Early Life History Dynamics and Survivorship of Vagrant Tropical Fish in Temperate Environments

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Certificate of Original Authorship

I, Alexander Rigg declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of the Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Thesis Structure & Publication

This thesis adheres to the guidelines for a thesis by compilation format. It is organised as a unified document consisting of chapters and works either published or under review.

The experimental chapters (Chapters 2-5) are formatted in line with the specific requirements of the journals to which the manuscripts have been submitted or published. Despite these variations, a consistent formatting style has been maintained throughout the thesis to the extent possible

List of Publications, Contributions, and Permissions.

Chapter 2

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I conducted the fieldwork, laboratory work, dissections, microscopy, data analysis, and graphics and wrote the manuscript. CB contributed to fish feeding. AF provided feedback and general guidance on the manuscript. DB provided oversight, direction, and feedback on the manuscript

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Chapter 3

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Thesis Abstract

Climate change is driving the poleward expansion of tropical marine species into temperate ecosystems globally, a process known as tropicalisation. This thesis examines the life history traits, behavioural strategies, and environmental conditions that influence the settlement, persistence, and winter survivorship of a vagrant poleward expatriating tropical fish species in southeastern Australia. By addressing the biological and environmental factors underpinning range expansions, this research contributes to understanding how tropical species may establish populations in temperate environments as oceans continue to warm.

Firstly, in Chapter 2, I validated otolith microstructure analysis as a reliable age and growth estimation method in *Abudefduf vaigiensis*, confirming daily increment deposition. The study demonstrated the potential negative effects of high concentrations of Alizarin Red S on otolith growth, highlighting the need for consideration when applying chemical marking techniques to study fish population dynamics.

In Chapter 3, I demonstrated, using otolith microstructure, that faster larval growth rates and shorter pelagic larval durations improve post-settlement persistence in *A. vaigiensis* in Sydney, while size at hatching and size at settlement showed no significant relationship with persistence. These results challenge the traditional assumption that larger sizes confer survival advantages during early life stages in temperate environments.

Next, in Chapter 4, I investigated how temperature, habitat complexity, and fish size interact to influence the behaviour of the range-expanding vagrant, *A. vaigiensis*. The study demonstrated that fish exhibit reduced activity levels and increased reliance

on structured habitats below a critical thermal threshold of 17°C, a key survival mechanism in temperate waters. Larger fish showed predator avoidance strategies using habitat, while habitat complexity did not significantly impact behaviour but influenced bite rates. These findings suggest ontogenetic shifts in behaviour as fish grow and face new environmental challenges such as overwintering, demonstrating behavioural plasticity in response to temperature.

Finally, in Chapter 5, I examined the factors driving winter survival, identifying size at hatching, settlement temperature, and body condition as key predictors. Fish that settled under lower ocean temperatures had higher odds of winter survival, likely due to the less acclimation before winter ocean temperatures and predation. Larger fish, with higher body condition at the time of settlement, were better equipped to endure the physiological demands of overwintering in temperate waters. Fish that wintered were also larger at hatching and settlement. Early growth was shown to be an important factor, with wintered fish having elevated trajectories in the first 50 days. These findings suggest that some early life history traits are selected for survival into winter, potentially mediating the physiological impacts of declining water temperature.

This research synthesises the key stages of *A. vaigiensis* temperate existence to provide evidence of the biological and environmental factors influencing the success of tropical vagrant species in temperate ecosystems. By integrating otolith analysis, behavioural trials, and environmental factors, I demonstrated how tropical fish may persist in temperate environments as ocean temperatures continue to rise.

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Chapter 1 General Introduction

1.1 Climate Change Impacts on Marine Ecosystems

Climate change is reshaping marine ecosystems globally, driving significant alterations in species distributions and community structures (Pecl et al., 2017). Rising ocean temperatures, which have increased by approximately 0.13°C per decade since 1900 (Intergovernmental Panel on Climate, 2023), are particularly evident in certain regions known as ocean warming hotspots, such as the southeast coast of Australia (Ridgway, 2007, Suthers et al., 2011). These hotspots are experiencing temperature increases at two to three times faster than the global average, often due to the strengthening of adjacent western boundary currents (Wu et al., 2012) . The East Australian Current (EAC, a western boundary current) has strengthened and extended southward in recent years, driving warmer waters into the temperate ecosystems of southeastern Australia (Ridgway, 2007). Projections suggest that ocean temperatures in this region may rise by 2–3°C by 2100 (Sun et al., 2012), significantly affecting temperate marine environments.

1.2 Tropicalisation of Temperate Ecosystems

Tropicalisation refers to how temperate ecosystems shift towards more tropical characteristics, driven primarily by invading tropical taxa via dispersal in poleward currents and rising ocean temperatures (Zarzyczny et al., 2024). This process involves the poleward expansion of tropical species, including fish, invertebrates, and other marine organisms, into environments inhabited by species adapted to temperate conditions (Gervais et al., 2021). As tropical species move into these cooler receiving environments, profound shifts in community composition, habitat structure, and ecosystem function can occur (Vergés et al., 2014, Wernberg et al., 2016, Kingsbury, 2019, Kingsbury et al., 2020b, Nagelkerken et al., 2023). The increasing suitability of temperate waters for tropical species due to warming is a key aspect of tropicalisation, and its effects are becoming more pronounced in climate change hot spots like southeastern Australia and Japan (Nakamura et al., 2013a, Vergés et al., 2014).

In temperate ecosystems, tropicalisation often leads to complex novel interactions, often at the demise of native temperate species. For example, tropicalisation can lead to significant shifts in fish assemblages and habitat structures. Newly established coral beds, like the *Pocillopora aliciae* coral observed in south-eastern Australia, are attracting both range-expanding tropical and local temperate fish species, with fish densities on the coral up to 48 times higher than on nearby temperate rocky reefs (O'Connell et al., 2023). This suggests that coral habitats may concentrate fish populations and support tropicalisation by providing suitable habitats for tropical species, expanding their range. Additionally, tropicalised temperate reefs in Australia demonstrate higher biomass and productivity in herbivorous fish due to turf-dominated algal communities, which sustain greater feeding rates by tropical species such as surgeonfish (Pessarrodona et al., 2022). These tropical herbivores drive an energy shift from traditional kelp-based systems to turf and detrital pathways, fundamentally altering the ecosystem dynamics and often disadvantaging native temperate species.

1.3 Vagrant Fish and Tropicalisation

A key aspect of tropicalisation is the poleward expansion of "vagrant" tropical fish - species typically restricted to tropical waters now frequently observed in temperate regions (Booth et al., 2007, Feary et al., 2014). This global phenomenon has been documented from the North Atlantic to the South Pacific along Australian coasts (Poloczanska et al., 2013, Sorte et al., 2010). The establishment of vagrant tropical species in temperate ecosystems leads to the formation of hybrid communities, where both temperate and tropical species coexist (Nakamura et al., 2013a).

Under a changing climate, these mixed communities often exhibit shifts in resource use, predator-prey dynamics, and competitive interactions (Vergés et al., 2014, Coni et al., 2022b). For instance, the decline of temperate kelp forests, accelerated by warming waters and grazing pressure from range-expanding tropical herbivores, creates conditions more favourable for tropical fish adapted to simplified reef systems (Wernberg et al., 2011, Basford et al., 2015, Agostini et al., 2021). Such changes in habitat structure and species composition can fundamentally alter ecosystem functions, affecting biodiversity and fisheries.

Research into the factors that affect tropicalisation is particularly relevant in areas like southeastern Australia, where ongoing ocean warming will enable further expansion of tropical species. This influx has led to measurable ecological impacts, including alterations in species composition, disruptions of local food webs, and increased competition with native species (Agostini et al., 2021, Basford et al., 2015, McCosker et al., 2022, Mitchell et al., 2023, O'Connell et al., 2023, Osland et al., 2021, Pessarrodona et al., 2022). Investigating the determinants of successful establishment among vagrant fishes will enhance our understanding of the potential impacts on range-expanding species.

1.4 Dispersal Dynamics of Vagrant and Natal Fish

Many coral reef fish species in their natal tropical environment exhibit a 'open' bipartite life cycle. Adults spawn on the reef, producing pelagic larvae that disperse in ocean currents during their pelagic larval duration (PLD) phase (Mora, 2015). These larvae then settle onto reefs, which may be distant from their natal site, where they metamorphose into juveniles and eventually grow to adulthood and reproduce. Open cycles allow for connectivity between reef populations and contribute to genetic mixing across broad geographic areas.

In contrast, vagrant tropical fish in temperate regions represent the extreme of the dispersal kernel in an open life cycle (Fig. 1b). While larvae may successfully settle and survive in these areas during warmer months, they typically fail to complete the full cycle due to environmental constraints, such as low ocean temperatures (Booth et al., 2007, Figueira and Booth, 2010, Feary et al., 2014). Vagrant fish can overwinter, where individuals survive cooler winter temperatures but generally do not reproduce locally (Figueira and Booth, 2010). This results in an open population dynamic, where the presence of these species relies on continued larval supply from tropical source populations rather than local reproduction. The population becomes a demographic sink, maintained by repeated recruitment events and the ability of some individuals to overwinter but without contributing to the next generation in these temperate waters. This scenario creates an ecological situation where vagrant populations persist through physiological and behavioural adaptations to cooler conditions but remain reproductively isolated from their tropical source populations.

1.5 Poleward Expansion of Vagrant Fish in Eastern Australia

The distribution and persistence of vagrant tropical fish species along the east coast of Australia, as illustrated in Figure 1, show a latitudinal gradient reflecting varying degrees of recruitment, survival, and reproduction. Figure 1 is used to illustrate the concept of vagrant fish latitudinal larval supply derived from Figueira and Booth (2010), rather than provide a scaled or definitive map of recruitment patterns.

In the southernmost regions, the (1) Sporadic Larvae Only zone depicts occasional larval arrival, but colder winter temperatures prevent survival to the subsequent season. Moving further north, in regions like southern New South Wales, the (2) Regular Settlement, Occasional Overwintering zone demonstrates that larvae settle more consistently, with some individuals surviving winter, though this remains infrequent. As the gradient progresses northward towards central New South Wales, the diagram's (3) Small, Non-reproductive Adult Population area indicates that while adults may settle and survive, environmental conditions prevent them from reaching reproductive maturity or forming viable populations. In (4) Viable Populations, Export Larvae to Nonviable Populations, found in regions such as southern Queensland, the populations are self-sustaining and reproduce locally, exporting larvae to areas further south where conditions do not allow full establishment. Finally, in the northernmost zone, (5) Viable Populations, Export Larvae to Viable Populations, including regions like the Great Barrier Reef, populations are colonised, self-sustaining, and export larvae to other viable populations. This area is considered their natal range.

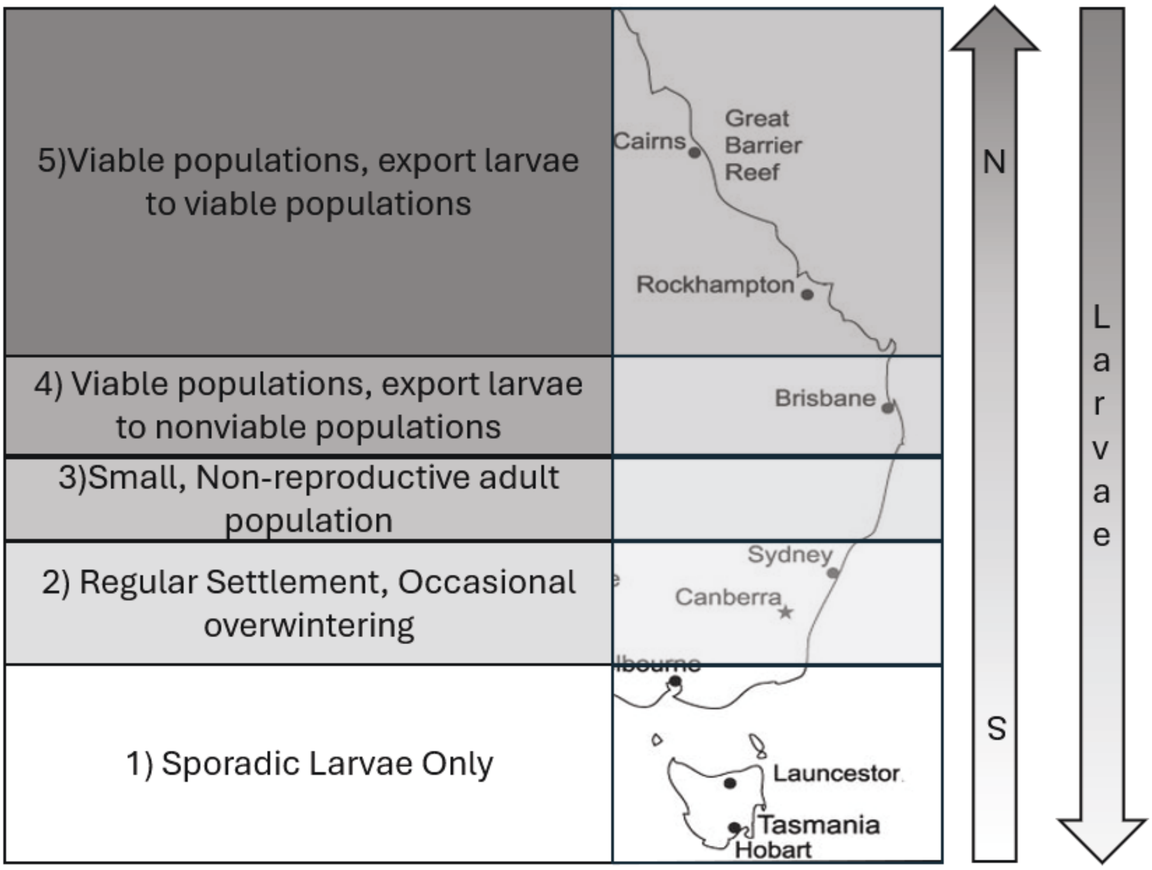

Figure 1.1 Latitudinal gradient approximating stages of population persistence and reproductive success for vagrant tropical fish species along Australia's east coast, adapted from Booth et al. (2011a). The diagram illustrates transitions from sporadic larval settlement in the south to fully established, self-sustaining populations with larval export in the north, with zones representing approximate population dynamics. The diagram is an approximation and not drawn to scale; it illustrates the general latitudinal gradient and transition in population dynamics along the Australian coastline rather than precise geographic or population boundaries

This conceptual framework demonstrates how a combination of environmental factors and early life histories, such as larval dispersal, settlement preferences, and temperature tolerance shapes the distribution of vagrant fish along Australia’s east coast. These traits influence the capacity of vagrant species to recruit, survive, and potentially establish in temperate regions, with each latitudinal zone reflecting unique thresholds for these life stages.

1.6 Early Life History Traits and Settlement of Coral Reef Fishes

Settlement and early post-settlement are high mortality periods that result in the selection of favourable characteristics (McCormick, 1998, Bergenius et al., 2002, Hoey and McCormick, 2004, Gagliano et al., 2007a). These critical life stages shape population dynamics through the survival of individuals with specific early life history traits (ELHTs). Known ELHTs that affect survivorship include size at hatching, pelagic larval duration (PLD), size at settlement, and growth rates.

Evidence demonstrates the importance of ELHTs in determining survival outcomes. For example, larger size at hatching may enhance survival prospects by improving predator avoidance and foraging capabilities (Gagliano et al., 2007a). The duration of the pelagic larval phase influences both dispersal distances from the natal environment and the physiological condition of settling fish, with direct implications for post-settlement survival (Bergenius et al., 2002, Sponaugle et al., 2006). Larger settlers commonly exhibit higher survival rates due to enhanced competitive abilities and reduced predation risk (McCormick and Hoey, 2004). Furthermore, accelerated growth during larval and early juvenile phases can increase survival by reducing individuals' time in the ontogeny most vulnerable to predation (Vigliola and Meekan, 2002, Searcy and Sponaugle, 2000).

For vagrant tropical fishes settling in temperate environments, the selective pressures acting on these ELHTs may differ from those in their native ranges. The novel ecological conditions in temperate habitats, including lower temperatures, different predator assemblages, and altered resource availability, could shift the value of these traits in temperate environments. However, to date, the specific advantages of ELHTs for vagrant species in temperate waters remain speculative.

1.7 Challenges for Vagrant Species in Temperate Environments

Vagrant tropical fish species face various novel challenges when settling in temperate environments. Temperate ecosystems have colder ocean temperatures, different prey availability and habitat types, and a new suite of possible predators and competitors than their native tropical regions. For species accustomed to the warmer, stable conditions of tropical ecosystems, the lower temperatures of temperate regions can impair physiological processes such as growth, metabolism, and immune function, potentially increasing mortality rates during the critical post-settlement phase (Rowe et al., 2018, Kingsbury et al., 2020a, Djurichkovic et al., 2019a). Food availability and type also differ significantly, requiring vagrant species to adapt to new prey types or foraging strategies (Kingsbury et al., 2020). Predation risks may also be heightened as vagrants encounter unfamiliar predators with different hunting behaviours, further affecting their survival in these novel ecosystems (Beck et al., 2016b).

These environmental challenges will likely alter the persistence of certain ELHTs and affect the survivorship of vagrant species. For example, traits such as faster pre-settlement growth rates and larger size at settlement, which confer survival advantages in tropical regions, may become even more critical in temperate environments. Growing quickly before entering these more challenging habitats could allow vagrants to reach thermal and size thresholds necessary for withstanding colder temperatures and competing for resources (Rankin and Sponaugle, 2011). However, prolonged periods of colder conditions may also be selected for individuals with traits that enhance thermal tolerance or enable them to endure extended periods of reduced metabolic activity (Donelson et al., 2019).

One of the most significant challenges that vagrant species face in temperate regions is surviving the winter months. Overwintering refers to the ability to survive through the winter season, to the subsequent summer. Overwintering poses a substantial barrier to establishing stable populations, as survival during this period is highly temperature-dependent (Figueira and Booth, 2010). For many vagrant species, current temperate winter temperatures are near the lower limits of their thermal tolerance (Djurichkovic et al., 2019a, Rowe et al., 2018). However, as ocean temperatures rise, these regions become more suitable for tropical species year-round (Wu et al., 2012). With increasing winter temperatures, vagrant species may establish permanent populations in temperate waters, following a trajectory in line with the invasion model of tropicalisation (Hellmann et al., 2008, Davidson et al., 2011, Bates et al., 2014). Once established, these species could spread, leading to long-term shifts in community composition and permanently altering the dynamics of temperate ecosystems (Pecl et al., 2017).

1.8 Deriving ELHTs from Otolith Microstructure

Otoliths are calcium carbonate structures of the inner ear in teleost fishes and can provide a chronology of individual life histories. Teleosts possess three pairs of otoliths - the sagittae, lapilli, and asterisci. These structures grow continuously throughout a fish's life, forming daily increments that reflect growth rates and environmental conditions (Panella, 1971). Daily increment formation has been validated across many species, allowing precise estimates of age in days (Wilson and McCormick, 1999, Campana, 2005). The microstructure of otoliths can provide detailed information on early life history events such as hatching, settlement, and metamorphosis, often marked by distinct changes in increment width or optical density (Fig. 2) (Panfili et al., 2009b). For studies of vagrant tropical fishes, otolith analysis offers a powerful tool to reconstruct early life histories and examine traits that may influence post-settlement survival in temperate waters. Importantly, relationships between fish size and otolith size allow back-calculation of size-at-age, enabling reconstruction of individual growth histories (Wilson et al., 2009). However, validation of daily increment formation and size-otolith relationships is crucial for ensuring the reliability of age and growth estimates derived from these structures.

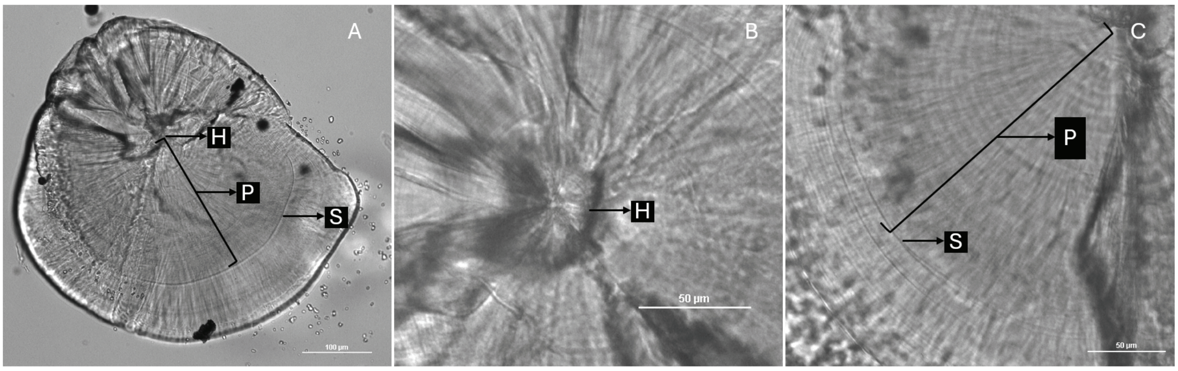

Figure 1.2 Microstructure of a lapillus otolith from *Abudefduf vaigiensis*. (A) The whole otolith view shows key developmental markers: H - hatch check, P - pelagic larval duration (PLD), and S - settlement mark at 200x magnification. (B) Magnified view (400x) of the otolith core region, highlighting the hatch check (H). (C) Close-up of the otolith edge region at 400x magnification, showing the transition from pelagic (P) to post-settlement (S) growth. Scale bars: 100 µm (A), 50 µm (B, C). These structures allow for accurate aging and reconstruction of early life history events in this vagrant tropical fish species.

1.9 Knowledge Gaps and Research Rationale

Despite increasing occurrences of vagrant tropical fish in temperate waters, our understanding of factors influencing their settlement, persistence, and winter survival remains limited. Much of the research surrounding vagrant biology has focused on interactions, competitive processes, and impacts on temperate marine ecosystems. This study takes a different approach, emphasising vagrant biology and population dynamics rather than heterospecific competitive processes. There is a significant gap in the literature regarding intrinsic factors that lead to successful establishment. Similarly, little is known about how vagrant fish utilise temperate habitats post-settlement, which differs vastly in structure and complexity from their natal ranges. The relationship between habitat use and temperature may play a role in mediating the establishment of temperate populations.

A significant area of uncertainty is how ELHTs that confer advantages in tropical environments translate to success in temperate waters. While faster growth rates and larger size at settlement are generally advantageous in tropical reefs (Gagliano et al., 2007a), it is unclear whether these traits provide similar benefits in challenging temperate conditions. Feary et al. (2014) found that tropical fish species with large body sizes, faster swimming ability, and large size at settlement are more likely to settle into temperate habitats successfully. However, it is important to note that this review primarily discusses factors affecting post-settlement abundance, not the settlement process or survival.

As tropical fish expand their ranges poleward due to warming oceans, they encounter different habitats and predator assemblages to their natal range. Coni et al. (2022a) found that tropical fish exhibit increased risk-averse behaviours at their poleward range limits. Specifically, these fishes increased their shelter use and had longer flight initiation distances, indicating heightened caution in the presence of potential predators. Despite these behavioural changes, they maintained their feeding rates and body condition, suggesting that adopting more cautious behaviours does not necessarily compromise their fitness. Instead, increased sheltering and reduced activity levels may be strategic adaptations to conserve energy and enhance survival in the cooler, predator-rich temperate waters.

Complex habitat plays a significant role in the recruitment and settlement success in temperate regions. Beck et al. (2017) demonstrated that temperate macroalgal habitats negatively impact tropical fishes' recruitment densities and species richness. Their surveys in southeastern Australia and western Japan revealed that non-macroalgal habitats, such as rocky reef barrens, supported over seven times greater densities of recruiting tropical fishes than macroalgal-dominated reefs. The presence of macroalgae may pose physical or ecological barriers to settlement, suggesting that habitat composition is a key factor influencing the initial stages of tropical fish establishment in temperate zones. Despite much research on habitat complexity and its impacts on tropical ecosystems (Almany, 2004, Connell and Jones, 1991, Komyakova et al., 2013, Nay et al., 2020), little has been explored in temperate habitats, particularly regarding the gradients of complexity associated with sandstone reefs. This knowledge gap is significant, as temperate sandstone reefs offer a range of habitat complexities that may influence the settlement and survival of vagrant tropical species differently from their natal coral reef habitats.

Surviving the winter bottleneck presents significant challenges for tropical vagrants in temperate regions. As water temperatures decline leading into winter, energetic demands of maintaining homeostasis increase, making it essential to find suitable habitat that provide shelter and food sources for survival. While winter temperatures pose a significant challenge to tropical species (Figueira & Booth, 2010), our understanding of how vagrants cope with these periods of stress remains limited. The adoption of risk-averse behaviours, preference for complex sheltered habitats, and ELHTs may collectively enhance the ability of vagrant fish to endure temperate winter conditions. However, we lack information on which specific traits or strategies might enhance survival during cooler ocean temperatures.

1.10 Thesis Study Species: *Abudefduf vaigiensis*

Abudefduf vaigiensis, the Indo-Pacific sergeant major, is a suitable model species for studying range expansion dynamics in marine fishes. Native to Indo-Pacific coral and rocky reefs, it exhibits a bipartite life cycle with a pelagic larval phase followed by benthic settlement (Wellington and Victor, 1989). At settlement, *A. vaigiensis* individuals range from 13 to 30 mm long, reaching up to 18 cm within their natal range (Bray, 2023). After settlement, *A. vaigiensis* displays strong site fidelity, often remaining within a specific reef area, which provides stability for studying localised behaviours and habitat interactions post-settlement. *A. vaigiensis* is seasonally abundant along the east coast of Australia's latitudinal gradient (Feary et al., 2014, Booth et al., 2007), with known occurrences in temperate regions and occasional overwinter survival (Figueira and Booth, 2010). These characteristics, combined with knowledge of temperature thresholds (Rowe et al., 2018, Djurichkovic et al., 2019a), position *A. vaigiensis* as a valuable model for understanding the mechanisms underlying range expansions in vagrant marine fishes under a changing climate.

1.11 Structure, Study Approach, and Aims

This thesis examines the biological and ecological factors that influence the settlement, survival, wintering, and potential establishment of vagrant tropical fish in temperate ecosystems, using *Abudefduf vaigiensis* as a model species. By integrating otolith microstructure analysis, field observations, and controlled experiments, this research provides an understanding of how early life history traits, environmental conditions, and behaviour interact to determine the establishment of tropical fish in novel temperate habitats. This study aims to enhance our ability to predict and manage the ecological consequences of climate-driven range expansions in marine ecosystems.

Specifically:

- Chapter 1 introduces relevant concepts to the research, reviews the current literature, and explores the knowledge gaps in our understanding of vagrant tropical fish in temperate ecosystems. This chapter sets the context for the thesis by examining the impacts of climate change on marine ecosystems, with a focus on the tropicalisation of temperate regions.
- Chapter 2 aims to validate otolith microstructure for *A. vaigiensis* to ensure it can be used as a valid proxy for daily growth patterns and other early life history traits. This involves capturing individuals, staining the otoliths with Alizarin Red S (ARS), and conducting otolith microscopy and microstructure analysis. This methodological foundation supports the subsequent analyses in Chapters 3 and 5.
- Chapter 3 assesses the early life history traits (ELHTs) of *A. vaigiensis* that influence early post-settlement persistence in temperate environments. The investigation uses a temporal cohort serial sampling approach, tracking a newly settled shoal of *A. vaigiensis* at Little Manly, Sydney Australia.
- Chapter 4 examines the interactive effects of temperature, habitat complexity, and fish size on the behaviour in a controlled aquarium study, building upon the post-settlement processes from Chapter 3 and provides a link to winter survival in Chapter 5. The aquarium experiment systematically manipulates temperature, habitat complexity, and fish size across behavioural trials.
- Chapter 5 evaluates the role of ELHTs in *A. vaigiensis* to survive into the winter. The investigation expands the temporal and spatial scale of the study, involving monthly sampling at various locations in Sydney over two years. This chapter uses otolith microstructure analysis to determine factors that contribute to surviving temperate winters.

- Chapter 6 synthesises the findings from the previous chapters to provide an understanding of the factors influencing the settlement, survival, and potential establishment of vagrant tropical fishes in temperate waters.

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Chapter 2 : Staining protocols affect use of otolith to estimate the demography of the damselfish sergeant major (*Abudefduf vaigiensis*)

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2.1 Abstract

This study assessed the otolith (sagittae, lapilli, and asterisci) increment deposition rate in the range-shifting damselfish, *A. vaigiensis*, using different concentrations of Alizain Red S and evaluated the impact of staining on increment width. Daily increment deposition was verified in all otolith types and presented clearer fluorescent markings in the lapilli and sagittae than the asterisci, with high stain concentration showing the best clarity. Higher stain concentrations were found to decrease increment width, suggesting care is needed when using stained otoliths as a proxy for growth for this species.

2.2 Introduction

The ongoing warming of marine environments facilitates the poleward expansion of species (Poloczanska et al. 2013). Throughout the austral summer and autumn, the East Australian Current delivers a suite of larval tropical marine fishes (vagrants) to temperate areas on the East Coast of Australia (Booth et al., 2007). This annual recruitment event currently ends in near-complete mortality, with winter temperatures acting as a bottleneck to survivorship (Figueira and Booth, 2010). Given winter water temperatures are expected to increase due to climate change, the annual recruitment phase is likely to result in the establishment of viable tropical vagrant populations on temperate reefs and increased winter survivorship, potentially affecting long term competitive processes with temperate species and food chains (Coni et al., 2022b). Despite the expatriation of tropical reef fish to temperate communities, little is known about their *in situ* performance, such as growth patterns and early life histories. Performance is mediated by temperature and potentially interacts with predation pressure to dictate survival above critical thermal minima. Given that age, growth, and mortality are key to understanding population dynamics of fish, ecologists need accurate methods to estimate these metrics.

Otoliths are calcium carbonate structures found within the inner ear of fish. Otolith growth is a continuous precipitous process that results in the deposition of concentric light and dark bands that can be observed microscopically. These increments are typically deposited both daily and annually, enabling accurate estimates of age to the resolution of a day (Panella, 1971). Increment widths can be coupled to somatic growth and thus daily increment width can be used as a proxy for growth (Molony and Choat, 1990). Microstructural analysis of otolith increments can reveal insights into ecological dynamics such as pelagic larval duration, larval dispersal, and early growth and survivorship. Otolith microstructural analysis in range-extension ecology can provide metrics that allow researchers to examine the drivers of success (or permanent residency) of vagrant populations under climate change (Booth et al., 2011a).

Most fish species have three pairs of otoliths: the sagittae, lapilli, and asterisci. Properties, like deposition rate and shape, can differ among the types of otoliths and among species for the same type of otolith. Due to the inter-species differences in the characteristics of each otolith, researchers need to confirm that a predictable relationship exists between otolith radius, increment size, and body size within all otolith types of a particular species prior to using microstructure as a demographic proxy. The three-dimensional shape, clarity of deposition, cracking, opaque zones and secondary growth patterns should influence a researcher's selection of otolith type for a species, yet sagittae are commonly used, often without validation or examination of other otolith types.

Otoliths should be validated to ensure that one increment represents a day of growth (Panfili et al., 2009a). A common technique to validate the otolith is to mark the otolith at two known timepoints and compare the number of visible increments with the number of days elapsed between events (Simon & Dörner 2005). One such chemical, Alizarin Red S (ARS hereon), is frequently used to mark otoliths as it has low toxicity (Bensimon-Brito et al., 2016), and is cost effective compared to other stains such as oxytetracycline (Niva et al., 2005). The red hue emitted under fluorescence is also superior for identifying faint marks relative to the green hue of other otolith stains such as oxytetracycline.

It is important to know whether marking affects somatic growth for experiments that attempt to both validate increment deposition and measure growth rates in laboratory settings. Despite the suggestion ARS does not impact growth (Liu et al., 2009, Bashey, 2004), others indicate growth inhibition occurs between different manufacturers (Jurgelėnė et al., 2022). As the use of stain extends beyond validation, such as bone mineralisation research, fish performance, and fish stock programs, it is important to establish whether ARS impacts growth and physiological processes that may interfere with the interpretation of growth from calcified structures, and whether a lower concentration should be used to minimise any potential effects.

Abudefduf vaigiensis (Quoy & Gaimard 1825) is frequently used in range-expansion studies as a model fish species (Figueira et al., 2009, Booth et al., 2011a, Feary et al., 2014, Vergés et al., 2014, Beck et al., 2014, Beck et al., 2016a) due to high reliable seasonal abundance and recruitment to temperate areas. Despite A. *vaigensis* daily increment in otoliths not being validated, numerous studies have assumed daily deposition lines in juvenile otoliths (Kingsbury et al., 2020b, Thresher and Brothers, 1989, Wellington and Victor, 1989) and annuli deposition in adults (Nakano et al., 2004). The current study aims to:

(1) Assess the increment deposition rate in juvenile A. *vaigiensis* in three otolith types;

(2) Compare and evaluate the chemical marking efficacy at different stain concentrations (low - 150 mg/L, medium - 250 mg/L and high -350 mg/L) in three otolith types;

(3) Assess the effect of stain on otolith increment width, used as a proxy for growth.

2.3 Methods

Twenty-four juvenile *A. vaigiensis* were collected from Little Manly (33°48'25.7"S 151°17'07.6"E) and Narrabeen (33°42'08.8"S 151°18'05.6"E), Sydney, Australia, during February – April 2022. Fish were collected in accordance with the NSW Department of Primary Industries collection permit (F94/696(A)-9.0) and University of Technology Sydney (UTS) Animal Ethics requirements (ETH-6609). For each stain-treatment, fish were separated into two 12 L tanks (6 fish per treatment, 3 fish per tank), containing fish with similar body size, ranging in total length from 17 mm-50 mm. Similar sample sizes have been used in previous studies to validate otoliths (Parkinson et al., 2012). Ocean temperatures ranged from 22 – 24°C at the time of collection, and fish were therefore acclimated over one day to 23°C in the laboratory. A 12 h light:12 h dark cycle was maintained throughout the investigation. Fish were fed 50ml of live Artemia twice a day and water temperature (23ºC), pH (8.1), dissolved oxygen (6-8mg/L), and salinity (35) were monitored twice daily, followed by the removal of debris and a 20% water change. Fish were immersed for 24 h in ARS (Sigma-Aldrich, A5533) at day 0 and 7 – 9 following acclimation. Stain treatments were 150 mg/L (low – 9 days), 250 mg/L (medium – 8 days), 350 mg/L (high – 7 days) and a control group which was kept under the same conditions and timeframe of experimentation (20 days) but did not experience staining. There were two mortalities after the first stain (one each for medium and high stain). Fish were euthanised using an ice bath at the conclusion of the investigation. The sagittae, lapilli, and asterisci were extracted from each fish and mounted dorsal side up on a microscope slide using

Crystalbond™ (509-1A). Upon inspection, daily increments were clearly visible and did not require polishing. A Nikon Eclipse *Ni-E* compound microscope (400x) equipped with fluorescence (emission λ wavelength 852nm, excitation wavelength 562nm) was used to view the markings. The increments visible between the two marks were counted and compared to the number of days elapsed between staining events. All samples were counted by two researchers. The mark quality was assessed following a 0-5 ordinal scale, where: 0 = no visible mark or fluorescence, 1 = weak fluorescence, 2 = mark easily visible under fluorescent light, 3 = shining brightly under fluorescence, 4 = mark visible under visible and fluorescent light; 5, mark distinct under visible and fluorescent light (Taylor et al., 2005). 20% of samples were randomly selected by a second researcher and evaluated for mark quality. If the score differed by one, the sample was discussed against the criteria and a score was decided upon. Increment widths were measured on the lapilli for all treatment groups, as the lapilli resulted in the clearest increment banding. Individual increments of each fish were measured to determine total increment width for the experimental period (20 days) and was measured on the same axis (dorsal view, from the centre of the primordium to the furthest edge along the anterior-posterior axis) using Nikon NIS-elements Advanced Research software (V5.02.02).

2.4 Statistical Analysis

For Aim 1, a one-sample t-test was used to test the hypothesis that increments would equal the number of days between marks in each otolith type and treatment. For Aim 2, two Kruskal-Wallis tests were conducted to compare the effect of stain concentration and otolith type. The post hoc Dunn's test was performed to conduct a pairwise comparison between the concentrations for the three stain treatments and otolith types. For Aim 3, the average increment width across the experimental period was compared among stain treatments and the control with a Kruskal-Wallis test, as the assumptions of the parametric ANOVA test were not met. A pairwise comparison was used to identify which pairs were significantly different. All data were statistically analysed using a significance level (α) of 0.05.

2.5 Results and Discussion

The number of increments between marking events was as expected for all treatments: the increment count equated to the days between marks in all otolith types ($p > 0.05$). Our findings thus support the hypothesis that increments were deposited daily in all otolith types.

Although our findings support the usefulness and validity in using all otoliths in aging and daily growth studies of A. *vaigiensis*, care should be taken when selecting which type of otolith to use. Few studies explore the validity of otolith type (David et al., 1994), with a bias in the literature towards using the sagittae as they are generally larger, easier to find, and have larger increment widths (Panfili et al., 2009a). Indeed, the larger size of the sagittae and increment widths therein was seen for A. *vaigiensis*, but we note clearer increments in the lapillus, which was also observed by Kingsbury et al. (2020a) and Soeparno et al. (2012). Although not used previously for A. *vaigiensis*, the asterisci often forms at settlement in demersal fish, which would underestimate pelagic larval duration. In many fishes, the complex three dimensional shape of the sagittae gives rise to secondary growth patterns that make readings along a single axis difficult (Morioka et al., 2006); this was observed for juvenile *A. vaigiensis*. Given these observations and constraints the lapillus is recommended in future ageing studies for A. *vaigiensis*.

Marking scores differed across otolith types (Figure 1, Kruskal-Wallis, H(3) = 9.87, p = 0.007) and across staining treatments (Figure 1 Kruskal-Wallis, H(3) = 9.44, p = 0.009). Dunn's post hoc comparisons for otolith type revealed significant differences in marking scores between the asterisci and lapilli (p=0.005) and asterisci and sagittae (p=0.009), but not the lapilli and sagittae (p=0.863). For stain concentration, Dunn's post hoc comparisons showed a significant difference between low and high concentration (p=0.008), but not for medium and high (p=0.273) and low and medium (p=0.061). In many fishes, the asterisci have been shown to have fainter and smaller daily increments (Tsukamoto and Kajihara, 1987) and more irregular concentric increments (Espino-Barr, 2019, Hoff et al., 1997). Although daily increment deposition in the asterisci of *A. vaigiensis* was validated, we hypothesise that the thinner depositions (1-2µm, as opposed to 3-5 µm for the lapillus and sagittae) provide less opportunity for the absorption of stain, or less visibility of the mark, resulting in lower marking scores. The lapilli were laterally flatter than the sagittae, resulting in clearer concentric markings. To determine the optimal concentration of stain required often includes making a compromise between research aims and fish health, immersion time, type of otolith and water conditions (Taylor et al., 2005). The results demonstrate that a concentration of 250mg/L ARS for 24 hours results in markings in the sagittae and lapilli that are at least as clear and reliable as 350mg/L concentration (p=0.818), whilst minimising wastage of product and avoiding fish exposure to higher concentrations of stain.

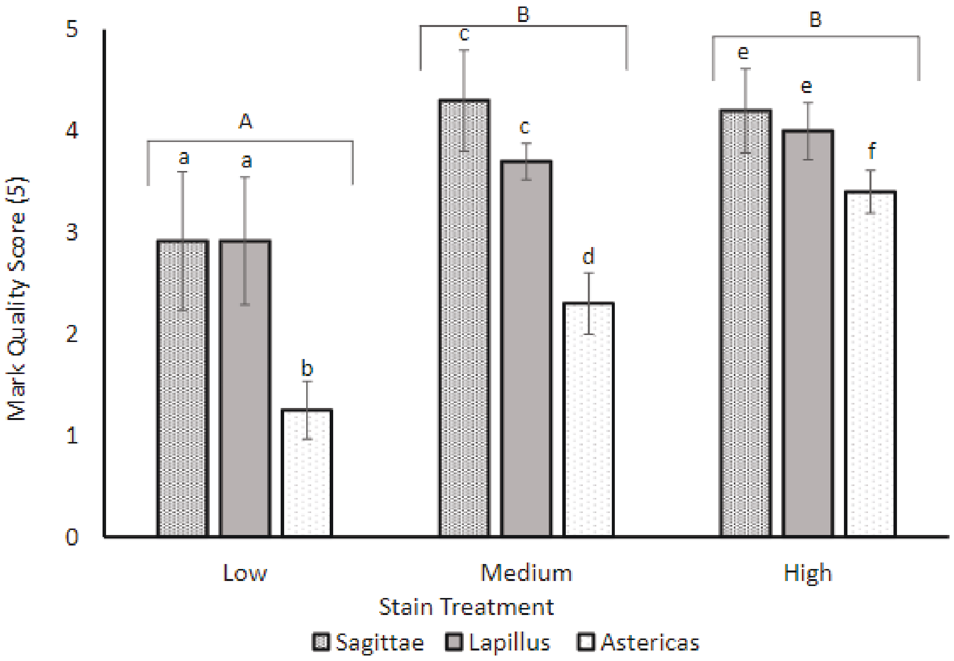

Figure 2.1 Mark quality in different concentrations of stain in the sagittae, lapilli, and asterisci. Error bars represent 95% confidence intervals and letters represent significant differences. Capitalized letters compare across treatments and lower-case letters compare within a treatment.

A Kruskal-Wallis test showed that higher ARS concentration results in smaller increment width (Figure 2, H(3) = 13.6, P = 0.004) in the lapillus. Post hoc tests revealed that the difference between low and medium treatments (H(3) = 10, P = 0.002) and low and high treatments (H(3) = 10, P = 0.002) was significant, whilst the difference between the control and low, medium and high were not significant (all comparisons, H(3) = 3.6, P = 0.058), suggesting higher stain concentrations may inhibit growth. These findings demonstrate that studies using ARS, such as fish stocking research, could introduce confounding effect when using otoliths to analyse growth-age relationships. Although we did not investigate the mechanism that caused the smaller increment widths, it may be explained by one of three different pathways: 1) inhibition of bone growth; 2) inhibiting of enzymatic pathways; and/or 3) causing a physiological and/or behavioural stress response. Although not fully explored in fish, ARS has been shown to inhibit growth and mineralisation in guinea pigs, rats and rabbits (Hoyte, 1960). Given otoliths are a calcified bone structure, at medium and high concentrations ARS could temporarily stunt growth. At the molecular level, ARS has been shown to bind to the active site of catalase (Hu et al., 2019) an enzyme involved in regulating oxidative damage and a fish's immune defence system (Kumari et al., 2014). These toxicants have been shown to result in lethargic movement, disrupt startle response, and impair swimming performance, indicating a possible mechanism for stunting growth at high concentration (*ibid*). Given the high concentrations of contaminates found in ARS by Jurgelėnė et al. (2022), the downstream effect on xenobiotic processes at high and medium concentrations should not be discounted. Physiological stress, like chemical and physical changes are well documented to have negative effects on fish growth and wellbeing (Barton, 2002). Despite no observations of changes in fish behaviour in the current study, it is possible that the 24-hour immersion at higher concentrations (involving less light, changes to water chemistry) could cause physiological stress to the fish, resulting in temporary decreases in growth.

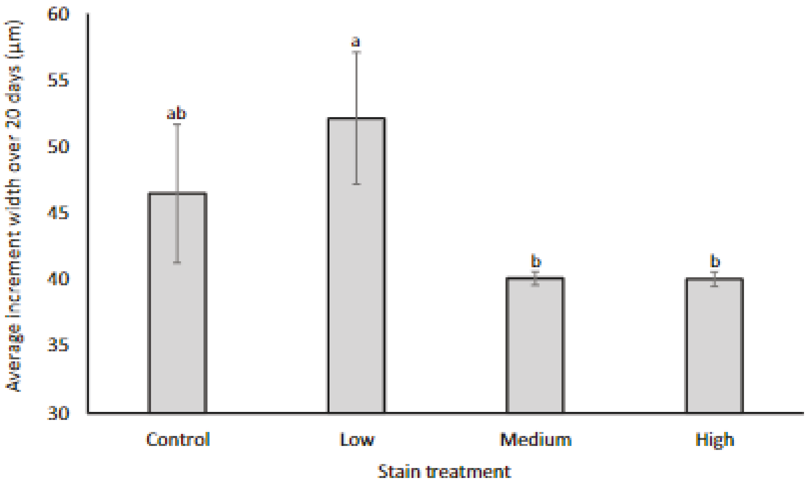

Figure 2.2 Average increment width (µm) during the experimental period in the lapillus at low, medium, and high stain concentrations and the control. Error bars represent 95% confidence intervals and letters represent significant differences. Lower-case letters represent significant relationships between concentration.

2.6 Conclusion

Overall daily otolith increments were verified in all three otolith types, but care needs to be taken when choosing stain concentration, as higher concentrations may affect somatic growth in fishes.

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Chapter 3 : Faster growth and shorter pelagic larval duration enhance the post-settlement persistence of a common range-extending coral-reef fish in a temperate ecosystem.

Under review for publication in the journal of Coral Reefs as:

Rigg, A; Fowler, A; Nagelkerken, I; Booth, D. (2024). Faster growth and shorter pelagic larval duration enhance the post-settlement persistence of a common range-extending coral-reef fish in a temperate ecosystem.

3.1 Abstract

Climate-induced ocean warming facilitates the poleward range expansion of tropical marine species into temperate waters. Such tropicalisation is reshaping marine ecosystems globally and has ecological implications. Our understanding of the factors influencing the establishment and persistence of vagrant tropical species in temperate waters remains limited. To address this knowledge gap, we examined which early life history traits affect the post-settlement persistence of a vagrant tropical fish, the sergeant major damselfish *Abudefduf vaigiensis*, in temperate waters off southeast Australia. Over an 8-week period, fish were collected, and otolith microstructure analysis indicated that early post-settlement persistence was linked to fast larval growth in the week prior to settlement and to shorter pelagic larval duration, but not body size at hatching or body size at settlement. We conclude that successful invasions of temperate

ecosystems by this vagrant tropical fish is likely mediated by demographic advantages these fish gain as early-stage larvae.

3.2 Introduction

Climate-induced ocean warming is transforming marine ecosystems worldwide, with temperate regions experiencing an influx of expatriated tropical fish species (referred to as ‘vagrant’ fish) (Fowler et al., 2018, Figueira and Booth, 2010, Nakamura et al., 2013a) . This phenomenon is particularly evident in climate change hotspots (Hobday and Pecl, 2014), where strengthening poleward currents facilitate the transport of tropical larvae to temperate habitats (Suthers et al., 2011). As a result, these regions serve as natural laboratories for studying the early life history dynamics and survival of vagrant fish species in novel environments.

One such hotspot is the south-eastern coast of Australia, where the strengthening of the East Australian Current (EAC) has increased the presence of vagrant tropical fish (Booth et al., 2018). However, this phenomenon is not unique to south-east Australia; similar observations have been made on the temperate west coast of Australia (Pearce et al., 2016) and in other temperate regions globally, such as Japan (Beck et al., 2017, Beck et al., 2016c), the Mediterranean Sea (Azzurro et al., 2011), and the western North Atlantic (Zarzyczny et al., 2024). These temperate ecosystems are gradually shifting towards tropical conditions, with the establishment of vagrant fish populations currently limited by their ability to survive the critical settlement phase and the cooler temperatures of winter to the next season (overwintering) (Figueira and Booth, 2010).

The settlement phase is a crucial period for reef fishes, particularly vagrant fish species arriving in temperate regions as they transition from pelagic larval stages to benthic juvenile stages and face novel challenges in terms of diet, habitat preference, and growth rates (Kimirei et al., 2013). Early life history traits (ELHTs), such as size at hatching, size at settlement, pelagic larval duration (PLD), and growth rates, are common metrics linked to the survival and success of individual fish during this critical phase (Bergenius et al., 2005, Bergenius et al., 2002, Vigliola and Meekan, 2002). A larger settlement size is often considered advantageous, as it can confer benefits in resource competition and predator avoidance (Hoey and McCormick, 2004). However, the relationship between ELHT and post-settlement survivorship is complex, with studies suggesting a decreasing risk of predation and rapid growth (Dingeldein and White, 2016).

In the context of vagrant tropical fish species settling in temperate environments, the importance of ELHT may be heightened due to the novel challenges they face in these non-native habitats. Factors such as thermal stress, novel habitats and ecological interactions may result in a shift in or strengthening of, the selective pressures acting on ELHT compared to those in their native range (Rankin and Sponaugle, 2011, Kingsbury et al., 2020a, Coni et al., 2022a). Temporal studies following individual cohorts offer a unique opportunity to investigate which factors influence the survival of vagrant fish species during settlement. This approach is particularly valuable for understanding the challenges faced by vagrant fish species in temperate environments, as the settling fish persistence is often limited to the warmer seasons, with few individuals successfully overwintering (Figueira et al., 2009).

Fish otoliths are small, calcified structures in fish's inner ears that grow in concentric rings, typically representing daily growth (Panella, 1971). The size and microstructure of these rings provide a historical timeline (proxy) of a fish's age, growth rate, and settlement timing. The validity of otolith analysis has been well-established (Panfili et al., 2009a), including for the species reported here, the sergeant major damselfish *A. vaigiensis* (Rigg et al., 2023). Temporal studies using otolith analysis offer a unique opportunity to investigate demographic factors influencing the growth and survival of vagrant fish species during settlement, as they allow for a focused examination of the relationship between ELHT and post-settlement persistence.

Here, we examine the relationship between ELHTs and early post-settlement persistence in the vagrant tropical damselfish, *Abudefduf vaigiensis*, to understand the traits that contribute to its successful settlement in temperate waters. We employed a site-specific temporal approach, tracking newly settled fish over 56 days at a single location throughout a high recruitment period in an ocean-warming hotspot off southeastern Australia. By analysing hatch size, pelagic larval duration (PLD), size at settlement, and growth rates, we identified ELHTs that could enhance post-settlement persistence. Understanding the factors that mediate the survival of a vagrant tropical fish during settlement is crucial for better predicting potential poleward range expansions and the establishment of warm-water populations in temperate regions.

3.3 Methods

This investigation was conducted following the University of Technology Sydney's Animal Ethics Committee Approval (ETH-6609) and under the NSW DPI permit (F94/696(A)-9.0).

Abudefduf vaigiensis was chosen as a model species because it is a common vagrant fish that often arrives in high numbers in temperate waters during summer-autumn recruitment seasons (Beck, 2014, Beck et al., 2014). Additionally, the ELHTs of *A. vaigiensis* have been previously validated through otolith analysis (Rigg et al., 2023) making it suitable for investigating the relationship between these traits and post-settlement persistence in a vagrant tropical fish.

The study was carried out on a shallow subtidal rocky reef at Little Manly, Sydney, Australia, over 56 days from the 4th of March 2021 to the 29th of April 2021 (coordinates: 33.8068° S, 151.2867° E). During this time, there was a significant influx of *A. vaigensis* larvae which led to the establishment of several shoals in the area. Fish were collected during seven sampling events over 8-weeks using hand nets, with each sampling event targeting randomly selected individuals from these shoals. The shoals consisted of fish of approximately the same size class during each event, ensuring a consistent sample in terms of size across the study period. Prior to sampling, fish were immobilized using a clove oil solution (1:5 dilution in ethanol). Following collection, specimens were euthanised using an ice bath.

The total length of each fish was measured to the nearest 0.1 cm using callipers, and mass was measured to the nearest 0.001 g. The lapillus otoliths were extracted and mounted because the lapillus was found to be flatter and resulted in clearer depositions and markings for analysis (Rigg et al., 2023). The mediolateral axis was oriented for analysis (Supplementary Source 2). To achieve a flat surface, samples were polished using lapping paper (3µm) and water to minimise marks generated during grinding. Measurements spanned from the primordium's centre to the furthest point on the anterior-posterior axis (Supplementary Source. 3).

A Nikon Eclipse Ni-E microscope was used at 200x magnification, with Nikon NIS-elements Advanced Research software (V5.02.02) for measurements. Cedarwood oil was applied during microscopy to improve clarity for reading otoliths.

A strong positive relationship between otolith radius and fish total length was established ($R^2 = 0.8668$, Supplementary Source 1), validating this study's use of otolith measurements as proxies for fish size and growth. Based on this relationship, the hatch radius, used as a proxy for size at hatch, was determined by measuring the distance from the centre of the primordium to the edge of the hatch check mark, expressed in micrometres (µm). Daily increment width was determined by measuring the distance between the edges of two consecutive increments. The settlement mark corresponds to the settlement day and was used to calculate the PLD by counting the increments between the hatch check and the settlement mark. The otolith radius at the settlement mark, measured from the hatch check, was used as a proxy for size at settlement. The growth rate immediately prior to settlement was calculated by averaging the increment widths for each fish seven days before settlement and was expressed in µm/day. To characterise the dependent variables, we examined their distributions. These histograms, coupled with density plots, highlighted the range, central tendency, and spread of the data for each trait (Supplementary Source. 3).

Otolith microstructure analysis methods followed the guidelines provided by Panfili et al. (2009b) to ensure the reliability of the readings. Fish were randomised and read blind to avoid potential biases. Otoliths were read twice; if counts of daily increments in those readings differed by more than 10%, a third reading was done. The reading closest to the third reading was considered more reliable. If the two readings were similar, they were likely correct and accepted. Discussions with another researcher on the otolith markings (i.e., hatch, PLD, etc.) were conducted to reach a consensus.

To investigate the relationship between ELHTs and post-settlement persistence, we conducted linear regression analyses using age (days post-settlement) as the independent variable and each of the following traits as dependent variables: size at hatch, pelagic larval duration (PLD), size at settlement, and pre-settlement growth rate. We also tested the relationship between PLD and growth rates using the same process. The average otolith increment width over seven days prior to settlement was used as a proxy for immediate pre-settlement somatic growth. This approach is commonly used to estimate growth and development during the early life stages of fish and has been employed in numerous studies exploring ELHTs (Searcy and Sponaugle, 2000, Sponaugle and Grorud-Colvert, 2006, Wilson and McCormick, 1999).

The regression models were evaluated using the coefficient of determination (R^2) to assess the proportion of variance explained by the relationship. A $P < 0.05$ was considered significant. Assumptions of linearity, homoscedasticity, independence, and normality of residuals were checked. Linearity and homoscedasticity were assessed visually using residual plots. The Breusch-Pagan test was used to statistically test for homoscedasticity, with $P > 0.05$ indicating homoscedastic residuals. The normality of residuals was assessed by visually inspecting Q-Q plots.

All statistical analyses were performed using Python (version 3.11.5) with the *statsmodels* and *scipy* libraries. Visualisations of the regression analyses were created using the *matplotlib* and *seaborn* libraries.

Table 3.1 Descriptive statistics for *A. vaigiensis* samples (n = 47), showing the range and mean (with standard deviation) for age, length, hatch size, Pelagic larval duration, size at settlement and pre-settlement growth rate. SD = 1 standard deviation.

Variable	Range	Mean ± SD
Age (days)	33 - 77	56.9 ± 14.3
Total body length (mm)	13.5 - 50.0	31.7 ± 9.7
Hatch size (µm)	7.5 - 16.9	11.4 ± 1.8
PLD (days)	21.0 - 29.0	26.0 ± 1.6
Total length at settlement (µm)	158.9 - 248.7	199.6 ± 22.1
Pre-settlement growth rate (µm/day)	4.4 - 13.3	7.7 ± 1.8

3.4 Results and Discussion Early life history dynamics have been shown to drive subsequent post-settlement population dynamics of reef fishes (Doherty and Fowler, 1994). However, this has rarely been assessed for vagrant range-extending species. Here, we examined a suite of ELHTs using an 8-week sampling approach to understand which ELHTs influenced the post-settlement persistence of *A. vaigiensis* at a temperate site. We defined post-settlement persistence as the duration an individual remained at the site after settlement, using days post-settlement as a proxy for this measure

We found a significant negative relationship between PLD and days post-settlement ($R^2 = 0.24$, $p = 0.0005$, Fig 1A), indicating that individuals with shorter PLDs persisted longer after settlement. The PLDs we observed (21-29 days, Tab. 1) were longer than those reported for *A. vaigiensis* in its native tropical eastern Pacific range (17-20 days, n=3) (Wellington and Victor, 1989). This finding suggests that the sampled individuals may have experienced extended duration in the plankton.

While the relationship between PLD and dispersal distance is complex and often inconsistent across larval marine species (Shanks, 2009, Feary et al., 2014), our findings suggest that fish with lower PLD hatched closer to those of this species in the native tropical PLD range may confer an advantage for this range-extending species in temperate environments. This advantage in post-settlement persistence might stem from these individuals retaining more energy reserves for post-settlement survival, possibly due to spending less time in the plankton. Additionally, these energy reserves could potentially support faster growth rates, which may contribute to better survival in the new environment. Alternatively, these results could indicate a mixed cohort from different origins, with fish from populations closer to the study site having shorter PLDs and better survival. Future studies using analysis of elemental isotopes in otoliths could reveal if fish had a common origin (Gillanders, 2005, Trueman et al., 2012). Similarly, LA-ICPMS could provide finer-scale insights into natal origins by analysing otolith microchemistry (Fairclough et al., 2011), while genomic approaches could assess genetic connectivity among populations (Gajdzik et al., 2021).

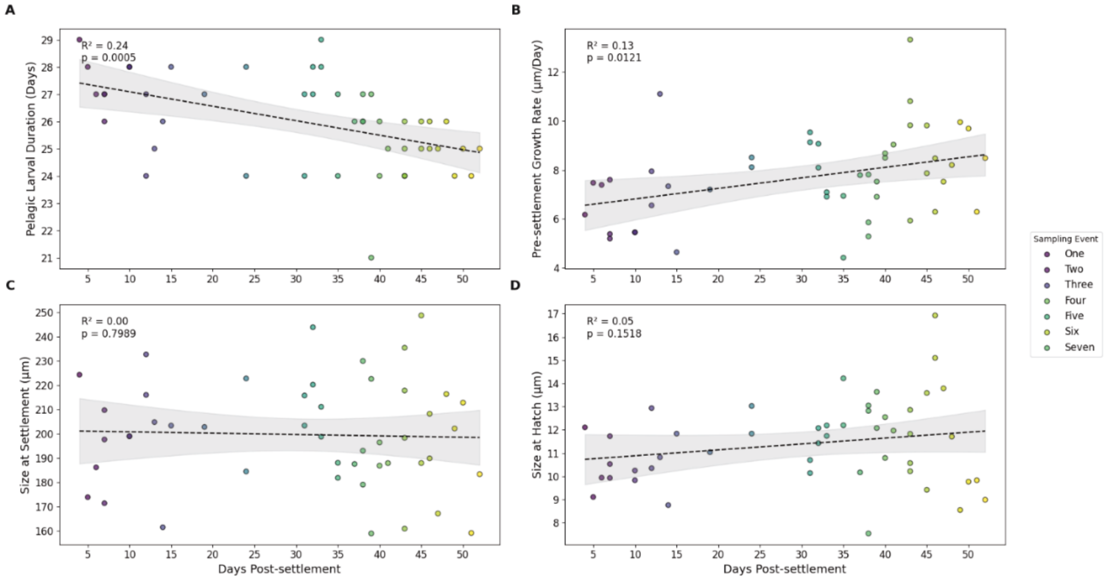

Figure 3.1 Relationships between days post settlement of *A. vaigiensis* and (A) Pelagic larval duration (days), (B) Pre-settlement growth rate (μm/day), (C) Size at settlement (μm), and (D) Size at hatch (μm). Dots represent individual fish and colours represent one of seven collection groups. The dashed lines indicate linear regression fits and the shaded area represents the CI 95% interval. R² values show the coefficient of determination, and p-values indicate the statistical significance of each relationship.

We found a significant positive relationship between pre-settlement growth rate and persistence ($R^2 = 0.13$, $p = 0.0121$, Fig. 1B). Fish with faster pre-settlement growth rates also tended to have shorter PLDs ($R^2 = 0.12$, $P = 0.0167$, Fig. 2). This aligns with the "growth-mortality hypothesis" (Anderson, 1988, Searcy and Sponaugle, 2001), which states that faster-growing larvae can achieve the minimum size required for settlement more quickly, potentially reducing their exposure to high mortality rates in the pelagic environment. Our results extend this concept to vagrant populations, suggesting that the ability to grow quickly and settle earlier might be an important factor for the successful establishment of this tropical species in temperate waters.

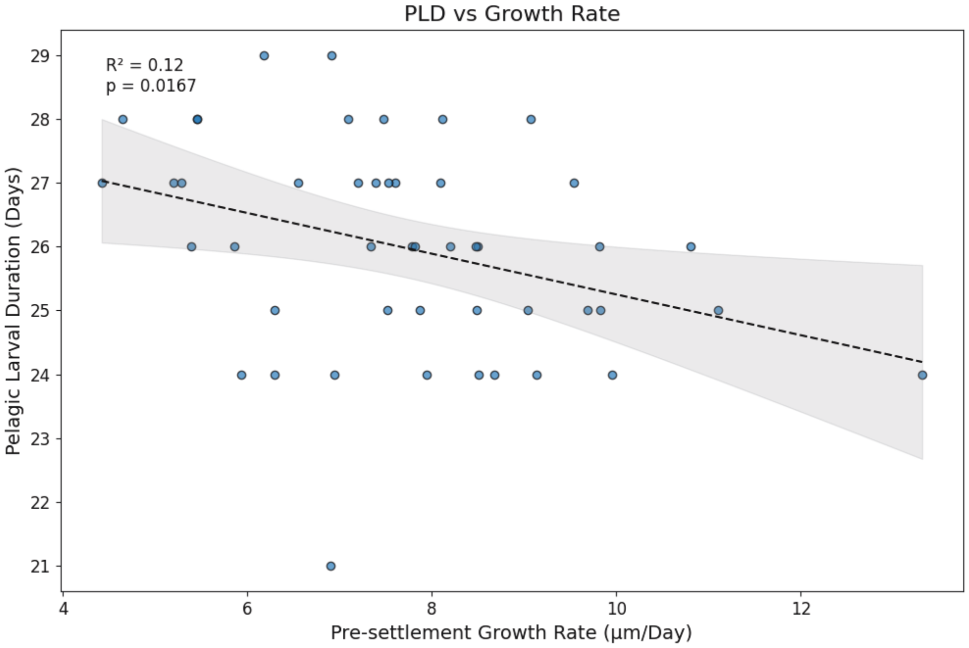

Figure 3.2 Relationship between pelagic larval duration (PLD) and pre-settlement growth rate. The x-axis shows pre-settlement growth rate (μm/day) and the y-axis shows pelagic larval duration (days). Each dot represents an individual fish. The dashed line indicates the linear regression fit and the shaded grey area represents the 95% confidence interval. R^2 value shows the coefficient of determination (0.12), and the p-value (0.0167) indicates the statistical significance of the relationship between PLD and pre-settlement growth rate.

Surprisingly, we did not find a significant relationship between days post-settlement and size at settlement ($R^2 = 0.00$, $p = 0.7989$, Fig 1C), suggesting that while faster-growing individuals settled earlier, they did not necessarily settle at a larger size. Slower growers may need to stay in the plankton for longer to reach the larger sizes required for successful settlement (Searcy and Sponaugle, 2001). This dynamic may explain our finding of lower persistence in fish with longer PLDs, as these individuals likely represent slower growers that remained in the plankton longer and possibly entered the settlement phase in a weaker condition compared to their faster-growing counterparts. This suggests that this range-extending tropical fish is subject to trade-offs in their novel ranges like their normal ranges, where body size at settlement is not a crucial mediator of their persistence, but growth rate and PLD are key factors influencing post-settlement survival (Gagliano et al., 2007a).

Our analysis revealed no significant relationship between days post-settlement and size at hatch ($R^2 = 0.05$, $p = 0.1518$, Fig 1A), contrasting with previous studies on other species within natal ranges where size at hatch influences early-life survival and recruitment success (Gagliano et al., 2007a, Vigliola and Meekan, 2002). However, our findings suggest that the relationship between size at hatch and early settlement may not hold for range-extending tropical fish in temperate waters for this species. The lack of correlation between size at hatch and post-settlement persistence in our study indicates that the factors influencing the survival of this vagrant species in novel environments may differ from those in their native ranges.

3.5 Conclusion

In conclusion, our study provides evidence that early life history traits, particularly shorter pelagic larval duration and faster pre-settlement growth rates, may confer advantages for individuals of *A vaigiensis* and possibly other vagrant tropical fish early persistence in temperate environments. As ocean warming continues to facilitate range expansions, understanding these traits amongst other species could help better predict which individuals and populations are more likely to successfully establish in new environments, with implications for ecosystem changes in temperate regions.

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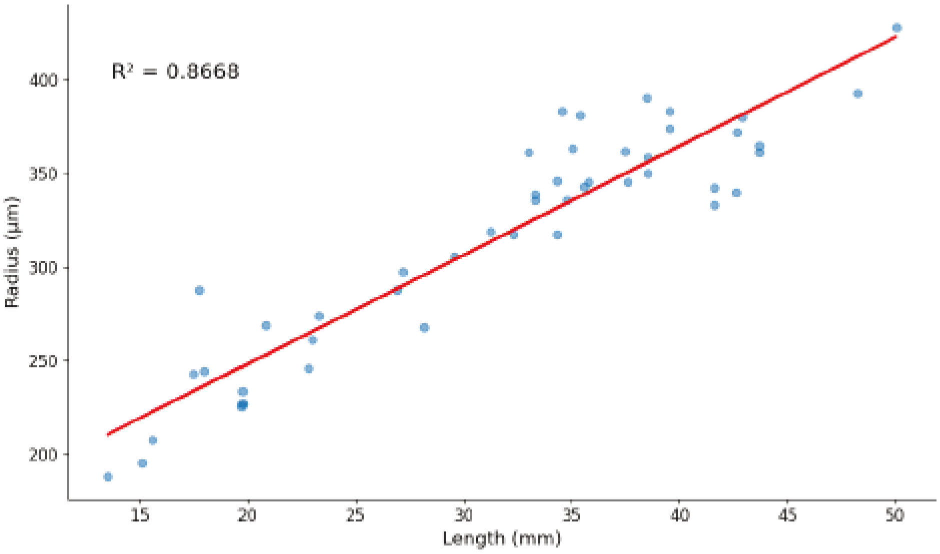
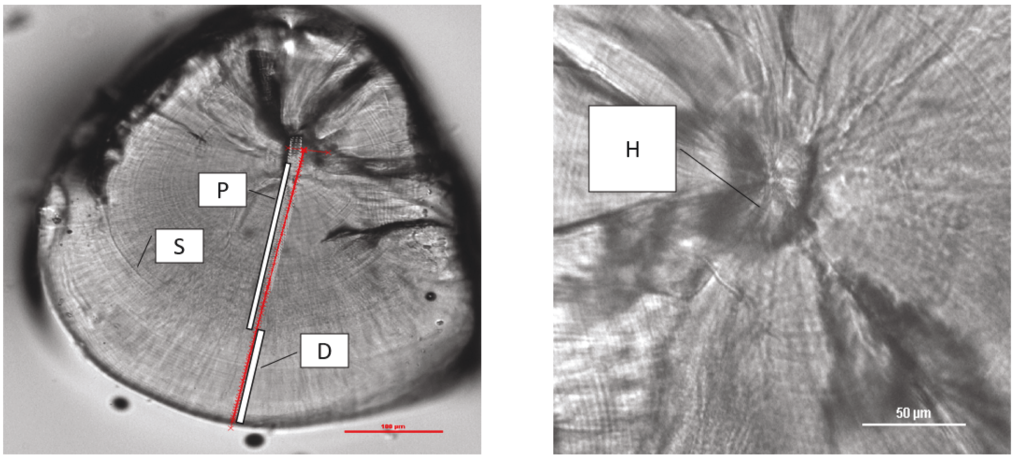
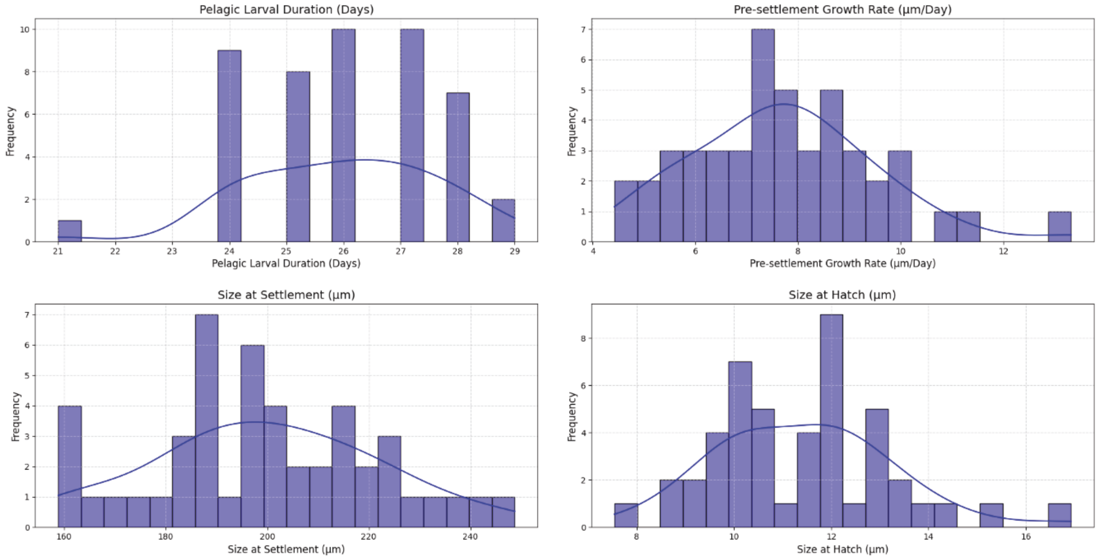
3.7 Supplementary



Supplementary Source 3.1 Relationship between fish total length (mm) and otolith radius (µm) in *Abudefduf vaigiensis*. Each point represents an individual fish (n = 47). The solid red line indicates the linear regression ($y = 5.8x + 131.9$, $R^2 = 0.8668$).



Supplementary Source 3.2 Photos of Lapillus and Key Markings. A) Depicts Pelagic Larval Duration (PLD), marked as 'P', characterised by larger growth increments originating from the primordia. 'S' indicates the settlement mark, and 'D' represents the post-settlement daily growth increments. B) A magnified view of a different sample, highlighting the hatch check on the lapillus, denoted by 'H'.



Supplementary Source 3.3 Distributions of dependent variables. Histograms and density plots illustrate the range, central tendency, and spread for each variable: (A) Pelagic Larval Duration, (B) Pre-settlement Growth Rate, (C) Size at Settlement, and (D) Size at Hatch

Chapter 4 : Habitat complexity and body size mediate feeding and risk-taking in a range-extending tropical fish under future warming

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4.1 Abstract

Understanding the ecological factors that mediate the establishment of tropical vagrant fishes at their cold-water range edges under a changing climate is important for predicting future range expansions and the dynamics of novel community structures. Using controlled laboratory experiments, we examined how water temperature, fish body size, and habitat complexity influence the behaviour of the common tropical vagrant fish *Abudefduf vaigiensis*. Based on temperature-dependent performance theory, we predicted higher activity and feeding at 22°C and 26°C (future winter temperatures at range edge) compared to 17°C (present-day winter temperature at cold range edge), with complex habitats potentially mitigating physiological performance at all temperatures by enhancing foraging opportunities. All fish size classes exhibited significantly higher activity levels and decreased shelter use at warmer temperatures compared to 17°C. Size-specific differences were only observed for larger fish during a simulated predator exposure, where they spent more time in shelter than smaller fish, irrespective of temperature. Bite rates increased linearly with temperature in complex

habitats but plateaued at 22°C and 26°C in less complex habitats. Here, we reveal how complex habitats might facilitate the successful establishment of tropical fish at temperate range edges under future ocean warming by increasing feeding rates at higher temperatures.

4.2 Introduction

Climate change is already significantly altering marine environments worldwide and driving tropicalisation—where subtropical and tropical marine species expand their ranges into temperate ecosystems (Horta e Costa et al., 2014). Western boundary currents transitioning from tropical to temperate waters, such as the East Australian Current in Australia and Kuroshio Current in Japan, serve as thermal corridors accelerating this process. These boundary regions are warming at two to three times the global average (Wu et al., 2012), creating hotspots for tropical species' poleward range expansions. While much attention has focused on the physiological tolerances of range-shifting species and the impacts on community dynamics, behavioural responses remain largely unexplored and might also be important in determining establishment success, particularly in surviving cooler winter temperatures at range edges.

Behavioural plasticity can determine whether species successfully establish themselves in novel environments (Davidson et al., 2011). For range-expanding marine species, behavioural characteristics influence habitat selection, predator avoidance, resource acquisition, and shoaling interactions in their new environments (Coni et al., 2022b, Paijmans et al., 2020, Paijmans et al., 2019, Kingsbury et al., 2020b). For example, temperate reefs transitioning from kelp to turf-dominated systems provide novel habitat opportunities for tropical species (Pessarrodona et al., 2022). The range expansion of the sea urchin *Centrostephanus rodgersii*, driven by warming waters and the East Australian Current, has led to the overgrazing of kelp forests and the creation of urchin barren habitat in certain parts of southeastern Australia (Andrew and O'Neill, 2000, Wernberg et al., 2011). This alteration of the seascape potentially creates a more favourable habitat seascape for tropical fish by reducing competition with kelp-associated species and opening up new settlement opportunities (Vergés et al., 2014, O'Connell et al., 2023, Coni et al., 2021), but only if these species can effectively modify their behaviour to utilise these habitats. Similarly, while physiological tolerances may permit survival at certain temperatures, behavioural limitations like reduced feeding or increased sheltering could prevent population establishment (Weetman et al., 1998).

Most tropical vagrant fishes arriving in temperate waters fail to survive winter temperatures, making winter survival a bottleneck for population establishment (Figueira and Booth, 2010). For example, in southeastern Australia, winter water temperatures regularly drop to ~17°C, a threshold associated with significant mortality in tropical species (Booth et al., 2018, Djurichkovic et al., 2019a) . However, with winter temperatures projected to exceed 17°C more frequently under climate change (Suthers et al., 2011, Intergovernmental Panel on Climate, 2023), understanding behavioural responses above this thermal threshold becomes crucial. For *Abudefduf vaigiensis*, a common tropical vagrant fish in temperate waters, future survival through the winter period could enable the establishment of permanent populations, fundamentally altering temperate reef communities (Vergés et al., 2014, Zarco‐Perello et al., 2021, Zarco‐Perello et al., 2020). While predation is often hypothesised as the primary cause of winter mortality (Beck et al., 2016a), the behavioural mechanisms that link temperature, predation risk, and survival remain unclear. Moreover, seasonal pulses of tropical fish recruitment mean that individuals of varying sizes face these winter conditions simultaneously (Figueira et al., 2009), potentially leading to size-specific survival strategies.

Temperature fundamentally influences fish behaviour through its effects on metabolic processes and enzyme function (Fry, 1971). Temperature-dependent performance theory predicts that as temperatures deviate from optimal ranges, behavioural changes often precede physiological failure (González-Ferreras et al., 2023, Payne et al., 2016). In fishes, these behavioural modifications typically manifest as decreased activity, reduced feeding rates, and increased sheltering behaviour (Coni et al., 2022c, Djurichkovic et al., 2019a). For tropical species entering temperate waters, 17°C appears to be a critical thermal minimum driving significant behavioural changes. However, the extent to which habitat complexity might mediate these temperature-dependent responses remains poorly understood. Complex habitats could allow fish to maintain higher performance at suboptimal temperatures by providing enhanced foraging opportunities and refuges that reduce the energetic costs of predator avoidance (Almany, 2004, Bonin et al., 2009).

The size of an individual may influence its behavioural responses to novel environments, particularly as winter temperatures approach. Vagrant tropical fishes typically arrive in temperate waters during warmer months when temperatures are within their thermal preferences. However, recruitment occurs in pulses throughout summer, resulting in fish of different sizes and ontogenetic stages - from recent settlers to adults - experiencing their first winter concurrently. These size differences likely affect individuals' abilities to respond to thermal stress, modify habitat use, and balance predation risk with foraging needs. However, most studies examining tropical vagrants focus on a narrow range of size classes (Djurichkovic et al., 2019a, Paijmans et al., 2020), limiting our understanding of how size-dependent behaviours might influence overwinter survival and eventual population establishment.

The structural complexity of temperate sandstone reefs vary, potentially influencing how these fish respond to temperature fluctuations and size-related behavioural changes. While climate-driven loss of kelp forests alters temperate reef habitats (Davis et al., 2022), this may benefit tropical vagrants by exposing preferred sandstone habitats. These sandstone rocky reefs vary considerably in structural complexity, from intricate formations with numerous small crevices to larger, more open structures. Complex sandstone crevices can provide refuge from predators and enhanced foraging opportunities (Almany, 2004, Bonin et al., 2009). However, we have limited understanding of how tropical vagrant fish utilise different levels of sandstone complexity, particularly as temperatures are increasing beyond winter thermal thresholds. The interaction between habitat complexity and temperature could be an important factor for survival, as fish may rely more heavily on complex habitat structures during periods of thermal stress. This relationship becomes increasingly relevant as climate change exposes more sandstone habitats, potentially creating wider settlement opportunities for vagrant fish while influencing their ability to persist through seasonal temperature fluctuations.

To address these knowledge gaps, we conducted a controlled laboratory study to explore the interacting factors influencing the behaviour of *Abudefduf vaigiensis*, one of the most common tropical vagrant species in southeastern Australia. We developed several hypotheses about how size, temperature, and habitat complexity would interact to influence fish behaviour in temperate waters. First, we hypothesised that all size classes would show higher activity and reduced shelter use at warmer temperatures (22°C, 26°C) compared to 17°C. We predicted that smaller fish would show higher activity levels and feeding rates at elevated temperatures compared to larger fish, reflecting different energetic demands across size classes. Second, we expected that more complex habitats would enhance feeding opportunities and provide better predator refuges, resulting in higher bite rates and more rapid recovery from predator exposure than less complex habitats. Third, we predicted elevated water temperatures would decrease shoal cohesion (measured through nearest neighbour distance and shoal area) as fish prioritise individual shelter-seeking over group formation, particularly in more complex habitats. Finally, we hypothesised that size-specific behavioural responses would be most pronounced during predator exposure, with larger fish showing stronger shelter-seeking behaviour and tighter shoaling due to their greater visibility to predators.

By addressing these objectives through controlled video-based experimental phases, we aim to enhance our understanding of the processes influencing the establishment of tropical fish species in temperate marine ecosystems and the role of habitat complexity in mediating fish behaviour under climate change. Our findings will provide insights into the potential behavioural mechanisms facilitating the range expansion of tropical vagrant species in warming temperate waters.

4.3 Methods

4.3.1 Permits and Ethics

This investigation was conducted following the University of Technology Sydney's Animal Ethics Committee Approval (ETH-6609) and under the NSW DPI permit (F94/696(A)-9.0).

4.3.2 Model Fish and Collection

To study the effects of environmental factors on the behaviour of the sergeant major damselfish, *Abudefduf vaigiensis*, we manipulated temperature, habitat complexity, and fish size in a laboratory environment. *A. vaigiensis*, a widely studied tropical reef fish known for its broad geographical distribution throughout the tropics, is an ideal model species due to its prominence in climate change ecology studies and reliable abundance in the austral summer and autumn (Figueira and Booth, 2010). This species is characterised by a larval dispersal phase, strong site fidelity, and the potential to grow to a size of 20cm (adult), making it a suitable model for examining the effect of environmental variables on fish behaviour.

A. vaigiensis, ranging in size from 15mm to 80mm, were collected from Little Manly (33.8067° S, 151.2870° E), Freshwater Beach (33°46'53"S 151°17'40"E), and Malabar Beach (33.9640° S, 151.2522° E) in Eastern NSW during January 2023 to April 2024. Capture was achieved using hand nets and fish were immobilised using a clove oil solution (Fernandes et al., 2017). Post-collection, the specimens were transported to the aquaria facility at the University of Technology Sydney, where they were acclimated within a 1°C margin of the site water temperature.

4.3.3 Water Chemistry and Husbandry

To maintain minimal habitat conditions during the pre-experimental phase, two 3-inch (7.6cm) PVC pipes were provided in each tank. Water chemistry was monitored twice daily, maintaining ~35,000 ppm salinity, DO 8mg/L, and a pH of 8.1. Additionally, ~25% water changes were conducted twice daily, including debris removal to ensure optimal water quality. Temperatures were achieved in glass tanks (80cmx45cmx60cm) using water heaters, with the temperature adjusted gradually at a rate of 1°C per day. Live artemia, hatched from cysts after two days, was used for feeding to maintain consistent prey density across the experiment. Feeding volumes were 40ml for small, 50ml for medium, and 60ml for large fish on non-experimental days.

4.3.4 Fish Size Treatments

The fish were categorised into three size classes: small (<30mm), medium (30-45mm), and large (>45mm). Size classes were determined based on ontogenetic stages and field observations of the species' growth cycle in temperate regions. Small individuals (<30mm) represented early settlers, medium (30-45mm) represented juveniles, and large (>45mm) represented adults, with the upper size limit determined by the seasonal growth patterns observed in temperate ecosystems. Five fish of varying lengths within the same size class were selected for each treatment group (size, temperature, and habitat) to maintain a similar mean size across the groups (Supplementary Source 1). Each fish was measured using callipers (to the nearest 0.1mm) and weighed (to the nearest 0.001g) within 24-48 hours of arriving at the aquaria facility. Subsequently, they were tagged on both sides of the body with visible implant elastomer (product 1VIFE000007, Northwest Marine Technology, Inc), using unique colour codes and tag positions for identification.

4.3.5 Temperature Treatments

Experimental temperatures were chosen to reflect key thermal thresholds relevant to the survival and behaviour of vagrant fish. The lower bound of 17°C represents a temperature known as the bottleneck to survivorship for these species in Eastern Australia (Figueira et al., 2009). This temperature marks the lower thermal tolerance limit for many vagrant species, making it a critical point for understanding stress responses. 22°C represents typical Sydney summer conditions, providing a baseline for normal physiological and behavioural activities in temperate conditions. The upper bound of 26°C anticipates the potential future scenarios under climate change, where temperate locations may experience elevated temperatures that are currently typical of the fish's tropical native habitats (Mitchell et al., 2022). Fish were acclimated by 1ºC per day until target temperatures were reached.

4.3.6 Habitat Complexity Treatment

The experimental habitats reflected the structural complexity of potential temperate settlement sites for vagrant tropical fish, as described in the literature Beck et al. (2017). The 'high complexity habitat' consisted of numerous smaller rocks, which provided increased complexity with smaller cavities, mirroring the crevices of sandstone reefs that offer shelter and foraging spaces. In contrast, the low-complexity habitat setup had larger sandstone boulders, creating a less intricate structure with wider gaps and fewer hiding spots. Both habitat types were standardised in height and breadth to ensure experimental consistency.

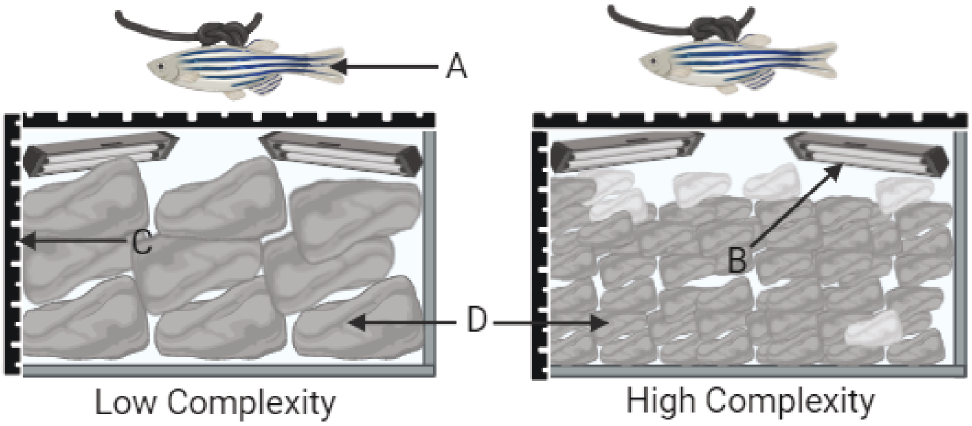

Figure 4.1 Experimental aquarium setup depicting two levels of habitat complexity for experimental phases. (A) A Halco MAX 130 lure rigged on a pulley system to simulate predatory threats, (B) UV lighting installed above to enhance the visibility of fluorescent Visible Implant Elastomer (VIE) tags for individual fish identification, (C) rulers mounted along the tank's perimeter for scale reference, and (D) habitat complexity ranging from low complexity with larger sandstone formations on the left to high complexity with smaller sandstone structures on the right.

4.3.7 Experimental Phases

Fish were added to the habitat in the afternoon, between 1400 and 1700, before the investigation the following day. Just before the investigation, UV lighting was used to enhance the visibility of the VIE tags to identify individual fish. Behavioural observations of the experimental phase were conducted within a 15-minute session in the morning (0600 – 0800) for each treatment group, filmed using a GoPro Hero 7 camera. We measured the behavioural proxies (Tab.2) over four consecutive experimental phases: baseline, feeding, predator simulation and post-predator simulation. The sequence was in a fixed order, meaning it was consistent across treatments and replicates. The purpose of the sequence was to simulate a series of events that fish might encounter *in situ*. Each experimental phase is as follows:

- **Baseline Phase (0-5 minutes):** Fish were filmed for a 5-minute period to acclimate without any external stimuli. The observer remained concealed, ensuring undisturbed baseline data was collected at the 2-minute mark (Tab.2).
- **Feeding Phase (5 - 10 minutes):** At the five-minute mark, treatments were provided 50ml of live artemia via a syringe from above the tank. 'Feeding' behavioural metrics were then recorded starting 30 seconds post-food introduction to capture the immediate response to feeding (Tab.2)
- **Predator Simulation Phase (at 10 minutes):** A simulated predatory threat was introduced using a Halco MAX 130 lure, deployed via a concealed pulley system. The predator simulation response phase focused on recording the fish's immediate behavioural metrics over a brief 15-second interval, synchronised with the lure's contact with the tank bottom, to capture the fear response to the simulated predator. Feeding metrics were not recorded in this phase (Tab.2)
- **Post-Predator Phase (13 minutes):** 3-minute after the Predator Simulation response, 'post-predator' recovery behavioural metrics were recorded, excluding bite rate (Tab.2). The post-fear assessment provides insights into the duration and extent of behavioural alterations following a stress event.

Table 4.1 Behavioural Metrics Measured During Experimental Phases.

Variable	**Shoal or Individual**	**Description**	**Expression**
Habitat Usage	Individual	The proportion of time spent by the fish less than 0.5 body lengths from the habitat over a 30-second period.	Average habitat usage (%)
Nearest Neighbour	Individual	Proximity of the target fish to the nearest fish (Paijmans et al., 2022). Measured from the fish's eyes using ImageJ. Measured 3 times over a 30-second period.	Average distance (mm and body lengths)
Feeding/Bite Rate	Individual	Frequency of feeding attempts within a 30-second observation period (Djurichkovic et al., 2019a).	Bites per minute (BPM)
Shoal Area	Shoal	ImageJ software measured fish shoal area (Schneider et al., 2012). Images were calibrated to real-world dimensions based on a known scale included in the image. Measured 3 times over a 30-second period to create an average shoal area value.	Average area (cm^2)
Activity	Individual	The amount of time over a 30 second period the fish was swimming (Coni et al., 2022a).	Activity %

4.3.8 Data Analysis

We used individual fish as the unit of replication in this study because fish behaviours can vary considerably among individuals, even within the same species and under similar environmental conditions (Mittelbach et al., 2014). Treating each fish as an independent replicate allowed us to account for this individual variation and obtain a more accurate representation of the population-level responses to the experimental factors. The selection of fish for each treatment was randomised, ensuring that any observed behaviour differences could be attributed to the experimental factors rather than pre-existing individual differences

The effects of temperature, habitat complexity, fish size, and experimental phase on the behaviour of *A. vaigiensis* were investigated using a series of permutational multivariate analysis of variance (PERMANOVA) tests. Analysis was conducted using the software package PRIMER v7 with the PERMANOVA+ add-on (Anderson et al., 2008; Clarke & Gorley, 2015).

Prior to analysis, the behavioural response variables were standardised to account for differences in scale. A Euclidean distance resemblance matrix was then calculated from the standardised data. The PERMANOVA model was constructed using a four-way design, with temperature (fixed factor; 3 levels: 17°C, 22°C, 26°C), habitat complexity (fixed factor; 2 levels: high, low), fish size (fixed factor; 3 levels: small, medium, large), and experimental phase (random factor; 4 levels: normal, feeding, fear, post-fear) as the main effects. We included all interactions of the model.

To account for the repeated measures design, where each fish's behaviour was measured across a sequence of events, the individual fish ID (FISHID) was included as a random factor nested within the combination of temperature, habitat complexity, and fish size. The PERMANOVA models were run using 9999 permutations of residuals under a reduced model, with statistical significance set at $\alpha = 0.05$. Pairwise comparisons were performed for significant main effects and interactions using the PERMANOVA t-statistic, with 9999 permutations.

A repeated measures ANOVA was used to determine whether there were significant differences in individual behaviours across the experimental phase and whether these differences varied as a function of temperature, habitat complexity, and fish size. Before conducting the repeated measures ANOVA, the assumption of sphericity was tested using Mauchly's Test of Sphericity. Sphericity assumes that the variances of the differences between levels of the within-subjects factor (experimental phases) are equal. When this assumption was violated, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom and ensure the validity of the F-statistic. Post-hoc comparisons were performed using Bonferroni-corrected pairwise tests to control for Type I errors for significant main effects and interactions. Statistical significance was set at $\alpha = 0.05$.

Bite rate was analysed separately from other behavioural metrics as it could only be measured during the feeding phase, while other behaviours were measured across all experimental phases. We analysed the effects of size class, habitat complexity, and temperature on bite rate using a three-way univariate permutational analysis of variance (PERMANOVA) with 9999 permutations. Factors were treated as fixed effects, and the resemblance matrix was calculated using Euclidean distances. We considered the main effects and all possible interactions. Significance was assessed at $\alpha = 0.05$.

We conducted post-hoc pairwise comparisons using PERMANOVA for significant main effects and interactions. These pairwise tests were performed with 9999 permutations and p-values were adjusted for multiple comparisons using the Monte Carlo method.

4.4 Results

The tropical vagrant fish *A. vaigiensis* exhibited distinct behavioural changes at 22°C and 26°C, compared to 17°C, in their baseline activity levels, shelter use (Fig 2), and bite rates (Temperature effect: Pseudo-F = 3.4276, $p = 0.0033$, Fig. 3, Supp. 2). Above this threshold (at 22°C and 26°C), fish showed similar behavioural patterns (22°C vs 26°C: $t = 0.77317$, $p = 0.5244$, Fig 2, Supp. 5).

Bite rates were analysed separately from other behavioural metrics as they could only be measured during the feeding phase. Temperature significantly affected both feeding behaviour and habitat use, with these responses varying by habitat complexity.

In complex habitats, bite rates increased from 17°C to 26°C (Temperature × Habitat complexity: Pseudo-F = 6.3028, $p = 0.0069$, Fig. 3, Supp. 6), while in simpler habitats, feeding activity plateaued above 22°C (Fig. 4). Smaller (<30 mm) and medium-sized fish (30-45 mm) had higher bite rates than larger individuals (>45 mm) ($p < 0.001$) (Fig. 3). Fish spent significantly less time outside shelter at 17°C compared to warmer conditions (22°C $p < 0.001$, 26°C $p < 0.001$, Fig. 2, Supp. 10), with activity levels following a similar pattern (22°C $p < 0.001$, 26°C $p < 0.001$, Fig. 3, Supp. 9). However, larger fish showed more conservative behaviour overall, spending significantly less time outside the shelter compared to small ($p < 0.001$) and medium fish ($p < 0.001$, Fig. 2, Supp. 10).

The presence of a simulated predator and food presence triggered contrasting behavioural responses (Experimental phase effect: Pseudo-F = 10.825, $p < 0.001$, Fig. 2, Supp. 2,3). During predator exposure, all fish reduced their time outside shelter ($p < 0.001$, Fig. 2, Supp 10), although this response was strongest in larger individuals. Feeding increased activity levels ($p < 0.001$, Fig. 2, Supp. 9) and time spent outside shelter across all size classes, but this effect was dampened at 17°C. Following predator exposure, fish at warmer temperatures (22°C and 26°C) returned to baseline activity levels compared to those at 17°C ($p < 0.001$, Fig. 2, Supp 9).

No significant effects of temperature, size class, or habitat complexity were found on shoaling behaviour (nearest neighbour distance and shoal area) across any experimental phases ($p > 0.05$, Fig. 2).

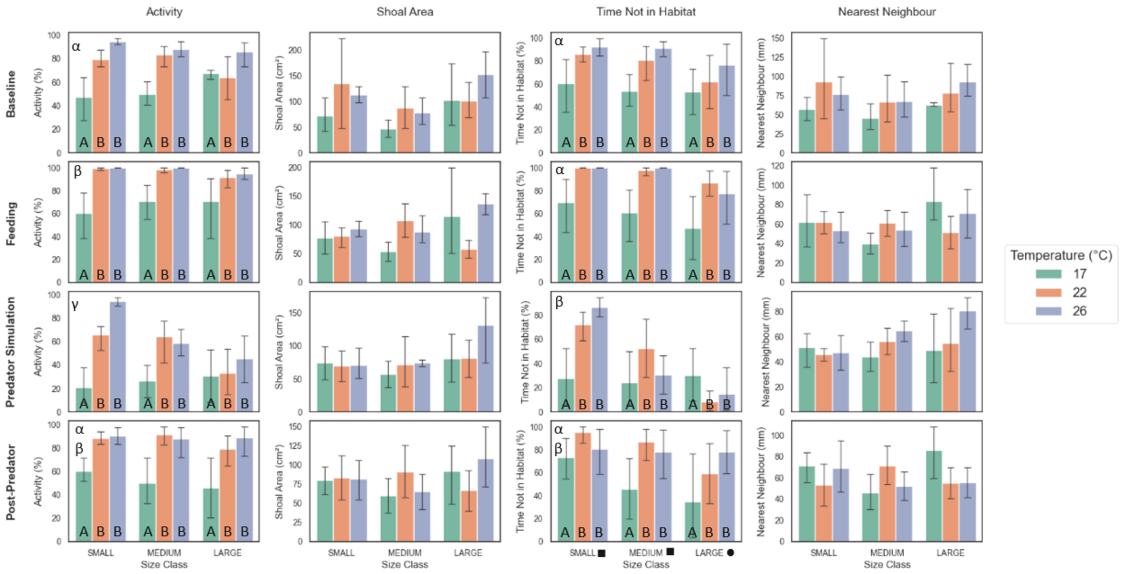

Figure 4.2 Effects of temperature, size class, and experimental phase on the behaviour of tropical vagrant fish *Abudefduf vaigiensis* under different conditions. Bar plots illustrate four measured behavioural responses: (A) Activity (%), the percentage of active behaviours; (B) Shoal Area (cm²), the area covered by the fish shoal; (C) Time Not in Habitat (%), the percentage of time spent outside designated habitat zones; and (D) Nearest Neighbour Distance (mm), the average distance to the nearest neighbouring fish. The rows represent four experimental phases, with differences significant differences indicated by Greek letters (α, β, γ). The columns show responses across three size classes, with significant differences indicated by shapes (■, ●). Bars are grouped by size class and coloured by temperature conditions, with letters denoting significant differences in temperature (A, B). Error bars indicate standard error (SE), and data represent mean ± SE for each group. Data for high and low habitat complexity treatments were pooled due to non-significant differences.

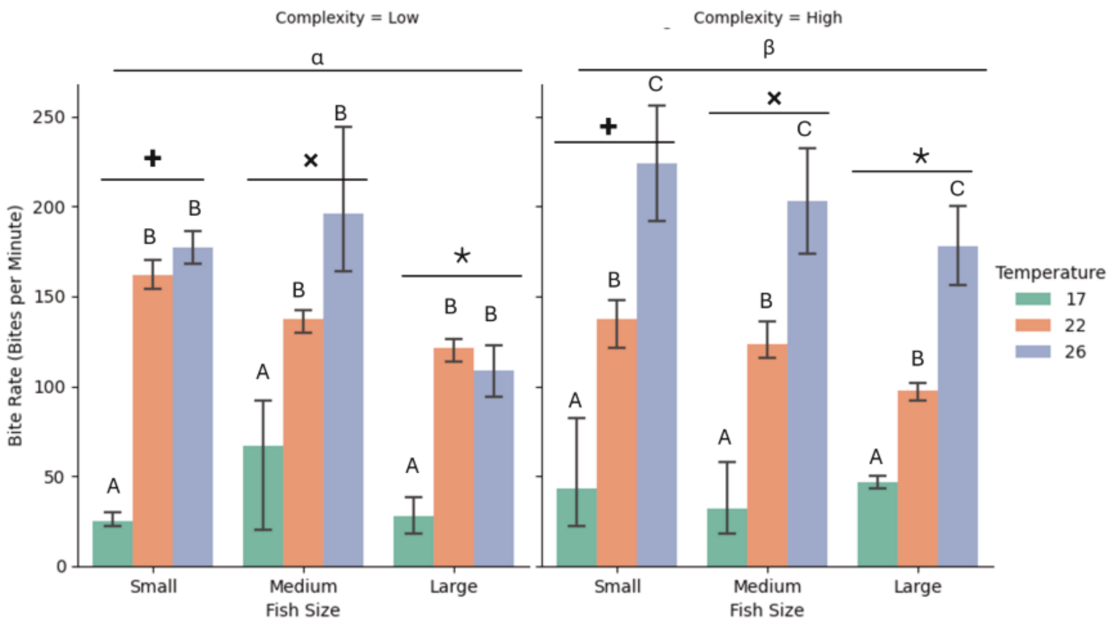

Figure 4.3 Bite rate variations in fish across different size classes, temperatures, and habitat complexities during feeding. Bars represent mean bite rates (bites per minute) with error bars showing 95% confidence intervals. Letters (A, B, C) above bars indicate significant differences in bite rates between temperatures within each size class and complexity level. Symbols above horizontal lines denote significant differences between size classes: '+' for small vs. medium, '×' for medium vs. large, and '★' for small vs. large. Greek letters α and β at the top denote significant differences between low and high-complexity habitats. Temperature conditions are represented by different colours: green (17°C), orange (22°C), and blue (26°C).

4.5 Discussion

Our study reveals that habitat complexity mediates the thermal constraints acting on range-expanding tropical fishes in temperate waters. Most notably, we found that structurally complex habitats enhanced feeding performance at warmer temperatures, with bite rates increasing linearly from 17°C to 26°C in complex environments but plateauing at 22°C and 26°C in simpler habitats. This habitat-dependent thermal response suggests that physical complexity may facilitate successful establishment of tropical vagrant fish at temperate range edges as waters continue to warm. Complex habitats likely provide enhanced foraging opportunities through increased surface area for algal growth and invertebrate prey (Bonin et al., 2009), while simultaneously offering nearby refuges that reduce the energetic costs of predator vigilance (Almany, 2004, Orpwood et al., 2008). The interaction between habitat structure and temperature appears particularly important given projected increases in exposed sandstone habitat due to climate-driven loss of kelp forests in temperate regions (Davis et al., 2022, Vergés et al., 2014). As kelp forests retract, the resulting mosaic of habitat complexities could create varying opportunities for tropical vagrants to persist through seasonal temperature fluctuations, with more complex areas potentially serving as thermal refuge zones that support sustained feeding activity.

Previous research on vagrant fish behaviour has largely focused on narrow size ranges, either examining settlers (16-35mm) (Djurichkovic et al., 2019a) or mature individuals (Beck et al., 2016b). Our study expands this understanding by examining behavioural responses across a broader size spectrum (16-80mm), revealing that the ontogenetic stage fundamentally influences responses to thermal stress and predation risk. This size-dependent plasticity manifests in different strategies: smaller fish maintain higher feeding rates and activity levels despite increased risk, while larger individuals adopt more conservative behaviours prioritising shelter use. These findings demonstrate that body size modulates behavioural responses to thermal stress, with important implications for understanding size-specific survival strategies at range edges.

The ontogenetic stage of vagrant fish strongly influenced their behavioural responses to temperature, particularly regarding the balance between feeding and shelter use. While all size classes showed reduced activity at 17°C compared to warmer temperatures, smaller (< 30 mm) and medium-sized fish (30-45 mm) maintained significantly higher bite rates than larger individuals across all temperatures. This size-dependent pattern likely reflects different energetic trade-offs: smaller fish must prioritise growth to improve survival prospects, while larger individuals can afford more conservative strategies prioritising immediate survival over growth (Basford et al., 2016, Hintz and Lonzarich, 2018). The more risk-averse behaviour of larger fish manifested as increased shelter use across all experimental phases, potentially reflecting their greater visibility to predators balanced against lower mass-specific metabolic demands compared to smaller conspecifics (Hintz and Lonzarich, 2018, Orrock et al., 2013) . These findings expand on previous work showing reduced performance of tropical vagrants at cool temperatures (Djurichkovic et al., 2019a), by demonstrating how body size modulates behavioural responses to thermal stress.

The size-dependent behavioural patterns we observed extended to predator-response strategies, demonstrating how body size influences risk assessment under thermal stress. When exposed to a simulated predator, larger fish showed a pronounced shift toward refuge use, particularly at 17°C. This response suggests that size-specific predation risk strongly shapes behavioural decisions at range edges. Such heightened antipredator responses in larger individuals reflect the fundamental trade-offs between predation risk and foraging gain—an idea central to optimal foraging theory (DeWitt et al., 1998, Orrock et al., 2013, Paijmans et al., 2020). Larger prey, being more conspicuous and potentially more profitable to predators, must weigh the benefits of feeding against a significantly elevated risk of predation.

In contrast, despite their inherent vulnerability, smaller fish displayed more flexible activity patterns and more rapid return to normal foraging behaviour after predator exposure at warmer temperatures (22°C and 26°C). This capacity to quickly resume feeding activity, even under threat, likely reflects the priority of maintaining energetic intake when metabolic demands are elevated by warming (Weetman et al., 1998). The improved behavioural recovery with increasing temperature may thus relax the constraints imposed by predation threat, potentially mitigating some of the mortality risks traditionally linked to cooler, winter conditions. Given the hypothesised role of predation as a primary cause of winter mortality in tropical vagrants (Beck et al., 2016b, Booth et al., 2018), these findings highlight the importance of thermal and size-dependent trade-offs in shaping survival outcomes at high-latitude range edges.

Notably, we found no significant effects on shoaling behaviour across temperatures or size classes, contrasting with previous field observations of increased shoal cohesion at range edges (Mitchell et al., 2022, Smith et al., 2018). This discrepancy might reflect the controlled laboratory setting of our study, where the absence of natural predators and conspecific cues could have reduced the immediate benefits of shoaling. Additionally, the confined space of experimental tanks may have restricted natural shoaling patterns by limiting the distances fish could maintain between each other. However, the consistent individual-level responses we observed across size classes suggest that basic behavioural mechanisms governing feeding and predator avoidance may operate independently of social dynamics in this species.

Our findings have important implications for understanding how tropical vagrants might establish populations in temperate waters under climate change. The interaction between habitat complexity and temperature in mediating feeding behaviour suggests that the availability of complex microhabitats could be crucial for overwinter survival, particularly as temperatures rise above current winter minima. This habitat-dependent thermal response may help explain the patchy distribution of vagrant fish populations in temperate regions, where successful establishment often correlates with areas of high structural complexity (Beck et al., 2017, O'Connell et al., 2023). Furthermore, the size-dependent behavioural strategies we observed suggest that larger individuals might be better equipped to survive their first winter through conservative shelter use, while smaller fish must balance growth requirements against predation risk through more active foraging.

These results should be considered within the context of several limitations. Our laboratory setting, while allowing precise control of environmental variables, may not fully capture the complexity of natural systems where multiple factors interact simultaneously. Additionally, our three-temperature design focused on key thermal thresholds but may not capture the full range of behavioural responses across the temperature gradient these fish experience. Future research should explore how diel and seasonal temperature fluctuations influence these behavioural patterns, particularly in increasingly variable climate conditions. Long-term field studies tracking survival and behaviour across multiple seasons would be valuable for understanding how these laboratory-observed patterns translate to natural systems.

As climate change continues to drive the tropicalisation of temperate marine ecosystems, understanding the mechanisms facilitating vagrant fish establishment becomes increasingly important. Our study reveals that successful establishment likely depends on the interaction between habitat availability, body size, and temperature, rather than thermal tolerance alone. This suggests that management strategies to facilitate or control range expansions should consider habitat complexity and population size structure alongside temperature changes. As winter temperatures in temperate regions increasingly exceed 17°C, complex habitats may become crucial stepping stones for tropical species expanding their ranges poleward, potentially leading to novel ecological interactions in temperate reef communities.

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4.7 Supplementary

Supplementary Source 4.1: of Mean Length and Weight of Fish Across Different Size Classes, Temperatures, and Habitat Types

Size Class	Temperature (°C)	Habitat Complexity	Mean Length (mm)	SD Length	Mean Weight (g)	SD Weight	Number of Fish
Large	17	Low	66.70	11.48	5.71	2.49	10
Large	17	High	69.52	15.31	7.09	4.77	10
Large	22	Low	53.30	6.00	2.74	1.01	15
Large	22	High	48.07	5.81	2.02	0.67	15
Large	26	Low	60.66	10.66	4.11	1.87	10
Large	26	High	58.88	5.51	3.84	1.07	10
Medium	17	High	37.38	4.69	0.94	0.32	15
Medium	17	Low	38.67	3.59	1.02	0.28	15
Medium	22	High	33.87	4.13	0.70	0.29	15
Medium	22	Low	33.87	3.14	0.68	0.23	15
Medium	26	High	36.30	4.62	0.88	0.32	10
Medium	26	Low	40.95	4.35	1.33	0.39	15
Small	17	Large	23.24	3.30	0.25	0.08	15
Small	17	High	24.88	3.68	0.30	0.14	15
Small	22	Low	25.62	2.74	0.32	0.09	10
Small	22	High	25.14	2.06	0.29	0.08	15
Small	26	Low	26.19	4.48	0.41	0.18	10
Small	26	High	24.69	2.75	0.29	0.09	15

Supplementary Source 4.2 PERMANOVA results showing the effects of experimental phase (Be), size class (Si), temperature (Te), and habitat complexity (Co), along with their interactions, on fish behaviour. The table includes degrees of freedom (df), sums of squares (SS), mean squares (MS), pseudo-F values, permutation-based p-values (P(perm)), number of permutations (perms), and Monte Carlo p-values (P(MC)). Significant main effects were found for Be (experimental phase), Si (size class), and Te (temperature).

						Unique	
Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Be	**3**	**8242.2**	**2747.4**	**10.825**	**0.0001**	**9948**	**0.0001**
Si	**2**	**5125.5**	**2562.8**	**3.6114**	**0.0018**	**9909**	**0.0141**
Te	**2**	**3994**	**1997**	**3.4276**	**0.0033**	**9913**	**0.0146**

Co	1	325.07	325.07	0.73783	0.6716	9928	0.6096
Be×Si	6	2071.1	345.18	1.4224	0.1743	9934	0.1656
Be×Te	6	1328.2	221.36	0.93888	0.5013	9917	0.4964
Be×Co	3	1057	352.33	1.4511	0.2004	9926	0.1898
Si×Te	4	1496.8	374.2	0.802	0.7332	9874	0.6953
Si×Co	2	1719.3	859.66	1.6407	0.1089	9914	0.1499
Te×Co	2	722.4	361.2	0.96893	0.4685	9906	0.4726
Be×Si×Te	12	4203.9	350.32	1.4421	0.1103	9901	0.1075
Be×Si×Co	6	1467.8	244.63	1.0296	0.4143	9932	0.4106
Be×Te×Co	6	1208.1	201.35	0.86083	0.5629	9914	0.5808
Si×Te×Co	4	1101.1	275.26	0.863	0.6497	9901	0.6459
Ta(Si×Co×Te)	**27**	**10930**	**404.8**	**1.7006**	**0.0114**	**9857**	**0.0098**
Be×Si×Te×Co	12	2161.5	180.12	0.77783	0.7323	9901	0.764
Ta(Si×Co×Te)×Be	81	19280	238.03	0.94872	0.5819	9924	0.5932
Res	8	2007.2	250.89				
Total	187	69900					

Supplementary Source 4.3 Experimental Phases (Be) PERMANOVA pairwise comparisons for the effect of experimental phases on fish behaviour. Results include t-values, permutation-based p-values (P(perm)), Monte Carlo p-values (P(MC)).

Term 'Be'				
Groups	t	P(perm)	Unique perms	P(MC)
FEEDING, NORMAL	**1.9847**	**0.0211**	**9945**	**0.0263**
FEEDING, POST_FEAR	1.1619	0.2502	9956	0.2352
FEEDING, FEAR	**5.0896**	**0.0001**	**9942**	**0.0001**
NORMAL, POST_FEAR	1.0143	0.3289	9952	0.334
NORMAL, FEAR	**3.3769**	**0.0004**	**9941**	**0.0004**
POST_FEAR, FEAR	**3.9658**	**0.0001**	**9941**	**0.0001**

Supplementary Source 4.4: Size Class (Si) PERMANOVA pairwise comparisons for the effect of size class on fish behaviour. Results include t-values, permutation-based p-values (P(perm)), and Monte Carlo p-values (P(MC)).

Term 'Si'				
Groups	t	P(perm)	Unique perms	P(MC)
MEDIUM, SMALL	1.304	0.1657	9941	0.1697
MEDIUM, LARGE	**3.4569**	**0.0001**	**9950**	**0.0002**
SMALL, LARGE	**3.373**	**0.0003**	**9947**	**0.0002**

Supplementary Source 4.5: Temperature (Te) PERMANOVA pairwise comparisons for the effect of temperature on fish behaviour. Results include t-values, permutation-based p-values (P(perm)), and Monte Carlo p-values (P(MC)).

Term 'Te'				
Groups	t	P(perm)	Unique perms	P(MC)
17, 22	**3.0571**	**0.0002**	**9950**	**0.0003**
17, 26	**3.1471**	**0.0006**	**9939**	**0.0006**
22, 26	0.77317	0.5244	9946	0.5194

Supplementary Source 4.6: PERMANOVA analysis for the effects of size class (Si), habitat complexity (Co), temperature (Te), and their interactions on bite rate. The table includes degrees of freedom (df), sums of squares (SS), mean squares (MS), pseudo-F values, and permutation-based p-values (P(perm)).

PERMANOVA table of results						

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	**2**	**8956**	**4478**	**7.7872**	**0.002**	**9959**
Co	1	553.81	553.81	0.96308	0.3374	9828
Te	**2**	**1.49E+05**	**74343**	**129.28**	**0.0001**	**9957**
Si×Co	2	2656.6	1328.3	2.3099	0.1183	9944
Si×Te	4	5638.8	1409.7	2.4515	0.066	9947
Co×Te	**2**	**7248.8**	**3624.4**	**6.3028**	**0.0069**	**9954**
Si×Co×Te	4	2604.8	651.19	1.1324	0.3642	9960
Res	29	16676	575.05			
Total	46	2.04E+05				

Supplementary Source 4.7: Pairwise Comparisons for Bite Rate by Complexity and Temperature (Co×Te): Pairwise PERMANOVA comparisons of temperature levels within high and low habitat complexity. Results include t-values and permutation-based p-values (P(perm)).

Term 'Co×Te' for pairs of levels of factor 'Temperature'			
Within level 'HIGH' of factor 'Complexity'			
Groups	t	P(perm)	Unique perms
17, 22	**8.416**	**0.0001**	**9824**
17, 26	**10.256**	**0.0002**	**9828**
22, 26	**6.9388**	**0.0002**	**9842**
Within level 'LOW' of factor 'Complexity'			
Groups	t	P(perm)	Unique perms
17, 22	**10.094**	**0.0001**	**9802**
17, 26	**7.8288**	**0.0002**	**9823**
22, 26	1.7284	0.1122	9823

Supplementary Source 4.8: Pairwise Comparisons for Bite Rate by Size (Si): Pairwise PERMANOVA comparisons of bite rate across size classes. Results include t-values and permutation-based p-values (P(perm)).

Term 'Si'			
Groups	t	P(perm)	Unique perms
SMALL, MEDIUM	0.19448	0.84	9853
SMALL, LARGE	**4.3659**	**0.0005**	**9815**
MEDIUM, LARGE	**3.285**	**0.0046**	**9836**

Supplementary Source 4.9: Repeated measures ANOVA on activity levels showing the effects of repeated measures (RM Factor 1, representing experimental phases: Normal, Feeding, Fear, and Post Fear), size class (SIZE_CLASS: Large, Medium, and Small), and temperature (TEMP: 17°C, 22°C, and 26°C) on the activity levels of *Abudefduf vaigiensis*. The results include within-subjects effects (behavioural changes across experimental phases), between-subjects effects (differences between size classes and temperatures), and post hoc comparisons. Statistical corrections were applied where necessary, including Greenhouse-Geisser corrections for violations of the assumption of sphericity (Mauchly's test).

Within Subjects Effects

Cases	Sphericity Correction	Sum of Squares	df	Mean Square	F	p
RM Factor 1	None	47.181[a]	3.000[a]	15.727[a]	48.579[a]	< .001[a]
	Greenhouse-Geisser	**47.181**	**2.199**	**21.454**	**48.579**	**< .001**
RM Factor 1 ✻ SIZE_CLASS	None	3.317[a]	6.000[a]	0.553[a]	1.708[a]	0.124[a]
	Greenhouse-Geisser	3.317	4.398	0.754	1.708	0.149
RM Factor 1 ✻ TEMP	None	1.757[a]	6.000[a]	0.293[a]	0.905[a]	0.494[a]
	Greenhouse-Geisser	1.757	4.398	0.400	0.905	0.472
Residuals	None	40.792	126.000	0.324		
	Greenhouse-Geisser	40.792	92.366	0.442		

Note. Type III Sum of Squares

[a] Mauchly's test of sphericity indicates that the assumption of sphericity is violated (p < .05).

Between Subjects Effects					
Cases	Sum of Squares	df	Mean Square	F	p
SIZE_CLASS	3.464	2	1.732	2.222	0.121
TEMP	**60.013**	**2**	**30.006**	**38.491**	**< .001**
Residuals	32.742	42	0.780		
Note. Type III Sum of Squares					

Post Hoc Comparisons - RM Factor 1								
			95% CI for Mean Difference					
		Mean Difference	Lower	Upper	SE	t	p_{bonf}	p_{holm}
Normal	**Feeding**	**-0.516**	**-0.833**	**-0.199**	**0.118**	**-4.368**	**< .001**	**< .001**
	Fear	**0.866**	**0.550**	**1.183**	**0.118**	**7.331**	**< .001**	**< .001**
	Post Fear	-0.119	-0.436	0.198	0.118	-1.006	1.000	0.316
Feeding	**Fear**	**1.382**	**1.066**	**1.699**	**0.118**	**11.698**	**< .001**	**< .001**
	Post Fear	**0.397**	**0.080**	**0.714**	**0.118**	**3.362**	**0.006**	**0.002**
Fear	**Post Fear**	**-0.985**	**-1.302**	**-0.668**	**0.118**	**-8.337**	**< .001**	**< .001**
Note. P-value and confidence intervals adjusted for comparing a family of 6 estimates (confidence intervals corrected using the bonferroni method).								
Note. Results are averaged over the levels of: SIZE_CLASS, TEMP								

Post Hoc Comparisons - SIZE_CLASS								
			95% CI for Mean Difference					
		Mean Difference	Lower	Upper	SE	t	p_{bonf}	p_{holm}
LARGE	MEDIUM	-0.252	-0.650	0.146	0.160	-1.577	0.367	0.244
	SMALL	-0.330	-0.735	0.075	0.162	-2.031	0.146	0.146
MEDIUM	SMALL	-0.078	-0.462	0.306	0.154	-0.507	1.000	0.615
Note. P-value and confidence intervals adjusted for comparing a family of 3 estimates (confidence intervals corrected using the bonferroni method).								
Note. Results are averaged over the levels of: TEMP, RM Factor 1								

Post Hoc Comparisons - TEMP		
	95% CI for Mean Difference	

		Mean Difference	Lower	Upper	SE	t	p_{bonf}	p_{holm}
TEMP17	**TEMP22**	**-1.045**	**-1.431**	**-0.660**	**0.155**	**-6.764**	**< .001**	**< .001**
	TEMP26	**-1.323**	**-1.727**	**-0.920**	**0.162**	**-8.188**	**< .001**	**< .001**
TEMP22	TEMP26	-0.278	-0.676	0.120	0.160	-1.742	0.267	0.089
Note. P-value and confidence intervals adjusted for comparing a family of 3 estimates (confidence intervals corrected using the bonferroni method).								
Note. Results are averaged over the levels of: SIZE_CLASS, RM Factor 1								

Supplementary Source 4.10: This analysis examines the effects of repeated measures (RM Factor 1, representing experimental phases: Normal, Feeding, Fear, and Post Fear), size class (SIZE_CLASS: Large, Medium, and Small), and temperature (TEMP: 17°C, 22°C, and 26°C) on the habitat use of *Abudefduf vaigiensis*. The results include within-subjects effects (behavioural changes across experimental phases), between-subjects effects (differences between size classes and temperatures), and post hoc comparisons. Greenhouse-Geisser corrections were applied where Mauchly’s test indicated violations of the assumption of sphericity.

Within Subjects Effects						
Cases	Sphericity Correction	Sum of Squares	df	Mean Square	F	p
RM Factor 1	None	46.307^a	3.000^a	15.436 ^{a}	33.603^a	< .001^a
	Greenhouse-Geisser	**46.307**	**2.606**	**17.771**	**33.603**	**< .001**
RM Factor 1 ✻ SIZE_CLASS	None	3.709^a	6.000^a	0.618^a	1.346^a	0.242^a
	Greenhouse-Geisser	3.709	5.211	0.712	1.346	0.249
RM Factor 1 ✻ TEMP	None	1.758^a	6.000^a	0.293^a	0.638^a	0.700^a
	Greenhouse-Geisser	1.758	5.211	0.337	0.638	0.678
Residuals	None	57.878	126.000	0.459		

	Greenhouse-Geisser		57.878	109.441	0.529		
Note. Type III Sum of Squares							
ᵃ Mauchly's test of sphericity indicates that the assumption of sphericity is violated (p < .05).							

Between Subjects Effects

Cases	Sum of Squares	df	Mean Square	F	p
SIZE CLASS	**18.850**	**2**	**9.425**	**11.073**	**< .001**
TEMP	**26.970**	**2**	**13.485**	**15.843**	**< .001**
Residuals	35.749	42	0.851		

Note. Type III Sum of Squares

Post Hoc Comparisons - RM Factor 1

		95% CI for Mean Difference					95% CI for Cohen's d		
		Mean Difference	Lower	Upper	SE	t	Cohen's d	Lower	Upper	p_{bonf}
Normal	Feeding	-0.289	-0.666	0.089	0.141	-2.050	-0.387	-0.903	0.130	0.254
	Fear	**1.040**	**0.663**	**1.417**	**0.141**	**7.388**	**1.393**	**0.833**	**1.953**	**< .001**
	Post Fear	0.079	-0.298	0.456	0.141	0.561	0.106	-0.399	0.611	1.000
Feeding	**Fear**	**1.329**	**0.951**	**1.706**	**0.141**	**9.439**	**1.780**	**1.259**	**2.301**	**< .001**
	Post Fear	0.368	-0.010	0.745	0.141	2.612	0.493	-0.032	1.017	0.061
Fear	**Post Fear**	**-0.961**	**-1.338**	**-0.584**	**0.141**	**-6.827**	**-1.287**	**-1.861**	**-0.713**	**< .001**

Note. P-value and confidence intervals adjusted for comparing a family of 6 estimates (confidence intervals corrected using the bonferroni method).

Note. Results are averaged over the levels of: SIZE CLASS, TEMP

Post Hoc Comparisons - SIZE CLASS

		95% CI for Mean Difference					95% CI for Cohen's d		
		Mean Difference	Lower	Upper	SE	t	Cohen's d	Lower	Upper	p_{bonf}
LARGE	**MEDIUM**	**-0.457**	**-0.873**	**-0.040**	**0.167**	**-2.736**	**-0.612**	**-1.186**	**-0.037**	**0.027**
	SMALL	**-0.798**	**-1.221**	**-0.375**	**0.170**	**-4.701**	**-1.068**	**-1.635**	**-0.502**	**< .001**
MEDIUM	SMALL	-0.341	-0.742	0.060	0.161	-2.122	-0.457	-1.008	0.094	0.119

Note. P-value and confidence intervals adjusted for comparing a family of 3 estimates (confidence intervals corrected using the bonferroni method).

Note. Results are averaged over the levels of: TEMP, RM Factor 1

Post Hoc Comparisons - TEMP

		95% CI for Mean Difference					95% CI for Cohen's d			
		Mean Difference	Lower	Upper	SE	t	Cohen's d	Lower	Upper	p_{bonf}
TEMP17	**TEMP22**	**-0.779**	**-1.182**	**-0.376**	**0.161**	**-4.824**	**-1.044**	**-1.593**	**-0.494**	**< .001**
	TEMP26	**-0.827**	**-1.248**	**-0.405**	**0.169**	**-4.894**	**-1.107**	**-1.678**	**-0.536**	**< .001**
TEMP 22	TEMP 26	-0.048	-0.464	0.368	0.167	-0.285	-0.064	-0.621	0.494	1.000

Note. P-value and confidence intervals adjusted for comparing a family of 3 estimates (confidence intervals corrected using the bonferroni method).

Note. Results are averaged over the levels of: SIZE CLASS, RM Factor 1

Chapter 5 : Early Life History Traits and Higher Growth Rates Linked to Winter Survival in Range-Expanding Coral Reef Fish

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5.1 Abstract

Climate change is driving the poleward range expansion of sub-tropical and tropical marine species into temperate ecosystems, with ocean-warming hotspots experiencing rapid shifts in species distributions. While recruitment to temperate waters by expatriating tropical vagrant fish is increasing, surviving through the cooler winters remains a significant barrier to their establishment. This study investigates the links between early life history traits and survival into winter in the Sergeant Major (*Abudefduf vaigiensis*), a tropical damselfish expanding its range into temperate southeastern Australia. Using otolith microstructure analysis, we reconstructed the early life histories of 122 individuals collected from rocky reef in Sydney (33.8ºS) Australia in 2022 and 2023 and assessed their early life history traits. Our results demonstrate that individuals surviving into the winter (hereafter "wintered fish") had a larger size at hatching, longer pelagic larval duration, and higher body condition than fish collected before winter. Lower settlement water temperatures were positively associated with winter survival, indicating that a settlement date closer to the onset of winter was favourable. Additionally, wintered fish, exhibited higher growth rates in early life compared to fish sampled prior to winter onset. These findings suggest that some early life history traits are selected for survival into winter, potentially mediating the physiological impacts of declining water temperature. If such traits increase overwinter survival they may facilitate the establishment of this range-expanding species in temperate rocky reef environments.

5.2 Introduction

Climate change is altering marine ecosystems globally, with one significant impact being the poleward range expansion of tropical species into temperate waters (Sunday et al., 2015, Munday et al., 2008, Pecl et al., 2017). Over the past century, ocean temperatures have risen significantly, with the global mean sea surface temperature increasing by approximately 0.13°C per decade since 1900 (Intergovernmental Panel on Climate, 2023). The warming trend is particularly pronounced in certain regions, known as ocean warming hotspots. These areas, including the southeast coast of Australia (Suthers et al., 2011), the Mediterranean Sea (Nykjaer, 2009), and waters off Japan (Wu et al., 2012) are experiencing temperature increases at two to four times faster than the global average. Such rapid warming is facilitated by the increase in western boundary currents, increasing tropical species into traditionally temperate ecosystems (Pecl et al., 2017), and as a result altering ecological dynamics and community structures in these regions (Nakamura et al., 2013b, Kingsbury et al., 2020b).

The range expansion process in tropical vagrant fishes can be conceptualised using the invasion pathway model, which involves distinct stages: transport, colonisation, establishment, and landscape spread (Hellmann et al., 2008). As global temperatures rise, more tropical fish species appear in temperate waters, far beyond their usual range (Figueira and Booth, 2010, Fowler et al., 2018) . Vagrant fishes are transported as larvae by strengthening ocean currents, such as the East Australian Current, which has shown increased southward penetration over the past 60 years (Booth et al., 2011b, Suthers et al., 2011, Ridgway, 2007). While this model provides a useful framework, it is important to note that the establishment and colonisation stages are currently bottlenecked by winter ocean temperatures in temperate waters (Booth et al., 2018). Seasonal mortality rates of vagrant fish approach 99% due to low water temperatures (Figueira & Booth, 2010), with most individuals only surviving the arrival season, although a small fraction persists. The high mortality represents a barrier to these species establishing in temperate locations. However, as ocean temperatures continue to rise, the potential for successful overwintering will likely increase, potentially removing a key barrier in the invasion pathway model. Our understanding of winter survival factors remains limited, particularly for range-expanding marine species.

Early life histories potentially play a significant role in individuals persisting near their lower thermal limits. Early life history traits (ELHTs), such as growth rates, pelagic larval duration (PLD), and size at key early life milestones, are well-established factors influencing post-settlement survivorship in tropical fish in natal environments (Rankin and Sponaugle, 2011, Sponaugle and Grorud-Colvert, 2006, Sponaugle et al., 2006, Vigliola and Meekan, 2002, Gagliano et al., 2007a). However, their specific role in facilitating survival to and through winter for range-expanding marine species in temperate ecosystems remains poorly understood.

Fish may have undergone a selective process during their pelagic larval phase, with certain phenotypes persisting through high mortality events such as hatching, PLD, and settlement (Garrido et al., 2015, Gagliano et al., 2007b). The surviving subset of fish may already possess traits that favour survival in general, but whether these traits are advantageous for overwintering in a temperate environment remains an open question. Studies on tropical reef fishes in their native ranges have identified several ELHTs affecting survivorship, including size at hatching, PLD, growth, and size at settlement (Rankin and Sponaugle, 2011, Sponaugle et al., 2006) and thus is plausible that they also influence persistence in cooler temperate environments.

The period between settlement, usually in warmer ocean temperatures, and the onset of winter ocean temperatures is likely crucial for the survival of vagrant fish through the winter months. As larvae arrive in cohorts throughout the austral summer and autumn, the duration of this window progressively shortens for later arrivals. During this time, fish can grow rapidly and build energy reserves before water temperatures decline. Rapid growth during early life stages is frequently associated with increased survival in fish, particularly for species with high early-life mortality rates (Vigliola and Meekan, 2002, Gagliano et al., 2007a). Larger individuals are often better equipped to compete for resources, avoid predators, and withstand environmental stressors (Dingeldein and White, 2016, Garrido et al., 2015, Meekan and Fortier, 1996). However, for tropical vagrants, this period of rapid growth coincides with their arrival in temperate waters and adaption to different environmental conditions, such as food and habitat, may differ substantially from their native ranges, potentially affecting their growth trajectories and subsequent survival.

This study investigates the links between early life history traits and survival into winter for the Sergeant Major (*Abudefduf vaigiensis*), a tropical damselfish expanding its range into temperate waters of Southeast Australia. This species was selected due to its documented range expansion into temperate waters, driven by warming ocean temperatures (Figueira et al., 2009, Beck et al., 2016a, Booth et al., 2018). The relatively high seasonal recruitment and seasonal mortality in temperate waters provide an ideal context for investigating the ELHTs that influence survival at the poleward thermal limits. Our objectives were:

(1) To determine which early life history traits, such as size at hatching, pelagic larval duration (PLD), and size at settlement, are associated with survival into winter in temperate ecosystems, and

(2) To evaluate how growth trajectories during early life stages affect the likelihood of survival into winter in a vagrant tropical fish in temperate waters

5.3 Methods

5.3.1 Permits and Ethics

This investigation was conducted following the University of Technology Sydney's Animal Ethics Committee Approval (ETH-6609) and under the NSW DPI permit (F94/696(A)-9.0).

5.3.2 Sample Collection

Abudefduf vaigiensis (n=122) samples were collected from shallow rocky reefs in Sydney, Australia, at four sites: Little Manly (33.8067° S, 151.2870° E), Malabar

(33.9679° S, 151.2511° E), Little Bay (33.9817° S, 151.2403° E), and Freshwater Beach (33.7813° S, 151.2906° E). These locations were chosen to provide a broader spatial representation of the species within temperate waters. Sampling was conducted between January and August of 2022 and 2023, with a monthly sampling regimen employed to obtain a variety of size classes (Fig. 1). The collected samples were approximately representative of the body length distribution of wild conspecifics (Supp. Tab.1).

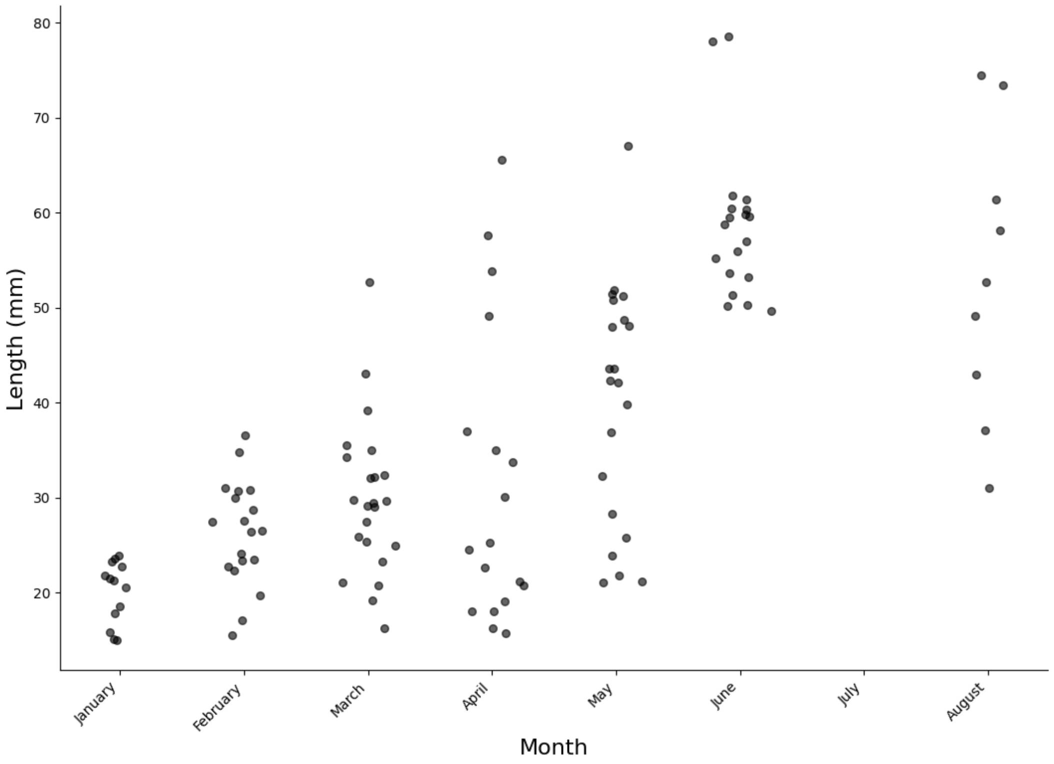

Figure 5.1 Distribution of Fish Size Across Months. Each data point represents the length of a single fish in millimetres (mm). Sample sizes per month were January (n = 12), February (n = 19), March (n = 23), April (n = 18), May (n = 21), June (n = 19), and August (n = 9). Fish were pooled across the four sampling locations and over two years

(2022 and 2023). Data are grouped by month to illustrate temporal variations in fish size distributions.

Fish were sedated and immobilised using a clove oil dilution protocol (1:4 ethanol) and then captured using hand nets (Fernandes et al., 2017). They were euthanised by placing them in an ice bath. All captured fish were transported to the University of Technology Sydney, where they were weighted (to the nearest 0.001g) and measured with callipers (to the nearest 0.1mm), then stored at -4°C until otolith analysis.

5.3.3 Otolith Analysis

Otoliths, the calcified structures found in the inner ears of fish, were used to collect information on ELHTs. The validity of using otoliths for such purposes has been well-documented in previous studies (Panfili et al., 2009), and a validation study has been conducted specifically for this species (Rigg et al., 2023). In Rigg et al. (2023) daily deposition of visible micro-increments (light and dark banding) was confirmed, meaning the number of increments in an otolith could be counted to determine the time elapsed in days and allow daily growth rates to be estimated. Fish were dissected under a stereomicroscope using a probing tool and scalpel to extract the lapillus otoliths. The otoliths were then mounted on microscope slides with the dorsal side facing up, using Crystalbond™ (509-1A) as the mounting medium. The samples were polished using 3-µm lapping paper to obtain a flat surface for the measurements. The lapilli were chosen for increment width measurements due to their distinct and clear increment banding (Rigg et al., 2023).

Several precautions were taken to ensure the accuracy and reliability of otolith readings. Fish were randomly selected and analysed without knowledge of their size or collection date to minimise potential reader biases. Each otolith was read twice by a single experienced reader, and if the two readings differed by more than 10%, a third reading was performed. The reading closest to the third reading was considered the most reliable (Wilson et al., 2009). Consultation with another experienced researcher was undertaken to reach a consensus on identifying key otolith markings, such as the hatch check, settlement mark, and daily growth increments. The methods employed adhered to the guidelines outlined by Panfili et al. (2009).

A Nikon Upright NI Series microscope was used, and measurements were taken using Nikon NIS-elements Advanced Research software (V5.02.02), spanning from the centre of the primordium to the furthest point on the anterior-posterior axis. The radius of the hatch check, daily increment width, settlement mark, and total otolith radius were recorded for each fish.

Key ELHTs examined using otolith microstructure included size at hatch, early life growth rates, PLD, body condition at capture, and size at settlement (Tab. 1). The analysis involved identifying and measuring specific otolith structures: the primordium, hatch check, daily growth increments, and settlement mark (Fig 2). Settlement marks were identified by an abrupt change in increment width, characteristic of a Type I settlement mark (Wilson and McCormick 1999). The position of this mark relative to the otolith edge varied predictably with fish size, appearing close to the edge in small, recent settlers and further from the edge in larger, older fish. This pattern was consistent with a settlement mark of similar size and duration among individuals. Increment width measurements and counts of these structures provided identification of individual growth histories and key life history transitions.

5.3.4 Body Condition at Capture

Body condition at capture was calculated using Fulton's K formula (K = weight (g)/length $(\mathrm{cm})^3$) x 100) (Fulton, 1904) (Tab. 1). The measure is frequently used to assess body condition, providing a standardised measure of the fish's condition at capture (Bolger and Connolly, 1989, Hamilton, 2008, Kingsbury et al., 2020a). To account for potential biases in K caused by allometry (the relationship between body length and plumpness) (Froese, 2006), we examined the relationship between K and body length. Allometry was not a significant factor within the size range we examined in this species, as indicated by a regression analysis ($R^2 = 0.012$, $P = 0.228$).

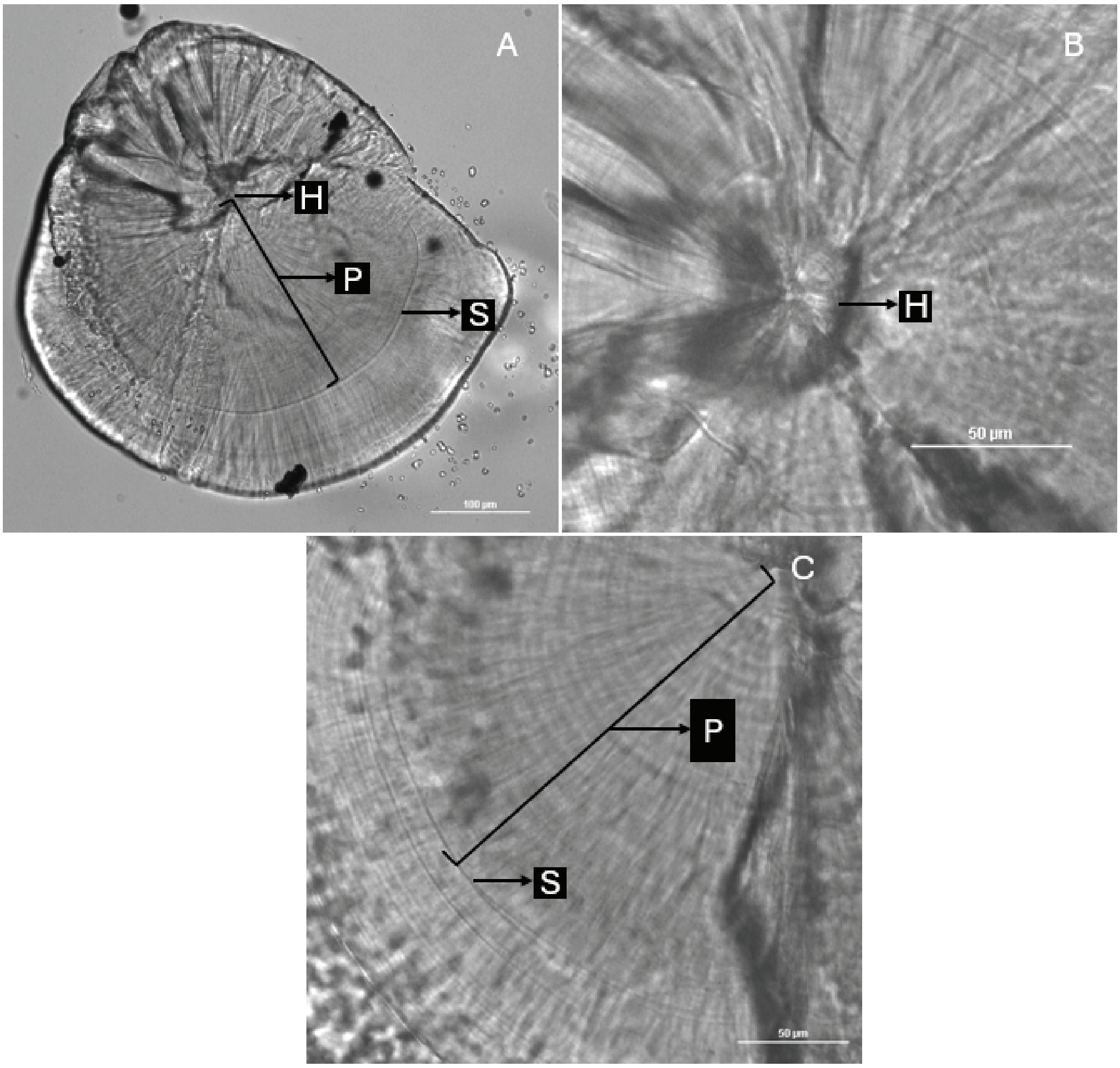

Figure 5.2 Microstructure of a lapillus otolith from *Abudefduf vaigiensis*. (A) Whole otolith view showing key developmental markers at 200x: H - hatch check, P - pelagic larval duration (PLD), and S - settlement mark. (B) Magnified view at 400x of the otolith core region, highlighting the hatch check (H). (C) Close-up at 200x of the otolith edge region, showing the transition from pelagic (P) to post-settlement (S) growth. Scale bars: 100µm (A), 50µm (B, C). These structures allow for accurate aging and reconstruction of early life history events in this vagrant tropical fish species.

Table 5.1 Summary of life history traits used to assess the impact of wintering in vagrant tropical fish. Traits, including hatch radius, daily increment width, pelagic larval duration (PLD), size at settlement, ocean temperature at settlement, and Fulton's K Body Condition. The raw otolith values were used in the size-at-age model to approximate fish size in millimetres at hatch radius, daily increment width, and size at settlement.

Early Life History Trait	Description
Hatch Radius (µm)	The distance from the centre of the otolith primordium (core) to the hatch check mark (Fig 2). Provides an estimate of the size of the fish at hatching.
Daily Increment Width (µm)	The distance between two consecutive daily growth rings on the otolith. The values were used to model growth trajectories of the fish.
Pelagic Larval Duration (days)	The number of daily increments between the hatch check and the settlement mark on the otolith (Fig. 2). This represents the fish's total days in its pelagic (open-water) larval stage before settling into a benthic habitat.
Otolith Radius at Settlement (µm)	The distance from the centre of the otolith primordium to the settlement mark (Fig.2). This serves as an indicator of the fish's size when it transitioned from the pelagic larval stage to the benthic juvenile stage.
Condition (Fulton's K) at Capture	A measure of the fish's overall condition, calculated as $K=W/L^3\times100$, where W is the fish's weight (g) and L is the fish's length (cm). This index reflects the robustness or fatness of the fish.
Temperature at Settlement (°C)	The ocean temperature at settlement was determined by backcalculating the time interval from the otolith microstructure (from the date of settlement to the date of capture) and using satellite data to estimate the temperature (see methods, *Water Temperature*).

5.3.5 Size-at-age Model

Following otolith microstructure analysis, size at each age was estimated using the Biological Intercept Model (BI model; Campana, 1990). The BI Model assumes a linear relationship between otolith and somatic growth, which was subsequently validated for *A. vaigiensis* during the early growth phase ($r^2 = 0.97$, Supp Fig. 1). For each fish, otolith intervals (distances between daily increments) were measured, and back-calculated body lengths (Lt) for each age were calculated using:

$$L_t = L_c - \left(\frac{R_c - R_t}{R_c}\right) \times \left(L_c - L_0\right)$$

where L_t is the length at age t, L_c is the capture length, R_c is the capture radius, R_t is the radius at time t, and L_0 is the initial length.

5.3.6 Water Temperature to Determine Winter Onset and Settlement Temperature

Sea surface temperature (SST) data for Sydney during 2022 and 2023 were obtained from the NOAA OISST V2 High-Resolution dataset (Huang et al., 2021). Shelly Beach (33.7992° S, 151.2983° E) was selected as the representative site for SST measurements across all four sampling locations. To justify the use of Shelly Beach data as representative for all sites, we assumed that SST patterns are broadly similar across the relatively small geographic range (~16km) of our study area due to the proximity of the locations. However, it is important to note that local variations may exist and were not directly measured in this study. The SST data were extracted and processed using Python with the *xarray* library, resampled to obtain daily mean temperatures, and compiled for further analysis.

5.3.7 Winter Onset Definition and Fish Survival Classification

The onset of winter conditions was defined using a sea surface temperature (SST) threshold of approximately 20°C, derived from synthesising physiological studies on tropical damselfish in temperate environments and satellite temperature data (as above). This threshold estimates the critical temperature at which *A. vaigiensis* begins to experience physiological stress. It delineated two groups of fish: 'prewinter' (those collected before winter onset) and 'wintered' (those that survived into winter). Several key studies informed this threshold: Djurichkovic et al. (2019b) demonstrated significant physiological effects below 22°C in tropical *Abudefduf* species; Eme and Bennett (2008) reported mean minimum acclimation temperatures of 19.3°C for Indo-Pacific damselfishes; Figueira and Booth (2010) estimated 17.1°C as the overwinter survival threshold for *A. vaigiensis*; and Booth et al. (2018) identified 15.6-19.8°C as overwintering temperatures for *Abudefduf* species. The 50th percentile of observed temperature distributions for 2022 (20.21°C) and 2023 (20.76°C) coincided with the onset of sustained cooler conditions, with winter onset identified as June 8th, 2022, and May 27th, 2023 (Supp. Fig. 2). This threshold closely mirrors our 2023 in situ temperature logger data collected at Shelly Beach (Unpublished data, 2023 only). The defined threshold captures the beginning of the rapid seasonal environmental changes wintering fish face, after which winter mortality increases due to the physiological thresholds of *A. vaigensis*.

5.4 Statistical Analysis

5.4.1 Early Life History Traits at Settlement

We conducted a logistic regression analysis to investigate the factors influencing fish survival. The response variable was Survivorship (binary: wintered or prewinter), and the predictor variables included size at hatching (mm), pelagic larval duration (PLD, days), size at settlement (mm), body condition at capture (Fulton's K), and ocean temperature at settlement (°C). All continuous predictor variables were standardised prior to analysis. A generalised linear model (GLM) with a binomial error distribution and logit link function was employed. The initial full model included all predictor variables. Manual backward elimination was performed using the Akaike Information Criterion (AIC) to select the most parsimonious model.

Given the small sample size of wintered fish in the 2023 data, including year as a random effect was not feasible. The 2023 model exhibited traits of overfitting, with extreme standard errors and unreliable coefficients. As a result, we pooled data from both 2022 and 2023 to strengthen the statistical power and reliability of the analysis. To examine whether data from different years might affect model outcomes, we compared the pooled model (2022 + 2023) with a model using only the 2022 data. Key predictors such as size at hatching, condition at capture, and temperature at settlement were consistent in both models, supporting the decision to pool the data.

The final model specification was:

$$logit(P(Survivorship)) = \beta_0 + \beta_1 * Size\ at\ Hatch + \beta_2 * PLD + \beta_3 * Size\ at\ Settl$$

Where Logit(P(survivorship)) is the logit function of the probability of survivorship, β_0 is the intercept term, and $\beta_1 - \beta_5$ are coefficients that quantify the relationship between each predictor variable and the log odds of survivorship.

Model diagnostics were performed using the DHARMa package (Hartig, 2018) to assess logistic regression assumptions. Variance Inflation Factors (VIF) were calculated to check for multicollinearity. The log odds ratios and their 95% confidence intervals were computed to interpret the effects of the predictor variables. All analyses were conducted in R version 4.2.

5.4.2 Early Growth Trajectories

We used Generalised Additive Models (GAMs) to investigate growth trajectories between fish that survived into winter and prewintered fish. The decision to use GAMs over Generalised Linear Models (GLMs) was driven by the potential for complex, non-linear relationships between fish size and day of observation, which could not be adequately captured by a standard linear model. GAMs allowed for more flexibility in modelling the non-linear growth patterns, that are typical of early growth in tropical fish with a PLD.

The models were fitted using the *mgcv* package in R (v4.2). Initially, we tested multiple models with different distributions, link functions, and interactions to account for non-linear growth patterns, heteroscedasticity, and non-normality in the data. The response variable was fish size (mm), and the predictor was day of observation (Day), with separate smooth functions (splines) for each group (wintered, prewinter).

Model selection was based on Akaike Information Criterion (AIC) values. The Gamma distribution with a log link function provided the best fit with the lowest AIC. We also ensured that overfitting was minimised using the Restricted Maximum Likelihood (REML) method for smooth parameter selection. Model diagnostics, including Q-Q plots, residuals versus fitted values plots, and histograms of residuals, confirmed an adequate fit of the final model.

We incorporated random effects in all models to account for variability between individual fish and the repeated growth measures. Random intercepts and random slopes were fitted for each fish to capture individual deviations in growth rates and starting sizes. These random effects were nested within the survival group (Survivorship), allowing each fish to have its own growth trajectory while accounting for the influence of survival status.

The final model specification was:

$$Size\ (mm) \sim s(Day,\ by = Survivorship\ , K =- 1,\ bs = "cs") + Survivorship\ + s(FishN$$

$$+\ s(Day,\ FishNumber,\ by\ =\ Survivorship\ =\ "fs")$$

Where s(Day, by = Survivorship, bs = "cs") represented a cubic spline fitted to the day of observation, allowing for an interaction between Day and Survivorship. This interaction enabled the model to fit separate growth trajectories for each group, accounting for differences in the relationship between Day and Size in pre-winter and wintered fish. The term s(FishNumber, by = Survivorship, bs = "re") captured the random intercepts for each fish, accounting for individual differences in the initial size within each survival group. The term s(Day, FishNumber, by = Survivorship, bs = "re") modelled the random slopes for each fish, allowing individual growth rates to vary over time within survival groups. The k parameter (number of knots, K=-1) was evaluated to ensure the smooth term was adequately flexible without overfitting.

5.5 Results

5.5.1 Early Life History Traits in Wintered Vagrant Fish

We conducted backward elimination using the Akaike Information Criterion (AIC) to select the most parsimonious model. All terms initially included in the full model—size at hatch, pelagic larval duration (PLD), size at settlement, condition at capture, and temperature at settlement—were retained based on the AIC selection process. The logistic regression analysis identified several significant predictors of surviving into winter (Fig. 3). Size at hatch was a strong positive predictor (OR = 8.03, 95% CI: 2.34–27.55, $p < 0.001$), indicating that larger hatch sizes substantially increase the odds of surviving into winter. Similarly, size at settlement (OR = 2.92, 95% CI: 1.06–8.05, $p = 0.038$) and condition at capture (OR = 3.07, 95% CI: 1.18–7.99, $p = 0.021$) were significant positive predictors. Conversely, temperature at settlement was a significant negative predictor (OR = 0.24, 95% CI: 0.09–0.68, $p = 0.003$), suggesting that higher temperatures at settlement reduce the odds of surviving into winter. Pelagic larval duration did not significantly predict surviving into winter ($p = 0.146$).

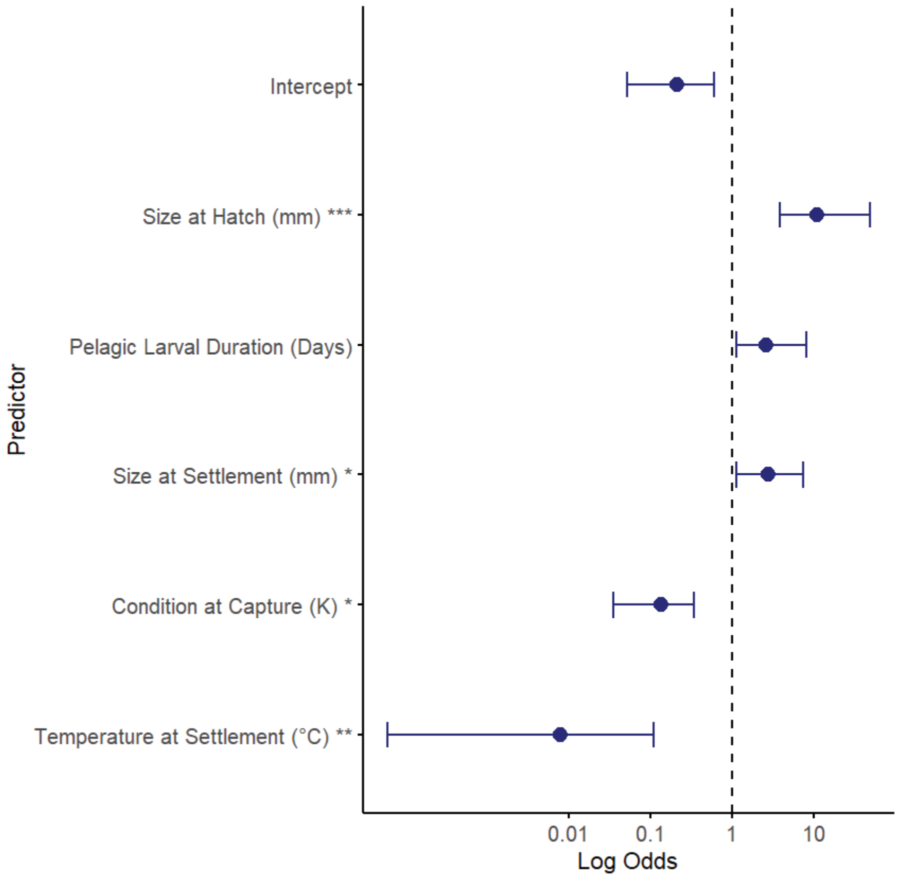

Figure 5.3 Forest plot showing the effect log odds (with 95% confidence intervals) from a Logistic Generalized Linear Model assessing the influence of five predictors on fish survival (wintered vs prewinter). Positive estimates indicate traits or conditions associated with increased odds of survival, while negative estimates suggest decreased odds of survival. The vertical dashed line at 0 represents no effect. Significance levels are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

5.5.2 Early Growth Rate

The model incorporating group-specific splines (wintered and prewinter) had a lower Akaike Information Criterion (AIC) value (27793.11) compared to the model without group-specific splines (AIC = 27856.20, Fig. 4). The reduction in AIC indicated that the growth trajectories between fish that survived into winter and prewintered fish differed, justifying the use of separate growth curves for each group. The parametric coefficient for survivorship in the model was positive and significant (estimate = 0.329, $p < 0.001$). This result suggests that, on average, fish that survived into winter had a larger body size than prewintered fish. Both groups exhibited non-linear growth patterns over time, as evidenced by the significant smooth terms for day ($p < 0.001$). The effective degrees of freedom (edf) for the smooth terms indicated moderately complex growth patterns, with edf = 8.77 for fish that did not survive and edf = 8.27 for fish that survived into winter. The random effects for individual fish were significant in both groups (prewinter: $p = 0.026$; wintered: $p = 0.098$). This finding indicated significant individual variability in growth among the fish within each group. Notably, the interaction between individual fish and time was significant for fish that survived into winter ($p = 0.038$) but not for the prewinter group ($p = 0.261$), indicating a higher degree of variability in growth patterns in the wintered group.

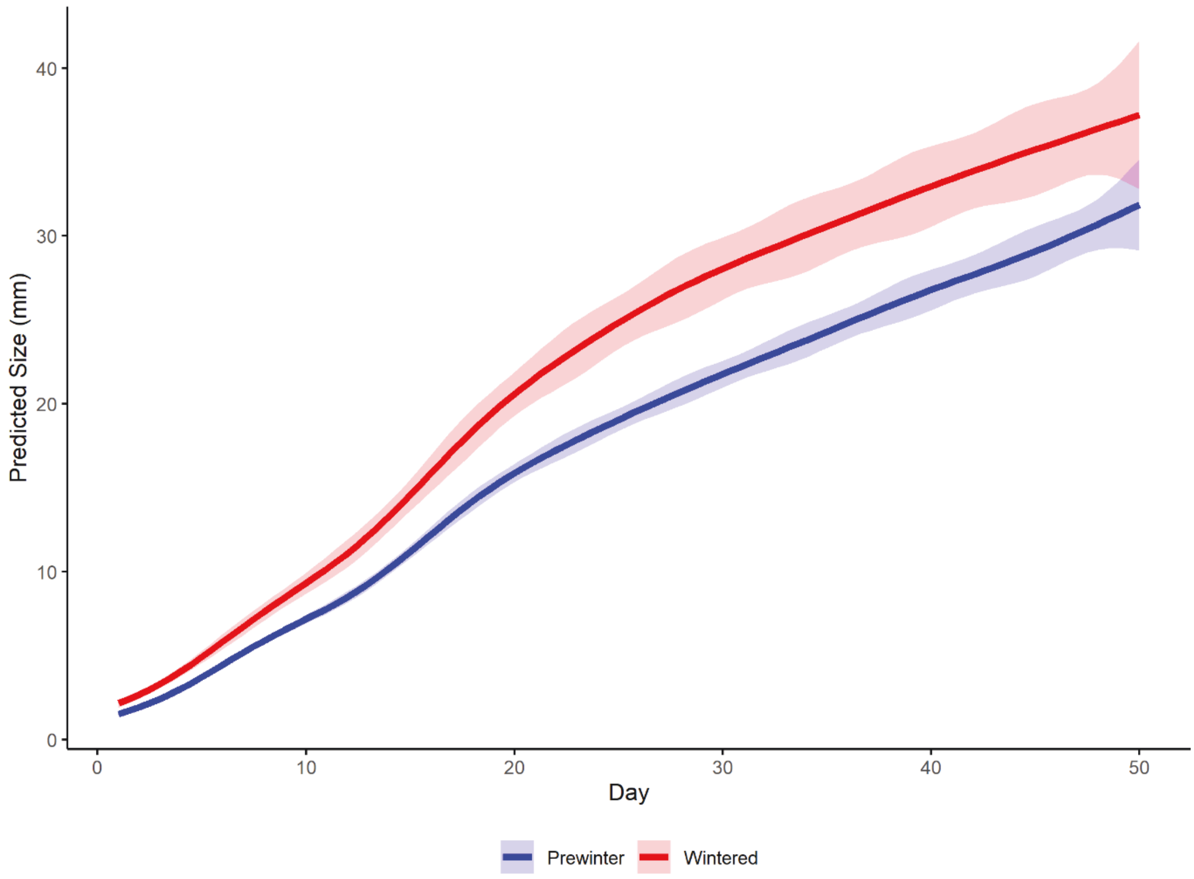

Figure 5.4 Growth trajectories predicted by the Generalised Additive Model, comparing wintered fish (red) and prewinter fish (blue). The x-axis represents time in days from hatching, and the y-axis represents the predicted size of fish in millimetres (mm). Shaded regions denote 95% confidence intervals of fixed effects, highlighting the divergence between the two groups' growth trajectories.

5.6 Discussion

Understanding the early life history traits (ELHTs) that enable tropical fish to survive into winter in temperate regions is crucial for predicting how range-shifting marine species will respond to climate change. Our study demonstrated that key ELHTs contribute to the likelihood of surviving into winter in range-expanding tropical fish, *A. vaigiensis*, in temperate southeastern Australia. We found that larger size at hatching and settlement, and higher body condition at capture were significantly associated with increased odds of surviving into winter, whilst higher temperatures at settlement were associated with decreased survival odds. Importantly, wintered fish exhibited faster growth rates during early life than prewinter fish and attained larger sizes at key stages, including hatching, PLD and settlement, often associated with high mortality.

The importance of size at both hatch and settlement has emerged as an important factor in surviving into winter for tropical vagrant fish in temperate environments, as supported by observations of tropical fish in their natal environments. For example, larger individuals at hatch experience faster growth and development, a pattern observed across numerous coral reef fish species (Vigliola and Meekan, 2002). This initial size advantage appears to compound throughout the larval stage and into settlement, likely creating a positive feedback loop of enhanced survival and growth.

The mechanisms underlying the size-based survival advantage observed in vagrant fish are likely multifaceted. Larger hatchlings possess more substantial yolk reserves (Coleman and Galvani, 1998) and better-developed sensory and swimming capabilities (Peck et al., 2012), providing significant advantages during early development. These enhanced capabilities typically result in higher growth rates and improved predator evasion (Gagliano et al., 2007a, Cowan Jr and Houde, 1992), with studies showing that larger hatchlings experience lower mortality rates during the critical first days post-hatching (McCormick and Hoey, 2004). This initial size advantage appears to initiate a cascade of benefits, providing an advantage to larger individuals for survival through both direct physiological advantages and indirect ecological effects and interactions.

These early advantages appear to compound in this study, creating cumulative benefits that extend into settlement. Larger individuals typically demonstrate a higher metabolism (Giguère et al., 1988), which may impact feeding behaviours, competition, and risk-taking behaviour. Larger fish are often more efficient and bolder foragers (Brown et al., 2007), allowing them to secure better nutrition during critical periods of growth (Dingeldein and White, 2016) and before reduced food availability in winter. The selective pressure favouring larger individuals creates a subset of the population that has passed through significant mortality filters before winter arrives, suggesting that winter survival is influenced by traits selected earlier in their development.

Post-settlement predation may also contribute to the observed size-based survival patterns, though this was not directly tested in our study. Size-selective predation typically targets smaller individuals (Almany and Webster, 2006, Cowen and Sponaugle, 1997), suggesting that larger fish may have better survival odds through enhanced predator avoidance capabilities and competitive advantages in securing resources (Brown et al., 2007). This predation-driven selection could contribute to the observed pattern where surviving fish are not only larger but also in better condition. The size advantage at early life stages may therefore serve as a proxy for multiple beneficial traits, such as enhanced foraging ability and predator avoidance, though further research is needed to test these mechanisms directly in the context of winter survival.

Our study associated body condition at capture with winter success, but this relationship may be more complex than it initially appears. Kingsbury et al. (2020) found that tropical fishes maintained their body condition in temperate environments despite reduced activity and feeding in cooler waters, suggesting a potential trade-off between growth and condition maintenance. However, our results did not demonstrate such a trade-off, as we observed higher growth rates and better body conditions in wintered fish. This discrepancy with previous findings warrants further investigation. It is important to note that while our study did not follow fish through the entire winter season, survivors are typically very rare and difficult to capture later in winter. Mitchell et al. (2023) proposed that some tropical species exhibit physiological plasticity in temperate winter conditions, potentially reallocating energy resources for long-term survival. While we did not directly measure physiological changes, we observed that growth and condition were maintained in wintered fish, suggesting that *A. vaigiensis* may have adaptive strategies to survive the cooler waters. Additionally, the relationship between body condition and survival may be influenced by selective predation, as predators tend to target individuals with low body condition during the settlement phase (Hoey and McCormick, 2004, Booth and Beretta, 2004). Selective pressure could have contributed to the higher body condition observed in our wintering fish. It is important to note that our body condition measurements were taken at capture and that we were unable to measure body condition before the drop in ocean temperature. The fish we captured had therefore successfully survived into winter and is possible that the body condition reflects the outcome of wintering rather than serving as a predictive factor for survival. Future studies that measure body condition before and after winter are thus needed to more accurately assess the role of body condition in winter survivorship.

The subset of vagrant fish that survived into winter grew faster and were substantially larger (39% on average) than those sampled prewinter, demonstrating that these growth advantages persist and compound throughout early life history stages. The larger size at each life stage reflects their ability to capitalise on available resources and avoid predation during the pelagic larval duration (PLD) phase. The complex, non-linear growth patterns observed in both groups suggest that growth trajectories are highly responsive to environmental conditions and ontogenetic transitions. Notably, the faster growth rates in wintered fish highlight the importance of achieving a size threshold before winter, giving them a significant advantage in surviving into cooler winter waters.

The significant individual variation in growth among survivors, indicated by the random effects and fish-by-day interactions, suggests there may be a broad range of viable growth trajectories in novel temperate environments. Rather than specific growth strategies being favoured, this variation suggests flexibility in the growth patterns that can lead to winter survival. Selection for higher growth in early life histories is documented in other tropical species (Bergenius et al., 2002, Gagliano et al., 2007a, McCormick and Hoey, 2004), but our results indicate that multiple growth trajectories may be compatible with winter survival. This flexibility in growth patterns could be advantageous for tropical vagrants establishing in temperate ecosystems, potentially supporting their range expansion under climate change.

Our results suggest a complex relationship between settlement timing, water temperature, and overwintering success in *A. vaigiensis*. The link between settlement temperature and time of year suggests that settlement timing could be a factor in interpreting this result. Specifically, fish settling at higher temperatures, typically earlier in the recruitment season, faced reduced survival odds in our study. This suggests that while warmer temperatures are common in their tropical range, settling in warm periods further from winter might be disadvantageous in temperate waters. Wintered fish, on average, settled approximately 50 days before the winter temperature drop, which may facilitate acclimation to the sudden drop in temperature change at the onset of winter. This is supported by the speed of temperature decline, not just the temperature itself, likely affects acclimation to cooler water temperatures (Reid et al., 2022). Fish therefore settling during cooling periods may cope better with environmental changes than those arriving in warmer summer conditions (Stewart et al., 2023). Later settlers in turn face fewer threats from predators and competitors, potentially saving energy for winter. They also settle in cooler water, which may require less physiological adjustment.

As ocean warming continues to facilitate the range expansion of tropical species into temperate waters, understanding the mechanisms behind their survival in these marginal environments becomes increasingly important. Our in-depth study provides a critical foundation for understanding the factors contributing to the winter survival of tropical fish in temperate environments. *A. vaigiensis* is one of the most common tropical vagrants, exhibiting some of the highest survival in temperate regions through winter (although it is still low), and further research is needed to determine how representative our results are of other species. Comparative studies with other range-expanding species, and multi-year studies that account for inter-annual variability in environmental conditions and capture fully overwintered specimens will thus be an important next step in assessing the generality of the observed patterns. Notably, our results highlight that due to their growth advantages and physiological resilience, larger individuals are better equipped to withstand the selective pressures imposed by cooler climates. By identifying these critical periods of vulnerability and understanding the growth dynamics, we can therefore more accurately predict not only the persistence of these species but also their competitive interactions and ecological impacts within temperate marine ecosystems.

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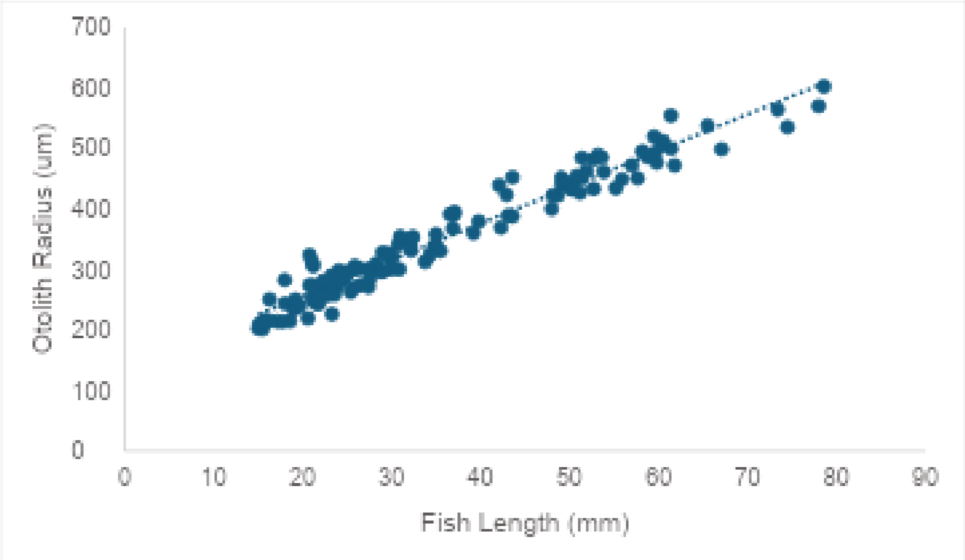
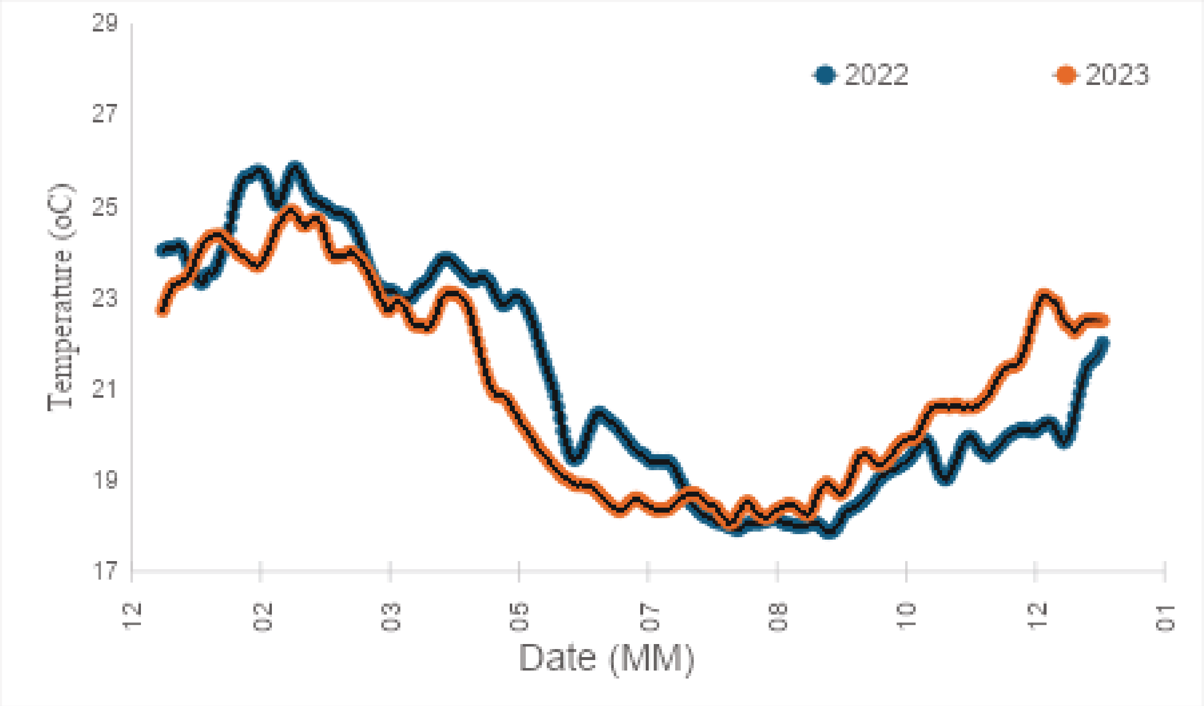
5.8 Supplementary

Supplementary Source 5.1 Comparison of study fish size class proportions and historical proportions across months. For each month, the table shows the number of fish sampled (n) and the percentages of fish in three size categories: Small (<25mm), Medium (25-50mm), and Large (>50mm). The table compares the proportions from the current study with historical data, showing trends in the size class distribution across different months.

Month	n	Study Small (<25mm)	Historical Small (<25mm)	Study Medium (25mm-50mm)	Historical Medium (25mm-50mm)	Study (50mm+)	Historical (50mm+)
January	13	100	58	0	38	0	4
February	19	58	24	42	74	0	2
March	23	30	12	65	86	4	2
April	18	28	22	61	74	11	4
May	21	24	10	57	77	19	13
June	19	0	1	16	75	84	24
July	0	-	0	-	37	-	63
August	9	0	0	33	20	67	80



Supplementary Source 5.2 Relationship between otolith radius (µm) and fish length (mm), showing a strong positive correlation. The dotted line represents the linear regression fit, with an R^2 value of 0.9478, indicating a high degree of explanatory power for fish length based on otolith radius measurements.



Supplementary Source 5.3 Seasonal temperature trends for Shelly Beach, Sydney, NSW, based on satellite data and presented as a rolling 3-day average for the years 2022 (blue) and 2023 (orange). The y-axis represents temperature in degrees Celsius (°C), while the x-axis denotes the date by month (MM). A notable rapid decline in temperature is observed in both years following the summer peak, particularly from the end of April to July, highlighting a pronounced cooling period at this location.

Chapter 6 : Overall Discussion

6.1 Introduction

Understanding the factors that influence species persistence and establishment in novel environments has become increasingly important as our oceans continue to warm due to climate change (Intergovernmental Panel on Climate, 2023, Pecl et al., 2017). Notably, the poleward expansion of tropical marine species, known as tropicalisation, is reshaping marine ecosystems globally, with profound ecological implications (Coni et al., 2021, Pessarrodona et al., 2022, Vergés et al., 2014). This thesis explores the physiological and behavioural traits contributing to the life histories, establishment of populations, and survivorship of tropical vagrant fish species in temperate ecosystems. By focusing on vagrant tropical species, I provide evidence of how vagrant fish persist and potentially establish populations in novel temperate environments.

Vagrant species serve as living indicators of the changing thermal regimes in our oceans and provide insights into the potential future composition of temperate marine communities (Fogarty et al., 2017). By focusing on the life history traits, behavioural strategies, and responses to winter onset in vagrant fish, this research contributes to understanding the mechanisms underlying successful range expansions in marine environments (Feary et al., 2014).

This study fills a critical knowledge gap, by moving beyond the impact of tropicalisation on native temperate species to directly investigate the physiological and behavioural characteristics that enable tropical vagrants to persist and potentially establish in novel temperate ecosystems. By tracking the ontogenetic trajectory of vagrant fish—from their settlement on temperate rocky reefs to their seasonal survival and winter survival—this research identifies key factors that contribute to their establishment. These findings increase our ecological understanding of vagrant biology and provide insights relevant to predicting tropical species' range expansions under current climate trends.

6.2 Summary of Key Findings

Chapter 2 focused on validating otolith microstructure characteristics in *A. vaigiensis*, confirming daily increment deposition across all three otolith types: sagittae, lapilli, and asterisci (Rigg et al., 2023). This established a valid approach for ageing and assessing early life history traits (ELHTs). The research also demonstrated that higher concentrations of Alizarin Red S (ARS) produced clearer fluorescent markings, particularly in lapilli and sagittae. However, an important finding was that increased ARS concentrations reduced increment width, potentially inhibiting otolith growth (Hu et al., 2019, Jurgelėnė et al., 2022). This finding has implications for future studies using chemical marking techniques, as it highlights the need to balance marking clarity with potential growth impacts when studying fish life histories and population dynamics.

Chapter 3 used the otolith analysis techniques validated in Chapter 2 to investigate the factors affecting the settlement process of vagrant tropical fish. Settlement is a high-mortality event in the life cycle of reef fishes, selecting specific characteristics that are favourable for survival. Chapter 3's investigation into early ELHTs and post-settlement persistence demonstrated that shorter pelagic larval duration

(PLD) was associated with longer post-settlement persistence. Similarly, faster pre-settlement growth rates correlated with higher post-settlement persistence. Surprisingly, neither size at settlement nor size at hatching showed a significant relationship with post-settlement persistence, challenging some conventional assumptions about the advantages of larger body size during early life stages in temperate waters (Walsh, 1985, Sponaugle and Grorud-Colvert, 2006, Almany and Webster, 2006, Gagliano et al., 2007a, Hamilton et al., 2008).

Chapter 4 examined the environmental drivers of behaviour in *Abudefduf vaigiensis*, revealing temperature, fish size, and habitat complexity as key factors shaping behavioural responses in temperate waters. The study identified 17°C as a critical temperature, where fish activity levels markedly decreased, and structured habitat use increased across all size classes, indicating a shift to energy conservation as temperatures decline. Behavioural differences by size class were also prominent: larger fish had a habitat reliance for predator avoidance, while smaller fish showed increased bite rates and activity at warmer temperatures, potentially to meet energy demands but at higher predation risk. While habitat complexity did not broadly alter behaviour, it significantly influenced bite rate, particularly in warmer temperatures, where complex habitats supported higher feeding rates, identifying an interaction between temperature and habitat structure in feeding efficiency. These findings demonstrate how vagrant tropical fish adjust behaviours like feeding, habitat use, and predator response to meet temperate environments' thermal and ecological demands, which may contribute to the persistence and establishment as winter approaches.

Chapter 5 examined ELHTs and their association with winter survival in *A. vaigiensis*, identifying size at hatching, size at settlement, settling water temperatures, and body condition at capture as factors that affected survival into winter. Larger hatch sizes and body condition at capture were linked to survival into winter, suggesting that these early advantages may facilitate survival in temperate waters. Additionally, fish that survived into the winter had faster and higher growth trajectories during their early life stages than their pre-wintered samples, indicating that rapid growth may offset the physiological challenges of cooler temperatures. Notably, higher temperatures at settlement were inversely correlated with winter survival, suggesting that later-settling cohorts are at a survival advantage into winter. These findings demonstrate the advantage conferred by early growth and suggest that ELHTs, particularly those increasing in body size and condition, may support the range expansion of tropical fish species into temperate ecosystems.

6.3 Synthesis of Research Outcomes

Based on the data chapters (Chapters 2, 3, 4, and 5), I synthesise research findings through the open life cycle of *A. vaigiensis* to illustrate how ELHTs, behavioural strategies, and environmental factors may influence survival, and thus the establishment of a population, at different stages of ontogeny. Given that survival strategies and selective pressures differ across life stages, organising the findings chronologically—from hatching to winter survival—provides a clearer understanding of how specific traits and behaviours contribute to survival at each stage.

6.3.1 Hatching

Hatching refers to the emergence of larvae from fertilised eggs, a stage marked by significant physiological and morphological transitions (Wootton, 1990). It is characterised by selective pressures, such as predation and environmental factors, occurring in the species' natal tropical range (Feary et al., 2014). Since mortality due to predation or other factors occurs long before larvae reach temperate regions, these selection pressures cannot be observed or directly measured from the receiving temperate environment. However, through otolith microstructure analysis, it is possible to analyse the effect of size at hatching on subsequent temperate settlement by examining attributes of the surviving subset of fish recruited to temperate locations. Using two different approaches, this thesis established that larger size at hatch was associated with survival into winter (Chapter 5) but not post-settlement persistence (Chapter 3).

Chapter 3 used a weekly sampling approach of a newly settled cohort to determine that size at hatch did not influence the post-settlement persistence of vagrant fish in temperate waters. This suggests that once larvae settle in temperate ecosystems, the survival advantages conferred by hatching size may be outweighed by other factors, such as pelagic larval duration (PLD) and growth rates, which play more prominent roles in determining immediate post-settlement survival. In contrast, Chapter 5, which used a two-year monthly sampling approach, identified size at hatching as a predictor of survival into the winter. Larger hatch sizes were associated with increased survivorship during the colder winter months. This suggests that early-stage development provides physiological advantages that become critical later in life, particularly when facing the cooler conditions of temperate winters.

This pattern is consistent with the concept of oppositional selection pressure, where a trait may be advantageous at one life stage but neutral or even disadvantageous at another. A similar dynamic was observed by Gagliano et al. (2007a), who found that selection pressures on growth rates in small tropical fish changed direction later during settlement. In the case of *A. vaigiensis*, size at hatching does not significantly influence post-settlement persistence, possibly because survival during this phase is influenced by a range of factors, such as sufficient food availability and predation pressures that may not strongly select for size. However, as winter approaches, larger size at hatching becomes significant for survival, as these fish require greater physiological resilience to endure colder temperatures and reduced food availability in temperate regions. This suggests that fish that were larger at hatching are more capable of withstanding the physiological demands of winter and avoiding predation. Thus, while size at hatching may not determine immediate post-settlement survival, it becomes a key factor for long-term survival in temperate environments, particularly during the winter onset period.

6.3.2 Pelagic Larval Duration

Pelagic larval duration (PLD) refers to the time fish larvae spend in the planktonic phase, where they grow and disperse before settling into a suitable habitat (Leis, 2010). This stage is critical for early development, as larvae experience significant growth and high mortality from predation and environmental factors. Larval fish tend to remain in the plankton until they reach a minimum size necessary for settlement, which betters their ability to compete for resources and avoid predation in the benthic environment (McCormick and Hoey, 2004).

In Chapter 3, a shorter PLD was associated with increased post-settlement persistence, as larvae settling sooner likely reduce exposure to pelagic risks. However, in Chapter 5, PLD was not a significant predictor of winter survival, suggesting that while a shorter PLD supports initial settlement, it does not directly impact survival through winter. This shift highlights selective pressures across life stages: shorter PLD may improve immediate post-settlement survival by allowing earlier arrival when resources are abundant and temperatures are optimal, facilitating rapid adaptation to benthic habitats. Additionally, recruitment from shorter dispersal distances may have influenced the settling survival dynamics, as vagrants with shorted PLDs likely originate from nearer tropical territories, reducing travel-related energy costs (Feary et al., 2014). The closer recruitment source might provide ecological advantages, with fish arriving in better condition and better prepared to adjust to temperate conditions, thereby increasing their likelihood of successful settlement.

In contrast, the lack of a direct link between PLD and winter survival suggests that other traits, particularly growth rate and body condition at settlement, are more influential for winter survival. This finding implies that while a shorter PLD may provide immediate post-settlement advantages, allowing larvae to settle sooner and avoid extended pelagic risks, the period between settlement and winter onset becomes a more decisive phase for survival. This dynamic indicates that while shorter PLD confers short-term benefits to survival, the benefit is not associated with, nor has long-standing advantages to survival, such as overwintering.

6.3.3 Early Post-settlement Period

During the settlement phase, vagrant tropical fishes transition from pelagic to temperate benthic environments. This settlement phase is complex as vagrants enter temperate environments that differ significantly from their native tropical habitats. Settlers are typically small, ranging from 12 to 30 mm in body size (Chapter 3). During this "searching phase," settlers actively explore potential settlement sites, guided by environmental cues such as habitat structure, water quality, and the presence of conspecifics (Stamps et al., 2005, Bogdan et al., 2020). The ability to quickly locate and secure an optimal habitat is critical for post-settlement survival, influencing their capacity to access resources, avoid predators, and persist in novel ecosystems.

The settlement of vagrant tropical fishes in temperate benthic environments is shaped by a progression of physiological and behavioural factors. This research demonstrates how these factors relate, demonstrating stage-specific attributes that impact survival. Chapter 3 demonstrated that faster larval growth correlates with post-settlement persistence, independent of size at settlement, suggesting that accelerated growth results in stronger metabolic systems and energy reserves crucial for the transition from pelagic to benthic life (Giguère et al., 1988, O'Connor et al., 2007, Sandblom et al., 2014). In the post-settlement phase, Chapter 4 illustrates that behavioural strategies of settling fish become important, particularly for smaller individuals facing heightened predation risk. Increased feeding rates allow for rapid energy acquisition while shoaling and strategic habitat use might mitigate predation risks and improve foraging success (Paijmans et al., 2020, Sackley and Kaufman, 1996). These behaviours compensate for the disadvantages of smaller size, enabling small settlers to build reserves and settle in temperate environments. However, as the overwintering stage approaches, Chapter 5 identifies absolute size at settlement as a contributor to winter survival, with larger fish possessing the energy reserves and physiological resilience needed to endure winter onset.

Chapter 3 showed that although size at settlement does not strongly predict survival, fast larval growth before settlement significantly enhances post-settlement persistence. Notably, Chapter 5 demonstrated that size at settlement becomes a predictor of overwintering success, highlighting a shift in selective pressures as fish mature. This demonstrates the concept of oppositional selection pressures, where traits like size at hatching and PLD provide varying benefits at different life stages. Larger fish at settlement likely build growth momentum that provides more energy reserves and physiological resilience and is better equipped to survive into the winter as temperatures approach the lower thermal limit of 17°C.

The interconnected progression of these factors—from rapid larval growth to post-settlement behaviours, and ultimately to achieving a size advantage before winter—illustrates how early developmental and behavioural adaptations have compounding effects on later survival. This sequential framework enhances our understanding of how vagrant tropical fish might survive and persist in temperate ecosystems. Plasticity in growth (Chapter 3, Chapter 5) and behaviour (Chapter 4) may enable vagrant fish to survive (or at least this successful vagrant species) the settlement process and eventually establish in novel environments, impacting population dynamics, ecosystem structure, and resilience under climate change. This research demonstrates stage-specific survival mechanisms and the need for an integrated understanding of post-settlement success as vagrant tropical species navigate environmental change and expand their range.

6.3.4 Juvenile, Adult, and Wintering Stages

The juvenile and adult stages of *A. vaigiensis* are developmental periods characterised by increased site fidelity, growth, and interactions with abiotic and biotic factors in the temperate ecosystem. These phases are typically marked by the transition from growth-focused survival to strategies prioritising energy conservation, predator avoidance, and habitat use (Wootton, 1990). My research identifies the benthic juvenile and adult phase as an important life stage, potentially mediating survivorship throughout temperature declines leading to winter. Factors such as body size, higher growth rates, physiological condition, and behavioural plasticity become increasingly important to survival as ocean temperatures decrease approaching winter. This section explores the relationship of these factors across the juvenile and adult phases to influence survivorship into the winter, highlighting the adaptive mechanisms and selective pressures that shape the persistence of tropical vagrants in temperate ecosystems.

The extended settlement of *A. vaigiensis* throughout the Austral summer and autumn results in fish of various size classes and ontogenetic stages by the time water temperatures begin to decline rapidly. Findings from Chapter 5 revealed that fish surviving into winter exhibited higher growth rates within their first 50 days and maintained better body condition than those sampled before winter onset. This subset of wintered fish avoided predation and continued feeding, indicating successful feeding strategies and habitat utilisation through the juvenile and sub-adult phases. Chapter 4 showed that medium-to-large juvenile fish, comparable in size to the wintered subset, demonstrated increased habitat use relative to smaller fish, particularly during feeding and predator events. This trend became more polarised in the 17°C treatment, where smaller fish exhibited riskier behaviours—such as shoaling, increased boldness, and less reliance on habitat structures—throughout feeding and predation events. These patterns suggest a size-selective mechanism in which larger individuals, having had more time to grow and accumulate energy reserves, are better equipped behaviourally and physiologically to withstand the winter conditions, whilst showing a level of behavioural plasticity and predator avoidance in response to cooling water temperatures.

Surviving the winter period is a key bottleneck for establishing *A. vaigiensis* populations in temperate ecosystems (Figueira and Booth, 2010). My research in Chapter 5 indicates that larger fish and those settling closer to winter are better positioned to accumulate the energy reserves essential for surviving winter. In contrast, smaller fish may struggle to survive this period due to behavioural disadvantages. Findings from Chapter 4 suggest that smaller fish tend not to use habitats as effectively, even at 17°C, as their larger counterparts, instead relying on shoaling as a primary mechanism of predator avoidance. While this strategy might be effective in warmer conditions, this behaviour may expose smaller fish more as temperatures decline, resulting in slower burst speeds to avoid predation. Without the shelter of complex habitats, smaller fish become easier targets for predators, reducing their likelihood of survival as winter approaches. Furthermore, the energy demands of active shoaling likely deplete their already limited reserves, preventing them from accumulating the body condition needed to withstand colder conditions. The lack of habitat use and reliance on high-risk behaviours contribute to size-selective pressures that favour larger, more conservatively behaving individuals through the winter.

Synthesising these findings, it is evident that physiological and behavioural adaptations differ among the juvenile, adult, and wintering stages. Effective habitat use, which supports predator avoidance and foraging efficiency, allows fish to maximise their body condition (Booth and Beretta, 2004, Kingsbury et al., 2020a) —a key predictor of overwintering success. Larger fish tend to remain stationary during predation threats and use habitat for cover, conserving energy and avoiding risks associated with more active strategies used by smaller fish. My research demonstrates that *A. vaigiensis*'s survival in temperate ecosystems is mediated by early growth advantages, behavioural flexibility, and the ability to effectively modify behaviour to decreased temperatures. Importantly, life history traits that may not provide immediate survival benefits at settlement become essential for overwintering, illustrating how size selectivity and trait persistence shape vagrant fish establishment.

6.4 Implications to Vagrant Biology Research

This research provides evidence of the factors influencing the survival and establishment of tropical vagrant fish in temperate ecosystems, with implications for understanding species persistence in a changing climate. The study reveals a complex relationship of stage-specific selective pressures, where traits like size at hatching, while not immediately beneficial, become critical for surviving into the winter. This highlights the importance of considering the delayed effects of ELHTs and adopting a life-stage-specific approach when studying vagrant species. Furthermore, behavioural plasticity, particularly the shift from shoaling to habitat use as fish grow, emerges as a key survival mechanism in fluctuating environmental conditions. This highlights the relationship between physiological and behavioural adaptations in determining survival outcomes and surviving into the winter. The findings also emphasise the influence of environmental factors, particularly temperature, on survival and potential range expansions. Successful establishment in novel environments depends on a combination of advantages of early life history, behavioural adaptability, and coping with changing environmental conditions. This knowledge can help inform other research into predicting species responses to climate change and improve the management of range-shifting species, including those with invasive potential.

6.5 Methodological Advancements

This section outlines the key methodological improvements that have resulted from my research. The methods discussed here provide a framework for future investigations, ensuring that subsequent studies can build on robust and validated approaches.

6.5.1 Validation of Daily Increment Deposition

Chapter 2 provided a methodological advancement by validating otolith analysis techniques for *A. vaigiensis*. Daily increment deposition was confirmed across all three otolith types: sagittae, lapilli, and asterisci. This validation ensured that age determination and growth rate calculations represented daily growth patterns. Validation allows for assessments of ELHTs and sets a precedent and foundation for future studies seeking to accurately track the age and growth of vagrant tropical fish through otolith microstructure.

6.5.2 Chemical Marking Techniques

Chapter 2 explored the use of Alizarin Red S (ARS) as a chemical marker to improve the visibility of otolith increments. Higher concentrations of ARS enhanced the fluorescent markings, particularly in lapilli and sagittae, facilitating more precise readings. However, a significant observation was that increased ARS concentrations led to a reduction in increment width, indicating a possible inhibitory effect on otolith growth. This finding emphasises the importance of balancing the clarity of chemical markings with the potential impact on growth, which is crucial for maintaining the accuracy of life history and population dynamics studies. Future research will need to consider this trade-off when utilising chemical marking techniques.

6.5.3 Methodological Advancements in Assessing Behaviour

Chapter 4 is the first study investigating the gradient of habitat complexities on rocky sandstone substrates and its effect on vagrant fish behaviour, particularly bite rates. This controlled laboratory study revealed that changes in habitat complexity could influence feeding bite rates in vagrant fish, suggesting that future research should consider the effect of habitat complexity on fish behaviour and performance. Additionally, this chapter addressed an often-overlooked aspect of vagrant fish biology: the different size classes and their corresponding behavioural differences. Many studies overlook ontogenetic shifts in vagrant species, likely due to vagrants only surviving the year of arrival. Furthermore, the study tested the interactive effects of size, temperature, and behavioural trials. Although no significant interaction was found, this approach was methodologically innovative, allowing future studies to consider these dynamics in greater detail.

6.5.4 Early Life History Traits in Vagrant Fish Biology

Chapter 3 assessed the role of ELHTs in post-settlement persistence of vagrant tropical fish, establishing a framework for understanding how early developmental traits influence settlement. Notable, my research approach marked a significant difference from prior research, which often relied on unvalidated proxies and provided more nuanced insights into how traits like growth rates and PLD contribute to the post-settlement persistence of vagrant fish in temperate waters. Building on this, Chapter 5 extended the methodological application of ELHTs to winter survival phase, linking these early traits to long-term survival under temperate conditions.

6.6 Limitations and Future Directions

6.6.1 Increasing Geographic Scope

My research was conducted in the temperate waters of Sydney, Australia, where *A. vaigiensis* is considered a vagrant species. Sydney's unique position at the boundary between subtropical and temperate marine ecosystems makes it an ideal location for studying range-expanding tropical species. Additionally, the region is recognised as a climate change hotspot, with sea surface temperatures rising faster than the global average (Wu et al., 2012). These characteristics provide valuable insights into the factors influencing tropical species' persistence in temperate environments.

However, while Sydney's unique position justifies its use as a focal study site, the findings may not be fully generalisable to other temperate regions. Environmental conditions, such as temperature regimes, habitat complexity, and food availability, vary significantly across latitudes, potentially influencing species' behaviours and survival strategies in different contexts.

Future research should expand the geographic scope by incorporating a latitudinal component, conducting similar studies at multiple sites ranging from subtropical to cool temperate waters. Comparative analyses of vagrant fish responses to environmental pressures across a broader geographic range could provide a better understanding of the species' range expansion dynamics. Additionally, investigating early life stage dispersal patterns will be crucial for understanding the connectivity between tropical source populations and temperate settlement sites. Otolith microchemistry could be used to trace the environmental history of individual fish, revealing the water masses larvae traverse and identifying key dispersal pathways.

6.6.2 Increasing Sample Size and Study Duration

Logistical challenges and the sporadic occurrence of vagrant *A. vaigiensis* constrained sample sizes for certain life stages and experimental conditions. For instance, in the study investigating temperature effects on fish behaviour (Chapter 4), the limited number of small individuals at the highest temperature (26°C) affected the ability to detect statistically robust trends. The restricted sample sizes in these instances reduced the statistical power, making it more challenging to identify biologically significant behavioural responses across different temperature conditions. Similarly, in analysing ELHTs and growth rates in Chapter 5, the low number of individuals surviving through the overwintering phase limited the depth of analysis on inter-annual variability within the timeframe of my PhD. The sporadic recruitment of vagrant fish into temperate waters made it difficult to assess survival patterns across multiple seasons. These constraints likely reduced the ability to capture the full range of behavioural and life history variability in *A. vaigiensis*, potentially increasing the likelihood of Type II errors, where real effects remain undetected due to insufficient sample size.

Future research should aim to increase sample sizes and duration, which would improve statistical power and allow for more robust conclusions. Expanding the study across multiple seasons and locations could capture a wider range of environmental conditions and recruitment events, providing a better understanding of inter-annual variability and the factors influencing the establishment and range expansion of vagrant species.

6.6.3 Applicability to Other Vagrant Species

This research focused on *A. vaigiensis*, providing valuable insights into its life history traits and behavioural plasticity. However, the findings may not directly apply to other vagrant tropical fish species, as different species exhibit unique life history strategies, physiological tolerances, and behavioural responses. Generalising these results to other species should be done cautiously, considering species-specific traits.

To address this, future research should expand to include other vagrant tropical fish species, testing the generalisability of patterns observed in *A. vaigiensis*.

Comparative studies could involve otolith microstructure analysis and behavioural experiments across multiple species to identify common traits that predict successful establishment in temperate environments. This broader research scope would not only help validate the findings of this study but also contribute to our understanding of tropicalisation and range expansion.

6.6.4 Behavioural Studies: Expanding and Linking the Field and Laboratory Studies

The behavioural studies in Chapter 4 were conducted under controlled laboratory conditions, which may not fully reflect the complexities of natural environments. Factors such as confinement stress, artificial habitats, and the absence of natural predators can influence fish behaviour, potentially leading to responses that differ from those observed in the wild. Thus, the behaviours observed in my laboratory experiments may not directly translate to field conditions. Future research should incorporate complementary field studies to better understand how vagrant fishes behave in their natural temperate environments, where more realistic predator-prey dynamics, shoaling dynamics, and habitat structures are present.

In addition to day-time behavioural observations, future studies should also investigate nocturnal behaviour and vulnerability of vagrant fish in temperate environments. While not included in this thesis, preliminary research suggests that vagrant fish shoals may disperse at night, with individuals seeking shelter in small crevices. Using non-invasive techniques, such as infrared cameras, would allow researchers to capture night-time activity, including changes in shoaling dynamics, resting site selection, and susceptibility to predation across different size classes and seasons.

Furthermore, the role of habitat complexity, particularly in temperate sandstone reefs, remains an important area for future research. Varying degrees of structural complexity can significantly influence the establishment and persistence of vagrant tropical species. Creating detailed 3D models of reef structures to quantify complexity and studying its effects on fish abundance, behaviour, and survival across different life stages would help clarify the relationship between habitat use and survival in these dynamic environments.

6.7 Concluding Remarks

This thesis provides evidence of the mechanisms that enable tropical vagrant fish, such as *Abudefduf vaigiensis*, to persist in temperate waters. It advances our understanding of marine ecology, particularly in the context of climate change, where the boundaries between tropical and temperate ecosystems are increasingly blurred. The persistence of tropical vagrants highlights the adaptive potential of range-extending species to respond to fluctuating environmental pressures.

My findings illustrate how the physiological and behavioural traits of tropical vagrants can support range expansions, fundamentally shaping ecosystem interactions in temperate regions. These findings align with foundational theories of ecological resilience, where species adaptively respond to new environmental pressures, potentially redefining local ecosystem structures. The persistence of *A. vaigiensis* in temperate environments could signify long-term shifts in community dynamics, potentially out-competing temperate species and altering ecosystem dynamics, such as the transition from kelp-based to detritus-based food webs observed in tropicalised ecosystems.

By examining the relationships between early life history traits, behavioural plasticity, and environmental factors, this study reveals how these elements collectively influence the survival and potential establishment of tropical vagrants in novel environments. The findings highlight the role of specific traits in mediating survival through seasonal challenges like wintering and the importance of physiological and behavioural plasticity in enabling species to persist across various environmental conditions.

Given projected ocean warming trends, these insights are especially pertinent. Understanding which traits facilitate the survival of vagrant species contributes to predicting future ecosystem shifts under climate change. Ultimately, this research contributes to understanding the complex selective pressures shaping the survival and persistence of vagrant species in temperate ecosystems, adding valuable context to ongoing studies of marine biodiversity shifts driven by global climate change.

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