

Larval drift and development of amphidromous  
fishes, particularly the bluegill bully (*Gobiomorphus  
hubbsi*)

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## Abstract

Amphidromy is a distinct life history strategy found in many aquatic organisms, involving a return migration ('drift') to a pelagic feeding habitat (usually the sea), undertaken by newly hatched larvae. The freshwater fish faunas of many Indo-Pacific islands are dominated by amphidromous species, yet they remain understudied, especially their larval stages. Amphidromous larvae hatch out exceptionally small and undeveloped, and so face a range of specific challenges during migration such as irreversible starvation and failed development if migration is delayed, as well as management difficulties due to their small size. Basic ecological knowledge such as timing and extent of migration remains unknown, but is crucial to the management of amphidromous species. It was therefore the aim of this thesis to further our knowledge on the larval ecology and migration of a number of New Zealand's amphidromous fish species. This thesis examines patterns of larval drift and development, focussing on the bluegill bully (*Gobiomorphus hubbsi*), an endemic eleotrid. A distinct diel and spatial drift pattern was documented, with the vast majority of fish larvae migrating to sea within a few hours of sunset. It is suggested that targeting conservation measures within this window of drift represents a potentially beneficial management strategy for amphidromous species. Development and starvation of larvae was also examined, both through field studies and lab experiments. No distinct pattern of starvation was observed in larvae during their seaward migration, however larvae retained in freshwater failed to develop to as complete a state as those transferred to seawater, implying delayed migration may adversely affect amphidromous fishes developmentally, ultimately reducing their success upon reaching the sea. These results indicate both threats to amphidromous fishes during their larval migration, and a potential approach which may prove beneficial in conserving these fascinating and vulnerable species.

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## Chapter 1: General Introduction

### Migration, diadromy and amphidromy

Movement is of great significance to animals, allowing them to better exploit spatial and temporal heterogeneities in resources such as food, shelter, and mates (Dingle & Drake, 2007). In many animals this manifests most obviously in migrations: behavioural traits or syndromes involving the persistent, undistracted movement of individuals (Sih *et al.*, 2004; Dingle & Drake, 2007). These are often spectacular, such as spawning migrations upstream in salmon (McDowall, 2007), and the migration of the arctic tern, which travels up to 71,000 km annually to take advantage of favourable summer conditions year-round (Egevang *et al.*, 2010). Reasons behind migrations are not always obvious, but migrations are the product of natural selection, and must therefore involve fitness benefits for migratory relative to non-migratory individuals (Dingle & Drake, 2007). Differences in the costs and benefits involved in different migrations result in the potential for the evolution of different life histories, and ultimately speciation (Dingle & Drake, 2007). Often these are direct benefits such as increased resources or reduced predation (e.g. Skov *et al.*, 2013), but benefits may be indirect, where the advantage is not directly gained by the migratory individual, but given to their offspring through reproducing in certain areas (Jorgensen *et al.*, 2008). This is likely the case in many migratory aquatic species, which often migrate between habitats specifically for the purposes of spawning.

Diadromy is a distinct migratory life history in aquatic animals, and involves a 'round trip' migration between freshwater and marine environments at particular life stages, and is crucial for reproduction or growth (Gross, 1987; McDowall, 1998). Less than 1.5% of known fish species are considered diadromous, but the life history is known from many fish families (McDowall, 1988), and is of great interest as diadromous species are often of conservation or fisheries significance, such as trout, salmon and freshwater eels (McDowall, 1988). Diadromy is also important to biogeography, and is thought to be the key factor determining freshwater fish distributions in New Zealand, with a downstream increase in species richness having been attributed to New Zealand's coastal streams being dominated by diadromous species (McDowall, 1998; Joy, Henderson & Death, 2000; Jowett & Richardson, 2003). Diadromous migrations can be further classified as either anadromous, catadromous or amphidromous based on differences in habitat and the life-history stages at which migrations occur (Gross, 1987; McDowall, 2007). Anadromous migrations involve reproduction in freshwater, but individuals undertake most of their growth at sea, before making an adult migration back to freshwater for spawning purposes, e.g. salmon and lamprey (McDowall, 1997). Conversely,

catadromous species reproduce in the sea and have a pelagic larval phase, and then migrate to freshwater for most of their growth before returning to the sea for spawning purposes, e.g. anguillid eels (McDowall, 1997). Amphidromy is similar to catadromy in that it features a pelagic larval stage, but unlike the other types of diadromy which involve an adult migration between biomes, an amphidromous life history involves a biome-shifting migration immediately after hatch. Adult fish live and spawn in freshwater rivers and streams, after which newly hatched larvae immediately migrate to a pelagic (usually marine) environment for a short period of feeding and growth before returning to freshwater as post-larvae or juveniles (McDowall, 2007; Closs, Hicks & Jellyman, 2013).

#### Amphidromy

An amphidromous life history strategy has been found in a number of aquatic invertebrates, and in many fish species from a range of fish families, including Cottidae, Galaxiidae, Gobiidae, Eleotridae, Pinguipedidae, Mugilidae, and Retropinnidae (McDowall, 2007; Closs, Hicks & Jellyman, 2013). Despite this, amphidromy is poorly understood relative to the other types of diadromy, and there has been debate over the usefulness of the term (McDowall, 2007). Much of this debate is thought to be the result of the lack of amphidromous species present in continental areas like Europe and the United States, which has resulted in confusion (or ignorance) of amphidromy among many researchers from these areas who may have little or no exposure to amphidromous fishes (McDowall, 2007). However, amphidromous fishes can dominate the freshwater fish faunas of small tropical and sub-tropical islands (Keith, 2003; McDowall, 2007), and the term is widely accepted in areas where the life history strategy is prevalent, particularly throughout the Indo-Pacific, and most well-known from Hawai'i, Japan and New Zealand (McDowall, 2010b).

#### Why be amphidromous?

Migratory life histories are a product of natural selection, and so there must be clear benefits to migratory individuals driving the evolution and maintenance of migration (Dingle & Drake, 2007). There are however a variety of costs and risks associated with an amphidromous life history, including energetically expensive migrations to and from the larval feeding habitat, and the physiological challenges of shifting between biomes (Webster & Dill, 2006; McDowall, 2007). Larval migration is also potentially dangerous, as larvae are thought to be unable to feed during their downstream migration, relying solely on endogenous energy sources, and so risk irreversible starvation, becoming unable to develop if they fail to reach their pelagic feeding habitat before the exhaustion of their yolk sac (Iguchi & Mizuno, 1999; Iida *et al.*, 2010).

Because of the costs and risks inherent to a larval migration between biomes, the benefits involved in the evolution and maintenance of an amphidromous migration has been the focus of debate (McDowall, 2007; Poulin *et al.*, 2012; Closs, Hicks & Jellyman, 2013). It has been suggested that amphidromy simply represents an evolutionary intermediate between a non-diadromous life cycle and other forms of diadromy (Gross, 1987). McDowall (2007) argued that there was no phylogenetic evidence to support this hypothesis, instead offering a range of advantages to an amphidromous life history, including enhanced dispersal and (re)colonisation of oceanic islands due to a marine larval stage, release from predation by leaving the marine plankton, and the maintenance of high fecundity by producing many small larvae. McDowall (2010b) went on to argue that wide oceanic dispersal and the associated colonisation benefits were likely the main strategic advantage to an amphidromous life history, and that the prevalence of amphidromous fishes throughout the Indo-Pacific islands likely reflected the colonisation of newly formed volcanic islands, or quick recolonisation of ephemeral island streams following extirpations. This idea that an amphidromous life history is the product of oceanic dispersal has been widely accepted, and is prevalent in the literature (e.g. Hickford & Schiel, 2011; Taillebois *et al.*, 2012). It has however been argued that this is illogical, as pelagic larvae could not evolve in response to unpredictable and catastrophic future events (Closs, Hicks & Jellyman, 2013), and there is increasing evidence that some amphidromous fishes have very limited dispersal abilities, remaining near freshwater plumes during their oceanic stage and returning to (or close to) their natal habitat (Sorenson & Hobson, 2005; Huey *et al.*, 2014). Non-diadromous recruitment (landlocking) has also been identified in several amphidromous species (McDowall, 2010a; Closs, Hicks & Jellyman, 2013), suggesting the marine phase (and thus its dispersal benefits) is not obligatory in at least some amphidromous species.

Instead, it has been suggested that rather than being driven by dispersal, the main benefits of an amphidromous life history is that these fishes maintain a competitive advantage over non-migratory species when near a pelagic habitat (whether the sea or a lake), through the production of large numbers of small pelagic larvae which can take advantage of the relatively productive pelagic environment (Closs, Hicks & Jellyman, 2013). This idea has been gaining support, with recent studies examining life history variation related to landscape in the amphidromous gobiid *Awaous acritosus* concluding that active dispersal and recolonisation are unlikely driving biome shifts (Huey *et al.*, 2014).

This debate as to the benefits of amphidromy highlights our poor understanding of amphidromy as a life history, especially relative to the other forms of diadromy (McDowall, 2007). Little is known of the early life history or larval ecology of amphidromous fishes in general, but this

knowledge is critical in understanding their distributions and ecological requirements, and in identifying potential conservation issues (Keith, 2003; Valade *et al.*, 2009; Closs, Hicks & Jellyman, 2013). This is especially true in New Zealand, where knowledge of early life-history and larval migration remains largely speculative, with the larvae of some species remaining unseen and undocumented during their seaward emigration (McDowall, 1995; McDowall, 2000). The wide acceptance of the ‘amphidromy = dispersal’ hypothesis, and the assumed open population dynamics and strong recolonisation abilities that would result, have steered the conservation and management of amphidromous species in New Zealand (McDowall, 1995; Leathwick *et al.*, 2009). A better understanding of larval ecology and migration is therefore necessary if we are to understand the reasons behind an amphidromous life history, in order to better inform conservation decisions.

#### Previous research

##### Larval drift

Little research has been conducted on amphidromous migrations in general, especially in New Zealand. Internationally, research has tended to focus on freshwater gobies (Gobiidae) throughout Asia. Larval drift has been examined in a number of gobies, particularly *Rhinogobius* species. Iguchi and Mizuno (1990) examined spatial and diel drift patterns of several goby species in several small rivers on Shikoku Island, Japan, and found a distinct peak in larval drift after sunset near the river mouth, while larvae drifted throughout the day further upstream. These differences in diel drift pattern have been related to the combined influence of larval behaviours and landscape, with larvae passively drifting all day in steep, swift flowing upper courses, and halting drift due to responses to light intensity in the slow flowing pools of the lower reaches (Iguchi & Mizuno, 1990; Iguchi & Mizuno, 1991). Diel and seasonal patterns of larval drift have also been investigated on Okinawa Island, Japan, again concluding that larval drift in a number of goby and pipefish species peaks just after sunset (Maeda & Tachihara, 2010). A small number of drift studies have also been conducted on amphidromous fish and shrimp throughout other areas such as examining patterns of drift in relation to urbanisation in Hawaii (Luton *et al.*, 2005), and threats to larvae during migration in Puerto Rico (Benstead *et al.*, 1999).

##### New Zealand larval drift studies

Larval migration in New Zealand’s amphidromous fishes has received little attention, and a poor understanding of the timing and location of spawning for New Zealand’s migratory fish has been noted (McDowall, 1995; McDowall & Suren, 1995). Research has generally focussed on migratory galaxiids (McDowall & Suren, 1995; Charteris, Allibone & Death, 2003), with little

known on other taxa. McDowall (1995) stressed the importance of understanding the patterns of fish migration in New Zealand's rivers, and presented a rough outline of seasonal pulses in migration, however for many species the timing of migration remained purely speculative. Perhaps the only in-depth research undertaken on larval drift in New Zealand has been a series of studies conducted on the lower Waikato River, examining larval drift to assess the impacts of a power station. These studies focussed on entrainment of larvae (Meredith *et al.*, 1987) as well as their distribution patterns (Meredith *et al.*, 1989) and spatial and annual variation in drift (Empson *et al.*, 1992; Meredith, Empson & Boubee, 1992). Additionally, limited work has been conducted in the lower reaches of the Taieri River and its estuary (Sutherland & Closs, 2001), examining spatial and seasonal variation in ichthyoplankton catches. It is important to investigate patterns of drift in different types of rivers due to differences in patterns of larval drift having previously been related to landscape (Iguchi & Mizuno, 1991). The Waikato and Taieri represent two relatively large rivers, with abundant estuarine habitat, and tidally influenced lower reaches (Sutherland & Closs, 2001). The drift patterns in New Zealand's smaller, fast flowing coastal rivers and streams (which often have limited estuarine habitat) remains unknown.

#### Larval survival and development

As well as the migration itself, knowledge on larval survival and development during migration is critical to understanding the early life history of amphidromous species (Keith, 2003; Closs, Hicks & Jellyman, 2013), and again research in this field is limited. Larval survival in some species is known to be influenced by temperature and salinity (Iida *et al.*, 2010). Larval starvation during downstream migration has been recognised as a large contributor to larval mortality, with a large proportion of larvae from the upper reaches of rivers starving irreversibly before reaching their feeding habitat (Iguchi & Mizuno, 1999). This starvation is thought to be important in limiting the distributions of amphidromous fishes, but has only been studied in *Rhinogobius* spp. in any detail (Iguchi & Mizuno, 1999; Moriyama *et al.*, 1998).

The development of some species has been found to only occur upon larvae reaching salt water (Iida *et al.*, 2010; Ellien *et al.*, 2011), though saltwater is thought to be detrimental to other species, resulting in faster starvation due to higher osmoregulation requirements exhausting yolk reserves quicker (Iguchi & Takeshima, 2011). Development of *Sicyopterus lagocephalus* in particular has been extensively studied, with developmental stages being characterised from hatch and larval migration to the sea (Valade *et al.*, 2009), through to post-larvae and juveniles returning to freshwater (Keith *et al.*, 2008). No studies exist on larval development or survival for any of New Zealand's amphidromous fishes.

## Thesis focus

It was the objective of this thesis to examine larval drift and development in the lower reaches of the Waianakarua River, South Island, New Zealand. The Waianakarua River is located in North Otago, and is a relatively short, swiftly flowing coastal stream which drains easterly into the Pacific Ocean. The Waianakarua River was chosen due to its relatively high diversity of amphidromous taxa, and its particularly high densities of bullies (*Gobiomorphus* spp.) and torrentfish (*Cheimarrichthys fosteri*) (NIWA 2014; M Warburton 2014, pers. comm.).

## Bullies

New Zealand is home to seven known bully (Eleotridae) species, all of which are endemic, and belong to the *Gobiomorphus* genus: common bully (*G. cotidianus*), Cran's bully (*G. basalis*), upland bully (*G. breviceps*), bluegill bully (*G. hubbsi*), tarndale bully (*G. alpinus*), redfin bully (*G. huttoni*), and giant bully (*G. gobioides*) (McDowall, 1990). Of these, four species are considered amphidromous: the bluegill bully, common bully, redfin bully and giant bully. Only one of these, the common bully, is facultatively diadromous, being capable of forming landlocked populations in freshwater systems (Closs *et al.*, 2003). The Waianakarua River is known to contain the common, redfin, bluegill, giant and upland bullies (New Zealand Freshwater Fish Database (NIWA, 2014)). The primary focus species of this study is the bluegill bully, selected due to its inability to form landlocked populations, as well as the nearby Waianakarua River supporting a large population, and larvae being readily available through the collection of egg plaques. Bluegill bullies are specialised in their habitat use, typically inhabiting (and reproducing in) swiftly flowing stream habitats such as riffles (McDowall, 1990). Though a relatively large adult population exists, the species is listed as 'declining' under the New Zealand Threat Classification System (Goodman *et al.*, 2014), with declines of 10-70% predicted in the next 10 years. The reasons behind these declines are largely unknown, and the species is considered 'Data Poor' (Goodman *et al.*, 2014). Little is known of the population, recruitment or larval ecology of any of the bully species (McDowall, 2010a).

## Torrentfish

The torrentfish is also endemic to New Zealand. The phylogeny of the species has been somewhat contentious, but the species represents either the sole member of its own family (Cheimarrichthyidae) or the sole freshwater inhabitant of the family Pinguipedidae, and is most closely related to a marine species, the blue cod (*Paraperca colias*) (McDowall, 2010a). Like the bluegill bully, torrentfish inhabits swift-flowing riffles, and a large population is present in the Waianakarua River. Torrentfish are not known to form landlocked populations, and larvae remain unseen and undocumented during their seaward migration (McDowall, 2010a).

## Objectives

Overall, this thesis aims to further our understanding of the downstream larval migration which is characteristic of many of New Zealand's freshwater fish species, and provide key knowledge on the larval ecology of some of these species, primarily the bluegill bully. The first data chapter (Chapter two) will examine diel and spatial patterns of larval drift in the species described above, and the implications for species management. The second data chapter (Chapter three) focusses on development and starvation of amphidromous fishes during their larval migration, to better understand the early life history of these species and amphidromous migrations in general, as well as potential effects of delayed migration.

## Chapter 2: Temporal and spatial patterns of drifting fish larvae in a South Island, New Zealand coastal stream

### Introduction

Migrations should be viewed not simply as an event in a single taxon, but as a behavioural, ecological and evolutionary phenomenon: they are the product of natural selection, and must involve clear benefits to migratory individuals (Dingle & Drake, 2007). The timing of migration is also the product of natural selection, and is evolutionarily selected so that individuals may best exploit heterogeneities in environmental conditions or resource availability, benefitting survival, growth and reproduction (Dingle & Drake, 2007; Jorgensen *et al.*, 2008).

It is well recognised that anthropogenic activities (particularly barriers) along migration routes can delay or completely prevent migration and alter environmental conditions along the route or at the destination, disrupting the evolutionarily selected timing of migration, and the associated benefits (Marschall *et al.*, 2011). Diadromous fishes, which migrate between freshwater and marine habitats are especially vulnerable due to the narrow migratory corridor formed by the river channel (Hall, Jordaan & Frisk, 2011), and are highly susceptible to the disruption of their migratory patterns due to the effects of river regulation (Miles *et al.*, 2014).

Amphidromy is a distinct sub-category of diadromy (McDowall, 2007). The defining feature of an amphidromous life history is a larval migration ('drift') downstream to a pelagic (usually marine, but sometimes lentic) environment, undertaken by exceptionally small and fragile larvae immediately after hatch (Closs, Hicks & Jellyman, 2013; McDowall, 2007). Amphidromous larvae face irreversible starvation and failed development if migration is delayed (Iguchi & Mizuno, 1999; Valade *et al.*, 2009; Iida *et al.*, 2010), as they are thought to be unable to feed or develop during their downstream migration, instead relying on endogenous energy sources until they reach their pelagic feeding habitat (Iguchi & Mizuno, 1991; McDowall, 2007). An amphidromous migration also increases the likelihood of larvae encountering water infrastructure (Brown *et al.*, 2014), and so migratory larvae are highly vulnerable to death and injury due to human constructs such as weirs, which can cause barotrauma (trauma due to rapid changes in barometric pressure (Baumgartner, Reynoldson & Gilligan, 2006; Brown *et al.*, 2014)), and irrigation systems which can directly entrain larvae (Benstead *et al.*, 1999; Ellsworth, Tyler & VanderKooij, 2010).

Amphidromous fish may therefore be further at risk during their migration relative to other diadromous species, facing a greater range of threats during their vulnerable larval stage.

Despite this, when it comes to amphidromous species, much of the research and management focus has been placed on upstream migrations of adult fish, while downstream larval migration has received little attention, even in New Zealand where amphidromous taxa dominate the freshwater fish fauna (Boubee *et al.*, 1999; Bonnett *et al.*, 2014). Little is known on the timing or extent of larval drift in amphidromous fishes, but this knowledge is of great importance, as it may indicate critical times when larvae are most vulnerable to adverse impacts (McDowall, 1995; Brown *et al.*, 2014), and conservation measures would therefore be most effective. This is of importance, as many amphidromous fishes are of conservation, recreational, commercial and cultural significance, both in New Zealand and throughout the Indo-Pacific (Keith, 2003; Goodman *et al.*, 2014).

A small number of studies have noted distinct diel patterns of migration in amphidromous fish larvae (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), with larval drift peaking around sunset and then rapidly declining. Differences in diel drift patterns along the course of a river have also been observed, and explained by the interaction between larval behaviours and differing environmental factors along river courses (Iguchi & Mizuno, 1991). Very little research on larval migrations of freshwater fish has been conducted in New Zealand. Larval drift on the lower Waikato - a large, sandy bottomed North Island river - has been examined (Meredith *et al.*, 1989), and seasonal patterns of larval drift of the lower Taieri River and its estuary has also been studied (Sutherland & Closs, 2001), though full diel sampling was not conducted. These studies both represent large river systems (the Taieri having significant lacustrine and estuarine influences), but it is important to examine different types of rivers, as larval drift may be influenced by the physical characteristics of water courses (Iguchi & Mizuno, 1990).

It was the objective of this study to examine the spatial and temporal patterns of larval drift in the lower reaches of a coastal New Zealand stream. The primary study species targeted was the bluegill bully (*Gobiomorphus hubbsi*), an endemic eleotrid of New Zealand, recognised as declining under the New Zealand Threat Classification System (Goodman *et al.*, 2014). Based on the few existing studies (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), it was hypothesised that a distinct diel drift pattern would be observed, with larval drift peaking around sunset, and that drift patterns may differ at different locations along the course of the river.

## Methods

### Field sites & equipment.

The study was conducted in the Waianakarua River, a coastal stream on the east coast of South Island, New Zealand, with abundant riffles and a bed largely comprised of cobbles. The site was chosen due to the relatively high diversity of amphidromous taxa present, and its large

population of bluegill bullies (NIWA, 2014; M. Warburton 2014 pers. comm.). Due to downstream variations in diel drift patterns observed in other studies (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), two sampling sites were selected: one site approximately 1.5 km from the sea ('downstream'), and one site approximately 10 km from the sea ('upstream') (Fig 2.1). Drift nets were used to capture larvae during their downstream migration. These nets had a mouth of 60 cm x 30 cm, a length of 1.2 m, and a mesh size of 250  $\mu\text{m}$ , and were placed in the water for c. 20 minutes each sampling period. Nets were equipped with a screw-off collection bottle attached to the cod end, so that samples could easily be removed and preserved. Preliminary sampling and nest searches indicated larval fish first appeared in December, and numbers were negligible by April, and so sampling was conducted from January to February 2014.

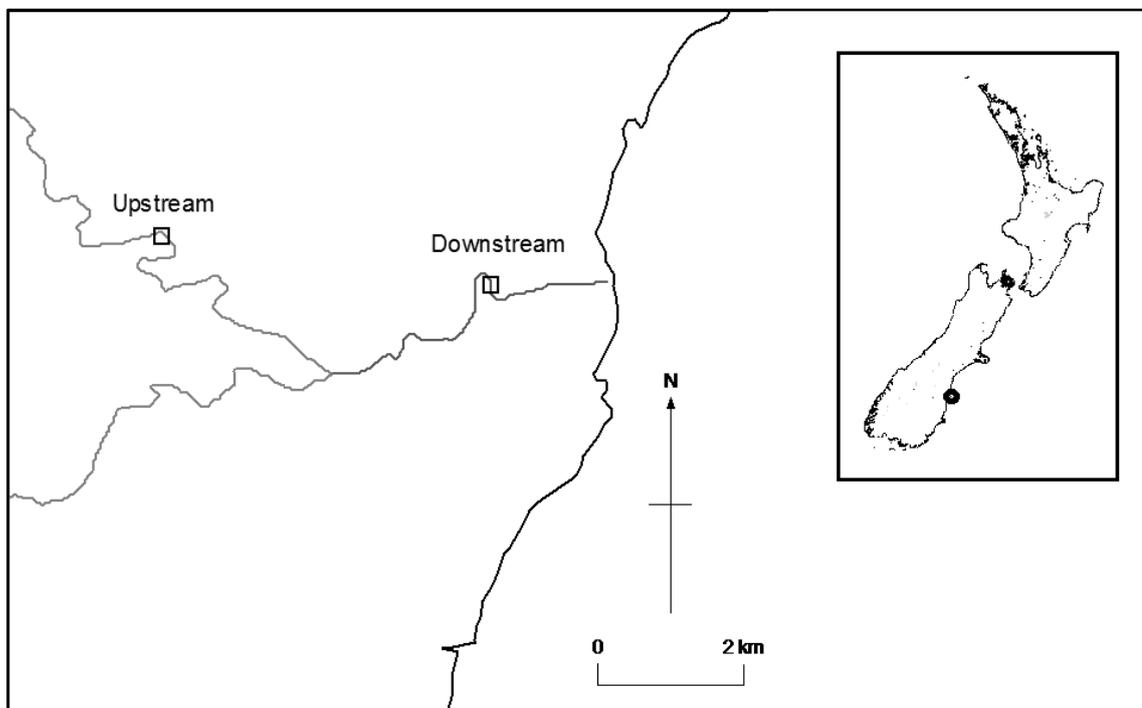


Figure 2.1: Sampling sites on the Waianakarua River, New Zealand.

#### Diel drift sampling

To determine the diel pattern of larval drift, sampling took place over three 24 hour periods: January 9-10, January 20-21, and January 25-26. Sampling was conducted six times over each 24 hour period. The following periods were chosen due to amphidromous larvae having been reported to display behavioural responses to light intensity (Iguchi & Mizuno, 1991; Iida *et al.*, 2010): before sunrise, after sunrise, midday, before sunset, after sunset, and midnight. To account for potential across channel differences in drift (Meredith *et al.*, 1989), three nets were set across the river from the river's edge into the main channel. Samples were immediately

preserved in 10% formalin, to later be processed in the lab. To control for any variations in flow between sampling dates and nets, water depth and velocity were measured at the mouth of each net, and used to convert larval counts into larvae per m<sup>3</sup> of water filtered.

To focus on the period predicted to contain a distinct peak in larval drift (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), additional fine-scale sampling centred around sunset was undertaken on February 4 2014. This sampling was conducted at the downstream site, using a single drift net identical to those described above. Beginning 20 minutes prior to sunset, samples were taken for each 20 minute period until midnight.

#### Lab

To simplify processing, large invertebrates and other particles captured in the drift nets were removed before samples were sorted. This was accomplished by sieving samples in a water bath for c. 30 seconds using a pair of sieves (one with a mesh of 2 mm, and another with a mesh of 125 µm), selected based on tests examining the mesh size by which all larvae would be retained. Any material that did not pass through the 2mm sieve was discarded, and all material collected in a 125 µm sieve was stained to aid in the identification of fish. Samples were then examined using a dissecting microscope, and larvae were sorted into different categories based on morphology and melanophore pattern, as similar small undeveloped fish larvae can be differentiated by melanophore arrangement (Yamasaki *et al.*, 2011). Larval fish were identified based on larval keys (McCarter, 1994) and adult fish assemblages (Luton *et al.*, 2005).

During processing, distinct differences were observed in the condition of captured larvae, with many larvae being clearly dead or damaged upon capture. As such, larvae were visually classified as either 'damaged' or 'undamaged' (Fig. 2.2). Larvae which were clearly dead upon capture, as evidenced by decay and/or the presence of fungal hyphae, were classed as damaged. In cases where larvae which were missing a tail or broken in half, but in otherwise good condition, damage was assumed to be caused by capture or sample processing, and these individuals were classed as undamaged.

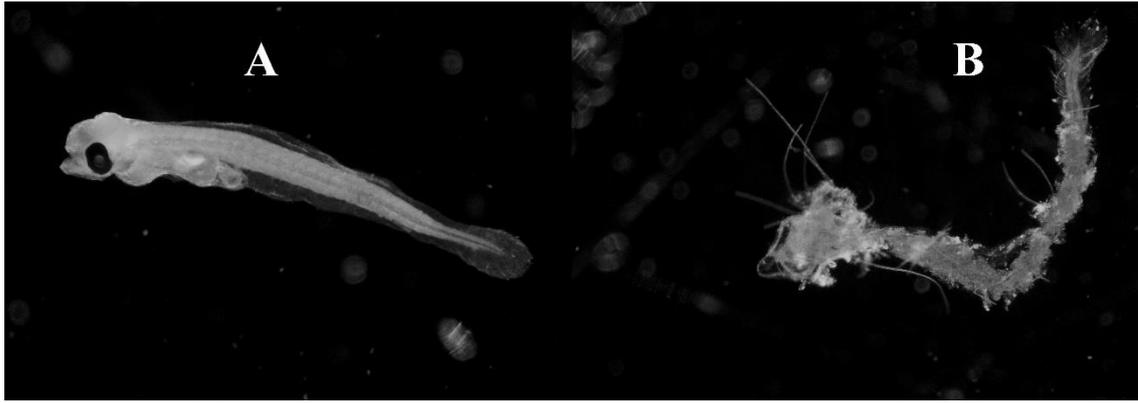


Figure 2.2: Examples of larvae classified as undamaged (A), and damaged (B).

## Results

### Larval Types

Four distinct larval types were identified during sorting of larvae – bluegill bully, common bully, an unidentified species (referred to hereafter as ‘species A’), and upland bully (Fig. 2.3). These larvae were distinguishable based on their size and melanophore patterns (Table 2.1). Upland bully are not amphidromous, and so were excluded from any analyses.

Table 2.1: Morphological differences between larval types observed in this study.

Species	Mean (+/- SE) notochord length ( $\mu\text{m}$ )	Pigmentation pattern	Other distinctive features
A - Bluegill bully	2579 +/- 6 (n = 774)	Melanophores all along ventral side	Upward jaw angle
B - Common bully	3422 +/- 8 (n = 306)	Two distinct patches of melanophores on ventral side	Upward jaw angle
C – Species A	2892 +/- 27 (n = 53)	Distinct row of melanophores above yolk sac and gut	Flat jaw angle, large yolk sac (mean depth = 447 $\mu\text{m}$ ) always present
D - Upland bully	5142 +/- 125 (n = 10)	Well pigmented all over	Upward jaw angle, well developed fins with fin rays present

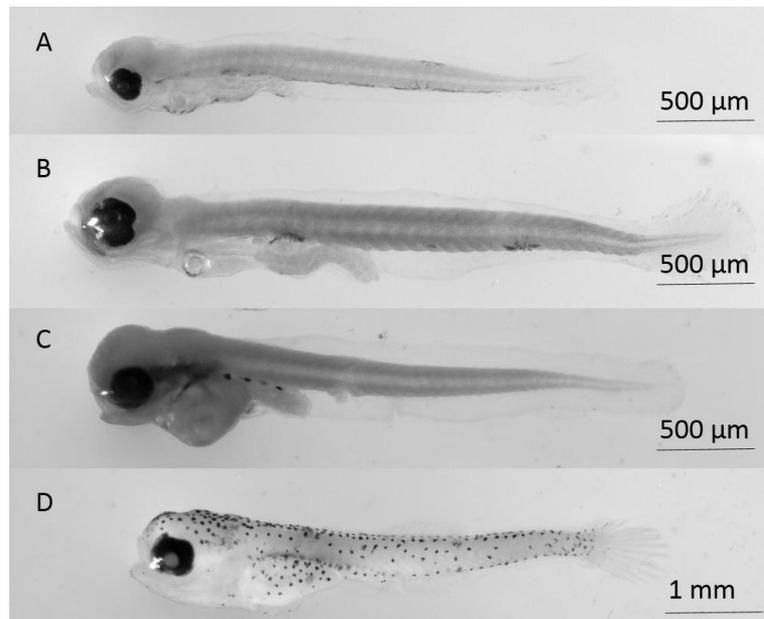


Figure 2.3: Examples of larval types. A = bluegill bully, B = common bully, C = Species A, D = upland bully.

#### Diel drift sampling

The sampling design resulted in 108 drift samples containing a total of 22252 larvae being processed to examine the diel drift pattern at the upstream and downstream sites (mean larvae per net = 209.92, SE = 58.58). Of these larvae, 13434 (60%) were identified as bluegill bully larvae, 8762 (39%) were identified as common bully larvae and 56 (<1%) were identified as species A.

Larval drift occurred at low levels throughout the day, and rapidly increased immediately after sunset, trailing off by midnight (Fig. 2.4). This pattern was observed at both the upstream and downstream sites, however the number of drifting larvae caught at the downstream site (21381 larvae, 96% of the total number caught) was far greater than at the upstream site (871 larvae, 4% of the total) (Fig. 2.4).

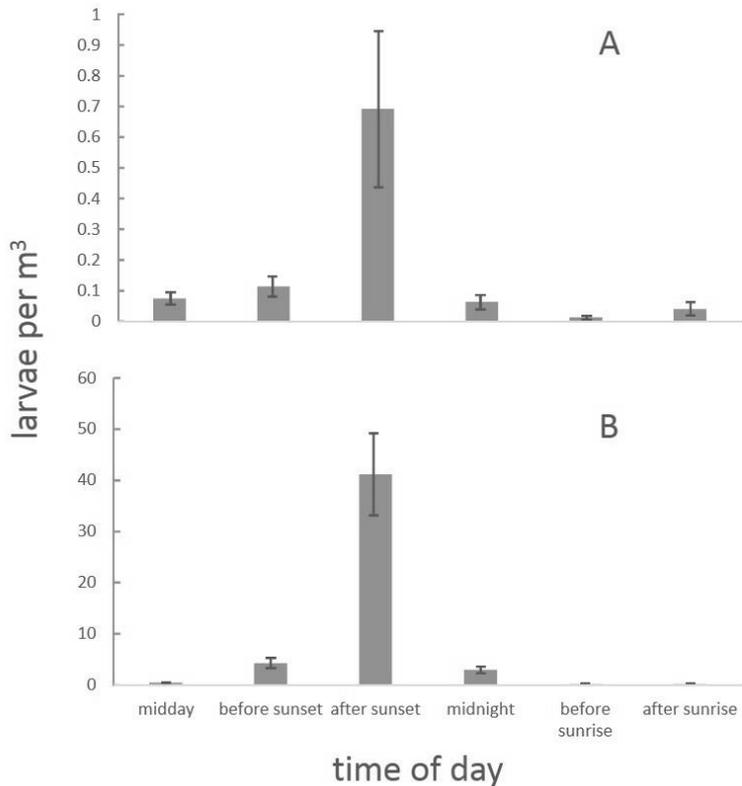


Figure 2.4: Combined diel drift pattern (mean  $\pm$  SE) of all fish larvae at the upstream (A) and downstream (B) site on the Waianakarua River. Note different Y-axis scales.  $n = 9$  for each bar.

#### Fine-scale sampling

For the fine-scale sampling around sunset, 8 samples were processed, containing a total of 7618 larvae. The results indicate that the period of peak drift began immediately following sunset, and lasted < 2 hours, dropping rapidly between 100 and 120 minutes after sunset (Fig. 2.5).

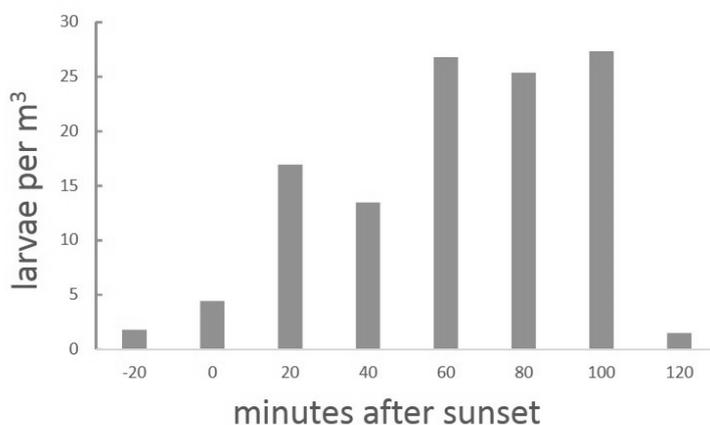


Figure 2.5: Sunset peak of total larval drift in the Waianakarua River on February 4 2014.  $n = 1$  for each bar.

#### Species specific drift patterns

The diel drift pattern of bluegill bully larvae mirrored that of overall drift, peaking just after sunset with a small amount of drift occurring throughout the rest of the day. This pattern was

again observed at both the upstream and downstream sites, with much greater numbers of larvae captured at the downstream site (Fig. 2.6).

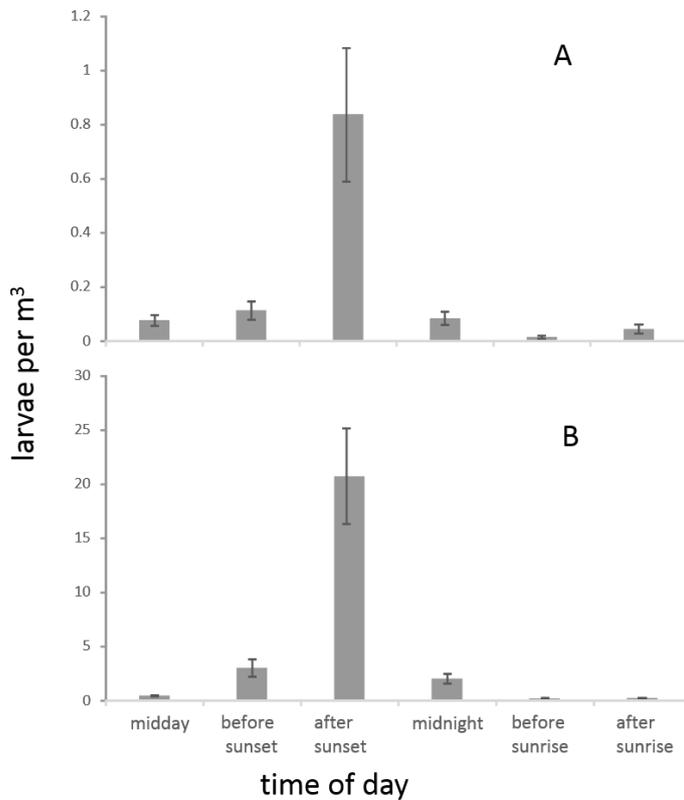


Figure 2.6: Diel drift pattern of the bluegill bully (mean  $\pm$  SE) at the upstream (A) and downstream (B) sites.  $n = 9$  for each bar.

Common bully also exhibited this diel drift pattern at the downstream site (Fig. 2.7), however at the upstream site only two larvae were caught, and so no pattern could be quantified. Species A larvae were restricted entirely to the downstream site, and showed a different pattern from the other species in this study, with a low level of drift throughout the day and a small peak around midnight (Fig. 2.7).

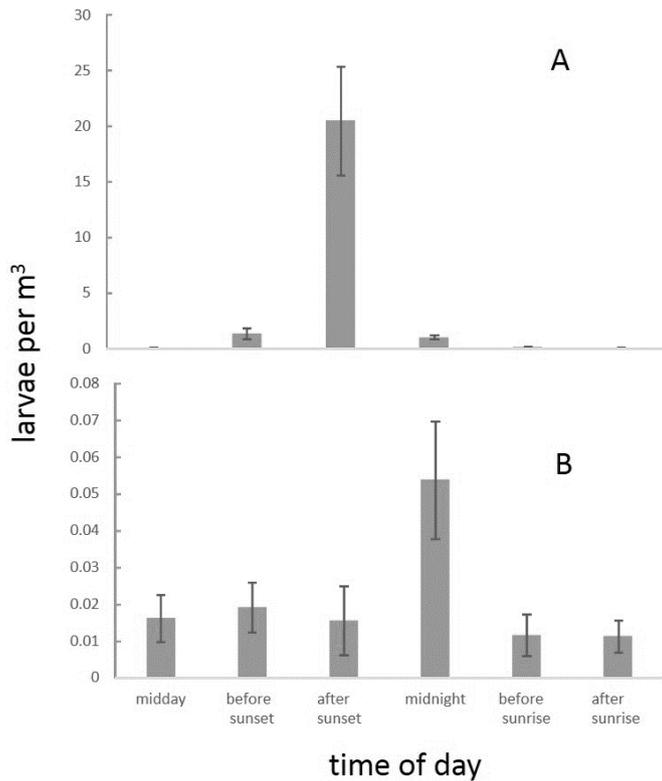


Figure 2.7: Diel drift pattern (mean  $\pm$  SE) of the common bully (A) and species A (B) at the downstream site.  $n = 9$  for each bar.

#### Condition of larvae

A distinct diel pattern was also observed in the proportion of larvae within a sample classified as damaged captured throughout the day. The proportion of damaged larvae increased at midnight, and declined at a slower rate than the drift of undamaged larvae, remaining relatively high throughout the night, a pattern consistent across sites (Fig. 2.8). These damaged larvae could not be identified to species, but when viewed alongside the diel drift pattern as a whole (Fig. 2.4), it can be seen that relatively low numbers of larvae captured during the peak period of drift were damaged (mean 7.9%), while immediately after (at midnight) a large proportion (mean 39.4%) were damaged (Fig. 2.8).

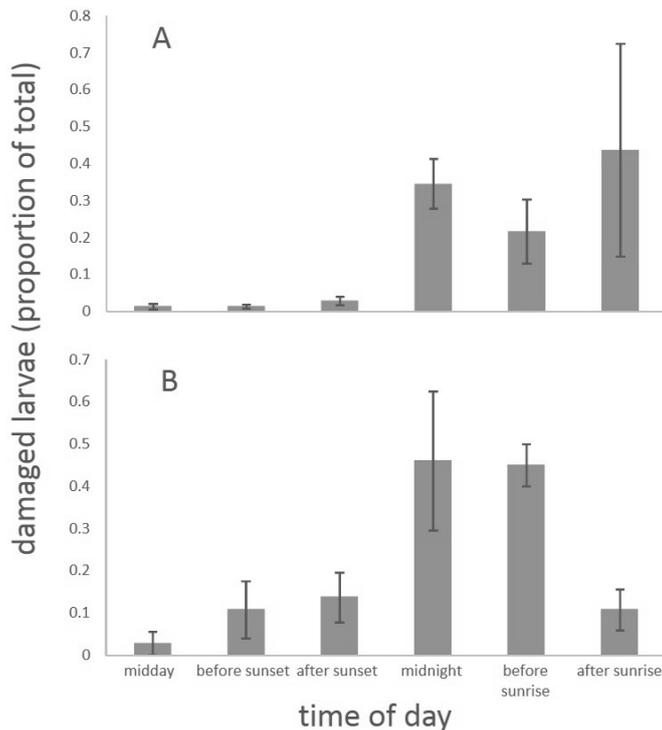


Figure 2.8: Diel pattern of the proportion of damaged larvae (mean  $\pm$  SE) captured at the downstream (A) and upstream (B) sites.  $n = 9$  for each bar.

## Discussion

### Larval types

Bluegill bully larvae were readily identifiable based on previous studies and the availability of experimentally hatched larvae collected through nest searches, but the other larval types captured during this study were not able to be identified definitively. The classifications used are inferences made based on the proximity to spawning sites and the presence of adult fish as indicated by electrofishing surveys (M. Warburton, 2014 pers. comm.), as larval drift is a function of the adult assemblages of streams (Luton *et al.*, 2005). Adult presence and spawning combined with larval keys (McCarter, 1994) supports the classification of the common and upland bully, and based on morphology, unidentified species A is neither a bully nor a galaxiid larvae. The only other alternative is that species A is the larvae of the torrentfish, a species common in the Waianakarua. This represents the first time torrentfish larvae have been documented during their downstream migration (McDowall, 2000). Genetic analyses of larval types will be conducted in order to definitively assign them to a species.

### Diel drift patterns

The results of this study suggest a distinct diel drift pattern is present in the larvae of bluegill bully and common bully in the Waianakarua River, with the vast majority of larval drift occurring between sunset and midnight, and limited drift throughout the rest of the day (and much of

that comprised of already dead larvae). As far as we know, this study represents the first description of a diel drift pattern for amphidromous fish in any of New Zealand's coastal streams.

The diel pattern of larval drift observed in this study compares closely with that found in the amphidromous goby *Rhinogobius brunneus* in Japan (Iguchi & Mizuno, 1990), which has been explained by a combination of behavioural responses of larvae to light and the influence of landscape as follows (Iguchi & Mizuno, 1991). In *R. brunneus* drift is low throughout the day as larvae exhibit a negative response to bright light. Peak drift is thought to be caused by larvae rising in the water column at sunset, attracted to the weak light of twilight, trailing off quickly as larvae sink due to negative buoyancy. Spatial differences in the diel drift pattern are thought to be explained by differences in landscape, with larvae drifting throughout the day in the steep upper courses where they are unable to halt migration, and showing clear diel variation in lower courses where larvae can halt migration in slow flowing pools. The idea of larvae actively halting migration in slow flowing areas is supported by the fact that in this study the period of peak drift dropped so suddenly, and that the larval catches at times after this peak were largely comprised of damaged individuals (i.e. those unable to halt their migration). However, the Waianakarua River lacks large pools in its lower reaches, and is characterised by swiftly flowing riffles throughout, and so it seems likely larvae may instead be halting migration in current refugia amongst the cobbles on the stream bed. This explanation is supported by the larval drift pattern in the sandy bottomed Waikato River, which has been found to have a much smaller peak in numbers of common bully larvae overnight, with comparatively high numbers drifting throughout the day (Meredith *et al.*, 1989).

Another proposed explanation which could lead to a diel drift pattern is synchronised hatch at dusk, which has been suggested for other amphidromous species, with hatch possibly encouraged by male parents which remain near the nest (Maeda & Tachihara, 2010). There is some evidence to support this, as bullies do defend nests (McDowall, 1990), and spontaneous hatch of bluegill bully nests can be induced by overturning rocks with nests on the underside, with seemingly little regard for the developmental stage of the larvae within (pers. obs.). Diel patterns of larval drift may therefore be a result of simultaneous hatch at sunset rather than (or in combination with) behavioural responses of already hatched larvae. The examination of hatching cues, swimming ability and larval behaviours may therefore provide an area of future research, shedding light on the mechanisms resulting in this distinct diel drift pattern.

The pattern of larval drift in torrentfish is less clear, exhibiting no clear peak at sunset, and only a relatively small peak in drift around midnight, however the low numbers of larvae collected limit my ability to accurately detect a pattern. While only a small number of larvae (53) were captured in the present study, torrentfish are abundant in the Waianakarua, and are highly fecund (Scrimgeour & Eldon, 1989), so should produce large numbers of larvae. The low numbers collected in the present study may be due to limited spawning occurring at the time of sampling, as the spawning season of torrentfish remains unknown (McDowall, 1995), and may not have been captured by the sampling design used. The majority of spawning may also have occurred downstream of the sampling sites, as torrentfish have been observed to undertake downstream migrations to the final few riffles before the sea (Scrimgeour & Eldon, 1989), likely for the purposes of spawning.

#### Spatial drift patterns

Larval drift was found to be largely restricted to the lower reaches (~ 10 km) of the river, with 96 % of total larvae being captured at the downstream site, as well as greater larval diversity. The only species consistently found at both sites, the bluegill bully, had a diel drift pattern which was comparable at both the upstream and downstream sites, which differs from the results of previous studies (Iguchi & Mizuno, 1990). It seems likely that this result is a product of both the ecology of the bluegill bully and the landscape of the Waianakarua, as in the Waianakarua River bluegill bullies do not spawn far enough inland for larvae to reach the steep courses typical of the upper reaches discussed by Iguchi and Mizuno (1990), explaining why the drift pattern observed in this study is consistent at both sites.

The other two amphidromous species, common bully and torrentfish, were found exclusively at the downstream site, with the exception of two common bully larvae. This is of interest, as the upstream site was only ~10 km from the sea, and suggests spawning in the Waianakarua is restricted almost entirely to the lower reaches. The idea that torrentfish undertake an adult migration downstream to spawn in the last few riffles before the sea (Scrimgeour & Eldon, 1989) is supported by the absence of torrentfish larvae caught at the upstream site.

#### Damaged larvae

The diel pattern observed in drifting larvae classified as damaged suggests that this pattern is related to injury before capture. If the damage was caused during processing or by the nets used, the proportion of damaged larvae should remain constant across the different times of day. This however was not the case, with clear diel variation in larval condition. While the cause of damaged larvae remains unclear, one possibility is that the extremely small and fragile larvae are vulnerable to barotrauma and/or physical damage during their downstream migration

(Baumgartner, Reynoldson & Gilligan, 2006; Brown *et al.*, 2014). The further larvae must drift, the higher chance they have of being damaged, and I suggest that the spatial extent of drift may be limited by this damage, restricting spawning (and therefore larvae) to the lower reaches of the river. The distance larvae can drift is also thought to be limited by starvation following the exhaustion of endogenous energy sources (Iguchi & Mizuno, 1999), and so a trade-off may exist between slow flowing 'safe' rivers (reducing risk of damage and increasing risk of starvation), and swifter, more 'dangerous' rivers (facilitating rapid migration to avoid starvation, but carrying a greater risk of damage). Another possible explanation is that the damage to larvae is the result of fungal infection of nests. During nest searches one such nest was found (Fig. 2.9), but larvae could still be induced to hatch. Many damaged larvae captured during this study appear to be partially decomposed and fungal hyphae were common on many of these fish, supporting this idea. This is of note, as fungal pathogens are known to be a significant threat to various fish species, both in aquaculture and under natural conditions (Czeczuga *et al.*, 2005; Wagner, Bartley & Oplinger, 2012), and fungal infection of egg masses in amphibians has been recognised as a contributor to species loss and decline (Blaustein *et al.*, 1994).

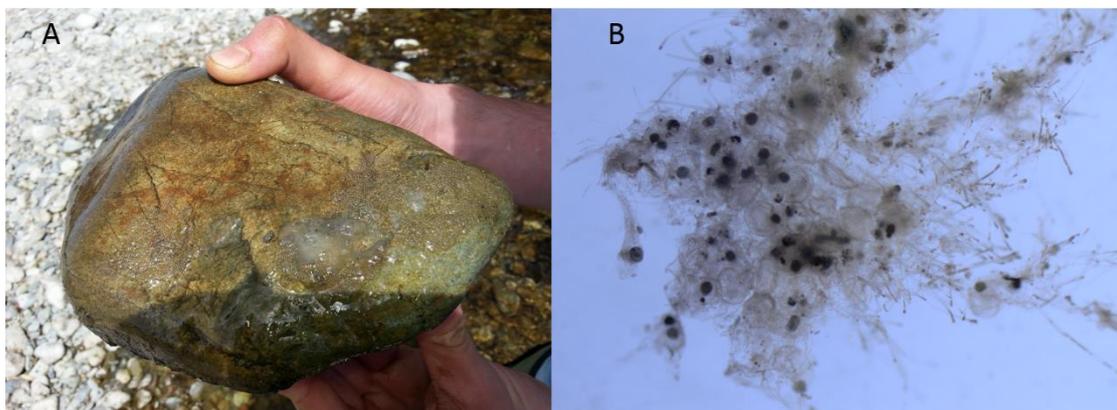


Figure 2.9: Bluegill bully nest with fungal infection (A), and a close up showing eggs and fungus (B).

#### Implications

Together, the spatial and temporal drift pattern of larvae suggests a critical window exists in coastal streams within which the greatest number and diversity of amphidromous larvae are migrating to sea, and are therefore most vulnerable to adverse impacts. This window (which contained 74.4 % of the total drifting larvae captured in this study) extends for only a few hours after sunset, and at most 10 km inland, and possibly much less. Minimising irrigation impact within this window may prove beneficial for populations of these vulnerable migratory fish. For example, irrigation withdrawals combined with summer low flows may act to delay or entirely prevent the seaward migration of larvae, and the entrainment of larvae into irrigation systems has been recognised as a threat to fish during their larval migration (Benstead *et al.*, 1999;

Ellsworth, Tyler & VanderKooi, 2010). No data was collected on larval fish entrainment in the present study, however numbers are likely considerable as other researchers have found that under normal conditions an average of 42% of drifting amphidromous larvae are entrained, with 100% of larvae being entrained during periods of low flow (Benstead *et al.*, 1999). Fish screens are normally used to mitigate entrainment of fish into irrigation takes, but amphidromous larvae are exceptionally small, and so screening is unlikely to be an effective option (Bonnett *et al.*, 2014). By identifying this critical window for larval fish migration, it is possible to focus conservation measures (e.g. shutting off water takes), minimising disruption to water abstraction while providing safe passage to a large proportion of migratory larval fish populations.

Whilst this study is restricted to a single river, similar temporal patterns of larval migration (that is, peaking rapidly immediately after sunset and declining by midnight) have been found in a small number of other amphidromous species outside of New Zealand, particularly freshwater gobies (Gobiidae) (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010). These fish exhibit similar spawning habits to species in this study, laying adhesive eggs in nests (mainly on the underside of stones) which are defended by the male parent (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), and as discussed above, this spawning habit may explain the sunset peak of larval drift. Freshwater gobies and related groups which exhibit this spawning habit dominate the freshwater fish faunas of streams throughout the Indo-Pacific (McDowall, 2007), and it is therefore suggested the diel drift pattern observed in this study may be widespread across amphidromous fishes throughout this region.

The same generalisations however cannot be made about the spatial and seasonal patterns of larval drift. Penetration inland is highly species specific, and seasonal differences in spawning between species have been found in the amphidromous fishes of Okinawa Island (Maeda & Tachihara, 2010; Yamasaki *et al.*, 2011), and suggested in New Zealand (McDowall, 1995), though for many species the actual timing of migration remains unknown. There may also be latitudinal differences in spawning season within species, based on temperature and photoperiod (Vinagre *et al.*, 2008a; Vinagre *et al.*, 2008b). Because of these potential differences, the drift patterns of other amphidromous fishes and river types warrants further examination, particularly when considering the effects of water infrastructure.

## Conclusions

This study has identified a clear diel pattern of larval drift and condition in some of New Zealand's amphidromous fishes in the Waianakarua River, providing valuable knowledge on their larval ecology, and further evidence that peak drift for a few hours after sunset may be a

consistent pattern across many amphidromous fishes. The results of this study suggest clear times when amphidromous fish are most vulnerable to impacts such as water abstraction, and allows for conservation measures to be targeted while minimising disruption to human activities. This is of great significance, as increased water abstraction in lowland areas combined with the difficulties of effectively screening larval fish from irrigation intakes poses a significant threat to the conservation of amphidromous fishes. Halting water withdrawals during this period of peak drift may therefore represent the best option for minimising larval entrainment of amphidromous fishes.

## Chapter 3: Development and starvation during the early life history of some of New Zealand's amphidromous fishes, particularly the bluegill bully (*Gobiomorphus hubbsi*)

### Introduction

Knowledge of all life history stages of fish species is critical to understanding their distributions and identifying potential conservation issues (Valade *et al.*, 2009; Closs, Hicks & Jellyman, 2013). The early life history stages of amphidromous species are poorly known, but greatly influence their habitat use and migration patterns (Iida *et al.*, 2010), and so are of importance in helping us understand the processes involved in determining their distributions and population dynamics, such as larval dispersal and recolonisation (Valade *et al.*, 2009). Amphidromous fishes contribute greatly to the diversity of fish communities throughout the Indo-Pacific (McDowall, 2007), and are of commercial, cultural and economic importance. There is therefore an urgent need to improve our knowledge of the early life history stages of these species to aid the management and conservation of amphidromous fishes (Keith & Marion, 2002; Valade *et al.*, 2009; Goodman *et al.*, 2014).

Amphidromous fishes hatch as extremely small and undeveloped larvae, and are thought to be unable to feed during their downstream migration due to the lack of suitable zooplankton prey in running water, instead relying on limited endogenous energy sources (i.e. their yolk sac) until they reach their pelagic feeding habitat (Iguchi & Mizuno, 1999; McDowall, 2007; Closs, Hicks & Jellyman, 2013). Little is known of the ability of amphidromous fish larvae to survive their downstream migration, with only a handful of studies having been conducted, and these focussing almost exclusively on *Rhinogobius* spp. (Moriyama *et al.*, 1998; Iguchi & Mizuno, 1999; Tamada & Iwata, 2005). These studies have generally concluded that amphidromous fishes are highly susceptible to irreversible starvation during their period of drift. It has been estimated that most newly hatched larvae from upstream reaches perish before reaching the marine environment under normal or low flows as a result of retention in eddies and pools along the river course (Moriyama *et al.*, 1998). Longer migrations (along longer rivers) have also been found to result in greater rates of mortality, with a comparison between comparatively short and long rivers finding that almost twice as many larvae (>50% of total drifting) died during migration on the longer course (Iguchi & Mizuno, 1999).

As well as starvation, laboratory studies have indicated that at least in some species, survival is lower and development is delayed until larvae enter marine habitats (Valade *et al.*, 2009; Iida

*et al.*, 2010). The results of these studies suggest that failed development and early starvation may play important roles in limiting the distributions and success of amphidromous fishes. This has serious implications for how downstream migrations may be influenced by human activities, which are known to have adverse effects on migratory fish, delaying or completely preventing migration (Marschall *et al.*, 2011), or altering migratory habitat (Iguchi & Takeshima, 2011). Understanding this early life history is therefore of critical importance in the management of these species and their habitat.

New Zealand's freshwater fish fauna is dominated by amphidromous species, yet almost nothing is known of the larval ecology of any of New Zealand's amphidromous fishes (McDowall, 2010a). The purpose of this study is to improve our understanding of the early life history of a number of New Zealand's amphidromous fishes, particularly the bluegill bully, by examining development and starvation of larvae during their migration downstream. There were two components to this study. The first component examined larval starvation and development in the lower reaches of a New Zealand river using larvae captured during their downstream migration. The second component utilised lab studies to examine larval development in freshwater and saltwater, in order to determine the habitat requirements of larvae, and the potential influence of delayed or blocked migration on larval development.

From the studies outlined above, a number of hypotheses were formulated regarding the developmental and starvation state of bluegill bully larvae along the course of the river. In regards to starvation, it was expected that more starved larvae would be captured downstream than upstream during the field study (Moriyama *et al.*, 1998; Iguchi & Mizuno, 1999). For larval development, it was expected that delayed development in freshwater would result in a relatively uniform developmental stage along the river. For the lab experiments, it was expected that larvae would develop faster in seawater relative to those larvae reared in freshwater, which may fail to develop at all (Valade *et al.*, 2009; Iida *et al.*, 2010), given there are no known examples of landlocked populations of bluegill bully (McDowall, 2010a).

## Methods

### Field study

The field study was conducted in the Waianakarua River, South Island, New Zealand. This field site was selected due to the high diversity of amphidromous fishes found in the river, and its particularly high densities of bluegill bullies (NIWA 2014; M Warburton 2014 pers. comm.). To examine patterns of development and starvation along the course of the river, two sampling sites were selected: one site approximately 1.5 km from the sea ('downstream'), and one site approximately 10 km from the sea ('upstream') (these sites were the same as those used in

chapter two, refer to Fig. 2.1). Larvae were captured simultaneously at these sites using drift nets, which had a mouth of 60 cm x 30 cm, a length of 1.2 m, and a mesh size of 250 $\mu$ m. Samples were immediately preserved in 10% formalin, to later be processed in the lab.

The sampling design (for a full explanation see chapter two) resulted in the collection of 108 drift samples (54 at each site), and for each sample, a maximum of 20 larvae were randomly selected to examine developmental and starvation state. To examine larval starvation, larvae were visually classified as either being in an endogenous feeding state or an irreversible starvation state (see Iguchi and Mizuno (1999), Fig. 3.1), and yolk sac depth and myomere width were measured. To examine development and growth, these as well as several other variables were measured including notochord length, eye diameter, and fin development (Kerrigan, 1997; Valade *et al.*, 2009; Iguchi & Takeshima, 2011) (Fig. 3.2).



Figure 3.1: Larvae classed as being in an endogenous feeding state (A) and irreversible starvation state (B). Note yolk sac consumption and reduced myomere thickness in the starved larva (7 days old).

#### Experimental procedures

To monitor larval growth and development in freshwater and saltwater, egg plaques were collected from the underside of stones at the downstream site in the Waianakarua River and placed in buckets on the stream bank. This stimulated hatching, and so larval hatch time was reliably known. Larvae were taken back to the lab and placed in 2 L aquaria containing either freshwater or seawater. Larval development is influenced by temperature (Iguchi & Takeshima, 2011), and so experiments were conducted simultaneously under the same conditions to control for this effect. Based on the methods of Iguchi and Takeshima (2011), up to 10 larvae (depending on availability due to mortality) were removed and preserved in 10% formalin after 2, 4, 7 and 10 days to later examine growth (notochord length) and development. Larval development was examined by measuring the same indicators as used for the field study (see Fig. 3.2).

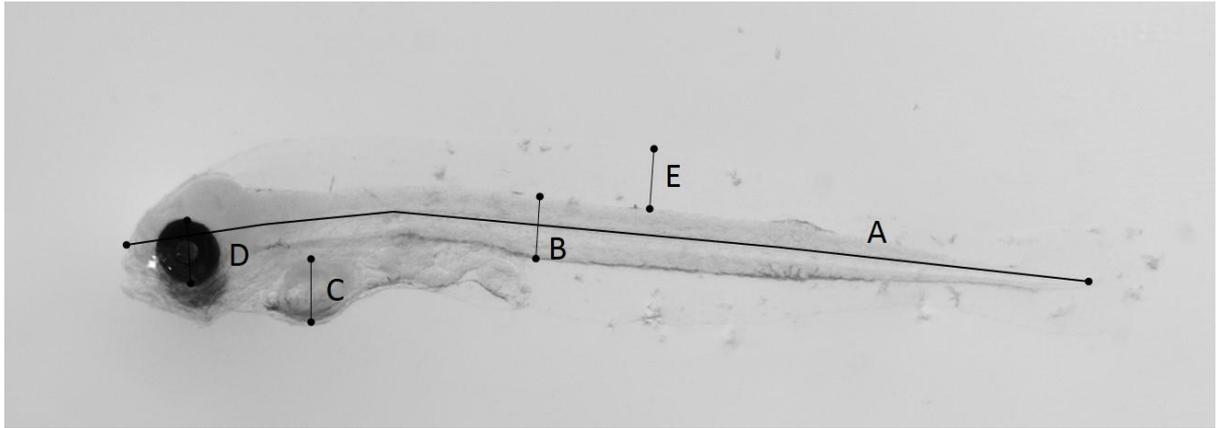


Figure 3.2: Measured distances on the surface of a bluegill bully larva. A = notochord length, B = myomere width, C = yolk sac depth, D = eye diameter, E = dorsal fin depth.

### Analyses

To examine differences in developmental variables between sites for the field study, independent samples t-tests were used. To determine whether larvae were utilising their yolk for growth during migration, linear regression was conducted on notochord length and yolk sac depth for larvae captured in the field. To examine the influence of freshwater retention and time on larval development in the lab experiment, two-way ANOVA were utilised. Statistical analyses were conducted using SPSS (IBM Corp., 2013) and R (R Development Core Team, 2008).

### Results

#### Field study

While four species were caught (see chapter two), only those which were considered amphidromous were included in the analyses: bluegill bully, common bully and torrentfish. For all species examined, a negative relationship was observed between notochord length and yolk sac depth (Fig. 3.3).

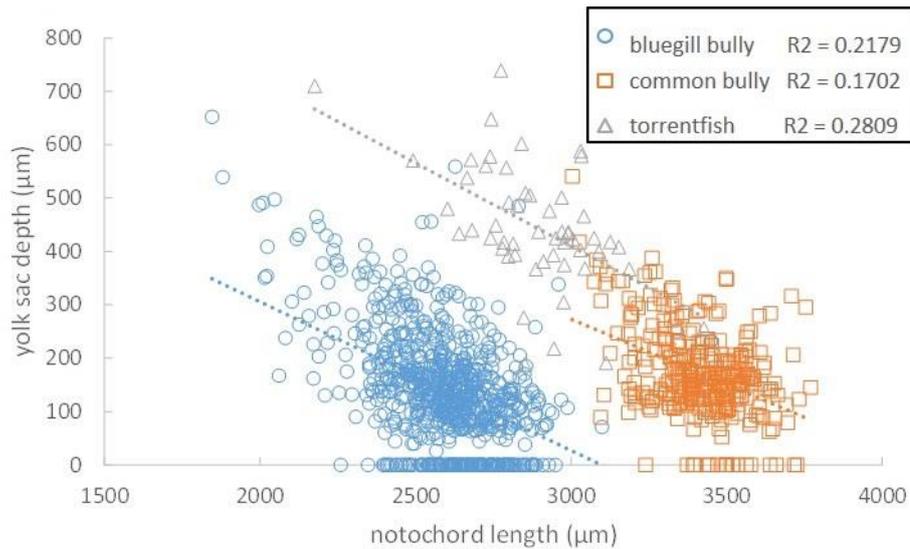


Figure 3.3: Relationships between notochord and yolk sac depth (including regression lines and  $R^2$  values) for the bluegill bully ( $n = 774$ ,  $F_{1,772} = 215$ ,  $p < 0.001$ ), common bully ( $n = 306$ ,  $F_{1,304} = 62.37$ ,  $p < 0.001$ ), and torrentfish ( $n = 53$ ,  $F_{1,51} = 19.92$ ,  $p < 0.001$ ) captured during their downstream migration.

Spatial patterns of larval starvation and development in the field could only be examined in the bluegill bully, as they were the only species of larvae consistently captured at both sites. Significant differences were present between sites in a number of developmental variables (Fig. 3.4). Yolk sac depth (Fig. 3.4a) was significantly higher at the downstream site (mean = 173.186  $\mu\text{m}$ ) than the upstream site (mean = 82.069  $\mu\text{m}$ ), with a difference in means of 91.1  $\mu\text{m}$  (95% CI 105.38 – 78.85,  $t = 12.5$ ,  $p < 0.001$ ). Notochord length (Fig. 3.4b) was significantly higher at the upstream site (mean = 2651  $\mu\text{m}$ ) than the downstream site (mean = 2560  $\mu\text{m}$ ), with an average difference of 91  $\mu\text{m}$  (95% CI 65 – 116,  $t = 7.038$ ,  $p < 0.001$ ). Eye diameter (Fig. 3.4c) was also significantly higher (mean difference 16.52  $\mu\text{m}$  (95% CI 11.4 – 22.6),  $t = 6.353$ ,  $p > .001$ ), and myomere thickness (Fig. 3.4d) was significantly lower at the upstream site (mean difference 4.07  $\mu\text{m}$  (95% CI 1.1 – 7.0),  $t = 2.7$ ,  $p = 0.007$ ), however these were biologically small differences.

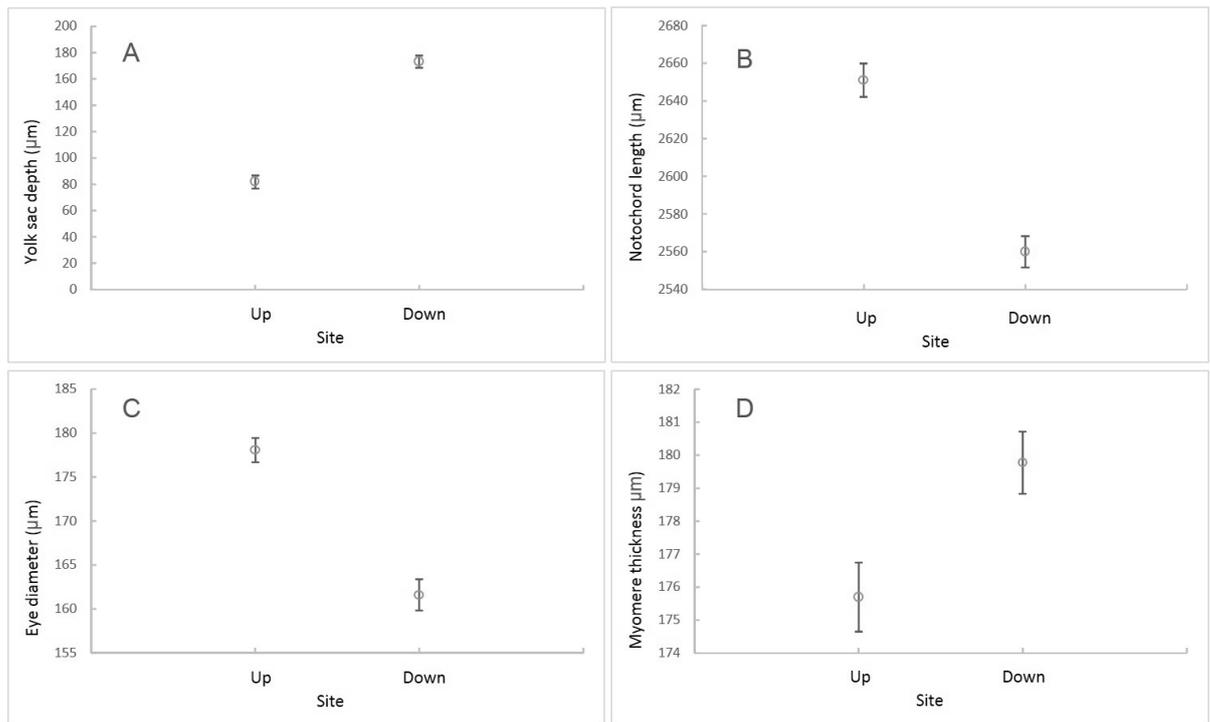


Figure 3.4: Variation in developmental variables (mean  $\pm$  SE) for the bluegill bully between upstream ( $n = 275$ ) and downstream ( $n = 499$ ) sites.

#### Lab study

Larval mortality was higher in freshwater than saltwater, with no larvae from the freshwater treatment surviving to ten days old. Upon hatch larvae were already relatively well developed, with the eyes, jaw, gut and chromatophores already present. There were significant differences in developmental variables between larvae reared in freshwater compared to those reared in saltwater. Larvae reared in saltwater exhausted their yolk reserves (Fig. 3.5a) more quickly than those in freshwater ( $F_{1,50} = 12.561$ ,  $p < 0.001$ ) and showed greater loss of muscle (as measured by myomere width, ( $F_{1,50} = 61.541$ ,  $p < 0.001$ ) over time (Fig. 3.5b). Conversely, larvae reared in seawater also exhibited greater growth of the notochord (Fig 3.5c,  $F_{1,50} = 65.371$ ,  $p < 0.001$ ), and developed much larger fins (Fig. 3.5d,  $F_{1,50} = 216.978$ ,  $p < 0.001$ ) than those in freshwater. No significant difference was observed in eye diameter (Fig. 3.5e) between treatments ( $F_{1,50} = 0.703$ ,  $p = 0.406$ ) or over time ( $F_{3,50} = 0.644$ ,  $p = 0.578$ ).

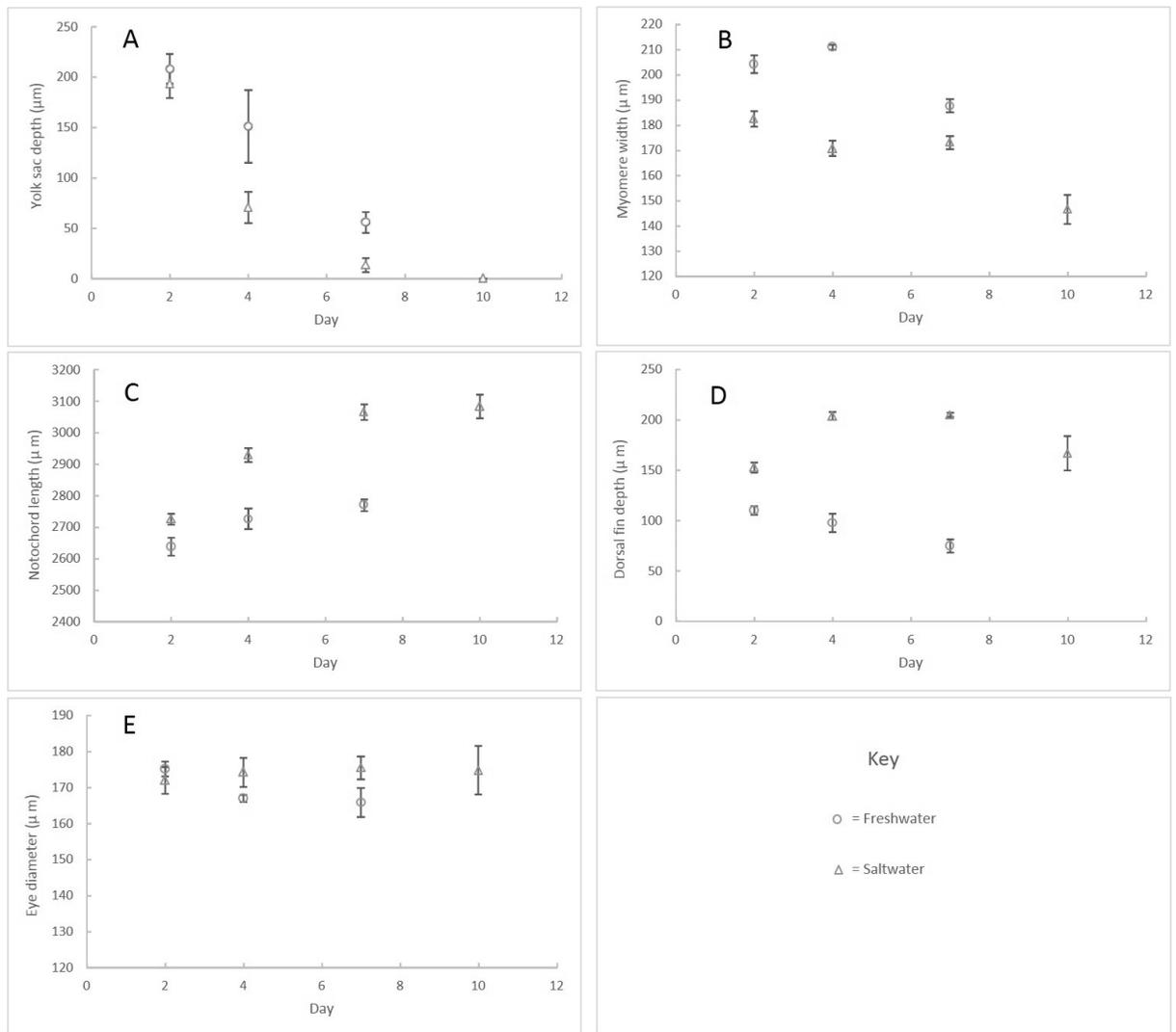


Figure 3.5: Changes over time in developmental variables (mean  $\pm$  SE) for larvae reared in freshwater and saltwater.  $n = 10$  except saltwater day 10 where  $n = 5$ .

## Discussion

### Spatial patterns

The results of the field study did not support the hypothesis that the upstream distribution of amphidromous fish in the Waianakarua River is limited by starvation occurring during their downstream migration. While larvae were captured which had no yolk remaining, these larvae did not appear to be starved, and their oil droplets were not yet absorbed. They still had relatively large muscle blocks, and had not yet entered a starvation state as indicated by larvae experimentally starved under lab conditions. Unexpectedly, larvae upstream generally had less yolk than those downstream. Upstream larvae were also significantly longer and had better developed eyes than those downstream, which together with the negative relationship between notochord length and yolk sac depth, indicates that they had used their energy reserves for growth and development. These upstream larvae also had significantly smaller

myomere widths than those downstream, perhaps indicating the onset of starvation. This pattern is the opposite of that predicted, as these larvae must still undergo a relatively long migration to the sea (i.e. approximately 10km).

I suggest that the reason no distinct starvation pattern was observed along the course of the river is that any starvation pattern was obscured by varying hatch times of larvae. Larvae captured during their downstream migration were caught across a range of developmental stages and sizes, ranging from small eyeless fish with large yolk reserves to well-developed fish with no yolk remaining. On the other hand, those hatched experimentally (all from plaques collected from the downstream site) were at a relatively uniform, well-developed stage. This may indicate larvae are capable of hatching out at an earlier, undeveloped stage to maximise their yolk reserves in order to avoid starvation. The cue(s) associated with hatch are unknown, though it has been suggested for other amphidromous fishes that hatch may be stimulated by the male parent (Maeda & Tachihara, 2010). In this study, larvae hatched immediately following the physical disturbance of nests, suggesting bed-moving flood disturbance may play a role in stimulating hatch. This adaptation would prove beneficial to amphidromous species, which rely on open access to the sea to complete their life cycle, and such disturbance events may be necessary to prevent river mouth closure. Alternatively, it may simply be that the Waianakarua River lacks the physical characteristics which would cause starvation to become a major issue for amphidromous larvae (such as length, gradient, eddies and pools), and larvae in this system are rapidly transported to the marine environment.

#### Lab study

Larvae were capable of some development in freshwater. Larvae captured during their migration (having spent their entire life in freshwater) often had well developed eyes, jaw, gut and pigmentation, and larvae reared in freshwater in the lab exhibited further growth and development following hatch. These results do not support the hypothesis that larvae would fail to develop in freshwater.

Development was accelerated in seawater, with larvae reared in seawater having increased notochord lengths, as well as additional features such as fins, when compared to those retained in freshwater. In this study, larvae exposed to seawater also exhibited increased yolk consumption, a pattern which has also been observed in the amphidromous ayu (*Plecoglossus altivelis*) (Iguchi & Mizuno, 1999). Iguchi and Mizuno (1999) suggested that greater osmoregulatory costs associated with the transition to saltwater result in an increased consumption rate of yolk, which together with other metabolic requirements would restrict the amount of energy available for growth. They concluded that seawater had an adverse effect on

newly hatched larvae, with this growth–osmoregulation trade-off ultimately influencing foraging efficiency and predator avoidance in larvae. However, the results of this study indicate at least some of this yolk is clearly being used for development and growth, not just osmoregulation.

The developmental differences observed in this study suggest that when in freshwater (and thus unable to feed), larvae invest their limited resources to develop only those features which are necessary (i.e. eyes, gut, and mouth), conserving yolk to prevent irreversible starvation. Upon reaching the sea (their pelagic feeding habitat), they begin to develop features which benefit them at this life stage. Growing larger results in greater swimming ability (Tsukamoto, Kajihara & Nishiwaki, 1975), and fins are also presumably adaptive for increased swimming ability. Swimming ability influences foraging efficiency and predator avoidance - factors which are likely less important to larvae during their downstream migration, yet critical upon reaching their pelagic feeding habitat.

#### Implications

Together, these results suggest that larvae of bluegill bully risk growth and developmental issues if migration is delayed, which can influence feeding and predator avoidance abilities in larvae, which could increase the risk of larval mortality upon reaching the sea (Iguchi & Takeshima, 2011). Due to their limited swimming ability, larval fish which must undergo an amphidromous migration are highly vulnerable to retention in freshwater. Even under natural conditions, retention of larvae by eddies and pools appears to have adverse effects on larval success (Moriyama *et al.*, 1998), and anthropogenic activities and structures may worsen the problem of freshwater retention of larvae. Dams and weirs are known to alter flow conditions, acting as barriers which can delay or entirely prevent migration (Marschall *et al.*, 2011). Water abstraction and diversion can also artificially reduce flows (Dewson, James & Death, 2007), which may also influence larval retention, as the poor swimming ability of larvae results in them being unable to effectively swim in turbulent freshwater systems, relying largely on the flow to sweep them out to sea (Iguchi & Mizuno, 1990; Iguchi & Mizuno, 1991). Anthropogenic activities which reduce or alter flows may therefore indirectly affect the success of amphidromous fishes by altering the likely developmental pathways for larvae.

This study, along with the results of previous research into the development of amphidromous fishes, highlights the importance of examining the ecology of the early life history stages of fishes. Some amphidromous species fail to develop when retained in freshwater (Valade *et al.*, 2009), while others (as in the present study) are capable of some development in freshwater, but ultimately benefit from rapidly entering seawater. Others still show reduced success in

seawater, relying on low salinity estuarine environments for development and survival (Iguchi & Takeshima, 2011). These differences ultimately represent different approaches to a trade-off between development and starvation, where fish can invest in early development and risk starvation, or delay development and maximise yolk reserves during migration in hopes of reaching their feeding habitat before starvation occurs.

These inter-specific differences in the influence of salinity on larval development support the hypothesis that amphidromy as a life history relies on a pelagic feeding environment (whether a lake, the sea or an estuary) rather than a strictly marine dispersal environment (Closs, Hicks & Jellyman, 2013). Some species may however rely on salinity as a cue for developmental changes, supporting the idea that biologically informative distinctions may be drawn between amphidromous species with high oceanic dependence where the marine stage is likely obligatory, and those with low oceanic dependence which may form landlocked populations (Watanabe *et al.*, 2014). Overall, these studies show that even when fish have a similar life history or adult habitat, larval requirements may greatly differ, and even contrast. Studies on specific requirements for larval development and habitat are therefore crucial in identifying potential conservation and management issues for amphidromous species.

## Chapter 4: General discussion

### Introduction

Understanding the benefits of migration is crucial in understanding the evolution of migratory life histories, and in recognising key threats to migratory species. New Zealand's freshwater fish fauna is dominated by species which undertake an amphidromous migration, as is the case with many species across the Indo-Pacific islands (McDowall, 2007). Throughout their geographic range, amphidromous fish are of recreational, cultural and conservation significance (Keith, 2003). Despite this, amphidromous fishes are understudied relative to their other diadromous fish species (particularly salmonids and eels), and the larval ecology of fishes is poorly understood in general. Critical to an amphidromous life history is a biome shifting migration, undertaken immediately after hatch by small, undeveloped larvae (McDowall, 2007). Few studies have been conducted internationally examining patterns of larval drift and development in amphidromous species. This study was the first in which larval drift has been examined in the swiftly flowing coastal streams of New Zealand, and represents the only study detailing any aspect of the larval ecology and development of the bluegill bully. In this chapter, I summarise the main conclusions of this research, address applications of the research to species management, and explore potential areas of future study.

### Review of findings

#### Patterns of larval drift

Understanding the evolutionarily selected patterns and timing of migrations is of critical importance in understanding the benefits of those migrations, and to best inform species managers on the specific management requirements of migratory species. In chapter two I used drift nets to identify the patterns of larval drift in the lower reaches of the Waianakarua River, finding a distinct spatial and diel pattern of drift in amphidromous fish larvae. The vast majority of larval fish were actively migrating downstream during the few hours after sunset, and larval drift was largely restricted to the lower reaches of the river, with larval catches being both more abundant and more diverse at the downstream sampling location. The drift pattern documented in this chapter is comparable to those of a number of other amphidromous species throughout the Indo-Pacific (Iguchi & Mizuno, 1990; Maeda & Tachihara, 2010), suggesting this pattern of peak sunset drift may be a feature of many amphidromous fishes.

#### Starvation and development

Amphidromous larvae are thought to be unable to feed during their downstream migration, instead being reliant on limited endogenous energy sources until they reach a pelagic feeding

habitat (Iguchi & Mizuno, 1999; Closs, Hicks & Jellyman, 2013). Prolonged migrations can result in larvae starving beyond a 'point of no return', and this irreversible starvation of larvae is thought to play an important role in limiting the success of amphidromous fishes (Iguchi & Mizuno, 1999). Some amphidromous larvae are also thought to be reliant on reaching the sea for developmental reasons, failing to develop if retained in freshwater (Valade *et al.*, 2009). Anthropogenic impacts which delay migration or promote the retention of larvae therefore pose a significant threat to amphidromous fishes.

In chapter three, I examined starvation and development along the Waianakarua River using a field survey, and experimentally examined the effect of salinity on development using lab experiments. No clear pattern of larval starvation was identified in the Waianakarua River, however this may be attributable to the larvae's apparent ability to hatch at variable stages of development, and differing lengths of time for which larvae had been drifting. Developmental differences were noted in the lab experiment, with larvae reared in saltwater exhibiting increased growth and the development of additional features relative to those retained in freshwater after hatch. Growth and development are related to success in fish larvae, with smaller undeveloped individuals at greater risk of mortality, suggesting activities which reduce flows and thus promote freshwater retention of larvae may have adverse effects on amphidromous fishes.

#### Amphidromy as a life history

The results of this study have implications for amphidromy as a life history. Amphidromy has been the focus of debate, with various authors discussing different mechanisms behind the life history, as well as questioning the relevance of the term as a whole (Gross, 1987; McDowall, 2007; Closs, Hicks & Jellyman, 2013). Central to this debate has been the widely accepted idea that the occurrence of amphidromy is related to the dispersal benefits inherent to an oceanic larval stage (McDowall, 2010b). Indeed, some species have been shown not to develop at all in freshwater, supporting the idea that an oceanic stage is critical to an amphidromous life history.

In chapter three, I examined the marine dependency of the bluegill bully by examining the development of larvae in freshwater and seawater. No known landlocked populations of bluegill bully exist, yet larvae were still capable of survival and development in freshwater. While larvae were not reared through into the juvenile stage, the results possibly indicate that an oceanic stage is not an obligate requirement for the bluegill bully. This has implications for the continued debate over the ecological significance of amphidromy, providing further support that oceanic dispersal is not the primary benefit of an amphidromous life history. Alongside the results of

other studies examining the development of amphidromous larvae which found differing and even contrasting developmental patterns in regards to salinity, there is evidence to suggest that biologically meaningful distinctions may be drawn between amphidromous species with high and low oceanic dependence respectively (Watanabe *et al.*, 2014). Amphidromy is viewed as a subcategory of diadromy, and some may use the habitat requirement differences between amphidromous species to argue against the classification of amphidromy as a distinct life history. I argue that while amphidromy may not be a distinct form of diadromy in the sense of a seawater-freshwater shift, it is still a distinct life history strategy, however perhaps (as suggested by Closs, Hicks and Jellyman (2013)) the categorisation should rely not on a migration between freshwater and the sea, and instead be based on those species which undertake larval migrations to a pelagic feeding habitat, regardless of whether this is marine or lentic. Amphidromy in this sense is still a biologically meaningful and useful term, as fishes which undertake such a migration will face unique biological and management requirements during their larval migration.

#### Management Implications

The combined results of the previous chapters indicate potential threats to amphidromous fishes (starvation, developmental problems resulting from anthropogenic activities), as well as opportunities to best mitigate these and other threats (targeting conservation measures during the period of peak larval drift). McDowall (1995) noted the importance of understanding the patterns of fish migration in rivers and streams to river managers, but suggested that amphidromous fish could naturally remedy any extirpations due to the dispersal benefits inherent to an oceanic larval stage. However, there is increasing evidence that amphidromy does not necessarily require a marine stage, and that benefits relating to dispersal and recolonisation are simply an 'accidental' consequence of a pelagic larval phase (Closs, Jellyman & Hicks, 2013; Huey *et al.*, 2014). It is thus of importance that threats are identified and appropriate conservation measures are considered to prevent population loss, rather than managers relying on amphidromous fishes recolonising quickly following extirpation.

One such threat to amphidromous fishes is the disruption of their migration routes, which can be the result of both barriers such as dams and weirs, and factors which increase larval mortality during migration, such as entrainment into irrigation intakes. These factors can greatly affect fishes with an amphidromous life history, the benefits of which rely on larvae safely reaching a pelagic feeding habitat. Allowing for the movements of migratory fish ('fish passage') has been a priority in the management of New Zealand's mostly diadromous freshwater fish fauna, but efforts have largely focussed on (mostly upstream) adult migrations, for example remediating

issues with fish passage at culverts (Boubee *et al.*, 1999; David *et al.*, 2014). Larval stages however are often ignored when it comes to management, perhaps arising from the difficulties inherent to studying and protecting them, but the consideration of all life stages is important when planning for conservation.

A significant threat to migratory fish larvae is increased lowland irrigation withdrawals. Fish screens are often used to attempt to exclude fish from irrigation systems, and are required for many intakes on New Zealand's rivers (Bonnett *et al.*, 2014). Mesh sizes of 3-5 mm are often used, however these will be ineffective at preventing the entrainment of many of New Zealand's larval and juvenile fish. This is particularly a problem for bullies and torrentfish, whose total length during their larval migration is often < 3 mm, and can pass through mesh sizes < 1 mm. Even if larval fish could be effectively screened using reduced mesh sizes, impingement on the screen remains a significant problem due to the limited swimming ability of larval fish (Boys, Baumgartner & Lowry, 2013). Due to the difficulties of protecting migratory fish larvae with conventional methods such as fish screens, I believe identifying critical spatial and temporal windows within which to apply conservation measures or halt water extraction represents a potentially beneficial alternative approach.

#### Future research directions

To determine the broad applicability of the results of this study, larval drift patterns should be examined in other streams and rivers of different sizes and locations throughout New Zealand. This is of importance, as drift may differ with factors such as landscape and substrate (Iguchi & Mizuno, 1991), and there may be latitudinal differences in drift season (Vinagre *et al.*, 2008a; Vinagre *et al.*, 2008b). Future research should also focus on assessing the benefits of applying conservation measures such as halting abstraction during periods of peak drift. Suggested future projects include quantifying the numbers of larval fish entrained at water intakes, particularly under different flows. Similar research has been conducted elsewhere (Ellsworth, Tyler & VanderKooi, 2010), but as with many other aspects of larval fish biology, little or no information exists for any of New Zealand's freshwater fish. Larval starvation and retention should also be investigated further. Quantifying the time taken for larvae to complete their migration, as well as the time until starvation beyond which further development is impossible would prove a valuable area of future research, having implications both for the management of amphidromous species, and understanding of amphidromy as a life-history strategy (Iguchi & Mizuno, 1999; Closs, Hicks & Jellyman, 2013). It is suggested that starvation be examined experimentally, in order to overcome difficulties with variable hatch and differing drift times as were seen in this study.

## Conclusion

Amphidromous species remain understudied relative to other diadromous fishes, and their larvae, on whose successful migration hinges the entire life history strategy, are largely unconsidered during species management. This lack of knowledge stems from multiple causes, from their geographic distribution to the difficulties in studying (and protecting) their tiny and fragile larval stages. A better understanding of the significance of an amphidromous life history, and particularly the ecology and biology of amphidromous larvae, is critical to understand (and so protect) these fish which undertake such a fascinating and complex migration.

## References

- Baumgartner L.J., Reynoldson N. & Gilligan D.M. (2006) Mortality of larval Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*) associated with passage through two types of low-head weirs. *Marine and Freshwater Research*, **57**, 187-191.
- Benstead J.P., March J.G., Pringle C.M. & Scatena F.N. (1999) Effects of a low-head dam and water abstraction on migratory tropical stream biota. *Ecological Applications*, **9**, 656-668.
- Blaustein A.R., Hokit D.G., Ohara R.K. & Holt R.A. (1994) Pathogenic fungus contributes to amphibian losses in the Pacific-Northwest. *Biological Conservation*, **67**, 251-254.
- Bonnett M., Bowie S., Meredith A.S., Reese P. & Webb M. (2014) Findings from field investigations of six fish screen irrigation intakes. NIWA. 36pp.
- Boubee J.A.T., Jowett I.G., Nicols S. & Williams E. (1999) Fish passage at culverts: a review, with possible solutions for New Zealand Indigenous Species. Department of Conservation. 22pp.
- Boys C.A., Baumgartner L.J. & Lowry M. (2013) Entrainment and impingement of juvenile silver perch, *Bidyanus bidyanus*, and golden perch, *Macquaria ambigua*, at a fish screen: effect of velocity and light. *Fisheries Management and Ecology*, **20**, 362-373.
- Brown R.S., Colotelo A.H., Pflugrath B.D., Boys C.A., Baumgartner L.J., Deng Z.D., Silva L.G.M., Brauner C.J., Mallen-Cooper M., Phonekhampeng O., Thorncraft G. & Singhanouvong D. (2014) Understanding barotrauma in fish passing hydro structures: a global strategy for sustainable development of water resources. *Fisheries*, **39**, 108-122.
- Charteris S.C., Allibone R.M. & Death R.G. (2003) Spawning site selection, egg development, and larval drift of *Galaxias postvectis* and *G. fasciatus* in a New Zealand stream. *New Zealand Journal of Marine and Freshwater Research*, **37**, 493-505.
- Closs G.P., Hicks A.S. & Jellyman P.G. (2013) Life histories of closely related amphidromous and non-migratory fish species: a trade-off between egg size and fecundity. *Freshwater Biology*, **58**, 1162-1177.
- Closs G.P., Smith M., Barry B. & Markwitz A. (2003) Non-diadromous recruitment in coastal populations of common bully (*Gobiomorphus cotidianus*). *New Zealand Journal of Marine and Freshwater Research*, **37**, 301-313.
- Czeczuga B., Bartel R., Kiziewicz B., Godlewska A. & Muszynska E. (2005) Zoosporic fungi growing on the eggs of sea trout (*Salmo trutta m. trutta* L.) in river water of varied trophicity. *Polish Journal of Environmental Studies*, **14**, 295-303.
- David B.O., Tonkin J.D., Taipeti K.W.T. & Hokianga H.T. (2014) Learning the ropes: mussel spat ropes improve fish and shrimp passage through culverts. *Journal of Applied Ecology*, **51**, 214-223.
- Dewson Z.S., James A.B.W. & Death R.G. (2007) A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *Journal of the North American Benthological Society*, **26**, 401-415.
- Dingle H. & Drake V.A. (2007) What is migration? *BioScience*, **57**, 113-121.
- Egevang C., Stenhouse I.J., Phillips R.A., Petersen A., Fox J.W. & Silk J.R. (2010) Tracking of Arctic terns *Sterna paradisaea* reveals longest animal migration. *Proceedings of the National Academy of Sciences*, **107**, 2078-2081.
- Ellien C., Valade P., Bosmans J., Taillebois L., Teichert N. & Keith P. (2011) Influence of salinity on larval development of *Sicyopterus lagocephalus* (Pallas, 1770) (Gobioidei). *Cybium*, **35**, 381-390.
- Ellsworth C.M., Tyler T.J. & VanderKooi S.P. (2010) Using spatial, seasonal, and diel drift patterns of larval Lost River suckers *Deltistes luxatus* (Cypriniformes: Catostomidae)

- and shortnose suckers *Chasmistes bevirostris* (Cypriniformes: Catostomidae) to help identify a site for a water withdrawal structure on the Williamson River, Oregon. *Environmental Biology of Fishes*, **89**, 47-57.
- Empson P.W., Meredith A.S., Boubée J.A.T. & Wightman K.H. (1992) Ichthyoplankton studies on the lower Waikato River. IV. Annual variations in distribution. *New Zealand Freshwater Fisheries Miscellaneous Report No. 130*. 34pp.
- Goodman J.M., Dunn N.R., Ravenscroft P.J., Allibone R.M., Boubée J.A.T., David B.O., Griffiths M., Ling N., Hitchmough R.A. & Rolfe J.R. (2014) Conservation status of New Zealand freshwater fish, 2013. *New Zealand Threat Classification Series 7*, Department of Conservation. 12pp.
- Gross M.R. (1987) The evolution of diadromy in fishes. *American Fisheries Symposium*, **1**, 14-25.
- Hall C.J., Jordaan A. & Frisk M.G. (2011) The historic influence of dams on diadromous fish habitat with a focus on river herring and hydrologic longitudinal connectivity. *Landscape Ecology*, **26**, 95-107.
- Hickford M.J. & Schiel D.R. (2011) Population sinks resulting from degraded habitats of an obligate life-history pathway. *Oecologia*, **166**, 131-140.
- Huey J.A., Crook D.A., Macdonald J.I., Schmidt D.J., Marshall J.C., Balcombe S.R., Woods R.J. & Hughes J.M. (2014) Is variable connectivity among populations of a continental gobiid fish driven by local adaptation or passive dispersal? *Freshwater Biology*, **59**, 1672-1686.
- IBM Corp. (2013) IBM SPSS Statistics for Windows. IBM Corp., Armonk, NY.
- Iguchi K. & Mizuno N. (1990) Diel changes of larval drift among amphidromous gobies in Japan, especially *Rhinogobius brunneus*. *Journal of Fish Biology*, **37**, 255-264.
- Iguchi K. & Mizuno N. (1991) Mechanisms of embryonic drift in the amphidromous goby, *Rhinogobius brunneus*. *Environmental Biology of Fishes*, **31**, 295-300.
- Iguchi K. & Mizuno N. (1999) Early starvation limits survival in amphidromous fishes. *Journal of Fish Biology*, **54**, 705-712.
- Iguchi K. & Takeshima H. (2011) Effect of saline water on early success of amphidromous fish. *Ichthyological Research*, **58**, 33-37.
- Iida M., Watanabe S., Yamada Y., Lord C., Keith P. & Tsukamoto K. (2010) Survival and behavioral characteristics of amphidromous goby larvae of *Sicyopterus japonicus* (Tanaka, 1909) during their downstream migration. *Journal of Experimental Marine Biology and Ecology*, **383**, 17-22.
- Jorgensen C., Dunlop E.S., Opdal A.F. & Fiksen O. (2008) The evolution of spawning migrations: state dependence and fishing-induced changes. *Ecology*, **89**, 3436-3448.
- Jowett I.G. & Richardson J. (2003) Fish communities in New Zealand rivers and their relationship with environmental variables. *New Zealand Journal of Marine and Freshwater Research*, **37**, 347-366.
- Joy M.K., Henderson I.M. & Death R.G. (2000) Diadromy and longitudinal patterns of upstream penetration of freshwater fish in Taranaki, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **34**, 531-543.
- Keith P. (2003) Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and the Caribbean regions. *Journal of Fish Biology*, **63**, 831-847.
- Keith P., Hoareau T.B., Lord C., Ah-Yane O., Gimonneau G., Robinet T. & Valade P. (2008) Characterisation of post-larval to juvenile stages, metamorphosis and recruitment of an amphidromous goby, *Sicyopterus lagocephalus* (Pallas) (Teleostei : Gobiidae : Sicydiinae). *Marine and Freshwater Research*, **59**, 876-889.
- Keith P. & Marion L. (2002) Methodology for drawing up a Red List of threatened freshwater fish in France. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **12**, 169-179.

- Kerrigan B.A. (1997) Variability in larval development of the tropical reef fish *Pomacentrus ambainensis* (Pomacentridae): The parental legacy. *Marine Biology*, **127**, 395-402.
- Leathwick J.R., Elith J., Rowe D. & Julian J. (2009) Robust planning for restoring diadromous fish species in New Zealand's lowland rivers and streams. *New Zealand Journal of Marine and Freshwater Research*, **43**, 659-671.
- Luton C.D., Brasher A.M.D., Durkin D.C. & Little P. (2005) Larval drift of amphidromous shrimp and gobies on the island of Oahu, Hawai'i. *Micronesica*, **38**, 1-16.
- Maeda K. & Tachihara K. (2010) Diel and seasonal occurrence patterns of drifting fish larvae in the Teima stream, Okinawa Island. *Pacific Science*, **64**, 161-176.
- Marschall E.A., Mather, M.E., Parrish, D.L., Allison, G.W., Mcenemy, J.R. (2011) Migration delays caused by anthropogenic barriers: modeling dams, temperature, and success of migrating salmon smolts. *Ecological Applications*, **21**, 3014-3031.
- McCarter N. (1994) A key to some larval fish from New Zealand fresh water. *NIWA ecosystems publication No. 10*. NIWA. 33pp.
- McDowall R.M. (1988) *Diadromy in fishes: Migrations between freshwater and marine environments*, Croom Helm, London.
- McDowall R.M. (1990) *New Zealand freshwater fishes: a natural history and guide*, Heinemann Reed, Auckland.
- McDowall R.M. (1995) Seasonal pulses in migrations of New Zealand diadromous fish and the potential impacts of river mouth closure. *New Zealand Journal of Marine and Freshwater Research*, **29**, 517-526.
- McDowall R.M. (1997) The evolution of diadromy in fishes (revisited) and its place in phylogenetic analysis. *Reviews in Fish Biology and Fisheries*, **7**, 443-462.
- McDowall R.M. (1998) Driven by diadromy: its role in the historical and ecological biogeography of the New Zealand freshwater fish fauna. *Italian Journal of Zoology*, **65**, 73-85.
- McDowall R.M. (2000) Biogeography of the New Zealand torrentfish, *Cheimarrichthys fosteri* (Teleostei: Pinguipedidae): a distribution driven mostly by ecology and behaviour. *Environmental Biology of Fishes*, **58**, 119-131.
- McDowall R.M. (2007) On amphidromy, a distinct form of diadromy in aquatic organisms. *Fish and Fisheries*, **8**, 1-13.
- McDowall R.M. (2010a) *New Zealand freshwater fishes: an historical and ecological biogeography*, Springer, Dordrecht.
- McDowall R.M. (2010b) Why be amphidromous: expatrial dispersal and the place of source and sink population dynamics? *Reviews in Fish Biology and Fisheries*, **20**, 87-100.
- McDowall R.M. & Suren A.M. (1995) Emigrating larvae of koaro, *Galaxias brevipinnis* Gunther (Teleostei: Galaxiidae), from the Otira River, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **29**, 271-275.
- Meredith A.S., Empson P.W. & Boubee J.A.T. (1992) Ichthyoplankton studies on the lower Waikato River. III. Downstream variation in distributions. *New Zealand Freshwater Miscellaneous Report No. 128*. 62pp.
- Meredith A.S., Empson P.W., Boubee J.A.T. & Mitchell C.P. (1987) Ichthyoplankton studies on the lower Waikato River. I. Entrainment at Huntly Power Station. *New Zealand Freshwater Fisheries Report No. 88*, 22pp.
- Meredith A.S., Empson P.W., Boubee J.A.T. & Mitchell C.P. (1989) Ichthyoplankton studies on the lower Waikato River. II. Larval distributions at Huntly. *New Zealand Freshwater Fisheries Report No. 109*. 58pp.
- Miles N.G., Walsh C.T., Butler G., Ueda H. & West R.J. (2014) Australian diadromous fishes - challenges and solutions for understanding migrations in the 21st century. *Marine and Freshwater Research*, **65**, 12-24.

- Moriyama A., Yanagisawa Y., Mizuno N. & Omori K. (1998) Starvation of drifting goby larvae due to retention of free embryos in upstream reaches. *Environmental Biology of Fishes*, **52**, 321-329.
- NIWA. (2014) NZ Freshwater Fish Database. Available at: <http://nzffdms.niwa.co.nz>. Accessed on: 17/03/2014.
- Poulin R., Closs G.P., Lill A.W.T., Hicks A.S., Herrmann K.K. & Kelly D.W. (2012) Migration as an escape from parasitism in New Zealand galaxiid fishes. *Oecologia*, **169**, 955-963.
- R Development Core Team. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.
- Scrimgeour G.J. & Eldon G.A. (1989) Aspects of the reproductive biology of torrentfish, *Cheimarrichthys fosteri*, in two braided rivers of Canterbury, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **23**, 19-25.
- Sih A., Bell A.M., Johnson J.C. & Ziemba R.E. (2004) Behavioral syndromes: an integrated overview. *Quarterly Review of Biology*, **79**, 241-277.
- Skov C., Chapman B.B., Baktoft H., Brodersen J., Bronmark C., Hansson L.A., Hulthen K. & Nilsson P.A. (2013) Migration confers survival benefits against avian predators for partially migratory freshwater fish. *Biology Letters*, **9**, 20121178.
- Sorenson P.W. & Hobson L.A. (2005) Stable isotope analysis of amphidromous Hawaiian gobies suggests their larvae spend a substantial period of time in freshwater river plumes. *Environmental Biology of Fishes*, **74**, 31-42.
- Sutherland D.L. & Closs G.P. (2001) Spatial and temporal variation in the abundance and composition of ichthyoplankton in a large South Island, New Zealand river estuary. *New Zealand Journal of Marine and Freshwater Research*, **35**, 1061-1069.
- Taillebois L., Maeda K., Vigne S. & Keith P. (2012) Pelagic larval duration of three amphidromous Sicydiinae gobies (Teleostei: Gobioidae) including widespread and endemic species. *Ecology of Freshwater Fish*, **21**, 552-559.
- Tamada K. & Iwata K. (2005) Intra-specific variations of egg size, clutch size and larval survival related to maternal size in amphidromous *Rhinogobius* goby. *Environmental Biology of Fishes*, **73**, 379-389.
- Tsukamoto K., Kajihara T. & Nishiwaki M. (1975) Swimming ability of fish. *Bulletin of the Japanese Society of Scientific Fisheries*, **41**, 167-174.
- Valade P., Lord C., Grondin H., Bosc P., Taillebois L., Iida M., Tsukamoto K. & Keith P. (2009) Early life history and description of larval stages of an amphidromous goby, *Sicyopterus lagocephalus* (Gobioidae: Sicydiinae). *Cybium*, **33**, 309-319.
- Vinagre C., Amara R., Maia A. & Cabral H.N. (2008a) Latitudinal comparison of spawning season and growth of 0-group sole, *Solea solea* (L.). *Estuarine, Coastal and Shelf Science*, **78**, 521-528.
- Vinagre C., Ferreira T., Matos L., Costa M.J. & Cabral H.N. (2008b) Latitudinal gradients in growth and spawning of sea bass, *Dicentrarchus labrax*, and their relationship with temperature and photoperiod. *Estuarine, Coastal and Shelf Science*, **81**, 375-380.
- Wagner E.J., Bartley M.S. & Oplinger R.W. (2012) Egg disinfection to improve conservation aquaculture of leatherside chub. *North American Journal of Aquaculture*, **74**, 199-207.
- Watanabe S., Iida M., Lord C., Keith P. & Tsukamoto K. (2014) Tropical and temperate freshwater amphidromy: a comparison between life history characteristics of Sicydiinae, ayu, sculpins and galaxiids. *Reviews in Fish Biology and Fisheries*, **24**, 1-14.
- Webster S.J. & Dill L.M. (2006) The energetic equivalence of changing salinity and temperature to juvenile salmon. *Functional Ecology*, **20**, 621-629.
- Yamasaki N., Kondo M., Maeda K. & Tachihara K. (2011) Reproductive biology of three amphidromous gobies, *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp., on Okinawa Island. *Cybium*, **35**, 345-359.

