030977	0.3295
Water Movement	-0.679634	0.6391108	9	-1.063406	0.3153
Time	0.073642	0.0140025	465	5.259231	0.0000*
Standard length	0.591087	0.0546343	465	10.818979	0.0000*
Water Type*Time	-0.066029	0.017932	465	-3.68222	0.0003*

Table 17. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm) and exposure time on the final body width of G. maculatus. Standard length was included as a covariate to account for fish size and Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and standard length, 7.09% of the variation in body width is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	-0.11539639	0.22068116	465	-0.522910	0.6013
Water Type	-0.17583677	0.10337007	9	-1.701041	0.1231
Water Movement	-0.10402183	0.05114942	9	-2.033685	0.0725
Time	0.00281999	0.00138472	465	2.036498	0.0423*
Standard length	0.04249391	0.00549224	465	7.737077	0.0000*
Water Type*Time	-0.00660503	0.00177565	465	-3.719783	0.0002*

APPENDIX 2

Table 18. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on signed asymmetry of otolith area for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, 1.54% of the variation in signed otolith area asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	2792.7	2011	226	1.3889447	0.1662
Water Type	-290.3	920	9	-0.3156677	0.7595
Water Movement	-548.7	689	9	-0.7959786	0.4465
Time	-39.1	22	226	-1.7935605	0.0742
Body condition	-1044322	5434373	226	-0.1921698	0.8478

Table 19. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on signed asymmetry of otolith perimeter for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, <0.001% of the variation in signed otolith perimeter asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	126.02	56.05	226	2.2484189	0.0255
Water Type	-38.84	24.91	9	-1.5593726	0.1533
Water Movement	-3.99	17.71	9	-0.2255317	0.8266
Time	-1.00	0.61	226	-1.637339	0.1030
Body condition	-222717.9	156303.1	226	-1.4249102	0.1556

Table 20. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on signed asymmetry of otolith length for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment

for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, 2.87% of the variation in signed otolith length asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	0.332	3.2	226	0.1037176	0.9175
Water Type	-1.004	1.503	9	-0.6675795	0.5212
Water Movement	0.703	1.166	9	0.6028213	0.5615
Time	0.021	0.035	226	0.5991796	0.5497
Body condition	-5868.252	8469.885	226	-0.6928373	0.4891

Table 21. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on signed asymmetry of otolith width for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, 0.53% of the variation in signed otolith width asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	1.2266	2.034	226	0.6029625	0.5471
Water Type	-0.296	0.913	9	-0.3242195	0.7532
Water Movement	0.0576	0.662	9	0.0868955	0.9327
Time	-0.0185	0.022	226	-0.8345962	0.4048
Body condition	-430.0566	5605.612	226	-0.0767189	0.9389

Table 22. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on unsigned asymmetry of otolith area for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and body condition, <0.001% of the variation in absolute otolith area asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	25.37	9.697	226	2.6166177	0.0095
Water Type	7.84	4.31	9	1.8182355	0.1024
Water Movement	2.02	3.063	9	0.6608365	0.5253
Time	0.24	0.106	226	2.2809024	0.0235*
Body condition	49379.7	27043.7	226	1.8259224	0.0692

Table 23. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on unsigned asymmetry of otolith perimeter for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and body condition, 4.44% of the variation in absolute otolith perimeter asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	7.4765	3.002	223	2.490787	0.0135
Water Type	4.7962	3.612	8	1.328013	0.2208
Water Movement	3.7322	3.211	8	1.162501	0.2785
Time	0.0107	0.043	223	0.24816	0.8042
Body condition	1865.332	4897.824	223	0.380849	0.7037
Water Type*Water Movement	-16.4244	6.207	8	-2.646255	0.0294*
Water Type*Time	-0.0783	0.057	223	-1.369753	0.1721
Water Movement*Time	-0.0459	0.052	223	-0.876471	0.3817
Water Type*Water Movement*Time	0.3083	0.125	223	2.472711	0.0142*

Table 24. Results of full linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on unsigned asymmetry of otolith perimeter for G. maculatus, after vateritic otolith removal. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. P-values marked with a '*' represent significance at the α=0.05 level. After accounting for treatment combination, time and body condition, 6.31% of the variation in absolute otolith perimeter asymmetry is due to bin-to-bin differences, after removal of vateritic otoliths.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	7.0262	2.986	219	2.353369	0.0195
Water Type	4.1324	3.584	8	1.152975	0.2822
Water Movement	4.0407	3.209	8	1.259181	0.2435
Time	0.0163	0.043	219	0.382783	0.7023
Body condition	1875.5706	4801.088	219	0.390655	0.6964
Water Type*Water Movement	-14.7539	6.482	8	-2.275983	0.0524
Water Type*Time	-0.0714	0.056	219	-1.269823	0.2055
Water Movement*Time	-0.0536	0.052	219	-1.031782	0.3033
Water Type*Water Movement*Time	0.2752	0.131	219	2.094639	0.0374*

Table 25. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on unsigned asymmetry of otolith length for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, <0.001% of the variation in absolute otolith length asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	1.6171	0.3698	226	4.372627	0.0000
Water Type	0.0961	0.1644	9	0.584385	0.5733
Water Movement	-0.0598	0.1168	9	-0.512061	0.6209
Time	0.0063	0.004	226	1.549667	0.1226
Body condition	738.3451	1031.4056	226	0.715863	0.4748

Table 26. Results of reduced linear mixed effects model investigating separate and joint effects of water type (salt versus fresh), water movement (turbulent versus calm), exposure time and body condition on unsigned asymmetry of otolith width for G. maculatus. Bin was included as a random effect in the model to account for the structure of my data (i.e., fish within the same bin may have correlated responses). Coefficient estimates are given relative to salt treatment for water type, and relative to turbulent treatment for water movement. After accounting for treatment combination, time and body condition, <0.001% of the variation in absolute otolith width asymmetry is due to bin-to-bin differences.

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value
Intercept	1.1099	0.3266	226	3.398732	0.0008
Water Type	0.1999	0.1451	9	1.377124	0.2018
Water Movement	-0.0061	0.1032	9	-0.058895	0.9543
Time	0.0046	0.0036	226	1.299167	0.1952
Body condition	1113.809	910.72	226	1.222998	0.2226

Effect	Coefficient Estimate	Std Error	DF	t-value	p-value

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