

THE INFLUENCE OF WATER TEMPERATURE AND SHOALING ON THE FITNESS, SURVIVABILITY AND RANGE EXPANSION POTENTIAL OF A TROPICAL VAGRANT FISH

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Presented for the degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Lucas Djurichkovic, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced 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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STATE INDICATING THESIS FORMAT

I, Lucas Djurichkovic, declare that this thesis is formatted and being submitted to the University of Technology Sydney is the format of a conventional thesis.

STATEMENT OF CANDIDATE CONTRIBUTION

I, Lucas Djurichkovic, declare that I was responsible for the data collection, study design, data interpretation and writing of this thesis (Chapter 1-6). My primary supervisor Professor David Booth provided guidance and assistance for study design (Chapters 3-5), data analysis (Chapters 3-5), data interpretations (Chapters 3-5) and editing of all chapters in this thesis (Chapters 1-6). My former PhD colleague Alexander Rigg also aided in data collection and editing of Chapter 4 and therefore is a co-author on this Chapter. I was responsible for all edits and changes made to this thesis.

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ETHICS STATEMENT

This research was conducted within accordance and approval of the UTS Animal Ethics Committee (ETH21-6609) under the NSW Department of Primary Industries scientific collection permit (No. F94/696(A) – 9.0 and OUT19/4387).

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Abstract

Climate change is the major driving force of the redistribution and range expansion of tropical marine species into temperate habitats, catalysing the process known as 'tropicalisation'. Several temperate marine ecosystems globally have become the sites of the seasonal displacement of coral reef fishes from lower latitude tropical ecosystems, facilitated by the action of poleward-flowing boundary currents. This results in the arrival of low-density populations of juvenile tropical fish larvae (termed 'vagrant' fishes); however their settlement is limited by environmental and ecological barriers, which has been shown to have adverse effects on the physiology and behaviour these tropical recruiting vagrants. This phenomenon is occurring in temperate coastal ecosystems of south-eastern (SE) Australia, and to such an extent the region has been referred to as a global 'hot spot' for climate change due to extensive localised rising sea surface temperatures. Previous research conducted in this region has focused on a particular species of schooling tropical vagrant, the Indo-Pacific Sergeant Major (*Abudefduf vaigiensis*), as it is the most commonly occurring vagrant throughout this region, using it as a model species to predict the ecological impacts of tropical fish recruitment in temperate marine ecosystems. Studies have shown that this species exhibits physiological and behavioural plasticity in response to cooler water regimes and novel interactions with local species to ameliorate the effects of recruiting to sub-optimal geographic ranges. This thesis focuses on the physiology and behaviour of *A. vaigiensis*, and how they are influenced by extrinsic factors namely latitude, water temperature and shoaling interactions. Chapter 1 is a general introduction summarising the topic of tropicalisation and the role it plays in SE Australia marine ecosystems. In Chapter 2 we critically review the subject of tropical vagrant shoaling, discussing all recent research relating to the range expansion of *A. vaigiensis* in SE Australia, assessing the physiological and behavioural traits that will either promote, or limit, their successful population establishment in temperate marine ecosystems. Chapter 3 focuses on the behavioural traits of *A. vaigiensis* in single-species and mixed-species shoals, where we carried out *in-situ* manipulative field experiments across a large area of their latitudinal range to determine the influence of latitude and shoal structure on their behaviour, primarily focusing on two

critical behavioural traits, to observe specific trade-offs that occurred when these fish were forced to make decisions regarding food intake and exposure to predation risk. Latitudinal gradients in behavioural performance were identified in both foraging and risk-averse behaviour, showing an inverse relationship at high latitude locations in range periphery populations; fish that were recorded further away from the reef expressed more risk averse behaviours and reduced boldness that consequentially led to reduced foraging performance. Shoaling dynamics also exhibit behavioural plasticity, with some beneficial trade-offs occurring in mixed-species shoals, including longer sheltering times post predator exposure. In chapter 4 we conducted laboratory experiments to determine how ambient water temperature and varying shoaling compositions influence the physiology and behaviour of *A. vaigiensis* and how this may initiate behavioural trade-offs within shoals that impact their fitness and survival probability. Testing varying shoal combinations of *A. vaigiensis* with a local temperate species *Microcanthus joyceae*, and exposure to a live predator the mourning cuttlefish (*Sepia plangon*), we determined that higher water temperatures correlated to an increase in performance of *A. vaigiensis*, resulting in greater growth rates, foraging rates, boldness and reduced predator-induced mortality in mixed shoals with *M. joyceae*. Results also indicated that novel predators induce the expression of risk-averse behaviours, as we observed reduced boldness when exposed to a live temperate predator *Sepia plangon*. In Chapter 5 we collected recently recruited juvenile *A. vaigiensis* from the same latitudinal regions used in Chapter 3, with the goal of determining the influence of latitude and shoal structure on post-settlement growth rates, primarily using body size and otolith ring increment widths as a proxy for physiological performance. Latitudinal location of *A. vaigiensis* and shoal type had a significant effect on the growth rates, as post-settlement ring increment widths were greater at lower latitude sites closer to their natal range. At the high latitude region, there was also an effect of mixed-shoaling, as *A. vaigiensis* collected from mixed-shoals yielded higher post-settlement increment widths when compared to those developing in single-species shoals, indicating possible advantages of shoaling with local species when expatriated outside of optimal geographic range. Chapter 6 is a general discussion, summarising the results obtained in this thesis, and indicates future

implications for the range expansion potential of tropical vagrant fish into SE Australia. Overall, there was clear evidence of physiological and behavioural plasticity in *A. vaigiensis* populations in single and mixed-species shoals along a latitudinal gradient within their geographical range, with trade-offs regarding foraging efficiency and predator avoidance as the predominant factors in mitigating cooler water temperatures and novel predators.

CHAPTER 1: General Introduction

1.1 Climate change and altered species distribution

Anthropogenic climate change is having profound effects on the physiology and behaviour of many animal taxa, resulting in adverse species and community level responses (Brierley *et al.*, 2009; Dijkstra *et al.*, 2011; Pacifici *et al.*, 2017; Foden *et al.*, 2019). All species respond to environmental temperature changes, as a strong relationship exists between organism function and ambient temperature, with alterations at metabolic levels manifesting into changes of physiological and behavioural functions in species that result in whole community level reactions (Pörtner and Knust, 2007). A multitude of studies have already shown the negative effects of temperature increases on organism function, including oxygen limitation, growth, muscle activity, reproduction and behaviour (Pörtner, 2002; Pörtner and Farrell, 2008; Tattersall *et al.*, 2012; Abram *et al.*, 2017; Knapp and Huang, 2022). Over the last century earth's climate has warmed by approximately 0.6°C, with current projections indicating that by the year 2100, global mean temperatures are predicted to increase by 2°C (Hoegh-Guldberg *et al.*, 2018), with maximum of 6°C (Kogan, 2023). The effects of warming global temperatures tend to have greater influence in marine ecosystems, as the world's oceans act as heat and CO² sinks for the earth's atmosphere (Rind and Chandler, 1991; Trenberth and Solomon, 1994; Brierley and Kingsford, 2009; Yao and Somero, 2014), absorbing between 80-90% of global heat produced by greenhouse gas emissions (Lindsey and Dahlman, 2020). This has initiated widespread thermal stress among many marine organisms (Brierley and Kingsford, 2009; Assan *et al.*, 2020), as even small temperature increases can have significant consequences in marine ecosystems due to the natural minimal variation in these environments, in addition to many marine organisms already existing close to their thermal thresholds (Pinsky *et al.*, 2020).

In response to warmer global temperatures, many species are shifting their geographical ranges to cooler ecosystems in response to prolonged thermal stress (Thuiller, 2004; Chen *et al.*, 2011; Poloczanska *et al.*, 2013; VanDerWal *et al.*, 2013).

The effects of warming climate regimes are intensified in marine ecosystems, due to the high rate of atmospheric heat absorption in the world's oceans, and the enhanced connectivity compared to terrestrial habitats due to currents, which has resulted in marine species' range shifts occurring at a rate approximately 20x greater than observed in terrestrial species (Lenoir *et al.*, 2020). Poleward range shifts have intensified in strength and occurrence over the past few decades due to rapid increase in sea surface temperatures (Kortsch *et al.*, 2015; Poloczanska *et al.*, 2016; Hastings *et al.*, 2020), further facilitated by the action of poleward flowing boundary currents, that enables the transportation of tropical marine organisms to higher latitude locations (Yang *et al.*, 2016; Yamano *et al.*, 2011; Hastings *et al.*, 2020) often resulting in a series of ecological interactions in recipient subtropical and temperate communities.

1.2 Tropicalisation of temperate ecosystems

Tropicalisation is defined as the warming of higher latitude subtropical and temperate marine ecosystems, characterised by the influx and poleward range expansion of tropical species that catalyse interactions in recipient communities (Vergés *et al.*, 2019; Zarzychny *et al.*, 2024), resulting in these ecosystems exhibiting more "tropical" characteristics. The occurrence of warm-affinity species in higher latitude locations due to the action of poleward flowing boundary currents and consequent tropicalisation, has been recorded in multiple regions globally, as seen in North America, the Mediterranean, Japan and Australia. Resultant changes in impacted ecosystems range from temporal ecological interactions to community phase shifts (Vergés *et al.*, 2019). Herbivory pressure tends to be greater in tropical regions than temperate regions (Steneck *et al.*, 2017), therefore tropicalisation can lead to an increased rate of spatial herbivory (Kumagai *et al.*, 2018). The increased intensity of herbivory can facilitate an ecosystem phase shift from a macroalgal dominated habitat to a rocky reef habitat (Yamano *et al.*, 2011; Vergés *et al.*, 2016). Occurrences of community phase shifts have been seen in both Japan and Australia, where displaced tropical herbivorous surgeonfish (Acanthuridae) settling into temperate habitats dominated by macroalgal experienced over-grazing of these habitat-forming species, subsequently catalysing phase shifts and facilitating the settlement of reef-building corals in Japan (Nakamura *et al.*, 2013; Vergés *et al.*, 2014). The effects of

tropicalisation are occurring in temperate south-eastern (SE) Australia's coastal marine ecosystems, which are characterised by rocky reefs and macroalgal dominated habitats that are subjected to the warm-water influence of the poleward flowing East-Australian Current (EAC) that transports tropical biota from the Coral Sea to high latitudes regions (Ridgway and Hill, 2009; Suther *et al.*, 2011). This region has been referred to as a global "hot spot" for climate change (Wernberg *et al.*, 2011; Hobday and Pecl, 2014) due to the strong influence of the EAC, which has caused an increase in sea surface temperature 6x greater than the global average (Ridgway and Hill, 2009), and the regular incursion and settlement of tropical marine species (Booth *et al.*, 2007; Feary *et al.*, 2014).

1.3 Tropical fish recruitment and shoaling in temperate marine SE Australia

Reproduction of fishes in the Great Barrier Reef (GBR) is highly seasonally, usually occurring between late spring and early December (Robertson, 1991; Munday *et al.*, 2007). During this spawning season, however, only a small number of larvae settle in suitable habitats, while the majority of larval prologues are dispersed and due to the actions of the EAC, can be transported 1000's of kilometres to high latitude locations (Booth *et al.*, 2007). They arrive in pulses in subtropical and temperate regions along the SE coastline of Australia annually between January and April (Booth *et al.*, 2007; Booth *et al.*, 2011; Feary *et al.*, 2014), dependent on factors including species-specifics planktonic larval duration (Booth *et al.*, 2011; Rigg *et al.*, 2025) and oceanographic factors that influence flow and magnitude of the EAC (Ridgway and Hill, 2009; Suther *et al.*, 2011). The most common species that are observed expanding their ranges into temperate SE Australia are damselfishes (Pomacentridae), surgeonfishes (Acanthuridae), butterflyfishes (Chaetodontidae) and wrasses (Labridae), with approximately 200 species being recorded in high latitude regions (Booth *et al.*, 2018). Post settlement they undergo rapid ontogenetic shifts while simultaneously adapting to novel temperate ecosystem dynamics and cooler water regimes, before the onset of winter-water temperatures, reducing tropical vagrant fish populations by approximately 99% (Booth *et al.*, 2007; Figueira and Booth, 2010). Common consensus is that winter-water temperatures remain the limiting factor in their survival and establishment of long-term populations.

Of recent interest has been the settlement and subsequent ecological interactions of a specific tropical recruiting fish species, the Indo-Pacific sergeant major (*Abudefduf vaigiensis*), due to its consistent recruitment into SE Australia rocky reefs and abundance during the Austral summer months (Booth *et al.*, 2007). This schooling planktivore has been recorded forming shoaling aggregations with conspecifics and heterospecifics of other tropical vagrant species or local temperate species (Smith *et al.*, 2018; Paijmans *et al.*, 2020). A range of behavioural trade-offs occur when *A. vaigiensis* form single-species shoals or join mixed-species shoals in novel temperate ecosystems, indicating that there is a need to balance the acquisition of food and avoiding predation to promote survival in range periphery habitats (Coni *et al.*, 2022b; Mitchell *et al.*; 2025). While previous literature has emphasised the physiological effects cooler water temperature has on *A. vaigiensis*, showing significantly reduced performance including metabolic rates, growth, feeding and burst swimming speed (Figueira *et al.*, 2009; Figueira and Booth, 2010; Djurichkovic *et al.*, 2019), the net benefits attained from shoaling, particularly with local temperate heterospecifics, may mitigate the effects of novel predator interactions and hostile environmental conditions (Smith *et al.*, 2018; Coni *et al.*, 2022a), enabling long-term survival and the establishment of permanent populations. While there is a growing body of published literature in this field, many knowledge gaps remain. While several studies have investigated the effects of water temperature on *A. vaigiensis* physiology and behaviour (Djurichkovic *et al.*, 2019), it is unknown whether this influence scales to shoal responses, and how it varies between single-species and mixed-species. Coni *et al.* (2022b) and Mitchell *et al.* (2025) both demonstrated how reduced foraging efficiency and increased exhibition of risk-averse behaviours of *A. vaigiensis* are more prevalent in range periphery habitats, however, these behaviours have not been simultaneously, and therefore it has not been tested whether *A. vaigiensis* will continue foraging post predator threat, and the potential latitudinal gradients that may exist with such behavioural traits. Smith *et al.* (2018) showed that *A. vaigiensis* in mixed-species shoals in Sydney (34°S) attained larger body sizes compared to those in single-species shoals over the course of the study, with the authors using *in situ* body size estimations to measure growth rates. Otolith analysis provides more accurate

method to assess life history characteristics, including post settlement growth rates, however, has not been researched in depth for *A. vaigiensis* in these regions, and compared between different shoaling types and latitudes. Finally, due to the complex nature of mixed-species shoaling, little research has been conducted on how specific ratios of conspecifics to heterospecifics may maximise performance, and if such thresholds exist in populations of *A. vaigiensis* in novel temperate ecosystems.

1.4 Thesis aims and outlines

The objective of this thesis was to investigate the influence of water temperature and shoal metrics on the physiological and behavioural performance of *A. vaigiensis* and how this may assist in their survival in novel temperate ecosystems. The main behavioural metrics and trade-offs assessed in this thesis were foraging efficiency and predator avoidance and how they vary when comparing single-species and mixed-species shoals of *A. vaigiensis*. There was also a focus on latitudinal gradients and associated water temperatures influence on the growth performance of recently recruited *A. vaigiensis* juveniles. Aims relating to these factors are outlined in a summary of each chapter below.

In Chapter 2, we review the subject of shoaling and subsequent trade-offs that occur in different shoaling forms, and the influence of water temperature on fish and scale this up to impacts on shoaling. This information is used to develop a foundation that can be related to the phenomenon of tropicalisation that is occurring in SE Australia and specifically apply this evidence to investigating how shoaling may enable the persistence of *A. vaigiensis* in range periphery habitats. We review current literature relating to the shoaling metrics and performance of *A. vaigiensis*, establishing a synthesis of the factors that may permit a successful range expansion and colonisation of temperate ecosystems.

In Chapter 3 we designed a manipulative field experiment to investigate the behavioural metrics of *A. vaigiensis* in single-species and mixed-species shoals to determine the influence of the foraging-predation trade-off on their performance and the extent to which latitudinal gradient exist in these specific shoaling behaviours. It was anticipated that at higher latitudes, *A. vaigiensis* would show reduced foraging

performance and exhibit more risk-averse behaviours, indicating a higher degree of behavioural plasticity in range periphery populations. The exposure to a predator stimulus while foraging will allow accurate assessments of the two most prominent factors that drive shoal formation: food acquisition and predator avoidance.

Chapter 4 builds on the foundations established in Chapter 3, assessing shoaling behaviours of *A. vaigiensis* in different shoal types, however, is carried out in a laboratory to separately test additional metrics including growth, direct predation and to establish whether a ratio of conspecifics to heterospecifics in mixed-species shoals can maximise performance across a range of temperatures chosen to simulate seasonal water temperatures experienced in SE temperate Australia. It was predicted that higher water temperatures would correlate with increased *A. vaigiensis* performance (growth, feeding and boldness), and reduce predation rates by a temperate predator (Mourning cuttlefish, *Sepia plangon*). We also anticipated that higher shoal ratios of conspecifics to heterospecifics (*Microcanthus joyceae*) would result in increased overall performance of *A. vaigiensis*.

At the latitudinal sites where research for Chapter 3 was conducted, additional surveys of shoals of *A. vaigiensis* were carried out, and recently settled juveniles were collected for otolith extraction to determine the influence on latitude on the post settlement growth rates of *A. vaigiensis*. In Chapter 5 we predicted that *A. vaigiensis* abundance would increase at lower latitude locations, and there would a higher occurrence of single-species shoals in these locations. In addition to this, we predicted that post-settlement growth rates (using increment width as a proxy for growth) would show increases at lower latitudes, and potential differences in the increment widths (a proxy for daily somatic growth) depending on whether *A. vaigiensis* recruited to a single-species or mixed-species shoal.

This research is designed to investigate the complex effects of shoaling behaviours and effects of a tropical vagrant coral reef fish expanding its range into cold-edge habitats facilitated by warming ocean temperatures. The findings will be essential for coastal marine managers and from a fishery perspective when managing coastal reef

ecosystems and the ecological interactions that govern them under climate change, as well as predicting long-term changes to temperate marine ecosystems.

CHAPTER 2: The shoaling functions of tropical range expanding fish: A review of shoal types, fitness benefits and links to foraging and predation risk

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Abstract

Shoaling functions occur in over 10,000 species of fish during some point of their ontogeny and can take a variety of forms from small freshwater single-species shoals to highly variable mixed-species shoals in marine environments comprised of a multitude of species. Shoaling plays a major role in the regulation and survival of many fish populations, providing overall fitness benefits through a series of trade-offs that maximise foraging and mitigate predation risk. Environmental parameters, predominantly water temperature, influence performance at species levels that scales to shoaling responses, therefore must be considered when reviewing shoaling functions and behaviours. In this review we assess the knowledge attained in previous bodies of literature regarding shoaling and water temperature and apply it to the complex nature of the range expansion of schooling coral reef fishes that are recruiting into temperate habitats. Specifically, we focus on the occurrence of coral reef fishes in temperate south-eastern Australia and focus on a vagrant tropical reef fish the Indo-Pacific sergeant major (*Abudefduf vaigiensis*), that has been previously recorded forming a variety of shoaling types with both conspecifics and heterospecifics in their cold-edge range. As cooler water regimes characterise the temperate regions they settle into, we summarise the effects water temperature has on these tropical vagrants and review all relevant literature that relates to their shoaling performance in temperate habitats. We assess the predominant trade-offs for *A. vaigiensis* in novel temperate ecosystems and evaluate the benefits and costs to this species forming single-species and mixed-species shoals. This review is designed for future researchers

investigating range expanding tropical reef fish and tropicalisation, as we explain possible mitigating factors that determine the success of tropical vagrant fish in temperate habitats and how shoaling can ameliorate the novel conditions of temperate ecosystems.

2. 1 Introduction

Animal behaviour is governed by interactions with their environment, including other animals (Okubo et al., 2001; Schmidt *et al.*, 2010; Gil *et al.*, 2017), and these interactions are perceived as information that drives decision making (Dall *et al.*, 2005). Interactions with other animals includes social interactions, temporary or otherwise, that drive complex social grouping behaviours (Sumpter, 2010). Social grouping behaviour is ubiquitous across a broad range of taxa (Giardina, 2008), between conspecifics and heterospecifics (Kotagama and Goodale, 2004) and includes a range of spatial and temporal aggregations (Greenberg, 2001). Foraging opportunities and predator avoidance can be greatly improved when animals form social groups (Gil *et al.*, 2017) that have evolved from trade-offs relating to an animals' fitness (Booth, 1995; Rubenstein and Kealey, 2012).

Key benefits of social grouping are well-studied: predator attack dilution (Delm, 1990; Lehtonen and Jaatinen, 2016), increased vigilance (Powell, 1974; Magurran, 1985; Taraborelli *et al.*, 2011), predator confusion (Landeau and Terborgh, 1986), the occupation of larger foraging areas (Cvikel *et al.*, 2015), increased ability to overcome competitors (Foster, 1985) and social learning and information transfer (Lindeyer and Reading, 2010; Webster and Laland, 2017). Concurrently, animal aggregations can incur costs to individuals and populations such as increased levels of competition, increasing conspicuousness to predators and the transfer of diseases (Giraldeau *et al.*, 2002; Stensland *et al.*, 2003; Beauchamp, 2013; Ward and Webster, 2016b). For example, Savannah ecosystems in Africa see the co-occurrence of large mammals forming complicated aggregations, such as between species of gazelle, water buffalo and zebra, as grouping behaviour changes temporally. During the dry season, group emergence is driven by interspecific resource competition, and by anti-predator advantages during the wet season (Kiffner *et al.*, 2014). Foraging performance can be correlated with population size, as seen in the African Penguin (*Spheniscus demersus*), where group foraging hunting strategies resulted in greater catch per-unit-efforts when compared to individual foraging excursions (McInnes *et al.*, 2017). For species of fish, social grouping, or aggregations, is often referred to as shoaling and occurs in around 10,000 species at some point during their life (Shaw, 1978). Considering the

vast array of group form and functions, fish shoals are a valuable model to investigate the parameters that influence shoaling behaviours, specifically environmental conditions, predation and foraging.

The life history traits of most fish species, characterised by bipartite life cycles and ontogenetic ecological shifts (Leis, 2006; Sánchez-Hernández *et al.*, 2019), drives the formation of diverse shoals, from small monospecific shoals in freshwater habitats to highly complex heterospecific pelagic shoals in marine ecosystems (Barber and Wright, 2001; Rieucau *et al.*, 2015). A large body of literature has identified and quantified the role that shoaling plays in the regulation and survival of fish populations, with a common consensus that net benefits outweigh costs that are produced through a series of trade-offs that arise from mitigating predation risk and increasing foraging efficiency. Extensive research has revealed that the net benefits attained from shoaling are more likely when phenotypic, physiological and behavioural similarities exist among members in single-species shoals (Shaw, 1978; Pitcher, 2012; Cattelan and Griggio, 2018). However, mixed-species shoals occur across a wide range of ecosystems, and in some cases, species show a preference over single-species shoals, attaining larger body sizes (Smith *et al.*, 2018), mitigating predator risk (Mathis and Chivers, 2003) and accessing previously unattainable foraging patches (Aronson and Sanderson, 1987) when shoaling with heterospecifics. This may mean that there are additional trade-offs that fish encounter, influencing their shoaling preferences and possibly the existence of a threshold relating to the ratio of conspecifics to heterospecifics.

Multiple factors must be considered when investigating shoaling behaviours. In addition to ecological variables such as predation, foraging and competition, environmental conditions may influence shoaling formation and behaviour. As fish are ectotherms, ambient temperature is a crucial factor governing their metabolism and therefore their ability to survive (Atkinson, 1996). Increases in temperature are directly correlated to accelerated metabolic rate at the cellular level, manifesting into altered physiological and behavioural responses including increased feeding rates, growth and reproduction (Johnson *et al.*, 2002; Person-Le Ruyet *et al.*, 2004; Pérez-Casanova *et al.*, 2009; Bowyer *et al.*, 2013;). As shoaling is a behavioural adaptation in response to

external stimuli, environmental temperature may affect shoaling parameters. Temperature-dependent responses of shoaling behaviour have been demonstrated in multiple studies, indicating changes to predation risk (Weetman *et al.*, 1999), shoal cohesiveness (Colchen *et al.*, 2017), anti-predator responses (Weetman *et al.*, 1998) and foraging patterns (Greenwood and Metcalfe, 1998). Indirect effects of rising sea surface temperatures due to anthropogenic climate change may further alter social grouping behaviours of fish, specifically warming ocean currents. This has led to a phenomenon known as tropicalisation; the process by which tropical biological matter is transported to temperate ecosystems through the action of poleward-flowing boundary currents (Ridgway, 2007; Vergés *et al.*, 2014). This can lead to the restructuring of temperate fish communities, as they encounter a diverse range of novel interactions (Kingsbury *et al.*, 2020a). These displaced tropical fish, termed ‘vagrants’, arrive and settle as juveniles in a temperate habitat that is closer to their thermal minimal temperatures for survival, and therefore to counteract this exposure, as well as novel predators and foraging resources, they form mixed-species shoals with local fish (Smith *et al.*, 2018; Kingsbury *et al.*, 2020a; Pajmans *et al.*, 2020).

Here, we provide an empirical review that highlights the relevance of shoaling to performance and survival, and the major factors that contribute to their formation under different conditions, with implications of climate change. We will investigate fundamental research papers to provide the background and then apply this to the shoaling behaviours exhibited by vagrant tropical fish in novel temperate habitats and summarise consequences shoaling this may have on their survival and patterns of future range expansion/change. The first section provides an overview of the benefits and costs of shoaling, and how this applies to both single and mixed-species shoals and hypothesise whether the ratio of conspecifics to heterospecifics within mixed-species shoals impacts performance. In the second section, we investigate how temperature affects shoaling behaviours, and how rising sea surface temperatures could affect shoal formations in the future. The third section synthesises the impact of predation on shoaling behaviours, and how it influences performance. Finally, using the information gathered in the preceding sections, we relate this to the displacement of tropical fish in south-eastern temperate marine ecosystems and the governing factors

that regulate shoal formation and survival (predominantly environmental water temperature, predation, foraging), and provide an innovative synthesis of the catalysts of shoaling in novel ecosystems in relation to climate driven ocean fish range redistribution.

2.2 Shoaling Behaviour: An Overview

2.2.1 The trade-offs that interact with shoaling

Shoaling behaviours have evolved through a synthesis of trade-offs, providing both benefits and costs, between predation risk and foraging efficiency (Rubenstein and Kealey, 2012; Paijmans *et al.*, 2019). While predation and foraging are considered the major contributors to driving shoal formation and maintenance, many other underlying mechanisms act concurrently that impact on social behaviours, including breeding and disease resilience. In the following section we will provide a summary of the mechanisms and trade-offs that arise from shoaling and the extent to which they drive shoaling decisions.

Predation risk is considered one of the major ecological forces that drives many social grouping behaviours (Magurran, 2005), and consequently grouping dynamics such as shoaling, may have developed as adaptations to avoid predators (Templeton and Shriner, 2004; Ioannou *et al.*, 2012). Empirical evidence shows that individuals experience reduced predation risk within shoals than remaining solitary (Pitcher, 1986; Pitcher, 2012; Paijmans *et al.*, 2019), commonly referred to as the “safety in numbers” hypothesis (Hager and Helfman, 1991). This antipredator advantage reduces the chance of individual fish encountering a predator when surrounded by other shoaling individuals, termed predator encounter dilution (Morgan and Godin, 1985). In addition to reducing individual predation chance, the formation of shoals allows for greater vigilance and the detection of predators sooner (Godin and Morgan, 1985; Magurran *et al.*, 1985; Lima, 1995). Vigilance and early prey detection are critical to survival, increasing the probability of escape from a predator, as tested in mosquitofish (*Gambusia holbrooki*) where both speed and accuracy of decision had positive feedback with increasing shoal size; these responses were predicted by the “many

eyes" prediction (Ward *et al.*, 2011). With increasing group size, it can make them more conspicuous to predators, however also fewer and more finite, decreasing the encounter rate between predator and prey aggregation (Krause and Godin, 1995; Ioannou *et al.*, 2011). Previous research has shown that when individual blue acara cichlid fish (*Aequidens pulcher*) were introduced into an experimental aquaria with varying size shoals of guppies (*Poecilia reticulata*), the cichlid had greater hunting success when attacking smaller shoals of guppies, indicating that these guppies gained an anti-predatory advantage by joining larger shoals (Krause and Godin, 1995). The aforementioned anti-predatory shoaling mechanisms operate before predators detect prey, reducing the chances of predator-prey interactions. Once a shoal has been detected, the confusion effect and efficient inter-shoal information transfer are strategies employed by shoals to avoid predation (Landeau and Terborgh, 1986; Ward *et al.*, 2011; Ioannou *et al.*, 2008). The confusion effect works by overloading the predator's visual analysis channels (Pitcher and Parrish, 1993), and as these channels rely on movement and trajectories, prediction accuracy and therefore predation is less successful when potential targets are higher in number and effective information transfer is occurring (Ioannou *et al.*, 2008; Frommen *et al.*, 2009). Indeed, the benefits of shoaling in regard to anti-predatory behaviours are well studied, however several costs can arise due to these trade-offs. Larger shoals can increase the detectability for predators during the encounter phase of predation, as observed in Botham *et al.* (2005), where 3 different types of predatory fish (Acara cichlids; *Aequidens pulcher*, Pike cichlids; *Crenicichla frenata* and Wolf-fish; *Hoplias malabaricus*) all showed a strong preference to attack a shoal of guppies compared to a solitary guppy. Shoaling can also reduce the availability of refuges, causing density-dependent mortality (Holbrook and Schmitt, 2002; Samhouri *et al.*, 2009). When shoals reach above low-density thresholds it can prevent the employment of adequate predator-escape behaviours and can reduce the amount of space available for shoals that aggregate in the open (Ford and Swearer, 2013).

While shoaling fish maintain vigilance to detect predators, they simultaneously consider foraging opportunities and success, which is a major determinant of individual and population fitness (Pitcher, 1986; Pitcher *et al.*, 1988; Ward and

Webster, 2016). As aforementioned, group vigilance is a direct benefit of shoaling behaviours, and while it is imperative to detecting predators, it concurrently decreases the amount of time dedicated in the search for food (Pitcher *et al.*, 1982). The acquisition of food sources is imperative to survival, and while the act of individual foraging can reduce competition with conspecifics or heterospecifics, foraging can promote the formation of shoals (Olson *et al.*, 2015) and enhances foraging efforts by enabling the occupation of larger patches of food, overwhelming competitors for resources, transferring information among shoal members and increasing food intake (Pitcher *et al.*, 1982; DeBlois and Rose, 1995; Laland and Williams, 1997; Lachlan *et al.*, 1998; Pitcher *et al.*, 1998). Foraging as a group can also reduce competition from neighbouring shoals or other species, which is observed in coral reef ecosystems, where larger shoal sizes can contribute to reduced aggression in competitors where trophic niche overlap (Foster, 1985, Aronson and Sanderson, 1987). Despite the multiple benefits, group foraging can also incur fitness-related costs, including within-shoal competition that directly reduce an individual's uptake of food (Robinson, 1994; Ward *et al.*, 2006; Thünken *et al.*, 2020). Increases in shoal size can also negatively impact individual fitness by limiting availability of food equally across shoaling fish. Desai *et al* (2025) demonstrated that in a laboratory a group size increase from 4 to 8 green chromides (*Etroplus suratensis*) resulted in reduced space and starvation, as well as reduced shoal cohesion. Access to food and foraging efficiency play major roles in the formation of shoals, however, much like predator avoidance, will vary within and between species.

2.2.2 Single-species vs Mixed-species Shoaling

Shoals comprised of one species, or single-species shoals, are often considered to be preferred by many fish species, as shoaling with familiar conspecifics that have a high degree of phenotypic similarity (e.g. body size) and are free of parasites has been shown in numerous studies (Ranta *et al.*, 1994; Griffiths and Magurran, 1997; Barber *et al.*, 1998; Svensson *et al.*, 2000; Farine *et al.*, 2015). The transfer of information within single-species shoals is theorised to be generally superior comparatively to mixed-species shoals. A faster rate of information transfer manifests into higher functioning shoaling behaviours, including early predator detection (Magurran *et al.*, 1985),

predator confusion (Magurran, 1990), greater shoal cohesion (Chivers *et al.*, 1995) and social learning cues that can enhance foraging efficiency (Chapman *et al.*, 2008). Fish living in mixed-species shoals tend not to possess the conventional advantages that single-species shoals provide, however, by shoaling with other species can achieve net fitness benefits through trade offs that were previously unattainable. A diversity of morphology, physiology and behaviours can drive fish to form mixed-species shoals. While inherent phenotypic oddity within a shoal may increase its susceptibility to predation (Ward *et al.*, 2002b), some species choose to shoal with heterospecifics that may have physiological disadvantages, in doing so becoming the less desirable shoal member to a predator. This was the case for brook sticklebacks (*Culaea inconstans*) that showed a preference to shoal with a nearby heterospecific, the flathead minnows (*Pimephales promelas*), when under predation threat (Mathis and Chivers, 2003). This response was presumably driven by the phenotypic differences between the species; the armoured sticklebacks reduced their probability of predation by shoaling with the non-armoured minnows. Anti-predator behaviours are species-specific and may catalyse mixed-species shoal formation, for example, early predator detection. This shoaling behavioural trait is employed by the cowtail stingray (*Pastinachus sephen*) when it shoals with the heterospecific the reticulate whipray (*Himantura uarnak*), as the whipray has been shown to exhibit early predator detection that acts as an alarm cue for the slower responding cowtail stingray (Semeniuk and Dill, 2006). Foraging advantages may also drive mixed-species aggregations (Lukoschek and McCormick, 2000), as they can promote resource partitioning concurrently reducing within shoal competition (Alatorre-Ramirez *et al.*, 2017). Species-specific spatial and temporal foraging behaviours within mixed-species shoals can prevent the overlap of trophic niches, allowing for success and survivorship (Kingsbury *et al.*, 2020a).

For shoaling fish species, and subsequent behavioural trade-offs of food acquisition and avoiding predation, are imperative to individual and populations fitness. The synergistic benefits of acquiring food and avoiding predation is well documented and regulates their survival. Environmental conditions, however, are often the limiting factor for fish growth and reproduction and therefore must be considered when investigating shoaling functions and behaviours.

2.3 The effects of temperature on shoaling dynamics

2.3.1 The relationship between temperature and fish performance

Due to the ectothermic nature of fish biology, there is a curve-linear relationship between ambient water temperature and individual fitness (Parmesan and Yohe, 2003; Nilsson et al., 2009; Toni *et al.*, 2025). Warmer temperature regimes can initiate thermoregulatory responses among fishes including geographic range shifts, increased metabolic rates, accelerated maturity, augmented growth and altered behaviour (Green and Fisher, 2004; Last *et al.*, 2011; Burraco *et al.*, 2020). Physiological and behavioural responses to warmer water temperatures however vary among species; as temperatures approach or exceed maximum thermal thresholds, metabolic reactions speed up subsequently increasing the necessity for oxygen consumption, limiting aerobic capacity which manifests into a decrease of higher functions such as swimming and growth (Pörtner and Knust, 2007; Munday *et al.*, 2008; Donelson et al., 2010; McDonnell and Chapman, 2016). In Munday et al (2008) a common coral-reef fish *Acanthochromis polyacanthus* was subjected to a series of temperature treatments that represented long-term minimum, average and maximum sea-surface temperatures that simulated conditions on the reef. At the maximum temperature treatment, growth rates declined comparatively to the other treatments, indicating that the capacity for growth is severely limited at higher water temperatures. As *A. polyacanthus* is a warm-adapted fish already living close to its maximum thermal threshold, predicted climate change scenarios and corresponding increases in sea-surface temperatures may have critical effects on the physiological and aerobic performance of many coral-reef fishes. For cooler-water adapted fishes, similar effects are seen when they are exposed to increased ocean temperatures, as seen in Mitchell *et al* (2023), where the authors found that a temperate fish species, *Atypichthys strigatus*, subjected to elevated summer conditions of 26°C resulted in higher oxidative damage and reduced energy storage when compared to individuals reared in cooler water treatments. While there is empirical evidence to suggest increasing ocean temperature due to climate change will negatively impact many fish species, more recent studies have shown similar adverse effects when water temperatures approach minimum thermal thresholds; a common scenario for warm-adapted fish species

displaced by the action of currents or extreme heat, resulting in the range expansion poleward of some populations into cooler latitudinal regions.

The effects of cold-water stress on fish physiology are far less studied than that of heat stress exposure (Reid *et al.*, 2022), however, cold water events are becoming more frequent due to anthropogenic climate change, poleward distribution shifts and altered natural events such as cold snaps and upwellings (Hsieh *et al.*, 2008; Hsu and Gwo, 2017; Wuebbles *et al.*, 2019). Significant neural damage can occur with rapid decreases in water temperature, affecting physiological and behavioural functions include growth rates, swimming and predator-escape responses, often having sublethal effects (Fuiman and Batty, 1997; Ward *et al.*, 2002; Preuss and Faber, 2003; Donaldson *et al.*, 2008). In Clarkson and Childs (2000) several species of freshwater juvenile fish were subjected to rapid decreases in cold water which severely affected their swimming performance, inducing cold coma in some individuals, with the authors concluding that such results would significantly increase their likelihood of predation in their natural habitats. While some cold-water events may be relatively sudden (in the cases of upwellings or industrial discharge), others may occur over larger temporal periods, such as in the case of displaced tropical fish that settle into sub-optimal temperate habitats due to the action of poleward-flowing currents. In such cases, warm-adapted tropical fish become subjected to winter-water temperatures, which often induce mass mortality events among these vagrant populations (Booth *et al.*, 2007; Booth *et al.*, 2011). A commonly occurring tropical fish vagrant species, the Indo-Pacific sergeant major (*Abudefduf vaigiensis*), experiences cold-shock during the onset of winter-water temperatures (approximately 17°C), resulting in reduced feeding rates and inhibited burst-escape speeds (Figueira *et al.*, 2009). Cold-water shock, much like heat stress, induces oxygen limitation in metabolic processes, catalysing the reduction in aerobic scope that in turn reduces the energy available for higher functions including muscle activity, growth and reproduction (Pörtner and Knust, 2007; Djurichkovic *et al.*, 2019). While there is clear evidence of effects of thermal stress on fish physiology and behaviour, how this manifolds into ecological functions such as shoaling is less understood.

2.3.2 Temperature and shoaling functions

The relationship between temperature and the individual fitness of fish would suggest that more complex behaviours such as shoaling will show altered functionality with changes in ambient temperature, having potentially critical consequences for schooling species. The mechanisms governing how temperature influences physiological and behavioural performance, from biochemical cellular pathways to whole organism function, the combined effects of individual fitness can have adverse implications for population-level dynamics (Bennett *et al.*, 2019). Suboptimal environmental temperatures may potentially inhibit the benefits of shoaling, including safety from predation, foraging efficiency and social learning (Hensor *et al.*, 2003; Kunz *et al.*, 2006; Chapman *et al.*, 2008), increasing the vulnerability of a shoal to disease and predation (Dadras *et al.*, 2017; Davis *et al.*, 2019).

Individuals in a shoal can show decreased metabolic performance when exposed to high temperature stress leading to unfavourable shoaling behaviours, as shown in Mukherjee and Bhat (2024), where several species of freshwater fish shoals were subjected to different temperature treatments based on current and predicted levels of environmental temperature change. In the highest water temperature regime at 31°C, some species exhibited elevated muscle glycogen and lower body weights, corresponding to reduced shoal cohesion. The interference of group cohesion in shoals can lead to less coordinated group movement and increased vulnerability to predation, as information transfer among shoal members is less efficient, reducing the confusion effect (Ward and Webster, 2016). Increases in temperature also lead to elevated metabolic rates, which must be met by higher rates of food acquisition, which increases within-shoal competition for food (Killen *et al.*, 2016), consequentially individual fish within the shoal will move apart reducing the anti-predatory benefits of shoaling.

Prolonged cold-water exposure has been shown to induce starvation through reduced food intake, reduce the effectiveness or exacerbate anti-predation behaviours, reduced swimming capabilities, impair senses and cause mass mortality among fish populations (Hsieh *et al.*, 2008; Bermejo-Nogales *et al.*, 2014; Cull *et al.*, 2015; Bai *et al.*, 2016). The growth of fish is governed by environmental temperature, and

regulated by food intake, meaning these factors are dependently intertwined. At cooler water temperatures, predator-prey interactions can be altered, as seen in Krause and Godin (1995), where shoals of juvenile guppies (*Poecilia reticulata*) were exposed to a live predator the blue acara cichlid (*Aequidens pulcher*) to measure hunting success on varying group sizes across several differing water temperatures. The authors found that *A. pulcher* exhibited a preference to attack smaller shoals over larger ones at cooler water temperatures, with the authors contributing this finding to reduced movement and activity amongst the shoal members, as the predators showed a preference to attack smaller and slower moving shoals. Cold-water temperatures are directly linked to reduced food uptake (Robinson and Pitcher, 1989; Mikheev *et al.*, 1992; Ahmad *et al.*, 2014), leading to lower energy availability and reduced behavioural performance, for example swimming abilities (Colchen *et al.*, 2017), which can impact shoal response times and predator-escape speeds.

When comparing the effects of temperature on single-species and mixed-species shoals, the benefits and costs incurred by trade-offs will vary due to altered shoal structure must be considered. In single-species shoals, despite potential unilateral differences, temperature would be expected to affect shoal members at a relatively constant rate, as physiological and behavioural traits would manifest into consistent shoaling responses; at temperatures that initiate stress responses among individuals, the shoal structure would exhibit vulnerability and less cohesion (Weetman *et al.*, 1999; Killen *et al.*, 2012; Killen *et al.*, 2017). For mixed-species shoals however, the influence of water temperature may have varying effects, as the shoal is made up of two or more species likely stress responses will not be uniform due to differences in phenotypes. The dynamic nature of mixed-species shoals, due to increased species oddity and niche preferences, may render them more vulnerable in suboptimal climatic conditions, due to one or more species exhibiting greater adverse reactions than others, potentially causing a collapse of shoal structure. The formation of mixed-species shoals may be driven by several factors, including enhanced foraging opportunities and predator avoidance (Mukherjee and Bhat, 2023), as associating with different species may provide greater information on foraging patches, suitable shelter or being the less desirable shoal member to a predator (Paijmans *et al.*, 2019).

Exposure to heat or cold stress may potentially have species-specific benefits or costs; some shoal members may exhibit beneficial trade-offs while others show decreased levels of performance. In the case of tropical fish species expanding their range into temperate regions, such multi-species shoal responses may occur as these vagrant fish recruit into novel habitats, and due to larval supply restrictions, utilise the niches occupied by local species, in turn forming mixed-species shoals that reshape ecological interactions for short and long-term temporal periods.

Recent studies have inferred benefits being gained from mixed-species shoals when certain thresholds of species exist within the shoal. Varying ratios of conspecifics to heterospecifics can result in improved performance for some species, as seen in Tang *et al* (2017) and Bai *et al* (2019), where the Chinese carp (*Parabramis pekinensis*) was shown to have elevated swimming and activity rates, and improved sociability (linked to shoal cohesion) when shoaling with heterospecifics and conspecifics in specific shoal ratios. Such findings may imply that for species that aggregate in mixed-species shoals, temporally or stable, may attain maximum fitness benefits and trade-offs at a threshold ratio. While the majority of studies that have researched shoaling compare how a species performs in either a single-species shoal or a mixed-species shoals, concluding that one shoaling function being more beneficial to the focal species than the other, very few have investigated whether different ratios of conspecifics to heterospecifics and how that might increase or reduce physiological and behavioural performance. Shoal properties differ depending on their function, and species responses in either the presence of conspecifics or heterospecifics show altered trade-offs to external stimuli (Magurran, 1986; Dill and Ydenberg, 1987; Goodale *et al.*, 2010). The existence of such a paradigm proposes interesting research questions, particularly in relation to climate change driven range expansion and the formation of shoals between species from different thermal niches.

2.4. Tropicalisation and shoaling functions in temperate marine ecosystems

2.4.1 Tropicalisation in temperate southern-eastern Australia

Increasing sea-surface temperature have catalysed the range expansion, retraction or relocation of many fish species globally, the commonality observed are that multiple tropical fish species are expanding their ranges into sub-tropical and temperate regions (Booth *et al.*, 2011; Feary *et al.*, 2014; Verges *et al.*, 2014). This phenomenon is regularly observed in temperate marine ecosystems along the coastline of south-eastern Australia, a region characterised by rocky reef ecosystems that receive annual influxes of settling tropical fish larvae (Booth *et al.*, 2007; Booth *et al.*, 2011) due to the action of the East-Australia Current (EAC), a poleward flowing boundary current which displaces tropical organisms from the coral sea to higher latitudes (Ridgway, 2007). Common species observed recruiting into these temperate regions include damselfishes (Pomacentridae), butterflyfishes (Chaetodontidae), wrasses (Labridae) and surgeonfishes (Acanthuridae) (Booth *et al.*, 2007; Booth *et al.*, 2018; Fowler *et al.*, 2018), which post-settlement begin initiating a series of ecological interactions with local species due to resources overlaps, as these tropical vagrants integrate themselves into temperate habitats and potentially compete with local species for foraging patches (Kingsbury *et al.*, 2020a). Novel environmental conditions and species will drive the formation of both single and mixed-species shoals of varying size, predominantly limited by temperature regimes and larval supply. The recruitment of juvenile tropical fishes into rocky reef habitats in SE Australia may also face aggressive competitive interactions with local heterospecifics, forcing them into suboptimal niche spaces, increasing their vulnerability to predation.

As previously mentioned, the commonly observed *A. vaigiensis*, a schooling species of damselfish, settle into rocky reefs along the SE Australian coastline, forming low-density populations during the Austral summer months (Booth *et al.*, 2007; Booth *et al.*, 2011; Booth *et al.*, 2018). Post-settlement these tropical fish initiate a change in local ecological interactions (Taylor and Figueira, 2021) as they form shoals with both conspecifics and heterospecifics, altering niche occupation and predator-prey dynamics in recipient communities. As their arrival in temperate SE Australia coincides with warmer-water temperatures, the gradual drops in water temperature due to the

onset of winter presents a major survival hurdle for these schooling vagrants, as their abundance is reduced by approximately 90% (Figueira and Booth, 2010; Booth *et al.*, 2018). This creates an interesting conflict regarding shoaling preferences in a novel ecosystem; shoaling with conspecifics will provide greater shoal cohesion, enhanced information transfer and reduced species oddity, however, shoaling with heterospecifics may provide access to and knowledge of local resources, and potentially maintain greater shoal densities during cooler periods, preserving anti-predatory mechanism such as predator encounter dilution as temperate species will naturally be less vulnerable to cooler water regimes.

2.4.2 *Abudefduf vaigiensis* in single-species and mixed species shoals

The formation of single-species and mixed-species shoals comprising the tropical vagrant *A. vaigiensis* will undoubtedly require behavioural trade-offs among shoaling strategies that will determine their successful settlement survivorship in the novel temperate ecosystems of SE Australia. The formation of single-species shoals has been shown to greatly improve information transfer among individuals, as well as enhanced shoal cohesion, both behavioural traits being highly linked with advanced predator warning, protection and improved coordinated movements that can cause the confusion effect (Makris *et al.*, 2006; Ioannou *et al.*, 2011; Ioannou *et al.*, 2012). Indeed, in Paijmans *et al.* (2021b), the authors found that in single-species shoals of *A. vaigiensis* exhibit great shoal cohesion and stronger alignment than mixed-species shoals containing the tropical vagrant. Improved shoaling strategies in novel temperate ecosystems would be highly advantageous to counteract exposure to novel predators and suboptimal environmental conditions. In Matis (2018) it was shown that latitudinal trends in shoal formations for a congeneric species of *A. vaigiensis*, the scissor-tail sergeant (*Abudefduf sexfasciatus*), concluding that at lower latitudes that are closer to their natal range, only single-species shoals were formed. This would indicate that *A. vaigiensis* in their natal geographic range, coral reefs in lower latitudes, are not constrained by environmental conditions and have access to primary foraging resources and mating opportunities, as would be expected for a coral reef fish (Ferreira *et al.*, 2004; Floeter *et al.*, 2004). In their native habitat where thermal and

ecological conditions would be optimal, *A. vaigiensis* shoaling with conspecifics would show net benefits regarding foraging success, predator avoidance strategies, enhanced information transfer and stronger shoal cohesion. Recent studies have already shown that warmer water temperatures ($\geq 25^{\circ}\text{C}$) that are closer to those experienced on coral reefs, greatly enhance the physiological and behavioural metrics of *A. vaigiensis*, including aerobic performance, growth rates, food uptake and burst swimming speed (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019). Such increases in overall performance and fitness of *A. vaigiensis* also reduce their chances of predation, with lower rates of predator-induced mortality being observed in Figueira *et al.* (2019). Such results were also observed in field-based studies such as Coni *et al.* (2021), linking *A. vaigiensis* enhanced performance with latitudes closer to their natal range. Considering the likely positive relationship between the higher occurrence of *A. vaigiensis* shoaling with conspecifics rather than heterospecifics, and greater performance, it raises questions as to why at range periphery locations are these tropical vagrants more often seen forming mixed-species shoals?

Tropical vagrant fishes recruiting to mixed-species shoals have been of recent interest in the field of tropicalisation, specifically along the coastline of SE Australia, with several studies already highlighting likely benefits attained for recently settled *A. vaigiensis* juveniles at higher latitude locations (Smith *et al.*, 2018; Coni *et al.*, 2022a, Mitchell *et al.*, 2025). Post-settlement processes in cold-range edge habitats will have a major influence over tropical vagrant survivorship, namely that of the behavioural trade-off between acquiring food resources and avoiding predation (Sexton *et al.*, 2009; Nagelkerken and Munday, 2016; Paijmans *et al.*, 2020). The decrease in foraging efficiency of *A. vaigiensis* towards their trailing range-edges has been recorded in multiple studies conducted in the SE Australian coastal marine regions (Coni *et al.*, 2021; Paijmans *et al.*, 2020), with the authors generally concluding that reductions in feeding are a consequence of novel conditions, as risk-averse behaviours such as increased sheltering and reduced boldness become more prominent in mixed-shoals (Coni *et al.*, 2022b; Mitchell *et al.*, 2025). While reductions in feeding will prolong time spent at smaller size-classes which are vulnerable to predation (Sogard, 1997), such behavioural plasticity may indicate additional trade-offs, as theorised in Kingsbury *et al.*

(2020b), where they concluded that *A. vaigiensis* juveniles likely exhibited seasonal energy partitioning which was responsible for a growth-body condition trade off to increase the chances of overwinter survival. Furthermore, the exhibition of plastic foraging behaviours may be an example of diet partitioning, which is a trait exercised in mixed-species shoals to reduce within shoal competition for resources (Mketsu, 2008; Varghese *et al.*, 2014; Paijmans *et al.*, 2019). Potential evidence of diet partitioning was found in Kingsbury *et al* (2020a), where the diet and niches used by *A. vaigiensis* did not generally overlap with temperate species they commonly form mixed shoals with at high latitude locations, including the stripey (*Microcanthus joyceae*) and the mado (*Atypichthys strigatus*). Such examples of trophic generalism and large-scale dietary plasticity indicate both tropical and temperate species exhibiting broad behavioural repertoires that allow for the co-existence of species and maintenance of mixed-species shoal integrity. Stronger evidence to support benefits attained by *A. vaigiensis* in mixed-species shoals as opposed to single-species shoals in trailing edge habitats was seen in Smith *et al* (2018), where the authors determined that recently recruited *A. vaigiensis* shoaling with temperate heterospecifics attained larger body sizes over the course of the recruitment season when compared to those shoaling with conspecifics. This would suggest that foraging efficiency is enhanced in range periphery locations when shoaling with local heterospecific species transfer knowledge of superior foraging patches.

Accessing foraging resources concurrently increases the risk of predation in most fish species (Pitcher, 2012; Ward and Webster, 2016; Paijmans *et al.*, 2019). Increased sheltering times and reduced boldness for *A. vaigiensis* shoaling in temperate habitats shows increased anti-predatory mechanisms being deployed to ameliorate the effects of survival in a novel temperate ecosystem. In Mitchell *et al* (2025) the authors found that at range periphery locations *A. vaigiensis* in single-species shoals spent significantly more time sheltering than those in mixed-species shoals. This concurs with similar studies conducted in the region, and would explain the overall reduced feeding rates, and simultaneously the increased growth rates of *A. vaigiensis* in mixed shoals in cold-edge range habitats. However, increased sheltering time and risk-averse behavioural plasticity may not fully mitigate the effects temperature have on

predator-induced mortality for newly settled *A. vaigiensis*. Figueira *et al* (2019) showed the strong relationship that exists between predation and temperature for *A. vaigiensis* juveniles, concluding that increases in burst-escape speed at a water temperature of 25°C was directly linked to reduced mortality by a local predator, the halfbanded seaperch (*Hypoplectrodes maccullochi*). In addition to latitude and temperature, predator threat has been shown to foraging rates of *A. vaigiensis*, as observed in Beck *et al* (2016), where the authors showed that predator presence significantly decreased bite rate in single-species shoals. While the influence of predators may regulate their shoaling behaviours across multiple shoal structures, they may not explain whether *A. vaigiensis* make active decisions to shoal with either conspecifics or heterospecifics, as seen in Paijmans *et al* (2021a), where juveniles in a laboratory mesocosm were exposed to a live predator (*Sepia plangon*) and given a choice to decide between shoaling with conspecifics or a shoal comprised of heterospecifics and conspecifics. The authors concluded that neither acute predation threat, nor species oddity, drive shoal choice.

The annual influx of tropical vagrant fishes in their cold-range edges will undoubtedly increase levels of competition with local species, particularly in mixed temperate and tropical shoals. Despite the lack of trophic niche overlap observed between *A. vaigiensis* and common temperate species they co-shoal with (Kingsbury *et al.*, 2020a), evidence suggests that the formation of mixed-species shoals containing *A. vaigiensis* may force both species into less preferred habitats, as their arrival into trailing edge habitats is driving multiple species away from optimal habitat usage (Taylor and Figueira, 2021), potentially altering behaviours and increase competition for resources. As these tropical vagrants are already recruiting to a suboptimal habitat, such findings would suggest that these incursions may cause costly trade-offs for local species that are in mixed-species shoals with *A. vaigiensis*. Co-shoaling with temperate species in trailing edge habitats has also been shown to exacerbate aggressive interactions among shoal members (Mitchell *et al.*, 2025), as potential niche overlaps may enhance competition and reduce shoal cohesion among mixed-species shoals. However, to counteract such effects, *A. vaigiesnis* has been observed to form single-species sub-groups within mixed-species shoals (Paijmans *et al.*, 2021b), which aided in

ameliorating shoal coordination that is normally less functional in mixed-species shoals.

The plastic shoaling behaviours exhibited by *A. vaigiensis* across multiple shoaling functions show benefits attained in both single-species (e.g. shoal cohesion) and mixed-species shoals, (e.g. improved growth rates). Based on this information, it would be reasonable to assume that in mixed-species shoals, a certain ratio of conspecifics to heterospecifics may improve *A. vaigiensis* performance through a series of trade-offs that can accommodate the acquisition of food and exhibiting appropriate anti-predatory behaviours in a novel temperate ecosystem where they are subjected to sub-optimal thermal regimes.

Table 2.1. Table summarising published articles that investigate the physiology and behaviour of *A. vaigiensis* recruiting to temperate habitats, as well as research relating to their shoaling functions and performance in novel temperate ecosystems.

Author/s	Focus: Individual, Shoaling or Population	Major findings
Figueira <i>et al</i> (2009)	Individual	Increased water temperatures result in increased performance
Figueira and Booth (2010)	Population	Critical minimal threshold of $\leq 17^{\circ}\text{C}$ for survival
Booth <i>et al</i> (2018)	Population	Overwinter ratio of 0.1 in temperate ecosystems (average over-winterers to total recruits)
Djurichkovic <i>et al</i> (2019)	Individual	Increased water temperatures increase metabolic rates. Cold water temperatures induce reduced fitness
Taylor and Figueira (2021)	Shoaling	Formation of mixed species shoals in trailing edge habitats can force species into suboptimal habitats
Figueira <i>et al.</i> , (2019)	Shoaling	Warmer water temperatures reduce mortality
Kingsbury <i>et al</i> (2020a)	Shoaling	Trophic niches show little overlap with commonly associated heterospecifics
Paijmans <i>et al</i> (2020)	Shoaling	Higher abundance of mixed-species shoals in poleward range limits, and enhanced foraging with more time spent further away from reef shelter

Paijmans <i>et al</i> (2021a)	Shoaling	Species oddity does not drive shoaling preferences in presence of a predator
Paijmans <i>et al</i> (2021b)	Shoaling	Enhanced shoal cohesion in single-species shoals, and formation of single-species subgroups in mixed-shoals
Coni <i>et al</i> (2021)	Shoaling	Reduction in foraging performance at temperate range edge
Coni <i>et al</i> (2022a)	Shoaling	Modified behavioural niches in presence of temperate heterospecifics
Coni <i>et al</i> (2022b)	Shoaling	Increased sheltering and reduced boldness at poleward range limits
Smith <i>et al</i> (2018)	Shoaling	Mixed-species shoal promote larger body sizes

2.5 Conclusions

Environmental temperature exerts a major influence over the physiology and behaviours of fishes, such effects often manifesting into shoaling functions that can alter the benefits and cost ratios that arise from fish forming aggregations such as foraging efficiency, predator avoidance, mate selection and information transfer. In the case of tropical coral reef fish expanding their ranges into temperate marine ecosystems, the formation of single-species and mixed-species shoals is critical to their survival in a novel habitat as they encounter cooler water temperatures and previously unencountered predators. Single-species and mixed-species shoals containing *A. vaigiensis* show no difference in occurrence in temperate-subtropical transition zones or cold-edge range habitats (Smith *et al.*, 2018; Paijmans *et al.*, 2020). A potential causation of this may not be preferences exhibited by *A. vaigiensis*, but simply larval supply issues, which becomes a limiting factor for the development of long-term populations at range periphery locations (Feary *et al.*, 2014). Based on the evidence collected, it can be concluded that the establishment of mixed-species shoals in temperate habitats can provide tropical vagrant fish with access to superior foraging resources and vital information regarding the foreign environmental and ecological interactions they are subject to. Behavioural adaptations are also a likely result of shoaling with temperate heterospecifics, as risk averse behaviours become more prominent in novel trailing edge habitats. However, abundance of suitable

heterospecifics to shoal with, along with accessible and viable habitats to colonise, may be additional limiting factors to recruit into mixed-shoals, possibly explaining why single-species of *A. vaigiensis* are equally as abundant as mixed-species in these regions. In consideration of the availability of suitable heterospecifics, and previous research highlighting benefits and costs attained from different shoaling structures, there may also exist a threshold of ratio of conspecifics to heterospecific regarding shoals containing *A. vaigiensis* in cold-edge ranges: where a certain number of temperate heterospecifics within a shoal may drive peak performance for one or both species. The honing abilities of pelagic larvae may also be a contributing factor to shoal selection, as larvae use olfactory and audio cues as indicators for suitable habitat choice (Booth, 1992). The aforementioned factors must all be considered when examining the shoaling behaviours of tropical vagrant fish recruiting into temperate habitats, it is safe to assume that shoaling strategies that provide overall net benefits are critical in range expanding coral reef fishes adapting and establishing long-term populations in higher latitude temperate marine ecosystems.

CHAPTER 3: THE EFFECTS OF LATITUDINE AND SHOAL STRUCTURE ON THE BEHAVIOURAL REPERTOIRE OF A RANGE-EXPANDING TROPICAL FISH

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Abstract

In response to a rapidly changing climate, an increasing number of animal taxa are shifting their geographical ranges, initiating behavioural plasticity as a response to exposure to novel environmental conditions. This is evident in the temperate rocky reef marine ecosystems of Australia's south-east coastline, an area subject to a strengthening poleward flowing boundary current, the East Australian Current (EAC). Consequent displacement and settlement of tropical vagrant reef fish into novel temperate habitats can modify ecological interactions in recipient communities. This study aimed to observe behavioural repertoires and degree of plasticity in the juvenile phase of a common vagrant tropical fish, the Indo-Pacific sergeant major (*Abudefduf vaigiensis*), to determine the influence of latitudinal gradients and shoaling forms (single-species vs mixed-species shoals) in range periphery populations. We focused on two key fitness-related behavioural traits, foraging efficiency and predator avoidance, and observed through manipulative field experiments specific behavioural trade-offs that occurred when these fish were forced to make decisions regarding food intake and exposure to predation risk. Latitudinal gradients in behavioural performance were identified in both foraging and risk averse behaviour, showing an inverse relationship at high latitudes (range periphery populations); fish that were recorded further away from the reef expressed more predator risk averse behaviours and reduced boldness that drove reduced foraging performance (decreases in grazing times and bite rates). *A. vaigiensis* juvenile also showed behavioural plasticity when shoaling with either conspecifics or heterospecifics, with some beneficial trade-offs occurring in mixed-species shoals, including 22% longer sheltering times post predator exposure,

providing evidence of learning, but also resulted in maladaptive responses, such as an increased rate of competitive interactions with heterospecifics. Overall, there was clear evidence of behavioural plasticity in *A. vaigiensis* populations in single and mixed-species shoals along a latitudinal gradient within their geographical range, with range periphery populations showing the highest rate of behavioural plasticity that may mitigate the impact of recruiting into a novel temperate ecosystem for a warm-adapted fish.

3.1 Introduction

Behavioural traits are intrinsic to the survival of all animals (Sumpter, 2006; Anthes *et al.*, 2010; Huntingford, 2012), defined by how they interact with their environment and other species, regulating functionality within an ecosystem (Barnard, 2012; Candolin *et al.*, 2023). Decision making within animal species dictates how they respond to environmental stimuli around them and provides plasticity regarding occupying and surviving in an ecosystem that will fulfill their physiological and behavioural needs (Fisher *et al.*, 2021). To cope with environmental factors, such as temperature, rainfall and water quality, and ecological variables including food resources, shelter and interactions with other species, animals have a suite of behavioural traits they can exhibit to enhance their chances of survival (MacArthur, 1984; Peterson and Lieberman, 2012). Within their normal range, animals exhibit a scope of behaviours that align with optimal functioning (Begon *et al.*, 2020), however this does not represent their full range of behavioural traits (Dingemanse *et al.*, 2010), referred to as behavioural plasticity. The mechanism of behavioural plasticity is defined as an individual animal possessing a broad and flexible repertoire of traits that not only allows them to thrive in their optimal range but also adapt to novel or dynamic ecosystems (Angert *et al.*, 2011; Chapple *et al.*, 2012; Slatyer *et al.*, 2013). The need for animals to exhibit plastic behaviours is becoming increasingly important to their susceptibility and resilience in a rapidly changing climate (Moritz and Agudo, 2013; Huey *et al.*, 2012; Buccholz *et al.*, 2019).

Climate change, specifically increasing global temperatures, has been predicted to significantly alter the physiology and behaviour of animals from a variety of taxa (Parmesan, 2006; Beever *et al.*, 2017), including insects, reptiles, birds, mammals and fish (Hickling *et al.*, 2006; Deutsch *et al.*, 2008; Kearney *et al.*, 2009; Righton *et al.*, 2010). Coupled with habitat loss, a mass behavioural change among vulnerable organisms is expected during the Anthropocene. Thermoregulation is a behavioural response to overheating or cold stress (Begon *et al.*, 2020), particularly for ectothermic animals that rely on ambient temperature to regulate metabolic function, which can require temporal or latitudinal range shifts when habitats are exposed to prolonged periods of environmental warming. Another common response among animal taxa to

rising global temperatures is to shift their range to more thermally suitable habitats (Walther *et al.*, 2002; Pinsky and Fogarty, 2012). A rapid rate of redistribution among organisms is occurring in marine taxa, specifically ectotherms, approximately six-times greater than observed in terrestrial species (Lenoir *et al.*, 2020). The susceptibility of marine taxa to climate change-induced range shifts can be attested to the connectivity of marine populations, largely driven by ocean currents. Poleward flowing boundary currents transport warm water and biota from low to high latitudes, facilitating the redistribution of marine organisms into novel ecosystems (Grothues *et al.*, 2002; Pörtner and Peck, 2010). However, this can catalyse negative ecosystem responses, such as the range expansion of a sea urchin (*Centrostephannus rodgersii*) in southern Australia, which catalysed the over-grazing and depletion of kelp forests which provided nurse ground and shelter for fish and crustacean species, several of which possess commercially significant value (Johnson *et al.*, 2011). Due to their life history characteristics, specifically pelagic larval dispersal, the geographical range of many fish species are influenced by these currents (Hutchins and Pearce, 1994; Yamano *et al.*, 2011), as the displacement of fish larvae can occur 1000's of kilometres from their home range (Booth *et al.*, 2007).

Tropicalisation, a phenomenon which occurs in temperate/tropical transitional zones, is characterised by the range expansion of tropical species into temperate marine ecosystems (Zarzyczny *et al.*, 2024), in some cases resulting in the retraction and decreased abundance of local temperate species (Nakamura *et al.*, 2013; Wernberg *et al.*, 2013). Temperate South-eastern (SE) Australia is experiencing the effects of tropicalisation in coastal marine habitats, as tropical matter is transported by the East-Australian Current (EAC) from the low latitude ecosystems of the coral sea to higher latitude locations resulting in the annual settlement of tropical fish species (Booth *et al.*, 2007; Figueira and Booth, 2010; Fowler *et al.*, 2018), leading to a diverse range of novel ecological reactions among displaced tropical fish and local temperate species. This marine region has been identified as a global “hot spot” for climate change (Booth *et al.*, 2011), warming at a rate of 0.023°C, which is over six-times greater than the global average (Ridgway, 2009), which currently now supports over 180 species of marine tropical fish (Booth *et al.*, 2018). These displaced tropical fish, referred to as

‘vagrants’, arrive annually as juveniles between January and April and recruit into rocky reef habitats along the New South Wales (NSW) coastline (Booth *et al.*, 2007; Booth *et al.*, 2011), expanding their historical ranges southwards into cooler regions. These settling tropical vagrants come from a variety of families include Pomacentrids, Acanthurids, Chaetodonts and Labrids.

The most prominent recruiting species of vagrant is the Indo-Pacific Sergeant Major, *Abudefduf vaigiensis*, with juveniles arriving in SE Australia in significant number during the Austral summer period, occurring at recruit densities between 10-100 times greater than other tropical vagrants (Booth *et al.*, 2018). Despite their abundance, the arrival of winter-water temperatures results in mass mortality among these juveniles, with rates of mortality as high as 90% (Booth *et al.*, 2007; Booth *et al.*, 2018), as studies have shown that their thermal-minimum threshold for survival is between 17-18°C (Figueira *et al.*, 2009; Figueira *et al.*, 2010). However, winter-water temperatures may not be the singular cause of mortality among the recently arrived *A. vaigiensis* recruits (Feary *et al.*, 2014; Figueira *et al.*, 2019), as they are subject to ecological variables such as temperate food regimes and novel predators. Therefore, the behavioural repertoire of this planktivorous schooling fish needs to exhibit plastic expressions as they alter their behaviour to adapt to a novel ecosystem

A common behaviour among fish species is shoaling, defined as the formation of at least 2 or more fish grouping together (Paijmans *et al.*, 2019), and can be as complex as the formation of 1000+ individuals grouping together in large marine pelagic shoals (Weber *et al.*, 2009). Shoaling can provide a suite of net benefits to individual fish, being attributed to increased vigilance reducing predation risk, foraging efficiency and social learning (Magurran 1990; Pitcher 1991; Lachlan *et al.*, 1998). Despite the multiple benefits, shoaling can incur costs (increased predator detection and competition), leading to a series of trade-offs that arise to balance the uptake of food resources and the risk of predation (Paijmans *et al.*, 2019). Single-species shoals possess the advantage of information transfer and predator confusion tactics (Ward *et al.*, 2011), whereas the formation of mixed-species shoals can reduce within-shoal competition between individuals (partitioning of food resources) and decrease predation by being the least desirable shoal member to predators (Mathis and Chivers,

2003; Alatorre-Ramirez *et al.*, 2017). The schooling tropical vagrant *A. vaigiensis* encounter these trade-offs as they recruit and settle into novel temperate ecosystems simultaneously facing immediate issues such as propagule pressure and arriving at a vulnerable size class (Figueria *et al.*, 2009; Coni *et al.*, 2021). To mitigate the obstacle of accessing food resources and suitable habitat while concurrently avoiding predation from novel predators, vagrant *A. vaigiensis* recruits form shoals with congeners and heterospecific species to ameliorate the pressure of surviving in a foreign habitat (Paijmans *et al.*, 2020; Coni *et al.*, 2022a). Recent studies have shown *A. vaigiensis* juveniles in SE Australian rock reefs exhibit behavioural plasticity in range periphery populations compared to populations closer to their natal range (Coni *et al.*, 2022b), showing increased shelter use and greater flight initiation distances (predator escape ability) at higher latitude locations, however simultaneously exhibiting lower feeding and activity rates compared to those observed at lower latitude locations. In Paijmans *et al* (2020) the authors found that *A. vaigiensis* in mixed-species shoals were more likely to occupy open habitats further away from the reef to access greater foraging patches of plankton and to counter-act predation risk, as the shoals were comprised of more individuals. Fundamentally, accessing foraging patches and avoiding predator-induced mortality are the predominant trade-off these tropical vagrants offset to increase their survivability in a novel temperate ecosystem and expand their ranges to higher latitudes, as they approach the bottleneck of approaching winter-water temperatures.

To enhance their survivability in a foreign ecosystem, shoaling with either conspecifics or heterospecifics is essential, however the formation of single-species or mixed-species shoals will come with their own benefits and costs, and these trade-offs are significantly influenced by latitudinal gradients (Coni *et al.*, 2022 a, Coni *et al.*, 2022 b). Vagrant *A. vaigiensis* juveniles in mixed-species shoals have been shown to attain larger body sizes (Smith *et al.*, 2018), exhibit bold behaviour accessing deeper water foraging regimes (Paijmans *et al.*, 2020) and form single-species subgroups within shoals (Paijmans *et al.*, 2021b) allowing for niche segregation and reduced within-shoal competition with heterospecifics (Kingsbury *et al.*, 2020a). These advantages have been linked to *A. vaigiensis* in mixed-species shoals, however there would be likely

trade-offs resulting in costs to individuals, including increased shoal consciousness to predators, reduced within-shoal information transfer and increased risk taking to access foraging patches. The trade-off of accessing food and avoiding predation may determine the survivability of *A. vaigiensis* that counteracts the limiting factor of winter-water temperatures, permitting successful colonisation of SE temperate rocky reef ecosystems and establish breeding populations in the future. While predation threat has been shown to have little influence on the decision making of *A. vaigiensis* juveniles concerning shoal type selection (single-species vs mixed-species) (Pajmans *et al.*, 2021a), there is lesser-known information regarding how likely they are to forage under predation threat, and do latitudinal gradients exist regarding this critical trade-off? Understanding the behavioural repertoire of these tropical vagrants, and latitudinally influenced behavioural plasticity, is a key determinant to predicting the range expansion potential of *A. vaigiensis* and future implications for ecological interactions in SE Australian marine ecosystems.

In this study we investigate the latitudinal patterns in behavioural trade-offs of *A. vaigiensis* of predator exposure and foraging in single-species shoals and mixed-species shoals to determine their range expansion potential. To determine this, we compare the behavioural traits of *A. vaigiensis* juveniles over an 800km latitudinal gradient at multiple sites identifying single and mixed-species shoals that contain *A. vaigiensis*. We predicted that 1) *A. vaigiensis* will exhibit more effective anti-predator behaviours in shoals composed of conspecifics rather than heterospecifics, including faster return times post predation threat and more risk averse behaviours. These behaviours will also show a latitudinal gradient in increased performance towards their natal range, 2) Foraging efficiency will be greater in shoals composed of conspecifics rather than heterospecifics, including increased bite rate and longer feeding times. Foraging performance will also increase with decreasing latitude and 3) There will be more aggressive interactions in shoals containing conspecifics than heterospecifics (independent of group size) due to resource overlap and enhanced competition within single-species shoals.

3.2 Methods

3.2.1 Study locations

This study was conducted along the SE coastline of Australia spanning across an approximate 800km latitudinal gradient encompassing the viable population range of *A. vaigiensis* at the northern sites and the range peripheries at the southern sites (**Figure 3.1**). Tweed heads (-28.169686° S, 153.552617° E, Little Duranbah beach and Marlo's beach) is within the viable population range of *A. vaigiensis*, this region acting as a control for their shoaling behaviours, given the assumption that their physiological and behavioural performance will be close to optimal. The sites within the middle latitude region are Nambucca (-30.647233° S, 153.010362° E) and South West Rocks (-30.884262° S, 153.038152° E), which are within the subtropical range of *A. vaigiensis*, comprised of small non-breeding adult populations that experience regular annual settlement (Booth *et al.*, 2011). The Sydney region was selected as the high latitude site, representing the range periphery where regular settlement and occasional overwintering of *A. vaigiensis* (Booth *et al.*, 2011) and considered as the potential range extension areas for these tropical vagrants. Sites within the Sydney region included: North Narrabeen lagoon (-33.702537° S, 151.306757° E), Freshwater (-33.781712° S, 151.294371° E), Little Manly (-33.807094° S, 151.285408° E), Long Bay (-33.964945° S, 151.255405° E) and Little Bay (-33.979440° S, 151.251579° E). Most of these sites are proven settlement areas for *A. vaigiensis* recruits (Booth *et al.*, 2007; Booth *et al.*, 2018; Coni *et al.*, 2021), therefore justifying their selection as research locations for this study. Data collection for the Sydney sites was conducted during the Austral summer months (Jan – April) between 2022 – 2024, and northern sites between November – April from 2024 and 2025 to keep temperature regimes relatively consistent. During data collection periods, the mean monthly water temperature for High latitude sites was 25.15°C (Range 22.5 – 26.6°C), Mid latitude sites was 23.86°C (Range 22.4 – 25.6°C) and Low latitude sites was 25.56°C (23.7 – 26.9°C) (**Figure 3.2**).

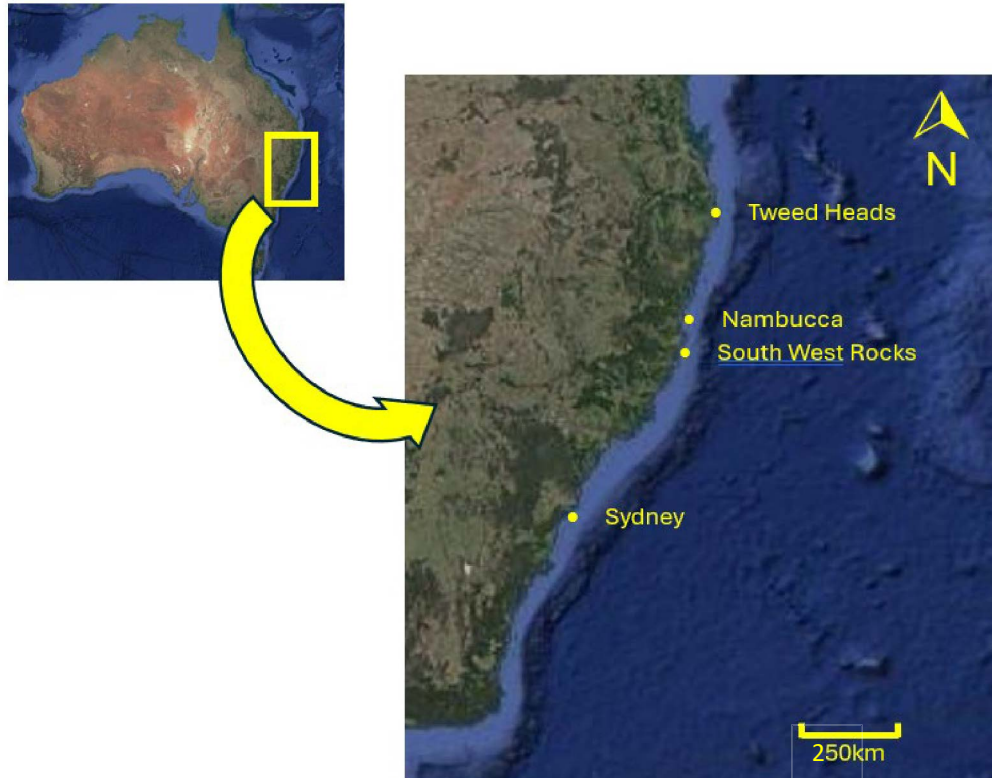


Figure 3.1. Satellite map of the geographical locations of the study sites along the SE coastline of Australia. High latitude data were collected from Sydney, Mid latitude data from Nambucca and South West Rocks and Low latitude data from Tweed Heads. Source: Google Earth © (2025).

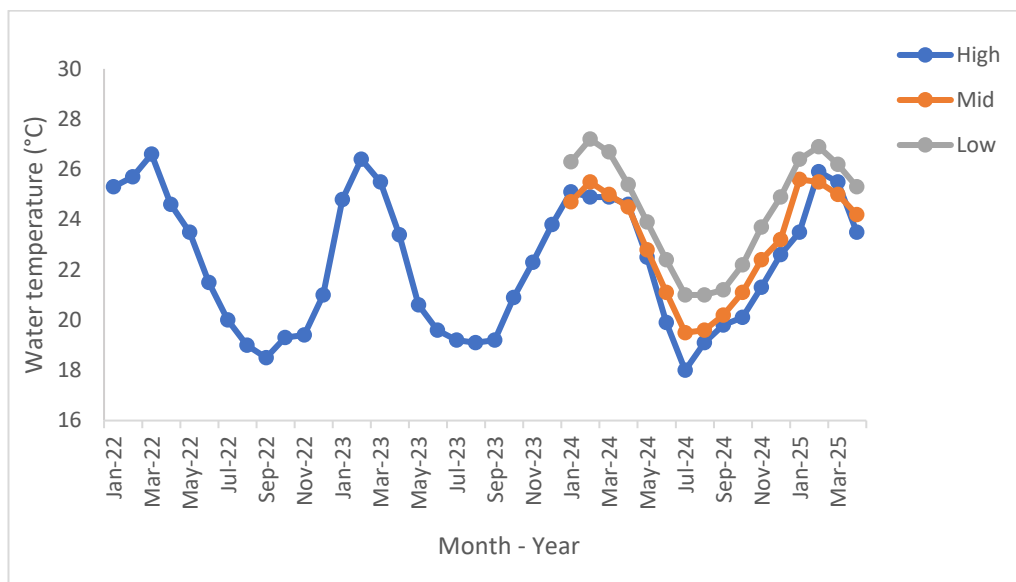


Figure 3.2. Water temperature time series accessed from IMOS (2025) – ‘National Mooring Network Facility - Temperature and salinity time-series’. Blue time series representing mean temperature per month within the High latitude sites across all years in which video data was taken (2022-2024). Orange

time series mean temperature per month within Mid latitude sites across period in which video data was taken (2024-2025). Grey time series mean temperature per month within Low latitude sites across period in which video data was taken (2024-2025)

3.2.2 Experimental design

To investigate the links between shoal composition and latitude on foraging and anti-predatory behaviours, manipulative field experiments were carried out as either feeding experiments and predator threat experiments. At study sites within each latitude region, survey transects were carried out along the shoreline embayment (depth ranging between 0-2m), spanning 2m wide and 250m long. During initial surveys all shoals with *A. vaigiensis* juveniles were identified, recording the following parameters: shoal type (single-species or mixed-species), shoal size (total number of individuals in shoal) and size of *A. vaigiensis* juveniles in shoals classified into 3 categories of small (S, 10-25mm), medium (M, 25-40mm) and large (L, > 40mm). Body sizes were visually assessed using a pre-cut sticks to estimate size classes.

When a suitable shoal was identified, which was comprised of 2 or more individuals shoaling together (containing at least 1 *A. vaigiensis* juvenile in either a single-species or mixed-species shoal) persisting for at least 15 seconds within 5 body lengths of each other (Herbert-Read *et al.*, 2011; Paijmans *et al.*, 2020), they were filmed using a SeaGis camera housing unit (containing 2 GoPro Hero Blacks 7's), placed approximately 2m away from the designated shoal; far enough so as to not disturb or alter their behaviour, and close enough to allow analysis of shoal parameters (Mitchel *et al.*, 2025). An acclimation period of 5min was carried out before filming to allow fish to become acclimatised to diver presence. Once this time has elapsed, 4mins of footage was recorded; 2mins of shoaling behaviours prior to feeding, and 2mins of footage once a food source had been dispensed by a large plastic syringe near the shoal (100ml of dead *Artemia* spp. solution). The predator threat experiments followed similar protocols to those mentioned previously, however included a model predator of a Mourning Cuttlefish (*Sepia Plangon*) attached to a 60cm pole used to probe the target individual. During the predator threat experiments, filming occurred for 2mins after food was dispensed, followed by aggressive exposure to the model predator, and then

was filmed for a 2mins post predator threat. An *S. plangon* model was selected as it has been recorded to predate on *A. vaigiensis* in laboratory conditions (Chapter 4) and does share overlapping habitats with the focal species in SE coastal marine ecosystems. The likelihood of pseudo-replication of individuals within shoals was low due to the spacing of temporal delays between site visitations and the nature of *A. vaigiensis* juveniles, well known for exhibiting strong site fidelity.

The total number of videos taken are as follows for normal behaviour: 32 for single species and 32 for mixed species at the high latitude sites, 29 for single and 26 for mixed-species shoals at the mid latitude sites and 32 for single and 20 for mixed-species shoals at the low latitude sites. For anti-predator videos: 35 for single species and 33 for mixed species at the high latitude sites, 26 for single and 28 for mixed-species shoals at the mid latitude sites and 29 for single and 22 for mixed-species shoals at the low latitude sites.

Across all three latitudinal regions fourteen species in total were observed shoaling in mixed-species shoals with *A. vaigiensis* during video analysis (**Figure 3.3**). There were 6 tropical species: *Abudefduf sexfasciatus*, *Abudefduf bengalensis*, *Abudefduf whitleyi*, *Mecaenichthys immaculatus*, *Acanthurus nigrofasciatus* and *Acanthurus triostegus*, and 8 temperate species recorded: *Microcanthus joyceae*, *Atypichthys strigatus*, *Trachinops taeniatus*, *Scorpius lineolata*, *Girella elevata*, *Parupeneus signatus*, *Cheilodactylus vestitus* and *Gnathanodon speciosus*. For videos analysed from high latitude sites, *A. vaigiensis* individuals comprised a mean of 37% of mixed-species shoals, 25% at mid latitude sites and 54% at low latitude sites. Combining all latitudes, *A. vaigiensis* comprised 38% of mixed-species shoals recorded in this study. The most prominent heterospecific recorded was the temperate *M. joyceae*, comprising a mean of 35% of all shoals recorded.

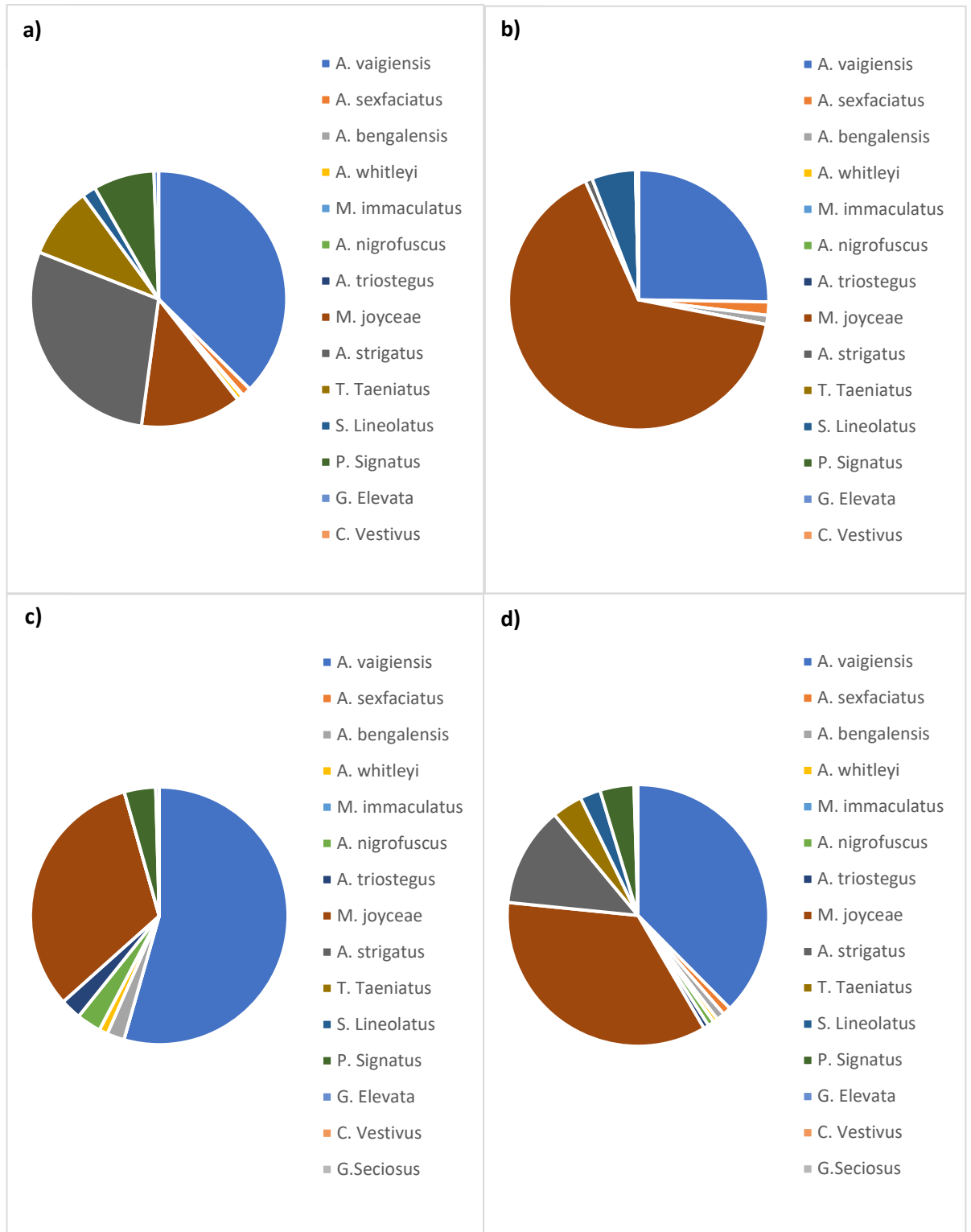


Figure 3.3. Pie chart breakdown of species observed in video recordings in mixed-species shoals containing *A. vaigiensis* across a) High latitude, b) Mid latitude, c) Low latitude and d) mean of all latitudes combined.

3.2.3 Behavioural performance metrics

To assess behavioural traits during feeding and predator threat experiments, we identified several behavioural traits that would be essential in defining how the behavioural repertoire of *A. vaigiensis* varies among shoal type and latitude. To analyse this the following behavioural traits were evaluated during feeding experiments: boldness (proportion of time spent away from rocky reef shelter), activity (proportion of time being active), grazing (proportion of time spent feeding) (Coni *et al.*, 2022a), time to start feeding (s), bite rate per minute and aggressive interactions between shoal members (total number of interactions recorded over video duration). During predator threat experiments the aforementioned behavioural traits were recorded prior to threat stimuli being introduced and post predator scare, however additional metrics were measured; time taken to return from shelter (s), time taken to resume feeding (s).

Behavioural videos were analysed using VLC video player, with one focal *A. vaigeisnis* randomly selected within the shoal and recorded per video. For behavioural analyses, videos were divided into 10 second intervals as per Coni *et al* (2021), which seems to be sufficient in analysing fish behaviour. When the video was paused at each 10 second interval (12 intervals per video; 10, 20, 30 40, 50, 60, 70, 80, 90, 100, 110, 120) the focal fish was identified as being bold or sheltering, active or inactive and feeding or not feeding, which was then converted into a percentage of time of performing the behaviour over the 2min interval. Boldness was defined as being ≥ 5 body lengths away from shelter (Coni *et al.*, 2022a. Activity rate was measured as a fish moving ≥ 5 body lengths between interval windows (Coni *et al.*, 2022a). For grazing state events, fish were considered to be grazing during a 10s interval if they were observed biting while swimming in the water or feeding on substrate. Bite rates were calculated as total bites taken of dispensed food over the 2min duration and converted to bites per minute. Antagonistic interactions were recorded as a total number of occurrences over video duration. Return time post predator stimuli was defined as the fish reemerging from hiding in shelter (so was not visible for a duration of time to seek shelter from predator) and return time to feeding recorded as the duration of time it took for focal fish to take bite post predator scare.

TABLE 3.1 Summary table of behavioural traits measured across the three latitudinal regions (High, Mid, Low) and shoal types (single-species and mixed-species). Brief descriptions provided for each behaviour and how they were analysed when gathering data from video recordings.

Behavioural trait	Definition	Analysis
Boldness	- More than 5 body lengths away from shelter - Fish described as either being bold or sheltering	- Event measured at 10s intervals (Proportion of total time, %)
Activity	- Moving more than 5 body lengths within states intervals (10s) - Fish described as either active or inactive	- Event measured at 10s intervals (Proportion of total time %)
Grazing	- Fish observed feeding on substrate or water column between state intervals	- Event measured between intervals (Proportion of total time %)
Bite rate	- Total number of feeding bites observed during video duration (2min) post food deployment	- Total number of bites (Bites/min)
Aggressive interactions	- Total number of chasing or chased responses observed - Rapid swimming towards or away from shoal member or biting individuals	- Total number of aggressive interactions
Response time to food	- Total time elapsed before first bite was taken post food deployment	- Time elapsed (s)
Return post predator stimuli	- Total time elapsed before fish returned from sheltering after exposure to predator stimuli	- Time elapsed (s)
Resume feeding post	- Total time elapsed for fish to take first bite of food post predator stimuli exposure	- Time elapsed (s)

3.2.4 Statistical Analysis

To measure the effect of latitude (High, Mid, Low) and shoal type (single-species or mixed species) on the shoaling behaviours of *A. vaigiensis*, separate Generalised Linear Models (GLMs) (with latitude x shoal type as an interaction) were run using each of the

behavioural traits as the response variable, latitude (3 levels) and shoal type (2 levels) as fixed factors, and shoal size as a covariate. Body size was not used as a covariate in analysis as all *A. vaigiensis* in targeted shoals were in the juvenile stages therefore body size was relatively consistent between videos. Within each latitude, data attained from sites was pooled together: High latitude data was obtained from Narrabeen, Freshwater, Little Manly, Long Bay and Little Bay, Mid latitude data from Nambucca and South West Rocks Data and Low latitude data from sites within Tweed Heads.

To compare behaviours while feeding vs post predator stimuli, additional GLMs models were run, (as there was a potential for predator manipulation to bias baseline behavioural results) with feeding vs post predator responses included as an additional factor (2 levels) with repeated measure tests to account for pseudo-replication of focal fish. The robust nature of GLMs models offer advantages when dealing with either non-parametric data or when complex relationships between variables are present (Gorosito *et al.*, 2018; de Melo *et al.*, 2022). To determine significant effects of the model on a dependent variable, Type III Wald chi-squared tests were conducted. All statistics were performed using IBM statistics software SPSS v31.

3.3 Results

3.3.1 Boldness

There was a significant interactive effect of latitude x shoal type on the exhibition of bold behaviours for all fish (**Table 3.4**, $p < 0.001$). Pairwise comparisons showed significant differences between all latitudes, with respective mean values of bold behaviour between sites reported as 47.1% (High), 55.2% (Mid) and 79.1% (Low) (**Figure 3.4**, all $p < 0.05$), with boldness decreasing with increasing latitude *A. vaigiensis* juveniles. Despite the significant effect of the covariate shoal size on bold behaviours, indicating that larger shoals were less risk averse ($p < 0.001$).

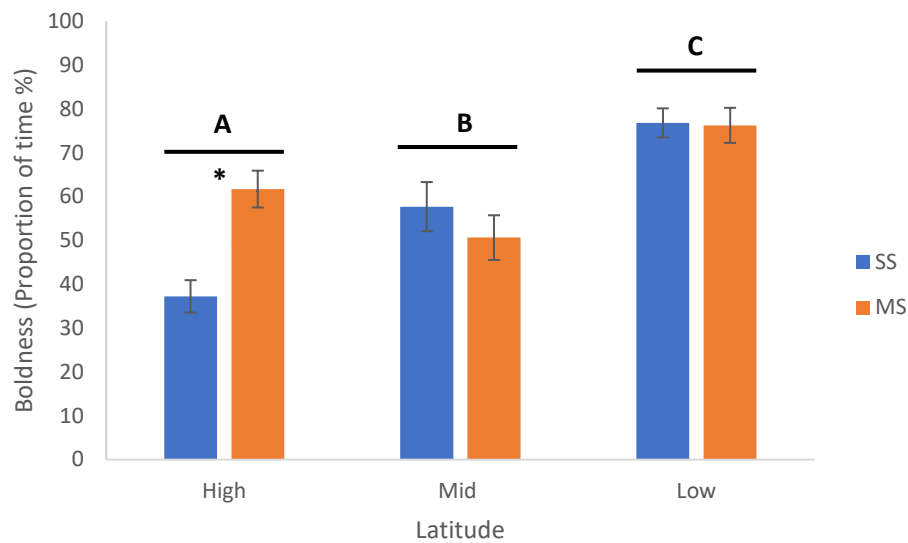


Figure 1.4. Mean ($\pm$ SE) proportion of time *A. vaigiensis* exhibited bold behaviour at latitudes (High: Sydney, Mid: South West Rock and Nambucca and Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

There was a significant effect on the bold behaviour of *A. vaigiensis* when comparing feeding and post predator exposure (**Table 3.3**, $p < 0.001$), with additional effects of latitude and shoal type (**Table 3.3**, all $p < 0.001$). *A. vaigiensis* in mixed-species shoals showed greater boldness when feeding 69% compared to 60% for single-species shoals (**Figure 3.5**, $p < 0.05$). Boldness was significantly reduced by predator exposure across all latitudes as revealed by pairwise comparisons ($p < 0.05$); while feeding *A. vaigiensis* spent 84.8% of their time exhibiting bold behaviour, and post predator stimuli, this value reduced to 44.1% (**Figure 3.5**). Shoal size had a significant effect on the exhibition on bold behaviours ($p < 0.001$).

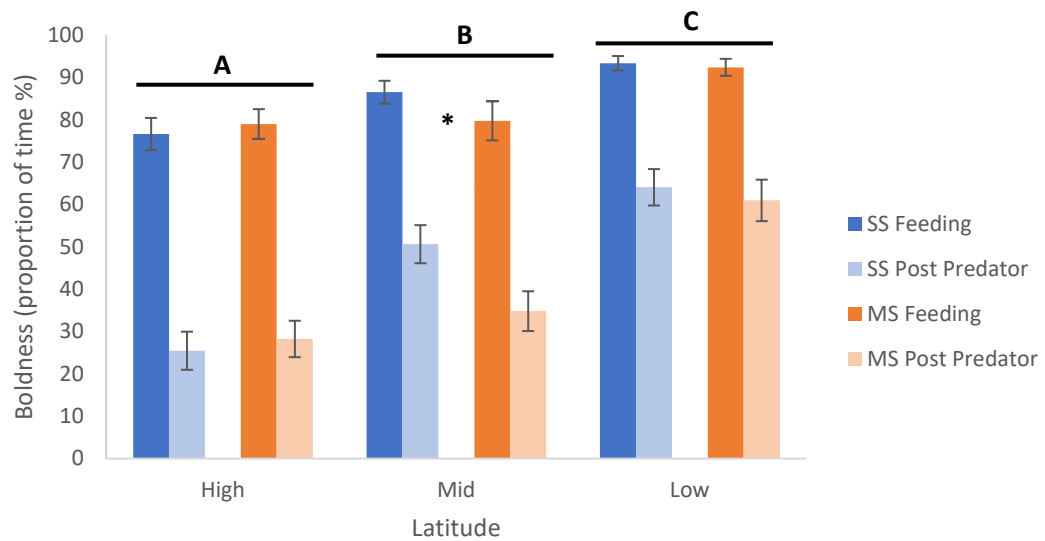


Figure 3.5. Mean ($\pm$ SE) proportion of time *A. vaigiensis* exhibited bold behaviour at 3 sites latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types with 2 levels of treatment; feeding vs predator exposure (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

3.3.2 Activity

Activity rates of *A. vaigiensis* juveniles were significantly affected by latitude (**Table 3.2**, $p < 0.001$), however there were no differences between single-species and mixed-species shoal types ($p = 0.096$). Pairwise comparisons showed significant differences in mean activity rates between latitudes, with *A. vaigiensis* recorded at the lower latitude having a significantly larger mean activity rates (85.9%) than at mid latitude (66.2%) and high latitude (66.6%), showing that closer to their home range *A. vaigiensis* spend more time being active (**Figure 3.6**). As the covariate of shoal size was significant on activity rates ($p = 0.002$), showing relationship between shoal size and activity rates.

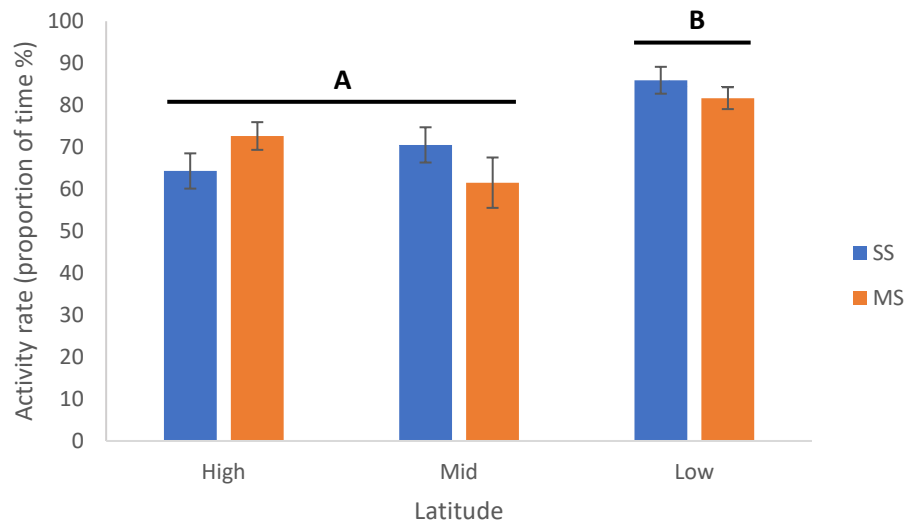


Figure 3.6. Mean ($\pm$ SE) proportion of time *A. vaigiensis* spent being active at 3 sites latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc tests indicated by letter groupings above lines.

Exposure to a predator stimulus while feeding had significant effects on activity rate (**Table 3.3**, $p < 0.001$), with an interactive effect of latitude x shoal type (**Table 3.3**, $p < 0.001$). Pairwise comparisons revealed significant differences between all latitudes and shoal types ($p < 0.05$). The exposure to the predator stimulus significantly reduced activity rates while feeding from 88.8% to 58% activity rate (**Figure 3.7**, $p < 0.001$). There was also a significant effect of the covariate shoal size ($p < 0.001$)

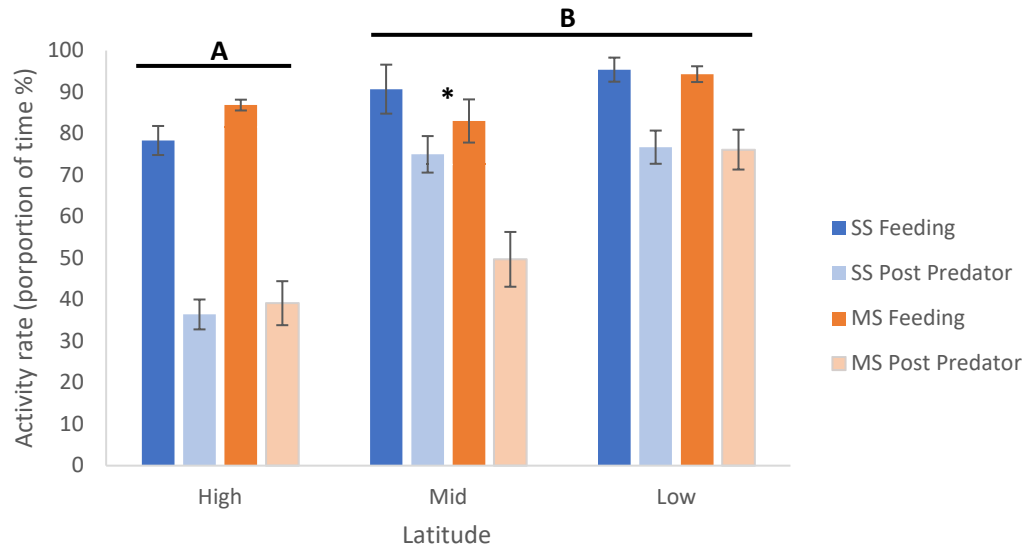


Figure 3.7. Mean ($\pm$ SE) proportion of time *A. vaigiensis* spent being active at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types with 2 levels of treatment; feeding vs predator exposure (SS: Single-species shoals, MS Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

Table 3.2. Generalised linear model results for 6 response variables: Boldness, Activity, Grazing, Bite rate, Feeding start time and Aggressive interactions. The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values.

Factor	<u>Boldness</u>			<u>Activity</u>			<u>Grazing</u>		
	df	X ²	p	df	X ²	p	df	X ²	p
Latitude	2	56.062	< 0.001	2	28.028	< 0.001	2	26.560	< 0.001
Shoal type	1	0.002	0.968	1	2.763	0.096	1	3.098	0.78
Latitude x shoal type	2	14.246	< 0.001	2	4.035	1.33	2	25.148	< 0.001
Shoal size	1	13.68	< 0.001	1	9.634	0.002	1	3.863	0.049
Error	164			164			165		
Factor	<u>Bite rate</u>			<u>Feeding start time</u>			<u>Aggressive interactions</u>		
	df	X ²	p	df	X ²	p	df	X ²	p
Latitude	2	156.378	< 0.001	2	15.914	< 0.001	2	4.934	0.111
Shoal type	1	0.287	0.592	1	3.331	0.068	1	9.210	0.002
Latitude x shoal type	2	1.244	0.537	2	9.581	0.008	2	1.980	0.372
Shoal size	1	11.980	< 0.001	1	11.629	< 0.001	1	1.587	0.208
Error	164			164			164		

3.3.3 Feeding start times

The time it took for *A. vaigiensis* juveniles to begin feeding after food deployment showed a significant interaction between sites and shoal types (**Table 3.2**, $p < 0.01$). Pairwise comparisons showed that feeding start times at the high latitude are significantly greater than both mid and low latitudes ($p < 0.01$). Overall mean feeding start times for *A. vaigiensis* juveniles showed an increase with latitude (**Figure 3.8**, as they were longer delays in the time it took to begin feeding at the high latitude (18.4s) and quicker responses to food deployment at the low latitude (12s). The covariate shoal size had a significant effect on feeding start times ($p < 0.01$).

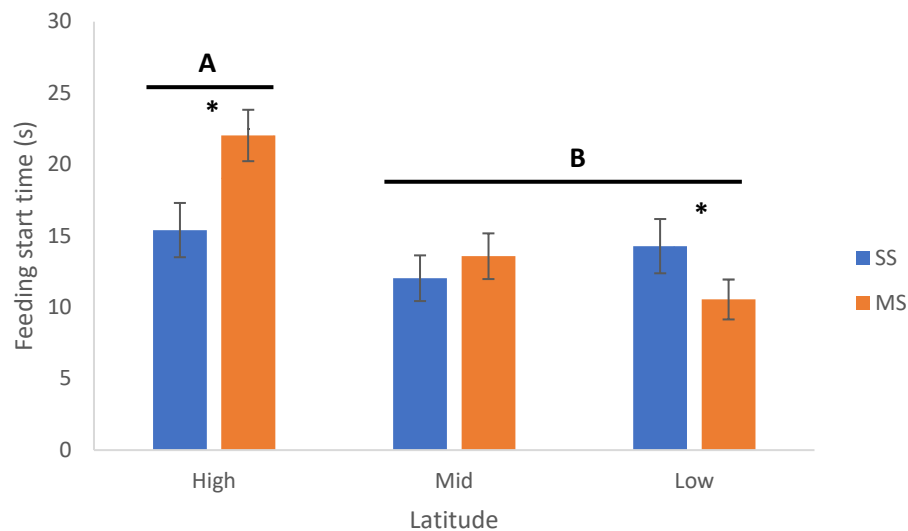


Figure 3.8. Mean ($\pm$ SE) feeding start of *A. vaigiensis* post food deployment at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

3.3.4 Grazing

The proportion of time spent grazing for *A. vaigiensis* juveniles showed a significant interaction between latitudes and shoal types (**Table 3.2**, $p < 0.001$). Pairwise comparisons showed that time spent grazing was highest at the lowest latitude (**Figure 3.9**, $p < 0.001$), recorded at (51.8%), but the other two latitudes did not significantly differ, with time spent grazing recorded at the mid latitude (32.3%) and high latitude

(37.1%) (**Figure 3.9**, $p = 0.201$). Shoal size had a significant effect on the variable of proportion of time spent grazing ($p < 0.05$).

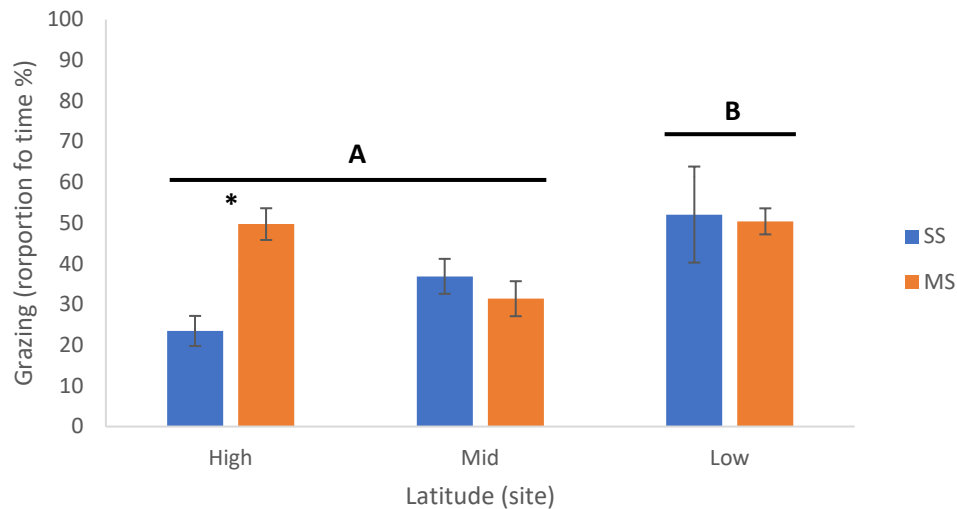


Figure 3.9. Mean ($\pm$ SE) proportion of time *A. vaigiensis* spent grazing at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

The exposure of a predator stimulus to shoals of *A. vaigiensis* while feeding had significant effects on grazing time (**Table 3.3**, $p < 0.001$). In addition to this the factors of latitude and shoal type (**Table 3.10**, all $p < 0.001$) had significant effects on the time *A. vaigiensis* individuals spent grazing during food deployment and after predator exposure. Pairwise comparisons showed that there was a significant reduction in grazing time post predator exposure ($p < 0.05$) when compared to grazing during feeding events; being 80.1% compared to 37.6% respectively (**Figure 3.10**). Shoal size had a significant effect on grazing time ($p < 0.001$).

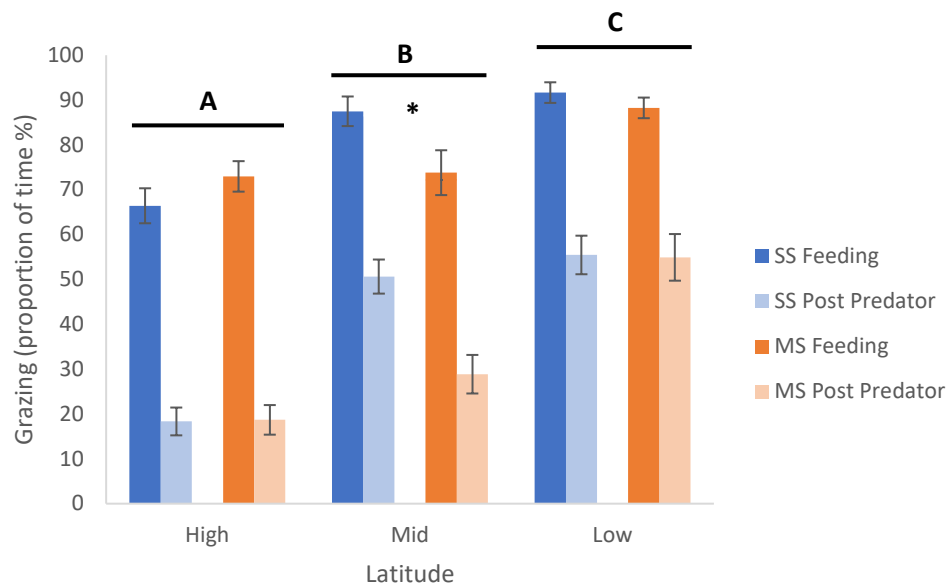


Figure 3.10. Mean ($\pm$ SE) proportion of time *A. vaigiensis* spent grazing at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types with 2 levels of treatment; feeding vs predator exposure (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

3.3.5 Bite rate

The bite rate of *A. vaigiensis* juveniles was significantly affected by latitude (**Table 3.2**, $p < 0.001$), however no significant effect was detected between single-species and mixed species shoals containing *A. vaigiensis* ($p > 0.05$). Pairwise comparisons showed significant differences between all latitudes ($p < 0.01$), with the overall trend indicating that bite rate increased as latitude decreased, being 22.2 bites.min⁻¹ at high latitude, 28.8 bites.min⁻¹ at mid latitude and 52 bites.min⁻¹ at low latitude (**Figure 3.11**). Covariate of shoal size had a significant effect on the bites taken per minute of *A. vaigiensis* ($p < 0.001$).

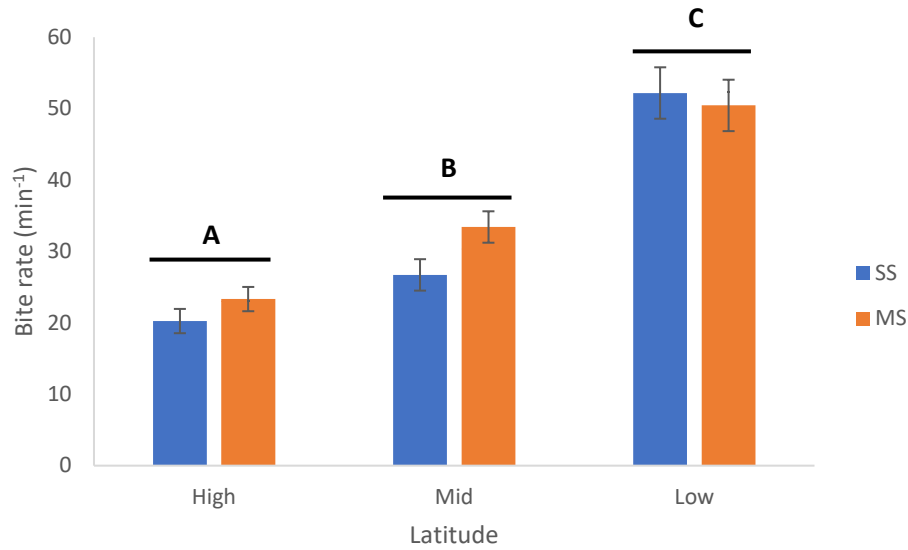


Figure 3.11. Mean ($\pm$ SE) bite rate per minute of *A. vaigiensis* at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines.

The exposure of a predator stimulus to shoals of *A. vaigiensis* while feeding had significant effects on the bite rate of *A. vaigiensis* by latitude ($p < 0.001$), with additional effects of latitude ($p < 0.001$). There was however no significant effect detected between single-species and mixed species shoals containing *A. vaigiensis* ($p > 0.05$). Pairwise comparison showed the effect of the predator treatment significantly reduced bite rates across all latitudes, with mean bite rate recorded at 39 bites.min⁻¹ decreasing by approximately 60% after predator exposure, recorded at 15.7 bites.min⁻¹ (**Figure 3.12**). Covariate of shoal size had a significant effect on the bites taken per minute of *A. vaigiensis* ($p < 0.001$).

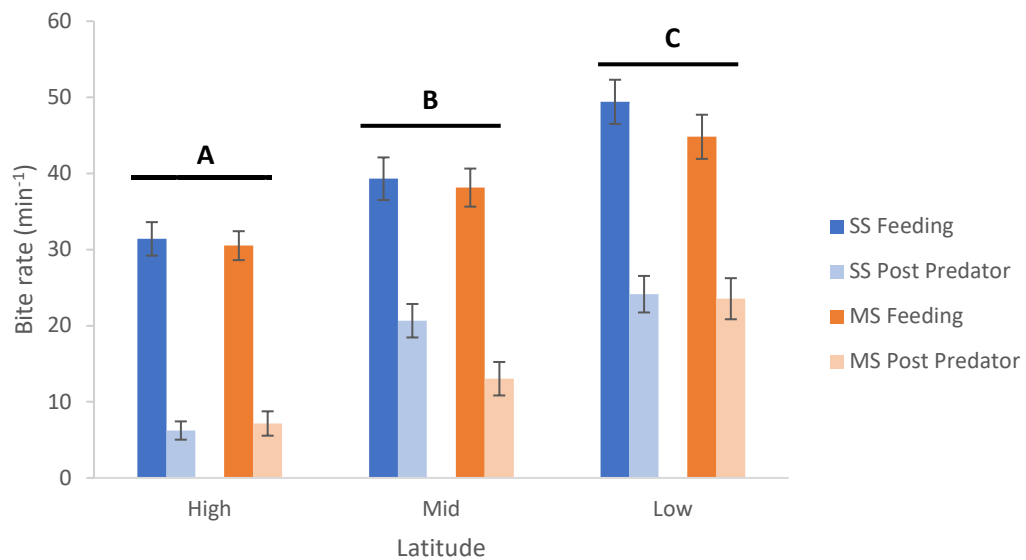


Figure 3.12. Mean ($\pm$ SE) bite rate per minute of *A. vaigiensis* at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types with 2 levels of treatment; feeding vs predator exposure (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines.

3.3.6 Fish return times post predator stimuli

Post exposure to a predator stimulus, *A. vaigiensis* juveniles showed significant differences in return times after sheltering between latitudes (**Table 3.3**, $p < 0.001$) and shoal types (**Table 3.3**, $p < 0.05$) Pairwise comparisons showed significant differences between all latitudes ($p < 0.001$) apart from mid and low latitudes ($p > 0.05$). The data showed that fish return time decreased at lower latitude locations, being 55.7s at high latitude, 20.3s at mid latitude and 12.6s at low latitude (**Figure 3.13**). Single-species shoals overall showed delayed return times compared to mixed-species shoals, with pairwise comparisons revealing significant differences between the two shoal types ($p < 0.05$); *A. vaigiensis* in single-species shoals had a mean return time post predator stimulus of 22.3s and in mixed-species shoals mean return times were 26.7s (**Figure 3.13**). Shoal size was shown to have a significant effect on return times ($p < 0.005$).

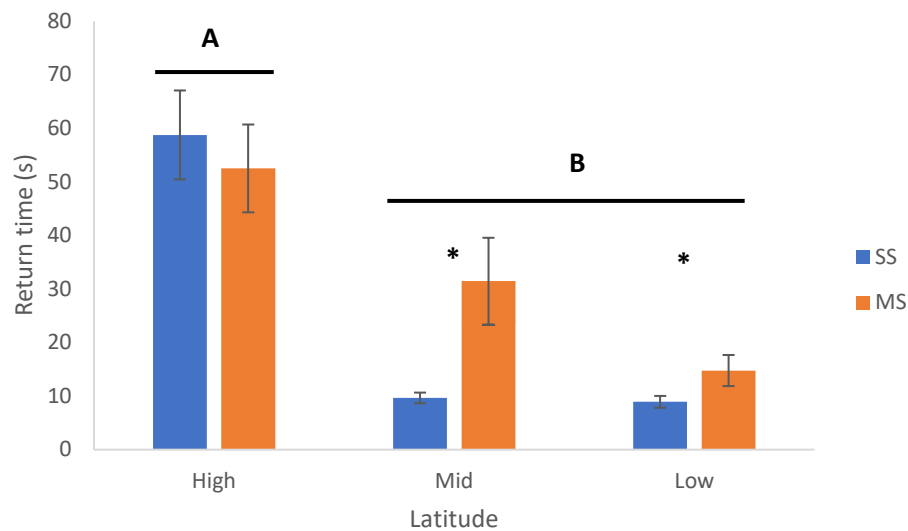


Figure 3.13. Mean ($\pm$ SE) return time of *A. vaigiensis* post exposure to a predator stimulus at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). Significant differences between latitudes ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

3.3.7 Aggressive interactions

Aggressive interactions within shoals containing *A. vaigiensis* were significantly different between single-species shoals and mixed-species shoals (**Table 3.2**, $p < 0.05$). For single-species shoals, mean aggressive interactions per shoal were recorded 0.1 interactions per shoal, and for mixed-species shoal they were significantly higher at 0.41 interactions per shoal (**Figure 3.14**). Overall interactions per shoal showed a decrease in aggression with decreasing latitude, being recorded at 0.37 at high latitude 0.29 at mid latitude and 0.12 interactions per shoal at low latitude (**Figure 3.14**).

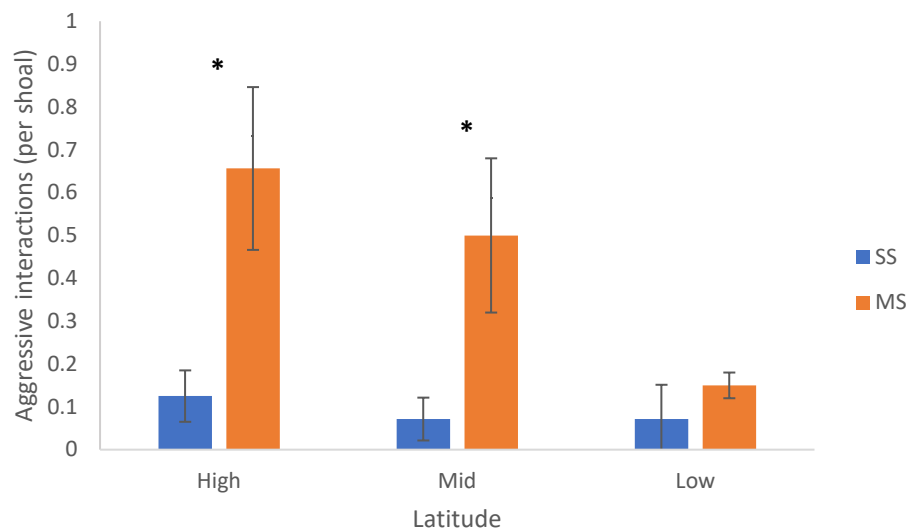


Figure 3.14. Mean ($\pm$ SE) of antagonistic interactions per shoal at 3 latitudes (High: Sydney, Mid: South West Rock and Nambucca, Low: Tweed Heads) and 2 shoal types (SS: Single-species shoals, MS: Mixed-species shoals). * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

Table 3.3. Generalised linear model results for 4 response variables measured during feeding vs predation testing: Boldness, Activity, Grazing and return time after predator exposure. The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values.

Factor	<u>Boldness</u>			<u>Activity</u>			<u>Grazing</u>			<u>Return time</u>		
	df	X ²	p	df	X ²	p	df	X ²	p	df	X ²	p
Latitude	2	81.50	<.001	2	63.07	<.001	2	123.00	<.001	2	56.49	<.001
Shoal	1	14.00	<.001	1	7.88	<.01	1	12.72	<.001	1	6.29	0.12
Feed vs Pred	1	340	<.001	1	138.44	<.001	1	403.7	<.001	-	-	-
Latitude x shoal	2	5.09	<.001	2	11.74	0.003	2	17.17	<.001	2	4.53	0.104
Shoal size	1	21.89	<.001	1	11.85	<.001	1	18.52	<.001	1	8.75	0.003
Error	335			338			335			166		

3.4 Discussion

Animal grouping behaviour is regulated by ecological parameters, forcing them to make decisions that lead to behavioural trade-offs, governing survival probability (Colchen *et al.*, 2017). Here we show that environmental and ecological factors such as geographical range, foraging requirements and predation risk drive complex social interactions within single-species and mixed species shoals of a tropical vagrant fish species *A. vaigiensis*. Consistent with our hypothesis, the exhibition of risk averse

behaviours such as reduced boldness and return times from sheltering post predator threat were more prominent at higher latitude locations, representing the southern range periphery of *A. vaigiensis* populations. Feeding behaviours including grazing time, bite rate and feeding start time were shown to be significantly affected by latitudinal gradients, aligning with our hypothesis, providing evidence of behavioural plasticity in range-edge populations of *A. vaigiensis*. Feeding behaviour results showed that at lower latitude locations such as Tweed heads, which is within the natal range of *A. vaigiensis*, bite rate was approximately 250% higher than it was in range periphery populations such as Sydney. Contrary to our hypothesis there was little confirmation to suggest that shoal type, single-species vs mixed-species, drives major differences in feeding behaviours and regimes, there was some evidence in regards to overall delayed feeding start times in mixed-species shoals, as well as reduced grazing times post predator stimulus exposure, to suggest some degree information transfer among mixed-shoals, and possibly trophic niche segregation to reduce competition within shoals while feeding (Kingsbury *et al.*, 2020). Regarding aggressive and competitive interactions within shoals, we were forced to reject our null hypothesis, as our results indicated that *A. vaigiensis* in mixed-species shoals experience significantly higher rates of aggressive interactions with shoal members. However, there was some evidence to suggest that aggressive interactions are reduced in lower latitude locations compared to the higher latitude range periphery populations of *A. vaigiensis*. Overall, these results build on the empirical evidence of behavioural adaptations and plasticity being employed by vagrant tropical fish populations to increase survivability in novel temperate ecosystems, and that location within geographical range, as well as shoal type, catalyse a series of behavioural trade-offs that drive decision making within shoals regarding significant ecological factors such as foraging and reducing predation risk. While previous related studies have focused on the behavioural repertoire of *A. vaigiensis* in range periphery habitats and how environmental factors regulate their colonisation of temperate habitats (Figueira *et al.*, 2009; Paijmans *et al.*, 2020; Coni *et al.*, 2021), here we focused on how specific behavioural trade-offs, using manipulative field experiments, predominantly foraging efficiency and predation risk, contribute to the functionality of tropical vagrant fish in novel temperate ecosystems. The exhibition

of behavioural plasticity, and the formation of large single-species or mixed-species shoals, essentially “hedging their bets”, will mitigate the negative effects of winter-water temperatures and novel predators capitalising on reduced physiological performance. Learning from local species and forming structured and phenotypically similar conspecific shoals will ameliorate the limiting factors that exist in a novel temperate ecosystem.

The exhibition of risk aversive behaviours is critical in determining the survival of grouping fish species (Gil *et al.*, 2017) by reducing predator interactions and exposure to environmental conditions, conversely however, may reduce resource acquisition consequentially reducing growth rates, extending the time fish spend at vulnerable size classes (Sogard, 1997; Lyon *et al.*, 2008). For recently recruited novel tropical fish settling into a temperate ecosystem, *A. vaigiensis* encounter the critical trade-off of acquiring food resources while simultaneously avoiding predation (Hulthén *et al.*, 2017; Coni *et al.*, 2022b). Using boldness as a proxy for risk taking and risk aversive behaviour, we showed that latitude has an influential effect on the expression of bold behaviours of *A. vaigiensis*, specifically fish were less likely to take risks when in poleward range limits, with over a 50% reduction in bold behaviour being recorded at a high latitude site compared to a low latitude location. We also found an interactive effect of latitude and shoal type, indicating that there was a difference in fish boldness between single-species and mixed-species at certain sites, most likely driven by the 50% difference recorded between *A. vaigiensis* juveniles recorded at our high latitude location and minimal differences at the lower latitudes. Results obtained while exposing fish to a model predator while feeding showed significant differences between shoal types and across sites, with boldness post predator threat showing a 2-fold decrease high latitude compared to low latitude. We also found that *A. vaigiensis* in mixed shoals showed longer return times after a simulated predator threat, following the same trend of reduced bold behaviour in low to high latitudinal trends. In Coni *et al* (2022b) the authors found that risk aversive behaviours of expatriated vagrant tropical fish (measured as flight-initiation distance) were higher at high latitude locations further away from their natal range, concluding that this was an adaptive trait to negate the effects of reduced food intake while existing in a lower-

temperature regime habitat. In addition to this, Beck *et al* (2016) showed that *A. vaigiensis* occupying temperate reefs spent more time sheltering and made shorter foraging excursions when compared to tropical populations. Our results concur with these studies, as cooler water regimes at high latitude sites result in reduced physiological performance of the warm-adapted *A. vaigiensis*, limiting energy uptake via food resources affected swimming speeds and growth rates, increasing their vulnerability to local predators (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019; Figueira *et al.*, 2019). Such limiting factors may promote the employment of risk aversion and *A. vaigiensis* spending more time closer to shelter, as energy is redirected into fish remaining more vigilant in a temperate novel ecosystem. Results also suggest that there are some benefits that arise from *A. vaigiensis* settling into or forming mixed-species shoals regarding reduced risk taking, which in Paijmans *et al* (2020) it was insinuated that such behaviours may be attributed to greater heterospecific vigilance (Semeniuk and Dill, 2006) or predator preference for more desirable shoal members (Mathis and Chivers, 2003). Such differences seen in longer return times to exposed shoaling seen in our study by *A. vaigiensis* in mixed shoals may also be attributed to social learning cues and information transfer between these tropical vagrants and local heterospecifics (Ward *et al.*, 2005; Ward and Webster, 2016).

The acquisition of foraging resources strongly influences the physiological and behavioural performance of fish species (Olson *et al.*, 2015; Paijmans *et al.*, 2019), and in populations of juvenile vagrant tropical fish subject to novel ecological conditions there is an essential need to search for foraging patches. Adaptive foraging behaviours and regimes are required when fish are dependent on sub-optimal foraging opportunities, which has been recorded in range-periphery populations of tropical vagrant fish species (Miranda *et al.* 2018; Kingsbury *et al.*, 2020b). Here we showed that a latitudinal gradient exists regarding the foraging performance of shoaling *A. vaigiensis*, demonstrating a positive relationship with increased foraging efficiency closer to natal range populations. Higher grazing times, increased bite rate and reduced feeding start times were strongly related to latitude; when comparing the low latitude and high latitude sites, we observed a 30% increase in grazing time, 230% in bite rate and 30% reduction in feeding start times. These were expected results, as *A.*

vaigiensis showed decreased prey intake in novel temperate latitudes (Kingsbury *et al.*, 2020b; Mitchel *et al.*, 2025). As temperatures increase fish metabolism accelerates (Donelson *et al.*, 2010), manifesting into increasing food requirements, which has been recorded in *A. vaigiensis* juveniles reared at high temperatures (Djurichkovic *et al.*, 2019). The warmer annual temperature regimes of the low latitude site used in this study (**Figure 3.2**) may attribute to the increase in foraging performance we observed in this study, as well as preferential food availability (Sih & Christesen, 2001) and regional differences in food regimes and consequential diets (Kingsbury *et al.*, 2020a). When comparing grazing behaviour before and after a simulated predator scare, shoal type was shown to have a significant effect, as well as a recorded interactive effect between site and shoal type regarding feeding start times of *A. vaigiensis*. The formation of mixed-species shoals may facilitate trophic niche segregation, resulting in diet partitioning and different feeding regimes within shoals (Krause, 1993; Kingsbury *et al.*, 2020a). Such behavioural adaptations may permit the long-term settlement of *A. vaigiensis* populations in temperate marine ecosystems, as the expression of behavioural plasticity and co-shoaling with local temperate species, providing information of novel food resources and predator alarm cues (Hoare & Krause 2003).

Aggregations of fish often result in density-dependent intraspecific competition (Pitcher, 1993; Robinson, 1994), and as individuals forage, they simultaneously compete to maximise food uptake. A high density of fish can create physical barriers for shoal members to forage and consequentially increase levels of aggression which has been observed in species of coral reef damselfish (Holbrook and Schmidt, 2002). In this study we found elevated rates of aggressive interactions among shoal members in mixed-species shoals compared to single-species shoals, with means interactions increasing at the high latitude region. For a tropical vagrant such as *A. vaigiensis* recruiting to cold-edge habitats, local temperate fish can indirectly inhibit their foraging efficiency by physically limiting access to food (Coni *et al.*, 2021). Some species of fish join shoals not primarily for anti-predatory benefits but show a preference to shoal with fish that exhibit low competitive ability in the form of aggressiveness, size or feeding rates (Metcalf and Thomson, 1995). While this is not confirmed in our study, *A. vaigiensis* are commonly seen shoaling with temperate

heterospecifics in range periphery locations such as the stripey (*Microcanthus joyceae*) and the mado (*Atypichthys strigatus*). Such shoaling arrangements may be driven by a lack of trophic niche overlap (Kingsbury *et al.*, 2020a) which in turn should reduce competition and aggressive interactions, however, joining these often-larger shoals may create physical feeding barriers for *A. vaigiensis*, initiating aggression among shoal members.

It is important to note during this study that the heterospecific presence within mixed-species shoals recorded was difficult to control, and they were not consistent between latitudes. The most common species observed shoaling with *A. vaigiensis* at high latitude sites was *A. strigatus* (mean 29%: *A. vaigiensis* 37%), at mid latitude sites was *M. joyceae* (mean 65%: *A. vaigiensis* 25%) and low latitude sites was *M. joyceae* (32%: *A. vaigiensis* 54%) (**Figure 3.3**). While many of the heterospecifics recorded exhibit similar patterns of behaviour that our target species does during juvenile stages, such as site attachment (Mitchell *et al.*, 2025), ecological niche overlaps do exist, and vary, among latitudes. For example, the higher proportion of *A. strigatus* at the high latitude sites may have altered the behavioural performance metrics of *A. vaigiensis*, as these two species have been shown to have overlapping ecological niches (Kingsbury *et al.*, 2020a Coni *et al.*, 2022a). Overall, the most commonly occurring heterospecific within mixed shoals across all latitudes was *M. joyceae* (mean shoal composition of 35%), and this species has been shown to have a rate of niche overlap with *A. vaigiensis* of less than 60%, representing a negligible behavioural shift among species (Guzzo *et al.*, 2013) and therefore likely having non-significant effects on the behavioural performance. The existence of trophic niche overlaps can drive competition within shoals (Coni *et al.*, 2022a), however *A. vaigiensis*, and many of the heterospecific species found shoaling with them (notably *M. joyceae*) in mixed-species shoals, exhibit relatively broad niches and dietary plasticity, indicating trophic generalism (Kingsbury *et al.*, 2020a) and are likely to co-exist without significant impacts on physiology and behaviour.

In this study we demonstrate clear evidence of latitudinal differences that exist regarding behavioural performance of *A. vaigiensis* and identify multiple key behavioural trade-offs that occur when they shoal with either conspecifics or

heterospecifics. Behavioural plasticity was apparent at higher latitude locations, which most likely is employed by *A. vaigiensis* to counteract the effects of cooler water regimes they are subject to at range periphery locations. Winter-water temperatures remain the predominant limiting factor for the establishment of long-term viable populations for range-expanding tropical fishes. However, the predicted increasing rates of global sea-surface temperatures may significantly reduce cold-stress related mortality, with winter-water temperatures becoming increasingly survivable by the year 2080 (Figueira and Booth, 2010). The key benefits attained by shoaling for recently recruited *A. vaigiensis* juveniles will undoubtedly increase the chances of survival in a novel temperate ecosystem, as a flexible behavioural repertoire will enhance foraging efficiency and predator avoidance. Future research directions investigating the role of shoaling behaviours in tropical range expanding fish species should direct focus to the influence of local predators and how they regulate behavioural repertoires during diurnal and nocturnal cycles.

Chapter 4: To eat or be eaten? The effects of water temperature and predation on behavioural and physiological metrics in single and mixed-species shoals of a tropical vagrant fish

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Abstract

Climate change is the major driving force in the redistribution and range expansion of tropical marine species into temperate habitats, catalysing the process known as 'tropicalisation'. Several temperate marine ecosystems globally have become the sites of the seasonal displacement of coral reef fishes from natal lower latitude tropical ecosystems, facilitated by the action of poleward-flowing boundary currents. This results in the arrival of low-density populations of juvenile tropical fish larvae (termed 'vagrant' fishes), which are subject to a series of extrinsic and intrinsic factors that restrict successful establishment, namely that of over-winter survival due to exposure to cold water. Previous research has shown that the vagrant tropical fish *Abudefduf vaigiensis* benefited from shoaling with temperate species through the mitigation of predation risk, allowing increased foraging opportunities and even attaining larger body sizes than when shoaling with conspecifics. This study aims to compare how ambient water temperature and different shoaling compositions of conspecifics and heterospecifics (local temperate fish species *Microcanthus joyceae*) influence the physiology and behaviour of *A. vaigiensis* in a laboratory experiment and how this may initiate behavioural trade-offs within shoals that impact their fitness and survival probability in a novel temperate ecosystem. Higher water temperatures correlate with an increase in performance of *A. vaigiensis*, resulting in greater growth rates, foraging rates, boldness and reduced chances of predation in mixed shoals with *M. joyceae*. Comparing the effects of single and mixed species shoals showed variable results, indicating that there are positive and negative trade-offs that occur. Overall, mixed shoal ratios of 100%, 75% and 50% *A. vaigiensis* showed improved performance metrics compared to 25% *A. vaigiensis*, indicating that higher presence of conspecifics

is correlated to enhanced fitness. Results also indicate that novel predators can induce the expression of risk aversive behaviours, as we observed reduced boldness when exposed to a live temperate predator *Sepia plangon*. Shoaling strategies may be a predominant factor in the range expansion potential of tropical fishes, and therefore it is imperative that we better understand how they are employed and what factors exert the greatest influence over them.

4.1 Introduction

As the climatic conditions of our world drastically change, largely due to increasing temperature regimes and extreme weather events associated with long-term anthropogenic activities (Thuiller, 2007; McNutt, 2013; Stott, 2016), the biogeographical landscape of species distributions is shifting (Parmesan, 2006; Poloczanska *et al.*, 2013; Pecl *et al.*, 2017). The expansion and redistribution in the range limits of species has been observed globally in a diverse range of biota (Moritz *et al.*, 2008; Johnson *et al.*, 2011; Pinsky and Fogarty, 2012), with marine ecosystems experiencing greater rates of change compared to terrestrial ecosystems as oceans act as heat-sinks for the global climate, and have greater connectivity between habitats due to the action of currents (Burrows *et al.*, 2011). Of particular interest are poleward-flowing boundary currents, which enable the transportation of warm water and organisms' native to tropical regions to higher latitude subtropical and temperate habitats (Ridgway, 2007; Seager *et al.*, 2016).

As poleward-flowing boundary currents facilitate the arrival of new species into a novel habitat, negative ecological impacts in the recipient communities may result (Wernberg *et al.*, 2013). For example, the Kuroshio Current off the coast of Japan is a poleward flowing boundary current that flows south-to-north, transferring bodies of warm water from higher-latitude subtropical regions of Ryukyu Islands (26°N) as far north as temperate coastal waters of Kochi 33°N (Hirata *et al.*, 2011; Yamano *et al.*, 2011; Soeparno *et al.*, 2013). Hirata *et al.* (2011) found that 136 tropical species were inhabiting temperate regions due to the actions of the Kuroshio current. This in turn has catalysed a tropicalisation of certain temperate habitats in this region, a phase shift of the temperate community as it undergoes a transformation into that of a tropical marine ecosystem. Nakaruma *et al.* (2013) reported that herbivorous tropical fish that were transported by the poleward current in response to increasing water temperature had begun to over-graze on the temperate kelp habitats, leading to the settlement of reef building corals in the region, which in this case of tropicalisation was irreversible. Similarly in Western Australia, as a result of the Leeuwin Current creating warming hotspots in higher latitude regions, the range expansion of tropical fish species and consequently the overgrazing of macroalgae dominant habitats has shifted

the state of the ecosystem (Verges *et al.*, 2014). On the eastern coastline of Australia, the East Australian Current (EAC) is responsible for the relocation of larvae into higher latitude regions (Ridgway, 2007), facilitating the range expansion of species into novel habitats. For example, the long-spined sea urchin (*Centrostephanus rodgersii*), larvae were being transported from locations in NSW coastal waters ($\approx 32^{\circ}\text{S}$) to the eastern coastline of Tasmania ($\approx 41^{\circ}\text{S}$) (Johnson *et al.*, 2004; Johnson *et al.*, 2005; Johnson *et al.*, 2011). In a report published by Johnson *et al.* (2005), the grazing of this invasive species on kelp habitats has triggered the formation of unproductive urchin barrens, forming a negative relationship with abundance of the commercially fished abalone (*Haliotis rubra*) and southern rock lobster (*Jasus edwardsii*), significantly impacting the quantity, yield and profit margins of these fisheries.

The EAC is driving community shifts in marine ecosystems along the eastern coastline of Australia, most notably the south-eastern (SE) region which has been labelled a global 'hot-spot' for climate change, with the rate of increasing sea-surface temperatures ($+0.023^{\circ}\text{C yr}^{-1}$) nearly 6 times greater than the global average (Lough *et al.*, 2009). The displacement and settlement of tropical fish larvae from the Great Barrier Reef is an annual occurrence in the south-eastern regions of Australia during the late summer-early autumnal months (Booth *et al.*, 2007), and the rise in species richness has occurred at an exponential rate; from 47 tropical fish species in 2007 identified in surveys (Booth *et al.*, 2007) to over 150 by 2018 (Booth *et al.*, 2018). The onset of winter-water temperatures results in high rates of mortality among these tropical vagrant fish, potentially as high as $\geq 90\%$ (Booth *et al.*, 2007; Booth *et al.*, 2018), however long-term data sets have indicated that the water temperatures in winter off the coastline of SE Australia may exceed the thermal-minimum of these tropical vagrants by 2080 (Figueira and Booth, 2010).

At the forefront of this "tropical invasion" are several species belonging to the damselfish (Pomacentridae) family, and the most commonly occurring and recorded being the sergeant major *Abudefduf vaigiensis*. These fish are generally territorial plankton feeders, exhibiting a high degree of site-fidelity, and have well-documented record of establishing population well outside their natal range, even as far south as Merimbula 37°S (Booth *et al.*, 2007). While *A. vaigiensis* tend to display the

characteristics of an ecological generalist, being able to feed across several functional groups (Kingsbury *et al.*, 2020a), as well as exhibiting phenotypic plasticity while adapting to cooler water temperatures (Djurichkovic *et al.*, 2019), they are exposed to a series of extrinsic and intrinsic processes that determine their settlement and survivorship in novel temperate ecosystems (Feary *et al.*, 2014). For a warm-affiliated fish species, water temperature will exert the greatest influence over their survival during settlement on their range-periphery, however behavioural traits also play a crucial role in their survivorship (Coni *et al.*, 2022b). Behavioural interactions with conspecifics and novel heterospecifics will regulate the rate of competition for resources and predation-induced mortality for newly settled *A. vaigiensis* juveniles (Paijmans *et al.*, 2020; Mitchel *et al.*, 2025), all the while arriving in an unfamiliar habitat at a vulnerable size-class. By forming aggregations with other individuals, termed shoaling, they can enhance their ability to find foraging patches and reduce the risk of predation (Paijmans *et al.*, 2019).

Shoaling is a common grouping behaviour among teleost species, varying from small monospecific shoals in freshwater habitats to highly complex heterospecific pelagic shoals in marine ecosystems (Barber and Wright, 2001; Rieucan *et al.*, 2015). A large body of literature has identified and quantified the role that shoaling plays in the regulation and survival of fish populations, with a common consensus that net benefits outweigh costs that are produced through a series of trade-offs that arise from mitigating predation risk and increasing foraging efficiency (Paijmans *et al.*, 2019). Net benefits attained from shoaling are more likely when phenotypic and behavioural similarities occur among shoal members (Pitcher, 2012; Cattelan and Griggio, 2018). Despite the multiple benefits of shoaling (predation encounter dilution, increased predator vigilance and collective foraging effort), there are also costs, such as increased competition and increased predator detection (Paijmans *et al.* 2019). In addition to ecological variables such as predation, foraging and competition, environmental conditions may influence shoaling formation and behaviour. As fish species are ectotherms, ambient temperature is a crucial factor governing metabolism and therefore ability to survive. Increases in temperature are directly correlated to accelerated metabolic rate at the cellular level, manifesting into altered physiological

and behavioural responses including increased feeding rates, growth and reproduction (Figueira *et al.*, 2009; Donelson *et al.*, 2010). As shoaling is a behavioural adaptation in response to external stimuli, environmental temperature may affect shoaling parameters. Temperature-dependent responses of shoaling behaviour have been demonstrated in several studies, indicating changes to predation risk (Weetman *et al.*, 1999), shoal cohesiveness (Colchen *et al.*, 2017), anti-predator responses (Weetman *et al.*, 1998) and foraging patterns (Greenwood and Metcalfe, 1998).

In the case of displaced tropical fish such as *A. vaigiensis* juveniles settling into the temperate marine habitats of SE Australia, novel shoaling interactions may determine their ability to navigate and survive in an unfamiliar and hostile habitat. As the larval propagules of these tropical vagrants arrive in low densities on the peripheries of their poleward range limits (Booth *et al.*, 2007), relative to that of the settlement rates in their natal range. This creates high propagule pressure (Lockwood *et al.*, 2005; Feary *et al.*, 2014), which can prove critical when the onset of winter-water temperatures induce mass mortality among the populations of these warm-adapted fish. A counter-active measure to increase their survival probability is to form shoals, enhancing foraging success and reduce vulnerability to predation through attack dilution (Paijmans *et al.*, 2020). Shoals comprised of one species (single-species shoals) provide net benefits namely from phenotypic similarities, which manifests into important social cues such as enhanced information transfer that enable the recognition of temporal foraging events and predator attacks (Paijmans *et al.*, 2019). Newly settled *A. vaigiensis* juveniles are just as likely to form shoals with local heterospecifics (mixed-species shoals) than they are with vagrant conspecifics (Smith *et al.*, 2018). Research has shown that over the course of the recruitment season in SE Australia temperate rocky reefs (January – April), *A. vaigiensis* in mixed-species shoals obtained larger body sizes compared to single-species shoals. Paijmans *et al* (2020) demonstrated that newly settled *A. vaigiensis* juveniles will exhibit a preference to shoal with a mixed-species stimulus compared to a shoal of conspecifics while exposed to a live predation threat. This evidence suggests that there are additional trade-offs that fish encounter influencing their shoaling preferences, and possibly the existence of a threshold relating to the ratio of conspecifics to heterospecifics.

Two major trade-off parameters of shoals are to increase foraging opportunities, stimulating growth rates, and decrease the risk of predation. Predation risk is one the major ecological forces that drive many social grouping behaviours (Magurran, 2005), and consequently grouping dynamics such as shoaling, have developed adaptations to avoid predators (Templeton and Shriner, 2004; Ioannou *et al.*, 2012), predominantly through 'safety in numbers' (Hager and Helfman, 1991). For *A. vaigiensis* juveniles settling into SE Australian rocky reef habitats, it is documented that there is a relationship between predation and temperature (Figueira *et al.*, 2019), as warm water temperature decreases the rate of predation-induced mortality, having an inverse relationship with temperate heterospecifics that are in the same family of fish (Pomacentridae). Therefore, there is a temperature dependency on mortality rates of *A. vaigiensis*, and this presents a major barrier in successful range expansion. As these tropical vagrants maintain vigilance to detect predators, they simultaneously consider foraging opportunities and success, which is a major determinant of individual fitness (Ward and Webster, 2016; Kingsbury *et al.*, 2020b). Upon encountering unfamiliar food patches in this novel habitat, competition for food sources may increase within vagrant shoals and neighbouring heterospecific shoals. Kingsbury *et al.* (2020a) found that sympatric tropical vagrants and native temperate species did not significantly overlap in their diet within temperate range edges, potentially reducing the competition within shoals, insinuating a partitioning of resources. The mechanisms of water temperature, shoaling structures and predation regulating the physiological and behavioural performance of the tropical vagrant *A. vaigiensis* in novel ecosystems is not fully understood and are the critically limiting factors in the long-term success establishment in their poleward range limits.

In a mesocosm laboratory experiment, we investigated how water temperature, tropical fish shoal density (relative to heterospecific density: shoal ratio) and predation regulate the *A. vaigeisnis* physiological (growth rates) and behavioural (feeding, aggression, boldness and predator avoidance) performance metrics. It was predicted that 1) A higher ratio of conspecifics will result in increased individual feeding rates and therefore growth, 2) A higher ratio of conspecifics to heterospecifics will result in increased individual boldness, 3) A higher water temperatures will correlate with an

increase *A. vaigiensis* performance, 4) Increases in water temperature will correlate to a decrease in likelihood of *A. vaigiensis* being predated on as a first choice by a live predator and 5) Survivorship rates of *A. vaigiensis* will increase in higher water temperature treatments.

4.2 Methods

4.2.1 Collection sites and study species

Fish collections began in early January 2023 and continued until early June 2023 from several rock reef habitats within the Sydney region (**Figure 4.1**); North Narrabeen lagoon (33.702537 S, 151.306757 E), Freshwater (-33.781712 S, 151.294371 E), Little Manly (-33.807094 S, 151.285408 E), Long Bay (-33.964945 S, 151.255405 E) and Little Bay (-33.979440 S, 151.251579 E).



Figure 4.1. Satellite map of the geographical locations of the 5 collection sites along the Sydney coastline, Australia. Source: Google Earth © (2025).

Post-settlement 'recruits' targeted were the Indo-pacific sergeant major damselfish (*Abudefduf vaigiensis*, **Figure 4.2a**) and the local temperate heterospecific the Stripey (*Microcanthus joyceae*, **Figure 4.2b**). Both species occupy similar habitat niches and are often seen schooling together among the rocky reefs that are prevalent along Sydney's coastline (Kingsbury *et al.*, 2020a). *A. vaigiensis* are the most common tropical vagrant in SE Australia and have been used successfully in laboratory experiments, being considered a 'model' species for range expanding tropical fish species (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019; Paijmans *et al.*, 2021a). *M. joyceae* occupies a similar biogeographical range that *A. vaigiensis* does, on occasion being spotted in rocky reef sites in the GBR (Mitchel *et al.*, 2025) yet is considerably more common in temperate waters. The Mourning cuttlefish (*Sepia plangon*, **Figure 4.2c**) was selected as the temperate predator as this species has been used in similar studies (Paijmans *et al.*, 2021a), and is often seen hunting along the edge-habitats of seagrass beds and rocky reefs during diurnal periods (Norman and Reid, 200). The two fish species were caught by divers using a hand net and a fish anaesthetic solution composed of clove oil and 95% ethanol in a ratio of 1:10 administered by a spray gun to aid in their capture. This method of capture has been used in previous studies (Figueria *et al.*, 2009, Figueira *et al.*, 2019; Paijmans *et al.*, 2021a) and in low quantities has been demonstrated to have minimal impacts on the health of the fish. Capture of the *S. plangon* was achieved using a large landing net and no anaesthetic solution as cephalopods have a delicate outer membrane and the effects of the solution on this species are unknown. Once captured species were transferred to a large plastic drum (60L) filled with seawater from the site they were collected from and taken to the UTS fish holding rooms for experimentation.

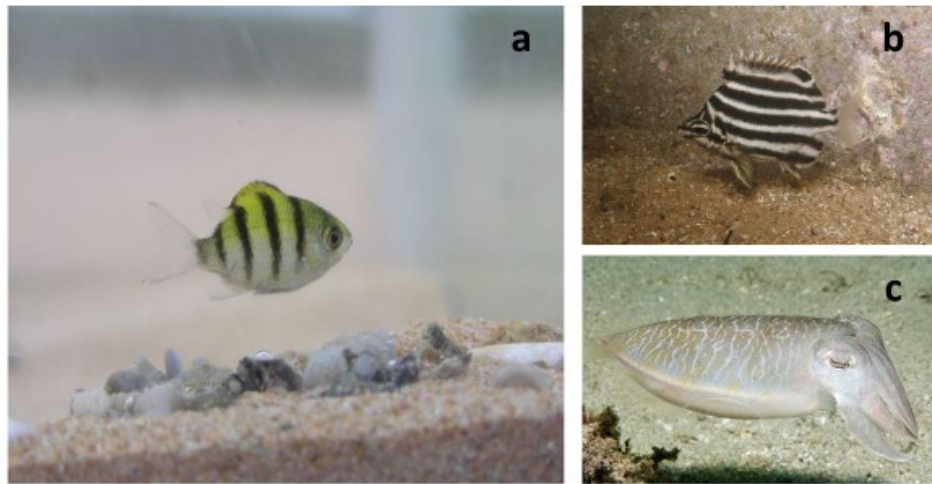


Figure 4.2. Model species used in experimentation: a) Indo-Pacific sergeant (*Abudefduf vaigiensis*), b) Stripey (*Microcanthus joyceae*) and c) Mourning cuttlefish (*Sepia plangon*). Image credits: Eric Keung, fishbase.se (a), Sarah Han-de Beaux, Sydneydives.com (b), Andrew Green, reeflifesurvey.com (c).

4.2.2 Fish housing

Upon arrival to UTS, fish were placed in a large glass aquaria (80x40x30cm) filled with 50L of seawater, which was matched to the sea surface temperature of the location the animals were captured from using two 50W water heaters. All fish species were placed together in one of these tanks with 5 PVC pipes (10cm section x 6cm diameter) to provide shelter and reduce stress, whilst the *S. plangon* was placed in a separate tank to avoid any predatory interactions, with 3 PVC pipes and plastic plants provided to best mimic its natural habitat. After a 3-day acclimation period (with temperature adjustments made depending on designated treatment temperature), all fish were measured (to the nearest 0.5mm standard and total length) using a 15cm perspex ruler and weighed (to the nearest 0.001g) on a digital balance. Total numbers of fish were as follows: *A. vaigiensis* N = 150, *M. joyceae* N = 90. For all fish total length (TL) ranged from 17 - 39mm (25.2 mean and $1.1 \pm \text{SE}$) and weight (g) ranged from 0.184 – 0.8427g (0.3895 mean and $0.09 \pm \text{SE}$). TL range for *S. plangon* was 90 - 160mm (not weighed due to potential stress caused and ink release affecting measurements). Post weighing and measuring, fish were tagged using a fluorescent elastomer polymer to mark for identification that was injected just under the skin. This method allows for each individual fish to be monitored and has been used in previous studies, where similar procedures were performed with minimal harm to the fish (Willis and Babcock, 1998;

Branconi *et al.*, 2019). One elastomer tag was injected into each fish to minimise damage, and while performing the tagging fish in a click-seal polyethylene bag with water for the injection process. Each fish had a unique tagging position per treatment as to allow identification while filming and tracking growth rates.

Once animals were weighed, they were transferred to treatment tanks in groups of 4 and size-matched within treatments to counteract the effects of any dominance hierarchy developing within shoals, and size-selectivity during the predation trials. Treatment tanks were filled with 50L of water and monitored daily for temperature to ensure that water temperature remained within $\pm 0.5^{\circ}\text{C}$ for the duration of the experiment. Daily water changes were carried out, removing between 10-20% of the total water to ensure all waste materials and excess food were siphoned off, along with any algae growth. Water quality was tested every 2 days using a WTW Multi Probe Meter that measured salinity (range 34.9 -36.2ppm), pH (range 7.9 - 8.1) and dissolved oxygen (DO, range 7.9 – 8.7), with ammonia being tested with an ammonia testing kit (range ≤ 0). To provide shelter five sections of PVC pipes (diameter 10cm) will be placed in each tank to reduce stress. Fish were fed to satiation using cultured live brine shrimp (*Artemia* species) twice daily, with excess food and excrement siphoned off during the daily water changes. The *S. plangon* was subject to similar treatment conditions and were fed daily with dead frozen lancefish (*Hypomesmus species*). All animals were starved for 12hrs before any testing to ensure there were no effect on the results due to metabolic processes (Figueira *et al.*, 2009). After a minimum of 6 days fish were weighed and measured to determine growth rates and then transferred to the predation treatment tank for predator presence trials, followed immediately by live-predation experimentation.

4.2.3 Experimental design

Post-acclimation, fish were randomly assigned to treatment tanks in groups of 4 to test the effect of temperature (17, 21 and 25°C), variations of the relative number conspecifics and heterospecifics referred to as shoal ratio (100% ; 4 *A. vaigiensis* vs 0 *M. joyceae*, 75% ; 3 *A. vaigiensis* vs 1 *M. joyceae*, 50% ; 2 *A. vaigiensis* vs 2 *M. joyceae* and 25% ; 1 *A. vaigiensis* vs 3 *M. joyceae*) and the presence or absence of a live predator (*S. plangon*). Each shoal ratio treatment (4) was run 5 times for each

temperature (3) over the course of the study, using newly collected fish each time to avoid pseudo-replication. These temperature treatments were selected to simulate the water temperature conditions in Sydney *A. vaigiensis* juveniles will be subject to during their ontogeny. The justification of the 25°C was to mimic the average early summer temperatures on the southern Great Barrier Reef (most likely source of vagrants in SE Australia) they experience during their spawning and matches the maximum sea surface temperature of Sydney's marine habitats during summer recruitment periods (IMOS, 2025). The selection of the 21°C temperature treatment was to simulate the average autumn seas surface temperatures of Sydney, corresponding to the arrival and settlement of juvenile *A. vaigiensis* (IMOS, 2025). Towards the end of the recruitment season in Sydney, and the onset of winter, sea surface temperature begins to drop averaging 18°C from early June onwards, justifying its selection as a temperature treatment in this study, and corresponds to their lower critical thermal limit of 17°C (Figueira and Booth, 2010). The use of different shoal ratio was used to represent possible aggregations scenarios these tropical vagrants may recruit to during their settlement and early-growth periods, and to determine the extent to which shoal members influence the physiology and behaviour of an individual fish. All behavioural testing was conducted in the absence and presence of the temperate predator *S. plangon*.

4.2.4 Feeding and predation experimental protocols

Behavioural response trials were filmed using a GoPro Hero Black 7 (mounted on a tripod) and conducted in separate testing tanks with the same dimensions as treatment tanks, with sea water, 1 PVC pipe placed on the far-right side of the tank, and a mesh separation box used to house the *S. plangon* attached to the left wall with suction cups (used in both predator absence and presence trials to prevent any placebo effects). During feeding trial experimentation, the GoPro was placed in front of the tank, and fish were given a 30min acclimation period to their new temporary habitat. During experimentation, fish behaviour was filmed for 7min before the administration of food, and a further 7min post food release. Food was administered on the left side of the tank above the mesh separation box, and an excess amount of live *Artemia* was used to ensure fish fed to satiation. The following responses will be

measured during the feeding trials; bite rate (bites^{-min}), response time to food (s), total feeding time (s), aggressive interactions between shoal members (total), boldness (% of time spent away from shelter) and total aggressive interactions between shoaling fish. Growth changes were assessed by calculating instantaneous growth rate (G_{INST}) (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019) using the wet weights recorded at the beginning (M_1) and end of the experiment (M_2) with the interval of length (Δt) in days: $G_{Inst} = \ln (M_2/M_1) / \Delta t$. G_{INST} results from this study showed variance among individuals measured, with a mean standard error ($\pm SE$) measured at 0.0038g d⁻¹.

The predator trials (**Figure 4.3**) followed a similar protocol, however with the addition of the live predator in the tank enclosed within the mesh separation box (which was used as a control in predator absence experimentation). The predator was introduced to the tank first, shortly followed by the shoal of fish and given a 30min acclimation period. Post this period, behaviour was filmed for 7min, and then the live *Artemia* was administered above the separation box containing the predator, and responses recorded for a further 7min. Once the predation trial had commenced, fish had their final lengths and weights measured and were placed in the testing tank. Fish were allowed to re-acclimate for a further 30mins, and then the mesh separation box was removed allowing the *S. plangon* to swim freely in the tank with the testing fish, commencing the predation-mortality phase of the experiment. Additional PVC tubing and plastic plants were added to simulate a more natural environment, allowing the *S. plangon* to hunt inconspicuously and provide the fish with shelter to avoid predation. These experiments were conducted over 24hrs to measure direct predation (survivorship rate per shoal the response variable) and the first fish to be predated upon. Surviving fish and the *S. plangon* were returned to the sites they were collected from once experimentation had concluded, as no animal was used more than once during the study.

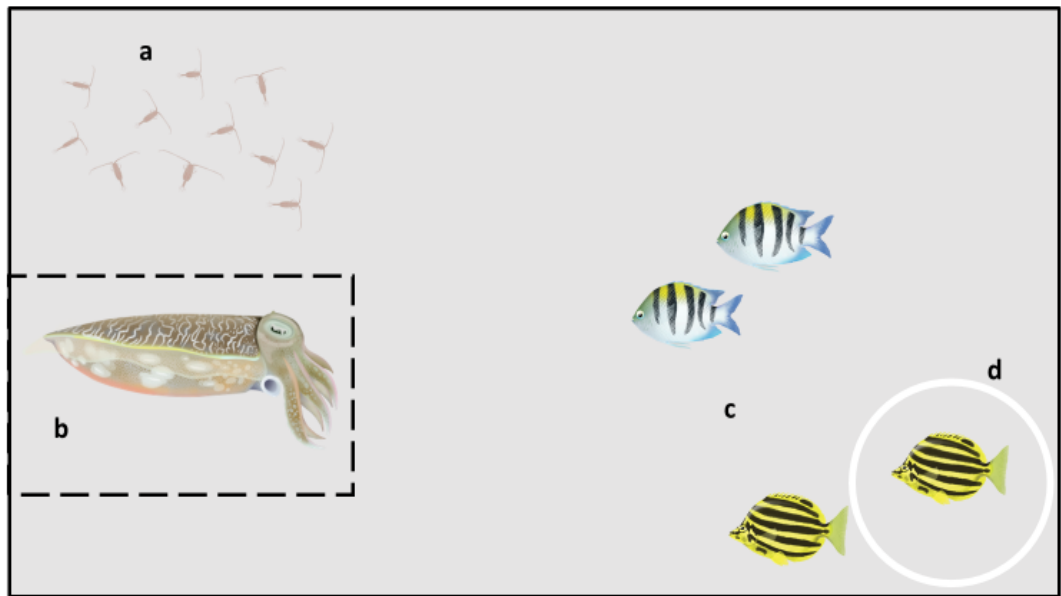


Figure 4.3. Feeding and predation trial set up (plain view of aquarium): a) Deployment area of food source *Artemia*, b) Predatory animal *S. plangon* enclosed within a mesh separation box, c) Treatment shoal of fish and d) Shelter (PVC pipe) providing a reference point to measure shoaling behaviours.

4.2.5 Video analysis

Behaviours were quantified using the software VLC 3.0.13 player, with 1 *A. vaigiensis* and 1 *M. joyceae* randomly selected per treatment (except for 100% shoal ratio treatment as it contained only *A. vaigiensis*, therefore only 1 fish was selected from these treatments) to be the focal fish for video behavioural analysis and statistical analysis. Video data was recorded 2min before the deployment of food, a further 2min after the first bite was taken, and each shoal was measured in the presence and absence of *S. plangon*. After the first bite was taken, videos were slowed down to 0.25 frames/second to count bite rate over a 2min interval and then calculated as bite rate. Boldness measurements were taken every 10 seconds before and during feeding for 2mins each way; boldness was defined as 5 body lengths distance away from shelter (Coni *et al.*, 2021), then calculated as a % of time exhibiting bold behaviours over the temporal period. Video analysis was not taken during predation-induced mortality trials, as any disturbance to the experiment would cause the cuttlefish to exhibit defensive behaviours towards the observer and bias results.

4.2.6 Statistical analysis

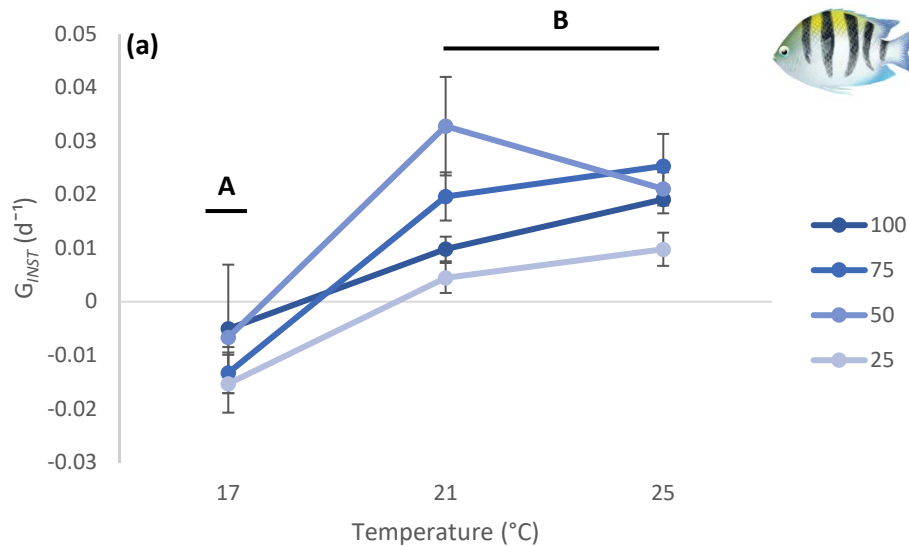
To measure the effect of water temperature (17°C, 21°, 25°C: 3 levels) and shoal ratio (100%, 75%, 50% and 25%: 4 levels) and predator presence/absence (2 levels) on the physiology and behaviours of *A. vaigiensis*, separate Generalised Linear Models (GLMs) (with temperature x shoal ratio as an interaction) were run using each of the previously mentioned performance metrics as a response variable. To test for effects on growth rates the GLMs model parameters were as follows: G_{INST} as the response variable, temperature (3 levels) and shoal ratio (4 levels) as fixed factors, species (1 level) as a random factor and initial weight (M_1) as a covariate to measure the effect of initial size on growth rates. The model for survivorship was run in a similar model, however the random factor of species was removed as the survivorship was measured per shoal, and the covariate was changed to the mean final weights of the shoal. For the response variables of bite rate and feeding start time, separate GLMs models were run with the following model parameters: Bite rate^{-min}/feeding start time (s) as the response variables, temperature (3 levels) and shoal ratio (4 levels) and predator presence/absence (2 levels) as fixed factors, species (2 levels) as a random factor and weight as a covariate. To analyse fish boldness, an additional factor of comparing behaviour before (normal) and during feeding was added to the following, with the model parameters run as follows; boldness (% of time showing bold behaviour) as the response variable, temperature (3 levels) and shoal ratio (4 levels), predator presence/absence (2 levels) and normal behaviour vs feeding behaviour (2 levels) as fixed factors, species (2 levels) as a random factor and weight as a covariate. The model for aggressive interactions was run in a similar fashion, however the random factor of species was removed as aggressive interactions were measured per shoal, and the covariate was changed to the mean weights of the shoal. The first prey fish to be consumed by the predator was analysed using a mixed effects linear model using the following parameters: 1st predated species as the response variable, temperature and ratio as fixed effects, total shoal numbers as the continuous variable, mean weight as the covariate and shoal ratio and temperature as the interaction. GLMs models are known to be robust and offer advantages when dealing with either non-parametric data or when complex relationships between variables exist. They are often used in

ecological data sets due to their flexible framework and allow for a wider range of response variable distributions (Ibrahim *et al.*, 2005; Gorosito *et al.*, 2018; de Melo *et al.*, 2022). All statistics were performed using IBM statistics software SPSS v31.

4.3 Results

4.3.1 Growth

Instantaneous growth rates were significantly affected by temperatures and species (Table 4.1., $p < 0.05$), however shoal ratio had no significant effect. Pairwise comparisons showed significant differences between the growth rates of fish reared at 17°C (-0.006 g d^{-1}) compared to 21 (0.012 g d^{-1}) and 25°C (0.0097 g d^{-1}) (Figure 4.4, $p < 0.05$). Overall *A. vaigiensis* juveniles showed significant higher growth rates than *M. joyceae*, with a mean instantaneous growth rate of 0.0085 g d^{-1} compared to -0.0009 g d^{-1} (Figure 4.4, $p < 0.001$).



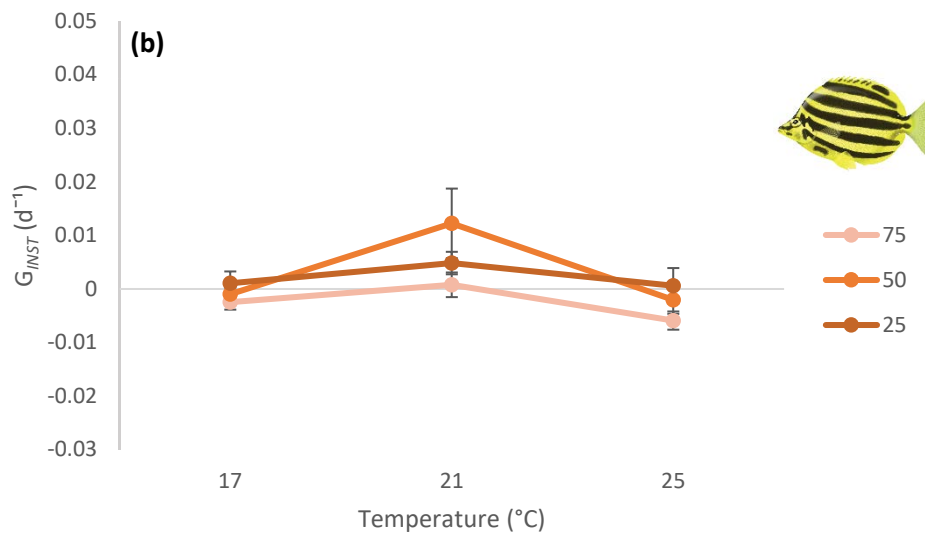


Figure 4.4. Mean ($\pm$ SE) Instantaneous growth rates (G_{INST} d⁻¹) for a) *A. vaigiensis* and b) *M. joyceae* (b) at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments. No 100% shoal ratio treatment taken for *M. joyceae*. Significant differences between temperature treatments ($p < 0.05$) from post hoc indicated by letter groupings above lines.

4.3.2 Feeding behaviours

The time it took for fish to start feeding was significantly affected by temperature, shoal ratio, species and predator presence and absence (**Table 4.1**, all $p < 0.05$). Pairwise comparisons indicated significant differences between 17°C (171.16s) temperature treatment and 21°C (90.54s) and 25°C (70.51s) (**Figure 4.5**, $p < 0.05$), with longer delays of feeding occurring at 17°C with a mean time of 171.16s. Shoal ratio treatments significantly differed between all groups ($p < 0.05$) with the exception of ratios and 75 (92.48s) and 100% (62.16s), indicating that feeding start times in shoal ratios progressively changes as the proportion of *A. vaigiensis* within the shoal changes. A ratio treatment of 25% *A. vaigiensis* causes the greatest mean delays in feeding time of 137.62s, over twice the time of a shoal ratio of 100%.

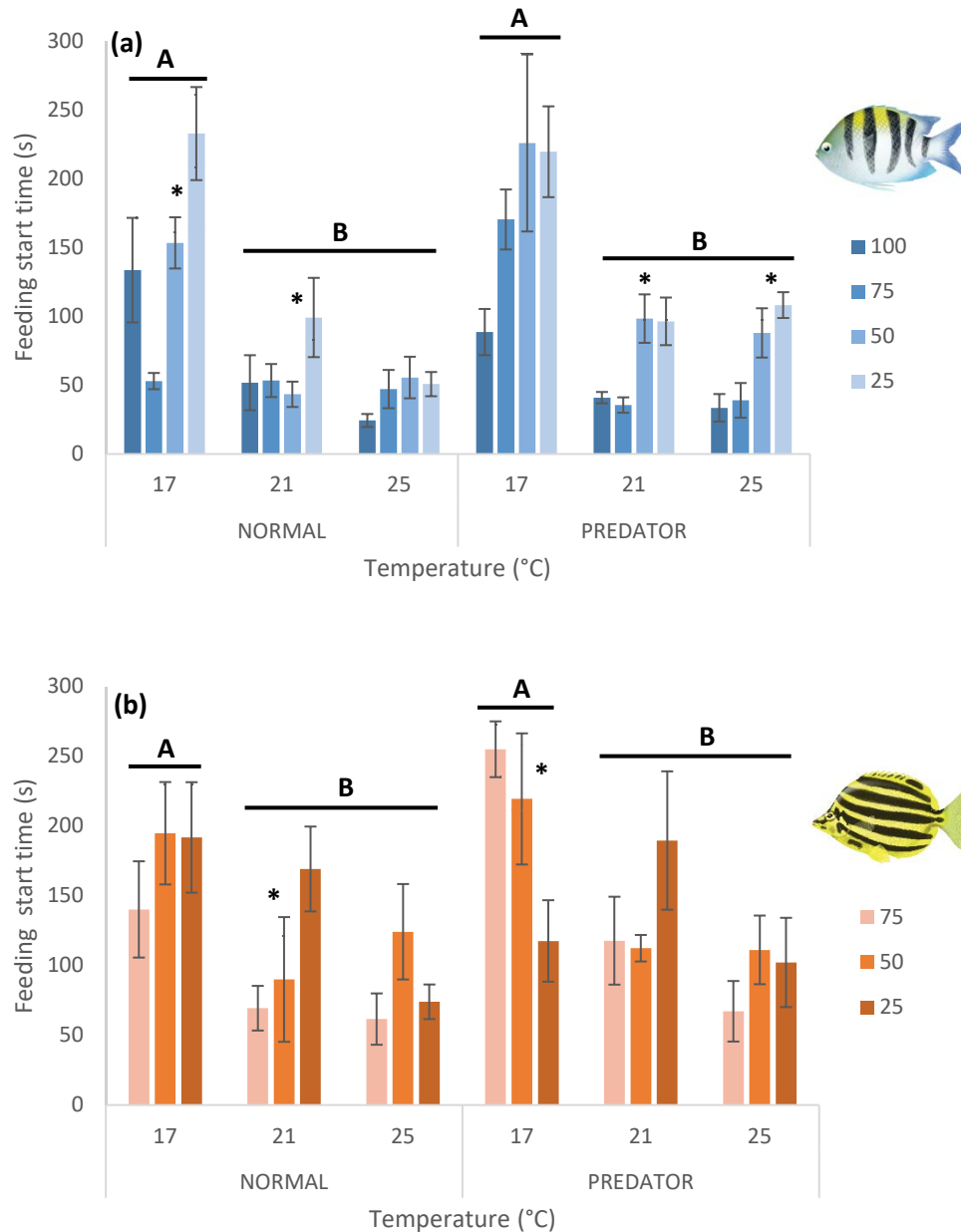
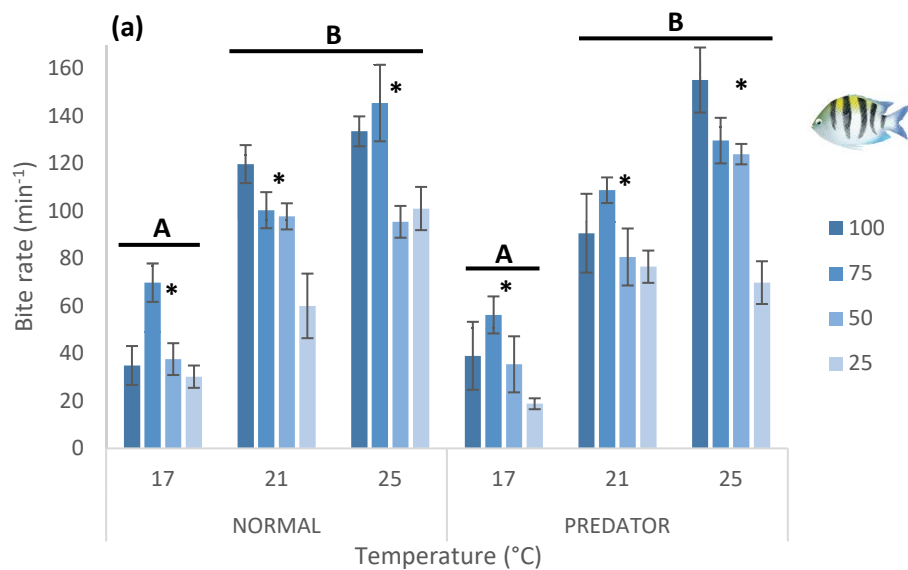


Figure 4.5. Mean ($\pm$ SE) feeding start times (s) for a) *A. vaigiensis* and b) *M. joyceae* at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments. No 100% shoal ratio treatment performed for *M. joyceae*. Feeding start time was measured in the absence of the predator (NORMAL) and in the presence of a predator (PREDATOR) *S. plangon*. Significant differences between temperature treatments ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal ratios ($p < 0.05$) from post hoc.

There was a significant effect of temperature, shoal ratio and species (**Table 4.1**, all $p < 0.05$) on the bite rate of fish. There was no effect when comparing predator presence and predator absence on the bite rate of fish (**Table 4.1**, $p = 0.525$). For temperature treatments, bite rate significantly differed when comparing the 17°C treatment temperature results with 21°C and 25°C ($p < 0.001$), with mean values indicating an overall increase corresponding to higher water temperatures; 34.31 bites.min⁻¹ at 17°C, 74.56 bites.min⁻¹ at 21°C and 96.88 bites.min⁻¹ at 25°C (**Figure 4.6**). Significant differences between ratio treatments were detected with pairwise comparisons between 25% and all other ratio treatments (all $p < 0.02$). Bite rate was greater at a ratio of 100% *A. vaigiensis* with 95.47 bites.min⁻¹, and lowest at 25% with 51.93 bites.min⁻¹ (**Figure 4.6**). Overall *A. vaigiensis* fed at a significantly higher rate than *M. joyceae* ($p < 0.001$), with 83.74 bites.min⁻¹ compared to 48.38 bites.min⁻¹.



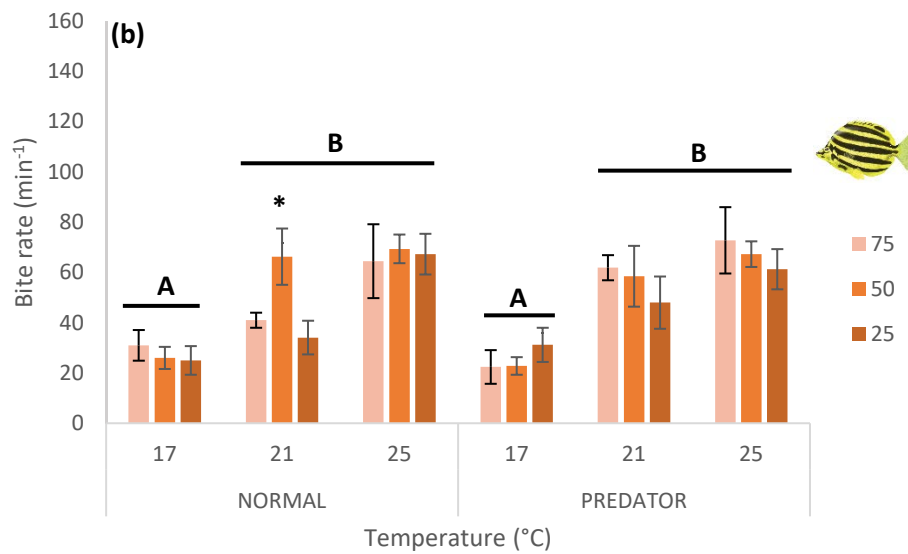
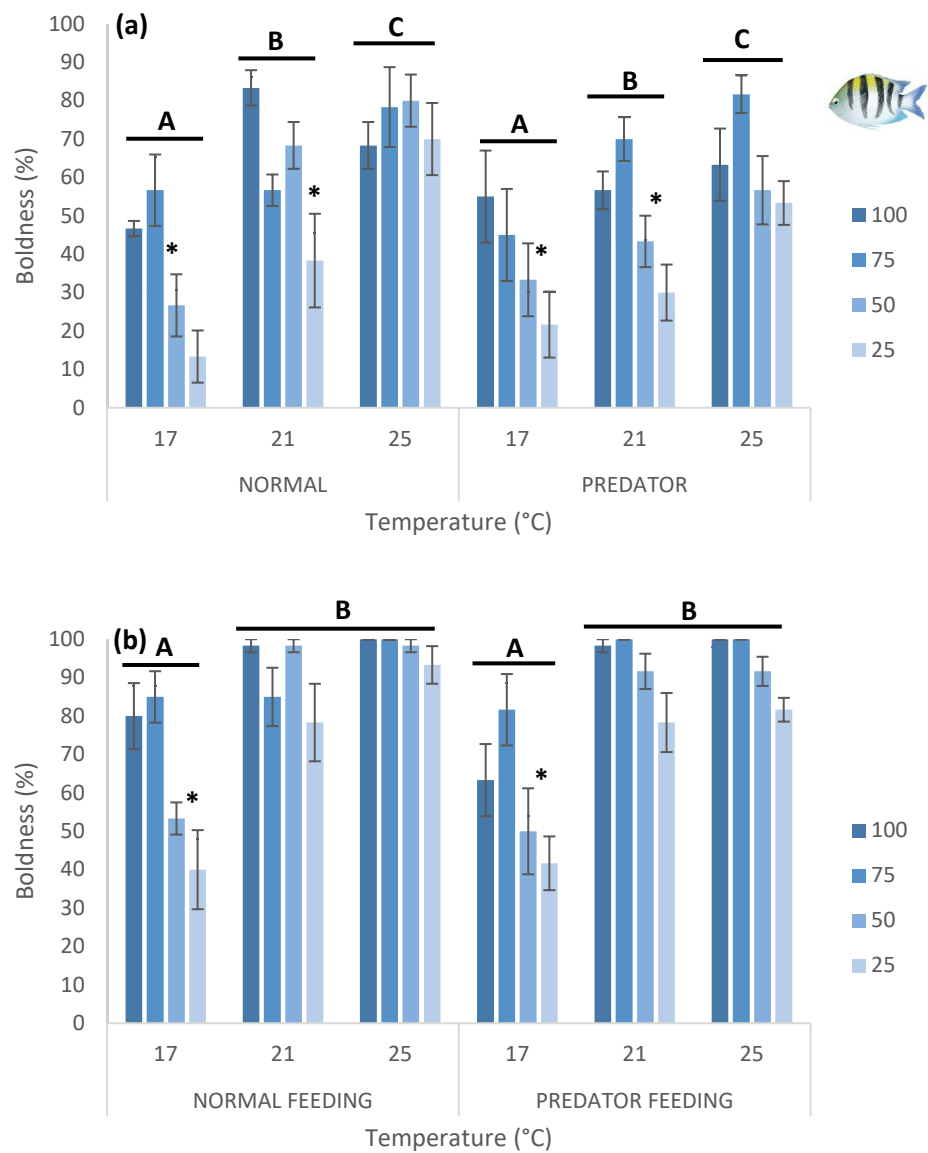


Figure 4.6. Mean ($\pm$ SE) bite rate^{min⁻¹} for a) *A. vaigiensis* and b) *M. joyceae* at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments. No 100% shoal ratio treatment performed for *M. joyceae*. Bite rate was measured in the absence of the predator (NORMAL) and in the presence of a predator (PREDATOR) *S. plangon*. Significant differences between temperature treatments ($p < 0.05$) from post hoc indicated by letter groupings above lines. * Denotes significant differences between shoal ratios ($p < 0.05$) from post hoc.

4.3.3 Boldness

Bold behaviour exhibited by fish showed significant interaction effects with temperature x shoal ratio (**Table 4.2**, $p < 0.01$). There were also significant effects between species and normal behaviours compared with feeding behaviours and in the absence and presence of a predator (**Table 4.2**, $p < 0.01$). For temperature treatments, pairwise comparisons showed that boldness significantly differed between all 3 temperatures ($p < 0.001$) and increased at higher water temperatures, with fish exhibiting bold behaviour 78.87% of the time at 25°C, 66.39% at 21°C and 44.68% at 17°C (**Figure 4.7**). Shoal ratio treatments significantly differed from one another ($p < 0.01$), with the only exception of no differences being detected between 75% (69.79%) and 50% (64.44%) for shoals of *A. vaigiensis* (**Figure 4.7a + b**). Overall fish displayed higher rates of bold behaviours at a ratio of 100% (76.32%) and lowest at 25% (49.02%) (**Figure 4.7**). Predator presence was shown to significantly lower boldness in both fish species, with mean boldness measured at 61.03% compared to 65.6% in the absence of a predator (**Figure 4.7**). *A. vaigiensis* showed significantly greater bold behaviours (p

< 0. 01) compared their temperate heterospecific *M. joyceae*, with 68.48% and 56.42% (Figure 4.7) respectively.



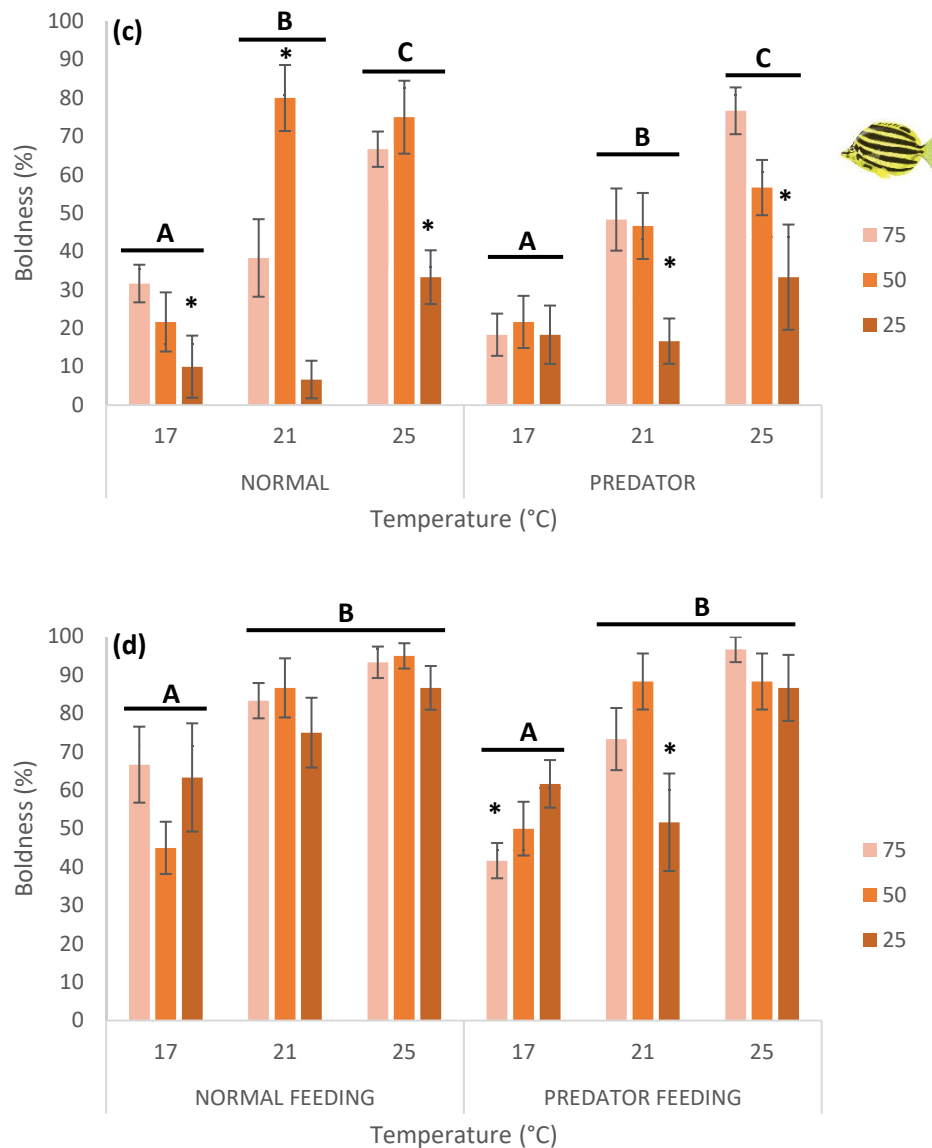
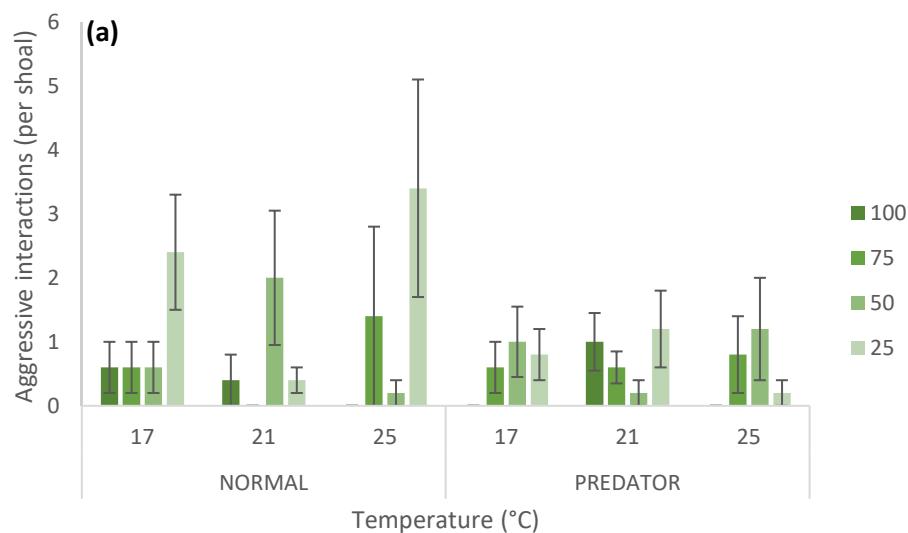


Figure 4.7. Mean ($\pm$ SE) Boldness (% of time) for a) + b) *A. vaigiensis* (Blue) and c) + d) *M. joyceae* (Orange) at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments. No 100% shoal ratio treatment performed for *M. joyceae*. a) *A. vaigiensis* boldness in the absence of the predator (NORMAL) vs in the presence of a predator (PREDATOR) *S. plangon*. b) *A. vaigiensis* boldness while feeding in the absence of the predator (NORMAL FEEDING) vs feeding in the presence of a predator (PREDATOR) *S. plangon*. c) *M. joyceae* boldness in the absence of the predator (NORMAL) vs in the presence of a predator (PREDATOR) *S. plangon*. d) *M. joyceae* boldness while feeding in the absence of the predator (NORMAL FEEDING) vs feeding in the presence of a predator (PREDATOR) *S. plangon*. Significant differences between temperature treatments ($p < 0.05$) from post hoc indicated by letter groupings. * Denotes significant differences between shoal ratios ($p < 0.05$) from post hoc.

4.3.4 Aggressive interactions

Aggressive interactions were not significantly affected by temperature or predator presence (**Table 4.2**, all $p > 0.05$), however there was a significant effect when comparing aggression before feeding (normal) and during feeding (**Table 4.2**, $p < 0.05$). There was an increase of approximately 4-fold when comparing aggressive interactions before and during feeding, with 0.82 interactions compared to 0.21 interactions per shoal respectively. Pairwise comparisons showed that a shoal ratio of 25% yielded significantly higher aggressive interactions per shoal when compared to all shoal ratio treatments ($p < 0.05$), apart from 75%. A shoal ratio treatment of 25% *A. vaigiensis* yielded the highest rates of aggression among ratio treatment, with 0.87 interactions per shoal, and lowest at 100% with 0.17 aggressive interactions per shoal (**Figure 4.8**).



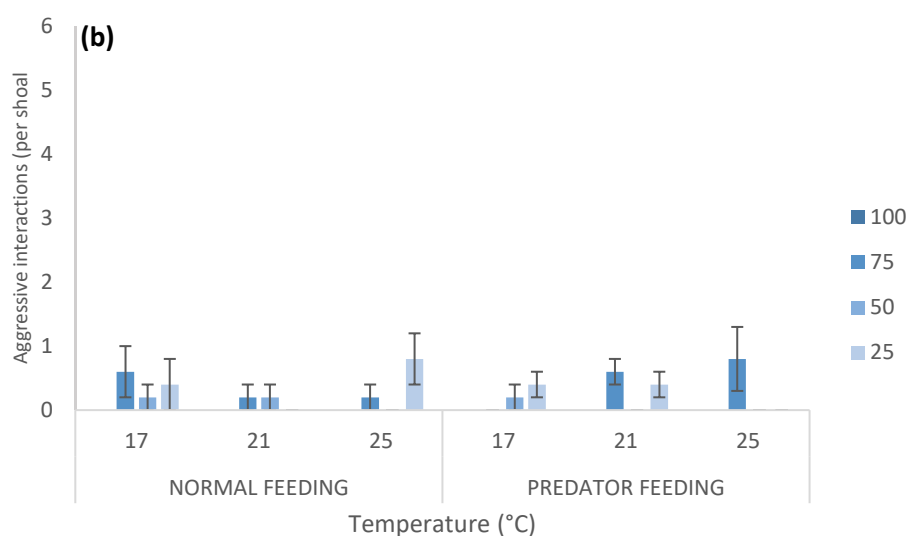


Figure 4.8. Mean ($\pm$ SE) aggressive interactions per shoal at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments. a) Aggressive interactions in the absence of a predator (NORMAL) and in the presence of a predator (PREDATOR) and b) Aggressive interactions while feeding in the absence (NORMAL) and presence (PREDATOR) of a predator *S. plangon*.

Table 4.1. Generalised linear model results for 3 response variables: instantaneous growth rate, feeding start time and bite rate. The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values.

Factor	<u>Growth</u>			<u>Feeding start</u>			<u>Bite rate</u>		
	df	X ²	p	df	X ²	p	df	X ²	p
Temperature	2	29.515	< 0.001	2	91.124	< 0.001	2	211.117	0.001
Ratio	3	4.761	0.190	3	37.523	< 0.001	3	10.642	0.14
Predation	-	-	-	1	6.6851	0.009	1	0.719	0.396
Species	1	5.827	0.016	1	4.706	0.030	1	24.076	0.001
Temperature x Ratio	6	7.769	0.255	6	5.338	0.501	6	11.799	0.067
Weight	1			1	0.564	0.454	1	0.300	0.584
Error	216			192			192		

Table 4.2. Generalised linear model results for 3 response variables: instantaneous boldness, aggressive interactions and survivorship rates. The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values.

Factor	<u>Boldness</u>			<u>Aggressive interactions</u>			<u>Survivorship</u>		
	df	X ²	p	df	X ²	p	df	X ²	p
Temperature	2	220.79	< 0.001	2	0.361	0.835	2	0.895	0.639
Ratio	3	109.514	< 0.001	3	15.839	0.001	3	7.268	0.064
Normal vs feeding	1	352.255	< 0.001	1	19.564	< 0.001	-	-	-
Predation	1	7.431	0.006	1	1.571	0.210	-	-	-
Species	1	27.335	< 0.001	1	-	-	1	-	-
Temperature x Ratio	6	33.262	< 0.001	6	3.262	0.775	6	7.162	0.306
Weight	1	1.449	0.229	1	3.661	0.056	1	3.630	0.057
Error	400			225			47		

4.3.4 Predation

There was a significant effect of temperature on the first species predated on by *S. plangon* ($F_{2,35} = 11.525$, $p < 0.001$), however there was no difference among the mixed-species shoal ratio treatments. Between temperature treatments, at 17°C there was an 86.7% probability that *A. vaigiensis* would be selected first for predation over *M. joyceae*, reducing to 66.7% at 21°C, and 13.3% at 25°C (**Figure 4.9**), which was statistically significant when comparing 25°C to 21 and 17°C ($p < 0.01$). The covariate of weight did not have a significant effect on the first species consumed by the *S. plangon* ($F_{1,35} = 0.52$, $p = 0.475$).

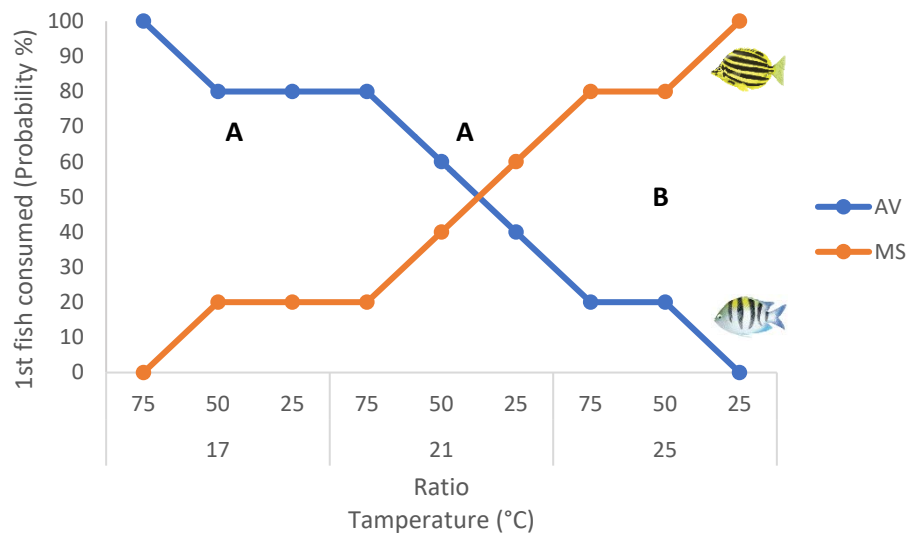


Figure 4.9. Mean ($\pm$ SE) % probability of 1st predation selection by *S. plangon* for *A. vaigiensis* (AV) and *M. joyceae* (MJ) at 3 temperature (17, 21 and 25°C) and 3 shoal ratio (75, 50 and 25% tropical fish per shoal) treatments. 100% ratio treatment excluded from analysis as it is a single-species shoal. Significant differences between temperature treatments ($p < 0.05$) from post hoc indicated by letter groupings.

Survivorship probability of a shoal over 24hr was unaffected by both temperature and shoal ratio (**Table 4.2.** $p > 0.05$). Comparison on means showed that survivorship was highest in a shoal ratio treatment of 25% (0.49) and lowest at 100% (0.35). Mean survivorship rates indicated that water temperature of 17°C results in lower rates of survival, with a mean survivors per shoal recorded at 0.43 (**Figure 4.10**).

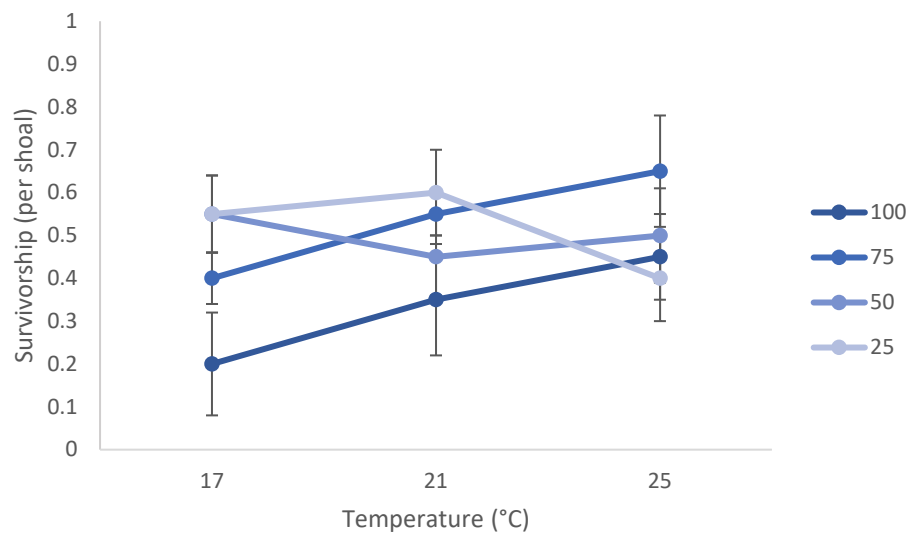


Figure 4.10. Mean ($\pm$ SE) survivorship per shoal when subjected to 24hr exposure to *S. plangon* at 3 temperature (17, 21 and 25°C) and 4 shoal ratio (100, 75, 50 and 25% tropical fish per shoal) treatments.

4.4 Discussion

The physiology and behaviour of fish are primarily governed by temperature (Neubauer *et al.*, 2019; Volkoff and Rønnestad, 2020), and results from this study have shown that in conjunction with shoaling composition and ecological factors can influence to varying degrees how fish behave in aggregations, further highlighting the complex nature of fish shoaling. Consistent with our hypothesis, higher water temperatures translated to an increase in physiological and behavioural performance of *A. vaigiensis*; augmented growth rates, bolder behaviour manifesting to greater feeding rates and reductions in predation selection. While water temperature increases resulted in enhanced boldness and feeding rates for the local temperate fish *M. joyceae*, it induced reductions in growth rates, and significantly increased their chance of being predated on by the local predator *S. plangon*. Shoal composition had varying effects on the behaviours of *A. vaigiensis*, and while there no effect on growth rates, there were impacts on feeding and boldness. These results were somewhat predicted in our hypothesis that higher ratios of conspecifics to heterospecifics would translate into increased *A. vaigiensis* performance, however there appeared to be a threshold reached when the majority of a shoal were conspecifics, with performance

metrics showing varying results across all tests performed. Under acute predation threat, variations in fish behaviour were limited, apart from a decrease in bold behaviours, as time spent closer to a shelter increased, which surprisingly did not manifest into a reduction in feeding rates. When comparing fish behavioural metrics of before feeding, or “normal behaviour”, to feeding behaviours, we showed that there was significant increase in time exhibiting boldness, and 4-fold reduction in aggressive interactions. These results provide further evidence that upon settlement in a novel ecosystem, tropical vagrant fish forming aggregations with either conspecifics or heterospecifics will result in a series of behavioural trade-offs that are influenced by not just by extrinsic temperature, but also intrinsic factors such as shoal composition, foraging behaviours and local predator-density. While previous studies in this field have highlighted the importance of water temperature in regulating the population of tropical vagrant fishes in SE Australian coastal marine ecosystems (Booth *et al.*, 2007; Figueira *et al.*, 2009; Feary *et al.*, 2014), the importance of shoaling cannot be understated, as it plays a major role in governing individual performance by providing enhanced foraging opportunities and anti-predatory defences (Paijmans *et al.*, 2019; Coni *et al.*, 2022). Overall, there was evidence provided in this study that specific physiological and behavioural traits of *A. vaigiensis* are enhanced when they shoal with local heterospecifics, which may contribute to greater settlement and survivorship success in the future. It is evident that the range expansion potential of tropical vagrant fish such as *A. vaigiensis* will be heavily influenced by rising water temperatures catalysed by climate change, and that this may negatively affect temperate fish species that occupy the same trophic niche, allowing these tropical fishes to outcompete the local species, and form viable and permanent populations.

Environmental temperature exerts a major influence over an ectothermic organism’s performance; changes that occur at cellular level that manifest into physiological and behavioural changes (Pörtner and Knust, 2007). As temperatures increase, metabolic processes speed up (Donelson *et al.*, 2011), increasing energy demands that are met through inflated feeding rates and consequently augmented growth rates. In this study we saw a correlation between increasing water temperature and daily growth rates of *A. vaigiensis* juveniles from 17°C to 21 and 25°C, which conforms to results obtained in

previous studies which examined the effects of temperature on the growth rates of *A. vaigiensis* (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019). As temperature approaches the lower thermal threshold-minimum, proteins and enzymes begin to destabilise, initiating the decline of oxygen delivery and thereby reducing aerobic scope (Pörtner, 2002). The aerobic scope is defined as the energy available for essential functions such as movement, feeding and growth (Pörtner, 2002; Pörtner and Knust, 2007); in this case we saw *A. vaigiensis* juveniles exhibit weight loss at 17°C, most likely due to a reduction in their aerobic scope (Djurichkovic *et al.*, 2019), coupled with reduced feeding rates. While not statistically significant, *A. vaigiensis* trended higher mean growth rates at a shoal ratio of 50% tropical and 50% temperate fish of 0.016g d⁻¹, in comparison to that of the 100% single-species shoal ratio which was 0.008g d⁻¹. In Smith *et al* (2018) the authors found that newly settled *A. vaigiensis* juveniles attained larger body sizes over the course of the recruitment season (Feb – May) when aggregating in mixed shoals compared to those in single species shoals. Shoaling with conspecifics has been shown to indirectly decrease individual fitness as competition for resources increases (Thünken *et al.*, 2020), possibly explaining why we observed body size decreases in this study when *A. vaigiensis* was reared in a shoal of conspecifics compared to heterospecifics. Growth rates of the heterospecific, *M. joyceae*, showed a bell-shaped performance with highest growth occurring at 21°C, with those reared at 17 °C and 25°C showing substantial losses in body weight regardless of shoal composition. While not ubiquitous across all temperate fish species, elevated water temperatures that reflect tropical water temperatures can result in the retardation of growth rates for temperate fish (O'Connor and Booth, 2021), and with current predicted temperature rises in temperate marine habitats, vagrant tropical fish may begin to thrive in these habitats while some temperate fish may shift in range or face local extinctions.

Foraging efficiency plays a fundamental role in determining individual fitness of fish (Sih and Christensen, 2001; Pajimans *et al.*, 2019) and is critical during the early life history of fishes as predation is often size-dependent, therefore acquiring food reduces their time spent at vulnerable size classes (Sogard, 1997). In this study both temperature and ratio had significant effects on both initial feeding start times and

bite rate, with faster feeding start times and bite rate occurring at 25°C. There is a positive relationship with increased temperature and feeding for most ectotherms, and as metabolic demand increases, this must be met with an increased uptake of food (Angilletta *et al.*, 2002). At 25°C bite rate was nearly 3 times that of 17°C, which concurred with results obtained that analysed the feeding rates of *A. vaigiensis* juveniles (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019). Higher water temperatures can result in the increased speed of neural conduction speed of fishes (Domenici and Hale, 2019), which may explain the shorter feeding times and aggressive feeding strategies employed by both species. A mixed-species shoal ratio of 75% tropical and 25% yielded higher feeding rates for *A. vaigiensis*, which may suggest there is behavioural segregation to allow persistence in a novel habitat with unfamiliar species (Coni *et al.*, 2022). Diet partitioning has been shown to be an important driver in the formation of mixed shoals, where species within the shoal may differ in feeding times and diet, reducing within-shoal competition (Alatorre-Ramirez *et al.*, 2017). This may explain why we observed ratio effect on feeding strategies, as intra-shoal competition may increase in single-species shoals, interfering with foraging behaviours.

The expression of conservative, or risk averse, behaviours is commonly seen in fish species to successfully avoid predation (Walker *et al.*, 2005), and in novel habitats, tropical fish exhibit these behaviours in response to their unfamiliar surroundings (Sih, 1992). The time fish spent exhibiting bold behaviour in this study showed that temperature, ratio, feeding vs no feeding behaviour, predator threat and species had significant effects. There is a critical trade-off fish must face between resource acquisition and predation risk (Rhoades and Blumstein, 2007), and as previously mentioned, higher temperature induces an increased metabolic demand that must be met by acquiring food resources, accounting for the increases in the expression of bold behaviours we observed for both species, correlating to increases in feeding performance. The reduction in bold behaviour under acute predation threat from *S. plangon* suggest a degree of behavioural plasticity. Anti-predatory behaviours exhibited by fish rely strongly on olfactory cues to assess the risk the predator presents and simultaneously evaluate escape options and proximity to shelter (Domenici, 2010), providing evidence of adaptive behavioural expression of fish observed in this study.

However, the significant increase in boldness rates during feeding, regardless of predation threat, suggests that resource acquisition in single and mixed-species shoals of *A. vaigiensis* is prioritised over risk aversion, and links to results obtained regarding feeding performance, where predator presence had no effect on their feeding initiation times and bite rate. This is a common behaviour that has been previously recorded for coral reef fish, where predation threat and simultaneously foraging opportunities are enhanced with increasing distance and time spent away from shelter (Sackley and Kaufman, 1996; Madin *et al.*, 2010; Beck *et al.*, 2016). We observed that *A. vaigiensis* juveniles spent more time away from shelter than their temperate heterospecific *M. joyceae*, which may be attested to the lack of recognition in olfactory cues of a temperate predator. This was further supported by the reduction in boldness as the ratio of *A. vaigiensis* in a shoal decreased. At higher proportions of *M. joyceae* in a mixed-shoal, bold behaviours decreased, indicating a degree of social learning and information transfer (Paijmans *et al.*, 2019) that influenced their behaviour that prioritised risk aversion.

Predation-induced mortality is regulated by many interacting factors including prey reaction time, escape direction and escape speed (Scharf *et al.*, 2003; Domenici, 2010), all of which are heavily influenced by temperature (Figueira *et al.*, 2009; Figueira *et al.*, 2019). At 25°C *A. vaigiensis* juveniles were able to avoid predation more successfully as the first fish consumed by *S. plangon* than at 17°C, being the first-choice selection for predation 13.3% of the time compared to 86.7% respectively. Water-temperatures below the thermal optima for *A. vaigiensis* have significantly detrimental impacts on their burst swimming performance (Figueira *et al.*, 2009; Figueira *et al.*, 2019; Djurichkovic *et al.*, 2019), results that are consistent with metabolic theory of swimming performance as cooler temperature cause a reduction in muscle contraction and tail-beat frequency (Batty and Blaxter, 1992). Simultaneously, *M. joyceae* juveniles experienced few occurrences of first-prey selection at 17°C and an 86.7% chance at 25°C. This significant increase in their inability to avoid predation at a higher water temperature may be due to a reduction in aerobic scope that occurs at either an animal's minimum, or maximum thermal limits (Pörtner and Knust, 2007; Donelson *et al.*, 2010). This manifests into a reduction of energy available for higher level

functioning and performance (Pörtner and Knust, 2002), in this case, escape speed and predator avoidance. Aerobic scope has been shown to be positively related to burst speed and boldness (Bellotto *et al.*, 2025), and coupled with reduced growth rates, increasing their time spent at a vulnerable size class (Sogard, 1997), may explain the negatively affected performance rates *M. joyceae* experienced at 25°C. In Figueira *et al* (2019) they compared the physiological performance and predation-induced mortality between the tropical vagrant *A. vaigiensis* and a commonly temperate-associated species *Atypichthys strigatus*. It was found that these species had inverse relationships regarding burst speed (predator escape ability), as the temperate *A. strigatus* showed reductions of nearly 50% from 17°C to 25°C water temperatures. The authors also found that mortality rates of *A. vaigiensis*, which were similarly observed in this study, reduced by nearly 100% when comparing 17°C to 25°C water temperatures, and while the results regarding *A. strigatus* predator-induced mortality results were complicated as the 25°C treatment was not run due to high levels of stress and reduced activity, the authors predicted that mortality rates would have been elevated had they been run. While the temperate species used in this study (*M. joyceae*) has a more northern distribution than *A. strigatus* (Kuiter, 2012) and likely shows elevated performance at warmer water temperatures comparatively, the results obtained in Figueira *et al* (2019) regarding reduced predator escape performance may explain the increased rates of first prey selection for *M. joyceae* at higher water temperatures.

It is important to note that fish individual behaviour can be influenced by factors other than temperature and social structure (in the case of this study, shoal ratio), for example stress (induced by capture or housing), sickness or life history. At the lowest water temperature treatment (17°C), both *A. vaigiensis* and *M. joyceae* showed reduced feeding (and consequentially slower growth rates/loss of weight) and boldness, which was evident through higher sheltering times and reduced social behaviour. This was most evident in fish that were housed in a shoal ratio of 25% for both species, and while it can be argued that a lack of conspecifics may facilitate reduced social behaviour, our results still showed increases in feeding and bold behaviour at higher temperatures for shoal ratios of 25%. While this study did not measure stress levels induced by possible effects of captivity, or reduced presence of

conspecifics, future studies could focus on cortisol levels and blood sampling to determine the impacts stress levels influence fish behaviour. A limiting factor of this experimental design was that fish housed in lower conspecific ratios (25%) were 1 of 4 fish in the treatment, meaning that there were no other conspecifics, which may have influenced both behaviour and physiological performance. A future improvement to similar experimental designs would be to include lower conspecific ratios that still had at least two of the same species, potentially reducing stress and abnormal social behaviours.

The temperate rocky reef ecosystems that characterise the coastline of SE Australia are undergoing rapid changes due to rising sea-surface temperatures, a strengthening poleward-sliding boundary current and the consequential arrival of tropical organisms that cause a shift in ecological interactions in their recipient community (Ridgway, 2007; Booth *et al.*, 2007; Feary *et al.*, 2014). Winter-water temperatures restore balance within these ecosystems, however there are extended periods of time during the summer and autumn months that coincide with the recruitment period of tropical vagrants such as *A. vaigiensis*, where water temperatures can exceed 24°C (AIMS, 2023). In this study we have shown how temperature regulates the physiological and behavioural performance of *A. vaigiensis* and *M. joyceae* across a range of temperatures experienced in temperate SE Australia, and how predation threat can alter performance metrics, and ultimately showing opposite trends in predation-induced mortality. A higher proportion of tropical vagrants in a mixed-shoal also showed to have significant effects on fish behaviour, often displaying enhanced performance metrics to a single-species shoal of *A. vaigiensis*. In Smith *et al.* (2018) *A. vaigiensis* juveniles were observed to attain larger body sizes when shoaling with temperate local species as opposed to vagrant tropical species and therefore increased their chances of survival in a novel habitat. Results gathered in this study parallel these findings, and while it is commonly agreed, regarding the range-expansion of tropical fish, that the ability to survive winter-water temperatures remains the prominent barrier in the establishment (Booth *et al.*, 2007; Booth *et al.*, 2011). However, with the predicted effects of climate change with anticipated global increases in sea surface temperatures, the over-winter survival rate of tropical vagrants in expatriating to

temperate habitats is projected to increase significantly. Indeed, it is predicted by the year 2080 that winter-water temperatures will significantly increase the survivorship of tropical vagrants to nearly 100% (Figueira and Booth, 2010). As more recruits survive the winter, this will drive changes in recruitment and settlement patterns. The increased chance of predation on local temperate species during these period of higher water temperatures, coupled with mass recruitment of tropical vagrants, may result in *A. vaigiensis* juveniles outcompeting their temperate heterospecifics as they more easily avoid predation through enhanced escape speeds and feeding rates. Enhanced performance metrics obtained in mixed-species shoals comprising a higher ratio of *A. vaigiensis* may initiate a preference for new recruits to shoal with heterospecifics thereby helping them adapt and survive, potentially overcoming a major barrier to the permanent establishment of tropical fish species in temperate habitat, and ultimately a successful range expansion into higher latitude marine ecosystems.

CHAPTER 5: THE INFLUENCE OF LATITUDE AND SHOAL COMPOSITION ON THE GROWTH AND SETTLEMENT PATTERNS OF A TROPICAL VAGRANT FISH

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Abstract

Increasing global temperatures have catalysed mass range expansion among species globally, with many animals forced to exhibit physiological and behavioural plasticity as they shift poleward. Range-extending tropical fish species have established low density populations in the south-eastern temperate marine ecosystems of south-eastern Australia, facilitated by a strengthening poleward-flowing boundary current the East Australia Current. Of these recently established tropical fish species the Indo-Pacific sergeant major (*Abudefduf vaigiensis*) are the most commonly occurring and successful tropical vagrant colonisers in these temperate ecosystems. The establishment of tropical fishes in temperate novel ecosystems however is limited by environmental and ecological barriers, which have been shown to have adverse effects on the physiology these tropical recruiting vagrants, predominantly reduced performance. Previous research has also shown that shoaling with local temperate species may enhance the growth rates of recently recruited *A. vaigiensis* juveniles therefore increasing their survivability in a foreign habitat. This study aims to determine the influence of latitude and shoal structure on growth rates, as well as settlement patterns, of *A. vaigiensis* along an 820km latitudinal gradient of their current range, primarily using body size and otolith growth structures as a proxy for post-settlement growth performance. The density of single-species shoals of *A. vaigiensis* was higher at lower latitudes, and these patterns decreased at higher latitudes. Latitudinal location of *A. vaigiensis* populations had a significant effect on the growth rates of recently recruited juveniles, as post-settlement daily ring

increment widths (proxy for somatic growth) were greater at lower latitude sites closer to their natal range. At the range-periphery site there was also an effect of mixed-shoaling, as *A. vaigiensis* from mixed-shoals yielded higher post-settlement increment widths (growth) compared to those developing in single-species shoals, indicating possible advantages of shoaling with local species when expatriated outside of optimal geographic range. Reducing time spent in predator-vulnerable size classes is a critical factor in tropical vagrant fish expanding their range into temperate habitats and establishing permanent populations in higher latitude locations. Environmental conditions and ecological processes will both play a major role in the tropicalisation of temperate marine ecosystems in SE Australia.

5.1 Introduction

Environmental conditions during the Anthropocene have greatly amplified the pressure of maintaining functioning and sustainable metabolic function in a wide array of organisms, predominantly affected by increasing global temperatures (Walther *et al.*, 2002; Hofmann and Todgham, 2010; Begon and Townsend, 2020). As organism function, whether endothermic or ectothermic, is closely linked to environmental temperature, projected global temperatures increases of 2°C by 2100 (Hoegh-Guldberg *et al.*, 2018) are anticipated to have profound impacts on organism physiology and behaviour. Many studies have already reported mass range shifts among species in response to rapidly changing climatic conditions (Poloczanska *et al.*, 2013; Lenoir *et al.*, 2020), predicting wide-spread local extinctions in the near future (Román-Palacios and Wiens, 2020).

The effects of increased environmental temperature begin at cellular level, catalysing accelerated metabolic processes that manifest into altered physiological and behavioural metrics (Pörtner and Knust, 2007), which has greater ramifications for ectothermic animals due to their positive homeostatic relationship with ambient temperature, which is primarily maintained through behavioural thermoregulation (Losos *et al.*, 2008; Begon and Townsend, 2020). Range shifts are a common ecological response in many animal taxa to warming environmental conditions (Parmesan *et al.*, 1999; Angilletta, 2009; Peterson and Lieberman, 2012; Pecl *et al.*, 2017), with recent studies showing accelerated rates of marine organism range shifts and extensions compared to terrestrial shifts at a rate approximately 20x greater (Lenoir *et al.*, 2020). Studies have shown marine fishes are expanding their historical ranges in response to rising sea surface temperatures (Drinkwater, 2005; Wernberg *et al.*, 2013; Fogarty *et al.*, 2017; Fowler *et al.*, 2018), often resulting in maladaptive responses in either the range-shifting species themselves, or the ecological functions of the recipient ecosystem. In 2005, Drinkwater (2005) reported a range shift of Atlantic cod (*Gadus morhua*) fishery to sub-arctic regions in response to rising sea surface temperatures, having major economic consequences. Raitso *et al.* (2010) showed that prolonged warming of the marine ecosystems in the Mediterranean Sea has led to the increased

settlement of foreign tropical fish species resulting in the over-grazing of habitat-forming algae species and the increase of low-productive sea urchin barrens.

Poleward-flowing boundary currents have facilitated the range expansion potential of many tropical fish species into novel temperate habitats (Yamano *et al.*, 2011; Verges *et al.*, 2016; Lefcheck *et al.*, 2024), triggering cascading ecological responses and the tropicalisation of sub-tropical and temperate marine regions in North America, Japan and Australia. For example, the Leeuwin Current and East Australia Current (EAC) transport tropical fish larvae from low latitude tropical regions to high latitude sub-tropical and temperate marine ecosystems, with recent studies showing shifting responses in the recipient communities (Cheung *et al.*, 2012), ranging from small scale species interactions to community phase shifts and the loss of habitat due to the increased influx of tropical herbivorous fish species (Wernberg *et al.*, 2013; Matis, 2018; Hayes *et al.*, 2024). The EAC, a poleward flowing boundary current extending over 4000km along Australia's eastern coastline, transporting tropical biota from the Great Barrier Reef (GBR) to southern temperate marine ecosystems within south-eastern (SE) Australia, has increased its poleward expansion potential by approximately 350km over the past 80 years, resulting in community changes of fish assemblages in south-eastern latitudes (Ridgway, 2007; Ridgway, 2009). The displaced tropical fish species, termed 'vagrants', are widespread over several tropical families; the most commonly abundant is the Indo-pacific sergeant major (*Abudefduf vaigiensis*), being recorded as far south as Merimbula at 37° latitude (Booth *et al.*, 2007; Coni *et al.*, 2022 a). Influxes of tropical vagrant fish settlement in SE Australia coastal rocky reef ecosystems occur predominantly during the Austral summer months (January – April), with post-larval recruits arriving in novel rocky-reef temperate habitats (Booth *et al.*, 2007; Booth *et al.*, 2011). Post recruitment, these juvenile tropical vagrants encounter a series of limiting environmental and ecological factors, the primary limiting factor to their survival being winter-water temperatures, annually resulting in mortality rates as high as 99% among these tropical fish species (Figueira *et al.*, 2009). Figueira and Booth (2010) demonstrated that cooler water temperatures of approximately 17°C is the critical thermal minima of most tropical vagrant fishes. Prolonged exposure to these temperatures resulted in the reduction of aerobic scope

(energy available for critical functions), and in *A. vaigiensis* has been shown to induce decreased feeding rates, burst swimming speed and somatic growth rates (Djurichkovic *et al.*, 2019).

The commonly-occurring tropical schooling vagrant *A. vaigiensis* often form mixed-species shoals with local phenotypically similar fish species including the Stripey (*Microcanthus joyceae*) and Mado (*Atypichthys Strigatus*) when recruiting into novel temperate ecosystems (Paijmans *et al.*, 2020; Kingsbury *et al.*, 2020a; Hayes *et al.*, 2024), triggering a series of behavioural trade-offs that result in both benefits and cost to individual fitness and therefore survival. Niche segregation, diet partitioning, formation of sub-shoals and the expression of behavioural plasticity of *A. vaigiensis* in mixed-species shoals in temperate range periphery populations provide strong evidence of the survival tactics employed that enable adaptation in a foreign habitat characterised by cooler water regimes (Kingsbury *et al.*, 2020a; Coni *et al.*, 2021; Paijmans *et al.*, 2021; Coni *et al.*, 2022b). As *A. vaigiensis* recruit into habitats as juveniles, they are settling into a novel ecosystem at a particularly vulnerable size class that is prone to predation, and as mortality in fishes is often size dependent (Sogard, 1997), food acquisition and consequential somatic growth rates are critical in permitting long-term survival. Smith *et al* (2018) reported that recently settled *A. vaigiensis* in mixed-species shoals attained larger body sizes over the course of the recruitment season compared to those shoaling with either conspecifics or other tropical vagrants, implying that there are energetic benefits to shoaling with heterospecific temperate species, significantly impacting their survivability and range expansion potential. While the authors noted that sizes were estimated *in situ*, the historical growth rates of fish can be accurately assessed by another methodology; the removal and counting of growth rings of their otoliths, a calcium carbonate ear stone structure that can assist in the quantifying of body condition and performance in fish species (Campana and Neilson, 1985; Morales-Nin, 2000).

The early stages of settlement in novel ecosystems are a critical event for juvenile tropical vagrant species such as *A. vaigiensis* as they face multiple physiological and ecological hurdles such as low water temperature, habitat availability and interactions with local species (Figueira *et al.*, 2009; Feary *et al.*, 2014; Beck *et al.*, 2016). The early

life history traits (ELHT) of *A. vaigiensis* recruits, such as planktonic larval duration (PLD), body condition, size-at-settlement and growth rates post-settlement during the settlement phase will significantly influence their survival and successful establishment in a temperate novel ecosystem (Smith *et al.*, 2018; Kingsbury *et al.*, 2020b; Rigg *et al.*, 2025). Analysis of the otolith microstructure can provide key insights into these ELHT physiological factors, allowing the assessment of age and growth rates by counting and measuring the daily increment rings (Panella, 1971). Fluctuations in environmental conditions have been shown to directly affect otolith formation, catalysing recurrent depositions of calcium carbonate crystal, resulting in the development of translucent opaque rings separated by zonation, referred to as growth increments (Morales-Nin, 2000). Environmental factors, predominantly temperature, influence the growth rate of the otolith (the increment widths), as the otolith size is directly linked to the fish's size; periods of faster growth (often associated with warmer temperature regimes) result in larger increment widths and periods of slower growth (cooler water temperatures) result in small increment widths (Morales-Nin, 2000). By counting these growth increments, the age of the fish can also be hindcasted, as many species exhibit daily depositions of growth increments (Pannella, 1971; Brunton and Booth, 2003). A recent study has shown that growth rings are deposited daily in *A. vaigiensis* otoliths (Rigg *et al.*, 2024) and indicated that persistence in a temperate habitat among *A. vaigiensis* population in range periphery locations is facilitated by faster larval growth prior to settlement and shorter PLD's (Rigg *et al.*, 2025). This study emphasises the importance of using the otolith microstructure as a proxy for growth performance and investigating ELHT's to make predictions regarding the range expansion potential of a tropical vagrant fish species.

In the present study we investigated the influence of latitude on shoaling patterns of tropical vagrant *Abudefduf vaigiensis*, and how this impacts their post settlement growth performance (using otolith analysis as a proxy for somatic growth). To determine this, we carried out surveys and extracted specimens for otolith extraction from single-species and mixed-species shoals at three latitudinal regions along an 820km latitudinal gradient encompassing a vast area of their geographic range. We predicted that 1) *A. vaigiensis* density will be higher at lower latitudes (closer to their

natal range), in addition to this there will be higher numbers of single-species shoals compared to mixed-species shoals, 2) Fish growth rates (using otolith increment widths as a proxy) will be higher for samples taken from the lower latitude region compared to the higher latitude regions, and will show a difference depending on whether the fish was taken from a single-species or mixed species shoal. Linear regression models of age vs standard length (SL) will be analysed to determine size-at-age, as we predicted that *A. vaigiensis* juveniles from lower latitudes will have a larger size-at-age compared to those from higher latitudes and therefore enhanced growth rates.

5.2 Methods

5.2.1 Study sites

Three latitudinal regions were selected among rocky reef and bund wall habitats along the SE coastline, spanning an area (approximately 820km in length) that encompasses a large range of habitats *A. vaigiensis* recruit and establish populations in (**Figure 5.1**): Tweed heads (-28.169686 S°, 153.552617° E), Nambucca (-30.647233° S, 153.010362° E) and South West Rocks (-30.884262° S, 153.038152° E) and several sites within the greater Sydney region (-33.876984, 151.295088). The low latitude region, Tweed Heads, is located within the viable population range of *A. vaigiensis* (Booth *et al.*, 2011), and because of its proximity to the likely source of the recruits, the GBR, and therefore closer to optimal environmental conditions. The mid-latitude region, Nambucca and South West Rocks, were selected along this latitudinal gradient due to the consistent annual settlement of recruits of *A. vaigiensis* that show evidence of regular over-wintering of juveniles that form non-breeding adult populations (Booth *et al.*, 2007; Coni *et al.*, 2021; Mitchell *et al.*, 2025). The high latitude region, Sydney, was selected due to its location being within the range-periphery of *A. vaigiensis* that experiences regular settlement of recruits and occasional over-wintering (Booth *et al.*, 2007). Sites within the Sydney region included: North Narrabeen lagoon (-33.702537 S, 151.306757 E), Freshwater (-33.781712 S, 151.294371 E), Little Manly (-33.807094 S, 151.285408 E) and Long Bay (-33.964945 S, 151.255405 E) and Little Bay (-33.979440 S, 151.251579 E). Survey sites to assess the abundance and structure of shoals containing

A. vaigiensis were conducted over 250m transects at 1 selected site within each latitudinal region (**Figure 5.2**); Little Duranbah beach (**Figure 5.2a**, -28.1686 S, 153.5514 E) on the northern side of the Tweed River entrance, the V-wall (**Figure 5.2b**, -30.6479 S, 153.0103 E) at the entrance of the Nambucca River and Little Bay (**Figure 5.2c**, -33.979440 S, 151.251579 E) located among Sydney's southern beaches. All survey data was carried out during 2024 and 2025: for the mid and low latitude, surveys were conducted in July, September and November of 2024, and an additional month in February 2025, and for the high latitude monthly surveys were carried out during the year of 2024.

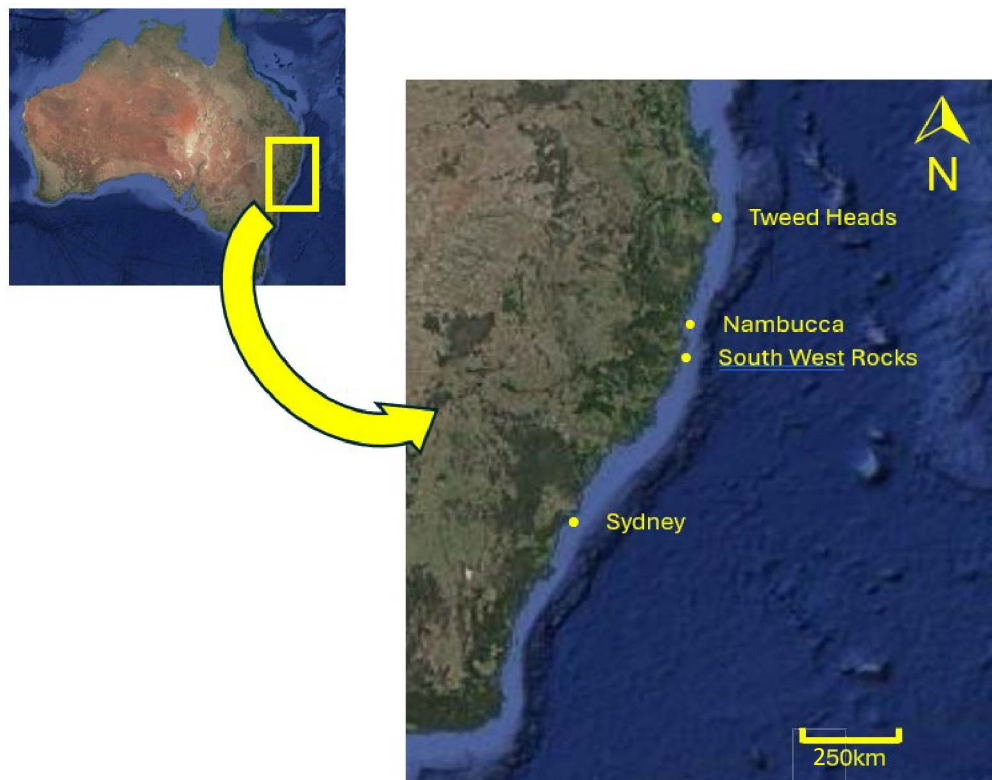


Figure 5.1. Satellite map of the geographical locations of the study sites along the SE coastline of Australia. High latitude data was gathered from Sydney, Mid latitude data from Nambucca and South West Rocks and Low latitude data from Tweed Heads. Source: Google Earth © (2025).



Figure 5.2. Satellite images with transect paths ($\approx 250\text{m}$ length $\times$ 2m wide) of surveys sites within each latitudinal region. a) Little Duranbah beach (Tweed Heads, Low latitude region, b) The V-wall (Nambucca, Mid-latitude region) and c) Little bay (Sydney, High latitude region. Source: Google Earth © (2025).

5.2.2 Experimental design

To investigate the effect of latitude on the shoal composition and abundance of *A. vaigiensis*, surveys were conducted at the 1 site within the three latitudinal regions (Low latitude region: Little Duranbah beach, Mid latitude region: the V-wall and High latitude region: Little Bay) along the shoreline embayment and artificial sea wall structures (depth ranging $0\text{--}2\text{m}$), spanning 2m wide and approximately 250m long (area covering $\approx 500\text{m}^2$). During surveys, shoals containing at least one *A. vaigiensis* were identified and recorded, with a shoal being defined as 2 or more individuals being in relative proximity to each other (within 5 body lengths) for a temporal period of 15s or more (Herbert-Read *et al.*, 2011; Paijmans *et al.*, 2020). When a shoal containing *A. vaigiensis* was identified, they were filmed for 2mins using a SeaGIS camera housing unit (containing 2 GoPro Hero Blacks 7's) approximately 2m away from the shoal for accurate analysis of shoal parameters to be conducted, and to allow for the possibility

of sheltering shoal members to be identified. The shoal parameters recorded during surveys were as follows; shoal type (single-species or mixed-species), shoal size (total number of individuals comprising shoal), species within the shoal (in mixed-species shoals), number of *A. vaigiensis* within the shoal (measured as a percentage in relation to total number in shoal) and the individual size of *A. vaigiensis* in shoals classified into 3 categories of small (S, 10-25mm), medium (M, 25-40mm) and large (L, > 40mm). Body sizes were visually assessed using a pre-cut sticks to approximately estimate size classes.

To investigate the effects of latitude and shoal structure on the post-settlement growth rates of *A. vaigiensis* juveniles, fish were harvested from the three latitudinal regions previously mentioned (**Figure 5.1**), captured by hand net after being anaesthetised using a solution of clove oil and 95% ethanol in a ratio of 1:10 to immobilise fish, administered by a spray gun atomiser that has been safely used in previous studies (Figueira *et al.*, 2009; Djurichkovic *et al.*, 2019). When a shoal containing *A. vaigiensis* was identified (either single-species or mixed-species), one *A. vaigiensis* juvenile per shoal was captured approximately 2 weeks after settlement (this was assessed by size and based on pilot survey data carried out prior to capture): this was to allow for sufficient growth to occur after settlement and to allow for potential effects of shoal type. Once the focal fish was captured, they were promptly euthanised by being transferred to a freshwater ice slurry, then placed in individual numbered zip-lock bags to ensure they could be easily identified from which shoal they belonged to in later analysis. In total there were 117 fish collected from the high latitude Sydney region (SS: n = 62, MS: n = 55), 82 from the mid-latitude region (SS: n = 39 SS, MS: n = 43) and 62 from the low-latitude region (SS: n = 40, MS: n = 22).

5.2.3 Otolith preparation

Post capture, fish were brought to UTS laboratories to be weighed (to the nearest 0.001g) and measured (to the nearest 0.1mm) for standard length (SL) and total length (TL). Recorded SL of *A. vaigiensis* juveniles harvested from the high latitude Sydney region ranged from 12mm to 31.1mm (mean 20.59mm, SE $\pm$ 0.49mm), from the mid-latitudes sites ranged from 16.1mm to 34.1mm (mean 23.88mm, SE $\pm$ 0.5mm) and

from the low latitude sites ranged from 12.3mm to 30.2mm (mean 19.46mm, SE $\pm$ 0.73mm).

Fish were dissected with both left and right sagittal otoliths removed from the vestibular labyrinth in the inner ear within the skull, after which they were mounted on a glass slide using a thermoplastic cement. Using a fine-grit lapping film (3 μ m) sagittal otolith were polished until a thin transverse section remained creating a planar surface, referred to as the incremental plane (Camapana, 1990). The mounted otolith was occasionally exposed to water during polishing to reduce heat created by polishing and to minimise scratching of the otolith surface. Polished otoliths were then analysed using a Nikon Eclipse Ci compound microscope with an attached TruChrome camera that captured images of the otolith in high definition at 400x magnification. A still image of the otolith was taken and using TCapture 5.1.1 software allowed clear photographs of the otolith to be taken, and permitted manual adjustments of zoom, light and saturation. To allow for measurements to be taken, a still image of a calibration slide was first taken to permit the calibration of distance measurements to be taken. To assess the growth performance of *A. vaigiensis* juveniles, several variables were measured:

- Total number of growth rings to determine age, which was later regressed against standard length
- The settlement mark (dark ring band followed by noticeably smaller ring increments) and the increment distance between rings

All measurements were taken along the same axis of the sagittal otolith, from the centre of the primordium to the furthest edge to ensure consistency in ring width increments. In *A. vaigiensis*, it has been shown that otoliths rings are deposited daily (Rigg *et al.*, 2024), therefore the age of the fish was determined by counting the total number of rings, as well as the number of days that had elapsed post settlement. Growth rate was determined by measuring the width of the rings beyond the post settlement mark (defined by the point at which growth rings rapidly decrease in width). For samples harvested from the high latitude site (Sydney) the post settlement rings varied between 9 – 29 days, at the mid latitude sites (South West Rocks and

Nambucca) 9 – 26 days and at the low latitude site (Tweed Heads) 9 – 18 days post settlement.

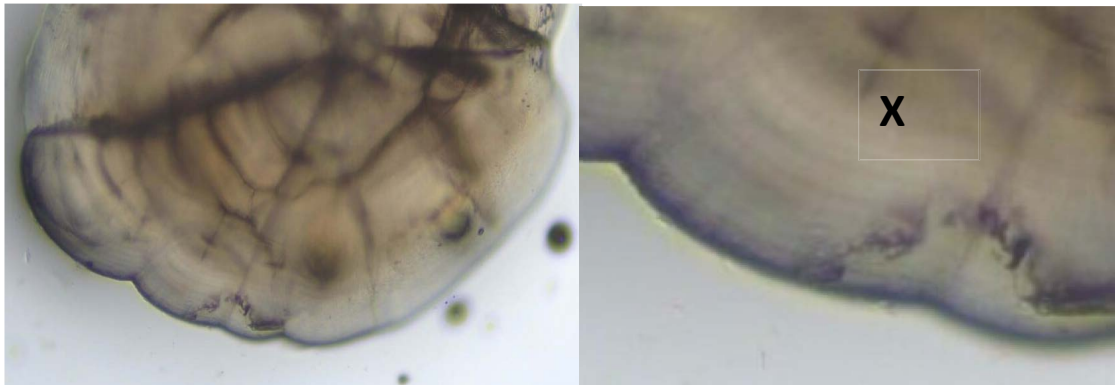


Figure 5.3. Captured image of *A. vaigiensis* otolith, with zoomed in image on right indicating the settlement mark (X) and the proceeding smaller post-settlement increment rings.

5.2.4 Statistical Analysis

To determine the influence of latitude on shoal structure and abundance of *A. vaigiensis*, a multivariate General Linear Model (GLM) was run comparing the survey results of the high latitude site during February, July, September and November 2024 and the mid and low latitude months of July, September, November 2024 and February 2025. The following shoal parameters were run in the model; using density of single-species shoals (SS), density mixed-species shoals (MS), total abundance of *A. vaigiensis* observed and total abundance of other species observed as response variables and latitudinal site and month as fixed factors. To measure the effect of growth performance across latitudinal range, a Generalised Linear Model (GLMs) was run using mean increment width post-settlement as the response variable, latitude as a fixed factor and total days post settlement and SL as covariates. To analyse the effects of latitude on size-at-age to support post settlement growth data, a linear regression model was run with a GLM using standard length (SL) as the response variable, latitudinal site as a fixed factor and fish age (total number of otolith rings, which was standardised to the youngest fish captured (9 days) at each location) as a covariate. All data was inspected for normality. All statistics were performed using IBM statistics software SPSS v31.

5.3 Results

5.3.1 Temperature data

Water temperature data accessed from IMOS (2025) – ‘National Mooring Network Facility - Temperature and salinity time-series’ indicated that average monthly water temperatures over the course of the study were highest at the low latitude region (annual mean $24.42^{\circ}\text{C} \pm \text{SE } 0.56$) and decreased progressively at mid (annual mean $23.12^{\circ}\text{C} \pm \text{SE } 0.55$) and high latitude region (annual mean $22.25^{\circ}\text{C} \pm \text{SE } 0.64$) (**Figure 5.4**).

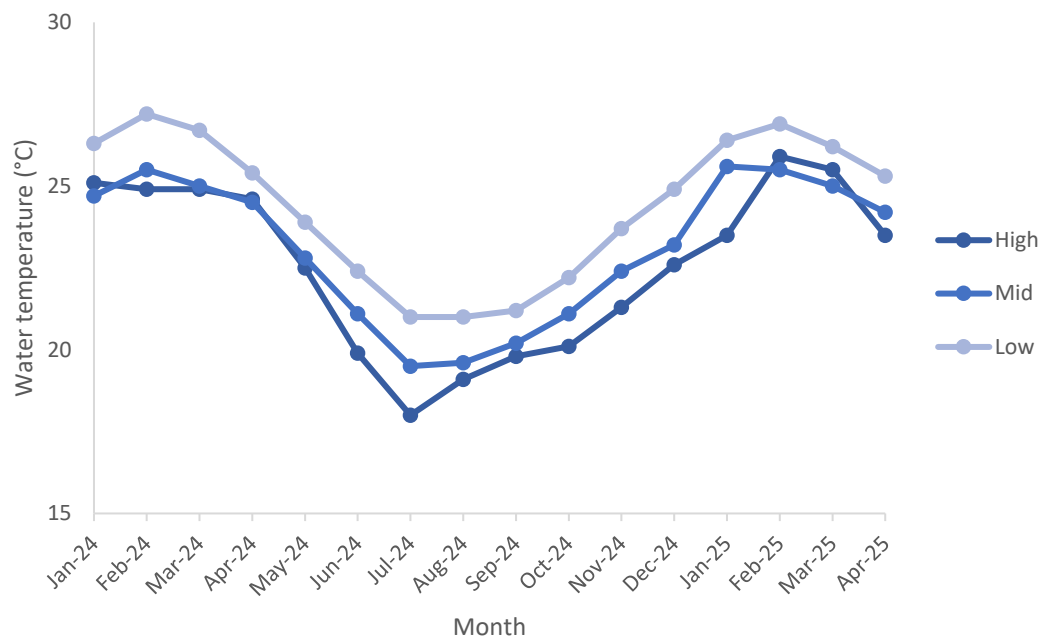


Figure 5.4. Mean monthly temperatures for High latitude (Sydney region), Mid latitude (Nambucca) and Low latitude (Tweed Heads). IMOS National Mooring Network Facility - Temperature and salinity time-series (2025).

5.3.2 Survey data: Density of *A. vaigiensis* shoals

Latitude had a significant effect on the density of *A. vaigiensis* single-species shoals (**Table 5.1**, $F_{2,11} = 40.22$, $p < 0.001$) and the total abundance of *A. vaigiensis* (**Table 1**, $F_{2,11} = 15.35$, $p < 0.05$). When comparing the presence of single-species shoals, the total number increased by over 6-fold, being 0.45 shoals/ m^2 at the high latitude and 2.95 shoals/ m^2 at the low latitude (**Figure 5.5**).

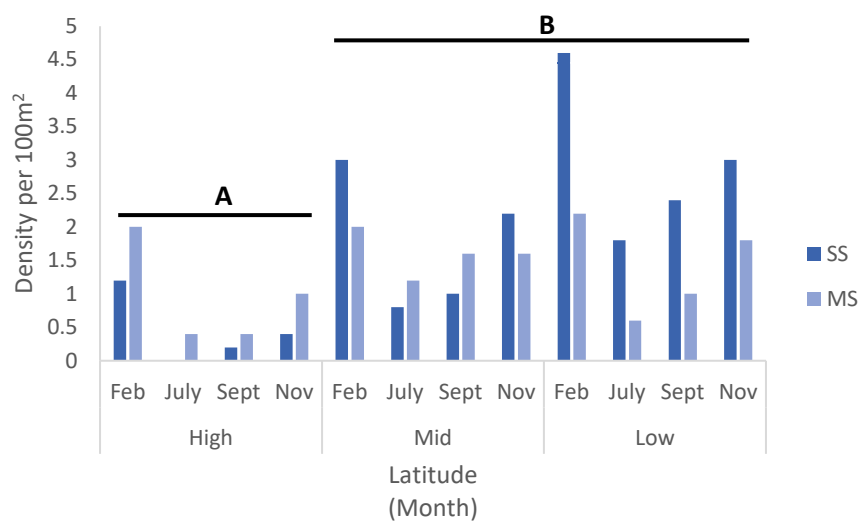


Figure 5.5. The total density per 100m² of single-species (dark blue) and mixed-species (light blue) shoals observed over the course of the survey period. Significant differences ($p < 0.05$) from post hoc indicated by letter groupings.

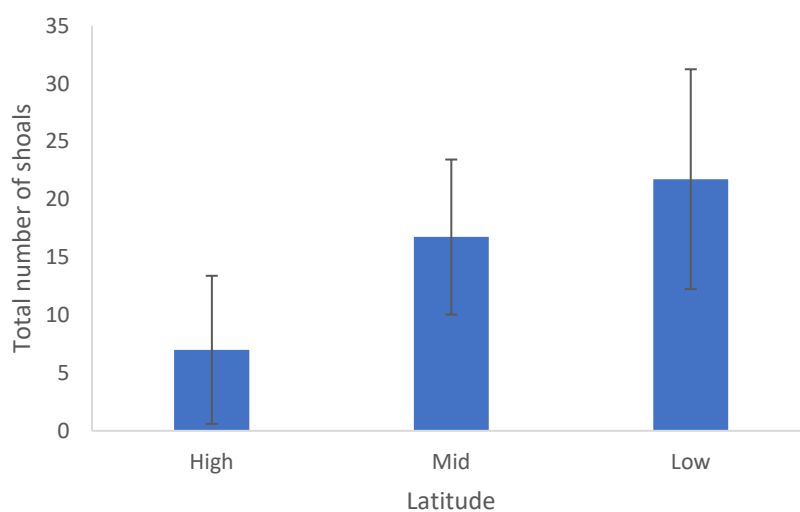


Figure 5.6. Mean (SE±) observed total of shoals (single-species and mixed-species) containing *A. vaigiensis* observed over the course of the survey period (all months combined).

Table 5.1. Multivariate GLM General linear model results for response variables: Single-species shoals of *A. vaigiensis*, Mixed-species containing *A. vaigiensis*, Total *A. vaigiensis* and Total of other species recorded shoaling with *A. vaigiensis*. The values displayed are the df followed by corresponding F statistic and p values.

Factor	<u>Single-species shoals</u>			<u>Mixed-species shoals</u>			<u>Total <i>A. vaigiensis</i></u>			<u>Total other species</u>		
	df	F	p	df	F	p	df	F	p	df	F	p
Latitude	2	40.2	<.001	2	4.807	0.057	2	15.35	0.004	2	7.59	0.023
Month	3	16.021	0.003	3	11.12	0.007	3	10.911	0.008	3	0.961	0.469
Error	11			11			335			11		

5.3.3 Size-at-age

Regression analysis showed that fish age and SL had a curve-linear relationship ($F_{2,260} = 66.315$, $p < 0.001$). A GLMs showed latitude a significant effect on the relationship between age and SL (**Table 5.2**, $p < 0.05$). Pairwise comparisons showed that significant differences between the high latitude compared to mid and low latitudes ($p < 0.001$), indicating that *A. vaigiensis* from the high latitude had a smaller size-at-age residual (**Figure 5.7**).

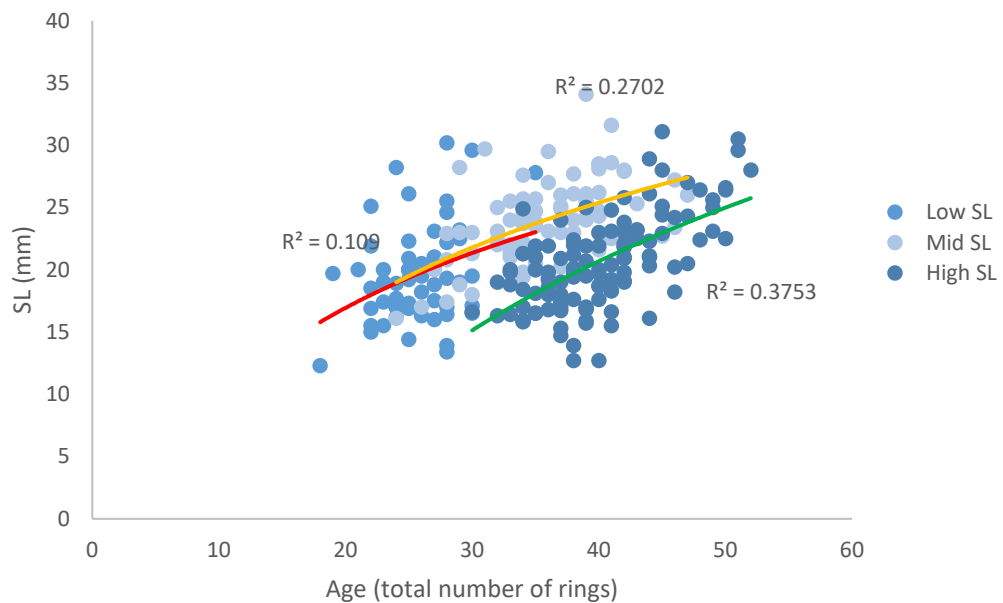


Figure 5.7. Age (total number of otolith rings) vs growth (fish SL mm) curve. *A. vaigiensis* juveniles from low latitude (Red linear curve), mid latitude (Yellow linear curve) and high latitude (Green linear curve).

Table 5.2. GLMs results for response variables: SL (Size-at-age). The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values

Factor	<u>SL (Size-at-age)</u>		
	df	χ^2	p
Latitude	2	117.084	< 0.001
Age	1	100.975	< 0.001
Error	257		

5.3.4 Post-settlement growth performance

There was an increase in post settlement increment widths with decreasing latitude, as the latitude was shown to have significant effect on the response variable (**Table 5.3**, $p < 0.001$). Pairwise comparisons showed that increment widths between all latitudes differed significantly ($p < 0.001$). Juveniles caught from the low latitude showed the highest mean post settlement increment widths at $4.54\mu\text{m}$ (range 2.25 to $7.53\mu\text{m}$, $\text{SE} \pm 0.1$), and lowest at the high latitude site with a mean value of $2.85\mu\text{m}$ (range 1.21 to $5.94\mu\text{m}$, $\text{SE} \pm 0.07$) (**Figure 5.8**). There was an additional effect of shoal type on post settlement increment (**Table 5.3**, $p < 0.05$). *A. vaigiensis* from mixed-species shoals had a higher mean post settlement increment width of $3.78\mu\text{m}$ compared to $3.57\mu\text{m}$ in single-species shoals (**Figure 5.8**).

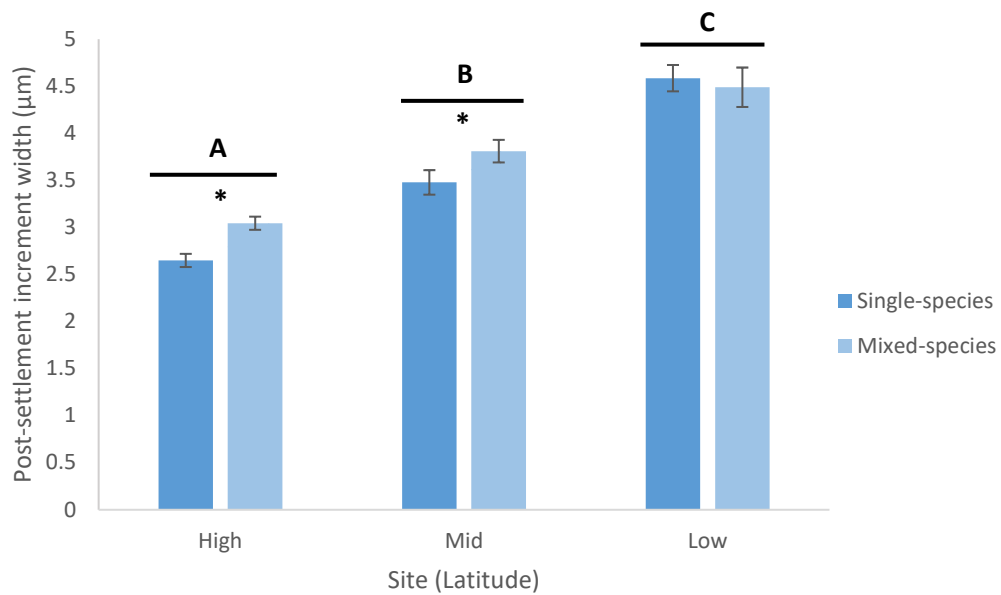


Figure 5.8. Mean (SE±) post-settlement ring increment widths of *A. vaigiensis* juveniles captured from 3 different latitudinal sites and 2 different shoal types; single-species shoals (dark blue) and mixed-species shoals (light blue). Significant differences ($p < 0.05$) between latitudes from post hoc indicated by letter groupings. * Denotes significant differences between shoal types ($p < 0.05$) from post hoc.

Table 5.3. Generalised linear model results for response variables: mean post settlement increment widths. The values displayed are the df followed by corresponding Type III Wald Chi-squared statistic and p values.

Factor	Increment widths		
	df	χ^2	p
Latitude	2	212.036	< 0.001
Shoal type	1	4.894	0.027
Latitude x shoal	2	4.596	0.062
SL	1	3.533	0.100
Error	255		

5.4 Discussion

The strong relationship between environmental temperature and ectothermic physiology and behaviour is well documented (Seebahcer, 2009; Schulte, 2015; Riemer *et al.*, 2018), regulating metabolic function that catalyses alterations of their

behavioural repertoire. Concurring with our hypothesis, we found that *A. vaigiensis* from the low latitude region grew faster than individuals. In addition to this, there was a greater overall proportion of single-species shoal formations of *A. vaigiensis*, and higher post-settlement rings increment widths when shoaling in mixed-species shoals in the high latitude region. This suggests that expansion in temperate ecosystems drives behavioural plasticity to support persistence in a habitat characterised by cooler-temperatures and novel ecological interactions. In line with our hypotheses, the density of *A. vaigiensis* single-species shoals was higher in the lower latitude region, with 2.95 shoals/m², 1.75 shoals/m² at mid latitude and 0.45 shoals/m² at the high latitude region. These results, while in agreeance with our hypothesis, may have potentially been driven by the lack of longer-term repeated surveys, possibly capturing smaller temporal trends that are influenced by temperature regimes and larval supply. Comparing the size of the fish (SL) to their age (total growth rings) did reveal a curve-linear relationship, and the position of the curve (far right) on the regression indicated that *A. vaigiensis* from high latitudes had a smaller size-at-age. These results may indicate the significance of pre-settlement growth rates and individual size-at-settlement in determining successful settlement during recruitment stages. Post-settlement growth rates also showed significant differences between sites, concurring with our hypothesis, suggesting that environmental and ecological processes following recruitment drives physiological performance for *A. vaigiensis* juveniles. At the high latitude sites within the Sydney region, recently recruited *A. vaigiensis* collected from mixed-species shoals showed higher mean ring-increments widths, suggesting that at range periphery locations shoaling with local species may yield fitness advantages of faster growth rates, reducing the time juvenile *A. vaigiensis* spend in vulnerable to predation size classes. Temperate and sub-tropical marine habitats are subject to greater variations in temperature regimes than the more thermally stable habitats in tropical latitudes (McGregor and Nieuwolt, 1998), with such variations potentially inducing oxygen limitation and thermal stress in organisms that possess relatively small thermal envelopes (Pörtner and Knust, 2007; Nilsson *et al.*, 2009). The displacement of tropical fish to temperate marine ecosystems may show susceptibility in an environment with greater temperature fluctuations that may place major stress

on physiological abilities, affecting body condition and growth (Relyea, 2002).

Environmental and ecological processes acting concurrently will partly regulate the range expansion potential of *A. vaigiensis* into SE Australia, however with predicted future increasing sea-surface temperatures inducing phenotypic and behavioural plasticity in these tropical vagrants is expected to alter local ecosystem mechanisms.

The early life history traits of fish, specifically planktonic larval duration, pre-settlement growth rates and size-at-settlement, drive post-settlement survivorship as pelagic larvae recruit into a benthic habitat results in multiple physiological and behavioural changes including growth rates, food preferences and competition for resources (Doherty and Fowler, 1994; Brunton and Booth, 2003; Meekan *et al.*, 2003). The effects of latitude on size-at-age analysis indicated that *A. vaigiensis* recruits have enhanced growth in low latitude regions, however this result may be driven by shooter PLDs and pre-settlement growth rates that determine size-at-settlement. Rigg *et al.* (2025) analysed the ELHT's of *A. vaigiensis* recruiting into SE Australia and insinuated that reduced PLD, coupled with faster pre-settlement growth rates may be the determining factors in long-term persistence in range periphery habitats. Faster growing larvae are more likely to be able to reduce time spent consuming algae in off-shore currents, thereby maintaining energy reserves, specifically aerobic scope, that will promote the allocation of energy into post-settlement growth rates that reduces time fish spend in a size class that is vulnerable to predation, which is often size-dependent (Sogard, 1997; Searcy and Sponaugle, 2001). The size of individual fish when they recruit into habitats therefore may not be truly indicative of their growth rates, and simply faster growing fish during larval stages may exhibit superior physiological advantages that increases their chances of survival. The variation we observed in size-at-age vs latitude may also indicate alternate sources for larvae, as previous studies observing tropical vagrant recruiting *A. vaigiensis* juveniles have theorised (Feary *et al.*, 2014; Matis 2018).

Otolith growth is isometric to somatic growth, so that in periods of augmented growth rates there will be a corresponding increase in ring increment widths (Gagliano and McCormick, 2004; Strelcheck *et al.*, 2003). The ectothermic nature of fish is defined by their positive metabolic relationship with temperature (Angilletta, 2009), and studies

have shown that otolith development is closely linked to their environmental temperature (Mosegaard *et al.*, 1988; Radtke and Shafer, 1992). Most tropical fish species have Type 1 post-settlement ring widths, with abrupt transitions in increment width profiles developing throughout their life history (Wilson and McCormick, 1999), primarily influenced by changes in environmental conditions and the metamorphic development of pelagic larvae into habitat-recruiting juveniles (Campana and Neilson, 1985; Wilson and McCormick, 1997). The settlement mark and corresponding post-settlement rings in otoliths of *A. vaigiensis* were easily identifiable in our analysis and showed significant differences in post-settlement growth rates among latitudes, indicating the existence of a latitudinal gradient with regards to physiological performance, increasing towards historical natal ranges in the tropics, which was supported by the water temperature results (IMOS, 2025). Warmer-water temperatures have been shown to increase the growth rates of *A. vaigiensis* juveniles (Figueira *et al.*, 2009); as temperatures approach a more optimum thermal envelope, the aerobic scope of *A. vaigiensis* increases allowing more energy to be allocated to feeding and consequent somatic growth (Djurichkovic *et al.*, 2019). This would explain the larger post-settlement ring increment widths we observed in juveniles harvested from lower latitude locations, with likely increased growth rates coupled with shorter PLD's possibly contributing to improved physiological performance post-settlement and increasing their likelihood of survivability (Gagliano *et al.*, 2007; Rigg *et al.*, 2025). At the highest latitude location, being the range-periphery site Sydney in this study, we also observed increased ring increment widths post-settlement in *A. vaigiensis* juveniles harvested from mixed-species shoals compared to those from single-species shoals. The formation of mixed-shoals has been shown to reduce within-shoal competition through sub-group formations and foraging niche segregation, reducing foraging pressure by altering temporal and spatial feeding regimes, promoting diet portioning (Krause, 1993; Alatorre-Ramirez *et al.*, 2017; Kingsbury *et al.*, 2020 a). In Smith *et al* (2018) the authors determined that *A. vaigiensis* schooling in mixed-shoals attained larger body sizes over the course of the recruitment season (February – April) in a range periphery habitat when compared to those in single-species shoals. The results attained in Smith *et al* (2018) concur with our study which specifically

measured growth rates, indicating that *A. vaigiensis* from mixed-species shoals in novel ecosystems are at an advantage in novel temperate ecosystems. In addition to this, *A. vaigiensis* shoaling with local temperate species may have access to higher rates of information transfer about a foreign habitat, giving access to superior foraging patches, sheltering resources and advanced predator detection (Paijmans *et al.*, 2019).

A major factor we could not control when designing and carrying out this study was potential of species, either the targeted *A. vaigiensis*, or heterospecifics, moving between shoals and influencing the behaviour and structure of nearby shoals. To control for this, we standardised the post-settlement ring increment width data to the first 9 days (youngest sample taken) post the settlement mark, thereby reducing the chance of fish moving between shoals. During early settlement phases and early juvenile life stages, our target species, and many of the tropical and temperate heterospecifics they commonly shoal with along the east coast of Australia (*M. joyceae*, *A. strigatus* and *A. sexfaciatus*) (Paijmans *et al.*, 2020; Coni *et al.*, 2022a), show strong site attachment (Mitchell *et al.*, 2025) and during this vulnerable phase where predator avoidance is paramount, the shifting of species between shoals is unlikely. In addition to this, surveys conducted within the latitudinal regions showed overall low occurrence of shoals (single-species or mixed-species) containing *A. vaigiensis* across the surveys transects: shoal density (per 500m²) within high latitudes was 3.2m², within mid latitude sites 5m² and low latitude sites 6.8m². For juvenile site attached species such sparseness of relevant shoals (to this study) reduced the likelihood of pseudo-effects that shoal member shifting may cause. It is important to note however, that such movement between shoals is still a possibility, however low, and may be a focus of future studies to fine-scale temporal surveys and collections to further reduce the chances of results being affected by movement between shoals.

In this study we have found evidence of growth and shoaling patterns along a latitudinal gradient that encompasses subtropical and range periphery populations of *A. vaigiensis*. The greater number of *A. vaigiensis* in single-species shoals in low latitude regions suggests both a preference for shoaling with conspecifics in optimal thermal conditions, and likely greater supply of pelagic larval settlement, and less abundance of common temperate heterospecifics that populate temperate rocky

reefs. The concurrent increases in growth performance with decreasing latitude is likely due to the optimal thermal conditions, and potentially superior food sources and shelter. Mixed-species shoaling has been shown to enhance growth rates for *A. vaigiensis* juveniles (Smith *et al.*, 2018), which was observed in this study using post settlement daily otolith rings as a proxy for growth, however the overall smaller ring increments observed at the high latitude regions suggests reduced fitness, with the formation of mixed-shoals potentially forcing species into suboptimal habitats (Taylor and Figueira, 2021). As sea surface temperatures rapidly increase in these regions, particularly in SE Australia, range expanding tropical fish will likely benefit, with permanent viable populations predicted to be established by the year 2080 due to increases in winter-water temperatures that will reduce cold stress mortality (Figueira and Booth, 2011).

CHAPTER 6: General Discussion

Environmental temperature is the primary factor that regulates animal behaviour and physiology, governing cellular reactions that manifest to individual species fitness, and scaling to populations dynamics that determine thermal geographic ranges (Schmidt-Nielsen, 1997; Pörtner, 2001; Bicego *et al.*, 2007; Bozinovic *et al.*, 2011). As climate change facilitates the redistribution and range expansion of animals into novel habitats, there will be subsequent ecological interactions with previously associated species, intensifying the rate of physiological and behavioural plasticity among impacted organisms and the restructuring of trophic and ecological dynamics (Le Roux and McGEOCH, 2008; Lancaster *et al.*, 2015; Poloczanska *et al.*, 2016; Usui *et al.*, 2023). For schooling fish, forming aggregations with local species may mitigate the effects of novel environmental conditions and enable successful range expansions into trailing edge habitats (Smith *et al.*, 2018; Pajmans *et al.*, 2020; Coni *et al.*, 2021; Taylor and Figueira, 2021; Chen, 2022). This thesis examined the role temperature, shoaling structures and behavioural trade-offs play in regulating the fitness and survival of a range expanding tropical reef fish at their trailing edge (Chapters 2, 3, 4 and 5), the Indo-Pacific sergeant major (*Abudefduf vaigiensis*), building on previous work to better understand predictive success regarding temperate rocky reef ecosystems management in the future. In Chapter 3 we investigated the existence of latitudinal gradients in shoaling performance of varying structures, with focus on trade-offs that occurred between foraging and predator avoidance, with our results suggesting that at trailing range edge habitats of their range, *A. vaigiensis* prioritised predator avoidance over food acquisition. In addition to this, forming mixed-species shoals with local temperate fish enabled specific benefits in high latitude locations regarding feeding and risk aversiveness, however, increased aggressive interactions likely due to competition for shelter and food. A laboratory experiment in Chapter 4 was designed to emulate the parameters investigated in Chapter 3, with additional focuses examining the relationship among water temperature, shoaling ratios (conspecifics to heterospecifics) and predation on the physiological and behavioural performance of *A. vaigiensis*. We showed that warm water temperature was a primary influencing factor

that governed the fitness of *A. vaigiensis* and increased their probability of survival when exposed to a live predator. Supplementary to these findings, we provided evidence of specific shoaling ratios in mixed-species shoals with a local heterospecific maximised several performance metrics. In chapter 5 we isolated growth as the dependent variable to determine the effects of latitude and shoal structure on the post settlement growth of *A. vaigiensis* using otolith increment width as a proxy. Furthermore, surveys of shoals containing *A. vaigiensis* were analysed at the same latitudinal location used in chapter 3 to determine trends in abundance and shoal structure. We found strong evidence that both latitude and shoal structure influence post settlement growth rates in *A. vaigiensis*, and that joining mixed shoals at high latitudes/trailing edge habitats results in increase growth rates and likely fitness. Single-species shoals and abundance of *A. vaigiensis* also increased with decreasing latitude, most likely due to proximity to core range habitats. Overall, this thesis found that both water temperature and shoal structure have strong influence on the physiology behaviour of *A. vaigiensis*, and that mixed-shoal formations enable additional fitness benefits, particularly towards range edges, that will be critical in their future settlement and range expansion of temperate ecosystems, which must be considered in the management of these coastal rocky reefs in the future.

6.1 The influence of water temperature on performance

Previous research in this field has highlighted the importance of water temperature on *A. vaigiensis* performance, concluding that warmer water temperatures result in increased metabolic, physiological and behavioural performance (Figueira *et al.*, 2009; Figueira *et al.*, 2019; Djurichkovic *et al.*, 2019). Our findings concurred with our hypotheses and were support by previous studies, with warmer water temperatures resulting in augmented growth rates (Chapters 4 and 5), increased foraging (Chapter 4) performance and enhanced predator avoidance strategies (Chapter 4) (**Figure 6.1**). Water temperature data in chapter 5 showed increases at lower latitude locations, which was mirrored by the post settlement growth increment widths of *A. vaigiensis* juveniles, indicating a positive relationship with water temperature and growth performance. In Chapter 4, the 25°C water temperature treatment also showed a reduction in the growth and consequent loss in body weight performance of the

temperate heterospecific *Microcanthus joyceae*, suggesting that at higher water temperatures tropical vagrants may outcompete cold-water affiliated species during the Austral summer months at high latitudes. This was also supported by the predation data, where *A. vaigiensis* and *M. joyceae* showed an inverse performance relationship with temperature when exposed to a cold-adapted predator *Sepia plangon*, concurring with the respective preferred thermal niches of each species as indicated by the somatic growth rates. High latitude locations in SE Australian coastal marine ecosystems are subject to increasing sea surface temperatures in the coming decades, which will suit the thermal envelopes of warm-affiliated species in summer seasons, possibly enabling a window of improved performance with respect to local temperate species and therefore larger settlement populations in the future.

Performance metric (vs Low latitude and 25°C)	Shoal type	NORMAL				PREDATOR			
		Factor				Factor			
		Latitude		Temperature		Latitude		Temperature	
		High	Mid	17	21	High	Mid	17	21
Growth rate		↙	↙	↙	No effect	Not measured			
Bite rate		↙	↙	↙	No effect	↙	↙	↙	No effect
Boldness		↙	↙	↙	No effect	↙	↙	↙	No effect
Growth rate		↙	↙	↙	No effect	Not measured			
Bite rate		↙	↙	↙	↙	↙	↙	↙	No effect
Boldness		↙	↙	↙	↙	↙	↙	↙	↙

Figure 6.1. Summary of results obtained from Chapters 3, 4 and 5 regarding the physiological (growth) and behavioural performance (Feeding and boldness) of *A. vaigiensis* (in single-species (two identical fish) and mixed-species (two different fish) shoals), comparing low latitude sites to mid and high latitude sites (Chapters 3 and 5) and highest water temperature treatment (25°C) to lower water temperature treatments (21°C and 17°C) (Chapter 4). Downwards arrows represent a significant decrease in performance compared to other treatments, and “No effect” represents no change compared to other treatments. Summary also compares data obtained from normal and feeding behaviours to behaviours observed post predator exposure. Note that for growth rates at latitudes refers to post-settlement ring increment widths (Chapter 5) and does not reflect the instantaneous growth rates that were measured in Chapter 4.

6.2 Latitudinal gradients in performance

Environmental conditions within species' core range reflect optimal thermal windows and subsequently maximise animal performance and fitness (Walczyńska *et al.*, 2016; Donelson *et al.*, 2019). For a coral reef dwelling species such as *A. vaigiensis*, core or natal ranges are primarily located within the tropics, therefore performance metrics are correlated with latitude, as has been in previous studies investigating the physiological and behavioural traits exhibited by *A. vaigiensis* (Paijmans *et al.*, 2020; Coni *et al.*, 2021; Coni *et al.*, 2022a; Coni *et al.*, 2022b; Mitchel *et al.*, 2025). In chapters 3 and 5 we found that populations of *A. vaigiensis* that were within proximity of the predicted core range showed increased growth and feeding rates, and towards trailing edges the exhibition of risk aversive behaviours became more dominant as fish showed plastic behavioural trade-offs in food uptake and predator avoidance (**Figure 6.1**). The faster growth rates observed (Chapter 5) at the low latitude site would suggest that foraging efficiency increases with correlated warmer water temperatures, which was support by the mean monthly temperature data for all latitudes. The higher abundance and densities of *A. vaigiensis* and the presence of single-species shoals (Chapter 5) suggests greater supply issues from natal regions and a possible preference of shoaling with conspecifics when there are higher influxes of larvae.

6.3 Single-species vs mixed-species shoals

The general consensus regarding shoal formation in fish species is that overall benefits attained outweigh the costs, including foraging, predator protection, mating opportunities and parasite resistance (Paijmans *et al.*, 2019). In the trailing edge habitats of the range expanding tropical vagrant *A. vaigiensis*, mixed-shoal formation has been shown to benefit predator avoidance (trade-off with foraging efficiency) and promote larger body sizes during early ontogeny (Smith *et al.*, 2018; Coni *et al.*, 2021; Coni *et al.*, 2022b; Mitchel *et al.*, 2025). Our results concurred with previous studies, as we found key differences in between single-species and mixed-species shoals across a suite of behavioural traits, identifying key trade-offs that were more prominent in trailing edge habitats (Chapter 3), namely reduced foraging in favour of predator avoidance. We also observed significantly higher rates of aggressive interactions

occurring in mixed-species shoals (Chapter 3) containing *A. vaigiensis*, suggesting that while there may be behavioural advantages of shoaling with local temperate species, it concurrently heightens competition for resources within shoals. In Chapter 4 specific shoal ratios (100%, 75%, 50% and 25%) containing *A. vaigiensis* were tested to investigate the degree to which shoal structures influences performance across a range of water temperatures. Similar to the results attained in chapter 3, mixed-species shoals showed reduced foraging performance (reduced bite rate and feeding start times) and increased risk-aversiveness, with a shoal ratio of 25% *A. vaigiensis* (tropical) and 75% *M. joyceae* (temperate) showing a trade-off between the shoaling behaviours. Growth rates were also enhanced for *A. vaigiensis* juveniles reared in mixed-species shoals, with the highest growth rates occurring at a shoal ratio of 50% tropical and 50% temperate, which confirmed results attained in Smith *et al* (2018). Overall results from this thesis showed the major influences shoal structure has on *A. vaigiensis* performance and suggests the significance of shoaling with local temperate heterospecifics will have on their long-term survival in trailing edge habitats.

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		NORMAL						PREDATOR					
		Factor						Factor					
		Latitude			Temperature			Latitude			Temperature		
		High	Mid	Low	17	21	25	High	Mid	Low	17	21	25
Performance metric	Shoal type												
Growth rate	 			No change	No change	No change	No change	Not measured					
Bite rate	 	No change	No change	No change	No change		No change	No change		No change	No change	No change	
Boldness	 		No change	No change	No change		No change	No change	No change	No change		No change	No change

Figure 6.2. Summary of results obtained from Chapters 3, 4 and 5 regarding the physiological (growth) and behavioural performance (Feeding and boldness) of *A. vaigiensis* (in single-species (two identical fish) and mixed-species (two different fish) shoals), comparing single-species vs mixed-species shoals between latitudinal sites (Chapters 3 and 5) and water temperature treatments (Chapter 4). Downwards

arrows represent a significant difference and increased performance of single-species shoals compared to mixed-species shoals, and upwards arrows represents a significant difference and decreased performance when comparing single-species to mixed species shoals. 'No change' represents no significant difference between single-species and mixed-species shoals. Summary also compares data obtained from normal and feeding behaviours to behaviours observed post predator exposure. Note that for growth rates at latitudes refers to post-settlement ring increment widths (Chapter 5) and does not reflect the instantaneous growth rates that were measured in Chapter 4.

6.4 Concluding remarks

Overall, this thesis investigated several knowledge gaps relating to the range expansion potential of a tropical vagrant fish, specifically investigating the influence of water temperature/latitude, shoal structure and predation on the physiology and behaviour of *A. vaigiensis*. This is also one of the few studies to decipher specific mixed-species shoal ratio trends that showed multiple benefits and costs regarding fish fitness and performance, and to expose mixed-species shoals to live predation to determine the potential effects of water temperature and shoal ratio. Results from this thesis can be used to make predictions regarding latitudinal thresholds in the range expansion of a tropical vagrant fish species, and infers how predation and local heterospecifics, in conjunction with water temperature, will regulate the survival and successful colonisation of range expanding tropical fishes in trailing edge habitats over the next few decades.

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