Effects of environmental and behavioural factors on fish swimming performance and passage success

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Abstract

Instream structures like culverts, dams, and other anthropogenic barriers fragment river networks worldwide, posing a significant threat to the connectivity and migration of freshwater fish communities. Designing effective fish passage solutions requires a comprehensive understanding of the swimming abilities, behaviours, and environmental tolerances of diverse fish species. This thesis aims to address this need by investigating the factors influencing the swimming performance and passage success of multiple migratory fish species in New Zealand, with implications for improving fish passage design and connectivity restoration efforts.

Two methods for assessing swimming performance, critical swimming speed (U_{crit}) and sprint swimming speed (U_{sprint}), were compared across two fish species. Results revealed no significant statistical difference between swimming speeds estimated using U_{crit} versus U_{sprint} protocols for the pelagic *Galaxias maculatus* and the benthic-associated *Galaxias fasciatus*. This suggests that shorter time-stepped swimming speeds tests can be used to measure swimming abilities of benthic-associated species, allowing comparisons across a broader range of fish for passage design.

Inter- and intraspecies variation in swimming speeds across nine migratory New Zealand fish species was quantified. *Galaxias brevipinnis, Galaxias argenteus,* and *Galaxias postvectis* exhibited the strongest swimming abilities. *Galaxias maculatus* was among the weakest swimmers. Body length was positively correlated with maximum speed, indicating that barriers select against weaker swimming species and smaller individuals within species. Maximum allowable culvert velocities should be significantly lower than previous standards to accommodate most individuals across species.

The impact of varying acute water temperatures on critical swimming speeds of four migratory species was investigated. At higher temperatures (26°C), three species (*Galaxias maculatus, Galaxias brevipinnis, Gobiomorphus cotidianus*) exhibited significant reductions in swimming performance compared to lower temperatures (8°C, 15°C). In contrast, *Galaxias fasciatus* showed no water temperature-related changes. These findings underscore the importance of designing fish passages to accommodate acute temperature fluctuations, to ensure successful migration under changing environmental conditions.

Potential benefits of collective navigation were explored using the small-bodied *Galaxias maculatus*. Experiments with an artificial velocity barrier revealed that fish swimming in groups had faster entry and passage rates, as well as lower metabolic rates indicating reduced energy expenditure, compared to solitary individuals. These findings highlight the importance of designing fish passes to facilitate movement of gregarious species by accommodating group dynamics.

The effect of repeated exposure on passage performance through an experimental raceway with high water velocities was examined. Over five consecutive days, passage success increased significantly, suggesting a role for cognition and spatial memory in improving passage performance. However, approach and entry rates did not improve, indicating other factors like attraction flows or fish physiology may be important for locating and entering structures.

This thesis provides insights into the factors influencing swimming performance and passage success for migratory New Zealand species. By studying variation across species in water temperature effects, group behaviour, and cognitive abilities, this research provides a comprehensive understanding to guide the development of more inclusive and effective fish passage solutions. The findings highlight the importance of accounting for many sources of variation when designing instream structures to facilitate unimpeded fish migration.

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Who knew that when I applied on a whim for a MSc scholarship to study fish passage in New Zealand, seen by my dad on Twitter, I would have ended up here – across the Pacific Ocean, one global pandemic and almost five years later. As they say, it really does take a village, and I don't think I would have made it through the events of the last few years without my village.

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—Douglas Adams, *Hitchhikers Guide to the Galaxy*

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Chapter 1

General introduction

Arch culvert, King County, Washington, USA

1.1. Connectivity

Freshwater connectivity is foundational for the survival of migratory species across various habitats including rivers, floodplains, wetlands, lakes and estuaries. Fragmentation of freshwater systems, caused by anthropogenic barriers and altered flows, poses a significant threat to connectivity (Castro-Santos and Haro 2010; Bunt et al. 2012; Fangue et al. 2015; Franklin and Gee 2019). This fragmentation triggers physicochemical shifts in freshwater processes: increased sedimentation, temperatures changes, altered oxygen, pH and flow (Fangue et al. 2015; Parasiewicz et al. 2023). The impacts of fragmentation extend beyond physical changes to the environment by creating barriers that disrupt the movement of species, gene flow and ecosystem services, leading to overall declines in biodiversity (Nicola et al. 1996; Lucas and Baras 2001; Daufresne and Boët 2007; Gough et al. 2012; Jellyman and Harding 2012; Neachell 2014; Radinger and Wolter 2014; Silva et al. 2018; Wilkes et al. 2019). Furthermore, altered flow regimes due to dams and other barriers disconnect floodplains, reducing species diversity and abundance through inbreeding (Raeymaekers et al. 2008; Wilkes et al. 2019; Thieme et al. 2023), although fragmentation and long-term isolation could also drive genetic drift (Sotola et al. 2017; Ruzich et al. 2019). Efforts to protect and restore connectivity, as outlined in the Emergency Recovery Plan for freshwater biodiversity loss (Tickner et al. 2020), are essential for preserving freshwater biodiversity and mitigating the impacts of fragmentation(Birnie-Gauvin et al. 2019; Thieme et al. 2023).

1.2. Overview of fish passage

For most of human history we have dammed, channelised and diverted freshwater systems to serve our needs, creating innumerable instream barriers. These barriers have restricted habitats and have been detrimental to migratory fish populations. Evidence suggests that humans began to notice fish population declines beginning in the mid-18th century (Katopodis and Williams 2012). The need to maintain these fish stocks gave rise to the first rudimentary fishways (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019) (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019). Robust research investigating the efficacy of fishways appears in early 20th century Belgium before spreading across Europe and into North America (Katopodis and Williams 2012; Silva et al. 2018). Fish passage research has continued to build on this foundation and now includes many passage structures such as steep-passes, pool-and-weir, vertical slot fishways, and other mechanisms (e.g. trap-and-haul and other more modern systems such as the sensationalised salmon cannon).

Many of the instream barriers in existence today were not initially designed with fish passage structures in place. Instead, fish passage structures are typically added to projects retroactively (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019). These passes and solutions tend to be based on engineering practices that take a reductionist approach to accounting for fish biology in the design process. These practices largely focus on equations solely involving average fish speed and water speed, without considering variations in physiology, migratory behaviour, or the environment (Roscoe and Hinch 2010; Katopodis and Williams 2012; Birnie-Gauvin et al. 2019). It is important to consider these individual variations in combination with physical, behavioural, and environmental factors to obtain a more holistic picture of passage success (Roscoe and Hinch 2010). A recent shift to this holistic view of fish passage has also precipitated a move away from the mindset of retroactive fishways implementation to a mindset of proactively including fish passage in new infrastructure designs (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019).

A recent meta-analysis of fish passage research supports the inclusion of fish biology into fish passage design. The study concluded that fishway type is not a significant predictor in passage success (Hershey 2021). This analysis also concluded that passage success is largely dependent on fish type, with pelagic rheophiles being the most successful (Hershey 2021). Hershey (2021) also noted that the methods used to evaluate fishways and passage success vary greatly across the field, making it difficult to compare the efficiency of the structures. To date, fish passage research is primarily focused on the upstream anadromous migration of adult salmon and trout populations, and generally does not address other life histories and fish species, or downstream migratory movements (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019).

Current fish passage solutions in New Zealand often prioritize climbing fish, using substrates like mussel spat ropes or ramps to help them ascend through structures (David et al. 2014). These solutions are typically implemented at higher elevations or further inland, where climbing species tend to penetrate more than non-climbing species. Another, more cost-effective approach involves adding baffles to create low-velocity zones, providing resting areas for fish and facilitating passage (Magaju et al. 2021).

1.3. Understanding fish swimming performance

In recent years, the importance of fish passage has become increasingly relevant across the world, with special emphasis placed on the ability of fishes to migrate upstream through manmade barriers to their spawning or rearing grounds. Human-made, instream structures can create habitat fragmentation that is detrimental to freshwater ecosystem health and services (Nicola et al. 1996; Daufresne and Boët 2007; Gough et al. 2012; Jellyman and Harding 2012; Neachell 2014; Radinger and Wolter 2014; Fangue et al. 2015; Silva et al. 2018). Fragmentation alters habitat quality and quantity by isolating populations of fish species, blocking access to upstream migration for diadromous fish, reducing dispersal, and ultimately reducing species richness (Castro-Santos et al. 2009; Castro-Santos and Haro 2010). Barriers can also cause changes in a stream's biogeochemical processes by increasing sedimentation, changing water temperatures and pH, backwatering and scouring, decreasing oxygen levels, and altering water velocities (Fangue et al. 2015; Washington Department of Fish and Wildlife 2019).

Successful fish passage is based on a suite of metrics, including the biological capabilities of the impacted fish species, as well as site-specific ecosystem characteristics (Castro-Santos and Haro 2010). A necessary component of the biological capabilities of fish is their swimming performance. In order to make upstream progress (Figure 1.1), a fish must swim faster than the downstream water speed they are exposed to (Mitchell 1989; Castro-Santos and Haro 2010). The speed at which a fish can swim depends on physiology, body shape and fin form, muscle function, metabolic costs, stamina, and even behaviour (Farrell and Steffensen 1987; Mitchell 1989; Rome 1990; Jayne and Lauder 1994).

Swimming is powered by aerobic musculature, anaerobic musculature, or a combination of the two (Beamish 1978; Castro-Santos et al. 2022). Swimming that is entirely maintained by aerobic musculature, can be maintained indefinitely, and is usually classified as > 200 min and called "sustained swimming" (Beamish 1978).

Swimming speed can be calculated through speed tests that measure fish swimming by their ability to hold station relative to flowing water at set velocities (Brett 1964). One way to calculate fish swimming speeds is through stepped speed tests, which gradually ramps up the water speed a fish is exposed to, and directly measures the swimming speed of the fish (Brett 1964). This test, referred to as critical swimming speed (U_{crit}), is thought to be a measure of maximum sustained swimming, reflecting the transition from aerobic to anaerobic respiration and ultimately resulting in exhaustion(Brett 1964; Beamish 1978; Plaut 2001). Sustained swimming is defined as any speed at which a fish can swim longer than 200 minutes and are typically lower swimming speeds that can be maintained indefinitely (Beamish 1978). While the original Brett (1964) U_{crit} test is meant to be a measure of sustained swimming, many researchers have adopted its use to measure minimum prolonged swimming speeds (Fisher et al. 2005; Laborde et al. 2016; Downie and Kieffer 2017a; Egger et al. 2020). Prolonged swimming is a combination of aerobic and anaerobic respiration, and ultimately results in fatigue, usually described as speeds that can be maintained between 20 s and 200 min (Brett 1964; Beamish 1978; Castro-Santos et al. 2022). Prolonged speeds decrease gradually with endurance time and use both slow-twitch red and fast-twitch-white muscles (Castro-Santos et al. 2022). Since most instream structures create velocity barriers, fish navigating these structures will likely employ a combination of aerobic and anaerobic respiration, or entirely anaerobic respiration, to swim fast enough through the structure to make forward progress.

Another measure of swimming speed is through fixed speed tests, which measure how long a fish can swim at a constant speed (Beamish 1978). This fixed speed test allows researchers to create endurance curves to determine at which speeds fish can maintain burst, prolonged, and sustained swimming (Beamish 1978). Burst swimming is fuelled primarily anaerobically and is defined as speeds a fish can only maintain for a matter of seconds (< 20 s) (Beamish 1978; Castro-Santos 2022). Burst speeds decrease rapidly with endurance times and use fast-twitch white muscles. The relative mass of red (slow-twitch) versus white (fast-twitch) muscles and the amounts of stored glycogen can indeed influence endurance times at burst speeds, as well as the buffering capacity of muscles during anaerobic respiration. Buffering capacity refers to the ability of muscles to neutralize or mitigate the accumulation of acidic by-products, such as lactic acid, produced during anaerobic metabolism. Muscles with higher buffering capacity can better withstand the effects of lactic acid buildup, delaying the onset of fatigue (Castellini and Somero 1981). Therefore, a fish with large glycogen stores but poor buffering capacity may struggle to maintain burst speeds for extended periods compared to a fish with higher buffering capacity (Castellini and Somero 1981).

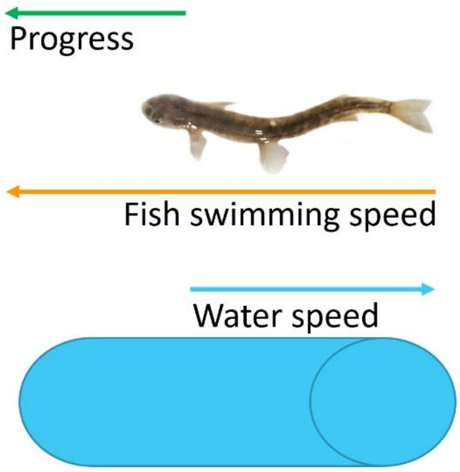


Figure 1.1: Fish progress overground is a combination of the speed of the water subtracted from the speed at which the fish is swimming (Gee and Crawford 2021).

Many researchers have used both stepped- and fixed-speed tests to assess how other variables, alongside water speed, affect fish swimming performance (Plaut 2001). These include environmental variables such as temperature, salinity, anoxia, and pollutant effects, and behavioural variables such as station-holding and swimming in groups (Plaut 2001; Bannon 2006; May and Kieffer 2017; Wiwchar et al. 2018; Crawford et al. 2024, *a*).

However, understanding fish passage performance in real-world scenarios requires more than just relying on swimming tests. Ideally, field observations involving the tracking of fish in their natural habitats would be conducted to determine the impact of various factors on passage performance. Considerable research has been devoted to studying passage of large-bodied species, such as salmonids, through fishways using biotelemetry (Bunt et al. 2012; Goerig and Castro-Santos 2017; Hershey 2021). These biotelemetry tracking methods are not universally applicable, especially for small bodied species that begin upstream migration as juveniles (Franklin et al. 2024). Unfortunately, many migratory fish in the Southern Hemisphere fall into this category, necessitating alternative approaches

(Franklin et al. 2024). There is a growing body of work dedicated to using machine learning tools to track these small-bodied fish in laboratory settings (Magaju et al. 2023). As these experiments take place in controlled laboratory settings, they offer the advantage of manipulating certain variables while allowing for the generation of more robust datasets. Laboratory experiments allow researchers to answer specific questions such as how different environmental or behavioural factors affect fish swimming ability and passage success, and this is the approach that has been adopted in this thesis.

1.4. Fish species studied

The fish genera of interest in the proposed research are migratory *Galaxias* species (*Galaxias maculatus*, *Galaxias fasciatus*, *Galaxias argenteus*, *Galaxias postvectis*, and *Galaxias brevipinnis*), migratory *Gobiomorphus* spp. (redfin and common bully *Gobiomorphus huttoni* and *Gobiomorphus cotidianus*), the common smelt (*Retropinna retropinna*), and an endemic eel species (*Anguilla dieffenbachii*). These species were chosen because they are all native to New Zealand, encompass a range of life history strategies and swimming abilities, and undergo upstream migration as part of their life histories (see Table 1.1) (McDowall 1990). All species were tested as juveniles because this is the life stage that begins migration upstream and is most likely to encounter unfavourable water velocities due to fish passage barriers (Franklin et al. 2018).

Juvenile migratory galaxiids are also known as whitebait in New Zealand and have both cultural and ecological importance (McDowall 2011). Galaxiids have amphidromous life history strategies, meaning that these fish migrate from freshwater to estuarine or ocean habitats, and then back to freshwater in spring. Common smelt are often referred to as whitebait species because they have similar timing of upstream migration as galaxiids and are caught as bycatch during whitebait season, even though they are not galaxiids. Swimming performance is expected to vary between these six species reflecting differences in morphology, physiology, and habitat preferences (McDowall 1990; McDowall 2003; Urbina et al. 2013; Augspurger et al. 2017). Scientific consensus generally portrays *Galaxias maculatus* as the weakest swimmers, and *Galaxias brevipinnis* are considered one of the strongest (McDowall 2011). Some *Galaxias* species can climb using their pectoral and pelvic fins, allowing them to penetrate far inland (e.g. *Galaxias brevipinnis*, McDowall 1990).

The genus *Gobiomorphus* (Family Eleotridae, known as bullies in New Zealand) contains several New Zealand amphidromous species (common, bluegill, redfin, and giant bullies). Migratory bullies spawn in freshwater, then migrate downstream as larvae, migrate back upstream as juveniles, then live in the pools and riffles of freshwater streams for up to two years until reaching maturity (McDowall 1990; Rowe 1999). Some *Gobiomorphus cotidianus* populations have a lacustrine life history, where they spend their lives entirely in freshwater systems (McDowall 1990, Rowe 1999). Bullies are generally benthic, using their pectoral fins to rest on or to hold onto bottom substrate, and some species also use their pectoral fins to climb (McDowall 1990). Research on the effect of bed slope on passage success found that redfin bullies have the ability to climb, with at least 15% of redfin bullies successfully navigating a ramp with a slope of 45° (Baker and Boubée 2006; Baker 2014).

There are two main *Anguilla* spp. in New Zealand, the longfin eel (*Anguilla dieffenbachii*) and shortfin eel (*Anguilla australis*). These species are referred to as tuna by indigenous New Zealanders (Māori) and are very important culturally and as a traditional food source. *Anguilla dieffenbachii* are endemic to New Zealand. Both *Anguilla dieffenbachii* and *Anguilla australis* are found throughout the country in most rivers, lakes, and streams. They tend to occupy slow moving water and large pools. Elvers are known for their climbing ability, successfully navigating waterfalls, and are even able to cross over land from one body of water to another. In anguilliform swimmers, fish whose forward motion is created through the undulation of trunk muscles, experience a trade-off between speed and efficiency due to the amplitude of the undulation (Lindsey 1978; Webb 1978; Anastasiadis et al. 2023).

1.5. Thesis aims and objectives

Current methods for remediation of instream barriers are primarily based on anadromous fish in the northern hemisphere, such as salmonids (Bunt et al. 2012; Katopodis and Williams 2012; Fangue et al. 2015; Silva et al. 2018; Birnie-Gauvin et al. 2019; Franklin and Gee 2019). However, this research is not necessarily transferable to the endemic species of New Zealand (Franklin and Gee 2019). Native New Zealand fish exhibit a range of life history strategies, swimming methods, and morphologies that differ to those of salmonids (McDowall 1990; Franklin and Gee 2019). The majority of New Zealand's endemic species exhibit amphidromous life histories and, in comparison to salmonids, are small, weak swimmers when they begin upstream migration (Franklin and Gee 2019; McDowall 1990). In addition to swimming, some New Zealand species can climb to overcome instream obstacles (Franklin and Gee, 2019; McDowall, 1990). Additionally, unlike in other parts of the world that prioritize large-scale fishways, here in New Zealand the most common fish passage structures are culverts, which often need to be replaced or retrofitted to improve fish passage.

Previous New Zealand research has examined a variety of swimming performance methods for several species of fish, however most of this research is based on non-standard methods or non-reproducible methods. Franklin and Gee (2019) highlight the need for detailed research specifically for New Zealand fish swimming performance. Using standard critical swimming speed (U_{crit}) protocol, previous research from Bannon (2006) demonstrated that *Galaxias maculatus* have a swimming speed range from 0.22 to 0.25 m s^{-1}, leading scientists believe *Galaxias maculatus* are one of the weaker swimming species in

New Zealand. Bannon (2006) measured *Galaxias maculatus* swimming performance following critical swimming speed methods outlined by Brett (1964). Nikora et al. (2003) conducted fixed speed tests on different life stages of *Galaxias maculatus*, aimed at understanding the transition from burst to prolonged swimming speeds. This research shows that the swimming ability of *Galaxias maculatus* increases with fish size, and juvenile *Galaxias maculatus* can swim up to 0.25 m s^{-1} for 5 min (Nikora et al. 2003).

Mitchell (1989) measured the 'sustained', 'prolonged', and 'burst' swimming speeds of several New Zealand fishes. His research found that *Gobiomorphus cotidianus* have a range of speeds from 0.24 to 0.60 m s^{-1} and *Galaxias fasciatus* have a range of speeds from 0.19 to 0.43 m s^{-1} (Mitchell 1989). These trials were carried out by placing a group of fish in a flume with a 1:15 slope, creating increasing water velocities as the fish swam upstream. Swimming speeds were measured visually to categorize their swimming speeds as 'burst,' 'steady,' or 'sustained.' (Boubée et al. 1999) also attempted to measure the 'burst' swimming speed of *Galaxias maculatus*. Boubée et al. (1999) found that juvenile *Galaxias maculatus* can maintain a speed of 1.09 m s^{-1} for 5 s and a speed of 0.60 m s^{-1} for 20 s. Burst swimming ability was assessed introducing fish into a 5-m-long culvert with varying slopes and timing how long they swam or took to reach the upstream end. However, it is essential to note that both Mitchell (1989) and Boubée et al. (1999) do not adhere to Brett's (1964) standard critical swimming speed (U_{crit}) protocol. Consequently, the results obtained from these methods are not comparable with each other or with standard (U_{crit}) results. Moreover, the definitions of 'burst,' 'steady,' and 'sustained,' swimming speeds used in these studies should not be considered equivalent to the standard definitions established by Beamish (1978).These existing studies on New Zealand fish passage lack reproducibility, underscoring the necessity for more robust methodologies. This becomes especially significant as the New Zealand Fish Passage Guidelines are undergoing a revision.

This thesis investigates sources of variation affecting swimming performance and passage success of migratory fish species in New Zealand. Across five research chapters, potential causes of variation are tested, including measurement techniques, species differences, environmental factors, behavioural differences, and cognitive abilities. The aim is to provide comprehensive and reliable information on how these sources of variation influence swimming performance and, ultimately, passage success. By accounting for these variation sources, more effective fish passage solutions can be created. Improved solutions will better accommodate variations in abilities and behaviours across species, ultimately supporting fish population conservation and restoration efforts, as well as enhancing habitat connectivity.

The principal aims of the thesis were therefore to:

1. Compare and review methodologies for assessing swimming performance.
2. Compare and benchmark swimming performance across nine New Zealand species.
3. Assess effects of temperature on fish swimming performance.
4. Determine how group swimming affects fish passage performance in an artificial raceway.
5. Examine how repeat exposure to an artificial barrier affects fish passage performance.

1.6. Thesis outline

This thesis consists of seven chapters, with chapters 2-6 comprising a series of five standalone studies, three of which have been published (Crawford et al. 2023, Crawford et al., 2024a, Crawford et al., 2024b), one is currently in review, and one is in preparation for submission to peer-reviewed scientific journal.

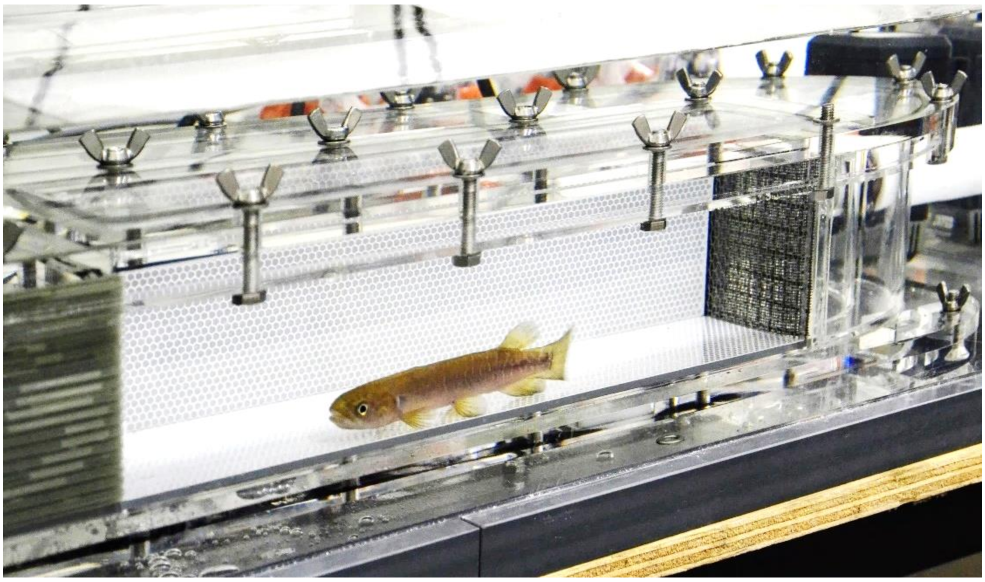
- **Chapter 2** investigates the methodology of two commonly used metrics for swimming speed, U_{crit} and U_{sprint} between benthic and pelagically swimming fish. The results indicate no significant difference in swimming speeds between the two methods, providing important methodological background for Chapters 3-4. Chapter 2 was published as by the Journal of Fish Biology under the title “No difference between critical and sprint swimming speeds for two galaxiid species,” by Rachel M.B. Crawford, Eleanor M. Gee, Deborah Dupont, Brendan J. Hicks, and Paul A. Franklin.
- **Chapter 3** describes the swimming ability of nine migratory New Zealand species, emphasizing the significant inter- and intraspecies variation observed. Our investigation led to the calculation of maximum allowable speeds for culverts based on the endurance abilities of the weakest swimmer, *Galaxias maculatus*. Chapter 3 was published by the Journal of Applied Ecology under the title “Accounting for interspecies and intraspecies variation in swimming performance for fish passage solutions.” The results of this study will be presented in the 2024 New Zealand Fish Passage Guidelines fish (Franklin et al. 2024) .

- **Chapter 4** investigates the impact of varying acute temperatures on the swimming speeds of many migratory New Zealand species. By comparing a range of temperature (8°C, 15°C, and 26°C) on swimming speeds, we can explore how natural changes in acute temperature might affect swimming and passage ability in the wild. Chapter 4 was published by the Journal of Conservation under the title “High water temperature significantly influences swimming performance of New Zealand migratory species.”
- **Chapter 5** studies how schooling behaviour enhances fish passage success in juvenile *Galaxias maculatus.* Laboratory trials were conducted to compare how groups versus solitary swimming fish pass an artificial speed barrier. Chapter 5 is currently in review by the Journal of Fish Biology.
- **Chapter 6** explores how repeated exposure to an artificial raceway improves passage success for juvenile *Galaxias maculatus* over time. Laboratory trials were set up to assess how fish, subjected to the same artificial speed barrier once daily for five consecutive days, were affected in their ability to successfully pass the barrier.

Since the chapters are structured as standalone studies, there may be some overlap in the methodological descriptions and background information provided. I took full responsibility for conducting the fieldwork, managing fish care, collecting and analysing data, and writing this thesis. I certify that all content is based on our own ideas, except when properly cited. Additionally, I participated in the data collection and analysis for a related publication that complements, though it is not part of, this thesis, published by Journal of Marine and Freshwater Research (2023) under the title "Impacts of VIE tagging and Rhodamine B immersion on two measures of performance for small-bodied fish” by Franklin et al. (see Appendix A1).

Chapter 2

No difference between critical and sprint swimming speeds for two galaxiid species



Galaxias fasciatus in swim tunnel, NIWA Hamilton, New Zealand
Photo credit: Eleanor Gee

This chapter has been published as a research article in the Journal of Fish Biology and permission has been granted to use this publication as part of this thesis. Citation: Crawford, R.; Gee, E.; Dupont, D.; Hicks, B.; and Franklin, P. (2023). No difference between critical and sprint swimming speeds for two galaxiid species. Journal of Fish Biology,102(5), 1141–1148. https://doi.org/10.1111/jfb.15355

2.1. Abstract

Researchers have used laboratory experiments to examine how fish might be affected by anthropogenic alterations and conclude how best to adjust fish passage and culvert remediation designs in response. A common way to document swimming performance for this purpose is measuring fish critical swimming speed (U_{crit}). However, the U_{crit} protocol as defined by Brett [(1964) Journal of the Fisheries Research Board of Canada, 21, 1183–1226] may be inappropriate for studying swimming performance and how it relates to upstream migration in benthic fish, as they may not actively swim throughout the entire U_{crit} test. An alternative method to estimate swimming performance is sprint swimming speed (U_{sprint}), which is suggested to be a measure of the burst speed of fish rather than maximum sustained swimming speed. We conducted comparative swimming performance experiments to evaluate whether U_{sprint} can be used to compare swimming performance of benthic species to pelagic, actively swimming species. We measured individual swimming speeds of īnanga (*Galaxias maculatus)*, an actively swimming pelagic species, and banded kōkopu (*Galaxias fasciatus)*, a fish that exhibits benthic station-holding behaviour, using both the U_{sprint} and U_{crit} test. Experiments revealed no significant statistical difference between swimming speeds estimated using the U_{crit} versus U_{sprint} test protocols for both *G. maculatus* and *G. fasciatus.* Our result suggests fish swimming speeds obtained using these two methods are comparable for the species in this study. By using U_{sprint} for benthic associated fish and U_{crit} for pelagic fish, we may be able to compare a broader range of species' swimming abilities for use in a fish passage context.

2.2. Introduction

Swimming performance of fish influences their ability to escape from predation and some forms of harvest to forage for and prey on food, and to migrate to new habitats (Drucker 1996; Laborde et al. 2016). Construction of instream structures can increase local channel gradients and create barriers to fish migration that fragment habitats(McIntyre et al. 2016). Specifically, structures like culverts can increase water velocities and restrict fish migration upstream to spawning and rearing habitats (Warren and Pardew 1998; Lucas and Baras 2001; Radinger and Wolter 2014; Silva et al. 2018). Many species have varying swimming modes and behaviours (e.g. climbers vs. swimmers, benthic vs. pelagic fish), which greatly affect fish swimming performance (McDowall 1990; McDowall 2003). To restore habitat connectivity, it is important to understand and compare a range of species' swimming speeds to inform future fish passage design criteria.

Laboratory experiments have been used to examine how fish might be affected by anthropogenic alterations and how best to adjust fishway design to account for fish swimming performance (Laborde et al. 2016; Tan et al. 2021a). A commonly used method to document swimming performance for this purpose is to measure fish critical swimming speed (U_{crit}) (e.g. Brett 1964; Peake et al. 1997; Laborde et al. 2016; Katopodis et al. 2019; Tan et al. 2021). While some researchers criticize this method's ecological relevance on the grounds that the water velocities and discharge do not accurately represent those found in the natural environment, the U_{crit} test is widely accepted for its ability to test a multitude of other variables alongside water velocity (Brett 1964; Johnston and Dunn 1987; Richardson et al. 1994; Schurmann and Steffensen 1997; Plaut 2001; Bannon 2006; Bunt et al. 2012).

Critical swimming speed experiments are conducted by exposing a fish to increasing water velocity in a stepwise fashion until this fish reaches fatigue (Brett 1964). This test is based on the transition from aerobic to anaerobic respiration (Beamish 1978) and is thought to estimate (but slightly overestimate) maximum sustained swimming speed (Beamish 1978; Plaut 2001). Sustained swimming speeds have been defined as any speed at which a fish can swim for longer than 200 mins, is fuelled entirely aerobically, and does not result in fatigue (Beamish 1978). Prolonged swimming is also fuelled primarily aerobically but lasts between 20 s and 200 mins and results in fatigue (Beamish 1978). Low to moderate swimming speeds (i.e. sustained, prolonged swimming) are powered by slow-twitch (red) muscles that are continuously supplied with oxygen and nutrients through the cardiovascular and respiratory systems (Bone 1978). During prolonged swimming, fatigue is caused by the transition from slow-twitch muscles to fast-twitch (white) muscles (Bone 1978). Fast-twitch muscle is fuelled anaerobically via glycolysis and glycogen stores (Bone 1978; McKenzie 2011). This results in fatigue once the nutrient stores are consumed (usually in a matter of seconds) and a recovery period is needed to renew nutrient levels (Beamish 1978; Bone 1978).

The initial U_{crit} methodology was based on research on salmonids and actively swimming fish typically found in the temperate Northern Hemisphere riverine systems (Brett 1964). Researchers have adopted U_{crit} experiments to determine the swimming performance of many fish species (e.g. Laborde et al. 2016; Tan et al. 2021). However, the U_{crit} protocol as defined by Brett (1964) may be inappropriate for studying swimming performance and how it relates to upstream migration in benthic fish because they may not actively swim throughout the U_{crit} test (Gilbert et al. 2016; Egger et al. 2020; Parisi et al. 2020; Raffini et al. 2020). For this reason, many researchers have adopted species-specific modifications of Brett's (1964) U_{crit} by varying the time intervals and velocity increments of the test (Farlinger and Beamish 1977; Beamish 1978; Kolok 1999; Fisher and Leis 2010; Dalziel and Schulte 2012; Downie and Kieffer 2017a; Illing et al. 2021). There is conflicting evidence about how these variations of the U_{crit} test parameters change the measured critical swimming speed of the fish. Some studies have found that increasing the time interval negatively affects U_{crit} (Farlinger and Beamish 1977; Beamish 1978; Fisher and Leis 2010; Downie and Kieffer 2017a; Illing et al. 2021), while other studies have found that changing time intervals does not affect U_{crit} (Fisher and Leis 2010; Dalziel and Schulte 2012; Illing et al. 2021). Overall, the results of these studies suggest that the response to the U_{crit} protocols is likely species- and life stage-specific (Farlinger and Beamish 1977; Beamish 1978; Downie and Kieffer 2017a; Illing et al. 2021).

An alternative method to estimate swimming performance is sprint swimming speed (U_{sprint}), which has been suggested as a measure of the burst speed of fish rather than maximum sustained swimming speed (Starrs et al. 2011). Burst swimming is fuelled primarily anaerobically, powered by fast twitch muscles, and lasts less than 20 s (Beamish 1978; Bone 1978; McKenzie 2011). The U_{sprint} test is carried out identically to the U_{crit} test except the stepped velocity increments occur more frequently (every ten seconds) compared to the longer-stepped velocity increments (typically minutes) in the U_{crit} tests (Starrs et al. 2011). The U_{sprint} test has been used by researchers studying the swimming performance of fish that do not actively swim (e.g. station-holding and benthic fish) during the total duration of a standard U_{crit} test, as the rapidly increasing water velocity forces more consistent fish swimming behaviour (Gilbert et al. 2016; Egger et al. 2020; Parisi et al. 2020; Raffini et al. 2020). While U_{sprint} has helped researchers in many cases, they have not assessed whether swimming during a U_{sprint} test is physiologically different from that of a U_{crit} test.

Starrs et al. (2011) maintain that U_{sprint} provides a measure of swimming performance that is more useful in the context of fish passage. The swimming mode that a fish exhibits when migrating through instream structures or turbulent water is more similar to the speeds and time observed in burst and sprint swimming (seconds) than in sustained swimming (> 200 mins), particularly in the case of small-bodied and benthic fishes (Peake et al. 1997; Peake and Farrell 2005; Starrs et al. 2011; Egger et al. 2020). Egger et al. (2020) suggested that U_{sprint} was a more accurate measure of swimming ability than U_{crit} for a station-holding species. When migrating through a model fishway, the fish actively swam throughout the duration of the U_{sprint} trial. Other researchers have also used the U_{sprint} methods as a measure of swimming abilities in the context of fish passage, focusing on invasive species control (Tierney et al. 2011; Gilbert et al. 2016).

Recent New Zealand policies require action to provide for and restore fish passage. This requires more robust swimming performance data, but consistent information is relatively scarce about fish swimming performance for New Zealand species (Franklin et al. 2018). Currently, available fish swimming performance data are mostly based on non-standard and non-reproducible methods(Mitchell 1989; Boubée et al. 1999). Mitchell (1989) tested swimming speeds by placing a group of fish into a 3-m-long flume tilted on a 1:15 slope to create a gradient of increasing water velocities as the fish swam “upstream.” “Burst,” “steady” and “sustained” swimming speeds were delineated based on qualitative visual assessments of behaviour and the water velocities “selected” by fish when in the flume. Boubée et al. (1999) tested the burst swimming ability of fish by placing them in a 5-

m-long culvert with varying slopes (and hence varying water velocities) and timing how long fish swam for or took to reach the upstream end. These methods are not considered to be variations of Brett's (1964) standard U_{crit} protocol, but unique protocols the results of which are not comparable with each other or with standard U_{crit} results. Furthermore, the definitions of "burst," "steady" and "sustained" swimming speeds used in these studies should not be considered equivalent to standard definitions (i.e. Beamish 1978).

Certain native New Zealand fish species, such as kōaro (*Galaxias brevipinnis*) and bullies (*Gobiomorphus* spp.), exhibit strong benthic, station-holding behaviours. These species can rest on the bottom of a swim tunnel for the entire duration of a U_{crit} trial (longer than 200 mins) (Dupont 2020). Consequently, U_{sprint} trials might better represent the swimming abilities of these station-holding species than the standard U_{crit} protocol since it may force the fish to swim for the duration of the trial. It is important, however, to keep in mind that changing the U_{crit} protocols for station-holding species can impact fish swimming behaviour, and as water speed increases there may be an increase in station-holding behaviours (Downie and Kieffer 2017b; May and Kieffer 2017). Furthermore, substrate is also an important factor in understanding swimming abilities of station-holding species. However, while swimming behaviours change with substrate roughness, the U_{crit} values do not differ significantly (Downie and Kieffer 2017b; May and Kieffer 2017).

To support work to establish a more robust understanding of the swimming performance of native fishes in New Zealand, including both species exhibiting station-holding and active swimming behaviours, we set out to determine the equivalency of swimming performance as determined using standardised U_{crit} and U_{sprint} tests. The objective was to compare the two swim-test methods to determine if they can be used interchangeably for the purpose of evaluating swimming performance to inform fish passage design. The null hypothesis was that there would be no statistically significant difference in swimming speed as calculated from the two different test methods. We measured individual swimming speeds of īnanga (*Galaxias maculatus)*, a native pelagic, actively swimming species, and banded kōkopu (*Galaxias fasciatus)*, a native fish that exhibits station-holding behaviour, (McDowall 1978, 1990; McDowall et al. 1994; Jowett and Richardson 1995), using both the U_{sprint} and U_{crit} test.

2.3. Methods

The fish collection for this research was carried out under NIWA's Ministry for Primary Industries Special Permit SP666-4. All experimental trials for this study were run with NIWA Animal Ethics Committee (AEC204) approval to manipulate live animals for research purposes in accordance with the requirements of section 83 of the New Zealand Animal Welfare Act 1999.

2.3.1. Fish collection and handling

Juvenile *Galaxias maculatus* were collected using fyke nets at the mouth of New Zealand's Rangitaiki River (37°54'34.6"S 176°52'53.0"E). Juvenile *Galaxias fasciatus* were collected using fyke nets from the Lilleburn Stream in the Hunua Ranges (37° 04' 55.6" S 175° 10' 02.9" E) and Waitawhara Stream in New Zealand's Glen Afton (37° 36' 20.6" S 175° 02' 12.8" E). Fish were transported to the NIWA laboratory in cool bins filled with aerated stream water from where they were collected. Fish were kept in the cool bins until the water temperature equilibrated with the laboratory tank water temperature. Fish were held in the NIWA Hamilton fish laboratory in a temperature-controlled room on a 12-hour day/12-hour night cycle. Fish were quarantined in 60 L tanks with 6 ppt salinity to prevent disease. After one week, fish were transferred to 60 L dechlorinated water tanks. These tanks were kept on a recirculating water system and the temperature was held at 15 ± 0.5°C. Fish were fed bloodworms every second day and were fasted for 24 hours before experimentation to ensure a postabsorptive state. Ammonia and pH levels were checked regularly, and water changes were completed if ammonia levels were higher than 0.25 mg L^{-1}.

2.3.2. Experimental setup

Swimming speed trials were carried out in a 10 L Loligo Systems Steffensen-type swim tunnel (Methling et al. 2011). The swim tunnel consisted of a 5 L swim chamber measuring 28 x 7.5 x 7.5 cm, a honeycomb matrix at the front of the chamber for flow straightening, variable frequency drive and pump for velocity control, and a chiller for temperature control. Body length, body width, and body depth measurements were recorded and entered in Autoresp v2.3.0 (Loligo Systems) to control water velocity in body lengths per second of the individual fish, and to compensate for the solid blocking effect (Kline et al. 2015). Temperature in the swim tunnel was kept at 15 ± 0.5 °C. The flush pump was kept running throughout the duration of the trial to maintain oxygen saturation levels. Dissolved oxygen levels were monitored throughout the trial and maintained at a mean of 80.4 ± 1% saturation. The flume was calibrated prior to commencing trials using a Hontzsch Flowtherm NT handset flow meter. A two-point calibration performed at two motor speeds (0.5 V and 4.6 V) corresponding to the minimum and maximum of the motor speed. A total of six measurements were taken across three depths at two locations in the flume. The swim tunnel was set up in a temperature-controlled room and operated remotely, allowing trials to be carried out without disturbance from the observer.

2.3.3. Experimental protocol

At the start of each trial, individual fish were placed into the swim tunnel and acclimated for 30 min at 0.5 bl s^{-1} (body lengths per second). This acclimation period was used to minimise the effects of handling stress on the swim trial. After acclimation, the water velocity in the swim tunnel was increased in a stepwise fashion by 1 bl s^{-1}. For U_{crit} trials, the water velocity was increased by 1 bl s^{-1} every 5 min. For U_{sprint} trials, water velocity was increased by 1 bl s^{-1} every 10 s. A trial was stopped after a fish reached fatigue and was resting against the mesh at the back of the tunnel for 3 s continuously or for a cumulative period of 10 s within a 30 s timeframe. Once the trial was stopped, fish were allowed to rest in the tunnel for 20 min before being placed back into the holding tank to ensure recovery. All trials were independent, with no fish re-used.

Galaxias maculatus and *G. fasciatus* were used to test critical swimming speed (U_{crit}) and sprint swimming speed (U_{sprint}; Table 2.1). Total length of the fish — which were all juveniles (the primary upstream migratory stage for these species) — was less than 5.5 cm. This was to minimise and control for possible effects of ontogenetic variation in migratory urge.

Table 2.1: Species data for swimming tests, including the number of individuals used and mean lengths.

Species	Test	N	Mean Length (cm)	Standard deviation (±)
G. maculatus	U_{crit}	19	4.40	0.38
	U_{sprint}	15	4.14	0.36
G. fasciatus	U_{crit}	17	4.23	0.32
	U_{sprint}	17	4.16	0.33

2.3.4. Analysis

The swimming speed was calculated using the following from Brett (1964):

$$U_{crit} \; or \; U_{sprint} \; = \; U_{f-1} + \Delta U \left(\frac{t_f}{\Delta t}\right)$$

Where U_{f-1} is the highest velocity the fish could sustain for a full-time interval (i.e. usually the second highest velocity), ΔU is the velocity increment (i.e. 1 bl s^{-1}), t_f is the time the fish swam at the highest velocity in s (i.e. always ≤ Δt), Δt is the time increment in s (i.e. 5 min for U_{crit}, 10 s for U_{sprint}). Swimming speed was recorded in body lengths per second.

An analysis of covariance model (ANCOVA) was used to statistically test for the differences in swimming speed for each species independently, with swimming test type (U_{crit} or U_{sprint}) as the categorical predictor variable and fish total length as a continuous predictor variable. The swimming speed (the value of U_{crit} or U_{sprint}) for individuals within each species was used as the response variable. This model was applied separately to both *Galaxias maculatus* and *G. fasciatus.* For *G. fasciatus,* fish capture location was added as a covariate to test for intraspecific variation between the two sampled populations, but was statistically insignificant and, therefore, removed from the final model. Two *G. fasciatus* outliers with lengths greater than 6.1 cm were removed from the analysis, as the desired fish lengths were between 3.5-5.5 cm. A Likelihood Ratio Test (LRT) was used to determine the model of best fit for predictor variables starting with a fully saturated model including interactions. The interaction between body length and swimming speed was not statistically significant for either species and was, therefore, removed from the models. All statistical analyses were carried out using the R statistical computing package v4.0.3 (R Core Team 2020). Figures were created using the R package ggplot (Wickham 2016).

2.4. Results

Galaxias maculatus U_{sprint} speeds had greater variation between individuals compared to U_{crit} speeds (U_{sprint}: 6.89 bl s^{-1} ± S.E. 3.96; U_{crit}: 6.45 bl s^{-1} ± S.E. 2.85; Figure 2.1). The *G. maculatus* ANCOVA confirmed there was no significant effect of swim test type on swimming speed (d.f. = 31, *F* statistic = 0.01, *p* = 0.90; Table 2.2, Figure 2.1). *Galaxias fasciatus* U_{sprint} speeds had greater variation between individuals compared to U_{crit} speeds (U_{sprint}: 7.49 bl s^{-1} ± S.E. 3.07; U_{crit}: 6.53bl s^{-1} ± S.E. 2.23; Figure 2.1). The *G. fasciatus* ANCOVA confirmed there was no significant effect of swim test type on swimming speed (d.f: 31, *F* statistic: 1.38, *p*: 0.25; Table 2.2, Figure 2.1). The co-variable body length did not have a statistically significant effect on swimming speed, using a confidence interval of 0.05 (*G. maculatus*: ANCOVA, d.f.: 31, *F* statistic: 2.29, *p*: 0.1; *G. fasciatus*: ANCOVA, d.f: 31, *F* statistic: 2.49, *p*: 0.12; Table 2.2, Figure 2.2). Using the ANCOVA model, we found no statistically significant effect of body length on swimming speed for either U_{sprint} or U_{crit} tests, for either species over the range of sizes tested. While the relationship between length and swimming speed was not significant, swimming speed showed a general decrease with length for *G. maculatus.* The opposite trend was observed with *G. fasciatus,* where swimming speed increased with length.

Table 2.2: Summary of Type III analysis of covariance with Satterthwaite's method comparing the difference between fish swimming ability determined using critical and sprint swimming speeds for both *Galaxias maculatus* and *G. fasciatus*. d.f. = degrees of freedom.

Species	*Source*	*Sum of squares.*	*Mean squares*	*Numerator d.f.*	*Denominator d.f.*	*F-value*	*p-value*
G. maculatus	Swimming test	0.20	0.19	1	31	0.01	0.9
	Length	25.30	25.3	1	31	2.29	0.14
G. fasciatus	Swimming test	9.44	38.03	1	31	1.38	0.25
	Length	17.04	17.04	1	31	2.49	0.12

Figure 2.1: Boxplots of critical swimming speed compared to sprint swimming speed, measured in body lengths per second. The points represent individual fish in each treatment group. Centre bar shows median, outer edges of the box show interquartile range ends, and whiskers show maxima and minima, an 'X' indicates mean. (A) *Galaxias maculatus* swimming speeds. (B) *Galaxias fasciatus* swimming speeds.

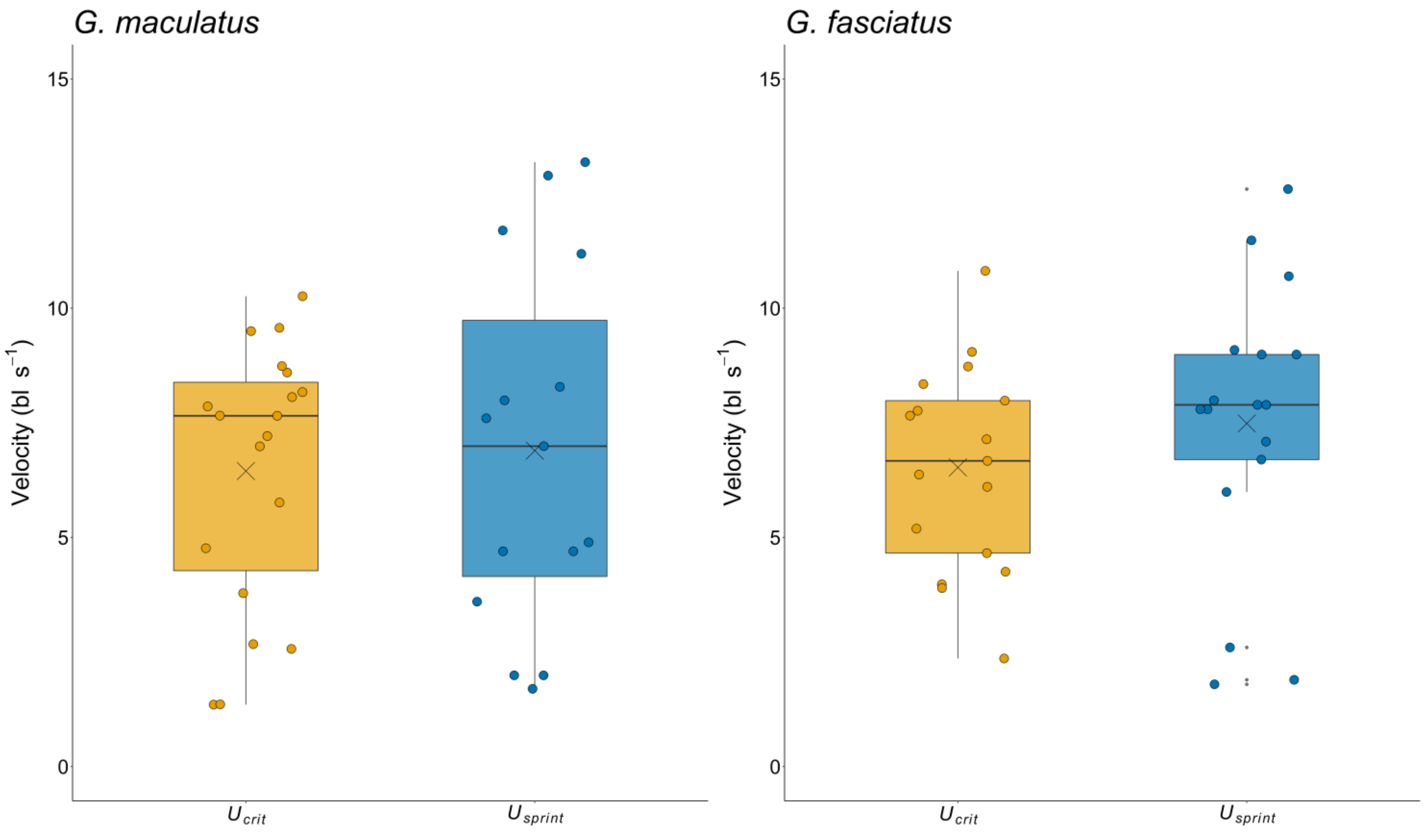
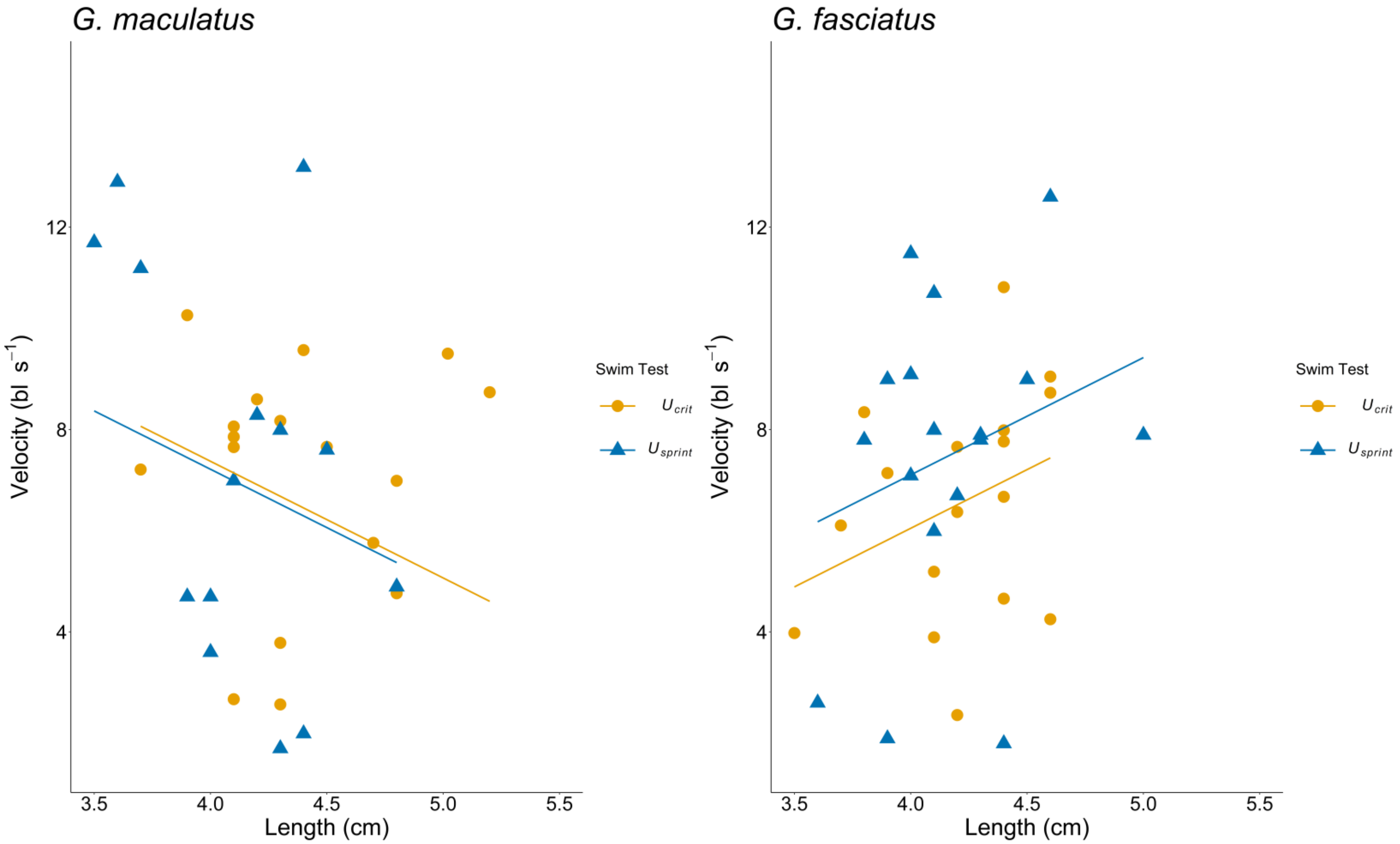


Figure 2.2: Scatter plot of individual length and swimming speed recorded in body lengths per second. Regression lines produced from the ANCOVA model predicted values are plotted for each swim test for each species. (A) *Galaxias maculatus* swimming speeds and plotted against fish length. Regression equation: y = 16.60 – 2.31*Length – 0.16*Swim Test Type, R^2 = 0.11. (B) *Galaxias fasciatus* swimming speed plotted against fish length. Regression equation: y = -3.22 + 2.32*Length + 1.06*Swimming Test Type, R^2 = 0.11.



2.5. Discussion

We found that there was no statistically significant difference between the results of the U_{crit} and U_{sprint} tests, which is consistent with our null hypothesis. This is important as understanding fish swimming performance is necessary for developing fish passage solutions for fragmented freshwater habitats. The interchangeability of these two methods will allow comparison of swimming performance across a wider range of species and will be valuable in designing future fish passage solutions.

For our comparison, any effect of fish length on swimming speed was expected to be negligible since swimming velocity units (bl s^{-1}) were standardised by the individual's body length and the length range was quite small (about 2 cm) for both species. The lack of influence of fish size observed here should be considered with caution as the range of lengths was quite small, and fish length is a factor known to influence swimming speeds (Beamish 1978; Wolter and Arlinghaus 2003; Cano-Barbacil et al. 2020). While fish weight was not recorded, it is also a known predictor of U_{crit} and should be recorded in future trials especially when comparing several fish species (Rubio-Gracia et al. 2020; Cano-Barbacil et al. 2020).

Our result suggests that fish swimming speeds obtained using these two methods are comparable for the species in this study. To the best of our knowledge, this is the first study to directly compare the U_{sprint} protocol (Starrs et al. 2011) to the U_{crit} protocol (Brett 1964) and find no statistically significant difference in measured swimming ability of the fish tested. Previous studies have compared sprint and critical swimming speed test types, resulting in a statistically significant difference between the two, but the methods used between the test types were not consistent (Egger et al. 2020; Raffini et al. 2020). Egger et al. (2020) prevented the fish in the U_{sprint} tests from resting against the back of the swim tunnel (unlike in the U_{crit} tests), possibly contributing to an increase in swimming speed when compared to the U_{crit} tests. In their U_{sprint} tests, Raffini et al. (2019) started the fish at an initial sprint speed double that of their critical swimming speed, deviating from the standardised U_{sprint} methods outlined by Starrs et al. (2011). Parisi et al. (2020) investigated the U_{sprint} and U_{crit} speeds of fish but did not compare the two swimming performance tests.

The previous research aimed to understand the differences between U_{crit} and U_{sprint} on the basis that they represent different swimming modes (prolonged and burst) and that different swimming modes are used for different activities (Starrs et al. 2011; Egger et al. 2020; Parisi et al. 2020). Prolonged swimming is used for activities such as foraging and migrating (Plaut 2001) and relies on a combination of aerobic and anaerobic respiration. The proportion of anaerobic respiration increases as swimming intensity increases, eventually resulting in fatigue (20 s to 200 min) (Beamish 1978; Brett 1964). U_{crit} is generally considered to be a measure of maximum speed fuelled primarily aerobically, and fatigue at the end of the U_{crit} test is thought to represent a switch from aerobic to anaerobic metabolism (Brett 1964; Pritchard et al. 1971; Webb 1986; Plaut 2001). Burst swimming is used for activities such as predator-prey interactions (e.g. predator-escape responses). Burst swimming relies on anaerobic respiration, using up oxygen stores and resulting in fatigue within seconds (< 20 s) (Beamish 1978; Brett 1964). This swimming mode allows fish to reach higher swimming speeds for shorter periods of time when compared to prolonged swimming. U_{sprint} as envisioned by Starrs et al. (2011) was intended to be a measure of burst swimming and thought to be fuelled primarily anaerobically.

Our research indicates no difference between the swimming speed calculated using the two protocols for two species. If burst swimming can only be sustained for a few seconds (Beamish 1978), we observe that this test of U_{sprint} may not be a good indicator for burst swimming as previously suggested by Starrs et al. (2011), as the fish exposed to U_{sprint} tests achieved the same swimming speed as those exposed to U_{crit} tests. This calls into question the relevance of using U_{sprint} as a measure of burst swimming.

If U_{sprint} is a true measure of burst swimming, it would be primarily anaerobic, using fast-twitch muscles (Brett 1964; Pritchard et al. 1971; Bone 1978; Webb 1986). If this were the case, we would expect to see significantly higher U_{sprint} speeds, as a fish can achieve higher speeds using burst swimming when compared to prolonged swimming (Beamish 1978). However, we saw no statistical difference between swimming speed between these two test types for the species tested and consequently hypothesise that U_{sprint} swimming is being powered primarily aerobically using slow-twitch muscle groups. It would seem other methods, such as assessing c-starts or startle tests, may be a more appropriate measure of burst swimming as they have been explicitly shown to activate the fast-twitch muscle groups (Bone 1978; Domenici and Blake 1997; McKenzie 2011; Marras et al. 2013). Further research is needed to examine changes in oxygen consumption and muscular use in U_{sprint} and U_{crit} trials to confirm this hypothesis.

While research has shown that varying time intervals cause physiological differences in fish due to metabolic demand (Farlinger and Beamish 1977; Beamish 1978), the reduction in time interval from 5 min to 10 s may not have impacted physiology significantly enough to create measurable differences in swimming speeds. This is supported by studies that show changes in the U_{crit} protocol have varying effects on swimming speeds, with some species and life stages more robust to these changes than others (Fisher and Leis 2010; Dalziel and Schulte 2012; Downie and Kieffer 2017a; Illing et al. 2021). Downie and Kieffer (2017a) showed that changing both the U_{crit} time interval (5 and 10 mins) and velocity increment resulted in statistically different speeds for two sturgeon species. Illing et al. (2021), in contrast, showed that changing the U_{crit} time interval (2, 5, 10, and 20 mins) did not result in statistically different speeds for *Lates calcarifer* (Bloch, 1790) larvae, but did result in significantly different U_{crit} speeds for *Amphiprion melanopus* (Bleeker, 1852) larvae. The standard U_{crit} protocol outlined by Brett (1964) is often adjusted depending on the species, life stage, and behaviour of the fish. This further highlights the need for preliminary trials to ensure that the U_{crit} protocols used during experimentation meet the unique needs of the research question (Farlinger and Beamish 1977; Fisher and Leis 2010; Downie and Kieffer 2017a, 2017b). Although previous researchers have not compared a time interval of seconds to an interval of minutes, our U_{sprint} method is effectively an abbreviated U_{crit} test.

The results of this study suggest that researchers can potentially save time by using U_{sprint} tests, which typically take a fraction of the time of a U_{crit} test, especially where the focus is on getting a general indication of swimming performance to inform fish passage design (as opposed to comparison of metabolism). In addition to reducing U_{crit} time requirements, using U_{sprint} tests could be seen as a more ethical test by reducing the time a fish is exposed to experimental conditions. However, while there is no significant difference in swimming speed outcomes based on test type, it is important to consider how the behaviour of the fish may be altered by decreasing the time interval of the velocity increments, and if there may be any physiological differences as a result. This becomes especially relevant when considering how results are translated into design criteria for fish passage.

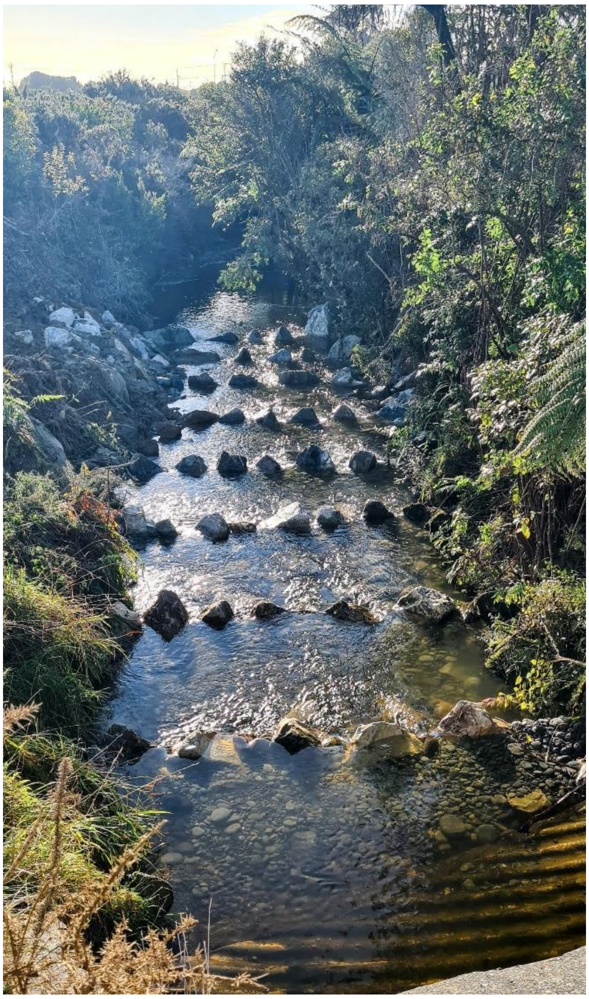
Swimming ability is important to understand to create benchmarks for both research and ecological management (Plaut 2001; Katopodis 2005). Traditionally, maximum sustained swimming speed tests are based on Brett's (1964) U_{crit} method and have been used as an ecological indicator for Darwinian fish survival and fitness, as migration and predator/prey interactions rely on prolonged swimming abilities (Reidy et al. 2000; Plaut 2001). This method was designed for salmonids, a pelagic species that exhibited constant swimming behaviour throughout the test. However, benthic-associated species do not always exhibit constant swimming behaviour, instead often holding station on the substrate (Tierney et al. 2011; Gilbert et al. 2016; Egger et al. 2020). In an attempt to prevent station-holding, U_{sprint} tests increase the velocity more frequently than U_{crit} tests (Starrs et al. 2011). By using U_{sprint} for benthic associated fish and U_{crit} for pelagic fish, we may be able to compare a broader range of species' swimming abilities for use in a fish passage context.

Galaxias maculatus is a pelagic species, but *G. fasciatus* is known to exhibit station-holding behaviours (McDowall 1978; Jowett and Richardson 1995). In our experiments, *G. fasciatus* swam constantly in both U_{crit} and U_{sprint} tests meaning our experimental methodology is driving behaviour that may not be consistent with natural swimming behaviour in this species. Studies have shown that changing U_{crit} protocols and adding rough substrate to flume design affects swimming behaviours but does not statistically alter swimming speeds (Downie and Kieffer 2017b; May and Kieffer 2017). Further research is needed to clarify how station-holding behaviour affects the outcomes of both U_{crit} and U_{sprint} trials and to understand the biological relevance of these two methods for other New

Zealand species. Some species, such as New Zealand's endemic torrentfish (*Cheimarrichthys fosteri,* Haast 1874), exhibit very strong station-holding behaviour that is likely to play a critical role in determining passage success at a migration barrier (Jowett and Richardson 1995). Consequently, particularly in the applied context of improving fish passage outcomes, research focusing on station-holding behaviour and the role it plays in upstream migration may be important for future fish passage design improvements.

Chapter 3

Accounting for interspecies and intraspecies variation in swimming performance for fish passage solutions



Newly built rock ramp on Mill Creek, Greymouth, New Zealand

Photo credit: Tim Marsden

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3.1. Abstract

Across the globe, there are millions of instream structures that fragment the world's river networks, acting as barriers that can impede the movements of fish. Designing effective solutions to accommodate fish communities requires information about the swimming abilities and behaviours of all species. This should account for different swimming modes, abilities, behaviours, and niches. We investigated the swimming speeds of nine migratory New Zealand species to assess both inter- and intraspecies variation. We then calculated maximum allowable speeds for culverts of a given length, based on the endurance abilities of our lowest performing species (*Galaxias maculatus*).

Our findings reveal significant interspecies and intraspecies variation in swimming speeds. Among the species studied, *Galaxias brevipinnis*, *Galaxias argenteus*, and *Galaxias postvectis* were the strongest swimmers. In contrast, *Galaxias maculatus* was one of the weakest swimmers. Body length positively correlated with U_{max} indicating that fish passage barriers select against the weakest swimming species, as well as smaller individuals within a species. Maximum allowable water speeds in a culvert should be significantly lower than 0.3 m s^{-1}, the previously assumed standard rule-of-thumb for New Zealand, to provide adequate passage for the majority of individuals. For example, a 15 m culvert should not have water speeds exceeding 0.2 m s^{-1}.

Previous maximum allowable water speeds for fish passage designs have been based on average swimming ability, but this approach only enables fish that are better than the average swimmers of their species to overcome barriers. This study highlights the importance of evidence-based designs for successful fish passage solutions to account for

the ability of all fish. By considering differences between and within species, rather than assuming a “one-size-fits-all” approach we can develop more effective passage solutions that better preserve fish communities.

3.2. Introduction

Across the globe, there are millions of instream structures that create fragmentation of the world’s river networks, acting as barriers that can impede the movements of fish (Grill et al. 2015; Belletti et al. 2020; Franklin et al. 2022). Many instream structures that exist today were not designed with fish passage in mind. Instead, fish passage structures or remediation are typically added to projects retroactively (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019). These passes and solutions tend to be based on engineering practices that take a reductionist approach to accounting for fish biology in the design process, representing fish as equations solely involving fish swimming speed and without considering variations within and between species and populations (Roscoe and Hinch 2010; Katopodis and Williams 2012; Birnie-Gauvin et al. 2019). It is important to consider these individual variations in combination with physical, behavioural, and environmental factors to obtain a more holistic picture of passage success (Roscoe and Hinch 2010).

To make upstream progress over ground, a fish must swim faster than the downstream water speeds they are exposed to (Peake et al. 1997; Castro-Santos and Haro 2010; Laborde et al. 2016). It is especially important to ensure that water speeds never exceed the swimming speeds of the fish and are ideally within the prolonged or sustained swimming speed range (Mitchell 1989; Castro-Santos and Haro 2010). Sustained swimming is thought to be any speed at which a fish is capable of sustaining swimming for a minimum of 200 min, prolonged swimming can be maintained between c. 20 s – 200 min, and burst speeds can only be maintained for a matter of seconds (< 20 s) (Beamish 1978). The speed at which a fish can swim relates to metabolic costs and stamina, and is highly dependent on the traits of an individual or species (Mitchell 1989; Laborde et al. 2016).

Fish passage design guidelines are typically based on a maximum water speed that should not be exceeded (Furniss et al. 2000). These guidelines often do not account for all the species present in a system, or the individual variation that occurs within a species. Within any given river community, there are several different morphologies, physiologies, behaviours, and habitat preferences encompassing a wide range of niches (Willis et al. 2005; Jones et al. 2020; Pang et al. 2020). Additionally, there is significant trait variation even among individuals of the same species (Taylor and McPhail 1985; Pakkasmaa and Piironen 2001; Tudorache et al. 2008; Jones et al. 2020). Despite this knowledge, guidelines for maximum water speeds through a culvert continue to ignore intra- and interspecies variations. Studies by Taylor and McPhail (1985), Tudorache et al. (2008), Pakkasmaa and Piironen (2001), and Webster et al. (2011) have highlighted the importance of considering inter- and intraspecies variation when developing fish passage solutions.

In the past, design predominantly focused on single species, with a particular emphasis on salmonids. However, there is now a growing recognition of the importance of considering other species and their various life stages, leading to a shift towards a more comprehensive, multi-species design approach (Katopodis and Williams 2012; Birnie-Gauvin et al. 2019).

In island nations, such as New Zealand, the majority of migratory species are small-bodied and amphidromous (Franklin and Gee 2019). Small-bodied species typically have lower absolute swimming speeds, blocking smaller fish from passing barriers that are usually surmountable by larger species. Consequently, designing effective solutions to accommodate fish communities requires information about the capabilities and behaviours of all species. This should consider different swimming modes, abilities, behaviours, and niches (Jones et al. 2020). The different modes and behaviours that native fish exhibit (e.g.: climbers vs. swimmers, benthic vs. pelagic; McDowall 1990) greatly impact fish swimming performance, and some species may be more able to migrate through fish barriers or passes.

In New Zealand, *Galaxias maculatus* has served as the standard benchmark for designing fish passages. It is thought to be one of the weakest swimming species, with a standard 'rule-of-thumb' suggesting that a design water speed of 0.3 m s^{-1} is sufficient for their passage, and consequently, for any species that can swim faster than *Galaxias maculatus*. This study marks the first attempt to compare swimming speeds among a variety of migratory species in New Zealand using consistent and repeatable methodology. Due to behavioural and logistical constraints we opted for a modified swimming speed protocol for benchmarking these species. We used shorter timesteps than the traditional U_{crit} test proposed by Brett (1964). While it may not be a true U_{crit} test, the shorter time intervals allowed us to assess species with diverse behaviours and morphologies, but still benchmark their swimming capabilities relative to each other using a standardised assessment methodology.

Seven native amphidromous species from New Zealand were used in this study. The species represent a variety of realized niches, swimming modes, and morphologies, including pelagic swimmers, benthic-associated species, climbing species, and anguilliforms:

Galaxias maculatus inanga, *Galaxias fasciatus* banded kokopu, *Galaxias brevipinnis* koaro, *Galaxias postvectis* shortjaw kokopu, *Galaxias argenteus* giant kokopu, *Gobiomorphus huttoni* redfin bully, *Gobiomorphus cotidianus* common bully. *Anguilla dieffenbachii* longfin eel, and *Retropinna retropinna* common smelt were also tested, but due to differences in swimming speed protocol were left out of our benchmarking comparison; results for these species can be found in supplementary material. The objective of this research was twofold: (i) to examine inter- and intraspecies variation in swimming speeds of native fish; and (ii) to model maximum water speeds for culvert structures for a low-performing species (*Galaxias maculatus*).

3.3. Methods

3.3.1. Fish collection and handling

Permits for fish collection and ethics were obtained from the relevant authorities (Ministry for Primary Industries Special Permit SP666-4; NIWA Animal Ethics Committee approval AEC204). *Galaxias maculatus*, *Galaxias fasciatus, Galaxias brevipinnis, Gobiomorphus huttoni, Gobiomorphus cotidianus* were collected from the wild across New Zealand's North Island streams. *Galaxias postvectis* and *Galaxias argenteus* were obtained from Manāki Farms, a whitebait hatchery in Warkworth, New Zealand. Further information regarding fish collection can be found in supplementary material (Table A1).

All fish were transported to the NIWA Hamilton laboratory in aerated water from where they were collected. On arrival at the laboratory, the fish were equilibrated to the holding tank water temperatures and placed in 60 L quarantine tanks containing water with a salinity of 6 ppt as a disease prevention measure. After a week, they were moved to 60 L tanks filled with dechlorinated water connected to a recirculating water system. Water temperatures were maintained at 15 ± 0.5C and fish were held on a 12-hour day/12-hour night cycle. The fish were fed bloodworms every other day and fasted for 24 hours prior to trials to ensure a postabsorptive condition. Regular monitoring of ammonia and pH levels took place. If ammonia concentrations exceeded 0.25 mg L^{-1}, water changes were performed to maintain water quality.

3.3.2. Experimental setup

Experimental set-up and protocol followed that of Crawford et al. (2023). Briefly, swimming speed trials were conducted using a Steffensen-type 10-L swim tunnel (Loligo Systems). The swim tunnel consisted of a 5-L swim chamber measuring 28 x 7.5 x 7.5 cm, a honeycomb matrix at the front of the chamber for flow straightening, variable frequency drive and pump for speed control, and a chiller for temperature control. Body length, body width and body depth measurements were recorded and entered in Autoresp v2.3.0 (Loligo Systems) (Table 3.1, Table A1) to control water speed in body lengths per second of the individual fish and to compensate for the solid blocking effect (Kline et al. 2015). Throughout the trial water temperatures within the swim tunnel were maintained at 15°C ±0.5°C. The flush pump was kept running throughout the duration of the trial to maintain dissolved oxygen saturation (80.4 ± 1%).

Table 3.1: Species data - summary of sample size and mean lengths, including number of each species. Additional information regarding fish characteristics, including collection information, can be found in Supplementary Material Table A1.

Species	N	Δ*t* (s)	Mean length (cm)	Standard deviation (±)	Min length (cm)	Max length (cm)
Galaxias maculatus	19	10	4.4	0.38	3.7	5.2
Galaxias fasciatus	17	10	4.4	0.31	3.6	5.0
Galaxias brevipinnis	20	10	5.2	0.60	4.3	6.1
Galaxias postvectis	17	10	5.7	0.54	4.8	6.5
Galaxias argenteus	20	10	4.9	0.28	4.2	5.3
Gobiomorphus huttoni	13	10	4.1	1.57	2.7	6.7
Gobiomorphus cotidianus	20	10	3.2	0.33	2.3	3.8

3.3.3. Experimental protocol

The stepped-speed test designed by Brett (1964) that measures fish critical swimming speed is frequently used to quantify swimming performance. Critical swimming speed (U_{crit}) represents the maximum sustained swimming speed, marking the transition from aerobic (red, slow-twitch muscles) to anaerobic swimming (white, fast-twitch muscles) that results in exhaustion due to the depletion of nutrient and oxygen stores (Brett 1964; Beamish 1978; Bone 1978; Hammer 1995; Farrell 2008; Castro-Santos et al. 2022). Many researchers have tailored Brett's (1964) U_{crit} protocol to specific species by adjusting time intervals and speed increments, with variations ranging from 2 to 60 min (see Downie and Kieffer 2017a, Table 3.1). The adjustments to the U_{crit} protocol are largely logistical, as traditional U_{crit} tests are time consuming, with testing times of > 10 hrs when time increments are 60 min (Brett 1967; Hammer 1995; Farrell 2008).

Modified stepped swimming speed tests were used to benchmark the swimming capabilities of different species. The modified test protocol was adopted for both logistical reasons and to account for differences in swimming behaviours between species. Given the limited information on how these protocol adjustments affect non-salmonid species' physiology, we refer to our swimming speed tests as U_{max} (Farrell 2008), acknowledging that our short time intervals likely measure prolonged rather than true maximum sustained swimming. Preliminary trials showed no significant difference in estimated swimming speeds between 10 s, 5 min and 30 min time increments (Crawford et al. 2023; NIWA unpublished data). Furthermore, station-holding behaviour in some species generated inaccurate estimates of swimming speeds at longer time intervals. Crawford et al. (2023) showed that adopting shorter time increments eliminated this behavioural influence on swimming speed estimates.

At the beginning of each trial, single fish were introduced into the swim tunnel and allowed to acclimate for a duration of 30 min at a water speed of 0.5 body lengths per second (bl s^{-1}). Fish that failed to maintain position against the flow at the start of the trial and subsequently did not reach the next speed increment (1.5 bl s^{-1}) were excluded from the data. Trials were concluded once a fish exhibited signs of fatigue, remaining stationary against the mesh at the rear of the tunnel for a continuous duration of 3 s, or for a combined duration of 10 s within a 30-s timeframe. At the conclusion of a trial, fish were rested for 20 min to facilitate recovery before being returned to the holding tank. All trials were conducted independently, with no fish being reused for subsequent trials.

For all species, the water speed in the swim tunnel was raised by 1 bl s^{-1} every 10 s. For fish that exhibited station-holding behaviours during the trial (i.e. *Galaxias brevipinnis*, *Gobiomorphus cotidianus*), the amount of time (s) the fish spent actively swimming throughout the trial was recorded and calculated into total percentage of time spent station-holding. Juvenile life stages of all species were used, to minimize the potential influences of developmental changes in migratory tendencies.

Swimming speeds of *Retropinna retropinna* and *Anguilla dieffenbachii* were also measured, but not modelled, and can be found in supplementary material (Table A2). These were removed from the model due to differences in methodology: the time step for *R. retropinna* was 30 s, while the velocity increment for *A. dieffenbachii* was 0.3 bl s^{-1}.

Endurance trials were conducted with *Galaxias maculatus*, as they were the fish that exhibited the lowest average U_{max}. Endurance trials are a fixed speed test, in which the swimming performance of the fish is measured as time to fatigue at a set speed. After acclimation, the water speed was increased to a set speed (0.2, 0.4, 0.5, 0.7, 0.8, or 1.0 m s^{-1}). We selected these water speeds to encompass the full range of velocities achievable within the swim tunnel. Our objective was to observe the swimming behaviour of the fish across a range of velocities to determine an endurance curve. Total length of the fish was between 5.2 to 11.9 cm due to collection timing (Table 3.2).

Table 3.2: *Galaxias maculatus* endurance data—summary of sample size and mean lengths.

Speed (m s^{-1})	N	Mean length (cm)	Standard deviation (±)	Minimum length (cm)	Maximum length (cm)
0.2	20	7.3	1.10	5.4	9.4
0.4	20	7.9	1.59	5.9	11.9
0.5	16	7.8	1.09	6.8	9.9
0.7	16	8.6	1.32	6.2	10.9
0.8	10	7.6	0.97	6.3	8.9
1.0	20	7.3	0.96	5.2	9.5

3.3.4. Analysis

U_{max} was calculated following Brett (1964):

$$U_{max} = U_{f-1} + \Delta U\left(\frac{t_f}{\Delta t}\right)$$

Where U_{f-1}: second-to-last water speed, ΔU: incremental speed change (1 bl s^{-1}), t_f: duration the fish sustained the maximum speed, measured in s ($0 \leq t_f < \Delta t$), Δt: time interval in s (10 s). Swimming speed was measured in units bl s^{-1}.

To compare swimming speed (U_{max}) across species, an analysis of covariance model (ANCOVA) was used. The categorical predictor variable was species, and the continuous predictor variable was total length of the fish. Two outliers from the *Galaxias fasciatus* dataset, with lengths exceeding 6.1 cm, were eliminated from the analysis, as the targeted fish lengths ranged from 3.5 to 5.5 cm. Three outliers from the *Galaxias postvectis* were eliminated, as they exhibited station-holding resulting in significantly higher U_{max} estimates. Model fitness was compared using a Likelihood Ratio Test starting with a fully saturated model with interactions. The interaction between body length and species was found to be statistically significant and retained in the model. To determine which species exhibited statistically distinct swimming speeds, a post-hoc Tukey-HSD test was administered. All statistical analyses were conducted in the R statistical computing software version 4.0.3 (R Core Team 2020). Figures were generated using the R package ggplot2 (Wickham 2016).

The percentage of time spent station-holding during a trial was calculated for *Galaxias brevipinnis* and *Gobiomorphus cotidianus* (the only species that exhibited station-holding behaviours throughout the trial). An ANCOVA was used to test the relationship between swimming speed and percentage of time spent station-holding for each species.

Swimming speed was the response variable and percentage of time spent station-holding and fish length were continuous predictor variables.

We used time-to-event analysis to assess fatigue rates of *Galaxias maculatus*, based on the methodology outlined by Winger et al. (1999). Time-to-event analysis enables the inclusion of censored data, allowing us to account for fish that did not fatigue by the end of the 90 min endurance trials and to calculate the likelihood a fish fatigue at a particular time for a given speed, given that it hasn't yet fatigued (i.e. the "fatigue rate"). The Cox regression model was used to model instantaneous rate of fatigue for each swimming speed (coxme, Therneau 2015) which was right censored and modelled as a function of time as shown in the following equation, as explained by Goerig and Castro-Santos (2017):

$$\lambda(t) = \lambda_0(t)e^{\mathrm{X}\beta+\mathrm{Z}b}$$

Where $\lambda(t)$ is the baseline hazard function (i.e. fatigue rate) modelled as a function of time (t), X represents the fixed effects, Z represents the random, β and b represent the fixed- and random-effect coefficients respectively. Speed (m s^{-1}) and fish length (cm) were the fixed effects. There was no random effect in our model. Based on this equation, hazard ratios (HR) were calculated, comparing the fatigue rates between swimming speeds. We then used the survfit function from the Coxme R package to calculate the proportion of fish that are predicted to fatigue at a given time (Therneau 2015). As time progressed, the predicted proportion of fish fatiguing decreased because the number of available fish decreased over time. The Likelihood Ratio Test, Wald Test, and Score (Logrank) Test were used to determine model fit.

Design curves (maximum allowable water speed)

The fatigue rates calculated from *Galaxias maculatus* endurance swimming were used to develop design curves to determine the relationship between water speed (U_{water}) and fish speed (U_{fish}). Design curves were calculated for fatigue rates of 0.1, 0.3, and 0.5 therefore allowing us to determine the maximum allowable water speed through a culvert for successful passage of 90%, 70%, and 50% of fish respectively. These curves were modelled for culvert lengths up to 60 m. We used the method describe by Peake et al. (1997) to calculate the relationship between water speed and fish speed for a fishway of a given length for each specified fatigue rate:

$$U_{water} = U_{fish} - \left(\frac{D}{t}\right)$$

Where U_{water} is the water speed in the fishway (m s^{-1}), U_{fish} is the swimming speed of the fish used in endurance tests (i.e. 0.2, 0.4, 0.5, 0.7, 0.8, 1.0 m s^{-1}), D is the length of the fishway (i.e. 5-60 m), and t is the endurance time of fish swimming at a given speed (U_{fish}) in s. Time (t) was extracted from the time-to-event analysis model, where each U_{fish} swimming speed crossed the fatigue rate threshold of either 0.1, 0.3, or 0.5. Any calculated U_{water} values at a given structure length that were negative indicate an impassable structure and thus were removed from the figure.

Maximum allowable water speed was then calculated by extracting maximum U_{water} values for each distance and fatigue rate combination:

$$U_{water,max} = \max\left(U_{fish} - \left(\frac{D}{t}\right)\right)$$

All analyses were carried out using R version 4.2.0 (R Core Team, 2020).

3.4. Results

3.4.1. Swimming speed

Swimming speed was highly variable between and within species (Table 3.3, Table 3.4, Figure 3.1, Figure A1). Species (d.f. = 6, *F* statistic = 9.99, $p < 0.0001$), body length (d.f. = 1, *F* statistic = 20.71, $p < 0.0001$), and the interaction between species and body length (d.f. = 6, *F* statistic = 3.03, $p = 0.0100$), were all statistically significant predictors of U_{max} (Table 3.3). Absolute U_{max} of *Galaxias maculatus, Galaxias fasciatus*, *Gobiomorphus cotidianus* and *Gobiomorphus huttoni,* were not statistically different from each other. *Galaxias postvectis*, *Galaxias brevipinnis*, and *Galaxias argenteus* also did not have statistically different U_{max} from each other, but all were significantly faster than all species in the group listed previously. All species exhibited intraspecies variation in speed, with *Galaxias brevipinnis*, *Galaxias argenteus*, and *Galaxias postvectis* individual U_{max} ranging across almost 1 m s^{-1}. U_{max} varied less for *Galaxias maculatus*, but still ranged over 0.37 m s^{-1}.

Table 3.3: Summary of Type III analysis of covariance using Satterthwaite's method, comparing the swimming abilities of different species as determined by their swimming speeds (m s^{-1}). d.f. = degrees of freedom.

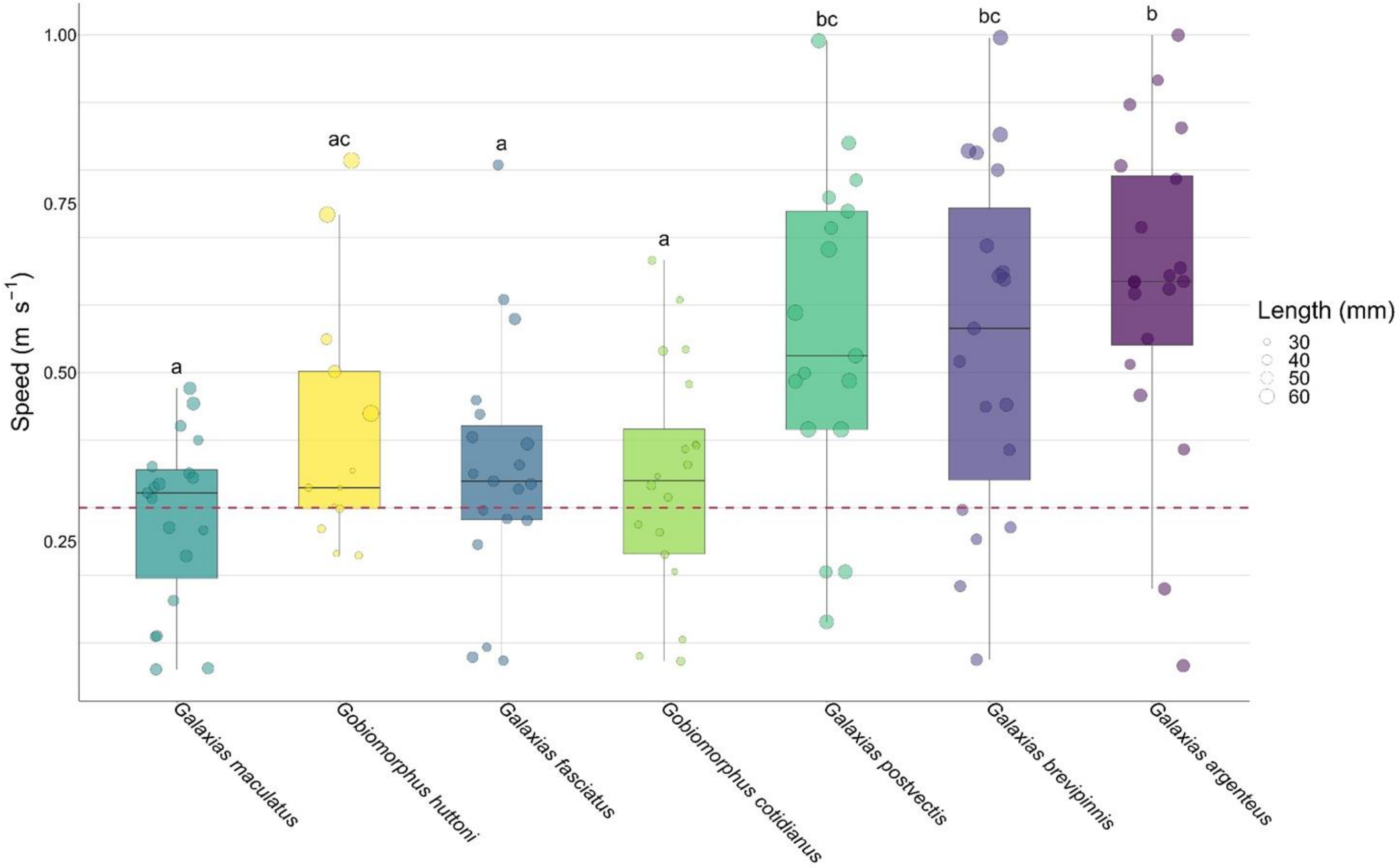
Source	Sum of squares	Mean squares	Numerator d.f.	Denominator d.f.	F-value	p-value
Species	1.97	0.33	6	113	9.99	<0.001
Length	0.68	0.68	1	113	20.71	<0.001
Species:Length	0.60	0.10	6	113	3.03	0.009

There was a statistically significant relationship between the proportion of time *Galaxias brevipinnis* and *Gobiomorphus cotidianus* spent actively swimming and U_{max}. As U_{max} increased, *Galaxias brevipinnis* spent significantly less time actively swimming (i.e. increasing station-holding behaviour) ($p < 0.01$). Conversely *Gobiomorphus cotidianus* exhibited the opposite behaviour, with an increase in active swimming as speed increased ($p < 0.01$).

Table 3.4: Summary of swimming speeds for seven New Zealand fish species.

Species	**Mean U_{crit}**	**Standard deviation**	**Minimum U_{crit} (m s^{-1})**	**Maximum U_{crit} (m s^{-1})**	**Range**
Galaxias maculatus	0.28	0.13	0.06	0.48	0.42
Galaxias brevipinnis	0.57	0.28	0.08	1.07	0.99
Gobiomorphus huttoni	0.41	0.19	0.23	0.81	0.58
Galaxias fasciatus	0.36	0.18	0.07	0.81	0.73
Gobiomorphus cotidianus	0.34	0.17	0.07	0.67	0.59
Galaxias argenteus	0.63	0.23	0.07	1.00	0.93
Galaxias postvectis	0.56	0.24	0.13	0.99	0.86

Figure 3.1: Swimming speeds of seven New Zealand species, measured in metres per second (m s^{-1}). The box indicates the interquartile range, whiskers extend 1.5 times the interquartile range, and the centre bar gives the median. The data points within each treatment group represent individual fish, and are sized based on fish length, with larger points representing larger fish. A red dashed line represents the current New Zealand rule-of-thumb guidance (0.3 m s^{-1}) for maximum water speed through a culvert. Tukey significance is symbolised with letters (e.g. a, b, c) displayed above the boxplots to indicate significant difference between group means



3.4.2. Endurance

The time-to-event analysis revealed a statistically significant effect of swimming speed on fatigue rates for *Galaxias maculatus*, with a positive relationship between speed and fatigue rate ($p < 0.001$; hazard ratio, HR = 292.00; Table 3.5, Figure 3.3). There was a statistically significant effect of fish length on fatigue rates, with a negative relationship between fish length and fatigue rate ($p = 0.001$; HR < 0.0001; Table 3.5, Figure 3.3).

Table 3.5: Estimation of parameters for the Cox proportional hazards model for fatigue rate.

Parameter	Coefficient	Standard error	Hazard ratio	p-value
Speed (bl s^{-1})	5.67	0.64	292.00	<0.001
Length (mm)	-37.90	11.60	3.47×10^{-17}	0.001

As the trials were terminated after 90 min, no true measure of sustained swimming was observed. At 0.2 m s^{-1} most fish were able to maintain swimming for 90 min. Even at this low speed some individuals swam for under 2 min. Prolonged swimming was observed between 0.2 and 1.0 m s^{-1}. Burst swimming (as defined by an endurance of < 20 s) was observed for a few individuals between 0.5 and 1.0 m s^{-1}. There were large variations in endurance time at speeds above average U_{max} (i.e. speeds of 0.4 m s^{-1} and greater), ranging from times typically associated with burst swimming (< 20 s) through to completing the 90 min trial without fatigue. At 0.4 m s^{-1}, eight individuals swam for 90 min, and three individuals swam for under 2 min. At speeds > 0.5 m s^{-1}, most individuals swam for < 2 min, with the proportion of individuals swimming less than 2 min increasing as speed increased (Figure 3.2).

Figure 3.2: Scatter plot of *Galaxias maculatus* endurance times against swimming speed. The data points within each treatment group represent individual fish. Grey horizontal dashed lines at 20 s and 5400 s represent burst and sustained swimming ranges respectively.

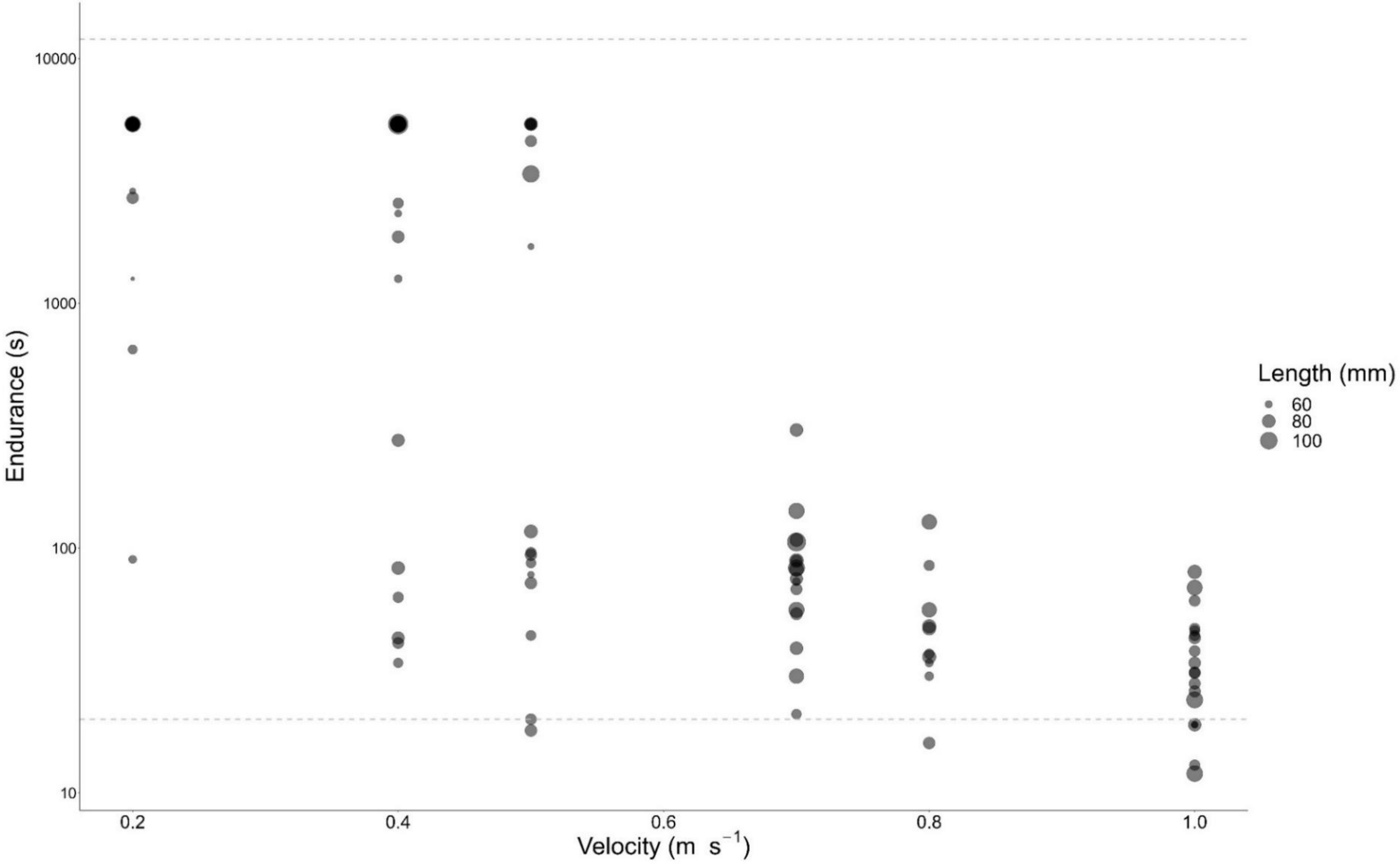
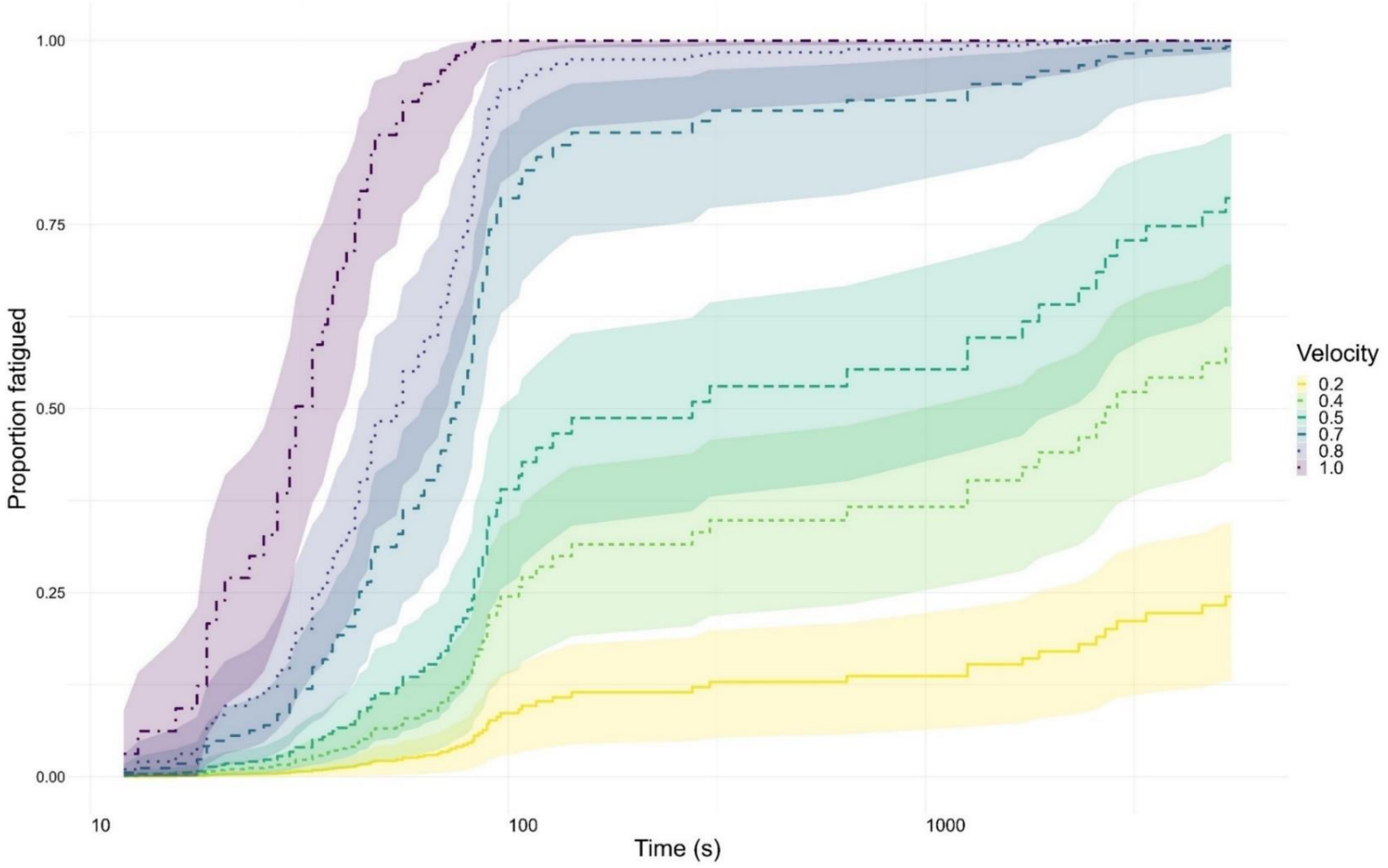


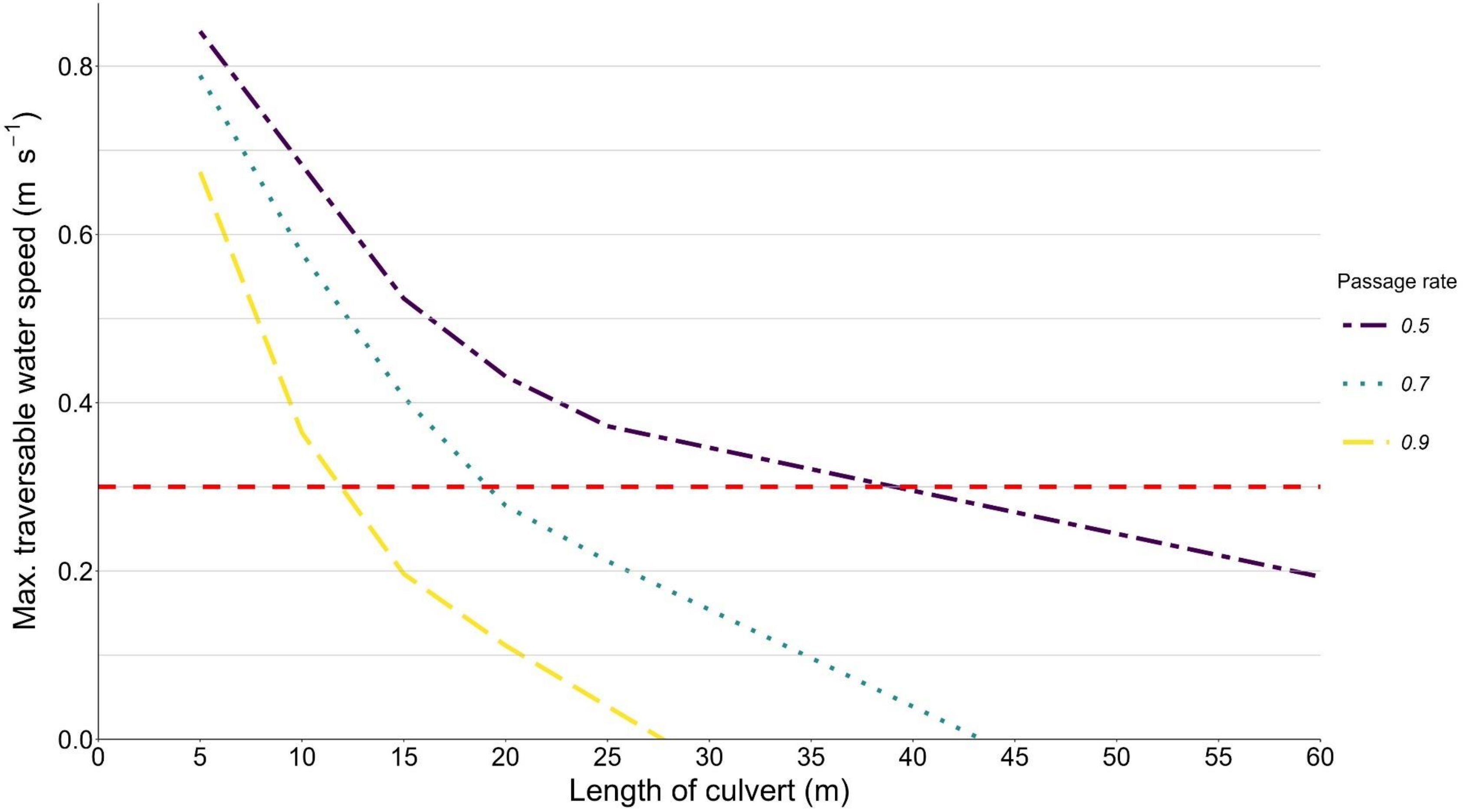
Figure 3.3: Predicted fatigue rates (proportion of individuals fatigued) across time for *Galaxias maculatus*. Data are stratified by fish speed (0.2, 0.4, 0.5, 0.7, 0.8, and 1.0 m s^{-1}). Zero on the y-axis represents that 0% of the population have fatigued at time (t), and 1 represents that 100% of the population have fatigued at time (t). Fish length was set at the mean length of all fish (76 mm). Shaded regions in corresponding colours represent 95% confidence intervals. Grey dashed lines represent the fatigue rates of 0.1, 0.3, and 0.5, which are later used to calculate the design curves outlined in Figure 4.



3.4.3. Maximum allowable water speed

Maximum allowable design water speeds, associated with three different fatigue rates, are represented in Figure 3.4. Maximum allowable water speed decreased with culvert length for all fatigue rates. To achieve a fatigue rate of 0.5 (i.e. 50% of fish fatigue after the end of the structure length), water speeds should not exceed 0.84 m s^{-1} for a 5 m culvert and 0.19 m s^{-1} for a 60 m culvert. To ensure a fatigue rate of 0.1 (i.e. 90% of fish fatigued after the end of the structure length and successfully pass through the culvert), water speeds should not exceed 0.67 m s^{-1} for a 5 m culvert. Furthermore, a 90% success rate at prolonged swimming speeds can only be achieved in culverts less than 30 m, as this is the point where maximum allowable water speeds reach 0 m s^{-1}.

Figure 3.4: Maximum allowable water speed versus culvert length calculated with fatigue rates for *Galaxias maculatus* representing the 0.1, 0.3, and 0.5 fatigues rates. A fatigue rate of 0.1 corresponds to 10% of fish fatiguing before the end of the structure length and not passing through the culvert. A fatigue rate of 0.5 corresponds to 50% of fish fatiguing before the end of the structure length and not passing through the culvert. Any calculated water speeds at a given structure length that were negative indicate an impassable structure and thus were removed from the figure.



3.5. Discussion

This study is one of the first to benchmark several native New Zealand fish swimming speeds using consistent and repeatable methodology. Our findings reveal significant interspecies and intraspecies variation in swimming speeds. Among the species studied, *Galaxias brevipinnis*, *Galaxias argenteus*, *Galaxias postvectis* were the strongest swimmers, with mean U_{max} of 0.57, 0.63, and 0.55 m s $^{-1}$, respectively. In contrast, *Galaxias maculatus* was one of the weakest swimmers, with a mean U_{max} of 0.28 m s^{-1}. Body length was a significant factor and positively correlated with U_{max}, supporting research that fish passage barriers select against the weakest species, as well as smaller individuals within a species (Jones et al. 2020).

Compared to other smaller-bodied fish, the species in our study demonstrated similar or slightly slower swimming speeds. A comparable range of swimming speeds was observed between the *Galaxias brevipinnis* individuals examined in this study and *Galaxias brevipinnis* in Australia, with mean U_{max} values of 0.57 m s^{-1} and 0.49 m s^{-1} respectively (Watson et al. 2019). Furthermore, the median swimming speeds reported by Watson et al. (2019) for other small-bodied Australian fishes (ranging from 0.2 m s^{-1} to 0.9 m s^{-1}) overlap closely with the median speeds observed in our study (0.3 m s^{-1} to 0.7 m s^{-1}). Even the strongest fish in our study had lower swimming speeds than *Cheirodon galusdae* and *Basilichthys microlepidotus*, two small-bodied Chilean sportfish with swimming speeds of 0.697 and 0.696 m s^{-1} respectively (Laborde et al., 2016). The range of speeds for these two Chilean species was similar to that of the species in this study (*C. galusdae*: 0.38-0.97 m s^{-1} and *B. microlepidotus*: 0.28-1.4 m s^{-1}) (Laborde et al*.,* 2016). *Galaxias fasciatus*, *Gobiomorphus cotidianus*, *Galaxias maculatus,* and *Gobiomorphus huttoni* had similar average swimming speeds to the goby *Neogobius melanostomus* (0.35 m s^{-1}) (Gilbert et al*.,* 2016).

Initially developed through research on salmonids and other actively swimming fish in the Northern Hemisphere, U_{crit} type experiments have been adopted by researchers to assess the swimming performance of various fish species and life stages (e.g. Fisher et al. 2005; Laborde et al. 2016; Egger et al. 2020). Several studies have found that shortening the time interval in U_{crit} tests does not result in significant differences in estimates of critical swimming speeds, indicating that there is some measure of physiological comparison that can be made across time intervals (e.g. 2-64 min, Hartwell et al. 1991; 2-10 min, Peake and Mckinley 1998; 2-5 min, Fisher et al. 2005; 10 s - 5 min, Crawford et al. 2023; 5-30 min, NIWA unpublished data). For example, Fisher et al. (2005) found no significant difference in U_{crit} when using time intervals of 2 and 5 min for 89 species of late-stage larval coral reef fishes. These late-stage larval fishes measured only marginally smaller than the juvenile fish lengths in our study. Moreover, the larval fishes exhibited a similar range of swimming speeds to those in our study (0.06 – 1.0 m s^{-1} and 0.07 – 0.99 m s^{-1} respectively) (Fisher et al. 2005).

Farlinger and Beamish (1997) found that reducing time increments in stepped velocity tests resulted in higher U_{crit} values in *Micropterus salmoides*, theorizing that shorter time increments contribute to faster lactate build-up due to increased anaerobic metabolism. As such, Farlinger and Beamish (1977) went on to warn that critical swimming speeds "should be measured under conditions in which fatigue is the result of swimming not of the method applied," to ensure that true maximum sustained swimming speeds are being measured. It is generally assumed that at shorter time intervals, researchers are measuring prolonged swimming speeds rather than fully aerobically-fuelled, sustained swimming speeds. Use of a shorter time interval raises questions about whether the point of fatigue at a trial's conclusion accurately reflects the same threshold of fatigue and prolonged swimming across all species (Farlinger and Beamish, 1977; Beamish 1978; Brett 1964; Facey & Grossman 1990). As we tested a shorter time step than the traditional U_{crit} test, we may not have a true measure of maximum sustained swimming speeds for our species, but rather a measure of prolonged swimming. We expect variations in physiology among different species, including differences in aerobic and anaerobic muscle composition and cardiovascular efficiency. Consequently, it may not be appropriate to assume that the standardised protocol initially set out by Farlinger and Beamish (1977) for estimating U_{crit} in *Micropterus salmoides* is directly transferable to other species with different physiology. Further research is, therefore, needed to understand how variations in time step interacts with the physiology of different fish species (and life stages) and what fatigue at the end of the trial represents in physiological terms. As such, we present our data as a collection of swimming speeds for various migratory species, serving as a baseline for countries like New Zealand, which lack consistent and repeatable swimming speed data for many species.

The use of laboratory derived swimming speeds as a measure of fish swimming ability may not fully capture the complexities of the natural world, such as how fish may respond to turbulence, varying speeds across culvert cross sections, or changes in water temperature (Plaut 2001; Castro-Santos et al. 2022). Laboratory tests offer the benefit of being able to control for confounding factors and overcome challenges of tracking live fish in field-based contexts (particularly relevant for small-bodied fishes). However, the artificial constraints placed on fish in a laboratory setting can alter fish behaviour and, therefore, introduce uncertainty regarding the real-world relevance of results. For example, forcing benthic-associated species to swim continuously rather than exploit typical station-holding behaviour may be unrepresentative of how they would navigate real-world obstacles. Future studies are required to determine the physiological implications of each time increment for different fish species, including benthic, station-holding, and pelagic species, which may differ from traditional salmonid physiology models.

In New Zealand, one of the most frequently encountered barriers to fish migration is road culverts. There is a growing need for engineers to have access to information that can help them design structures that provide optimal water speeds for effective fish passage. Current design approaches often rely on average swimming ability. Individual fish within a species can exhibit large trait variations, such as length, weight, metabolism, and variations in swimming ability (Jones et al*.* 2020). Designs that consider individual variation are crucial to successful fish passage solutions that promote the preservation of fish populations.

Setting maximum allowable water speeds for fish passage design based solely on average swimming ability restricts fish passage to only those individuals that excel beyond the average swimmers (Watson et al. 2019; O'Connor et al. 2022). Our results support other research findings that fish passage barriers create selection pressures, favouring the strongest and largest swimmers in a population (Taylor and McPhail 1985; Pakkasmaa and Piironen 2001; Webster et al. 2011; Jones et al. 2020). These selection pressures can have implications for population structure. For example, Egan et al. (2019) found that *Galaxias maculatus* have a prolonged spawning season, with larvae hatching from February to September. Size at time of upstream migration was negatively correlated with hatch date, with fish hatched earlier in the year beginning their upstream migration at a larger size (Egan et al. 2019). If barriers are selecting for larger and stronger fish (e.g. the strongest swimmers), then they may be selecting for fish hatched earlier in the year, skewing population dynamics to favour only a subset of life-history phenology.

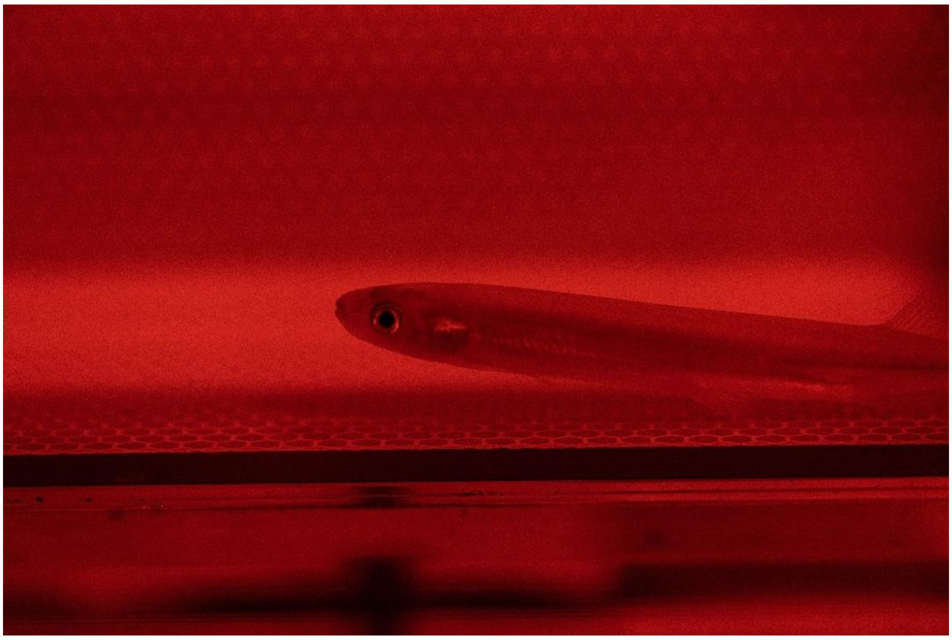
Lucas and Baras (2001) emphasize that structures should be passing 90-100% of diadromous and potadromous fish. To ensure successful passage for more than 90% of a population and reduce selection pressures, water speeds within culverts need to be much lower than previously thought for New Zealand's native fish community. Current rule-of-thumb guidance in New Zealand suggests that water speed should not exceed 0.3 m s^{-1} (Stevenson and Baker 2009; Franklin et al. 2018). However, to meet an objective of achieving passage success for 90% of *Galaxias maculatus* (based on swimming speeds), culvert length should be limited to less than 10 m at a maximum allowable water velocity of 0.3 m s^{-1}. To meet the object of achieving passage for at least 50% of fish, water speed through culverts should still be well below the 0.3 m s^{-1} rule-of-thumb. In-fact culverts should not exceed 25 m in length to successfully pass the majority of fish in a given population, unless water speeds are lower than all sustained swimming speeds. Laborde et al. (2016) also showed there can be significant differences in water speed design criteria, depending on whether the focus is on average swimming fish or those with minimal swimming abilities. Their research showed that suitable water speeds were comparable to ours for structure lengths between 5-60 m (0.4-0.6 m s^{-1} and 0.05-0.7 m s^{-1} respectively). Cumulatively, this research highlights the need to take into account individual variation within a population when designing fish passage solutions.

Conclusion

Contrary to previous assumptions, our findings suggest that maximum allowable speeds in culverts should be significantly lower in New Zealand than the commonly accepted standard rule-of-thumb (0.3 m s^{-1}). For example, a 15 m culvert should not have water speeds exceed 0.2 m s^{-1}, and culverts longer than 30 m should not have water speeds that exceed the critical swimming speeds of the fish population in that river system. This study emphasizes the need for designs that consider variation both within and between species, encompassing habitat preferences, morphologies, physiologies, and life history strategies. Adopting a tailored approach rather than a one-size-fits-all mindset will lead to more effective fish passage solutions, ensuring the preservation of fish populations in specific ecosystems.

Chapter 4

High water temperature significantly reduces swimming performance of New Zealand migratory species



Galaxias maculatus in swim tunnel, NIWA Hamilton, New Zealand
Photo credit: Stuart Mackay, NIWA

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4.1. Abstract

Anthropogenic structures in freshwater systems pose a significant threat by fragmenting habitats. Effective fish passage solutions should consider how environmental changes introduce variability into swimming performance. As temperature is considered the most important external factor influencing fish physiology, it is especially important to consider its effects on fish swimming performance. Even minor alterations in water properties, such as temperature and velocity, can profoundly affect fish metabolic demands, foraging behaviours, fitness, and, consequently, swimming performance and passage success.

In this study, we investigated the impact of three water temperature treatments on the critical swimming speeds of four migratory New Zealand species. Our findings revealed a significant reduction in critical swimming speeds at higher water temperatures (26°C) compared to lower ones (8°C and 15°C) for three out of four species (*Galaxias maculatus*, *Galaxias brevipinnis*, and *Gobiomorphus cotidianus*). In contrast, *Galaxias fasciatus* exhibited no significant temperature-related changes in swimming performance, suggesting species-specific responses to temperature. The cold temperature treatment did not impact swimming performance for any of the studied species.

As high-water temperatures significantly reduce fish swimming performance, it is important to ensure that fish passage solutions are designed to accommodate a range of temperature changes, including spatial and temporal changes, ranging from diel to decadal fluctuations. This research underscores the importance of incorporating temperature effects into fish passage models for habitat restoration, connectivity initiatives, and freshwater fish

conservation. The influence of temperature on fish swimming performance can alter migration patterns and population dynamics, highlighting the need for adaptive conservation strategies. To ensure the resilience of freshwater ecosystems it is important to account for the impact of temperature on fish swimming performance, particularly in the context of a changing climate.

4.2. Introduction

Anthropogenic instream structures fragment habitats, posing a significant threat to freshwater ecosystems (Castro-Santos et al. 2009; Bunt et al. 2012; Fangue et al. 2015; Franklin and Gee 2019). This fragmentation isolates fish populations and obstructs fish migration, limiting dispersal, and reducing species richness (Nicola et al. 1996; Lucas and Baras 2001; Daufresne and Boët 2007; Gough et al. 2012; Jellyman and Harding 2012; Neachell 2014; Radinger and Wolter 2014; Silva et al. 2018; Wilkes et al. 2019). These barriers to migration lead to global diadromous fish loss and degrade freshwater ecosystems (Bunt et al. 2012; Fangue et al. 2015; Franklin and Gee 2019).

Restoring river connectivity is a key restoration goal for freshwater ecosystems (Thieme et al. 2023; Franklin et al. 2024). Approaches to designing more effective fish passage solutions should consider not only inter- and intraspecies variability in behaviour and capabilities (Crawford et al.2024 *a*), but also how changes in the environment introduce variability into swimming performance (Jones et al. 2021). Changes in water temperature and velocity can have profound effects on fish metabolic demands, foraging behaviours, and survival (Green and Fisher 2004; Fangue et al. 2015) and subsequently, fish swimming performance and passage success.

Water temperature plays a crucial role for fish by regulating physiological and cellular processes (Brett 1971; Schurmann and Steffensen 1997; Claireaux and Lagardère 1999). Water temperature exerts a direct influence on fish metabolism because it increases the standard metabolic rate (SMR) and maximum metabolic rate (MMR), affecting predator avoidance, foraging, and swimming (Brett 1964; Claireaux and Lagardère 1999; Sandblom et al. 2014). Although increasing water temperatures have been shown to exponentially increase standard metabolic rate, maximum metabolic rate may either continue to increase, plateau, or even decrease depending on fish species and life stage (McKenzie and Claireaux 2010; Norin and Clark 2016). Additionally, changes in water temperature influence oxygen availability and consumption by organisms, further affecting fish swimming ability (Schurmann and Steffensen 1997).The effects of water temperature on fish swimming vary by species, with some species more susceptible to colder temperatures and others to warmer temperatures depending on whether they are temperate, tropical, or cold-water species (Johnston and Dunn 1987; Fangue et al. 2015; Parisi et al. 2020; Muhawenimana et al. 2021). Water temperature also serves as an ecological factor influencing niche partitioning, determining the habitat preferences and distributional limits for fish populations (Magnuson et al. 1979; Richardson et al. 1994).

Freshwater systems are highly dynamic and are characterised by thermal heterogeneity often driven by atmospheric conditions, topography, stream discharge, and stream bed characteristics (Caissie 2006; Pander et al. 2024). These drivers are influenced by various environmental factors such as transitions from sea to freshwater environments, temperature differentials where tributaries meet larger systems, and differences in riparian shade versus direct solar radiation (Caissie 2006; Tague et al. 2007). Additionally, small-scale temperature changes occur due to groundwater inputs, including hyporheic flows, springs, and seeps (Hatch et al. 2006). Steep topography, such as stream slope, has been linked to cooling water, as it facilitates increased mixing between surface water and interstitial water, and increased evaporative cooling (Pander et al. 2024).

Anthropogenic activities further contribute to stream thermal heterogeneity (Caissie 2006). Land use changes resulting in differences in riparian canopy cover significantly influences water temperature, with shaded sections of stream having lower water temperatures than sections with open canopy (Somers et al. 2013; Pander et al. 2024). Water abstraction and removal practices can elevate river temperatures beyond normal levels (Booker and Whitehead 2022) and in urban systems, stormwater runoff from impervious surfaces on heat islands leads to dramatic increases in stream temperature not found in forested streams (Somers et al. 2013). Hot water released from power stations and cold-water released from dams are significant contributors to water pollution in riverine systems (Parisi et al. 2020). Impoundments exacerbate temperature stratification in water columns that are typically well mixed, resulting in the loss of suitable fish habitat (Parasiewicz et al. 2023). Thermal heterogeneity affects fish behaviour and habitat use, with fish known to seek out cold water refugia when stream temperatures exceed their thermal optima impacting fish during migration (Sutton et al. 2007).

New Zealand's diverse geographic features, varied landscapes, and significant latitudinal gradients, contribute to a wide range of water temperatures in its freshwater systems (Warrick et al. 2001). Amphidromous species are prevalent within New Zealand’s native fish communities, beginning upstream migration as small-bodied juveniles. These species undertake extensive journeys inland and to high elevations during migration, experiencing a wide range of temperatures (McDowall 1990; Richardson et al. 1994;Franklin and Gee 2019). During upstream migration, water temperatures across the country can span from < 1°C to > 26°C, with average water temperatures ranging from 9°C to 18°C (Mosley 1982: New Zealand National Water Quality Monitoring Network, 2024; Table 4.1).

Table 4.1: Meteorological seasonal water temperatures (℃) across New Zealand, including minimum, maximum and average temperatures from 1989 to 2024, from New Zealand National Water Quality Monitoring Network. Spring: September – November; Summer: December – February; Autumn: March – May; Winter: June – August.

Season	Mean	Minimum	Maximum	Standard deviation (±)
Spring	12.8	3.5	26.8	3.25
Summer	18.1	7.1	31.4	3.43
Autumn	14.4	4.5	25.7	3.42
Winter	9.1	0.4	21.0	2.54

New Zealand's fish are susceptible to acute temperature changes during their migration periods, arising from both natural and anthropogenic factors. For example, increased land use for agriculture and forestry has resulted in more extreme water temperature fluctuations and seasonal water temperature variation (Quinn et al. 1992; Harding et al. 2001; Collier and Bowman 2003; Larned et al. 2020). Furthermore, thermal and geothermal power stations on New Zealand's large rivers are known to create hot water plumes, heating river temperatures downstream from the point source (e.g. Wairakei Geothermal Power Station, Waikato River; Baker et al. 2021). These natural and anthropogenic factors result in seasonal and spatial changes in water temperature and diel fluctuations. Despite the critical role of water temperature in fish physiology and ecological dynamics, limited research has been conducted on the impacts of temperature on fish swimming ability in New Zealand considering the number of species.

The primary objective of this research is to investigate the influence of acute water temperature treatments on the swimming performance of native diadromous New Zealand fish species. We conducted critical swimming speed measurements on *Galaxias maculatus* (inanga), *Galaxias fasciatus* (banded kokopu), *Galaxias brevipinnis* (koaro), and *Gobiomorphus cotidianus* (common bully), at three distinct water temperatures, 8°C, 15°C, and 26°C, that span the range of average summer temperatures in New Zealand's rivers. These four species were chosen as they all have widespread populations across the country, ranging in both latitude and elevation, and varying landscapes. These diadromous species must all migrate from the sea through the lowlands, exposing them to a range of water temperatures on their migration upstream.

By investigating how these acute water temperature differences affect fish swimming abilities, we can develop fish passage design strategies that consider the thermal challenges fish face in their habitats. It is important to design fish passage solutions that encompass the range of water temperatures fish communities will encounter now and into the future to ensure continuous accessibility for upstream migration (Birnie-Gauvin et al. 2019; Lynch et al. 2023). As climate change is expected to amplify temperature fluctuations, it is especially important to understand the ecological implications of acute temperature effects for future-proofing conservation and management strategies (sensu Lynch et al. 2023).

4.3. Methods

4.3.1. Fish collection and handling

Ethics approval and collection permits were secured from the relevant authorities (Ministry for Primary Industries Special Permit SP666-4; approval from NIWA Animal Ethics Committee, AEC204).

Juvenile *Galaxias maculatus* were collected in spring using fyke nets at the mouth of New Zealand’s Rangitaiki River (37°54’34.6” S 176°52’53.0” E). Juvenile *Galaxias fasciatus* were collected in spring using fyke nets from the Lilleburn Stream in the Hunua Ranges (37° 04’ 55.6” S 175° 10’ 02.9” E) and Waitawhara Stream in New Zealand’s Glen Afton (37° 36’ 20.6” S 175° 02’ 12.8” E). Juvenile *Galaxias brevipinnis* were also collected in spring using fyke nets from the Lilleburn Stream in the Hunua Ranges (37° 04’ 55.6” S 175° 10’ 02.9” E). Juvenile *Gobiomorphus cotidianus* were collected in autumn using seine netting from Waiotapu Stream (38°25’11.4” S 176°20’43.7” E). While water temperature was not measured at time of fish collection, maximum daily air temperature was between 10°C and 15°C across all fish collections.

All fish were transported to NIWA Hamilton laboratory in well-oxygenated stream water. Upon arrival, the fish were acclimated to the holding tank's temperature (15°C) and placed in 60 L quarantine tanks containing water with 6 ppt salinity as a precaution against disease. After a week, they were moved to 60 L tanks with dechlorinated water connected to a recirculating water system. Water temperature was maintained at 15 ± 0.5°C, and fish were kept on a 12-hour day/12-hour night cycle. Fish were fed bloodworms every second day starting once they were transferred into holding tanks and were subjected to a 24-hour fast prior to trials to ensure a postabsorptive state. Regular monitoring of ammonia and pH levels was carried out, and water changes were conducted to maintain water quality if ammonia concentrations exceeded 0.25 mg L^{-1}.

4.3.2. Temperature acclimation

The trial water temperatures selected for this study were 8°C, 15°C, and 26°C as they are environmentally relevant temperatures representing the typical range found in New Zealand stream systems (New Zealand National Water Quality Monitoring Network, 2024). Neither 8°C nor 26°C falls within the lethal water temperature range for the fish under investigation (Richardson et al. 1994).

To avoid the impacts of sudden changes in water temperature, the fish were acclimated to the trial temperature prior to testing. Once fish were initially acclimated to the laboratory as described above, the water temperature in the fish holding tanks was incrementally increased or decreased by 2°C every day until the desired water temperature was reached. Fish were then held for a minimum of 48 hours at the trial temperature before placing them in the swim tunnel (which was set to the trial temperature) and undertaking trials. As this length of time was not sufficient to truly acclimate the fish to the trial temperature, the following experiments tested the effects of acute temperature exposure on swimming performance. Once acclimated to the trial temperature, fish in the 8°C and 26°C temperature treatments were held for a maximum of 3 days before testing. Fish in the ambient treatment of 15°C were held for a maximum of 15 days. An analysis of covariance showed that there was no significant effect of treatment holding time on critical swimming speed ($p < 0.05$), for each species*,* where swimming speed was the response variable and fish length, water temperature, and holding time were predictor variables.

4.3.3. Experimental setup

The experimental setup and procedure mirrored that of Crawford et al. (2023). Critical swimming speed trials were conducted using a 10 L swim tunnel (Steffensen type swim tunnel from Loligo Systems). Water speed within the tunnel was controlled using Autoresp v2.3.0 (Loligo Systems), which also compensated for the solid blocking effect (Kline et al. 2015). Throughout the trials, Water temperature in the swim tunnel was kept at 8 ± 0.5 °C, 15 ± 0.5 °C, or 26 ± 0.5°C depending on temperature treatment. Dissolved oxygen percent saturation within the swim tunnel was maintained at 92.3 ± 1%.

4.3.4. Experimental procedure

At the start of each trial, a single fish was measured (total length) and then placed into the swim tunnel and acclimated for 30 min at a water speed of 0.5 body lengths per second (bl s^{-1}). Trials concluded when a fish displayed signs of fatigue, remaining stationary against the mesh at the back of the tunnel for a continuous period of 3 s or for a total of 10 s within a 30 s window. After a trial, fish were given a 20-min rest period to aid recovery before being returned to the holding tank. Each trial was conducted independently, with no reuse of fish for subsequent trials.

Modified critical swimming speed (U_{crit}) trials were conducted to accommodate the distinct behaviours of the species under study (Crawford et al. 2023). For pelagic swimming species (*Galaxias maculatus*), water speed was increased by 1 bl s^{-1} every 300 s. For station-holding and benthic-associated species (e.g. *Gobiomorphus cotidianus, Galaxias fasciatus*, and *Galaxias brevipinnis*), water speed was raised by 1 bl s^{-1} every 10 s. The U_{crit} test was conducted at each of the water temperature treatments (8, 15, and 26°C) for each species.

Juveniles of all species were used with total length less than 7 cm for *Galaxias maculatus*, *Galaxias fasciatus*, and *Galaxias brevipinnis* (Table 4.2). Total length of *Gobiomorphus cotidianus* was less than 4.7 cm (Table 4.2).

Table 4.2: Species data for temperature trials, including the number of species tested at each temperature, their mean total lengths, and standard deviation.

Species	Temperature (°C)	N	Mean length (cm)	Standard deviation (±)
Galaxias maculatus	8	17	4.6	0.48
	15	19	4.4	0.38
	26	18	4.2	0.21
Galaxias fasciatus	8	19	4.4	0.60
	15	20	4.3	0.53
	26	20	4.2	0.28
Galaxias brevipinnis	8	20	5.8	0.54
	15	20	5.2	0.60
	26	19	5.7	0.49
Gobiomorphus cotidianus	8	20	3.3	0.35
	15	20	3.2	0.33
	26	19	3.6	0.44

The critical swimming speed (relative to fish length) of each fish was calculated using the following equation from Brett (1964):

$$U_{crit} = U_{f-1} + \Delta U \left(\frac{t_f}{\Delta t}\right)$$

Where U_{f-1} represents the penultimate water speed the fish was subjected to in bl s^{-1}, ΔU represents the incremental change in water speed (i.e. 1 bl s^{-1}), t_f is the time the fish swam at the highest speed in s ($0 \leq t_f < \Delta t$), Δt represents the time interval in s (i.e. 300 s or 10 s depending on species). Swimming speed units were recorded in bl s^{-1}.

To test the effects of water temperature on fish critical swimming speed for each individual species, an analysis of covariance (ANCOVA) was used. Water temperature was used as a categorical predictor and fish length as a continuous predictor variable. Swimming speed in body lengths per second (the value of U_{crit}) was used as the response variable. To assess model fit, a Likelihood Ratio Test was used, starting with a fully saturated model including interactions. The interaction between body length and water temperature treatment was not statistically significant for any species and was removed from the models. For each species, length was not statistically significant, but was kept in the models as length is known to influence U_{crit} (Beamish 1978; Cano-Barbacil et al. 2020). A post-hoc Tukey's Honestly Significant Different (HSD) test was used to determine, per species, which temperature treatments had statistically different critical swimming speeds from one another. All statistical analyses were performed using R statistical computing software v4.0.3 (R Core Team, 2020).

Maximum metabolic rate

Due to logistical constraints, we focused our further metabolic experiments on *Galaxias maculatus*. This species is commonly used as a benchmark for designing fish passes in New Zealand because of its slow swimming speeds compared to other migratory species (Crawford et al. 2024 *a*). This species was also chosen because of its cultural and commercial significance in New Zealand, its widespread distribution in the temperate Southern Hemisphere, and its tendency to experience higher temperatures due to its adult habitat being in low-lying areas closer to the coast (McDowall 1990). Maximum metabolic rate

(MMR) of *Galaxias maculatus* was recorded immediately after termination of U_{crit} trials at 15°C and 26°C.

Galaxias maculatus was used as our model species for measuring maximum metabolic rate (MMR) as it exhibited the lowest Ucrit (bl s^{-1}) when compared to a range of native species (Crawford et al, 2024a). Immediately after fatigue, post-exercise maximum metabolic rates of Galaxias maculatus individuals were measured using a closed circuit respirometry unit, following the methods of Parisi et al. (2020). Fish length and weight were recorded before (MMR) trials. The closed circuit respirometry unit consisted of a 115 mL plastic container connected to a recirculating flow system. The respirometer was placed in a water bath held at the trial temperature (either 15°C or 26°C). Maximum metabolic rate was measured for 16 *Galaxias maculatus* at 15°C and 20 *Galaxias maculatus* at 26°C. The respirometer was connected to a Loligo System oxygen sensor, which measured oxygen concentration (% air saturation) every minute for 15 min. The metabolic rate of the fish was calculated using the following equation from Schurmann and Steffensen (1997):

$$\text{MMR} = -1 \times \Delta O_2 \times V \text{ x } M_{\text{fish}}^{-1} \times \beta O_2$$

Where: ΔO_2 is percent change in air saturation per hour); V is volume of the respirometer in litres, without the mass of the fish (assuming a density of 1 g ml^{-1}); M_{fish} is the mass of the fish in grams; βO_2 is the solubility of oxygen (mg L^{-1}) at the trial temperature (10.08 mg L-^{1} at 15°C and 8.11 mg L^{-1} at 26°C) (YSI, 2019).

An ANCOVA was used to test for the effects of water temperature on oxygen consumption in *Galaxias maculatus*. Maximum metabolic rate calculated as oxygen consumption (mg O_2 g^{-1} h^{-1}) was the response variable, with water temperature as the categorical predictor variables and length, weight, and critical swimming speed as the continuous predictor variables. To assess model fit, a Likelihood Ratio Test was used, starting with a fully saturated model including interactions. The interaction between water temperature and weight was statistically significant and kept in the model. Average length and critical swimming speed were not statistically significant but were kept in the model as these variables are known to influence MMR (Norin and Clark, 2016).

4.4. Results

Post-hoc pairwise comparisons using Tukey's Honestly Significant Difference (HSD) revealed that critical swimming speed was reduced significantly in *Galaxias maculatus*, *Galaxias brevipinnis*, and *Gobiomorphus cotidianus* at 26℃ compared to the 8℃ or 15℃ treatments (Table 4.3, Figure 4.1). There was no significant difference in critical swimming speed at any of the three temperature treatments for *Galaxias fasciatus*. There was also no statistically significant effect of fish length on critical swimming speed for any of the species, however low p-values were recorded. The lack of significance may be a result of small sample size reducing statistical power or reflect the relatively narrow range of fish lengths tested.

Table 4.3: Summary of Type III analysis of covariance with Satterthwaite's method comparing the difference between critical swimming speed with temperature and length for each species. d.f. = degrees of freedom.

Species	Source	Sum of squares	Mean squares	Numerator d.f.	Denominator d.f.	F-value	p-value
Galaxias maculatus	Temperature	46.2	23.1	2	50	3.43	0.01
	Length	20.5	20.5	1	50	3.05	0.09
Galaxias fasciatus	Temperature	44.6	22.3	2	55	0.99	0.38
	Length	3.7	3.8	1	55	0.17	0.68
Galaxias brevipinnis	Temperature	201.9	101.0	2	56	2.49	0.09
	Length	53.9	53.9	1	56	1.33	0.25
Gobiomorphus cotidianus	Temperature	233.2	116.6	2	56	6.32	0.003
	Length	3.3	3.4	1	56	0.18	0.67

Water temperature had a significant effect on Ucrit of Galaxias maculatus ($p = 0.01$). Pairwise comparisons showed that mean Ucrit was statistically significantly different at 26°C (4.35 bl s^{-1}, SD 2.44) when compared to both the 8°C and 15°C treatments (6.11 bl s^{-1}, SD 2.62 and 6.45 bl s^{-1}, SD 2.85 respectively; Table 4.3, Table 4.4, Figure 4.1). There was no statistically significant difference in mean Ucrit between the 15°C and 8°C treatments for Galaxias maculatus (Table 4.4). The effect of fish length on Ucrit was not statistically significant at the predetermined significance threshold ($p < 0.05$); however, a p-value higher than the threshold was recorded ($p = 0.09$; Table 4.3).

Water temperature had no statistically significant effect on Ucrit for Galaxias fasciatus ($p = 0.38$; 8°C: 10.34 bl s^{-1}, SD 3.31; 15°C: 8.28 bl s^{-1}, SD 4.27; 26°C: 9.78 bl s^{-1}, SD 6.03; Table 4.3, Figure 4.1). Pairwise comparisons showed no statistically significant difference between each of the three temperature treatments (Table 4.4, Figure 4.1). The effect of fish length on Ucrit was not statistically significant ($p = 0.68$; Table 4.3).

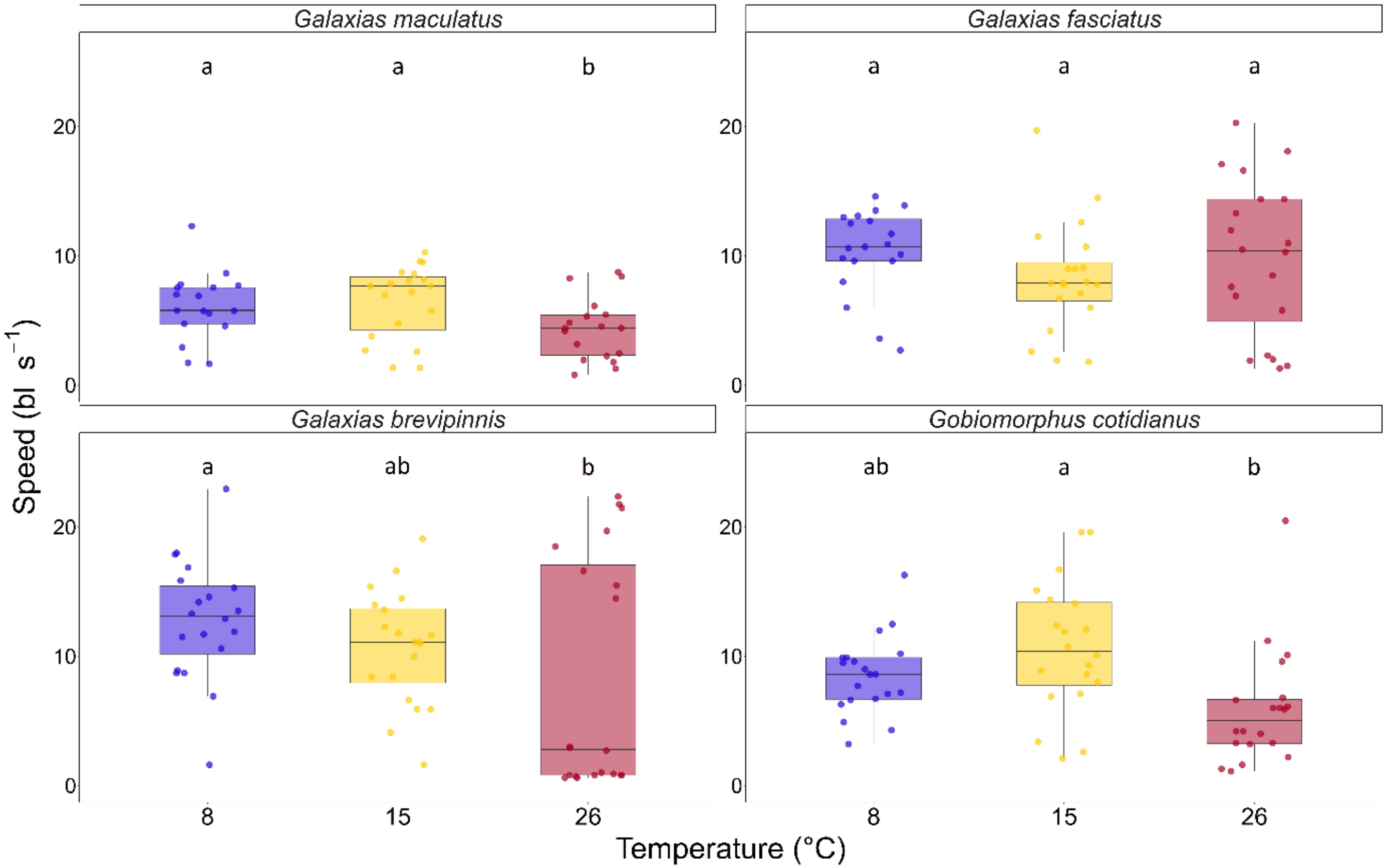
Water temperature had no statistically significant effect on U_{crit} of *Galaxias brevipinnis* at the 0.05 threshold ($p = 0.09$; Table 4.3, Figure 4.1). Pairwise comparison showed mean U_{crit} was statistically significantly different at 26°C (8.29 bl s^{-1}, SD 9.01) when compared to 8°C (12.79 bl s^{-1}, SD 4.65; Table 4.4, Figure 4.1). There was no significant difference between the 15°C treatment (10.64 bl s^{-1}, SD 4.39) and the 26°C, or between the 15°C and the 8°C treatment (Table 4.4). Fish length had no statistically significant effect on critical swimming speed ($p = 0.25$; Table 4.3).

Water temperature had a statistically significant effect on U_{crit} of *Gobiomorphus cotidianus* ($p = 0.003$; Table 4.3, Figure 4.1). Pairwise comparisons showed mean U_{crit} was statistically significantly different at 26°C (5.85 bl s^{-1}, SD 4.47) when compared to 15°C (10.67 bl s^{-1}, SD 5.04; Table 4.4). There was no statistically significant effect of the 8°C treatment on U_{crit} when compared to 15°C (8.5 bl s^{-1}, SD 3.02 and 10.67 bl s^{-1}, SD 5.04 respectively) or between the 8°C and the 26°C treatment (Table 4.4). Fish length had no statistically significant effect on critical swimming speed of *Gobiomorphus cotidianus* ($p = 0.67$; Table 4.3).

Table 4.4: Results of Post-hoc Tukey HSD tests for each species.

Species	Temperature comparison (℃)	Mean difference	p-value
Galaxias maculatus	8 – 15	0.23	0.96
	8 – 26	-2	0.07
	15 – 26	-2.23	0.03
Galaxias fasciatus	8 – 15	-2.06	0.37
	8 – 26	-0.54	0.93
	15 – 26	1.5	0.58
Galaxias brevipinnis	8 – 15	-2.15	0.54
	8 – 26	-4.49	0.04
	15 – 26	-2.35	0.48
Gobiomorphus cotidianus	8 – 15	2.18	0.25
	8 – 26	-2.64	0.13
	15 – 26	-4.82	0.002

Figure 4.1: Boxplots of critical swimming speed for to each temperature treatment (8℃, 15℃, 26℃) for each species, measured in body lengths per second. Individual fish in each treatment group are represented by points. The centre bar of each box corresponds to the median, while the box outer edges indicate the interquartile range. The whiskers extend to 1.5 times the interquartile range. Within each plot, letters above the bars represent temperature treatments that are significantly different from one another ($p < 0.05$). Groups sharing the same letter are not significantly different ($p > 0.05$).



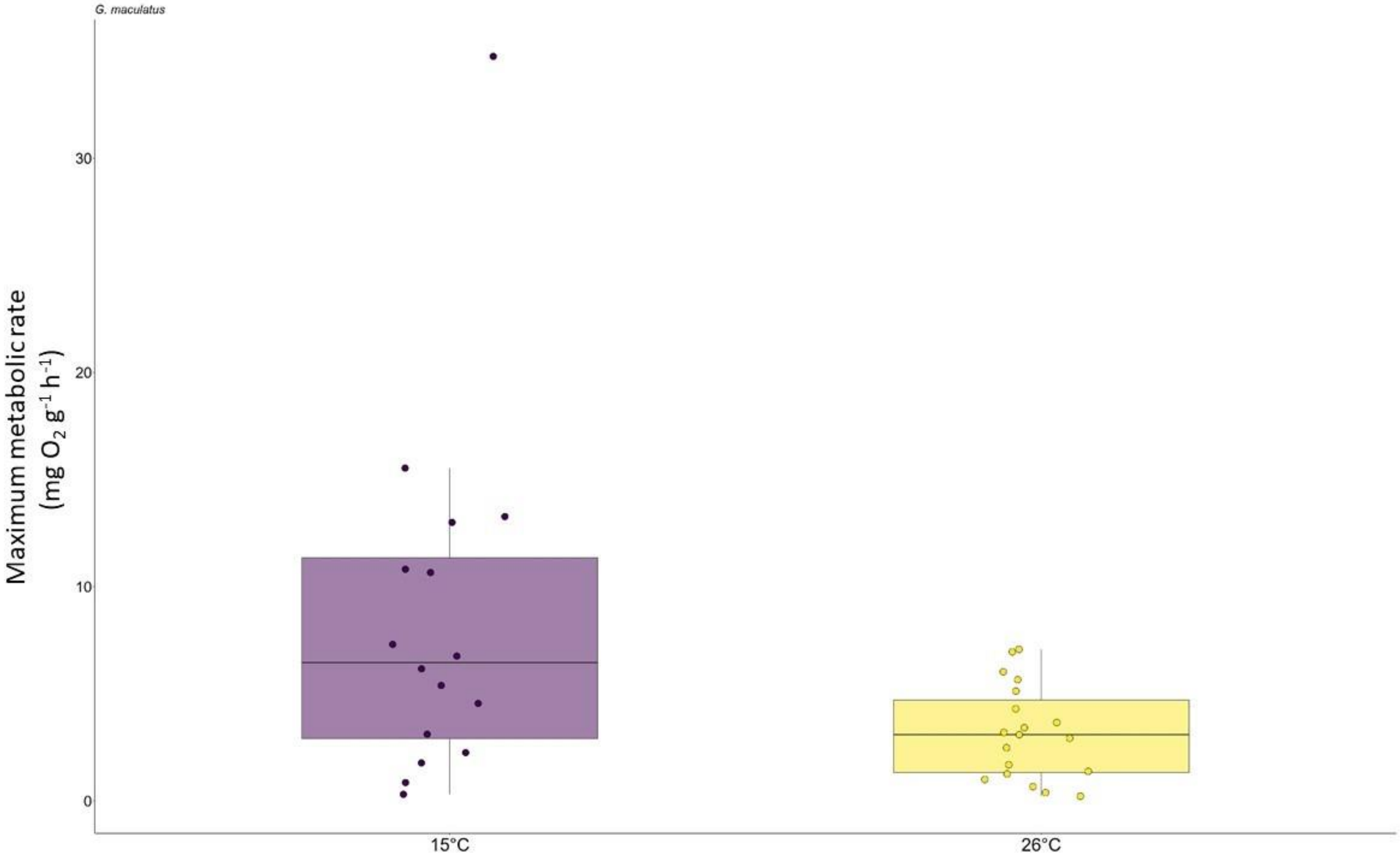
Maximum metabolic rate

Maximum metabolic rate was significantly reduced (p = 0.006) in *Galaxias maculatus* at 26℃ (3.19 mg O_2 g^{-1} h^{-1}, SD 2.19) when compared to 15℃ (8.54 mg O_2 g^{-1} h^{-1}, SD 8.43) (Table 4.5, Figure 4.2). There was greater variation in oxygen consumption at 15℃, with maximum metabolic rates ranging from 0.31 to 15.54 mg O_2 g^{-1} h^{-1} (with an outlier at 34.76mg O_2 g^{-1} h^{-1}) and a larger standard deviation, when compared to the 26℃ treatment (ranging from 0.23 to 7.08 mg O_2 g^{-1} h^{-1}). We assume there was a methodological issue with the outlier, as the fish had an average U_{crit} (5.1 bl s^{-1}) and was of average length. When the outlier was removed from the analysis, MMR at 15℃ was still significantly higher than 26℃. Fish weight was statistically significant (p = 0.034) and so was the interaction between water temperature and fish weight (p = 0.01).

Table 4.5: Summary of Type III analysis of covariance with Satterthwaite's method comparing the difference between *Galaxias maculatus* post-exercise oxygen consumption (mg O_2 g^{-1} h^{-1}) at 15℃ and 26℃.

Source	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F-value	p-value
Temperature	236.5	236.49	1	29	8.63	0.006
Weight	135.5	135.46	1	29	4.94	0.034
Length	19.8	19.78	1	29	0.72	0.402
Critical swimming speed	8.3	8.34	1	29	0.30	0.585
Temperature:Weight	205.5	205.55	1	29	7.50	0.010

Figure 4.2: Boxplots of post-exercise maximum metabolic rate (mg O2 g^{-1} h^{-1}) compared to each temperature treatment (15℃ and 26℃) for *Galaxias maculatus.*



4.5. Discussion

Our research sought to understand the effect of acute water temperature on swimming performance of several different migratory fish species native to New Zealand, encompassing a range of physiological, morphological, and life history traits. *Galaxias maculatus* and *Gobiomorphus cotidianus* had significantly reduced critical swimming speeds at 26℃ when compared to 15℃. There was no observed difference in swimming ability between the 8℃ and 15℃ treatments for either species. This corresponds with previous research by Bannon (2006), who showed that *Galaxias maculatus* had reduced swimming performance at high temperatures. There was no observed difference in swimming ability between 26℃ and 8℃ for *Gobiomorphus cotidianus* and *Galaxias brevipinnis.*

At the highest water temperature *Galaxias brevipinnis* critical swimming speeds exhibited a bimodal distribution and a large variation of swimming performance among individuals, which was not observed at the other two temperatures. This may indicate an intraspecific effect of temperature on individuals, whereby some individuals have a higher thermal tolerance than others. Or this may be an indicator that 26℃ is on the threshold of the upper thermal tolerance for this species (Taylor et al. 1997; Norin and Clark 2016). Previous research has shown that there are significant variations in swimming speeds both within and between populations of a species, due to variations in individual traits such as length, weight, and metabolism (Beamish 1978; Boily and Magnan 2002; Ojanguren and Brana 2003; Metcalfe et al. 2016; Jones et al. 2020; Crawford et al. 2024 *a*). This is consistent with the range of metabolic rates that we observed in *Galaxias maculatus* at both the temperate and high temperature treatments (Supplementary material: Table 4.5, Figure

4.2). However, it should be noted that while each *Galaxias brevipinnis* individual was uniquely identified, there is a possibility of misidentification, which may have contributed to the bimodal response observed in the temperature treatment.

Temperature effects on fish swimming performance typically follow a bell-shaped curve, with a peak in swimming performance at an optimum temperature range, and lower performance at temperature extremes (Brett 1971; Beamish 1978; McKenzie and Claireaux 2010). This is often referred to as a thermal performance curve. In our study, we did not observe a significantly lower swimming performance at our low temperature treatment (i.e. 8°C) for any of our test species and life stages. The 8°C treatment falls within the normal range of what these species might experience in the wild, particularly *Galaxias brevipinnis* as they can penetrate further inland to higher elevations and may be adapted to cope with these cooler temperatures (McDowall 1990; Richarson et al. 1994). However, lower temperatures than we studied may elicit a negative response in swimming performance, for example, Bannon (2006) showed that the larval life stage of *Galaxias maculatus* had significantly reduced swimming performance at 5°C.

Research has demonstrated that increasing temperatures come with increased energy costs for fish (Dickson et al. 2002; McKenzie et al. 2007; McKenzie and Claireaux 2010). Higher temperature affects metabolic processes, consistently leading to an exponential increase in maintenance metabolism and standard metabolic rates across species, due to the energy demands of maintaining homeostasis (Brett 1971; Fry 1971; Taylor et al. 1997). Temperature also affects maximum metabolic rate (MMR), which is the highest attainable level of aerobic metabolism (McKenzie and Claireaux 2010). As temperatures rise, MMR typically increases and is strongly correlated to increased aerobic swimming ability due to increased energy fluxes and metabolic demands (Rome 1990; Taylor et al. 1997). Typically, as temperatures approach species' upper thermal limits, MMR may plateau or even decrease. This decrease in MMR is attributed to the detrimental effects of high temperatures on convective oxygen transport and is correlated to a reduction in aerobic swimming ability (Jones 1971; Taylor et al. 1997). The upper thermal limit is species and life stage specific, whereby some species are more susceptible to high temperatures than others, leading to differences in species MMR and swimming ability (McKenzie and Claireaux 2010). For instance, *Oncorhynchus mykiss* had both a reduction in aerobic swimming ability and a reduction in MMR at high temperatures, indicating that high temperatures significantly constrain aerobic swimming ability for this species (Taylor et al. 1996). Conversely, *Dicentrarchus labrax* exhibited increasing MMR and increasing aerobic swimming ability at high temperatures, indicating that high temperatures enhance aerobic capacity (Claireaux et al. 2006).

In our study, we observed both a decrease in critical swimming speed and a decrease in maximum metabolic rate in *Galaxias maculatus* when exposed to a high-temperature treatment (26°C). Notably, *Galaxias maculatus* prefers temperatures between 18-20°C, with a lethal limit recorded at 30.5°C (Richardson et al. 1994). This suggests that 26°C approaches the upper limit of thermal tolerance for this species and likely the other Galaxiids studied. This is supported by Simons (1986), who proposed that Galaxiids should not be exposed to temperatures above 26-27°C, as fish exhibited behavioural abnormalities and increased mortality beyond this range. If we consider 26°C as the upper thermal limit for Galaxiids, we expect both a decrease in swimming performance and maximum metabolic rate. At these elevated temperatures, at the upper end of thermal tolerance, fish are compelled to allocate more energy to oxygen uptake and routine maintenance (SMR), reducing aerobic capacity for other activities (Taylor et al., 1997; Norin and Clark, 2016). This shift in energy allocation has been shown to result in reductions in critical swimming speeds in other species (Claireaux et al. 2006; Norin and Clark 2016).

Fish, particularly migratory species, frequently encounter temperature fluctuations, especially in freshwater systems as they migrate between habitats. This natural thermal heterogeneity is increasingly exacerbated by human alterations to freshwater environments (e.g. cold-water releases from dams, excessive abstraction, hot water discharges, urban stormwater runoff) (Caissie 2006; Somers et al. 2013; Parisi et al. 2020; Pander et al. 2024). Temperature during time of migration has been found to be a significant factor for migratory success and successful passage through instream structures (Haro et al. 2004; Meixler et al. 2009; Caudill et al. 2013). Fish encountering higher temperatures due to instream structures often delay migration through these structures (Caudill et al. 2013). When temperatures in fishways approach or exceed thermal limits, fish experience high stress levels. Multiple barriers with water temperature changes are expected to compound adverse effects on fish fitness, ultimately reducing migratory success due to increased energetic costs associated with migration. However, many models that assess fish passage barriers and barrier prioritisation have not previously addressed the temperature effect on migrating fish (Meixler 2009). A new model put forward by Meixler (2021) highlights the importance of including temperature as a factor when assessing fish passage barriers and the need to understand the effects of temperature on fish swimming abilities to determine passage success.

Potential delays in migration become especially evident when we calculate the expected time for a fish to pass through a structure of a given length. For instance, considering the average length of culverts in New Zealand (20 m) and the average water velocity through a culvert (0.37 m s^{-1}) (Franklin et al. 2022; New Zealand Fish Passage Assessment Tool Database, https://fishpassage.niwa.co.nz/, retrieved 15 March 2024) along with the mean U_{crit} of *Galaxias maculatus* swimming at 15°C, we would anticipate the fish to pass through the culvert in approximately 197 s. Under the same conditions, but with the average U_{crit} of *Galaxias maculatus* swimming at 26°C, we would no longer expect them to successfully traverse the culvert due to the water speed exceeding U_{crit} at this temperature, meaning they make negative progress overground. This reduction in migration ability is supported by Caudill et al. (2013), who found that a temperature gradient of over 1°C across a fish ladder corresponded to changes in fish body temperature, indicating departure from acclimation temperature during passage.

Climate change, along with escalating habitat modification and land use alterations, will result in heightened discharge variability, elevated average stream temperatures, and shifting temperature and runoff patterns (Paul and Meyer 2001; Morgan and Cushman 2005). Additionally, it will amplify the severity and frequency of droughts and floods (Milly et al. 2005; Alcamo et al. 2007). All these factors will lead to greater extremes in thermal heterogeneity that may be hard to predict. Restoring connectivity is one of the six actions identified in the Emergency Recovery Plan for global freshwater biodiversity (Tickner et al. 2020). To restore connectivity, it is important to consider temperature effects on fish dispersal and swimming ability. If maximum swimming speeds decrease as temperature increases, we need to design fish passes for lower swimming speeds than have previously been measured. This reduction in swimming ability could culminate in reductions in species' dispersal abilities, with implications for their ongoing ability to access suitable habitats.

Previous research has shown that temperature effects on fish are species specific. For example, some studies have demonstrated that cold water (more so than warm water) has significant negative impacts on temperate and tropical fish swimming performance (Johnston and Dunn 1987; Fangue et al. 2015; Parisi et al. 2020). Muhawenimana et al. (2021) observed that in a temperate location, warmer waters improved invasive species' swimming performance. This species-specific response to temperature is highlighted by our research. There was no observed effect of water temperature on *Galaxias fasciatus* swimming performance, but there was a significant reduction in swimming performance at high temperatures for both *Galaxias maculatus* and *Gobiomorphus cotidianus*. In *Galaxias brevipinnis*, we observed a bimodal distribution in swimming performance at high temperature, indicating that temperature effects also vary within a species.

We have demonstrated the importance of accounting for individual variation in swimming ability when developing water velocity design criteria for fish passage (Crawford et al. 2024 *a*). Water temperature adds an additional dimension of variability that should be accounted for to consider future fish passage design criteria.

Chapter 5

Group swimming significantly increases passage efficiency rate for a galaxiid species

Galaxias maculatus; Photo credit: Rachel Crawford

This chapter has been submitted as a research article in the Journal of Fish Biology:

Crawford, R.M.B.; Gee, E.M.; Hicks, B.J.; Franklin, P.A. (2024). Group swimming significantly increases passage efficiency rate for a galaxiid species.

5.1. Abstract

While there is a growing body of research exploring the relationship between fish social behaviour and fish passage success, most studies focus on the potential negative effects of fish density rather than the positive benefits of group swimming. In addition, much of the existing research concentrates on large-bodied species, such as salmonids, which may not be transferable to small-bodied species. We conducted laboratory experiments to explore the potential benefits of collective navigation for a small-bodied migratory species, *Galaxias maculatus*. An artificial velocity barrier was created to compare swimming success of groups of fish to solitary individuals. Swimming success was measured using several metrics including binomial success models and time-to-event analysis. Active metabolic rates were also measured as indices of energetic expenditure. Our findings revealed that fish swimming in groups have faster entry and passage rates compared to solitary individuals. Successful fish in groups displayed lower metabolic rates, suggesting the potential benefit of reduced energy expenditure for schools of fish. These findings underscore the importance of designing fish passes that accommodate gregarious species, ensuring improved success for fish populations overall. By considering the advantages of group navigation, fish passage structures can be tailored to better facilitate the movement of diverse fish species through aquatic environments.

5.2. Introduction

Globally, millions of instream structures create fragmentation in river networks, acting as impediments to fish movements (Grill et al. 2015; Belletti et al. 2020; Franklin et al. 2022). These impediments to upstream access for fishes have major ecological consequences, drastically changing fish populations, causing population declines, and even extirpation (Warren and Pardew 1998; Lucas and Baras 2001; Radinger and Wolter 2014; Silva et al. 2018). Consequently, it is common to see retroactive additions of fish passage structures or remediation efforts aimed at increasing the upstream migration of fishes. (Katopodis and Williams 2012; Silva et al. 2018; Birnie-Gauvin et al. 2019). Meta-analyses have continued to show highly variable and often poor passage efficiency (e.g. Roscoe and Hinch 2010; Bunt et al. 2012; Hershey 2021). To improve passage efficiency of fishways, we need to consider both environmental and behavioural factors influencing swimming performance of fishes as they swim upstream (Roscoe and Hinch 2010).

Swimming in groups, also known as schooling, has been shown to provide several benefits to fish including hydrodynamic advantages, navigational aids, and physiological effects that may have important implications for improving performance through fishways (Weihs 1973; Johansen et al. 2010; Ward et al. 2011; Marras et al. 2015; Okasaki et al. 2020). Schooling fish gain hydrodynamic advantages, which may help them overcome velocity challenges created by instream barriers. Some schooling fish experience reduced drag and increased lift, potentially reducing overall metabolic demand (Parker 1973; Marras et al. 2015; Nadler et al. 2016). There are three potential hydrodynamic benefits to explain this: the vortex hypothesis, the channelling hypothesis, and the wingtip-uplift hypothesis (Weihs 1973; Abrahams and Colgan 1987). The vortex hypothesis states that the vortices within the group reduce velocity relative to the individual. This is supported by more recent research showing that fish actively alter their body mechanics when swimming in vortices created by other members of the school, which reduces the energy required to swim at a given speed (Lindsey 1978; Liao 2003; Marras et al. 2015). The channelling hypothesis states that neighbouring fish in a group improve the thrust efficiency of an individual. The wingtip-uplift hypothesis states that neighbouring wakes provide lift to an individual (Weihs 1973). These methods of decreasing metabolic demands in schooling fish could increase their ability to overcome speed constraints.

Group size or local density may also influence the motivation of individual fishes to swim through an instream structure. When local density is high, some fishes may be less motivated to swim upstream (Dominy 1973; Goerig and Castro-Santos 2017). This is particularly problematic when large-bodied fishes like salmon are overcrowded and less likely to migrate through an instream structure (Dominy 1973; Johnson et al. 2012; Lemasson et al. 2014; Goerig and Castro-Santos 2017). Many large-bodied species can have strong social hierarchy, with larger individuals occupying dominant positions that make them less likely to swim upstream as density increases (Goerig and Santos 2017). Yet the role of social hierarchy on motivating the movement of smaller or habitually schooling fishes remains unknown. Migratory juvenile galaxiids, such as *Galaxias maculatus*, are relatively similar in size and school in the thousands, which likely reduces the effect of social hierarchy. Alternatively, some adult life stage galaxiids, like *Galaxias fasciatus,* are territorial with large adults dominating territories that may motivate non-dominant individuals to try to find other regions of the stream to inhabit (David and Stoffels 2003).

In terms of navigational benefits, group travel during migration can have major benefits for a fish’s ability to navigate to its intended destination by reducing navigational errors (Larkin and Walten 1969). Social interactions and group cohesion significantly enhance each individual's ability to align toward and reach a target or direction, particularly in environments with low levels of environmental turbulence (Codling et al. 2007). This may be due in part to individuals improving accuracy by combining their individual estimates, referred to as the “many wrongs” hypothesis (Larkin and Walton 1969). Other features of collective navigation include emergent sensing, leadership, social learning, and collective learning (Berdahl et al. 2018). However, it has been found that as environmental turbulence increases, individuals may benefit more from travelling on their own (Codling et al. 2007).

Schooling behaviours improve each individual’s fitness and survival (Weihs 1973; Johansen et al. 2010; Marras et al. 2015; Nadler et al. 2016). Schooling reduces predation risk and increases foraging and migration success (Abrahams and Colgan 1987). Physiologically, swimming in groups can have “calming effects” on fish, reducing stress levels and potentially improving overall performance (Parker 1973; Lefrançois et al. 2009; Nadler et al. 2016). The presence of conspecifics may provide a sense of safety and familiarity, as it both lowers stress hormones as well as increases regulation of antioxidant genes that mitigate stress (Gilmour and Bard 2022; Schumann et al. 2023). This effect is known as “social buffering” (Gilmour and Bard 2022; Schumann et al. 2023). These findings, taken together, suggest that fish living in groups experience reduced stress, with overall lower metabolic demand and reduced energy expenditure compared to solitary individuals (Parker 1973; Lefrançois et al. 2009; Nadler et al. 2016). However, the benefits of schooling fish compared to solitary individuals are compromised by anthropogenic disturbances that force typically schooling fish into social isolation that could cause negative physiological consequences (Nadler et al. 2016). Thus, poorly designed fish passes may be not just ecologically, but also physiologically detrimental to the fish that they block (Jones et al. 2021).

The failure to comprehend the importance of fish behaviour and, consequently, the variability in fish swimming performance, poses a challenge in designing effective fish passage solutions. Collective navigation can have a major effect on passage through dams, with lasting implications for survival, reproductive success, and population stability (Okasaki et al. 2020). However, current practices often neglect the nuances of social interactions in fish passage design. Experimental data collected from studying individual fish swimming isolation raises questions about the transferability of such findings to real-world conditions. In natural environments, the behaviour and physiology of groups of fish may differ significantly from solitary fish.

The limited information on how social interactions influence barrier passage highlights the need to understand the interplay of physiology, behaviour, and the environment on passage success. This understanding is particularly important when assessing how behaviours like schooling impact successful passage of instream structures. This is especially true for gregarious amphidromous and catadromous species (Coutant and Whitney 2000). Island freshwater fish communities have a high prevalence of obligatory amphidromy and in New Zealand, many migratory species are both amphidromous and small-bodied at migration (Franklin and Gee 2019). This life history and size constraint poses additional challenges to migration through anthropogenic structures. As there is a lack of

New Zealand specific research focusing on the interplay of fish biology and behaviour, it is important that we explore these mechanisms to create truly effective fish passage solutions. In this study, we tested whether schooling improves the ability of fish to traverse an artificial barrier. We used *Galaxias maculatus* as an experimental species as they have a wide distribution throughout the Southern Hemisphere and are an amphidromous species often used as a benchmark in New Zealand for evaluating fish passage barriers (Franklin et al. 2018; Crawford et al. 2024 *a*). Additionally, *G. maculatus* display gregarious behaviour, schooling in the thousands during their upstream migration as small-bodied juveniles. We hypothesised that the schools of fishes would be more successful at traversing a barrier than individual fishes.

5.3. Methods

The fish collection for this research was carried out under NIWA’s MPI special permit SP666-4. All experimental trials for this study were run with approval to manipulate live animals for the purpose of research by the NIWA Animal Ethics Committee (AEC204) in accordance with the requirements of section 83 of the New Zealand Animal Welfare Act 1999.

5.3.1. Fish collection and handling

Juvenile *Galaxias maculatus* were collected using fyke nets and Gee minnow traps from Parker Stream, Waikato (37°36'10.6"S 175°08'44.5"E). Fish were transported to the NIWA Hamilton fish laboratory in coolers filled with aerated water from where they were collected. Fish were kept in the coolers until the water temperature equilibrated with the laboratory tank water temperature and were then transferred to 60 L quarantine tanks with

6 ppt salinity to prevent disease. After one week, fish were transferred to 60 L freshwater tanks. Fish were held in a temperature-controlled room and kept on a 12-hour light, 12-hour dark cycle. The tanks were kept on a recirculating water system and the temperature held at 18℃ ± 0.5℃. Fish were fed bloodworms every other day and were fasted for 24 hours prior to experimentation to ensure a postabsorptive state. Ammonia and pH concentrations were checked regularly, and water changes were completed when ammonia levels were greater than or equal to 0.25 mg L^{-1}.

5.3.2. Experimental setup

Experiments were conducted using an indoor recirculating flume as previously detailed in Miller et al. (2002). The flume was constructed from acrylic plastic and measured 7.5 m in length, 0.5 m in width, and 0.5 m in depth, holding up to 1,500 L of water in the working section of the flume, with a return pipe of 0.4 m diameter running beneath it. A flow straightener consisting of stacked PVC tubes measuring 0.02 m in diameter and 0.3 m long was placed at the upstream end of the flume. The water speed was controlled by an impeller located in the descending section of the return pipe. This impeller was driven by a variable-speed AC motor. Spot water temperature measurements were taken at the beginning and end of each trial using a handheld thermometer. Temperature ranged from 17.9 – 22.2°C with a mean of 21.1°C (SD: 0.96) across all trials.

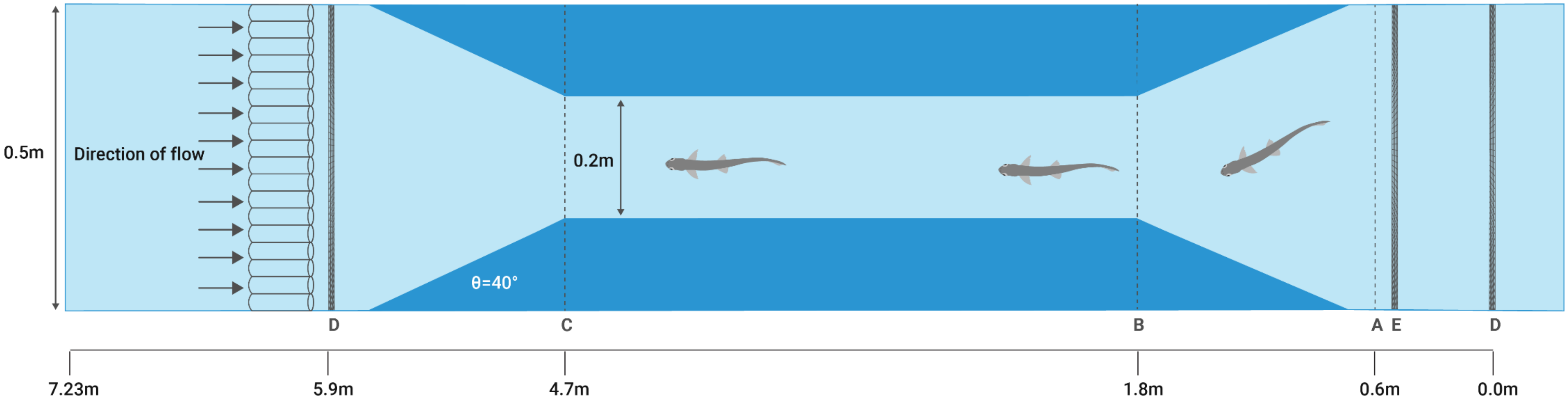
A raceway was installed to constrict the flow within the flume to create a water speed challenge for the fish. The raceway was designed so that the maximum water speed in the constricted section ranged between 0.45 and 0.50 m s^{-1}. This was based on the findings of Crawford et al. (2024 *a*), which showed that only the top 10% of *G. maculatus* individuals achieved critical swimming speeds of greater than 0.45 m s^{-1}.

The test section was delineated from 0 m to 5.9 m in the working section of the flume. The raceway was constructed of PVC (Figure 5.1) and consisted of two sets of 1.2 m long wingwalls on the upstream and downstream ends of the raceway set at a 40° angle relative to the flume wall. The body of the raceway was 2.9 m long and 0.2 m wide. The depth of water in the raceway was maintained at 0.20 m throughout all trials. Mesh screens were placed upstream and downstream of the raceway to keep the fish inside the test area. The base of the raceway was marked with tape every 0.1 m starting from zero at the entrance to the downstream wingwalls. The raceway had additional markings denoting the approach, entry, and passage thresholds in the raceway (0.6, 1.8, and 4.7 m respectively), marked from the end of the downstream working section within the flume (Figure 5.1).

For analysis of passage, the raceway was delineated at three points (Figure 5.1):

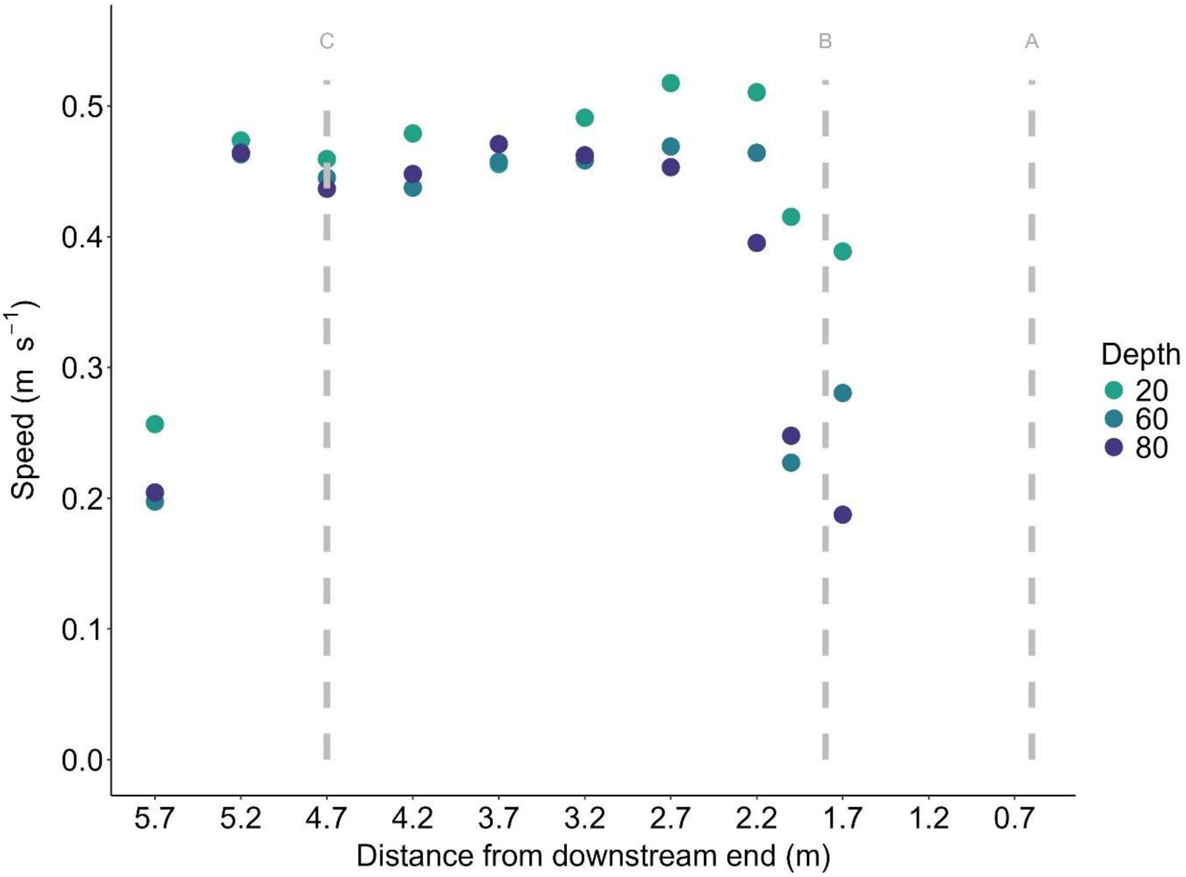
- Approach threshold – marking the point where fish crossed the threshold into the approach of the raceway (Figure 5.1, A).
- Entry threshold – marking the point of entry into the raceway (Figure 5.1, B).
- Passage threshold – marking the point where fish pass through the upstream end of the raceway (Figure 5.1, C).

Figure 5.1: Aerial view experimental setup, focusing on the working section containing the raceway. (A) Line marking the "approach" to the raceway, (B) line marking the "entry" to the raceway, (C) line marking successful "passage" of the fishway, (D) mesh screens (visible as dark bars) to confine fish within of the flume, E) removeable mesh screen, containing fish in the "starting pool" for acclimation before the start of the trial.



To characterize the variation in water speed throughout the raceway, a Sontek Flow Tracker 2 Acoustic Doppler Velocimeter (ADV) was used. Three-point vertical profiles of water speed were made at 0.5 m intervals along the centre of the channel, along the axis of the flume in the direction of flow. Measurements were made at heights of 20%, 60%, and 80% of the water column above the raceway floor, starting at 0.2 m from the downstream end of the raceway. At each height, speed measurements were collected for 40 s at a sampling frequency of 10.0 MHz, measured in m s^{-1}. After sampling, data were downloaded in FlowTracker2 desktop software, where water speed was averaged across each depth at each station, to map out the water speeds experienced by the fish (Figure 5.2).

Figure 5.2: ADV measurements throughout the raceway taken at 20%, 60%, and 80% depth of the water column. The grey dashed lines labelled A, B, and C correspond to the approach, entry, and passage lines depicted in Figure 5.1.



Five Swan security cameras were set up 1.1 m above the flume floor, evenly distributed across the length of working section, creating a slight overlap between camera frames. The video recording of every camera was synchronized throughout the duration of the trial to track fish movement. Video was recorded in colour, and all cameras were set at 30 frames per second.

5.3.3. Experimental protocol

Due to fish availability and timing of migration, a total of 37 group trials of five fish in each trial, and 58 solitary trials of a single fish were conducted (Table 5.1; Supplementary Tables D1-D4). Before being placed in the flume, body length and weight of all fish were measured. Condition factor of the fish was calculated using Fulton's condition factor (Froese 2006):

$$K = 100 \frac{W}{L^3}$$

Where K is the condition factor, W is the mass of the fish in g, and L is the length of the fish in cm.

Fish were placed in the downstream pool before the start of the trial, with a mesh screen preventing them from entering the raceway (Figure 5.1). The fish were allowed to acclimate in the pool for 30 min in still water to reduce the effects of handling stress.

After acclimation, the cameras were turned on to record fish movement for the 30 min trial. The impeller motor was set to 31 Hz, resulting in a water speed between 0.45 and 0.5 m s^{-1} in the working section of the raceway. Once the speed in the flume was set, the downstream mesh screen was removed. During the trial, the times that fish approached the raceway, entered the raceway, and finished the raceway were recorded. For trials using groups of fish, the time of each event was collected for every individual. The times at which the fish crossed the approach, entry, and passage thresholds were recorded from the time-stamped video recordings to the nearest second. Only the first attempt for each fish was recorded to prevent duplicating data but in general fish did not show multiple attempts for each threshold. For fish swimming in groups, we tracked individuals and recorded the behaviour of each fish. To minimise the effect of holding time on swimming performance, both group and solitary experiments were usually conducted each trial day. While this was not a fully randomized trial, it represented an effort to mitigate the effects of holding time and variations in migratory urges across treatment groups.

Table 5.1: Data for group vs. solitary trials, including the number of replicates tested in each category, mean total lengths, mean total weight, mean condition factor, water temperature, and standard deviations. Further detailed information can be found in Supplementary material, Tables D1-D4.

Category	Number of replicates	Mean length (mm)	Mean weight (g)	Mean condition factor (k)	Mean water temperature (°C)
Group	37	57.83 ± 4.05	0.81 ± 0.22	0.41 ± 0.05	21.45 ± 0.52
Solitary	58	54.36 ± 7.62	0.62 ± 0.38	0.34 ± 0.10	20.14 ± 1.34

5.3.3.1. Active metabolic rate

At the end of each trial, fish that successfully traversed the raceway were placed into a closed circuit respirometry unit to measure post-exercise active metabolic rate, following the methods of Parisi et al. (2020). For each group trial, all successful fish from that group were placed in a single respirometer together. The respirometer consisted of a 115 mL plastic container connected to a recirculating flow system. The respirometer was placed in a water bath held at ambient trial temperature. A Loligo System oxygen sensor was connected to the respirometer, measuring oxygen concentration (% air saturation) at one-minute intervals for a period of 15 min

The metabolic rate of solitary swimming fish was calculated using the following equation from Schurmann and Steffensen (1997):

$$MR = -1 \times \Delta O_2 \times V \text{ x } M_{fish}^{-1} \times \beta O_2$$

Where ΔO_2 represents the rate of change of oxygen (% air saturation per hour); V represents volume of the respirometer in L, without the mass of the fish (assuming a density of 1 g ml^{-1}); M_{fish} represents the mass of the fish in g; βO_2 represents the solubility of oxygen in water (mg L^{-1}) at the ambient trial temperature ranging from 9.47 L^{-1} at 18°C to 8.11 mg L^{-1} at 22°C.

5.3.4. Analysis

All analyses were carried out in R version 4.2.0 (R Core Team, 2020).

5.3.4.1. Binary success model

Success was modelled with binary logistic regression, where a fish was given a 1 for successfully crossing a threshold or a 0 for failing to cross a threshold. To model the effect swimming in groups had on the success of fish passing each threshold (approach, entry, passage), we compared the success of groups to individuals, with the response variable defined as the proportion of fish in each replicate that were successful (i.e. groups: 0, 0.2, 0.4, 0.6, 0.8, or 1; solitary: 0 or 1) A binomial generalized linear mixed effects model (GLMM) model was used, with proportion successful as the response variable and trial number as a random predictor variable. Fish length and weight were not included in the model due to our inability to uniquely identify these characteristics for individual fish within groups in the flume. For this model, a logit link function was selected without adjusting for overdispersion. A separate model was applied to the approach, entry, and finish thresholds of the raceway. Analysis was carried out using the glmmTMB package in R (Brooks et al. 2017). A Hosmer-Lemeshow test was used to assess model goodness of fit using the Resource Selection R package (Lele 2009).

5.3.4.2. Time-to-event analysis

We used time-to-event analysis, also known as survival analysis, to assess the rate of fish attempting the various stages within the raceway (approach rate, entry rate, and passage rate) (Castro-Santos and Haro 2003; Goerig and Castro-Santos 2017). Time-to-event analysis enables the inclusion of censored data, allowing us to account for fish that did not successfully pass each threshold of the raceway by the end of the 30 min trial. Time-to-event analysis calculates the likelihood that a fish will cross a threshold within the raceway at a particular time, given that it had not yet crossed that threshold (i.e. the event rate). We refer to the event rate at the approach threshold, entry threshold, and passage threshold as the “approach rate,” “entry rate,” and “passage rate,” respectively. The Cox regression model was used to model instantaneous event rate at each threshold, which was right censored and modelled as a function of time as shown in the following equation, and explained by Goerig and Castro-Santos (2017):

$$\lambda(t) = \lambda_0(t)e^{\mathrm{X}\beta+\mathrm{Z}b},$$

Where $\lambda(t)$ is the baseline hazard function (i.e. event rate) modelled as a function of time (t), X represents the matrix of fixed effect, Z represents the matrix of random effects, β and *b* represent the fixed- and random-effect coefficients respectively. Group status (group vs solitary fish) was the fixed effect, with trial number included as a frailty term to account for random effects. Based on this equation, hazard ratios (HR) are calculated for each treatment (group status), comparing the event rates between groups and solitary fish. A hazard ratio greater than 1 indicates that the treatment (group) has a higher event rate compared to the reference (solitary); a hazard ratio less than 1 indicates the treatment (group) has a lower event rate compared to the reference (solitary); a hazard ratio equal to 1 indicates no difference in event rate between either treatment.

These models were implemented using the Coxme package in R version 4.2.0 (R Core Team 2020; Therneau 2015). Fish length and weight were not included in the model due to our inability to uniquely identify these characteristics for individual fish within groups in the flume. The Likelihood Ratio Test, Wald Test, and Score (Logrank) Test were used to identify model fit. We developed three distinct models to answer specific questions:

1. The first model addresses the approach rate: the likelihood of a fish to cross the approach threshold per unit of time.
2. The second model addresses the entry rate: the likelihood of a fish to cross the entry threshold per unit of time.
3. The third model addresses the passage rate: the likelihood of a fish to cross the passage threshold per unit of time.

We then used the survfit function from the Coxme package to calculate the proportion of fish that are predicted to cross each threshold at a given time for each event rate model. As time progressed, the predicted proportion of fish crossing the threshold decreased because the number of available fish diminished over time.

5.3.4.3. Active metabolic rate

An ANCOVA was used to test for the effects of group vs solitary swimming on post-exercise oxygen consumption. Oxygen consumption (mg O_2 g^{-1} h^{-1}) was the response variable, with group as a categorical predictor variable, and average fish weight and average fish length as the continuous predictor variables. A Likelihood Ratio Test (LRT) was used to determine the model of best fit for predictor variables starting with a fully saturated model including interactions. All possible interactions between body length and fish weight were not statistically significant and were removed from the model.

5.4. Results

5.4.1. Modelling binary success

Of the 37 groups of fish, 31 groups had at least one fish successfully approach the raceway (84 %). Twelve groups had all five fish approach the raceway and six groups of fish had no individuals approach the raceway (Table 5.2). Of the 58 solitary fish, 42 individuals successfully approached the raceway (72 %), and 16 individuals did not. There was no statistically significant effect of group on the proportion of fish that successfully approached the raceway (p = 0.41; Table 5.3).

Table 5.2: Comparison of the success of groups to individuals, with the response variable defined as the proportion of fish in each replicate that were successful (i.e. groups: 0, 0.2, 0.4, 0.6, 0.8, or 1; solitary: 0 or 1). The number of replicates of each proportion for each raceway threshold are represented below.

Raceway threshold	**Group proportion**						**Solitary**	**Proportion**
	0	**0.2**	**0.4**	**0.6**	**0.8**	**1**	**0**	**1**
Approach	6	1	5	4	9	12	16	42
Entry	6	1	8	2	9	11	26	32
Passage	6	1	8	2	9	11	34	24

Thirty-one groups had at least one fish successfully enter the raceway (84 %). Eleven groups had all five fish enter the raceway and six groups of fish had no individuals enter the raceway (Table 5.2). Of the 58 solitary fish, 32 individuals successfully entered the raceway (55 %), and 26 individuals did not. There was no statistically significant effect of group on the proportion of fish that successfully entered the raceway (p = 0.54) (Table 5.3).

Thirty-one groups had at least one fish successfully pass the raceway (84 %). Eleven groups had all five fish pass the raceway and 6 groups had no fish pass the raceway (Table 5.2). Of the 58 solitary fish, 24 individuals successfully completed the raceway (41 %). There was no statistically significant effect of group on the proportion of fish that successfully completed the raceway at the p = 0.5 threshold (p = 0.06; Table 5.3). All three models fit model assumptions based on the Hosmer-Lemeshow test (Chi^2 values that fall below the 95% confidence interval, and high p- values).

Table 5.3: Model summaries for each of the three binary logistic regression models, predicting the likelihood of success for groups of fish compared to solitary fish, for each raceway threshold.

Threshold	Variable	Coefficient estimates	Standard error (±)	Z score	p-value
Approach	Group	0.38	0.45	0.83	0.41
Entry	Group	-0.27	0.43	-0.62	0.54
Passage	Group	-0.82	0.43	-1.91	0.06

5.4.1.1. Time-to-event analysis

Of the 95 trials in this study, 6 groups of fish and 16 individuals failed to approach the raceway but were still included in the analysis and censored at the maximum trial time. There was no statistically significant effect of group status on approach rate (p = 0.59; Table 5.4). Groups of fish had a slight increase in approach rate compared to solitary fish, with a hazard ratio (HR) of 1.14, with a confidence interval of 0.54 – 1.41. This indicates that groups are 14% more likely than solitary fish to cross the approach threshold by the next point in time. However, the confidence interval spanning 1 suggests insufficient evidence to support a significant difference in approach rates between groups and individuals. Groups of fish had a median approach time of 645 s and solitary fish had a median time of 997 s (Figure 5.3).

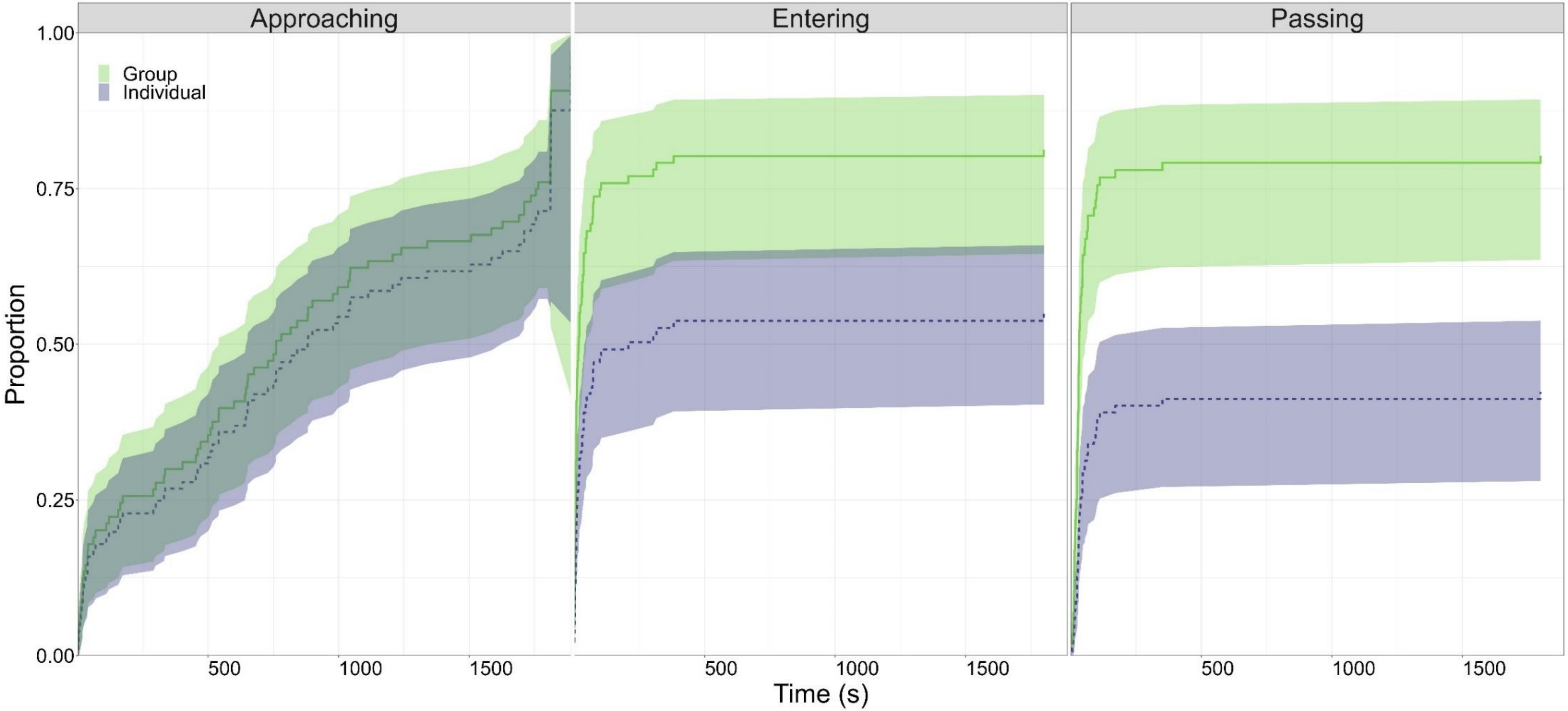
Table 5.4: Estimation of parameters for the selected model of event rate model for each raceway threshold. The models included group status as a predictor variable and trial number as a frailty term.

Threshold	Parameter	Coefficient ± SE	Hazard ratio	p-value
Approach	Group	0.13 ± 0.24	1.14	0.59
	Frailty (Trial)	-	-	0.93
Entry	Group	0.74 ± 0.26	2.10	0.004
	Frailty (Trial)	-	-	0.93
Passage	Group	1.08 ± 0.28	2.95	< 0.001
	Frailty (Trial)	-	-	0.93

Six groups of fish and 26 individuals failed to enter the raceway and were censored at the maximum trial time. There was a statistically significant effect of group status on entry rate (p = 0.004; Table 5.4, Figure 5.3). Groups of fish had a higher entry rate with a HR of 2.22, indicating groups were 122% more likely than solitary fish to cross the entry threshold by the next point in time. Groups had a median entry time of 15.8 s compared to solitary fish with a median entry time of 207 s (Figure 5.3).

Six groups of fish and 34 individuals failed to pass the raceway and were censored at the maximum trial time. There was a statistically significant effect of group status on passage rate ($p < 0.001$; Table 5.4, Figure 5.3). Groups of fish had a higher passage rate, with a HR of 2.49, indicating groups were 149% more likely to cross the passage threshold by the next point in time than solitary fish. Groups had a median passage time of 34.5 s. However, fewer than 50% of solitary fish crossed the passage threshold meaning that we were unable to determine a median passage time (Figure 5.3).

Figure 5.3: Event rate models for each raceway threshold, representing the proportion of fish crossing each threshold as a function of time. Zero on the y-axis represents that 0% of the population have crossed the threshold at that time (t), and one represents that 100% of the fish have crossed the threshold at time (t). Data are stratified by group status, where groups of fish are represented by the solid green line and solitary fish are represented by the dashed purple line. Shaded regions in the corresponding colours represent the 95% confidence intervals. Frailty terms are not included in the figure model.



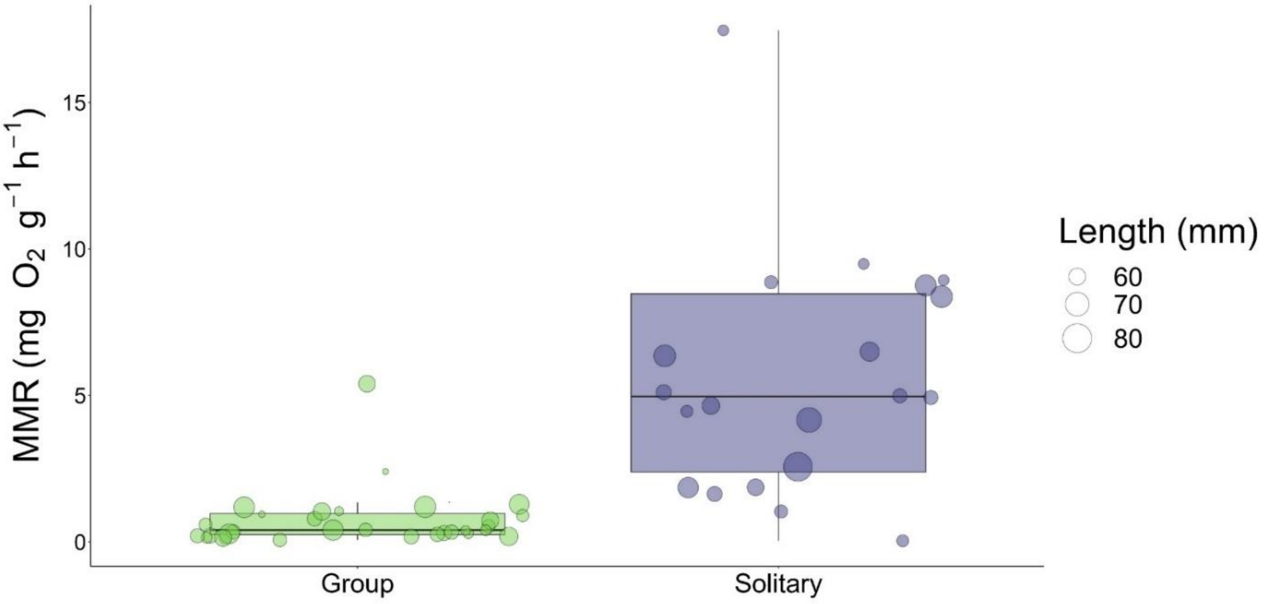
5.4.2. Active metabolic rate

Successful fish swimming in groups had significantly lower active metabolic rates (MMR) than successful solitary fish ($p < 0.001$; Table 5.5, Figure 5.4). Fish weight did not have a statistically significant effect on MMR ($p = 0.12$) but was kept in the model as weight is a known factor influencing metabolic rates (McKenzie and Claireaux 2010; Norin and Clark 2016).

Table 5.5: Summary of Satterthwaite's Type III analysis of covariance comparing the active metabolic rates of group vs solitary swimming fish. d.f = degrees of freedom

Source	Sum of squares	Mean squares	Numerator d.f.	Denominator d.f.	F-value	p-value
Group	115.6	115.6	1	49	21.47	<0.001
Weight	18.1	18.1	1	49	2.50	0.12

Figure 5.4: Boxplots of active metabolic rates (mg O_2 g^{-1} h^{-1}) of fish swimming in groups compared to individual solitary swimming fish. The data points within each treatment group represent individual replicates, and are sized based on average fish length, with larger points representing larger fish. Green represents groups and purple represents solitary fish.



5.5. Discussion

This study used binomial success and event rate, as indices of successful fish passage, and active metabolic rates as an index of energetic expenditure. Our study design offered an experimental approach to assess the effects of group swimming on successful passage in an artificial raceway, with the ability to manipulate group size, water speed, and to control for variables such as water temperature, dissolved oxygen, and turbulence. The results of this study found that fish swimming in groups had higher entry rates and higher passage rates when compared with solitary swimming fish. However, there was no effect of group on approach rates. There was also no measurable effect of group on the approach success, entry success, or passage success probability, at the $p = 0.05$ threshold.

Possible explanations for the higher passage rates include physiological or social benefits associated with group swimming (Weihs 1973; Parker 1973; Lefrançois et al. 2009; Nadler et al. 2016; Berdahl et al. 2018), as indicated by the reduced active metabolic rates. We observed that successful fish from groups had significantly lower metabolic rates when compared to successful solitary fish. However, as we did not measure the metabolic rates of all fish individually, we cannot definitively say whether the fish in groups had inherently lower active metabolic rates compared to the solitary individuals tested.

Group swimming did not seem to influence the overall probability of approach or entry success. For *Galaxias maculatus*, it appears that group behaviour may not serve as an explanatory factor for fishway attraction or for motivation to enter the raceway. However, the advantages of group swimming become apparent during the successful traversing of the raceway. We observed significantly faster times for groups crossing the passage threshold, with the majority of fish in groups passing the raceway in under 60 s.

For the initial stages of entry and approach, individual differences appear to play a more significant role than collective behaviour. We theorise that personality may be involved, and that bolder individuals exhibit a higher likelihood of approaching a challenge compared to shyer ones, consistent with previous studies (Mensinger et al. 2021). It has been observed that bold individuals tend to commit quickly to actions and exhibit less variability in their movements (Dahlbom et al. 2011). This may contribute to why we did not observe any benefit of group swimming on approach rates or probability of approaching or entering the raceway. However, once in the raceway, schooling behaviour demonstrates more pronounced benefits for the overall success and time reduction in completing the raceway. Given that the length of the raceway was greater than that of the approach or entrance (reflecting conditions in the field), we suggest that fish may gain more collective and hydrodynamics advantages when traversing the raceway itself compared to the approach or entrance, as the length of the raceway may amplify any potential benefits of collective navigation.

Studies on the impacts of social interactions on fish passage success have largely focused on salmonid species (Dominy 1973; Johnson et al. 2012; Lemasson et al. 2014; Goerig and Castro-Santos 2017; Okasaki et al. 2020). These studies typically focus on existing fishways using fish density as a predictor variable for passage success. Since these structures have predetermined size constraints, much of this research has focused on the negative effects of high fish densities on passage, rather than potential benefits of collective navigation. In contrast, our research took an alternative approach by using an experimental raceway where we controlled the number of individuals in each trial, effectively removing any confounding effects of overcrowding within the structure. This setup also allowed us to directly compare the passage performance of solitary fish to groups of fish, which is not as easily done in a real-world fishway. The combination of previous research highlighting the negative effects of overcrowding in fishways and our findings demonstrating the positive effect of group swimming on passage success, supports our management recommendation. Specifically, our results suggest that fish passage structures should be designed with dimensions that accommodate the collective navigation of large schools.

Additionally, it is important to understand how social interactions impact collective navigation and passage success for a variety of species to create more tailored fish passage solutions (Birnie-Gauvin et al. 2019). While researching density effects of salmonids in a fishway, Okasaki et al. (2020) found that the effects of social interactions are species-dependent. Their findings revealed that chinook salmon benefit from higher densities during passage, while sockeye salmon did not show any density-dependent response. They suggest that some fish species, like chinook salmon, might use their conspecifics as navigational guides or homing signals, enhancing their ability to negotiate challenging passages. In contrast, sockeye salmon may not have relied on such social cues for navigation, leading to a lack of positive effects from increased density.

Given that group swimming does not seem to offer benefits in terms of approach or entrance rates, it suggests a need for improved strategies in fishway entrance design to cater to more individualized behaviour. This could involve creating features that specifically attract less bold fish, ensuring that entrances are located where fish aggregate and flows attract fish to the entrance (Tan et al. 2021b). However, the significant positive impact of group swimming on the overall success of fish passage highlights the importance of designing fishways to accommodate group movement. Practical examples may include constructing larger resting pools or low-speed zones capable of accommodating entire schools of fish simultaneously. Haro et al. (1998) found that the ability of Atlantic salmon and American shad to pass through weirs decreases as group size increases, which suggests that artificial structures not specifically designed to accommodate large groups of fish may pose challenges for successful passage of these groups. Given that many published studies underscore overcrowding as a hindrance to successful fish passage, there is a particular emphasis on designing fish passes that accommodate the natural school sizes of fish. This approach aims to mitigate the negative impacts associated with overcrowding, ensuring that fish can move through passage systems more effectively and continue to benefit from schooling. A more nuanced approach to the design of fishways could enhance their efficiency for both individual and group-oriented swimming behaviours.

Conclusion

Our study used a controlled experimental design to assess the impact of group swimming on passage success at a water speed barrier. The results showed that group swimming improved the proportion of fish successfully traversing the barrier. This was coupled with group swimming resulting in significantly faster times to successfully pass the barrier. These findings along with the observation that groups had significantly reduced active metabolic rates, suggest physiological and behavioural advantages contribute to the success of collective navigation. Although group swimming did not influence the initial stages of approaching and entering the raceway, schooling significantly improved overall success in raceway passage.

Chapter 6

Repeated exposure to a velocity barrier improves passage performance

Galaxias maculatus; Photo credit: Stuart Mackay, NIWA

6.1. Abstract

Instream structures like culverts and dams can impede upstream fish migration, acting as environmental filters that only allow onward migration of individuals that can successfully pass them. Cognition and learning ability may be an important factor in determining if a fish can successfully traverse such structures. This study investigated the effect of repeated exposure on passage performance of juvenile *Galaxias maculatus* through an experimental raceway. Over five consecutive days, individual fish were subjected to the same high-speed (0.45-0.5 m s^{-1}) conditions within the raceway, and performance on each day was recorded. The proportion of fish successfully passing the barrier increased significantly from 40% on Day 1 to 63% on Day 5. Time-to-event analysis further revealed that by Day 5, fish successfully passed the barrier at a significantly faster rate compared to Day 1. However, repeated exposure did not significantly improve approach or entry rates into the raceway. Fish length influenced approach rates, but not entry or passage rates. These findings suggest that cognition and spatial memory play a role in improving passage performance through velocity barriers, but other factors such as attraction flows may play a more important role in successful passage outcomes through velocity barriers.

6.2. Introduction

Instream structures such as culverts, dams, and weirs are known to act as “environmental filters” (Egger et al. 2020), selecting only the individuals that can swim past, with potentially drastic effects on fish populations above and below the barrier, including declines and even extirpation (Castro-Santos and Haro 2010; Bunt et al. 2012; Silva et al. 2018; Egger et al. 2020; Jones et al. 2021). Even a single cascade barrier exerts significant selection pressure and cumulative passage effects on the local fishes (Jones et al. 2021). These pressures favour specific phenotypic traits that promote barrier passage across morphology, sex, physiology, and swimming performance (Haugen et al. 2008; Volpato et al. 2009; Maynard et al. 2017; Jones et al. 2021). These effects are magnified by the separation of populations by such barriers, which has the potential to create phenotypically distinct populations above barriers compared to the populations below. Recent research has suggested that instream structures create selective pressures not just on physical traits, but on behavioural traits as well. For instance, eels with more exploratory personalities have greater success traversing fish passages than less exploratory eels, suggesting that behavioural filtering at fish passage barriers is a migratory impediment for cautious individuals (Mensinger et al. 2021).

Cognition, which is the process by which animals acquire, store, process, learn, and act on information, could also play a role in a fish’s ability to traverse a barrier (Shettleworth 2001; Sih and Del Giudice 2012; Thornton and Lukas 2012; Lucon-Xiccato and Bisazza 2017b; Boogert et al. 2018). Cognition is required for spatial navigation, a crucial task for fish migrating through riverine habitat or instream structures (Lucon-Xiccato and Bisazza 2017b).

Learning is a type of cognition in fishes that has been observed across various contexts, including foraging routes and behaviours, social behaviour, avoidance and recognition processes, spatial and landmark orientations, and migration (Werner et al. 1981; Myrberg and Riggio 1985; Dodson 1988; Suboski 1988; Quinn and Dittman 1990; Kieffer and Colgan 1992). Learning allows more flexibility in behaviour in a changing environment (Dodson, 1998), and may be cognitively preprogramed and adapted to different environmental factors (Lorenz et al. 1998; Brown and Laland 2003; Odling-Smee and Braithwaite 2003; Fernö et al. 2011).

The ability of fishes to learn their spatial environment and monitor it is essential to their daily survival. Many fishes live in complex freshwater habitats, where water characteristics (pools and riffles), changes in substrate, woody debris, and vegetation make spatial cognition an important adaptation for fish (Lucon-Xiccato and Bisazza 2017b). When fish encounter a stimulus outside of their previous experience, they may use Hebbian learning, a process where simultaneous activation of neurons strengthens the connection between them, to associate their behaviour and the environment (Fernö et al. 2011). Fish must continuously traverse through their environment and monitor their location with respect to external reference points, suggesting that they have significant spatial learning capacity (Odling-Smee and Braithwaite 2003). Likewise, the ability of fish to detect environmental changes (Welker and Welker 1958) and display organized exploration patterns when introduced to new environments (Kleerekoper et al. 1974) suggests the presence of spatial memory, which aids in their ability to adapt and thrive in their surroundings. Fishes are known to use this memory to both solve and remember spatial problems; for example, multiple exposures of guppies to a maze decreased the time and errors required to navigate it (Petrazzini et al. 2015; Lucon-Xiccato and Bisazza 2017a). These observed effects of complex maze learning may also be due in part to classical conditioning. In experimental systems fish received food and social rewards upon successful navigation of the maze, and in natural environments fish traverse complex landscapes to access food and conspecifics (Bull 1935; Overmier and Hollis 1990; Fernö et al. 2011; Lucon-Xiccato and Bisazza 2017a).

When encountering anthropogenic changes in the environment, it is hard to predict how fish might respond cognitively, and their ability to learn and overcome these challenges varies by situation, species, or individual (Fernö et al. 2011; Lucon-Xiccato and Bisazza 2017b; Dougherty and Guillette 2018). Anthropogenic instream structures may require particularly complex spatial learning that combines response learning (going left or going right) and place learning (memorization of mental representation and landmarks, i.e. create a cognitive map) (Kareklas et al. 2017). However, fishes appear to have some ability to learn and remember anthropogenic structures, which can mitigate the detrimental effects of anthropogenic stream structures and even save populations when human induced changes to migratory routes cause population decline (Fernö et al. 2011). Fish captured upstream of a fishway, having previously traversed it, are more efficient at later traversing the fishway compared to downstream-captured fish that are naïve to the fishway (Roscoe and Hinch 2010; Cooke and Hinch 2013; Hershey 2021). Individual learning may therefore provide a crucial pathway for fishes to survive in a changing environment.

The role of learning as a response to anthropogenic structures is mainly understood in marine fisheries, classic model species, and migration of charismatic, large-bodied, freshwater fishes like salmonids, with little insight into obligately migratory amphidromous or catadromous species (Franklin and Gee 2019, Roscoe and Hinch 2010). Yet the capacity for learning to improve outcomes for fishes whose migration routes have been disturbed by artificial barriers suggests that it is important to understand the interaction between fish cognition and the navigation of structures in streams.

Our research aimed to determine how repeated exposure influences an individual's ability to traverse a barrier such as a culvert with high water speed. In particular, we aim to improve understanding of the implications of multiple barriers on the migratory success of fishes. We studied the ability of learning to improve passage through a barrier in an obligate migratory fish, *Galaxias maculatus,* that migrates upstream as a juvenile, and is widely distributed in the temperate Southern Hemisphere (Augspurger et al. 2017; Ramírez-Álvarez et al. 2022).

6.3. Methods

Galaxias maculatus, or inanga, are often amphidromous and migrate upstream from the sea as juveniles (Augspurger et la. 2017), so we used migratory juveniles for this work. The collection of fish for this research was conducted under NIWA's MPI special permit SP666-4. All experimental trials were conducted with approval from the NIWA Animal Ethics Committee (AEC204), adhering to the guidelines outlined in section 83 of the New Zealand Animal Welfare Act 1999.

6.3.1. Fish collection and holding

Juvenile *Galaxias maculatus* were collected using whitebait nets and Gee minnow traps from the Waikato River, New Zealand (-37° 18' 8.7156"S, 174° 50' 7.4688"E). The fish were transported to the NIWA Hamilton fish laboratory in coolers containing aerated water sourced from their collection site. They remained in the coolers until the water temperature matched that of the laboratory tank water, after which they were transferred to 60 L quarantine tanks containing 6 ppt salinity. After one week, fish were transferred to 60 L freshwater tanks equipped with a recirculating water system. These tanks were located in a temperature-controlled room with a 12-hour light, 12-hour dark cycle. The water temperature was maintained at 18°C ± 0.5°C. Fish were fed bloodworms every other day, with a 24-hour fasting period before experimentation to ensure they were in a postabsorptive state. Regular monitoring of ammonia and pH concentrations was conducted, and water changes were performed when ammonia levels reached or exceeded 0.25 mg L^{-1}.

6.3.2. Experimental setup

Experiments were conducted using an indoor recirculating flume, as described in Miller et al. (2002). The flume, constructed from acrylic, had dimensions of 7.5 m in length, 0.5 m in width, and 0.5 m in depth, capable of holding up to 1500 L of water in its working section. Beneath the flume ran a return pipe measuring 0.4 m in diameter. To ensure smooth flow, a flow straightener made of stacked PVC tubes, each 0.02 m in diameter and 0.3 m long, was positioned at the upstream end of the flume. Water speed was regulated by an impeller driven by a variable-speed AC motor installed in the descending section of the return pipe. Spot water temperature measurements were taken using a handheld thermometer at the beginning and conclusion of each trial. Across all trials, flume water temperatures ranged from 17.9°C to 22.2°C, with a mean of 21.1°C (standard deviation: 0.96°C).

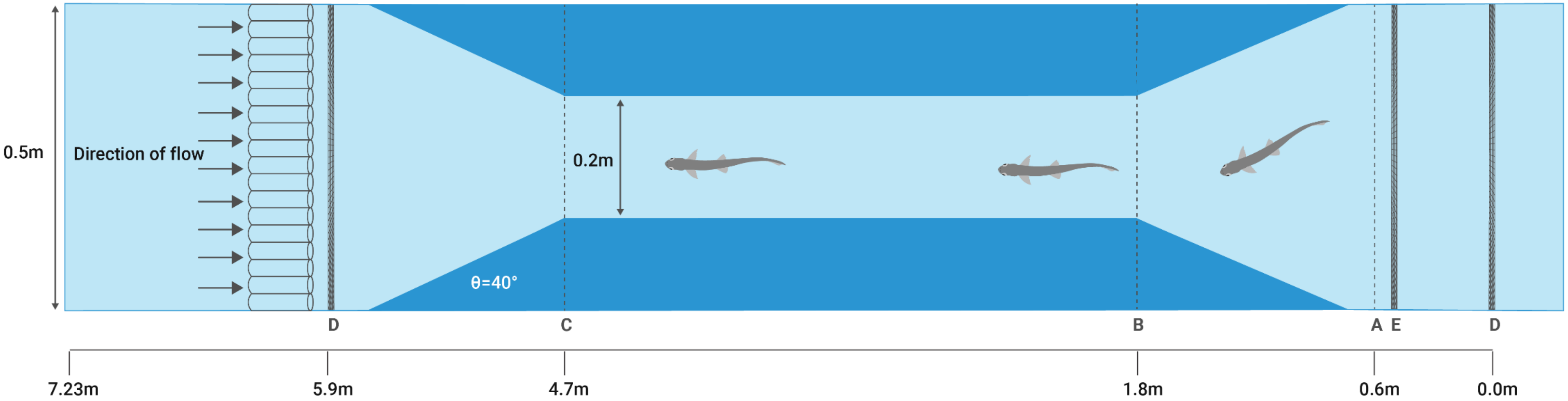
A raceway was built in the flume to constrict the flow, creating a water speed challenge for the fish. The design ensured that the maximum water speed in the constricted section ranged from 0.45 and 0.50 m s^{-1}. The water speed was chosen based on Crawford et al. (2024 *a*), which demonstrated that 90% of *G. maculatus* individuals had critical swimming speeds of 0.45 m s^{-1} or less.

The test section spanned from 0.0 m to 5.9 m within the working section of the flume. Constructed from PVC (see Figure 1), the raceway comprised two sets of angled wingwalls, each 1.2 m in length, positioned at a 40° angle relative to the flume wall, both upstream and downstream. The body of the raceway measured 2.9 m in length and 0.2 m in width, with a consistent water depth of 0.2 m maintained throughout all trials. Mesh screens were installed upstream and downstream of the raceway to contain the fish within the experimental area. The base of the raceway was marked with tape at 0.1 m intervals, starting from 0.0 at the entrance to the downstream wingwalls. Additional markings denoting the approach, entry, and passage thresholds within the raceway (0.6, 1.8, and 4.7 m, respectively) were positioned from the end of the downstream working section within the flume (Figure 1).

For analysis of passage, the raceway was delineated at three points (Figure 6.1):

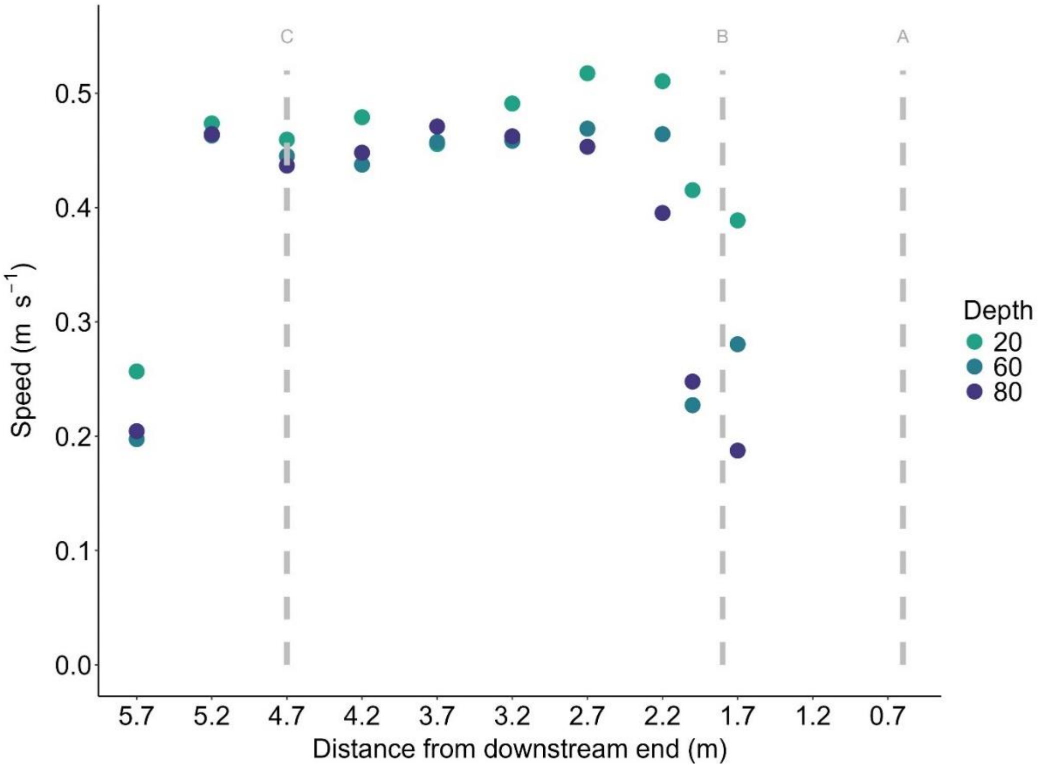
- Approach threshold - marking the point where fish crossed the threshold into the approach of the raceway (Figure 6.1, A).
- Entry threshold - marking the point of entry into the raceway (Figure 6.1, B).
- Passage threshold – marking the point where fish pass through the upstream end of the raceway (Figure 6.1, C).

Figure 6.1: Aerial view experimental setup, focusing on the working section containing the raceway. (A) Line marking the "approach" to the raceway, (B) line marking the "entry" to the raceway, (C) line marking successful "passage" of the fishway, (D) mesh screens (visible as dark bars) to confine fish within of the flume, (E) removeable mesh screen, containing fish in the "starting pool" for acclimation before the start of the trial



To assess the variation in water speed within the raceway, three-point vertical profiles of water speed were measured at 0.5 m intervals along the centre of the channel, starting 0.2 m from the downstream end of the raceway, along the axis of the flume in the direction of flow. These profiles were generated using a Sontek Flow Tracker 2 Acoustic Doppler Velocimeter (ADV). Measurements were taken at three heights: 20%, 60%, and 80% of the water column above the raceway floor. Water speed data, measured in m s^{-1}, were collected for 40 s at each height, with a sampling frequency of 10.0 MHz. Following data collection, the FlowTracker2 desktop software was used to average the water speed across each depth at each station and visualize the water speeds experienced by the fish (Figure 6.2).

Figure 6.2: ADV measurements throughout the raceway taken at 20%, 60%, and 80% depth of the water column. The grey dashed lines labelled A, B, and C correspond to the approach, entry, and passage lines depicted in Figure 6.1.



Five Swan security cameras were positioned 1.1 m above the flume floor, evenly spaced along the length of the working section with a slight overlap between camera frames. To track fish movement accurately, video recording from each camera was synchronized throughout the trial duration. All recordings were captured in colour, with all cameras set to a consistent frame rate of 30 frames per second.

6.3.3. Experimental protocol

A total of 40 trials were conducted, each trial consisted of an individual fish and no fish were reused: mean length of 51.7 mm ± 8.97; mean weight 0.48 g ± 0.32; mean condition factor 0.26 ± 0.09. Each trial was conducted over five consecutive days, where fish were exposed to the same experimental conditions once per day over this five-day period. Prior to their introduction into the flume, the body length and weight of each fish was recorded. The condition factor of each fish was calculated using Fulton's condition factor formula (Froese 2006):

$$\mathrm{K} = 100\ \frac{\mathrm{W}}{\mathrm{L}^3}$$

Where *K* is the condition factor, *W* is the mass of the fish in g, and *L* is the length of the fish in cm. Fish weight and condition factor were not included in any of the statistical model due to high correlation with length (0.85 and 0.76 respectively). At the start of the trial, fish were placed in the downstream pool, with a mesh screen in place to prevent them from entering the raceway (Figure 1). The fish were acclimated in the pool, where the water speed was maintained at 0.0 m s^{-1}, for a duration of 30 min to mitigate the potential effects of handling stress on the fish.

The cameras recorded fish movement for the duration of each 30-minute trial. The impeller motor was set to 31 Hz, ensuring water speed was between 0.45 and 0.5 m s^{-1} within the working section of the raceway. Once the speed in the flume was constant through the working section, the downstream mesh screen was removed. Throughout the trial, the times at which fish crossed the approach, entry, and passage threshold were recorded, based on time-stamped video footage to the nearest second. To avoid duplicating data, only the first attempt for each fish was recorded, although fish generally did not make multiple attempts at each threshold.

6.3.4. Analysis

All analyses were carried out in R version 4.2.0 (R Core Team 2020).

6.3.4.1. Binary success model

Success was modelled with binary logistic regression, where a fish was given a 1 for successfully crossing a threshold or a 0 for failing to cross a threshold. To model the effect that repeat exposure to a barrier had on the success of fish passing each threshold (approach success, entry success, passage success), a binomial Generalized Linear Mixed Effects Model (GLMM) model was used. Success (0 or 1) was the response variable, day (representing number of exposures) and fish length were fixed predictor variables, and fish number was included as a random predictor variable. For these models, a logit link function was selected without adjusting for overdispersion. A separate model was applied to each threshold of the raceway. Analysis was carried out using the glmmTMB package in R (Brooks et al. 2017). A Hosmer-Lemeshow test was used to assess model goodness of fit using the Resource Selection package (Lele 2009).

6.3.4.2. Time-to-event analysis

We used time-to-event analysis, also known as survival analysis, to assess the rate of fish attempting the various stages within the raceway (approach rate, entry rate, passage rate) (Castro-Santos and Haro 2003; Goerig and Castro-Santos 2017). Time-to-event analysis enables the inclusion of censored data, allowing us to account for fish that did not successfully pass each threshold of the raceway by the end of the 30 min trial. Time-to-event analysis calculates the likelihood that a fish will cross the threshold within the raceway at a particular time, given that it hasn't yet crossed that threshold (i.e. the event rate). We refer to the event rate at the approach threshold, entry threshold, and passage threshold as the "approach rate," "entry rate," and "passage rate," respectively. The Cox regression model was used to model instantaneous event rate at each threshold, which was right censored and modelled as a function of time as shown in the following equation, and explained by Goerig and Castro-Santos (2017):

$$\lambda(t) = \lambda_0(t)e^{\mathrm{X}\beta+\mathrm{Z}b},$$

Where $\lambda(t)$ is the baseline hazard function (i.e. event rate) modelled as a function of time (t), X represents the matrix of fixed effects, Z represents the matrix of random effects, β and *b* represent the fixed- and random-effect coefficients respectively. Day and fish length were the fixed effects, with fish number included as a frailty term to account for random effects. Based on this equation, hazard ratios (HR) were calculated for each treatment, comparing the event rates between days over time (representing the number of exposures). A hazard ratio greater than 1 indicates that the treatment (repeat exposure) has a higher event rate compared to the reference; a hazard ratio less than 1 indicates the treatment (repeat exposure) has a lower event rate compared to the reference (day 1); a hazard ratio equal to 1 indicates no difference in event rate between treatments.

These models were implemented using the Coxme package in R version 4.2.0 (R Core Team, 2020; Therneau, 2015). The Likelihood Ratio Test, Wald Test, and Score (Logrank) Test were used to identify model fit. We developed three distinct models to answer specific questions:

1. The first model addresses the approach rate: the likelihood of a fish to cross the approach threshold per unit of time.
2. The second model addresses the entry rate: the likelihood of a fish to cross the entry threshold per unit of time.
3. The third model addresses the passage rate: the likelihood of a fish to cross the passage threshold per unit of time.

We then used the survfit function form the Coxme R package to calculate the proportion of fish that are predicted to cross each threshold at a given time for each event rate model. As time progresses, the predicted proportion of fish crossing the threshold decreases because the number of available fish diminishes over time.

6.4. Results

6.4.1. Modelling binary success

Seventy-five percent of fish successfully approached the raceway on Day 1, compared to 78% on Day 5 (Table 6.1, Figure 6.3). There was an increase in fish successfully approaching the raceway on Day 2 (90%), however this increase was not significant and did not hold steady throughout the rest of the days. Statistical analysis using a binomial generalized linear mixed-effects model (GLMM) revealed no statistically significant effect of Day on approach success (p = 0.98, Table 6.2). Fish length was found to be a statistically significant factor influencing approach success (p = 0.01, Table 6.2).

On Day 1, 58% of fish successfully entered the raceway, compared to 63% on Day 5 (Table 6.1, Figure 6.3). Despite efforts to identify a best-fit model for entry success, none of the models, starting with a fully saturated model, passed the Hosmer-Lemeshow goodness of fit test. There was no statistically significant effect of day on entry success (p = 0.38, Table 6.2), and no statistically significant effect of fish length on entry success (p = 0.72, Table 6.2).

On Day 1, 40% of fish successfully passed the raceway compared to 63% on Day 5 (Table 6.1, Figure 6.3). There was a statistically significant effect of day on passage success (p = 0.017, Table 6.2), indicating that subsequent exposures to the barrier positively affected passage success. There was also a statistically significant effect of fish length on passage success (p = 0.017, Table 6.2). Further examination of the data revealed that there was a higher percentage of passage success on Day 3 and Day 4 compared to Day 1. However, this increase appeared to asymptote and even decrease after Day 4, with no significant difference between Day 4 and Day 5.

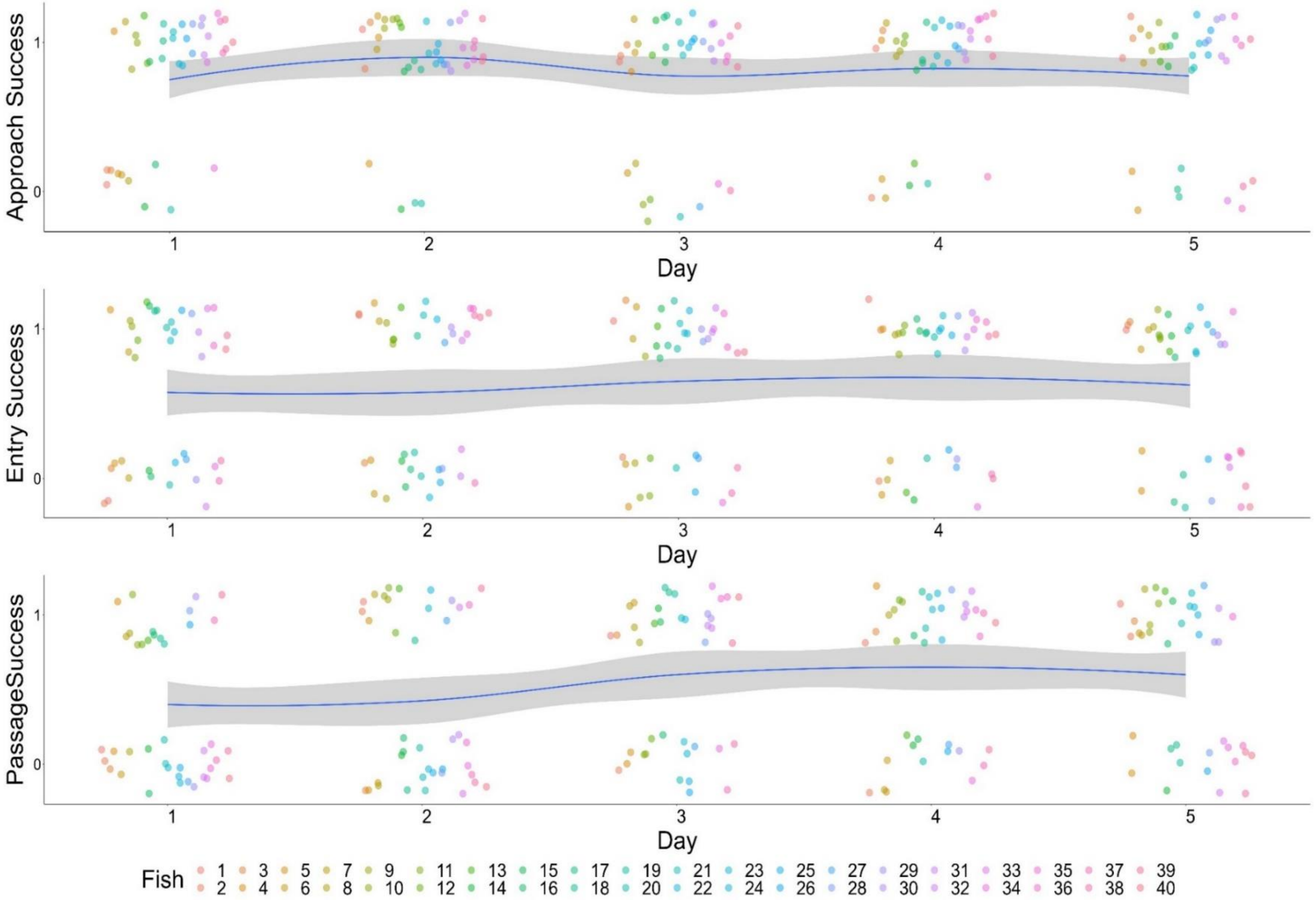
Table 6.1: Percent success of *Galaxias maculatus* replicates across all five trial days.

Day	Percent success		
	Approach	Entry	Passage
1	0.75	0.58	0.40
2	0.90	0.58	0.45
3	0.78	0.65	0.58
4	0.83	0.68	0.70
5	0.78	0.63	0.63

Table 6.2: Results of the binary logistic regression model predicting the likelihood of success across trial day for each raceway threshold.

Threshold	Parameter	Coefficient estimates	Standard error (±)	Z value	p-value
Approach	Day	0.003	0.13	-0.02	0.98
	Fish length	-0.08	0.03	-2.59	0.01
Entry	Day	0.10	0.11	0.88	0.38
	Fish length	-0.01	0.02	-0.35	0.72
Passage	Day	0.25	0.11	2.38	0.017
	Fish length	0.05	0.02	2.38	0.017

Figure 6.3: Binomial logistic regression of approach success, entry success, and passage success across five days. Each fish is represented by a point.



6.4.2. Time-to-event analysis

An average of 8 out of 40 fish failed to approach the raceway each day (range: 4-10 fish) but were still included in the analysis and censored at the maximum trial time (30 min). There was no statistically significant effect of repeat exposure (day) on approach rate (p = 0.99, HR = 1.0; Table 6.3, Figure 6.4). Day 1 fish had a median approach time of 325 s and Day 5 fish had a median time of 312 s (Table 6.4, Figure 6.3). The was a statistically significant effect of fish length on approach rates (p = 0.002), but the hazard ratio was indicated neither a positive nor negative effect of fish length on outcome (HR = 0.96; Table 6.3).

An average of 15 out of 40 fish failed to enter the raceway each day (range: 13-17 fish) but were still included in the analysis and censored at the maximum trial time. There was no statistically significant effect of repeat exposure (day) on entry rate (p = 0.46, HR = 1.03; Table 6.2, Figure 6.4). Day 1 fish had a median entry time of 548 s and Day 5 fish had a median time of 207 s (Table 6.4, Figure 6.3). The was not statistically significant effect of fish length on entry rates (p = 0.97, HR = 1.14; Table 3).

An average of 18 out of 40 fish failed to pass the raceway each day (range: 12-24 fish) but were still included in the analysis and censored at the maximum trial time. There was a statistically significant effect of repeat exposure (day) on passage rate (p = 0.021, HR = 1.17; Table 6.3, Figure 6.4). Median passage time was greater than the trial length on Day 1, and Day 5 fish had a median time of 42 s (Table 6.4, Figure 6.3). There was no statistically significant effect of fish length on passage rates at the p < 0.05 threshold (p = 0.11, HR = 1.02; Table 6.3). Passage success rates reached an asymptote by Day 5.

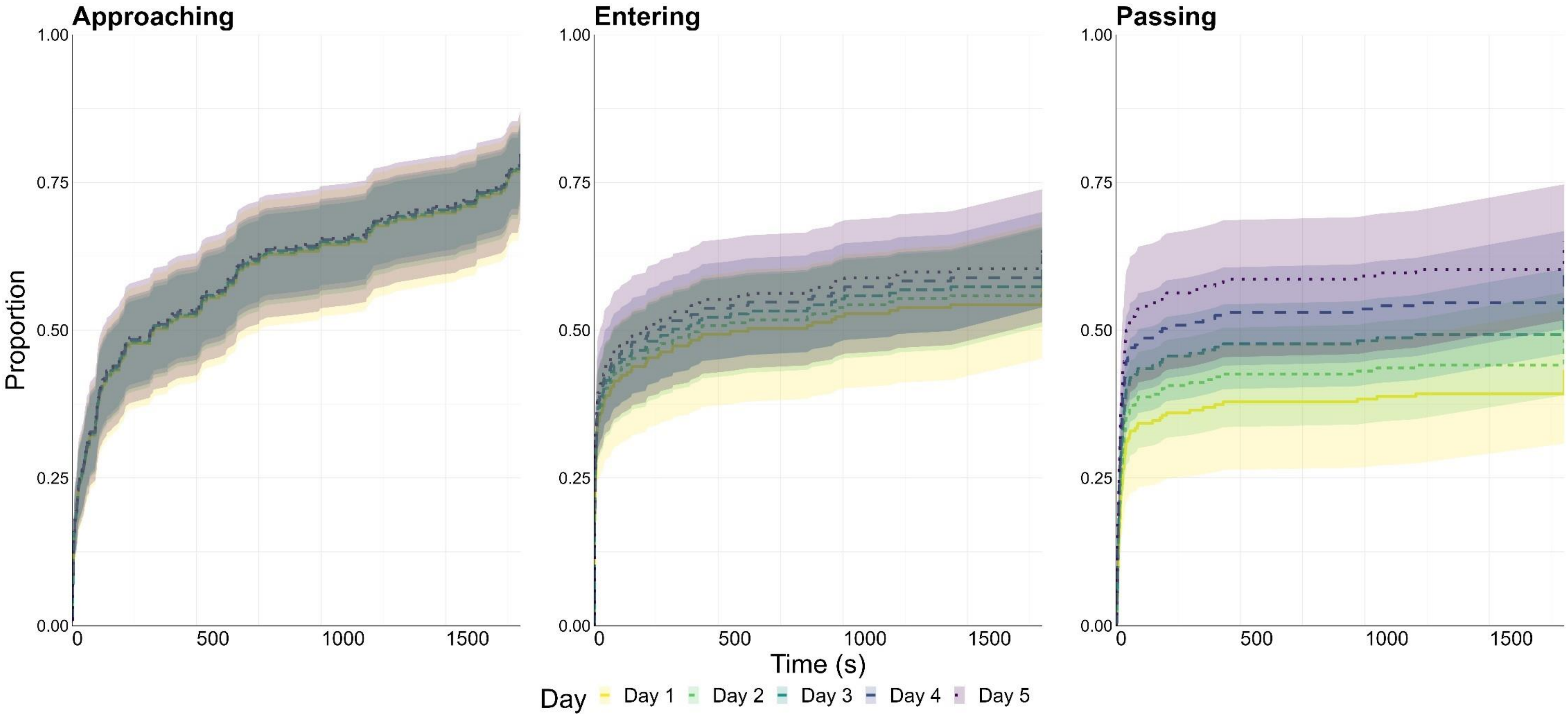
Table 6.3: Estimation of parameters for the selected model of event rate for each raceway threshold. The models included trial day and fish length as predictor variables, and fish number was included as a frailty term.

Threshold	Parameter	Coefficient ± SE	Hazard ratio	Chi^2	Degrees of freedom	p-value
Approach	Day	-0.0004 ± 0.06	1.00	0.00	1	0.99
	Fish length	-0.03 ± 0.007	0.97	9.90	1	0.002
	Frailty (Fish number)	-	-	21.04	13.52	0.09
Entry	Day	0.047 ± 0.06	1.03	0.54	1	0.46
	Fish length	-0.0004 ± 0.01	1.14	0.04	1	0.97
	Frailty (Fish number)	-	-	11.76	9.03	0.23
Passage	Day	0.15 ± 0.07	1.17	5.35	1	0.02
	Fish length	0.02 ± 0.01	1.02	2.59	1	0.11
	Frailty (Fish number)	-	-	0.00	0	0.92

Table 6.4: Median time to threshold values for the selected event rate model across all days. Null values in the table represent days where less than 50% of fish were successful. Total trial time was 1800 s.

Exposure Day	Median time to threshold (s)		
	Approach	Entry	Passage
Day 1	325	433	-
Day 2	325	390	-
Day 3	315	315	1205
Day 4	315	217	177
Day 5	312	207	42

Figure 6.4: Event rate models for each raceway threshold, representing the proportion of fish crossing each threshold as a function of time. Zero on the y-axis represents that 0% of the population have crossed the threshold at that time (t), and one represents that 100% of the fish have crossed the threshold at time (t). Data are stratified by exposure day (Day 1-5). Shaded regions in the corresponding colours represent the 95% confidence intervals. Frailty terms are not included in the figure model.



6.5. Discussion

Human modifications of freshwater systems have been increasing the challenges faced by the millions of fishes migrating each year. In particular, fish migrating upstream to their spawning or rearing grounds may face several instream structures along a single river network that either completely or partially impede their upstream migration, creating fish passage barriers (Franklin et al. 2022). As migrants encounter several of these barriers on their way upstream, it is important to understand how previous exposure to a barrier may influence subsequent exposures. We exposed local, obligately migratory fishes to a velocity barrier and found that individuals were more successful at passing through the barrier when they had been exposed to it multiple times. Specifically, a higher proportion of individuals successfully pass the barrier on Days 3 and 4 when compared to Day 1, and overall passage rates on Days 3, 4, and 5 were higher when compared to Day 1. The higher passage rates were especially evident when looking at the median time threshold, where one Day 1, 50% of fish failed to pass the barrier during the 30 min trial, whereas 50% of fish on Day 5 successfully passed the barrier within just 42 s. This suggested that not only do more fish successfully pass the barrier after repeated exposure, but they also pass the barrier more quickly. Our study looked at relatively short passage periods of 30 min compared to other research that looks at passage time over longer timescales in fishways. However, passage time through a barrier has been found to be an important factor influencing fish survival and migration success. Prolonged delays in passing barriers have been linked to higher rates of predation, increased energy demand, decreased migration speed, and decreased motivation (McCormick et al. 1999; Muir et al. 2006; Caudill et al. 2007; Marschall et al. 2011; Norrgård et al. 2013; Nyqvist et al. 2017).

The significant increase in passage success and passage rates observed is consistent with a pattern in which fish learn the raceway through spatial learning or landmark cues. Cognition in fishes is best understood as a combination of spatial learning and problem-solving, as it relates to visual cues and well-structured habitats (Kotrschal et al. 1998; Brown and Laland 2003; Odling-Smee and Braithwaite 2003 p. 200; Fernö et al. 2011). Fishes are routinely observed learning and remembering mazes in laboratory settings, where the structural complexity of the experimental setup simulates the complex aquatic habitat that they must migrate through (Petrazzini et al. 2015; Lucon-Xiccato and Bisazza 2017a). These indicators of spatial learning and memory in cognition, are supported by observations of faster navigation times upon repeated exposures to the maze (Petrazzini et al. 2015; Lucon-Xiccato and Bisazza 2017a). Our results suggest that, consistent with maze-based studies, repeated exposure to an instream barrier or culvert may improve passage outcomes in the wild. However, it is important to note that the use of an experimental raceway may not fully replicate the complexities of real-world structures and flow conditions.

Our study indicates that prior exposure to a barrier can enhance passage success. As such, where fish encounter multiple barriers along a migration route there is potential that previous experience may improve the likelihood of passage at successive barriers. However, in the real world, the characteristics of each barrier are unique. It remains unclear how the observed improvement in passage success following repeat exposure to the same barrier would translate to scenarios involving different barriers.

We observed a 50% increase in fish passage success over the five-day trial, suggesting memory and cognition played a role in success. However, the fact that passage success never reached 100% indicates other factors influenced fish ability to traverse the barrier as well. Physical and behavioural traits, such as fish size and health, are known to affect swimming performance and may have also affected passage success (Beamish 1978; Wolter and Arlinghaus 2003; Cano-Barbacil et al. 2020; Jones et al. 2021). Although we did not observe a significant effect of fish length on passage rates at the p = 0.05 threshold, we observed that fish length significantly influenced both approach rates and overall success of approach and passage. These results support the idea that physical characteristics may play an important role in passage success.

The positive effects of subsequent exposure on success were not mirrored in the approach and entry to the raceway. The overall rates of fish approaching the fishway remained consistent over time, and the median time to the approach threshold did not vary by more than 13 s throughout the five-day trial. Although we did not directly assess fish boldness or aversion, the observed pattern suggests that repeated exposure to the fishway may intensify the effects of shyness and aversion in individuals less inclined to approach or enter the raceway. It appears that spatial cognition did not significantly influence the outcome at the approach threshold. This is consistent with past studies of eels, which found that more exploratory eels had higher passage rates than less exploratory individuals, though there was no explicit link between boldness and passage motivation (Mensinger et al. 2021). The lack of improvement in approach and entrance success over time highlights a key issue raised in Hershey's (2021) meta-analysis on fishway efficiency. They demonstrated that passage efficiency was higher than attraction efficiency across species, migratory stages, and ecological niches. Hershey (2021) also found that efficiency was especially low for small-bodied fish. This has been corroborated by Sun et al. (2023), whose meta-analysis found that fishway type significantly influences the attraction efficiency of non-salmonids and that these fish are more sensitive to hydrological variations, such as attraction flow, backflow, hydraulic jump, and large-scale vortices. This suggests that for the majority of species, locating and entering a fishway may pose a greater challenge than successfully traversing it (Hershey 2021). Efforts to attract small-bodied, non-salmonid fish for successful passage require greater attention, especially given the global use of large-scale fishways in areas where non-salmonid species are present. There is also a notable lack of research on attraction to smaller-scale structures, such as culverts, despite their potential suitability for the species of interest.

Conclusion

In this study, we highlight the complex dynamics of fish passage through anthropogenic barriers in freshwater systems. We found that repeated exposure to a barrier improves passage rates and time to success, suggesting that fish possess a capacity for learning to traverse such barriers, potentially through spatial learning or landmark cues. However, while this positive effect of repeated exposure on passage success was statistically significant, it was marginal, indicating that other factors, including non-learned behaviours or physiological traits, such as fish size, may also play a role in passage success. Our findings highlight the importance of considering both learned and innate factors when assessing fish passage through barriers. We also highlight the challenges of attracting fish to fishway structures, particularly for small-bodied species. Efforts to improve attraction efficiency for small-bodied fish are essential for ensuring successful passage through anthropogenic barriers.

Chapter 7

Thesis synthesis

Construction of rock ramp on Mill Creek, Greymouth, New Zealand
Photo credit: Tim Marsden

7.1. Contextual overview

Fragmentation of rivers, caused by instream structures, poses a significant risk to freshwater biodiversity by altering habitat characteristics and creating physical barriers within ecosystems (Thieme et al. 2023). These barriers present a considerable challenge for migratory freshwater fish, which require unrestricted movement between marine and freshwater environments to complete their lifecycle. Hindering access to important spawning or rearing areas leads to population declines or even extirpation of species. Restoring freshwater connectivity is, therefore, important due to extensive global fragmentation caused by human activities (Tickner et al. 2020; Thieme et al. 2023).

While removing barriers is the preferred solution, it is often impractical due to logistical and cost limitations. Consequently, efforts focus on remediation or retrofitting existing instream structures to address fish passage concerns. However, effective solutions should account for variability in fish swimming abilities, behaviours, cognition, and environmental responses.

Although considerable research has been conducted on fish passage, early studies predominantly focused on salmonid species in the Northern Hemisphere (Bunt et al. 2012; Katopodis and Williams 2012; Fangue et al. 2015; Silva et al. 2018; Birnie-Gauvin et al. 2019; Franklin and Gee 2019). This limited the transferability of findings to other species, particularly small-bodied or those with distinct life histories (Roscoe and Hinch 2010; Katopodis and Williams 2012; Silva et al. 2018; Birnie-Gauvin et al. 2019; O’Connor et al. 2022). While there is increasing research on fish passage solutions for other species, significant knowledge gaps remain. In tropical and temperate island nations, amphidromous small-bodied species are prevalent in migratory fish populations and begin their upstream migration as juveniles (Franklin and Gee 2019). There is currently a lack of repeatable and consistent research on fish passage solutions for these species (Franklin and Gee 2019).

Current engineering practices tend to take a reductionist approach when designing passage structures, focusing on average fish swimming speed (Birnie-Gauvin et al. 2019). Like humans, fish exhibit considerable phenotypic variation, even within populations of the same species. Accounting for this individual variation in traits like swimming performance, environmental tolerances, motivation, cognition, and group dynamics is important for ensuring successful passage.

This thesis combined multiple approaches across five research chapters to examine how physiological, environmental, behavioural, and cognitive variation influences fish passage success for New Zealand’s migratory freshwater fish. I demonstrated that physiological differences resulted in significant variations in swimming ability both between and within fish species. I examined how the impact of environmental variation, in the form of acute changes in water temperature, significantly influenced fish swimming performance. I showed that behavioural variation, in the form of group swimming, influenced fish passage success in an artificial raceway. And finally, I explored how learning, as a measure of cognitive variation, influenced fish passage success. In this concluding chapter, I provide a synthesis of the key findings of this thesis.

7.2. Research chapter highlights

Chapter 2 compared how two different swimming speed test protocols, the critical swimming speed test (Brett 1964) and the sprint swimming speed test (Starrs et al. 2011), influenced measured swimming speeds for two galaxiid species: *Galaxias maculatus*, an actively swimming pelagic species, and *Galaxias fasciatus*, known for exhibiting station-holding behaviours. The study found no statistically significant difference in the measured swimming speeds obtained using either test protocol for both species. This result suggests that the swimming performance measures derived from these two tests are comparable.

The interchangeability of the critical and sprint swimming speed tests is important because the shorter time-step in the sprint test encourages fish with station-holding behaviours, like *Galaxias fasciatus*, to actively swim, allowing for direct measurement of their swimming performance. This provided the context for Chapters 3 and 4 to explore comparisons in swimming abilities across a wider range of species, assessing swimming speed variation among species.

Chapter 3 investigated the variation in swimming speeds across nine migratory New Zealand fish species, representing a range of physiological traits, behaviours, morphologies, and ecological niches. Additionally, it calculated maximum allowable water speeds in culverts based on the endurance abilities of the weakest swimmer, *Galaxias maculatus*. The study revealed significant interspecies and intraspecies variation in swimming speeds. *Galaxias brevipinnis*, *Galaxias argenteus*, and *Galaxias postvectis* exhibited the strongest swimming capabilities, while *Galaxias maculatus* was among the weakest swimmers. The model for maximum allowable water speeds demonstrated that water velocities through culverts should be significantly lower than the current 0.3 m s^{-1} rule-of-thumb to accommodate the variation in swimming performance across individuals. For example, a 15 m culvert should not have water speeds exceeding 0.2 m s^{-1}.

Traditional fish passage designs based solely on average swimming ability, allow passage for only above-average swimmers, failing to account for weaker individuals and species. This chapter highlighted the importance of fish passage designs that consider both interspecies and intraspecies variations to develop more effective fish passage solutions, preserving entire fish communities instead of a 'one-size-fits-all' approach.

Chapter 4 explored the impact of varying acute water temperatures on the critical swimming speeds of four migratory New Zealand fish species: *Galaxias maculatus*, *Galaxias brevipinnis, Galaxias fasciatus,* and *Gobiomorphus cotidianus*. The results demonstrated a significant reduction in critical swimming speeds at higher water temperatures (26°C) compared to lower temperatures (8°C and 15°C) for *Galaxias maculatus*, *Galaxias brevipinnis,* and *Gobiomorphus cotidianus*. In contrast, *Galaxias fasciatus* exhibited no significant difference in swimming performance across the three temperature treatments. As high-water temperatures can significantly reduce fish swimming performance, when attempting to pass an instream structure, fish may not be able to make positive progress overground or pass the structure at all compared to lower water temperatures. This may lead to potential delays in migration This research highlighted the importance of incorporating acute water temperature effects into fish passage solutions, particularly given the significant influence of human activities and climate change on water temperature fluctuations in freshwater systems.

Chapter 5 used laboratory experiments to explore the potential benefits of collective behaviour for the small-bodied migratory species, *Galaxias maculatus*, by comparing fish passage success and metabolic rates of groups versus solitary individuals through an artificial velocity barrier. My findings revealed that fish swimming in groups had faster entry and passage rates compared to solitary individuals, and successful fish in groups displayed lower metabolic rates. Increased passage success for groups of fish may be due to either hydrodynamic or behavioural benefits of swimming with conspecifics. However, there was no effect of group swimming on approach rates, or approach or entry success. These findings highlighted the importance of designing fish passage solutions that accommodate large schools of gregarious species to ensure improved migration success. When designing fish passes for gregarious species, engineers should incorporate larger resting pools or refuge areas and create lower velocity zones that can accommodate entire schools of fish simultaneously. As group swimming had no significant effect on locating or entering the raceway, fish passage solutions should focus not only on ensuring successful passage through structures but also on facilitating the ability of fish to find and enter structures. By considering the advantages of collective behaviour, fish passage structures can be tailored to better facilitate the movement of diverse fish species, including small-bodied ones, through aquatic environments.

Chapter 6 investigated the effect of repeated exposure on the passage performance of juvenile *Galaxias maculatus* through an experimental raceway with high water speeds over five consecutive days. The proportion of fish successfully passing the barrier increased significantly from the first exposure to the fifth exposure. Furthermore, time-to-event analysis revealed that by Day 5, fish successfully passed the barrier at a significantly faster rate compared to Day 1. This suggested that cognition and spatial memory play a role in improving passage performance through velocity barriers. While repeated exposure improved passage rates, it did not significantly affect approach or entry rates, indicating that other factors like fish physiology or environmental conditions may be more important for locating and entering instream structures. These findings highlighted the potential role of cognitive abilities and learning in improving fish passage performance, while also underscoring the need to consider multiple factors, including flow conditions and physical characteristics, when designing effective fish passage solutions.

7.3. Synthesis

Maintaining biodiversity and connectivity requires a shift in how we design fish passage structures (Silva et al. 2018; Thieme et al. 2023). Currently, most engineering practices are designed to pass the average fish and do not consider variation in fish swimming performance or behaviour. The collective findings from this thesis underscore the inherent variation that exists in fish passage success across species and within species. I have demonstrated differences in behaviours, cognitive abilities, and the influence of external factors like water temperature. Accounting for this variation is important when setting water speed criteria and design parameters for passage designs (Silva et al. 2018; Jones et al. 2020). New engineering solutions should be designed to the lowest common denominator, to reduce selection pressures of instream structures and ensure population survival. Factors influencing variation in passage success should be considered to design to the lowest common denominator.

7.3.1. Interspecies and intraspecies variation

Chapters 3 and 4 both revealed significant interspecific variation in swimming performance. At 15°C, fish swimming speeds varied across species likely due to differences in physiology, morphology, and behaviour (Beamish 1978; Hammer 1995; Boily and Magnan 2002; Nelson et al. 2003; Ojanguren and Brana 2003; Castro-Santos 2006; Laborde et al.

2016; Metcalfe et al. 2016; Jones et al. 2020). Within one genus (*Galaxias* spp.), swimming performance varied significantly, with *Galaxias argenteus*, *Galaxias postvectis*, and *Galaxias brevipinnis* being some of the strongest swimmers and *Galaxias maculatus* being one of the weakest. When exposed to high water temperature, the four species tested (*Galaxias maculatus*, *Galaxias fasciatus*, *Galaxias brevipinnis*, and *Gobiomorphus cotidianus*) exhibited different temperature dependant responses. For example, *Galaxias fasciatus* did not have reduced swimming speeds at the high-water temperature treatment. In contrast, the other three species tested (*Galaxias maculatus*, *Galaxias brevipinnis*, and *Gobiomorphus cotidianus*) all had significantly lower swimming performance. I also found that *Galaxias brevipinnis* exhibited a bimodal distribution at the high-water temperature, which was not observed among the other species. However, it should be noted that while each *Galaxias brevipinnis* individual was uniquely identified, there is a possibility of misidentification, which may have contributed to the bimodal response observed in the temperature treatment. These species-specific responses to temperature variation have been observed by other researchers (Johnston and Dunn 1987; Fangue et al. 2015; Norin and Clark 2016; Parisi et al. 2020; Muhawenimana et al. 2021). Substantial intraspecific variation in swimming ability was also observed. Species with the highest mean swimming speeds, such as *Galaxias argenteus* and *Galaxias postvectis*, also exhibited the largest ranges, spanning nearly 1 m s^{-1}. This intraspecific variation differed across species and was particularly high for *Galaxias brevipinnis* when exposed to high water temperatures.

7.3.2. Behavioural variation

The benchmarking of species in Chapter 3 revealed significant variation in swimming behaviour among the species tested. Several distinct patterns were observed between species even within a single behaviour type, such as station-holding. For instance, *Galaxias brevipinnis* exhibited an increase in station-holding as water speeds increased, while the opposite was true for *Gobiomorphus cotidianus*, where station-holding decreased with increasing speeds. Other species, like *Galaxias fasciatus*, are known to exhibit station-holding behaviour in the wild but did not display it during experimentation, potentially due to the choice of experimental methods, which was intended to minimise station-holding behaviour. *Anguilla dieffenbachii* had relatively low swimming speeds, consistent with their anguilliform swimming motion. These findings are consistent with Gilbert et al. (2016), who found significant intraspecies variation in swimming behaviour for the round goby during critical swimming speed tests. Some individuals relied primarily on station-holding behaviour to achieve critical swimming speeds, while others relied on a burst-and-coast swimming (Gilbert et al. 2016). For my experiments, the observed variations in station-holding behaviour are likely influenced by differences in morphology, habitat preferences, physiology, and external factors such as water velocity.

Many migratory fish species are gregarious, exhibiting schooling behaviour during upstream migration (Weihs 1973; Johansen et al. 2010; Ward et al. 2011; Marras et al. 2015; Okasaki et al. 2020). My research found that for the experimental species, *Galaxias maculatus*, swimming in groups resulted in higher passage rates compared to individuals swimming in isolation. However, not all groups were successful, nor were all fish within each group successful.

The observed station-holding behaviours and group dynamics highlight the importance of carefully considering how laboratory findings translate to real-world contexts. Both behaviours have implications for passage success and the swimming patterns that fish exhibit when traversing instream structures. Potential fish passage solutions to accommodate station-holding behaviours may be similar to those required to accommodate large groups of fish. Species exhibiting station-holding tend to display more burst-and-hold swimming patterns, suggesting fish passage facilities should incorporate more resting pools and low velocity zones. For gregarious species, these resting areas should be sized adequately to pass entire schools of fish. Passage structures designed to accommodate large groups should also be designed with enough space so as not to enforce negative effects of group density on passage success. If groups can pass barriers more successfully than individual fish, it may reduce the selective pressure favouring the largest and strongest individual fish. Conversely, if fish passage structures are too small or lack adequate refugia to accommodate entire schools, groups may become fragmented. This could reinforce selectivity for only the fittest individuals within a population (Jones et al. 2021). To maintain natural population structure and biodiversity, it is important to design fish passage solutions that facilitate the successful migration of entire schools (Okasaki et al. 2020; Jones et al. 2021; Nyqvist et al. 2024). Failing to account for gregarious behaviours could lead to unintended selective pressures and long-term impacts on fish populations.

When multiple stressors are present, their combined impact can result in different interactive effects. The effects can be additive (i.e. the combined impact is equal to the sum of the parts), or they can be subtractive (i.e. the positive effects cancel out negative effects). Stressors can also interact in a synergistic or antagonistic manner. Synergistic effects occur when the combined impacts of each stressor are greater than the sum of the individual stressors, while antagonistic effects occur when the combined impacts are less than expected (Piggott et al. 2015). When it comes to fish passage, this means accounting for how different sources of variation may interact and compound or mitigate negative effects of swimming performance or passage success. Multiple stressors will likely have differing effects among fish species given their diverse physiologies, morphologies, behaviours, and life histories. For example, *Galaxias maculatus* is one of the weakest swimming species and thus may experience compounded challenges from multiple sources of variation. The swimming performance of *Galaxias maculatus* is significantly reduced at high water temperatures, making it especially vulnerable to potential synergistic effects relative to fish passage design. Current designs are usually based on average swimming speeds and typically don't take into account the effects of environmental stressors (Meixler et al. 2009). However, I demonstrated that when accounting for the weakest individuals, the modelled maximum water speeds through a culvert were significantly lower than that of the average swimming individuals. The models in Chapter 3 only accounted for individual variation in swimming performance at 15°C. Based on the results in Chapter 4, if the model were to be recreated for high water temperatures, even lower maximum allowable water speeds would be expected. These results contribute to the growing body of evidence showing the importance of accounting for variation in fish passage performance (Peake et al. 1997; Laborde et al. 2016; Birnie-Gauvin et al. 2019; Jones et al. 2020; Meixler 2021). Other researchers have shown that additional environmental conditions such as hypoxia, high turbidity, and pollution also significantly reduce swimming performance (Brett 1967; Hammer 1995; Heath 1995; Kieffer et al. 1998; Plaut 2000; Bannon 2006). Given the cumulative impacts observed from temperature on already slow swimming species, the introduction of these added stressors may further constrain swimming abilities both between and within species.

The positive effect of group swimming may provide an antagonistic effect that mitigates other negative factors impacting swimming performance. For solitary *Galaxias maculatus* swum at high water temperatures (26°C), I observed reductions in both swimming speeds and maximum metabolic rates. This concurrent decline in swimming ability and aerobic capacity suggests that 26°C approaches *Galaxias maculatus*' upper thermal limit, where more energy must be allocated to oxygen uptake and maintenance rather than aerobic swimming. However, for groups of *Galaxias maculatus*, passage success metrics improved while active metabolic rates decreased for successful individuals in a group compared to solitary individuals. This indicates group swimming confers hydrodynamic or behavioural advantages that reduce individual energy expenditure (Weihs 1973; Parker 1973; Lefrançois et al. 2009; Nadler et al. 2016). It raises the question of whether group swimming could mitigate the negative effects of high-water temperatures on swimming performance. When fish are near the upper limit of their thermal tolerance and have limited energetic resources available for aerobic swimming, the reduced energy demands associated with group behaviour may offset the increased maintenance costs and constrained aerobic performance caused by high temperatures. The benefits of schooling may allow fish to conserve limited energetic capacity for prolonged swimming under high water temperatures.

7.3.3. Limitations

The research for this thesis was conducted entirely in a laboratory setting. While this approach allowed for precise control and manipulation of specific variables, it does not fully replicate the real-world conditions that fish encounter in natural freshwater environments. Ideally, experiments would have been conducted in natural stream conditions to more accurately assess how fish negotiate fish passage barriers in the wild. However, the current lack of advanced technology capable of tracking the movements of small-bodied fish in turbulent waters limits the ability to conduct such experiments in natural settings.

Several limitations arise from the use of laboratory settings, particularly regarding the transferability of results to natural environments. Three of the five research chapters used modified critical swimming speed (U_{crit}) tests as a measure of fish swimming performance. While U_{crit} tests have been used to assess swimming performance for fish passage design (Russon and Kemp 2011; Laborde et al. 2016; Tan et al. 2021a), questions have been raised about the transferability of these tests to the natural environment (Plaut 2001; Castro-Santos and Haro 2003; Castro-Santos et al. 2022). Plaut (2001) describes several limitations of U_{crit} tests. The swim tunnel is a uniform setting where artificial constraints are placed on the fish. The turbulence in the swim tunnels is minimised. The tunnel walls, floor, and ceiling create a boundary layer that the fish can exploit during trials, potentially allowing them to swim for longer at lower speeds than the average water speed. The solid blocking effect also changes water speed and pressure around the fish, although many researchers, including me, correct for this during experimentation (Kline et al. 2015).

A growing body of work suggests that critical swimming speed data underestimates the true maximum sustained swimming speeds of fish (Peake and Farrell 2004; Tudorache et al. 2008; Castro-Santos et al. 2013). The small swim tunnels I used may restrict fish movement, often preventing burst-and-coast behaviour and gait transitions (Kern et al. 2018). This has led Castro-Santos et al. (2013) to suggest the need to study swimming performance in open chambers that allow for volitional movement. However, the endurance swimming data collected on *Galaxias maculatus* in Chapter 3, supports the critical swimming speed findings for this species. The average U_{crit} for Galaxias maculatus was 0.28 m s^{-1}; for the endurance trials, the majority of individuals were able to sustain swimming for the duration of the 90-minute trial at 0.2 m s^{-1}, with fewer individuals sustaining swimming at 0.4 m $s^{-1.}$ This suggests that average critical swimming speeds may be a good indicator of maximum sustained swimming speeds.

While the experimental setup in Chapters 5 and 6 allowed fish to swim volitionally in an open chamber, there are still limitations to this laboratory setup. There are more complex boundary layers and a greater turbulence in the real world, which may affect fish hydrodynamics or behaviour as they try to negotiate an instream structure. Additionally, the number of fish in the system, temperature, flow, oxygen levels, and turbidity were all kept constant throughout the trials. Under natural conditions, these variables may fluctuate, potentially leading to different outcomes.

The short time steps used in the modified critical swimming speed tests for benthic and station-holding species raise questions about manipulating species' behaviour by forcing them to continuously swim. Researchers have referred to U_{crit} tests as "critical station-holding ability" for benthic-associated species (Hoover et al. 2003; Tierney et al. 2011), highlighting that different species achieve critical swimming speeds with different strategies. “Critical station-holding” speeds measure a fish’s ability to maintain its position (via station-holding) against flow for an extended length of time, rather than measuring active swimming ability. If these values are interpreted as critical swimming speeds for station-holding fish, it could overestimate the water velocities they can successfully swim against while making positive progress overground. Furthermore, the experimental equipment, velocity, and time steps all play a role in U_{crit} measurements (Farlinger and Beamish 1977; Hoover et al. 2003; Tierney et al. 2011; Gilbert et al. 2016).

Another limitation to consider is the sample size and species used in the experiments (Wenger and Olden 2012; Filazzola and Cahill Jr 2021). For Chapters 2, 3, and 4, the maximum number of replicates in each trial was 20 individuals. This relatively small sample size raises concerns about the transferability of results to wider fish populations and the generalisability beyond the specific populations sampled. In Chapter 2, preliminary trials were conducted with *Galaxias fasciatus* caught from two different locations to assess potential differences in swimming performance across populations. However, no significant differences were observed. Nevertheless, it is important to note that all species tested throughout this thesis were caught in the upper North Island of New Zealand. Variations in swimming performance might be expected in populations from other latitudes or altitudes due to potential physiological or environmental differences.

The primary experimental species used throughout this work was *Galaxias maculatus*. This limitation is particularly relevant for the maximum allowable water speed model in Chapter 3, the maximum metabolic rate data in Chapter 4, and the passage success trials in Chapters 5 and 6, which all used *Galaxias maculatus* as the focal species. The decision to primarily use *Galaxias maculatus* was driven by several factors, including the availability of the species, its status as the slowest swimmer among the tested species, its cultural significance, its ubiquity throughout the temperate Southern Hemisphere, and its frequent use as a baseline for New Zealand fish passage work. However, as highlighted in Chapter 3, significant variation in swimming performance and behaviour was observed across the tested species, even within one genus. Significant differences in swimming speeds were observed between Galaxiid species, with *Galaxias maculatus* being one of the slowest species of the four genera tested, while *Galaxias argenteus* and *Galaxias postvectis* were among the fastest. This interspecies variation was further supported in Chapter 4, where *Galaxias brevipinnis* exhibited a bimodal response to high water temperature treatments, which was not observed in the other three species tested, including *Galaxias maculatus*. These findings suggest that the results obtained for *Galaxias maculatus* might not be transferable to even its most closely related species, let alone to other New Zealand migratory species tested or across environmental conditions.

Even within the *Galaxias* genus, species exhibit different behaviours, with four out of the five migratory galaxiids exhibiting station-holding ability. If the results for *Galaxias maculatus* are not necessarily transferable to its own genus, it highlights the need for a species-specific approach. These findings emphasise the need to replicate these tests with other genera, such as *Anguilla* spp. or the benthic-dwelling *Gobiomorphus* spp. The issue of transferability to other species is further questioned by other researchers who have found significant inter- and intraspecies variation in swimming performance (Castro-Santos 2006; Katopodis and Gervais 2016).

7.4. Theoretical implications

My research in Chapters 2 to 5 raised several methodological questions about measures of critical swimming speeds. The critical swimming speed (U_{crit}) protocol was initially developed for studying actively swimming salmonid fishes in the Northern Hemisphere (Brett 1964). However, its application to other species with different physiologies and swimming modes raises questions about the transferability and ecological relevance of the results (Plaut 2001). One major consideration is how preserving the original velocity increments and time steps from the U_{crit} protocol, based on salmonids as set out by Brett (1964), impacts the interpretation of results for species like galaxiids. Salmonids have a large proportion of aerobically-powered muscles, while there is a significant knowledge gap regarding galaxiids' musculature and aerobic capacity, leaving us to speculate on the transferability of swimming speed tests and whether they represent the same physiological point of fatigue. Current research reveals some significant physiological differences between salmonids and galaxiids, suggesting that the critical swimming speed tests, as a measure of a specific physiological endpoint of exhaustion for salmonids, may not be transferable to galaxiids. Research on the oxygen consumption of *Galaxias maculatus* reveals that these species are oxygen conformers, meaning their oxygen consumption has a linear relationship with environmental oxygen availability (Farrell and Richards 2009; Barnes et al. 2011; Urbina et al. 2012). Conversely, most species are oxygen regulators, maintaining a relatively stable rate of oxygen uptake even as environmental oxygen levels decrease (Urbina et al. 2012). *Galaxias maculatus* is also one of a few species that use their skin as a respiratory tissue, with around 31% of oxygen uptake coming from diffusion through the dermis, owing to their scaleless bodies and high surface area to volume ratio (Busse 1993; Urbina et al. 2011). Additionally, the subadult stages of galaxiids lack haemoglobin (Busse 1993). Since juveniles begin the upstream migration, this has potential implications for their physiological response to aerobic and anaerobic respiration and corresponding swimming ability, especially compared to adult salmonids. These physiological differences between salmonids and galaxiids, particularly in terms of respiratory mechanisms and life histories, raise questions about the transferability of swimming speed test protocols and interpretations of results across these groups of fish.

In Chapter 2, I tested a modified U_{crit} protocol, comparing time steps of 10 s and 5 min. The results showed no significant difference in swimming speeds for *Galaxias maculatus* and *Galaxias fasciatus* between these two timesteps. Additionally, previous research found no significant difference in swimming speed for *Galaxias maculatus* between a 5 min and 30 min time step (NIWA unpublished data). While even the 30 min time step has been questioned as a true measure of maximum sustained swimming speeds (Farlinger and Beamish 1977; Castro-Santos et al. 2022), it has been widely used by researchers to measure critical swimming speeds (see Table 1, Down & Kieffer, 2017a). As there was no significant difference between the three timesteps for *Galaxias maculatus* (10 s, 5 min, or 30 min), this suggests these tests may be close to measuring maximum sustained swimming speeds. However, more physiological research is needed to accurately determine where on the continuum of prolonged swimming speeds these tests are measuring for galaxiids. The lack of significant difference in swimming speeds across varying timesteps does not necessarily imply that these tests accurately capture the maximum sustained swimming speeds for this species.

7.5. Practical applications and management suggestions

Taken together, this thesis stresses the need to account for variation when designing effective fish passage solutions. Continuously basing fish passage solutions on the average swimming abilities subjects populations to selective pressures, by potentially restricting the passage of weaker swimmers through these structures (Silva et al. 2018; Jones et al. 2021;

Thieme et al. 2023). It is important to also consider the needs of all species present in an ecosystem when designing fish passage structures. Historically, in the Northern Hemisphere, structures were tailored to salmonids, potentially hindering the migration of other fish species. Similarly, in New Zealand, solutions often focus on climbing species by enhancing climbing substrates, neglecting the preference of climbing species to swim as well as the needs of non-climbing species.

Ideal solutions for fish passage would be to construct bridges or implement stream simulation designs (Cenderelli et al. 2011). Nature-like fishways are currently considered the most effective at passing fish (Bunt et al. 2012; Hershey 2021). They involve creating a continuous channel through the structure, maintaining characteristics (depth, velocity, pathway) similar to the natural stream conditions. As noted by Cenderelli et al. (2011), these designs are not subject to uncertainties associated with fish swimming performance data. Nature-like fishways simulate natural substrates, flow, and gradients, while bridges maintain the original stream characteristics. These solutions are advantageous given the observed variation in swimming abilities across species, as they incorporate a diverse range of conditions that different species and individuals can make use of. This approach supports the results from my thesis, which highlight the importance of accounting for multiple sources of variation to make fish passage structures most effective. Additionally, they would minimally alter environmental conditions beyond what occurs naturally, eliminate unnecessary pressures on the weakest and smallest individuals, allow large groups to migrate together, and potentially leverage the cognitive abilities of fish already familiar with natural stream conditions.

Globally, the most common fish passage solutions are technical fishways, especially for large-scale structures such as dams (Castro-Santos et al. 2009; Hershey 2021). Vertical slot fishways are advantageous as they have been found to have some of the highest rates of attraction efficiency, likely due to their higher attraction flows (Bunt et al. 2012). However, this approach may not be universally appropriate or feasible across all contexts, including structure sizes and fish populations. Vertical slot fishways have areas of high turbulence and water velocities that can exceed the capabilities of smaller-bodied species, impacting their successful passage. In New Zealand, road culverts represent the majority of instream structures, requiring design solutions tailored for these smaller structures that differ from the approaches used for larger structures like dams. Removing and replacing these current instream structures is costly and logistically challenging. Consequently, the most cost-effective and timely approach is to retrofit existing structures using mitigation measures, such as rock ramps or the addition of baffles. For gregarious species, the resting areas and spacing between internal structures should be sized adequately to allow entire schools of fish to pass through.

It is important to consider the swimming mode that fish will employ when traversing instream structures. Some fish can pass through structures using primarily anaerobic respiration, swimming at high speeds but only for short periods. Burst swimming, involving short fast bouts of speed maintained for a matter of seconds, is typically used for predator-prey interactions or during startle events (Beamish 1978; Bone 1978; Plaut 2001). While fish may sometimes need to employ burst swimming during migration in the wild (e.g. navigating step pools or cascades), inducing unnecessary anaerobic stress through anthropogenic barriers should be avoided. These bursts of anaerobic swimming require recovery periods due to the depletion of oxygen and nutrient stores, and the accumulation of lactate (Beamish 1978; McKenzie 2011).

Extensive periods of burst swimming and associated stress can result in post-exercise mortality (Black 1958; Kieffer 2000). While most post-exercise mortality research has focused on marine environments in response to fishing trawls, similar risks may arise if fish exhaust themselves trying to migrate upstream through high-velocity barriers. Post-exercise mortality can occur when excess physical activity causes a buildup of metabolic byproducts that overwhelm the physiological capacity for timely clearance from muscle tissue (Black 1958; Kieffer 2000). Even if metabolites can eventually be cleared, exhaustion can still lead to equilibrium imbalances and as a result fish can suffer reduced swimming abilities leaving them more susceptible to other threats such as predation (Kieffer 2000; Jain and Farrell 2003; Danylchuk et al. 2007; Brownscombe et al. 2013; Raby et al. 2014).

Chapter 5 revealed that solitary fish that successfully traversed a barrier exhibited significantly higher active metabolic rates compared to successful groups of fish traversing the same barrier. I hypothesize that these elevated metabolic rates in solitary individuals may result from expending more energy resources typically reserved for anaerobic burst swimming during predator evasion situations. For instance, fish that successfully pass a high-velocity barrier may be too energetically depleted to effectively evade predators or contend with other challenges encountered in the upstream environment, raising concerns about the appropriateness of design velocities that necessitate excessive anaerobic effort. In contrast, fish swimming in groups can allocate less energy towards barrier passage, potentially conserving resources for other important activities like predator avoidance. Thus fish passage structures should be able to accommodate large groups of fish migrating together. Ideally, structures should be designed for sustained swimming speeds, ensuring that fish can pass through using only aerobic respiration and without exerting unnecessary stress. However, this may not always be logistically viable. In such cases, designing for prolonged swimming speeds, where fish might use a combination of aerobic and anaerobic swimming, could be a more practical approach. Prolonged swimming, fuelled with a combination of aerobic and anaerobic respiration, results in fatigue but may be less stressful than burst swimming. By designing for prolonged swimming speeds, fish passage structures can minimize the need for burst swimming and the associated risks of exhaustion and post-exercise mortality. Ultimately, the goal should be to design fish passage structures that minimize anthropogenic stress on fish and allow for safe and efficient passage using primarily aerobic swimming modes. While burst swimming may sometimes be necessary, it should not be the primary mode of navigation through anthropogenic structures. By considering the physiological limitations and swimming modes of different species, fish passage solutions can be optimized to promote successful migration and ecological connectivity.

7.6. Future research

One area for future research highlighted by this thesis is improving attraction to passage structures. Chapters 5 and 6 showed neither benefits from group dynamics nor spatial learning significantly enhanced approach or entrance success into an experimental raceway. This aligns with meta-analyses identifying poor fishway attraction efficiencies as a major bottleneck limiting fishway effectiveness across species and life stages (Hershey 2021; Sun et al. 2023). For most species, locating and entering a fishway may pose a greater challenge than navigating it successfully, with particularly low attraction rates for small-bodied non-salmonid fish (Hershey 2021). These species are more sensitive to hydrological factors like attraction flow, backflow, hydraulic jumps, and vortices around fishway entrances. Future research should prioritise understanding the motivational, behavioural, and physical factors influencing fish ability to locate and enter fishways across various scales, from small culverts to large technical fishways.

Another area of future research highlighted by this thesis is understanding how benthic-associated and station-holding species volitionally traverse fishways. Research has demonstrated that the measurement of critical swimming speeds for station-holding species does not truly reflect critical swimming speed - it could instead be considered more similar to a "critical station-holding" speed test (Hoover et al. 2003; Tierney et al. 2011). Laboratory tests evaluating critical swimming speeds may not accurately demonstrate the ability of station-holding species to successfully navigate instream structures. Given the global prevalence of migratory benthic species, research should focus on investigating active swimming and station-holding behaviours, aiming to determine the burst swimming speeds, durations, and resting intervals these species require to effectively negotiate fish passage structures.

Finally, this thesis suggests understanding the relationship between fishes’ cognitive ability and personality, along with the impact they have on passage success. My research shows that higher cognitive ability is correlated with increased passage success, while other research has shown that personality impacts passage (Mensinger et al. 2021). Given that cognitive ability and personality both impact passage success, it may be important to understand the relationship between these factors and the influence they may have on one another. Additionally, it may prove beneficial to understand if cognitive ability and personality play a role in group dynamics. Research should focus on understanding how these factors influence positioning in a group and whether this positioning has an impact on the passage success of the group.

7.7. Conclusion

From a practical standpoint, the findings of this thesis have important implications for the design and retrofitting of fish passage structures globally. By accounting for variations in swimming performance, behaviour, cognitive abilities, and environmental factors like temperature, fish passage solutions can be tailored to specific species and communities present in a given ecosystem. This targeted approach aims to minimize selective pressures, facilitate the passage of weaker individuals, accommodate collective migration patterns, and promote overall connectivity for migratory fish populations. Below are some major takeaways:

- To the best of my knowledge, this is the first study to directly test cognitive ability and learning capacity in the context of fish passage.
- My work questions the current rule-of-thumb guidance in New Zealand that water speeds through culverts should not exceed 0.3 m s^{-1}. Instead, I suggest that water speeds through culverts should be significantly lower than previously thought, to accommodate both inter- and intraspecies variation in swimming performance and passage success across fish communities.
- I provide one of the first collections of New Zealand migratory species' swimming performance using standard and repeatable methods.

Chapter 8

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Appendix A: Chapter 3 Supplementary Material

Table A1: Fish collection information including collection method, site, date, coordinates and physical characteristics of the fish.

Species	**Collection method**	**Collection Site**	**Date**	**Coordinates**	**N**	**Mean length (cm)**	**Mean body depth (cm)**	**Mean body width (cm)**
Galaxias maculatus	Fyke nets	Rangitaiki River	17/09/2020	37°54'34.6"S 176°52'53.0"E	19	4.4	0.50	0.40
Galaxias fasciatus	Electro-fishing	Lilleburn Stream, Hunua Ranges	22/09/2020	37° 04' 55.6" S 175° 10' 02.9" E	17	4.4	0.51	0.42
Galaxias brevipinnis	Electro-fishing	Lilleburn Stream, Hunua Ranges	22/09/2020	37° 04' 55.6" S 175° 10' 02.9" E	20	5.2	0.51	0.42
Galaxias argenteus	Aqua-culture	Mahurangi Technical Institute	1/12/2022	-	20	4.9	0.60	0.60
Galaxias postvectis	Aqua-culture	Mahurangi Technical Institute	1/12/2022	-	20	5.7	0.50	0.50
Gobiomorphus cotidianus	Seine netting	Waiotapu Stream	14/03/2024	38°25'11.4"S 176°20'43.7"E	20	3.2	0.41	0.40
Gobiomorphus huttoni	Fyke nets	Waitawhara Stream	9/03/2024	37° 36' 20.6" S 175° 02' 12.8" E	13	4.1	-	-
Retropinna retropinna	Seine netting	Waikato River	26/11/2019	37°48'24.4"S 175°18'19.1"E	18	7.4	-	-

Anguilla dieffenbachii	Trap and transfer	Lake Karipiro	1/02/2022	37°55'32.3"S 175°32'35.8"E	19	11	0.59	0.40

Table S2: Summary of swimming speeds for *Retropinna retropinna* and *Anguilla dieffenbachii*

Species	**Δ*t* (s)**	**Δ*U* (bl s^{-1})**	**Mean U_{crit}**	**SD**	**Min U_{crit}** (m s^{-1})	**Max U_{crit}** (m s^{-1})	**Range**
Retropinna retropinna	300	1	0.61	0.1	0.44	0.88	0.44
Anguilla dieffenbachii	10	0.3	0.32	0.11	0.13	0.51	0.38

Appendix B: Chapter 5 Supplementary Material

Table B1: Complete list of fish lengths (mm) for each group, including standard deviations and averages.

Length (mm)							
Trial	**Fish 1**	**Fish 2**	**Fish 3**	**Fish 4**	**Fish 5**	**Average**	**Standard deviation**
1	61	52	55	55	57	56	3.32
2	63	64	55	57	50	58	5.81
3	75	63	59	68	62	65	6.27
4	52	68	57	64	52	59	7.2
5	56	70	54	50	55	57	7.62
6	72	55	63	74	53	63	9.56
7	57	64	67	68	64	64	4.3
8	57	67	60	70	50	61	7.98
9	58	60	54	63	54	58	3.9
10	57	59	53	68	49	57	7.16
11	54	54	63	65	50	57	6.46
12	62	61	68	72	56	64	6.26
13	56	52	54	54	54	54	1.41
14	60	52	51	55	50	54	4.04
15	54	54	68	64	58	60	6.23
16	60	58	48	57	55	56	4.62
17	54	55	50	50	57	53	3.11
18	64	47	76	54	60	60	10.92
19	58	74	58	78	56	65	10.35
20	58	52	64	47	53	55	6.46
21	61	60	49	58	54	56	4.93
22	53	49	48	56	51	51	3.21
23	54	69	68	49	50	58	9.77
24	54	68	55	55	50	56	6.8
25	56	58	60	50	54	55.6	3.85
26	54	53	52	37	55	50.2	7.46
27	48	50	53	54	58	52.6	3.85
28	65	60	56	47	58	57.2	6.61
29	70	37	57	69	57	58	13.3
30	74	70	70	54	58	65.2	8.67
31	66	57	59	51	64	59.4	5.94
32	62	47	53	49	53	52.8	5.76
33	60	54	53	61	52	56	4.18
34	58	64	49	75	54	60	10.02
35	71	56	59	67	57	62	6.63

Table B2: Complete list of fish lengths (mm) for solitary fish.

Length (mm)					
Trial	**Length**	**Trial**	**Length**	**Trial**	**Length**
1	58	21	60	41	44
2	61	22	60	42	47
3	58	23	58	43	45
4	59	24	57	44	47
5	56	25	54	45	44
6	54	26	62	46	42
7	70	27	63	47	48
8	64	28	55	48	49
9	57	29	65	49	45
10	55	30	55	50	45
11	60	31	58	51	45
12	56	32	55	52	46
13	72	33	57	53	42
14	61	34	58	54	50
15	54	35	66	55	45
16	56	36	67	56	46
17	47	37	54	57	48
18	67	38	56	58	46
19	60	39	47		
20	53	40	44		

Table B3: Complete list of group weights (g), including averages and standard deviations.

	Weight (g)						
Trial	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Average	Standard deviation
1	1.31	0.51	0.57	0.42	0.6	0.68	0.36
2	1.21	0.96	0.43	0.72	0.36	0.74	0.36
3	1.61	1.02	0.84	1.79	0.98	1.25	0.42
4	0.52	1.21	0.86	1.02	0.67	0.86	0.27
5	1.63	0.62	0.48	0.63	0.63	0.8	0.47
6	1.62	0.61	0.89	1.55	0.46	1.03	0.53
7	0.65	0.83	1.39	1.39	1.04	1.06	0.33
8	0.71	1.5	0.77	1.16	0.37	0.9	0.44
9	0.9	1.01	0.53	0.93	0.67	0.81	0.2
10	0.6	0.67	0.64	1.33	0.39	0.73	0.36
11	0.63	0.66	1.17	1.21	0.47	0.83	0.34
12	0.95	0.98	1.29	1.48	0.58	1.06	0.35
13	0.6	0.58	0.57	0.62	0.58	0.59	0.02
14	0.8	0.4	0.35	0.32	0.39	0.45	0.2
15	0.49	0.63	1.31	1.11	0.72	0.85	0.34
16	0.9	0.71	0.27	0.79	0.79	0.69	0.25
17	0.68	0.4	0.35	0.33	0.74	0.5	0.19
18	1.02	0.47	2.1	0.55	0.97	1.02	0.65
19	0.75	1.78	0.77	1.78	0.79	1.17	0.55
20	0.82	0.54	0.98	0.36	0.6	0.66	0.24
21	0.74	0.68	0.55	0.59	0.62	0.64	0.08
22	0.77	0.43	0.43	0.69	0.55	0.57	0.15
23	0.86	0.99	0.85	0.44	0.51	0.73	0.24
24	0.67	0.65	0.53	0.57	0.59	0.6	0.06
25	0.81	0.6	0.91	0.87	0.56	0.75	0.16
26	0.66	0.44	0.34	0.83	0.84	0.62	0.23
27	0.23	0.61	0.42	0.32	0.78	0.47	0.22
28	1.22	0.77	0.58	0.3	0.78	0.73	0.34
29	1.25	1.49	0.59	1.27	0.79	1.08	0.37
30	1.71	1.53	1.51	0.63	0.83	1.24	0.48
31	1.38	0.77	0.72	0.69	1	0.91	0.29
32	0.96	0.26	0.6	0.46	0.56	0.57	0.26
33	1.03	0.52	0.52	1.04	0.5	0.72	0.29
34	0.81	0.91	0.33	1.82	0.61	0.9	0.56

Table B4: Complete list of solitary fish weights.

Trial	Weight (g)	Trial	Weight (g)	Trial	Weight (g)
1	0.76	21	0.99	41	0.2
2	0.92	22	0.71	42	0.18
3	0.77	23	1.08	43	0.24
4	0.77	24	0.55	44	0.2
5	0.77	25	0.56	45	0.13
6	0.57	26	0.89	46	0.18
7	1.37	27	1.19	47	0.24
8	1.22	28	0.58	48	0.28
9	0.68	29	1.27	49	0.21
10	0.77	30	0.67	50	0.23
11	0.86	31	0.66	51	0.18
12	0.61	32	0.68	52	0.18
13	1.69	33	0.64	53	0.24
14	1.08	34	0.82	54	0.27
15	0.56	35	0.75	55	0.23
16	0.71	36	1.19	56	0.25
17	0.45	37	0.48	57	0.27
18	1.5	38	0.7	58	0.16
19	0.81	39	0.2		
20	0.61	40	0.2		

Appendix C: Published co-authored manuscript

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Impacts of VIE tagging and Rhodamine B immersion staining on two measures of performance for a small-bodied fish

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ABSTRACT

Context. Passage efficiency is an important metric for quantifying the success of fish-passage remediation. Mark–recapture methods are typically employed to estimate fish-passage efficiency. Although biotelemetry methods have become a benchmark standard for such studies, they remain unfeasible for many small-bodies species because of the excessive size of electronic tags. **Aims.** This study compares two commonly deployed marking methods that are potentially suitable for estimating passage efficiency for small-bodied species, namely, visible implant elastomer (VIE) tagging and batch immersion staining with Rhodamine B. **Methods.** The critical swimming speeds and passage efficiency at a remediated culvert of *Galaxias maculatus* were compared for VIE-tagged, Rhodamine B-stained and control treatments. **Key results.** Both critical swimming speeds and passage efficiency at the culvert were significantly lower in VIE-tagged *Galaxias maculatus* than in both control fish and fish stained with Rhodamine B. **Conclusions.** Rhodamine B may be a suitable batch immersion stain for short-term (<4 day), non-destructive mark–recapture studies in small-bodied fishes, but VIE-tagging methods may result in underestimation of passage efficiency. **Implications.** It is important to evaluate the impact of marking techniques on the outcomes of mark–recapture studies to ensure that estimates of passage efficiency are accurate.

Keywords: fish migration, fish passage, *Galaxias maculatus*, immersion staining, mark–recapture, passage efficiency, Rhodamine B, visible implant elastomer.

Introduction

Restoration of river connectivity is a key goal for protecting and enhancing diadromous fish communities (Thieme *et al.* 2023). However, the effectiveness of many fish-passage solutions remains poorly understood (Silva *et al.* 2018; Hershey 2021). Biotelemetry has been held up as the gold-standard for evaluating the performance of different fish-passage solutions and for quantifying fish-passage efficiency (Bunt *et al.* 2012; Noonan *et al.* 2012; Hershey 2021). Although the information that can be captured from biotelemetry studies is invaluable and ever improving (Cooke *et al.* 2013; Hussey *et al.* 2015), applying biotelemetry methods is not physically possible for some species and life stages characterised by small body sizes (Watson *et al.* 2019).

Amphidromous fishes undertake their primary upstream migration as small-bodied (15–60-mm total length, TL) juveniles and are a key component of the fish communities of many island nations across the globe (Franklin and Gee 2019). Owing to their small size at migration, these species can be highly susceptible to the impacts of instream infrastructure on river connectivity, with vertical drops of as little as 10 cm shown to severely impede the upstream passage of some species (Baker 2003; Jones *et al.* 2021). Owing to their susceptibility to river fragmentation, evaluating the effectiveness of different fish-passage solutions for these species is essential, yet the use of biotelemetry is infeasible.

As with larger fishes, a wide range of methods has been applied to evaluate the effectiveness of different fish-passage solutions for smaller-bodied fishes. Many of these focus on trapping of fish entering or exiting fishways or other instream structures (e.g. culverts), or on surveys comparing fish community composition upstream and downstream of structures, or before and after remediation (Rolls *et al.* 2013). For example, Marsden and Stuart (2019) evaluated the effectiveness of three different fishway types for small-bodied species in Australia by using paired upstream and downstream trapping methods. They were able to demonstrate that, for empire gudgeons (*Hypseleotris compressa*), there was no difference in size between those entering and those exiting any of the fishways, but for bony herring (*Nematalosa erebi*) there was a significant difference in the sizes of fish entering and those exiting two of the fishways. However, because of the schooling nature of the target species (meaning that the abundance of fish passing can be highly temporally variable), they were unable to compare the abundance of fish entering versus fish exiting the fishway and, hence, estimate passage efficiency in the traditional sense. Similarly, Rolls *et al.* (2018) compared fish community composition and size structure between downstream control reaches and at the exit of two fishways by using passive trapping methods. They found significant differences in community composition, with a range of small-bodied species being absent from the samples collected at the fishway exits.

Unfortunately, the trapping methods highlighted above do not alone allow for accurate quantification of passage efficiency, that is, an estimation of the proportion of fish arriving at the structure that successfully pass. Although not being the only way that fish-passage effectiveness can be measured, passage efficiency is an important metric for understanding the relative performance of different solutions and is frequently used as a performance measure against which fish-passage projects are evaluated (Bunt *et al.* 2012; Wilkes *et al.* 2018; Hershey 2021).

Mark–recapture methods, whereby fish are marked somehow, liberated, and subsequently recaptured or observed later, offer a means of estimating the proportion of a sample population that passes a fishway or instream structure. Although contemporary biotelemetry technologies allow for a wide range of information to be captured about fish behaviour and movements from the tags (Hussey *et al.* 2015; Lennox *et al.* 2017), in essence biotelemetry studies are a form of the mark and recapture method. The tags act as unique identifiers for individual fish and allow collection of accurate information on the timing and extent of fish movements. As such, biotelemetry has become the go to approach for quantifying passage efficiency at fishways (Bunt *et al.* 2012; Noonan *et al.* 2012; Hershey 2021). Although increasing refinements and miniaturisation of the technology are enabling biotelemetry methods to be applied to species with increasingly smaller body sizes (Tummers *et al.* 2016; Baker *et al.* 2017; Watson *et al.* 2019), they remain impractical for many small-bodied species (Franklin and Gee 2019; Sandford *et al.* 2019). For example, Watson *et al.* (2019) showed 100% mortality rates for two small-bodied species (*Pseudomugil signifier* and *Macquaria ambigua*) following tagging with microPIT tags. Consequently, alternative marking methods are required for estimation of passage efficiency in these species.

Two examples of such marking methods that have been identified as having potential application for small-bodied species are visible implant elastomer (VIE) tags and immersion staining. VIE tags consist of a fluorescent liquid elastomer that is injected into the tissue of the fish that sets to form a longer-term biocompatible mark. Immersion staining involves total body immersion of the fish in an aqueous dye solution to impart a dye to the fish. In this study we compared VIE tags and immersion staining for their suitability for undertaking mark–recapture studies of a small-bodied fish to estimate passage efficiency at instream structures. To do this, we compared critical swimming speeds (U_{crit}) and field-based estimates of passage efficiency through a culvert for unmarked, VIE-tagged, and immersion-stained *Galaxias maculatus* individuals, a small-bodied amphidromous species with a widespread distribution in the temperate Southern Hemisphere.

Materials and methods

Fish capture and husbandry

Juvenile *G. maculatus* individuals were captured during their upstream migration by using either fine-mesh rigid frame whitebait traps or Gee-minnow traps from the Waikato River catchment in the North Island of New Zealand. Following capture, fish were transferred to aerated containers and transported either to the NIWA Hamilton laboratory (for the U_{crit} trials) or directly to the study site on Bankwood Stream, Hamilton (for the passage-efficiency trials).

On arrival at the laboratory, fish were held in a temperature-controlled room on a 12:12 h day:night cycle. Fish were quarantined in 60-L tanks with a salinity of 6 to prevent disease and transferred to 60 L of dechlorinated water tanks after 1 week. These tanks were kept on a recirculating water system at a temperature of 15 ± 0.5°C. Fish were fed bloodworms every other day and underwent a 24-h fasting period before trials to ensure a post-absorptive state. Fish used in the passage-efficiency trials were transferred to flow-through live bins and held within Bankwood Stream under ambient conditions prior to tagging.

All experimental trials for this study were conducted with approval to manipulate live animals for research by the NIWA Animal Ethics Committee (AEC204), in accordance with the requirements of Section 83 of the New Zealand *Animal Welfare Act* 1999.

Fish tagging

Fish to be used for the U_{crit} trials were tagged or stained within the laboratory, whereas fish used for the passage-efficiency trials were tagged or stained on site. Fish for the U_{crit} trials were captured using hand-nets and randomly allocated to the three different treatment groups (VIE-tagged, stained and control) to try and reduce the potential confounding impact of capture order. Fish in the first field-based passage-efficiency trial were all VIE tagged, whereas fish for the second field-based passage-efficiency trial were randomly allocated between the stained and control groups. All fish to be marked with VIE tags (Northwest Marine Technology, Anacortes, WA, USA) were first sedated using Aqui-S (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand), then fish length was recorded and the tags were injected into the dorsal surface of the fish. Staining was achieved by batch immersion of the fish in a solution of Rhodamine B (0.2 g L^{-1}; Sigma–Aldrich, Darmstadt, Germany). To optimise survival and stain retention, aquarium salts were added to the solutions to produce a salinity of ~15. The staining solution was aerated and, for the fish that were stained on site, ice was added to the solution as necessary to maintain the same temperature as the stream. The fish were removed from the stain after 2 h by using a dip net. To allow for recovery, all tagged and stained fish were subsequently transferred either to the laboratory holding tanks (for the U_{crit} trials) or flow-through live bins held within Bankwood Stream (for the passage-efficiency trials) for a minimum of 24 h prior to trials commencing. A randomly selected subgroup of VIE-tagged fish were held for 21 days prior to undertaking critical swimming tests to evaluate the influence of time since tagging. The control group of unmarked fish were retained in separate holding tanks to act as a control for the effect of the tagging–marking procedure (Table 1).

Critical swimming speeds

Critical swimming-speed trials were conducted in a 10-L Loligo Systems Steffensen-type swim tunnel (Loligo Systems, Viborg, Denmark), following the methods of Crawford *et al.* (2023). Briefly, water temperature in the swim tunnel was maintained at 15 ± 0.5°C and dissolved oxygen concentrations were maintained throughout the trials at a mean of 98.0 ± 0.9% saturation. At the beginning of each trial, individual fish were introduced into the swim tunnel and acclimated for 30 min at 0.5 bodylengths s^{-1}. The water velocity in the swim tunnel was then incrementally raised by 1 bodylength s^{-1} every 5 min. A trial was concluded once a fish displayed signs of fatigue, characterised by resting against the mesh at the back of the tunnel for a continuous duration of 3 s or for a cumulative total of 10 s within a 30-s timeframe. At the end of the trial, fish were rested in the tunnel with no velocity for 20 min before being returned to the holding tank to ensure recovery. Each trial was conducted independently, without any fish being re-used and all fish were tested once (Table 1).

Table 1. Number and size of *G. maculatus* individuals used in the critical swimming-speed trials.

Marking method	*n*	Mean total length (mm)	s.d. (mm)	Minimum total length (mm)	Maximum total length (mm)
Control	19	44.0	±3.85	37	50
Rhodamine B stain	19	48.6	±3.45	42	58
VIE tag	19	56.2	±4.65	48	67
VIE tag (21 days)	9	50.2	±2.64	47	55

Critical swimming speed was calculated following Brett (1964) as:

$$U_{\text{crit}} = U_{f-1} + \Delta U\left(\frac{t_f}{\Delta t}\right)$$

where U_{crit} is the critical swimming speed (bodylengths s^{-1}), U_{f-1} is the penultimate speed the fish experienced (bodylengths s^{-1}), ΔU represents the speed increase (1 bodylength s^{-1}), t_f is the duration of time the fish swam at the highest speed (s; $0 \leq t_f < \Delta t$), Δt represents the time increment (300 s).

Culvert passage efficiency

The passage-efficiency trials were conducted at the River Road culvert on Bankwood Stream in Hamilton, New Zealand. It is a 73.8-m-long single-barrel 1.5-m diameter concrete culvert with a mean gradient of 0.8° (range 0.3–2.55°). The culvert has been retrofitted with a rock-ramp fishway and spoiler baffles to improve fish passage as described in Franklin and Bartels (2012).

Passage trials were initially conducted using VIE-tagged fish and reported in Franklin and Bartels (2012). The initial trial was undertaken during the migratory period for *G. maculatus* in December 2009 under base-flow conditions. In total, 177 VIE-tagged inanga individuals were released in the receiving pool at the outlet of the culvert, which had been sealed with fine-mesh netting to prevent fish from escaping downstream. A fine-mesh rigid whitebait trap was then set and sealed at the top of the culvert to capture all fish successfully passing the culvert. The trial was run for 24 h to account for any diel variations in migratory motivation. Passage success was defined as being present in the upstream trap at the completion of the trial. The experimental design of Franklin and Bartels (2012) was subsequently replicated at the same site under equivalent base-flow conditions in March 2015 using fish batch stained with Rhodamine B and a control group of unmarked fish (Table 2). The second trial was run for

Table 2. Number and size of *G. maculatus* individuals used in the culvert passage-efficiency trials.

Marking method	*n*	Mean total length (mm)	s.d. (mm)	Minimum total length (mm)	Maximum total length (mm)
Control	200	64.1	±7.4	47	85
VIE tag	177	58.0	±6.3	44	81
Rhodamine B stain	200	65.2	±7.4	50	83

48 h, with the number of successful fish (i.e. those in the upstream trap) quantified after 24 and 48 h.

Statistical analyses

Analysis of covariance (ANCOVA) was used to test for differences in mean critical swimming speed (U_{crit}) between the three treatments. Marking treatment was used as a categorical predictor variable with three levels (control, stained, VIE) and fish total length was used as a continuous predictor variable. Individual critical swimming speeds were used as the response variable. To determine the model of best fit, a likelihood ratio test (LRT) was used, starting with a fully saturated model including interactions. A *post hoc* Tukey's HSD test was used to determine which treatments had statistically different critical swimming speeds.

Culvert passage success was compared among treatments by using a binomial generalised linear model (GLM) using the glm() function and a logit link in the base package of R. Individual passage success (pass or fail) was used as the response variable. Marking treatment and individual fish lengths were again used as predictor variables. A fully saturated model including interactions was fitted and stepwise selection was used to remove non-significant predictors. All statistical analyses were conducted using the R statistical computing software (ver. 4.3.1, R Foundation for Statistical Computing, Vienna, Austria, see https://www.r-project.org/).

Results

Critical swimming speeds

Mean critical swimming speeds (±s.d.) of the control, stained and VIE-tagged *G. maculatus* individuals were respectively 6.45 ± 2.85, 7.08 ± 2.67 and 3.14 ± 3.70 bodylengths s^{-1} (Fig. 1). For the fish 21-days post-VIE tagging, mean critical swimming speed (±s.d.) was 2.98 ± 2.23 bodylengths s^{-1} Fish length did not have a statistically significant effect on critical swimming speed ($F = 0.047$, $P = 0.829$), but there was a significant difference in critical swimming speed among treatments ($F = 7.779$, $P < 0.001$). Mean U_{crit} of the VIE-tagged fish was significantly lower than that of both the control ($P = 0.007$) and stained fish ($P < 0.001$), but there was no statistically significant difference between the control and stained fish ($P = 0.918$). Swimming speeds of the 21-day post-VIE tagging fish were significantly different from those of the control fish ($P = 0.030$), but not statistically different from those of the VIE-tagged fish tested 24 h after tagging ($P = 0.999$).

Culvert passage efficiency

Overall passage success after 24 h was 27.0% for the control fish, 28.0% for the stained fish and 6.2% for the VIE-tagged fish (Fig. 2). Fish length had no statistically significant effect on passage success ($P = 0.440$) and was removed from the GLM model. There was no statistically significant difference

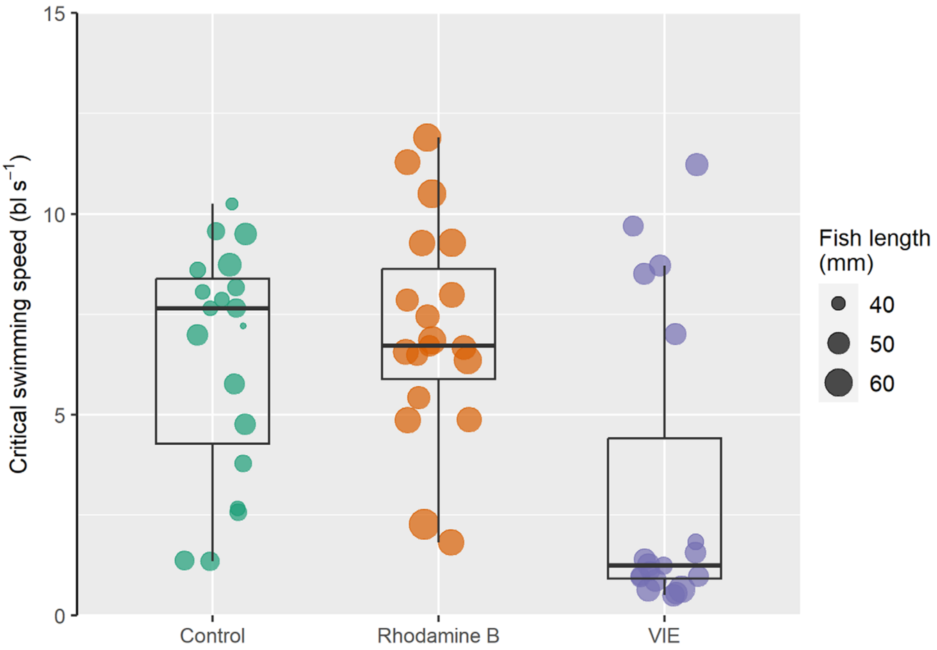

Fig. 1. Critical swimming speeds of non-marked and marked *G. maculatus* individuals. The data points correspond to individual fish within each treatment group. The centre bar displays the median, whereas the outer edges of the box display the interquartile range. The whiskers show 1.5 times the interquartile range.

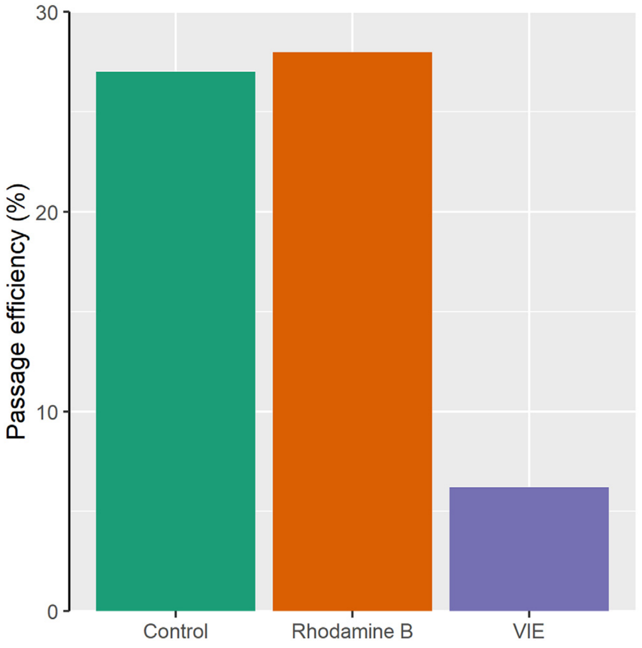

Fig. 2. Twenty-four-hour passage efficiency for *G. maculatus* through the 73.8-m River Road culvert with spoiler baffles.

in passage success between the control and stained fish ($P = 0.823$), but passage success was significantly lower for the VIE-tagged fish than both the control and stained fish ($P < 0.001$). For the control and stained fish, the trial was extended to 48 h, after which overall passage success had increased to 33.5% for both treatments.

Discussion

Passage efficiency is an important metric for evaluating and comparing the effectiveness of different fish passage solutions. In this study, we have demonstrated that marking method can have a significant impact on assessments of passage efficiency for a small-bodied fish, *G. maculatus*, using mark–recapture methods. Passage efficiency of *G. maculatus* stained with Rhodamine B was equivalent to that of non-marked control fish. However, passage efficiency was substantially lower for *G. maculatus* marked with VIE tags than for fish in the control group. These differences in passage efficiency were also apparent in critical swimming speeds, with no difference in U_{crit} between *G. maculatus* stained with Rhodamine B and control fish, but significantly lower swimming speeds being observed for the fish marked with VIE tags. What is more, the impacts of the VIE tags on critical swimming speed were still present 21 days post-tagging. In light of these results, it is clear that the passage-efficiency estimates previously presented by Franklin and Bartels (2012) by using VIE-tagged fish for this remediated culvert are an underestimation of true passage efficiency for *G. maculatus* at this site.

VIE tags have often been identified as being suitable for individual-based or batch marking and tracking of fish and other aquatic organisms, although numerous potential shortcomings have also been identified with respect to tag misidentification, impacts on survival, and alterations to behaviour (Jungwirth *et al.* 2019; Moore and Brewer 2021). We have demonstrated negative effects of VIE tagging on fish swimming speeds compared with control fish that are apparently translated into poorer fish-passage performance in a field-based setting for *G. maculatus*. Although a range of studies have evaluated the impacts of VIE tagging on growth and survival of different species (e.g. Astorga *et al.* 2005; Bolland *et al.* 2009; Moore and Brewer 2021), and these generally show minimal or no negative effects, there appear to be few studies that have addressed the potential impacts on locomotive capabilities of fish. Imbert *et al.* (2007) looked at the impacts of VIE tags on locomotive behaviour of juvenile European eels (*Anguilla anguilla*) and found no difference between tagged and untagged individuals. However, the water velocities within their test flume were low (0.09 m s^{-1}) compared with elver swimming capabilities (sustained and prolonged swimming threshold ~0.3 m s^{-1}; McCleave 1980; Vezza *et al.* 2020) and so may not have presented a significant physical challenge for the fish. Other studies that have evaluated fish swimming speeds and passage performance by using small-bodied VIE-tagged fish (e.g. Jones *et al.* 2020; Jones *et al.* 2021) have not reported comparisons with untagged (i.e. control) fish. Given our results, we suggest that in future studies it is essential that a control group of untagged fish is included as part of trials so that any potential tagging effects that may arise can be accounted for.

In contrast to the VIE tagged fish, we observed no significant effect on either critical swimming speed or passage success of fish batch stained using Rhodamine B when compared with control fish. A weakness of batch staining compared with some alternative marking methods is that it does not allow for individual identification of fish. However, in the context of determining overall passage efficiency at instream barriers, batch marking offers an effective solution. Another limitation of staining is the duration for which the stain is visible. In our experience, we have found that stained fish can be reliably identified for 3 days post-staining, but by Day 4 it can become more difficult to confidently identify all stained fish. Consequently, applications should be limited to short-term studies that can be completed in <4 days. *G. maculatus* is scaleless and at the larval life-stage is translucent, which may make it particularly suitable for staining. However, Rhodamine B has previously also been found to be an effective immersion marker for the scaled *Gambusia affinis* (O'Grady and Hoy 1972). Where longer tag-retention periods are required, Rhodamine B staining will not be suitable. Fluorochrome dyes (e.g. calcein, alizarin red) have been shown to produce more persistent (>100 days) detectable marks on bony structures in a range of fishes (Lü *et al.* 2015; Kullmann *et al.* 2018; Afentoulis *et al.* 2022;

Gaillard *et al.* 2022), put poor survival has been reported for some small-bodied species during and following the dying process (Afentoulis *et al.* 2022) and detection of the marks is generally destructive.

Marking fish with VIE tags requires considerably more handling of the fish than do the batch immersion methods. Furtbauer *et al.* (2015), for example, reported notable post-VIE tagging physiological stress responses for three-spined stickleback *Gasterosteus aculeatus*. We hypothesise that the greater handling of the fish required for VIE tagging imparts higher stresses than does immersion staining that, at least over the short term, imposes a physiological burden on fish that affects swimming capabilities. This may make VIE tagging less suitable for short-term (hours to days) studies using small-bodied fish species than the immersion staining method tested here. However, we also found that critical swimming speeds remained impaired even 21 days post-VIE tagging, suggesting potential prolonged impacts. These effects need to be explored further to understand the potential implications for studies using VIE tags.

Conclusions

We have demonstrated that VIE tagging had a significant negative effect on the critical swimming speed of the small-bodied *G. maculatus* and that this negative effect is sustained 3 weeks post-tagging. Furthermore, the negative effect of VIE tagging on swimming speeds was also reflected in a measurably lower passage success past a remediated culvert. Consequently, the passage-efficiency results for the remediated culvert previously presented by Franklin and Bartels (2012) are an underestimation of true passage efficiency for *G. maculatus* at this site.

The results of marking studies may be incorrect if the marks and or associated marking procedures change the behaviour, condition or survival of fish compared with unmarked individuals (Cooke *et al.* 2013; Sandford *et al.* 2019). Consequently, it is important that different marking methods are evaluated to determine their suitability for different species and applications. Biotelemetry tagging methods remain ineffective for some small-bodied fishes, meaning that alternative methods are required to evaluate fish-passage efficiency. On the basis of our results, batch immersion staining using Rhodamine B is a more suitable marking method than VIE tagging for short-term studies of *G. maculatus* passage efficiency.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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Franklin PA, Crawford R, van Ravenhorst WB, Baker CF (2024) Impacts of VIE tagging and Rhodamine B immersion staining on two measures of performance for a small-bodied fish. Marine and Freshwater Research 77, MF23187.

Nature of contribution by PhD candidate	Conducted laboratory research trials and conducted preliminary data analysis.
Extent of contribution by PhD candidate (%)	30

CO-AUTHORS

Name	Nature of Contribution
Paul Franklin	Conducted field trials and led drafting of manuscript
Bastiaan Ravenhorst	Conducted mark and recapture field trials
Cindy Baker	Conducted field trials and contributed to drafting of manuscript

Certification by Co-Authors

The undersigned hereby certify that:

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- that the candidate wrote all or the majority of the text.

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Chapter 2: No difference between critical and sprint swimming speeds for two galaxiid species

Nature of contribution by PhD candidate	Co-conceived and designed the study, conducted trials, managed data and analysis, wrote the manuscript
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

Name	Nature of Contribution
Eleanor Gee	Co-conceived and designed the study, and helped edit the manuscript
Deborah Dupont	Conducted trials
Brendan Hicks	Co-conceived and designed the study, and helped edit the manuscript
Paul Franklin	Co-conceived and designed the study, and helped edit the manuscript

Certification by Co-Authors

The undersigned hereby certify that:

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Chapter 3: Accounting for interspecies and intraspecies variation in swimming performance for fish passage solutions

Nature of contribution by PhD candidate	Co-conceived and designed the study, conducted trials, managed data and analysis, wrote the manuscript
Extent of contribution by PhD candidate (%)	85%

CO-AUTHORS

Name	Nature of Contribution
Eleanor Gee	Co-conceived and designed the study, and helped edit the manuscript
Deborah Dupont	Conducted trials
Dana Nolte	Conducted trials
Brendan Hicks	Co-conceived and designed the study, and helped edit the manuscript
Paul Franklin	Co-conceived and designed the study, and helped edit the manuscript

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Chapter 4: High water temperature significantly influences swimming performance of New Zealand migratory species

Nature of contribution by PhD candidate: Co-conceived and designed the study, conducted trials, managed data and analysis, wrote the manuscript

Extent of contribution by PhD candidate (%): 90%

CO-AUTHORS

Name	Nature of Contribution
Eleanor Gee	Co-conceived and designed the study, and helped edit the manuscript
Deborah Dupont	Conducted trials
Brendan Hicks	Co-conceived and designed the study, and helped edit the manuscript
Paul Franklin	Co-conceived and designed the study, and helped edit the manuscript

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Paul Franklin		21/05/2024
Eleanor Gee		21/05/2024
Brendan Hicks	Brendan	23/05/2024
Deborah Dupont		24/05/2024