

Ocean Acidification and Seasonal Temperatures Counter Positive Novel Species Interaction and Warming Effects on Tropicalising Temperate Fish Communities



Angus Mitchell

Presented for the degree of Doctor of Philosophy

School of Biological Sciences
The University of Adelaide



THE UNIVERSITY
of ADELAIDE

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

The author acknowledges that copyright of published works contained within the thesis resides with the copyright holder(s) of those works.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Angus Mitchell

31/03/2023

Acknowledgements

Great thanks go to my supervisors, Professor Ivan Nagelkerken and Professor David Booth for providing me with the opportunity to pursue my passion, and for guiding me to become the scientist I am today.

Thank you to my fellow lab group PhD students, Vittoria Cipriani, Ericka Coni, and Minami Sasaki for sharing their valuable knowledge, and developing my scientific skills throughout our time together.

A special thank you to my parents, Elizabeth and Rodney, and my brother, Oscar, for your ongoing support, kindness, and enduring belief in me.

Thank you to Chloe, for helping me achieve my goals and aspirations, for always being by my side, and putting up with my fish related dinner table chats. Without you I would not be the person I am today.

Thank you to the Australian Research Council Discovery Project (grant no. DP170101722), for supporting the research produced during my PhD and The University of Adelaide for awarding me with the Australian Government Research Training Program Scholarship.

Finally, to Leo, for all the runs and walks we have adventured on together. You are a source of immense happiness.

Table of Contents

Abstract	1
Chapter I	2
The Global Redistribution of Species Under Climate Change	3
References	15
Chapter II	21
Manuscript	23
Supplementary Information	37
Chapter III	49
Manuscript	52
Supplementary Information	63
Chapter IV	72
Manuscript	75
Supplementary Information	84
Chapter V	95
Abstract	98
Introduction	99
Materials and Methods	103
Results	113
Discussion	119
Conclusion	122
References	124
Supplementary Information	128
Chapter VI	143
General Discussion	144
Future Directions	151
General Conclusion	154
References	157

Abstract

Anthropogenic warming has facilitated the redistribution of species globally. Many tropical species have shifted their ranges poleward either to escape thermally unsuitable conditions or to invade previously inaccessible temperate environments. Climate-driven species redistributions have facilitated range-shifting and local species to interact for available resources. These novel species interactions can modify the pace of species range extensions. In Australia, over 150 tropical fish species have been observed moving poleward into temperate marine ecosystems but often fail to establish due to hostile conditions (winter temperatures and environmental novelty). However, ocean warming could minimise lethal winter effects on range-extending tropical fishes, allowing them to establish at temperate latitudes in the near future. This thesis provides empirical and experimental evidence that current and future climatic conditions (ocean acidification and warming) and novel species interactions can modify the pace of tropicalisation in temperate marine ecosystems and highlights the capacity of local temperate fish communities to respond to tropical fish range extensions. By using a climate-manipulated laboratory experiment, I reveal that ocean acidification might slow tropicalisation through degradation of the shoaling performance of novel tropical-temperate fish shoals (Chapter 2). In addition, I show that future winter conditions negatively affect tropical fish behavioural repertoire and physiological function in temperate ecosystems (Chapters 3 and 4). In contrast, I demonstrate that the temperate fish behaviour remained unchanged but experienced increased growth and physiological performance under future winter conditions compared to current and future summer conditions (Chapters 3 and 4), which could seasonally modify tropical-temperate interactions. I further reveal that tropical fish entering novel temperate environments trade off foraging efficiency for predator vigilance, independent of novel shoaling interactions (mixed-species shoaling). However, novel species interactions can enhance tropical fish foraging, which may allow them to perform better at their cold-temperate range edges (Chapter 5). While present-day lethal winter temperatures and novel temperate environmental conditions slow tropicalisation, the overall effect of ocean warming and positive species interactions will facilitate the tropicalisation of temperate waters in a future ocean. However, future winter temperatures and ocean acidification could limit tropical fish performance at temperate latitudes.

Chapter I

General Introduction



The Global Redistribution of Species under Climate Change

Anthropogenic warming of the Earth's climate over the last century (IPCC, 2021) has altered 80% of Earth's ecological processes, which determine all species' historical biogeographic distributions (Scheffers et al., 2016). In response to anthropogenic warming, 59% of the world's aquatic and terrestrial species have undergone biogeographic redistributions across altitudinal and latitudinal extents (Parmesan & Yohe, 2003; Chen et al., 2011; Pecl et al., 2017). Under projected future climatic conditions, many more species are expected to either move towards higher latitudes, altitudes, or deeper waters to escape the effects of warming or take advantage of new environments which were previously thermally inaccessible (Walther et al., 2002; Walther, 2010; García Molinos et al., 2015). Species entering new environments often interact with local species for available resources (Alexander et al., 2015; Coni et al., 2021a). Interactions between range-shifting and local species can modify the pace of range-shifts (Smith et al., 2018; Pajmans et al., 2020) and the persistence of local species (Twine et al., 2022) in recipient ecological communities. Novel species interactions can also modify ecosystem function (Pessarodona et al., 2022), community structure (Ling et al., 2008) and biodiversity (Gilman et al. 2010). However, the novel biotic interactions that occur during the initial stages of species range-shifts can be modified under by future climatic conditions (Tylianakis et al., 2008; Alexander et al., 2015) and therefore alter range-shift outcomes. Hence, concurrently understanding both the direct effects of climatic stressors and the indirect effects of species interactions upon recipient communities is critical to revealing the ecological, economical and sociological implications of species range shifts under climate change.

Poleward Range Shifts

Under anthropogenic warming, species are most commonly observed redistributing poleward to higher latitudes (Parmesan & Yohe, 2003; Chen et al., 2011; Pecl et al., 2017). This directional shift along latitudinal gradients is characterised by median rates of approximately 1.69 km/yr, contrasting significantly with the slower median rate of 0.011 km/yr observed along altitudinal gradients (Chen et al., 2011). Notably, regions experiencing temperatures rising above average levels of warming show the fastest rate of poleward species range shifts (Chen et al., 2011; Pecl et al., 2017). Nonetheless, the pace and extent of poleward range shifts are multifaceted and often mediated by concurrent factors including species traits (García Molinos et al., 2022), ecosystem complexity (Bates et al., 2013; Tekwa et al., 2022) and local environmental conditions (Pinsky et al., 2013). These complex interactions may explain how rapid average poleward range shifts result from a wide array of species level responses (Chen et al., 2011), whereby the idiosyncrasies of individual species responses can result in asymmetrical responses at a community-level (Soler et al., 2022), facilitating the emergence of novel biological communities at higher latitudes.

The reshuffling of high latitude biological communities is most pronounced in marine ecosystems (Pecl et al., 2017), where concurrent strengthening of boundary currents and higher connectivity in marine environments (compared to terrestrial environments) have caused marine organisms to move poleward faster than terrestrial organisms (Burrows et al., 2011; Chen et al., 2011; Poloczanska et al., 2013). This poleward movement of low latitude marine species into temperate ecosystems has facilitated a widening of the tropical belt known as tropicalisation (Seidel et al., 2007). The tropicalisation of temperate biological communities, has resulted in higher abundances of warmer-associated taxa and coinciding declines in local

cold-adapted species (Soler et al., 2022), leading to the thermophilization of shallow marine temperate ecosystems.

Thermophilization is rapidly occurring in high latitude marine ecosystems connected to lower latitudes by western boundary currents (e.g. East Australian Current in Australia, Kuroshio Current in Japan, Agulhas Current in South-east Africa, and Gulf Stream in North-east America; Sorte et al., 2010; Wu et al., 2012 Nakamura et al., 2013). These temperate regions have warmed three to four times faster ($0.037^{\circ}\text{C}/\text{yr}$) than global averages (Lough et al., 2008) and are termed ‘climate change hotspots’ (Hobday & Pecl, 2014). Temperate climate change hotspots are most often invaded by thermally sensitive ectotherms (Gervais et al., 2021). For example, tropical fishes (due to their narrow thermal ranges and thermal sensitivity; Harbary et al., 2017; Nati et al., 2021) are often observed at the forefront of marine range extensions into temperate ecosystems (Booth et al., 2007; Nakamura et al., 2013; Gervais et al., 2021). In Japan, the Kuroshio Current has driven the poleward expansion of tropical corallivores (Nakamura et al., 2013), which has coincided with the poleward expansion of multiple coral species along Japan’s east coast (Yamano et al., 2011). Furthermore, tropical species entering temperate ecosystems have facilitated community phase shifts through habitat modification (e.g. overgrazing kelp forests; Pessarrodona et al., 2022) in southeastern Australia (Ling, 2008), Japan (Vergés et al., 2022) and the Mediterranean (Vergés et al., 2014; 2016). Such evidence establishes the vulnerability of temperate ecosystems to tropical range-extending species. However, little is known about whether biotic interactions between tropical range-shifting fishes and recipient temperate communities mediate range-shift outcomes.

Lethal Winter Effects on Range-Shifting Species

Hostile winter conditions in high-latitude regions can limit the range extension success of warm-adapted species (Figueira et al., 2009; Ljungström et al., 2021). Winter sea surface temperatures often fall below the thermal minima of many range-shifting fishes (Booth et al., 2010), and can jeopardise tropical fish range shifts through physiological impacts (e.g., reduced growth; Figueira et al., 2009; Djurichkovic et al., 2019) and facilitate negative behavioural outcomes in temperate ecosystems (e.g., decreased feeding, Coni et al., 2021a; increased sheltering, Coni et al., 2022; slower predator escape response, Djurichkovic et al., 2019; Figueira et al., 2019). Reductions in light availability can also lead to greater depletion in energetic stores of warm-adapted fish during winters at higher latitudes (Ljungström et al., 2021). In addition, novel risks posed by temperate ecosystems can degrade the fitness of range-shifting species (Beck et al., 2016; Coni et al., 2022), slowing down tropical fish range shifts into temperate latitudes. However, under increasing ocean warming, environmental novelty and winter limitations on range-extension success are predicted to dissipate in the near future (Figueira & Booth, 2010), allowing tropical species to establish in temperate ecosystems permanently.

Marine Range Shifts in Southeastern Australia

In Australia, over 150 tropical fish species have been observed dispersing into southeastern temperate ecosystems via the East Australian Current (Booth et al., 2007; Feary et al., 2014). These fishes are commonly termed ‘vagrants’ (Booth et al., 2011). Tropical vagrants often fail to overwinter at their temperate cold-range edges as winter temperatures fall below their critical thermal minima (Figueira et al., 2009; Booth et al., 2011). However, as marine climate change hotspots (including southeastern Australia) continue to warm, many

species of tropical vagrants are expected to establish in high-latitude ecosystems (Booth et al., 2011; Gervais et al., 2021). Tropical vagrants are commonly observed shoaling with seventeen temperate fish species (Smith et al., 2018; Paijmans et al., 2020). Shoal-forming tropical damselfish species (*Abudefduf vagiensis*) are the most abundant tropical fish species observed entering southeastern Australian temperate latitudes and form shoals with morphologically similar local temperate species (Booth et al., 2007; Smith et al., 2018). Mixed-species shoaling can provide tropical vagrants with fitness benefits (e.g., larger body size and decreased mortality; Smith et al., 2018; increased predator avoidance and foraging efficiency; Paijmans et al., 2020), which can extend tropical vagrants persistence and lower mortality rates during winters in temperate ecosystems (Smith et al., 2018). Indeed, not all interactions with temperate species are beneficial for tropical vagrants, (see Beck et al., 2016; Coni et al., 2021a) and involve both physiological (Kingsbury et al., 2019) and behavioural trade-offs (Coni et al., 2022), which may limit establishment success at higher latitudes. Previous research shows that tropical vagrant's express risk-averse behaviour at their cold-temperate range limits (Coni et al., 2022), yet it remains unknown whether these behavioural shifts are adaptive or simply an artefact of environmental novelty.

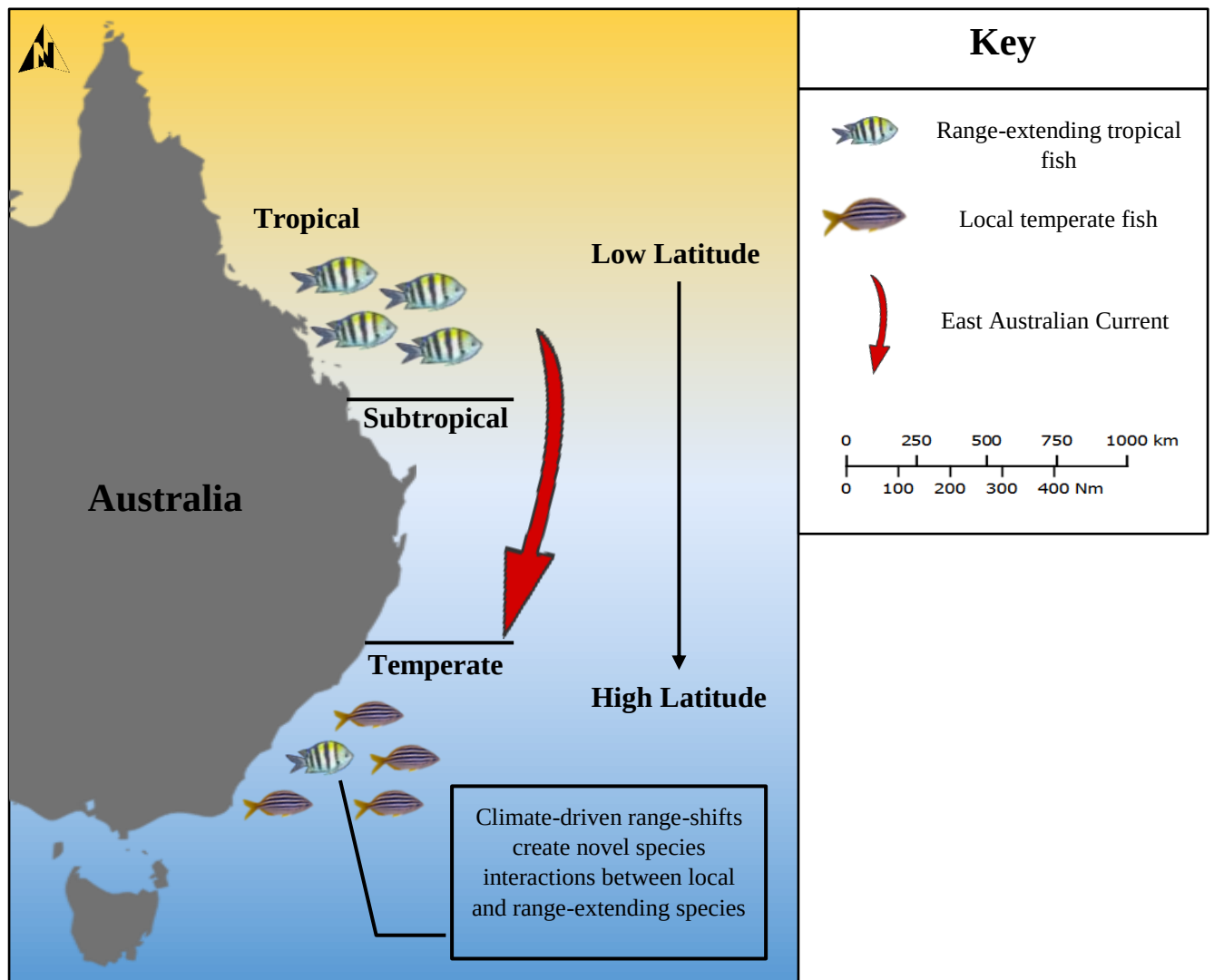


Figure 1: Conceptual diagram illustrating the intensification of the East Australian Current and ocean warming, which facilitate poleward range-extension of tropical fish species into temperate southeastern Australia, a global warming hotspot. The arrival of range-extending tropical fishes in temperate ecosystems creates novel species interactions, as local species are forced to interact with range-extending species for resources.

Range-Shift Effects on Local Species

Our understanding of novel biotic interaction effects on resident temperate fishes remains limited (see Coni et al., 2021a). At current, temperate species can outcompete co-shoaling tropical fish at temperate latitudes for prey items (Coni et al., 2021a), yet ocean

warming projected for temperate latitudes may mediate shifts in competition outcomes between co-shoaling temperate and tropical fishes (Figueira et al., 2009, 2019; Djurichkovic et al., 2019). However, the wider thermal niches of temperate fishes may provide fitness advantages over competing tropical fishes which show narrower thermal ranges and lower resilience to climatic variability in a future ocean (Nati et al., 2021). Nonetheless, present-day fitness advantages temperate fishes exert over co-shoaling tropical vagrants could be modified under projected future climatic scenarios, making it critical to understand: (1) if temperate species currently benefit from biotic interactions with tropical fishes (compared to temperate single-species shoals); and (2) how future climatic scenarios (e.g., ocean warming and acidification) could modify novel tropical-temperate interaction outcomes.

Plasticity in New and Changing Environments

Range-shifting species expressing high phenotypic plasticity have a greater chance of establishing in new environments than species exhibiting low plasticity (Donelson et al., 2019). Phenotypic plasticity is often considered a species' first response to environmental change (Munday et al., 2013). For example, behavioural plasticity can allow range-shifting species to modify their behaviour (Coni et al., 2021b) and increase persistence in new poleward environments (Coni et al., 2021a; Smith et al., 2018). Similarly, recipient community species must also possess some degree of behavioural plasticity to either coexist with (Smith et al., 2018) or compete against novel range-extending species (Twinaime et al., 2022). Behavioural plasticity can also allow resident species to adjust their behaviour at their range trailing edge to (1) adapt to trailing range-edge habitats which are becoming warmer than the species' current realised niche (Donelson et al., 2019), (2) compete against arriving novel competitors (Coni et al., 2021a; Twinaime et al., 2022) and (3) respond to the arrival of novel predator and prey species (Beck et al., 2016; Jones et al., 2020). However, not all phenotypic responses to

new or changing environmental conditions are adaptive and maladaptive responses can create lags in the poleward establishment of range-extending species or speed up range contractions at local species' range trailing edges (Angilletta, 2009; Donelson et al., 2019). Indeed, plasticity is context-dependent (Donelson et al., 2019) and may convey no advantage to range-shifting tropical fish in the presence of superior temperate competitors (Coni et al., 2021a), if for example, tropical and temperate fishes compete for a limited resource (e.g. prey items). Changes in the competitive performance of competing tropical and temperate fishes under ocean acidification and warming may concurrently undermine plasticity in a future ocean. In addition, warming and ocean acidification could also modify ecological contexts in space and time (Nagelkerken & Munday, 2016; Agostini et al., 2021; Coni et al., 2021c), and therefore mediate changes in plastic responses of higher latitude fish communities in a future ocean. Nonetheless, predicting whether phenotypic responses mediated by range shifts are beneficial to either resident or range-shifting species is critical to assessing if species are adapted to high latitude environmental conditions under current and future climatic scenarios.

Novel Species Interactions Can Mediate Range Shift Outcomes

Novel species interactions between local and range-shifting species can dictate the pace of species range shifts (Davis et al., 1998) and can contribute to responses at both warm- and cold-range edges (Sirén & Morelli et al., 2019; Lyu & Alexander, 2022). Some novel species interactions can enhance range shift success (Smith et al., 2018), while others, such as shifts in predator and competitor identity (Alexander et al., 2015; Beck et al., 2016; Twiname et al., 2022), can limit or slow species invasions (Coni et al., 2021a; Twiname et al., 2022). In addition, future climatic conditions could modify novel species interactions (Figueira et al., 2019). Novel species interactions can also change across species distributions, where

interacting warm- and cold-adapted species ranges overlap (Alexander et al., 2015; Coni et al., 2021a). Furthermore, shifts in trophic interactions (Tekwa et al., 2022), local abiotic conditions (Pinksy et al., 2013), climatic variability (Monaco et al., 2021) and a mismatch in competing species fitness (Kingsbury et al., 2019; Coni et al., 2021a) can concurrently modify the biotic interactions which shape range-extension success. Therefore, prospective research must attempt to concurrently incorporate future climatic conditions and novel species interactions to determine range-shift outcomes under climate change.

Ocean Warming and Acidification Effects on Range Shift

Outcomes

Previous research on marine range shifts has often focussed on species responses to ocean warming (Figueira et al., 2009; Beck et al., 2016; Djurichkovic et al., 2019; but see Coni et al., 2021c). However, local conditions (Pinksy et al., 2013), warming, currents and species traits all contribute to the initial stages of marine range shifts (Garcia Molinos et al., 2022). Ocean acidification also can modify species- and community-level responses to ocean warming, which could alter range-extensions across space and time (Nagelkerken & Munday, 2016; Coni et al., 2021c). High-latitude marine ecosystems undergoing species range shifts are often subjected to elevated rates of warming (Lough et al., 2008) and acidification (Scanes et al., 2020). In addition, these concurrent climatic stressors can, directly and indirectly, alter species interactions through shifts in behavioural (Nagelkerken & Munday, 2016), neurological (Nilsson et al., 2012; Andreassen et al., 2022), developmental (Baumann et al., 2012; Frommel et al., 2012; Moore et al., 2023) and sensory functions (Rossi et al., 2016) of marine fishes. Therefore, understanding how biotic interactions between tropical and temperate species are

modified by ocean warming and acidification is necessary for predicting the true pace of tropicalisation.

Shoaling as a Function of Range-Extension Success

Shoaling species are often more successful at range-extending than solitary species due to the various benefits provided by shoaling behaviour, such as social learning (Hoare & Krause, 2003; Ward et al., 2011; Smith et al., 2018), information transfer (Pitcher, 1983), anti-predator avoidance (Couzin et al., 2002), and enhanced foraging (Paijmans et al., 2020). Studies have shown that shoaling can enhance metabolic performance (Nadler et al., 2016) and reduce predation risk in both familiar and novel environments (Pitcher, 1983; Paijmans et al., 2020). In addition, range-extending species that shoal with local species can increase prey access (Paijmans et al., 2021). However, climatic stressors can destabilise the benefits of shoaling behaviour and alter shoaling dynamics (Lopes et al., 2016; Domenici et al., 2017; Zanghi et al., 2023). Thus, future climatic conditions could affect the shoaling performance of range-extending fishes and local co-shoaling species, ultimately modifying range-shift outcomes in high-latitude ecosystems.

Aims and Background

This thesis aims to understand how range-extending tropical and local temperate fish adjust respectively to novel and changing environmental conditions in their rapidly warming temperate ranges and to investigate how future range-extension outcomes along the southeastern coast of Australia could be modified under ocean warming and acidification.

In my first data chapter (**Chapter 2**), I investigate how ocean warming and acidification degrade the shoaling dynamics and lateralisation of range-extending tropical and co-shoaling

temperate fishes. I used a confined laboratory experiment to evaluate three shoaling proxies which reveal the shoaling performance of temperate-only and novel mixed-species shoals under ocean warming and acidification. I also test how the lateralisation and boldness of tropical and temperate fishes are altered by future high-latitude conditions (ocean warming and acidification). I then developed a conceptual model to demonstrate how under ocean warming, acidification, and novel shoaling interactions, the shoaling dynamics and lateralisation of co-shoaling tropical and temperate fishes are degraded and may slow the range-extension of gregarious tropical fish into temperate fish communities.

In **chapter 3**, I assess how future seasonal temperatures and ocean acidification affect the individual behavioural repertoire and growth of the co-shoaling tropical and temperate fishes studied in **chapter 2**. I used passive video recordings collected in Chapter 2, to assess how four behavioural domains (aggression, activity, boldness, and feeding) shift under future conditions and how might these behavioural changes alter the growth of co-shoaling tropical and temperate species. How competing tropical and temperate fish perform under projected conditions is an important determinant of species persistence and performance in temperate fish communities in a future ocean.

In **chapter 4**, I reveal how future seasonal temperatures and ocean acidification alter the physiological performance of competing range-extending tropical and resident temperate fishes. Following 35-40-days exposure, I used the tissues of the tropical and temperate fishes tested **chapters 2 and 3** to evaluate how future summer vs future winter temperatures, ocean acidification, and novel species interactions alter 2 cellular biomarkers, long- and short-term energy storage, and body condition of co-shoaling. How the physiological function of competing tropical and temperate fish, which can determine competitive outcomes, is altered both positively and negatively by future seasonal temperatures and ocean acidification is

critical to understanding the persistence of tropical and temperate fishes at their respective leading and trailing range edges.

Chapter 5 uses *in situ* field experiments to test how tropical and resident temperate fish adjust their shoaling dynamics, laterality, and anti-predator behaviour across shoaling contexts and their respective biogeographic range extents. At the cold-temperate range extent of tropical fish, these ecosystems pose as novel environments, with novel competitors, habitats, predators, and lethal winter temperatures, potentially preventing the establishment of tropical fish. Using *in situ* video analysis and manipulated field experiments, I compare the lateralisation and anti-predator behaviour of tropical and temperate fish and the shoaling dynamics of mixed-species, tropical-only, and temperate-only shoals along a 2000-km latitudinal gradient from southern NSW to northern Queensland in Australia. How social interactions (e.g. shoaling) are altered by shoal type and concurrent shifts in the novelty of cold-range environments is an important determinant of species performance and a key indicator of the adaptive capacity of tropical fish to establish at their current cold-range limits successfully.

In **Chapter 6**, I discuss the implications of my research (**Chapters 2–5**) in a broad ecological context and future research directions. By using field and laboratory-based experiments, my research chapters reveal how current and future climatic conditions alter range-shift outcomes, in southeastern Australian marine ecosystems, through the modification of novel species interactions and the behavioural and physiological responses which underpin these interactions.

References

- Alexander, J., Diez, J., Levine, J. (2015). Novel competitors shape species' responses to climate change. *Nature*. 525(7570). pp. 515–518. <https://doi.org/10.1038/nature14952>.
- Andreassen, AH., Hall, P., Khatibzadeh, P., Jutfelt, F., Kermen, F., (2022). Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. *Proceedings of the National Academy of Sciences*. 119(39). e2207052119. <https://doi.org/10.1073/pnas.2207052119>.
- Angilletta, MJJ. (2009) *Thermal adaptation: a theoretical and empirical synthesis*. Oxford, UK: Oxford University Press.
- Baumann, H., Talmage, SC., Gobler CJ., (2012). Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change*. 2. pp. 38–41. <https://doi.org/10.1038/nclimate1291>.
- Bates, AE., Barrett, NS., Stuart-Smith, RD., Holbrook, NJ., Thompson, PA., Edgar, GJ. (2014). Resilience and signatures of tropicalization in protected reef fish communities. *Nature Climate Change*. 4(1). pp. 62–67. <https://doi.org/10.1038/nclimate2062>.
- Beck, H., Feary, D., Fowler, A., Madin, E., Booth, DJ. (2016). Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Marine Biology*. 163(4). 70. <https://doi.org/10.1007/s00227-016-2844-8>.
- Booth, DJ., Bond, N., Macreadie, PI. (2011). Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research*. 62(9). pp. 1027–1042. <https://doi.org/10.1071/MF10270>.
- Booth, DJ., Figueira, WF., Gregson, MA., Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine Coastal and Shelf Science*. 72(1–2). pp. 102–114. <https://doi.org/10.1016/j.ecss.2006.10.003>.
- Burrows, M., Schoeman, D., Buckley, L., Moore, P., Poloczanska, E., Brander, K., Brown, C., Bruno, J., Duarte, C., Halpern, B., Holding, J., Kappel, C., Kiessling, W., O'Connor, M., Pandolfi, J., Parmesan, C., Schwing, F., Sydeman, W., Richardson, A. (2011). The Pace of Shifting Climate in Marine and Terrestrial Ecosystems. *Science*. 334(6056). pp. 652–655. <https://doi.org/10.1126/science.1210288>.
- Chen, IC., Hill, JK., Ohlemüller, R., Roy, DB., Thomas, CD. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*. 333(6045). pp. 1024–1026. <https://doi.org/10.1126/science.1206432>.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2021). Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef fish. *Coral Reefs*. 40(5). pp. 1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>.
- Coni, EOC., Booth, DJ., Ferreira, C., Nagelkerken, I. (2021b). Behavioural generalism could facilitate coexistence of tropical and temperate fishes under climate change. *Journal of Animal Ecology*. 91(1). pp. 86 – 100. <https://doi.org/10.1111/1365-2656.13599>.
- Coni, EOC., Nagelkerken, I., Ferreira, C., Connell, SD., Booth, DJ. (2021c). Ocean acidification may slow the pace of tropicalisation of temperate fish communities. *Nature Climate Change*. 11(3). pp. 249–256. <https://doi.org/10.1038/s41558-020-00980-w>.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2022). Coral-reef fishes can become more risk-averse at their poleward range limits. *Proceedings of the Royal Society B: Biological Sciences*, 289(1971). 20212676. <https://doi.org/10.1098/rspb.2021.2676>.

- Couzin, I., Krause, J., James, R., Ruxton, G., Franks, N. (2002). Collective Memory and Spatial Sorting in Animal Groups. *Journal of Theoretical Biology*. 218(1). pp. 1–11. <https://doi.org/10.1006/jtbi.2002.3065>.
- Davis, A., Jenkinson, L., Lawton, J., Shorrocks, B., Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature*. 391(6669). pp. 783–786. <https://doi.org/10.1038/35842>.
- Djurichkovic, L., Donelson, J., Fowler, A., Feary, D., Booth, DJ. (2019). The effects of water temperature on the juvenile performance of two tropical damselfishes expatriating to temperate reefs. *Scientific Reports*. 9(1). <https://doi.org/10.1038/s41598-019-50303-z>.
- Donelson, JM., Sunday, JM., Figueira, WF., Gaitán-Espitia, JD, Hobday, AJ., Johnson, CR., Leis, JM., Ling, SD., Marshall, D, Pandolfi, JM., Pecl GT, Rodgers, GG., Booth, DJ., Munday PL. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philosophical Transactions of the Royal Society B*. B3742018018620180186. <http://doi.org/10.1098/rstb.2018.0186>.
- Feary, DA., Pratchett, MS., Emslie, MJ., Fowler, A., Figueira, WF., Luiz, OJ., Nakamura, Y., Booth, DJ. (2014). Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*. 15(4). pp. 593–615. <https://doi.org/10.1111/faf.12036>.
- Figueira, WF., Biro, P., Booth, DJ., Valenzuela Davie, VC. (2009). Performance of tropical fish recruiting to temperate habitats: role of ambient temperature and implications of climate change. *Marine Ecology Progress Series*. 384. pp. 231–239. <https://doi.org/10.3354/meps08057>.
- Figueira, WF., Booth, DJ. (2010). Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Global Change Biology*. 16(2). pp. 506–516. <https://doi.org/10.1111/j.1365-2486.2009.01934.x>.
- Figueira, WF., Curley, B., Booth, DJ. (2019). Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes?. *Marine Biology*. 166. 73. <https://doi.org/10.1007/s00227-019-3521-5>.
- Frommel, AY., Maneja, R., Lowe, D., Malzahn, AM., A.J. Geffen, AJ., Folkvord, A., Piatkowski, U., Reusch, TBH., Clemmesen, C. (2012). Severe tissue damage in Atlantic cod larvae under increasing ocean acidification *Nature Climate Change*. 2. pp. 42–46. <https://doi.org/10.1038/nclimate1324>.
- García Molinos, J., Halpern, BS., Schoeman, DS., Brown, CJ., Kiessling, W., Moore, PJ., Pandolfi, JM., Poloczanska, ES., Richardson, AJ., Burrows, MT. (2015). Climate Velocity and the Future Global Redistribution of Marine Biodiversity. *Nature Climate Change*. 6. pp. 83–88. <https://doi.org/10.1038/nclimate2769>.
- García Molinos, J., Hunt, HL., Green, ME., Champion, C., Hartog JR., Pecl, GT. (2022). Climate, currents and species traits contribute to early stages of marine species redistribution. *Communications Biology*. 5. 1329. <https://doi.org/10.1038/s42003-022-04273-0>.
- Gervais, C., Champion, C., Pecl, G. (2021). Species on the move around the Australian coastline: A continental-scale review of climate-driven species redistribution in marine systems. *Global Change Biology*. 27(14). pp. 3200–3217. <https://doi.org/10.1111/gcb.15634>.
- Gilman SE., Urban MC., Tewksbury J., Gilchrist GW., Holt RD. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution*. 25(6). pp. 325–331. <https://doi.org/10.1016/j.tree.2010.03.002>.
- Habary, A., Johansen, JL., Nay, TJ., Steffensen, JF., Rummer, JL. (2017). Adapt, move or die—how will tropical coral reef fishes cope with ocean warming? *Global Change Biology*. 23. pp. 566–577. <https://doi.org/10.1111/gcb.13488>.

- Hoare, DJ., Krause, J. (2003). Social organisation, shoal structure and information transfer. *Fish and Fisheries*. 4. pp. 269–279. <https://doi.org/10.1046/j.1467-2979.2003.00130.x>.
- Hobday, AJ., Pecl, GT. (2014). Identification of global marine hotspots: Sentinels for change and vanguards for adaptation action. *Reviews in Fish Biology and Fisheries*. 24. pp. 415–425. <https://doi.org/10.1007/s11160-013-9326-6>.
- IPCC. (2021). Climate change 2021: The physical science basis. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.) Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press. In Press.
- Jones, NT., Symons, CC., Cavalheri, H., Pedroza-Ramos, A., Shurin, JB. (2020) Predators drive community reorganisation during experimental range shifts. *Journal of Animal Ecology*. 89. pp. 2378– 2388. <https://doi.org/10.1111/1365-2656.13289>.
- Kingsbury, K., Gillanders, B., Booth, DJ., Nagelkerken, I. (2019). Trophic niche segregation allows range-extending coral reef fishes to coexist with temperate species under climate change. *Global Change Biology*. 26(2). pp. 721–733. <https://doi.org/10.1111/gcb.14898>.
- Krause, J., Ruxton, G., Krause, S. (2010). Swarm intelligence in animals and humans. *Trends in Ecology & Evolution*. 25(1). pp. 28–34. <https://doi.org/10.1016/j.tree.2009.06.016>.
- Landeau, L., Terborgh, J. (1986). Oddity and the ‘confusion effect’ in predation. *Animal Behaviour*. 34(5). pp. 1372–1380. [https://doi.org/10.1016/S0003-3472\(86\)80208-](https://doi.org/10.1016/S0003-3472(86)80208-).
- Ling, SD. (2008). Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. *Oecologia*. 156. pp. 883–894. <https://doi.org/10.1007/s00442-008-1043-9>.
- Ljungström, G., Langbehn, TJ., Jørgensen, C., (2021). Light and energetics at seasonal extremes limit poleward range shifts. *Nature Climate Change*. 11(6). pp. 530–536. <https://doi.org/10.1038/s41558-021-01045-2>.
- Lopes, A., Morais, P., Pimentel, M., Rosa, R., Munday, PL., Gonçalves, E., Faria, A. (2016). Behavioural lateralization and shoaling cohesion of fish larvae altered under ocean acidification. *Marine Biology*. 163(12). pp. 1–10. <https://doi.org/10.1007/s00227-016-3026-4>.
- Lough, J. M. (2008). Shifting climate zones for Australia’s tropical marine ecosystems. *Geophysical Research Letters*. 35. <https://doi.org/10.1029/2008GL034634>.
- Lyu S, Alexander JM. (2022). Competition contributes to both warm and cool range edges. *Nature Communications*. 6(13). 2502. <https://doi.org/10.1038/s41467-022-30013-3>.
- Monaco, C., Booth, DJ., Figueira, W., Gillanders, B., Schoeman, D., Bradshaw, C., Nagelkerken, I. (2021). Natural and anthropogenic climate variability shape assemblages of range-extending coral-reef fishes. *Journal of Biogeography*. 48(5). pp. 1063–1075. <https://doi.org/10.1111/jbi.14058>.
- Moore, B., Jolly, J., Izumiyama, M., Kawai, E., Ryu, T., Ravasi, T. (2023). Clownfish larvae exhibit faster growth, higher metabolic rates and altered gene expression under future ocean warming. *Science of The Total Environment*. 873. 162296. <https://doi.org/10.1016/j.scitotenv.2023.162296>.
- Munday, PL., Warner, RR., Monro, K., Pandolfi, JM., Marshall, DJ. (2013) Predicting evolutionary responses to climate change in the sea. *Ecology Letters*. 16. pp. 1488–1500. <https://doi.org/10.1111/ele.12185>.
- Nadler, LE., Killen, SS., McClure, EC., Munday, PL., McCormick, MI., (2016). Shoaling reduces metabolic rate in a gregarious coral reef fish species. *Journal of Experimental Biology*. 219(18). pp. 2802–2805. <https://doi.org/10.1242/jeb.139493>.

- Nagelkerken I, Goldenberg SU, Ferreira CM, Russell BD, Connell, SD. (2017). Species interactions drive fish biodiversity loss in a high-CO₂ world. *Current Biology*. 27. pp. 2177–2184. <https://doi.org/10.1016/j.cub.2017.06.023>.
- Nagelkerken, I., Munday, PL. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Global Change Biology*. 22(3). pp. 974 – 989. <https://doi.org/10.1111/gcb.13167>.
- Nakamura, Y., Feary, D., Kanda, M., Yamaoka, K. (2013). Tropical fishes dominate temperate reef fish communities within western Japan. *PLoS One*. 8(12). e81107. <https://doi.org/10.1371/journal.pone.0081107>.
- Nati, JJH., Svendsen, MBS., Marras, S., Killen, SS., Steffensen, JF., McKenzie, DJ., Domenici, P. (2021). Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. *Scientific Reports*. 11(1). 21272. <https://doi.org/10.1038/s41598-021-00695-8>.
- Nilsson, GE., Dixon, DL., Domenici, PL., McCormick, MI., Sørensen, C., Watson, S., Munday, PL., (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change*. 2. pp. 201–204. <https://doi.org/10.1038/nclimate1352>.
- Paijmans, K., Booth, DJ., Wong, MY. (2020). Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *Journal of Fish Biology*. 96(3). pp. 806–814. <https://doi.org/10.1111/jfb.14277>.
- Paijmans, KC., Booth, DJ., Wong, MY. (2021). Single-species subgroups form within mixed-species shoals of tropical and temperate fishes. *Environmental Biology of Fishes*. 105(42). pp. 1–14. <https://doi.org/10.1007/s10641-021-01188-3>.
- Parmesan, C., Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*. 421(6918). <https://doi.org/10.1038/nature01286>.
- Pecl, G., Araújo, M., Bell, J., Blanchard, J., Bonebrake, T., Chen, I., Clark, T., Colwell, R., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R., Griffis, R., Hobday, A., Janion-Scheepers, C., Jarzyna, M., Jennings, S., Lenoir, J., Linnetved, H., Martin, V., McCormack, P., McDonald, J., Mitchell, N., Mustonen, T., Pandolfi, J., Pettorelli, N., Popova, E., Robinson, S., Scheffers, B., Shaw, J., Sorte, C., Strugnell, J., Sunday, J., Tuanmu, M., Vergés, A., Villanueva, C., Wernberg, T., Wapstra, E. and Williams, S. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*. 355(6332). <https://doi.org/10.1126/science.aai9214>.
- Pessarrodona, A., Vergés, A., Bosch, N., Bell, S., Smith, S., Sgarlatta, M., Wernberg, T. (2022). Tropicalization unlocks novel trophic pathways and enhances secondary productivity in temperate reefs. *Functional Ecology*. 36. pp. 659–673. <https://doi.org/10.1111/1365-2435.13990>.
- Pinsky, M., Worm, B., Fogarty, M., Sarmiento, J., Levin, S. (2013). Marine taxa track local climate velocities. *Science*. 341(6151). pp. 1239–1242. <https://doi.org/10.1126/science.1239352>.
- Pitcher, T., Magurran, A. (1983). Shoal size, patch profitability and information exchange in foraging goldfish. *Animal Behaviour*. 31(2). pp. 546–555. [https://doi.org/10.1016/S0003-3472\(83\)80078-5](https://doi.org/10.1016/S0003-3472(83)80078-5).
- Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, Brander K, Bruno JF, Buckley LB, Burrows MT, Duarte CM. (2013). Global imprint of climate change on marine life. *Nature Climate Change*. 3(10). pp. 919–925. <https://doi.org/10.1038/nclimate1958>.

- Rossi, T., Nagelkerken, I., Pistevidos, J.C., Connell, S.D. (2016). Lost at sea: ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification. *Biology Letters*. 12. 20150937. <https://doi.org/10.1098/rsbl.2015.0937>.
- Scanes, E., Scanes, P., Ross, P. (2020). Climate change rapidly warms and acidifies Australian estuaries. *Nature Communications*. 11(1). 1803. <https://doi.org/10.1038/s41467-020-15550-z>.
- Scheffers, B.R., De Meester, L., Bridge, T.C., Hoffmann, A.A., Pandolfi, J.M., Corlett, R.T., Butchart, S.H., Pearce-Kelly, P., Kovacs, K.M., Dudgeon, D., Pacifici, M. (2016) The broad footprint of climate change from genes to biomes to people. *Science*. 354(6313). aaf7671. <https://doi.org/10.1126/science.aaf7671>.
- Seidel, D., Fu, Q., Randel, W., Reichler, T. (2007). Widening of the tropical belt in a changing climate. *Nature Geoscience*. 1(1). pp. 21–24. <https://doi.org/10.1038/ngeo.2007.38>.
- Smith, S., Fox, R., Booth, D.J., Donelson, J. (2018). Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion frontline. *Global Change Biology*. 24(4). pp. 1663–1672. <https://doi.org/10.1111/gcb.14016>.
- Sirén, A.P., Morelli, T.L. (2020). Interactive range-limit theory (iRLT): An extension for predicting range shifts. *Journal of Animal Ecology*. 89(4). pp. 940–954. <https://doi.org/10.1111/1365-2656.13150>.
- Soler, G.A., Edgar, G.J., Barrett, N.S., Stuart-Smith, R.D., Oh, E., Cooper, A., Ridgway, K.R., Ling, S.D., (2022). Warming signals in temperate reef communities following more than a decade of ecological stability. *Proceedings of the Royal Society B*. 289(1989). 20221649. <https://doi.org/10.1098/rspb.2022.1649>.
- Sorte, C.J.B., Williams, S.L., Carlton J.T. (2010). Marine range shifts and species introductions: Comparative spread rates and community impacts. *Global Ecology Biogeography*. 19(3). pp. 303–316. <https://doi.org/10.1111/j.1466-8238.2009.00519.x>.
- Tekwa, E., Watson, J., Pinsky, M. (2022). Body size and food–web interactions mediate species range shifts under warming. *Proceedings of the Royal Society B: Biological Sciences*. 289(1972). 20212755. <https://doi.org/10.1098/rspb.2021.2755>.
- Twine, S., Fitzgibbon, Q., Hobday, A., Carter, C., Oellermann, M., Pecl, G. (2022). Resident lobsters dominate food competition with range-shifting lobsters in an ocean warming hotspot. *Marine Ecology Progress Series*. 685. pp. 171–181. <https://doi.org/10.3354/meps13984>.
- Tylianakis, J., Didham, R., Bascompte, J., Wardle, D. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*. 11(12). pp. 1351–1363. <https://doi.org/10.1111/j.1461-0248.2008.01250.x>.
- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, M., Hoegh-Guldberg, O., Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*. 416(6879). pp. 389–395. <https://doi.org/10.1038/416389a>.
- Walther, G.R. (2010). Community and ecosystem responses to recent climate change. *Phil. Trans. R. Soc. B* 365:2019–2024. <http://doi.org/10.1098/rstb.2010.0021>.
- Ward, A.J.W., Herbert-Read, J.E., Sumpter, D.J.T., Krause, J. (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proceedings of the National Academy of Sciences*. 108(6). pp. 2312–2315. <https://doi.org/10.1073/pnas.1007102108>.
- Wu, L., Cai, W., Zhang, L., Nakamura, H., Timmermann, A., Joyce, T., McPhaden, M., Alexander, M., Qiu, B., Visbeck, M., Chang, P., Giese, B. (2012). Enhanced warming over the global subtropical western boundary currents. *Nature Climate Change*. 2(3). pp. 161–166. <https://doi.org/10.1038/nclimate1353>.
- Vergés, A., Steinberg, P.D., Hay, M.E., Poore, A.G.B., Campbell, A.H., Ballesteros, E., Heck, K.L., Booth, D.J., Coleman, M.A., Feary, D.A., Figueira, W., Langlois, T., Marzinelli,

- EM., Mizerek, T., Mumby, PJ., Nakamura, Y., Roughan, M., van Seville, E., Sen Gupta, A., Smale, DA., Tomas, F., Wernberg, T., Wilson, SK. (2014). The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society of B: Biological Sciences*. 281(1789). 20140846. <https://doi.org/10.1098/rspb.2014.0846>.
- Vergés, A., Doropoulos, C., Malcolm, HA., Skye, M., Garcia-Piz, M., Marzinelli, EM., Campbell, AH., Ballesteros, E., Hoey, AS., Vila-Concejo, A., Bozec, YM., Steinberg, PD. (2016). Long-term empirical evidence of ocean warming leading to tropicalisation of fish communities, increased herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences of the United States of America*. 113(48). pp. 13791–3796. <https://doi.org/10.1073/pnas.1610725113>.
- Vergés, A., Lanham, BS., Kono, M. Okumura, S., Nakamura, Y. (2022). Differences in fish herbivory among tropical and temperate seaweeds and annual patterns in kelp consumption influence the tropicalisation of temperate reefs. *Scientific Reports*. 12. 21202. <https://doi.org/10.1038/s41598-022-24666-9>.
- Yamano, H., Sugihara, K., Nomura, K. (2011). Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophysical Research Letters*. 38(4). L04601. <https://doi.org/10.1029/2010GL046474>.
- Zanghi, C., Munro, M., Ioannou, CC. (2023). Temperature and turbidity interact synergistically to alter anti-predator behaviour in the Trinidadian guppy. *Proceedings of the Royal Society B*. 290. 20230961. <https://doi.org/10.1098/rspb.2023.0961>.

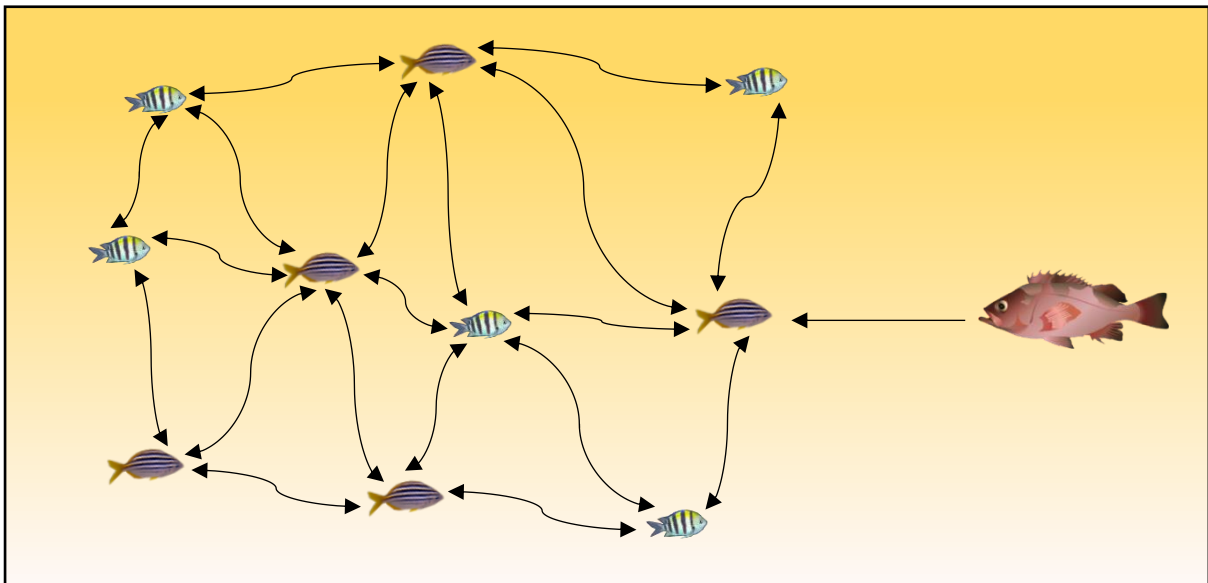
Chapter II

Ocean warming and acidification degrade shoaling performance and lateralization of novel tropical–temperate fish shoals

Angus Mitchell¹, David J. Booth², Ivan Nagelkerken¹

¹Southern Seas Ecology Laboratories, School of Biological Sciences and The Environment Institute, The University of Adelaide, DX 650 418, Adelaide, SA, 5005 Australia

²School of the Life Sciences, University of Technology Sydney, Ultimo, NSW, 2007, Australia



Statement of Authorship

Title of Paper	Ocean warming and acidification degrade shoaling performance and lateralization of novel tropical-temperate fish shoals
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	First published 17 December 2021

Principal Author

Name of Principal Author (Candidate)	Angus Mitchell		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	27/02/23

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	David J. Booth		
Contribution to the Paper	Study design and writing		
Signature		Date	27/02/23

Name of Co-Author	Ivan Nagelkerken		
Contribution to the Paper	Study design and writing		
Signature		Date	27/02/23

Please cut and paste additional co-author panels here as required.

Ocean warming and acidification degrade shoaling performance and lateralization of novel tropical–temperate fish shoals

Angus Mitchell¹  | David J. Booth²  | Ivan Nagelkerken¹ 

¹Southern Seas Ecology Laboratories, School of Biological Sciences and The Environment Institute, The University of Adelaide, Adelaide, South Australia, Australia

²School of Life Sciences, University of Technology Sydney, Ultimo, New South Wales, Australia

Correspondence

Ivan Nagelkerken, Southern Seas Ecology Laboratories, School of Biological Sciences and The Environment Institute, The University of Adelaide, DX 650 418, Adelaide, SA 5005 Australia.
Email: ivan.nagelkerken@adelaide.edu.au

Funding information

Australian Research Council, Grant/Award Number: DP170101722

Abstract

Gregarious behaviours are common in animals and provide various benefits such as food acquisition and protection against predators. Many gregarious tropical species are shifting poleward under current ocean warming, creating novel species and social interactions with local temperate taxa. However, how the dynamics of these novel shoals might be altered by future ocean warming and acidification remains untested. Here we evaluate how novel species interactions, ocean acidification and warming affect shoaling dynamics, motor lateralization and boldness of range-extending tropical and co-shoaling temperate fishes under controlled laboratory conditions. Fishes were exposed to 1 of 12 treatments (combinations of three temperature levels, two $p\text{CO}_2$ levels and two shoal type levels: mixed species or temperate only) for 38 days. Lateralization (a measure of asymmetric expression of cognitive function in group coordination and predator escape) of tropical and temperate species was right-side biased under present-day conditions, but side bias significantly diminished in tropical and temperate fishes under ocean acidification. Ocean acidification also decreased shoal cohesion irrespective of shoaling type, with mixed-species shoals showing significantly lower cohesion than temperate-only shoals irrespective of climate stressors. Tropical fish became bolder under ocean acidification (after 4 weeks), and temperate fish became bolder with increasing temperature, while ocean acidification dampened temperate fish boldness. Our findings highlight the direct effect of climate stressors on fish behaviour and the interplay with the indirect effects of novel species interactions. Because strong shoal cohesion and lateralization are key determinants of species fitness, their degradation under ocean warming and acidification could adversely affect species performance in novel assemblages in a future ocean, and might slow down tropical species range extensions.

KEYWORDS

climate change, lateralization, ocean acidification, ocean warming, range extension, shoaling, species interactions, tropicalization

1 | INTRODUCTION

Climate change has facilitated marine and terrestrial taxa to undergo poleward range extensions, shifts and contractions (Chen et al., 2011; Poloczanska et al., 2016). Range shifts are often seen as an

attempt to escape the effects of warming or an opportunity to take advantage of colder conditions at higher latitudes/deeper waters now suitable for invasion (Hoegh-Guldberg & Bruno, 2010; Walther et al., 2002), or could simply be an artefact of strengthening boundary currents moving dispersive larvae further poleward. Ectotherms

such as fish are particularly susceptible to ocean warming and are frequently observed at the forefront of marine range extensions (Booth et al., 2011; Walther et al., 2002). Marine fishes entering new environments often modify their behaviour through novel interactions with resident species (Coni et al., 2021b; Nagelkerken & Munday, 2016). These novel interactions can determine the rate of range extension and, ultimately, a species range-extension success. Previous studies assessing these interactions between marine range-extending and native species have focused on ocean warming effects but have not evaluated how the concurrent effects of ocean acidification may modify the effects of range extensions into high latitudes (Figueira et al., 2009, 2019).

Marine organisms shift biogeographically faster than terrestrial species (marine species: 28 km/decade; terrestrial: 6.1 km/decade; Burrows et al., 2011). Poleward boundary currents are the primary driver of marine biographic range shifts (Sorte et al., 2010; Wu et al., 2012). Ocean warming has made range shifts possible by increasing the accessibility of previously thermally unsuitable environments for dispersive tropical marine organisms (Walther et al., 2002). The concurrent effects of ocean warming and intensification of boundary currents have increased the dispersal volume of tropical organisms into higher latitudes (Parmesan & Yohe, 2003; Wu et al., 2012), inducing a widening of the tropical belt known as tropicalization (Seidel et al., 2007).

Regions exposed to poleward boundary currents (e.g. Kuroshio in Japan, East Australian Current in Australia and Gulf Stream in North-east America) have warmed three to four times faster ($0.037^{\circ}\text{C}/\text{yr}$) than global averages (Wu et al., 2012). Globally, poleward boundary currents have facilitated tropicalization. In eastern Africa, an inflow of fishes into sub-tropical latitudes has been observed directly due to the strengthening of the Agulhas Current (Lloyd et al., 2011). In eastern Japan, the Kuroshio Current has propagated poleward extensions of tropical fishes, especially corallivores (Nakamura et al., 2013). The arrival of tropical reef fishes in eastern Japan has aligned with the poleward movement of coral species, which form suitable habitats for many of the arriving tropical fishes observed (Yamano et al., 2011). In Australia, the East Australian Current has acted as a direct dispersal mechanism for over 150 tropical fish species and multiple species of tropical corals (Booth & Sear, 2018; Feary et al., 2014). Range-shifting tropical ecosystem engineers such as herbivorous fish have also mediated community phase shifts through habitat modification in regions including the Mediterranean, Japan and Australia (Verges et al., 2014, 2016). Such evidence demonstrates how invading tropical fishes travel to temperate latitudes, yet few studies have assessed how interactions between range-shifting and native species may mediate tropicalization (but see Coni et al., 2021a, 2021c).

Shallow marine ecosystems susceptible to tropical invasions are often exposed to above-average rates of ocean warming (Lough, 2008; Verges et al., 2014; Wu et al., 2012) and acidification (Scanes et al., 2020). These climatic stressors can, directly and indirectly, alter species interactions through shifts in behaviour (Malavasi et al., 2013; Nagelkerken & Munday, 2016), decision-making (Domenici

et al., 2014), learning (Briffa et al., 2012), neurological function (Nilsson et al., 2012), development (Warren et al., 2016), metabolism (Ishimatsu et al., 2005) and sensory function (Munday et al., 2009; Rossi et al., 2016) of the interacting species. Oceans are expected to experience a pH reduction of ~ 3.3 units and warm by up to 4.5°C by 2100 (Caldeira & Wickett, 2003; Feely et al., 2009; IPCC, 2021). Hence, it is necessary to assess how ocean warming and acidification affect novel species interactions to predict range-extension outcomes and their effects on marine ecosystem function.

Shoaling with natives may be an advantageous strategy for invading tropical species for a few reasons: (1) social learning from heterospecifics (Santiago-Arellano et al., 2021; Smith et al., 2018); (2) reduced predation pressure as temperate predators more often target native species (potentially overriding oddity effects; Mathis & Chivers, 2003); and (3) increased foraging efficiency (Camacho-Cervantes et al., 2014; Pajmans et al., 2020). However, because ocean warming and acidification can modify a wide range of behaviours, these benefits during the initial stages of range extension could be lost under future warming and acidification, driving functional shifts in shoaling dynamics of vagrant and native fishes. This could lead to decreased species performance, consequently altering range-extension outcomes in marine ecosystems.

Gregarious fish species often possess some degree of brain asymmetry, whereby they turn to one side more often than the other under predation stress (Miletto Petrazzini et al., 2020). For shoaling species, such lateralization can be an effective strategy to minimize predation risk through dilution and confusion effects (e.g. when an entire shoal turns right, it reduces the focal fish's probability of predation (Turner & Pitcher, 1986). Furthermore, lateralized individuals have increased cognition (Bibost & Brown, 2013; Bisazza & Brown, 2011) and form more cohesive and coordinated shoals than non-lateralized fish (Bisazza & Dadda, 2005). Hence, cohesive shoals inherently possess a competitive advantage over less cohesive and less lateralized shoals through superior performance of behavioural tasks such as predator vigilance, predator escape reactivity and prey acquisition (Bisazza & Dadda, 2005; Dadda & Bisazza, 2006; Dadda et al., 2010). Ocean warming and acidification have been shown to alter lateralization (Domenici et al., 2012, 2014; Vila Pouca et al., 2018), shoaling dynamics (Colchen et al., 2016; Lopes et al., 2016; Nadler et al., 2016) and anti-predator performance (Dadda et al., 2010; Domenici et al., 2019) in fishes. Thus, the performance of novel species shoals composed of vagrant and native fishes could strongly modulate the persistence of species undergoing range shifts.

Here we used a controlled laboratory experiment to test the interactive effects of ocean warming, ocean acidification and novel species interaction on the lateralization, shoaling dynamics and anti-predator performance of a common tropical vagrant and temperate co-shoaling fish species across a 1-month exposure period. Video recordings were used to analyse shoaling behaviour, while motor lateralization was repeatedly tested over 4 weeks using a standard detour test. We aimed to test how climate change stressors and novel interactions might alter the shoaling dynamics, boldness and lateralization of tropical vagrant fish and native co-shoaling temperate fish.

We then discuss how changes to these behavioural proxies might facilitate or restrict tropicalization in high latitude ecosystems.

2 | MATERIALS AND METHODS

2.1 | Fish collection and acclimation

In Australia, strengthening of the East Australian Current and faster than average ocean warming at high latitudes (34°S) along the east coast (Lough, 2008; Ridgway, 2007; Ridgway & Hill, 2009) has acted as a direct dispersal mechanism for dispersive larval-stage tropical fishes from the Great Barrier Reef into temperate south-eastern Australian waters (Booth et al., 2007, 2011). Southeastern Australia's cooler conditions have so far restricted tropical fish from establishing breeding populations, as temperatures drop below their thermal minima in winter (~17°C; Figueira et al., 2009; Figueira & Booth, 2010). However, ocean warming in southeastern Australia (Ridgway, 2007) is expected to facilitate the successful establishment of breeding tropical populations (Feary et al., 2014). These range-extending fish species are commonly termed 'vagrants'. One of the most common species of vagrants, Indo-Pacific Sergeants (*Abudefduf vaigiensis*), is often observed shoaling with temperate species, including Eastern Haulfish (*Trachinops taeniatus*) and, more frequently, Australian Mado (*Atypichthys strigatus*) (Smith et al., 2018).

A. vaigiensis is omnivorous, exhibits a dispersive larval stage, has high site fidelity in the adult stage, and reaches up to 20 cm in length (Beck et al., 2016). Co-shoaling temperate species, *A. strigatus*, grows up to 25 cm in length and is also omnivorous (Kuitert, 2000).

Flow-through 20-litre transparent tanks (IKEA product number: 898.914.70; dimensions: 39 × 28 × 28 cm, height × length × width; $n = 120$) were installed across two adjacent flowthrough temperature/pH control rooms at the Sydney Institute of Marine Science. Two 0.5 cm (diameter) holes were inserted 4 cm from the top of each tank (lengthways) to allow water to flow bilaterally out of each tank. Each pairing tank was allocated a tank identifier and ocean acidification (OA) treatment (Table S1). Tanks were randomly assigned one of the two CO₂ × 3 temperature treatments ($N = 6$ treatments; Table S1). The ocean warming and ocean acidification treatments reflected those forecasts during summer and winter for the New South Wales coastline by 2100 under an RCP 8.5 scenario (20°C: project future winter temperature, 23°C: current summer temperature and 26°C: projected future summer temperature; Ridgway, 2007). We added 10 cm lengths of PVC piping (diameter: 10 cm) to each tank for shelter (Figure S1). Tanks were maintained at experimental target temperatures and pH units throughout the experiment (Table S1). Solenoid feedback systems were used to maintain the designated temperature within a range of $\pm 0.25^\circ\text{C}$ of the tank target temperature. Ocean acidification treatments were reached by bubbling pure CO₂ into header tanks (one header tank for every four fish tanks) to reflect future values. Header tank water flowed into fish tanks at a rate of 150 ml·min⁻¹. pH was decreased at a rate of 0.06 pH/day

by gradually increasing the pCO₂ concentration in header tanks and monitored daily until target pH levels were reached.

Sixty *A. vaigiensis* and 180 *A. strigatus* were collected during 7–24 March 2020, using barrier and hand nets at Little Manly Cove (–33.806771°S, 151.285644°E) and Freshwater Beach (–33.781688°S, 151.294088°E) at depths of 0.1–2 metres. Following collection, fish were transported to ambient holding tanks (size: 100 litres) at the Sydney Institute of Marine Science.

Tropical vagrants benefit from shoaling with temperate residents, growing faster and surviving longer into winter than conspecific tropical shoals in temperate ecosystems (Smith et al., 2018). Hence, the rationale for selecting temperate + temperate and tropical + temperate pairs lies in understanding how novel co-shoaling species in temperate ecosystems (i.e. range-extending tropical) might alter temperate fish performance in mixed-species shoals (i.e. future conditions) compared to temperate-only pairs (i.e. present-day conditions), and how this is mediated by the direct effect of climate change stressors. This comparison of shoaling types can inform us about potential declines of temperate fishes in tropicalization hotspots and the rate of tropicalization in a future ocean. As such, and also because of space limitations, we did not include pairs of tropical species.

Fish were randomly assigned to pairing tanks (tropical vagrants, $N: 60$; mixed-species temperates, $N: 60$; temperate-only paired fish, $N: 120$) across climate-manipulated rooms. Temperate-only fish were paired with individuals of similar body sizes to mitigate competitive advantages that larger fish could develop in obtaining food and shelter. However, mixed-species shoaling fish displayed different body sizes to reflect the current field situation of tropical vagrants being smaller than their co-shoaling temperate species (Smith et al., 2018; Table S2). Before commencement of the experiments, all individual fish had their initial wet weight measured on digital scales (to the nearest 0.01 g) and initial standard length measured (to the nearest 0.01 mm) with a sliding calliper from the tip of the upper lip to the beginning of the caudal fin. Initial wet weight (mean $\pm$ SE) was: tropical fish 0.31 ± 0.04 g; mixed-species temperate fish 1.24 ± 0.15 ; temperate-only fish 4.04 ± 0.20 . For initial standard length, this was: tropical fish 18.61 ± 0.68 mm; mixed-species temperate fish 29.34 ± 1.52 mm; temperate-only fish 53.28 ± 0.80 mm).

Fish were acclimated from ambient temperature conditions by raising or decreasing temperatures by 0.5°C per day to minimize stress generated from rapid temperature changes (Alfonso et al., 2020). Fish introduction and acclimation to experimental tanks were staggered to prevent fish in control treatments (23°C, pH 8.1) from gaining an acclimation advantage over fish in treatments that required more acclimation steps (e.g. 20°C and pH 7.7 or 26°C and pH 7.7). Fish were exposed to climate treatments for 6 days before the commencement of motor lateralization testing. APITM Stress Coat solution was added to each tank daily during acclimation and experimental periods. No fish died during the acclimation period. All fish were fed under a restricted feeding regime of 2.86 g of Ocean NutritionTM frozen *Artemia* mixed into 60 ml fresh seawater twice daily. Fish were exposed to treatment conditions for a maximum length of 40 days.

2.2 | Water chemistry

Total alkalinity values were estimated by Gran titration (Metrohm 888 TitrandotM) from 60 ml water samples from all tanks at days 24 and 25. Mean $p\text{CO}_2$ values were calculated using CO2SYS (Pierrot et al., 2006) for Excel with constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) (Table S1).

2.3 | Lateralization test

Motor lateralization was evaluated using a standard detour test (T-test) (Bisazza et al., 1998). The detour test is commonly used to evaluate behavioural asymmetries in fish (Miletto Petrazzini et al., 2020). The arena used in this study was based on a design used previously by Bisazza et al. (1998; Figure S2; note: our arena design differed slightly, as we removed the visual stimulus cues at each end of the arena used in Bisazza et al., 1998), and it consists of a two-way T-shape runway which allows scoring the direction of the turn (e.g. left or right) of each individual over consecutive trials. The experimental arena consisted of a transparent plastic tank (IKEA product number: 198.856.46; $39 \times 28 \times 14$ cm, length $\times$ width $\times$ height), with a runway in the middle (20×8 cm, length $\times$ width). At both ends of the runway, plywood barriers ($12 \times 12 \times 1$ cm) were positioned perpendicular to the runway. The runway was created by placing custom wooden plywood inserts onto each side of the arena to form the runway ($20 \times 8 \times 15$ cm). Water in the arena was 15 cm deep and ran directly from a flowthrough control system into both sides of the arena, at a rate of $150 \text{ ml} \cdot \text{min}^{-1}$, to maintain target temperature and pH in the arena. Two parallel 0.5 cm (diameter) holes were added at each end of the runway to minimize bias in directional water flow.

At the start of each trial, a single fish was introduced from a pairing tank (tropical + temperate or temperate + temperate) into the experimental arena and left to acclimate to the novel arena environment for 2 min. We conducted 12 consecutive turning trials for each individual. During each trial, we chased the focal fish with a handheld net along the runway to force a left or right turning choice at the end of the runway (see Vinogradov et al., 2021). The experimenter was always positioned at the same end of the arena to minimize side bias. Upon completion of the 12 turning decisions, fish were returned to their pairing tank. The experimental arena was emptied and refilled with treatment seawater following each trial until all treatments had been tested.

Mean relative lateralization (L_R) index was calculated from the 12 turning decisions (per fish, per trial week) and used to identify the strength of laterality and turn preference (e.g. bias in left or right turns). For the L_R index, individuals were classified between the extreme values of '+100' (fish that turned right on all 12 turning decisions) and '-100' (fish that turned left on all 12 turning decisions). An individual L_R close to zero indicates that a fish is neither left- nor right biased in its turning tendency (Bisazza et al., 1998). Because individuals could be left biased (all negative scores) or right biased (all positive scores), the average L_R across replicate fish within a

treatment could hypothetically be zero (i.e. even number of fully left- vs. right-biased fish). This would not reveal the presence of a strong lateralization response that is independent of turning direction. Therefore, the absolute lateralization (L_A) index was also calculated (Domenici et al., 2014). This, in contrast to L_R , evaluates individual lateralization strength irrespective of the direction of their turns. The L_A index corresponds to the absolute value of L_R , thus ranging from 0 (an individual that turned in equal proportion to the right and the left) to 100 (an individual that turned right or left on all 12 trials).

Each fish performed 12 turning decisions at alternating ends of the runway, on trial days 6, 17 and 25, from which the mean score of the turning direction (L_R) and the mean L_A for each fish were obtained for each trial day. Lateralization tests were not performed on fish showing signs of stress (i.e. irregular swimming pattern).

Tropical and temperate fish were tested between 7 am and 10 pm on days 6, 17 and 25 of the experiment. One researcher set up the treatment water in the experimental arena on each trial day. The sequence of treatments being tested was selected randomly. The second person who performed the scoring of lateralization was blinded to the treatment type to minimize observer bias.

2.4 | Video observations of shoaling dynamics and startle escape response

On days 19 and 27 (recording days selected to minimize repeated experimental stress following T-tests on days 17 and 25) of treatment exposure, we filmed all fishes' shoaling dynamics (inter-individual distance [IID], amplitude variability and oscillation frequency) and startle escape distance (SED) in 9-min video recordings. Paired fish (mixed-species or temperate-only pairs) were kept in their pairing tanks during video observations (Figure S1). Tanks were illuminated by LED strip lighting (750 lumens) placed above the water surface outside the tank. White sheeting surrounded the back of the tank to minimize any external influences on tested fish during video recordings. We placed GoPro Hero 7 Silver cameras 30 cm away from pairing tanks and commenced recording. Video recordings lasted for 9 min, excluding 3 min of acclimation time to the cameras, and were split into three 3-min periods (period 1: shoaling dynamics, period 2: food administration and period 3: SED). Researchers were blinded to treatments of each video recording during video analysis until data collection was completed.

2.4.1 | Period 1: Shoaling dynamics

We measured three different proxies for shoal dynamics of each fish (mixed-species and temperate-only pairs) during the first 3-min period of the video analysis:

1. Inter-individual distance. The distance between individuals within each tank was measured every 3 s for 3 min ($N = 60$ measurements per tank) of video recording. The average distance

between fish was calculated to obtain the average distance for shoals (mixed species or temperate only; Figure S1). When both fish in a tank were observed residing within the shelter, the IID was scored as a distance of 0 cm. If both fish could not be observed simultaneously, a 'not applicable' distance value was given for that time point.

2. Amplitude variability. From the 60 repeated measurements of shoaling distance within each tank, the standard deviation of IID was calculated for each tank, and the mean, standard deviation for each treatment type was calculated to determine amplitude variability.
3. Oscillation frequency. The IID of shoaling fishes is not fixed (Miller & Gerlai, 2008) but oscillates between a maximum and minimum distance. We measured the time (seconds) elapsed between sequential maximum peak distances across the 60 IID samples taken across the 180 s shoaling video period to calculate the mean time between maximum distance points (the oscillation frequency).

Paired fish often were aligned with each other at the front of the tank (the side nearest to the camera), reducing the overall effect that tank depth had on calculating the distance between the individuals.

2.4.2 | Period 2: Food administration

Approximately 1.43 g of Ocean Nutrition™ frozen *Artemia* mixed into 60 ml fresh seawater was administered via a syringe into each tank to draw fish out of the shelter in preparation for SED testing. Tanks were left undisturbed for 3 min following the addition of food to tanks.

2.4.3 | Period 3: Startle escape distance

We used a SED test as a proxy to measure the boldness and anti-predator behaviour of paired fish. Startle escape distance was defined as 'the distance at which a fish initiated a rapid movement away from the simulated threat'. We used a white ceramic weight (size: 3 × 3 × 3 cm; weight 500 g) as the simulated threat.

After 6 min of video recording, a ceramic weight was handheld above a tank (to mimic a looming visual stimulus). The ceramic weight was then released from 3 cm above the water surface between the two paired fish to induce a startle escape response. In case a fish did not elicit a startle escape response or was within the shelter provided, a 'not applicable' score was given.

During video analysis, videos were paused to measure the distance between the focal fish and the ceramic weight at which startle escape response was initiated. Distance calibration during video analysis was accomplished by using the shelter size (10 cm) as a reference.

2.5 | Statistical analysis

Using Primer version 6, a permutational multivariate analysis of variance (MANOVA) was used to examine the effect of temperature,

pCO₂ and pairing type treatments on the various non-independent behaviours, that is, those measured from the same individuals (L_R , L_A , IID, mean distance amplitude, oscillation frequency, and SED) for the tropical and temperate fishes. A four-way MANOVA was performed for the tropical fish (factors: ocean acidification (OA), temperature (Te), time (Ti) and fish ID (Fi)), and a five-way MANOVA (addition of fixed factor: pairing type (Pa)) for the temperate fish. Fixed factors were temperature, ocean acidification and pair type. Fish ID was nested within all factors. Time was set as a repeated measure random factor. Fish which scored 'not applicable' scores in SED tests were excluded from MANOVAs. The data were first standardized (to adjust the values of the different behavioural types to a similar scale). The MANOVA datasets were square-root transformed and converted into a Euclidean distance resemblance matrix. Permutations were set to 9,999. Permutational MANOVAs were performed, followed by pairwise post hoc tests (significance accepted at $p < .05$; Anderson, 2001).

Lateralization (L_R and L_A) and startle response distance were also tested separately for the two species among the 12 treatments using four-way ANOVAs for tropical fish (ocean acidification (OA), temperature (Te), time (Ti) and fish ID (Fi)) and five-way ANOVAs (addition of fixed factor: pairing type (Pa)) for temperate fish. Fish ID was set as a random repeated measure factor and nested within the main factors (OA, Te and Pa). Values for lateralization and startle response distance were square-root transformed. Proxies of shoaling dynamics (IID, mean distance amplitude and oscillation frequency) were tested using five-way ANOVAs. Pairing type, temperature and ocean acidification were set as fixed factors. Tank ID was nested within the main effect factors. Time was set as a repeated random measure factor. Values for shoaling amplitude and inter-peak oscillation frequency were square-root transformed. The permutation method used was a permutation of residuals under a reduced model. A Euclidean distance dissimilarity resemblance matrix was used for univariate statistical analyses. The number of replicates for the above ANOVAs was larger than that used in the MANOVAs, as the latter could only include individuals for which all six behaviours were measured, excluding those with missing values for a particular behaviour, while the former included all fish.

We performed ANCOVAs to test how both IID and SED were related to relative laterality (i.e. did an increased IID correlate with decreased laterality). For ANCOVAs, we first tested for homogeneity of slopes by including interactions between covariates and main factors. In cases where interactions were not significant, these were removed from the final test statistics. Other assumptions for ANCOVAs (linearity, normality) were tested via residuals and box-plots. We found no association between laterality and shoaling or SED and hence did not discuss this result in detail (see the statistical output in Table S3-S6).

On R version 3.6.2, we used the function 'rpt' located in R package 'rptR' (Stoffel et al., 2017) to test the relative repeatability (L_R) across the three trial series for individuals of each species across treatments. This differed from testing the 'time' effect in the repeated measures ANOVAs because R package 'rptR' provides a measure of the consistency of L_R scores of individual fish

among treatments across time, while repeated measures ANOVAs testing time effects on L_R analyse differences in a samples mean L_R across time. Mean repeatability values for species across treatments ranged from 0 (non-repeatable) to 1 (fully repeatable) and provided a standardized measure of the consistency of phenotypes across time (Nakagawa & Schielzeth, 2010). We specified Gaussian error distributions and 1,000 bootstrapping and permutation iterations to calculate 95% confidence intervals (CIs). Repeatability was calculated separately for species (tropical or temperate), pCO_2 levels (control or OA), temperature (20, 23 or 26°C) and pair types (mixed species or temperate only). Fish ID was added as a random effect, as per Roche et al. (2020). The raw data used in the statistical analysis are publicly available (Mitchell et al., 2021).

3 | RESULTS

3.1 | Multivariate analysis of behaviours

Behaviours of the tropical fish were significantly altered under ocean acidification at 20°C (20°C: control vs. OA, $p = .049$; Tables S7, S9) and differed between 20°C and 26°C under ocean acidification ($p = .023$; Tables S7, S8). Behaviours of temperate-only paired fish significantly shifted from 20°C to 26°C ($p = .013$; Tables S10, S11). Temperature had no effect on the behaviours of mixed-species paired temperate fish ($p > .05$, Table S11). Behaviours also differed between temperate-only and mixed-species temperate fish at 20°C and 26°C (temperate only vs. mixed species, 20°C: $p = .022$, 26°C: $p = .015$; Table S12), but not at 23°C ($p > .05$; Table S12).

3.2 | Individual behaviours

Both the tropical and temperate fish showed a right-side turning bias at population level (L_R), which was significantly reduced under ocean acidification (Figure 1a,c,e, Figure S3a,c,e; tropical: $p = .003$, Table S15; mixed- and temperate-only paired temperate: $p = .004$, Table S8). Mixed-species paired temperate fish showed significantly greater L_R than temperate-only paired fish (Figure 1c,e, Figure S3c,e; $p = .037$, Table S13). Absolute lateralization was not affected by climate treatments in temperate or tropical fish (Figure 1b,d,f, Figure S3b,d,f; Tables S14, S16). Time had no effect on L_R or L_A ($p > .05$; Table S13–S16).

Mixed-species shoals showed significantly greater IID than temperate-only pairs (Figure 2a vs. 2b; $p < .001$, Table S17). Ocean acidification significantly increased IID in both pairing types ($p = .036$, Table S17). The mean amplitude of shoaling distance of mixed-species and temperate-only pairs was not affected by climate treatments but was greater in mixed-species pairs than temperate-only pairs (Figure 2c vs. 2d; $p < 0.001$, Table S18). In both shoal types, mean amplitude only decreased from day 19 to day 27 ($p = .016$; Figure 2c,d, Table S18). The oscillation frequency of IID of mixed-species and temperate-only pairs was not affected by ocean

acidification or temperature (Figure 2e,f) but was longer in mixed-species pairs than temperate-only pairs ($p < .001$, Table S19).

Startle escape distance of tropical fish exposed to ocean acidification significantly decreased from day 19 to day 27 (Figure 3a; $p = .030$, Tables S26, S30). In temperate fish (both pairing types), SED was significantly shorter under warming (20°C vs. 23°C and 26°C, $p < .001$, Tables S25, S26); however, temperate fish exposed to ocean acidification showed a greater SED than fish not exposed to ocean acidification, irrespective of warming, shoal type and time (Figure 3b,c; $p = .047$, Table S20). In mixed-species temperate fish, SED significantly increased from day 19 to day 27 across all climate treatments (Figure 3b; $p = .044$, Tables S20, S25).

3.3 | Repeatability of lateralization

Relative lateralization of individuals was not significantly repeatable over time in tropical or temperate fish across treatment types ($p > .05$, Table S31) except in tropical fish at 26°C under control pCO_2 levels ($R = 0.38 \pm 0.193$, $CI = 0-0.701$, $p = .038$, Table S31). In contrast, repeated measures ANOVAs showed no effect of time on the reduced lateralization observed under ocean acidification (OA $\times$ Ti interaction: $p = .563$ for tropical and $p = .390$ for temperates, Tables S13, S15).

4 | DISCUSSION

Here we show that ocean warming and acidification can disrupt the lateralization and shoaling behaviour of temperate fish pairs as well as novel mixed-species pairs composed of tropical and temperate fishes in climate range-shifting hotspots. Changes to critical behavioural traits, such as reduced lateralization, shoal cohesion and startle escape distance (only in temperate fish under warming) under ocean warming or acidification, could decrease temperate and tropical fish performance (e.g. feeding and predator avoidance). Reduced lateralization of tropical vagrants and co-shoaling temperates under ocean acidification could decrease individual cognitive performance (Bibost & Brown, 2014) and performance of novel shoals, increasing predation risk of both tropical and temperate fish. Ocean acidification may buffer tropicalization directly through reduced lateralization in tropical vagrants and indirectly through degrading the shoaling dynamics of novel tropical-temperate pairs (Bisazza & Dadda, 2005; Dadda et al., 2010; Lopes et al., 2016). The latter could reduce behavioural plasticity and learning (e.g. novel prey and predators; Bibost & Brown, 2014) of vagrants entering high-latitude ecosystems. In addition, ocean acidification could switch currently dominant habitat types at high latitudes from barrens to turf-dominated habitats, reducing the recruitment of tropical fishes and hence retard the rate of tropicalization at high latitudes (Coni et al., 2021c). We conclude that ocean acidification may decrease the performance of common temperate and tropical co-shoaling species through disruption of their lateralization and shoaling dynamics,

Lateralisation

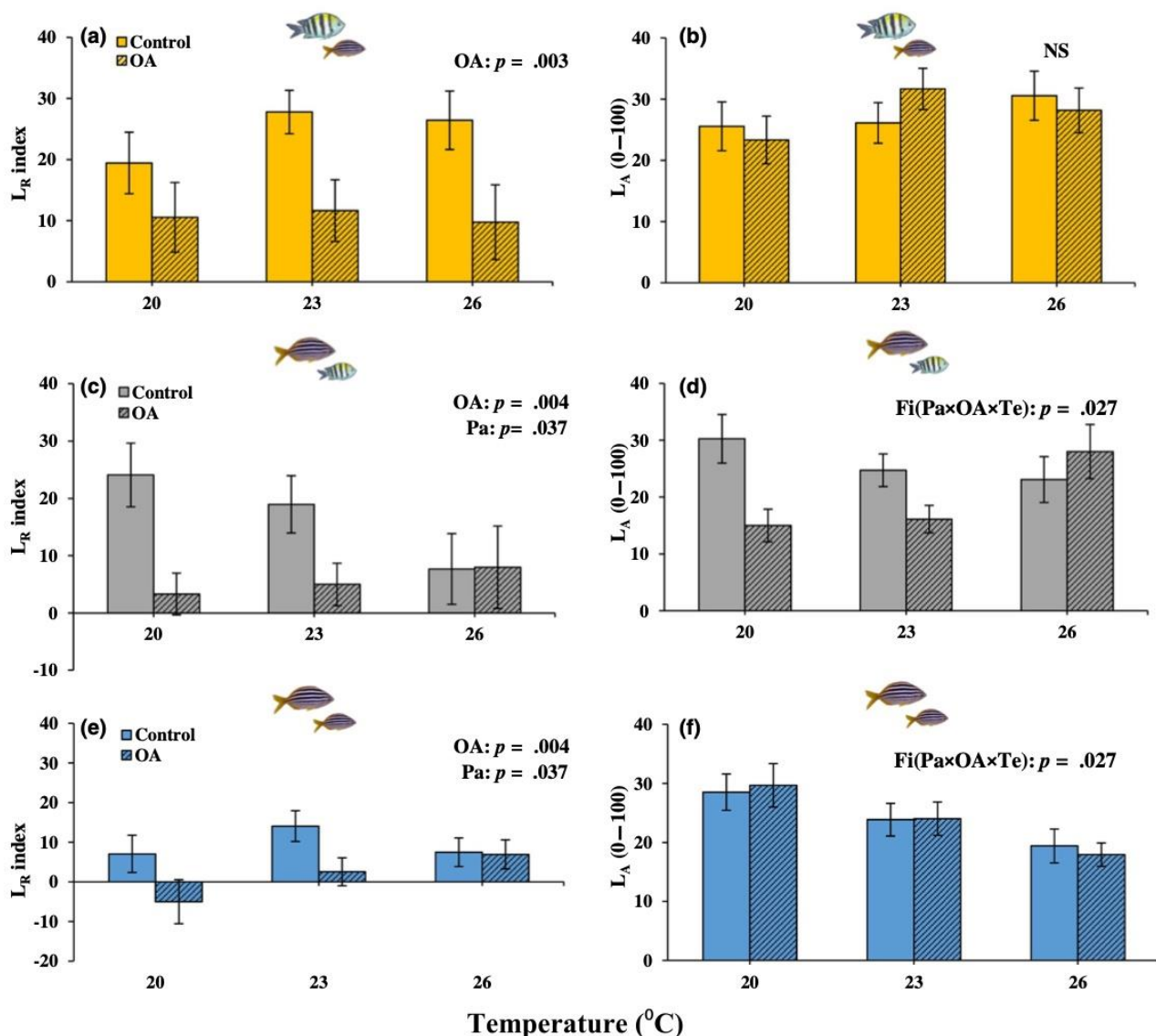


FIGURE 1 Mean difference ($\pm$ SE) in relative lateralization (L_R) and absolute lateralization (L_A) across three repeated trials for mixed-species shoaling *A. vaigiensis* (a and b), mixed-species shoaling *A. strigatus* (c and d), and temperate-only shoaling *A. strigatus* (e and f) among temperature (Te) treatments (20°C: project future winter temperature, 23°C: current summer temperature and 26°C: projected future summer temperature) and ocean acidification (OA) treatments (control pH: 8.1, projected future pH: 7.7; Tables S13–S16). NS = not significant, Fi = Fish individual, Pa = Pair Type. Error bars represent standard errors [Colour figure can be viewed at wileyonlinelibrary.com]

which might slow the rate of tropicalization in SE Australia and other temperate ecosystems.

Ocean warming increased the boldness (i.e. reduced startle escape distance) of temperate fish irrespective of shoaling type, while the combination with ocean acidification appears to dampen the boldness of the temperate fish. This, together with a decrease in shoal cohesion and lateralization under ocean acidification, could compromise the survival of temperate species at their current equatorward distributions (i.e. trailing edges) in a future ocean. Reduced shoaling cohesion is likely to decrease anti-predator shoal performance by extending the time taken before a minimum

relative number of shoal mates initiate a critical behaviour (e.g. left turn to evade a predator; Lemasson et al., 2018; Ward et al., 2008). Lateralized, more cohesive, and polarized shoals benefit from faster information transfer between shoal mates (Bisazza & Dadda, 2005; Ward et al., 2018). Hence, a destabilization of shoal cohesion and L_R can slow down information transfer time between shoal mates, thus decreasing decision-making and reaction time in critical fast-event situations (e.g. predator attacks). Altered shoaling dynamics could further modify the performance of temperate species via shifts in behavioural trade-offs (e.g. foraging activity vs. predator vigilance) typically observed in shoaling fish (Hintz & Lonzarich, 2018). Under

Shoal cohesion dynamics

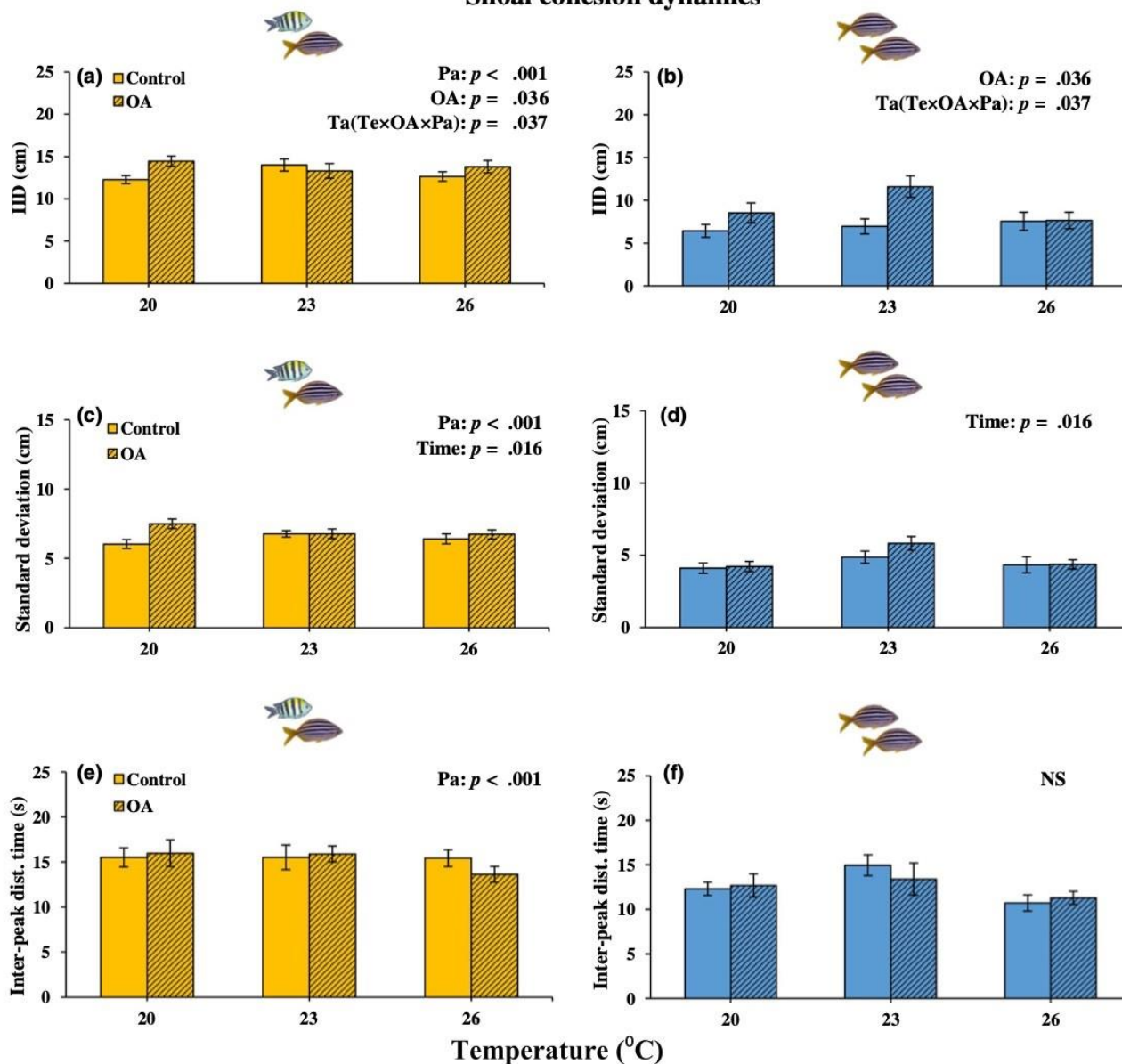


FIGURE 2 Mean inter-individual distance (IID, cm), mean distance amplitude of IID (standard deviation, cm) and the inter-peak distance oscillation frequency of IID (sec) across mixed-species pairs (panels: (a), (c) and (e)) and temperate-only pairs (panels: (b), (d) and (f)) shoals (Tables S17–S19). Data for video trials at days 19 and 27 were pooled for visualization purposes. NS = not significant. Pa = paring type (tropical + temperate vs. temperate only), Ta = Tank ID. Error bars represent standard errors [Colour figure can be viewed at wileyonlinelibrary.com]

ocean warming, bolder temperate fish may benefit from increased prey access (Weetman et al., 1998). However, boldness benefits may be dampened by decreased lateralization and altered shoaling dynamics (this study), concurrently increasing predation mortality risk and decreasing foraging efficiency (Miletto Petrazzini et al., 2020). Such decreases in startle escape distance and altered shoaling dynamics of temperate fish as a consequence of novel species interaction and climate change are likely to reduce the performance of shoaling temperate fishes, possibly speeding up range contraction rates at the trailing range edges of temperate residents

and lowering their overall abundance in temperate tropicalization hotspots.

The dampening of right-side bias of co-shoaling tropical and temperate fish under ocean acidification may have negative ecological implications on species performance. Our lateralization results align with previous work showing a decrease from right-side bias L_R in fish exposed to ocean acidification (Domenici et al., 2014). Strong L_R is more common in gregarious species, as lateralization can provide benefits relating to cognition (Bibost & Brown, 2014), shoal cohesion (Bibost & Brown, 2013; Bisazza & Dadda, 2005) and escape

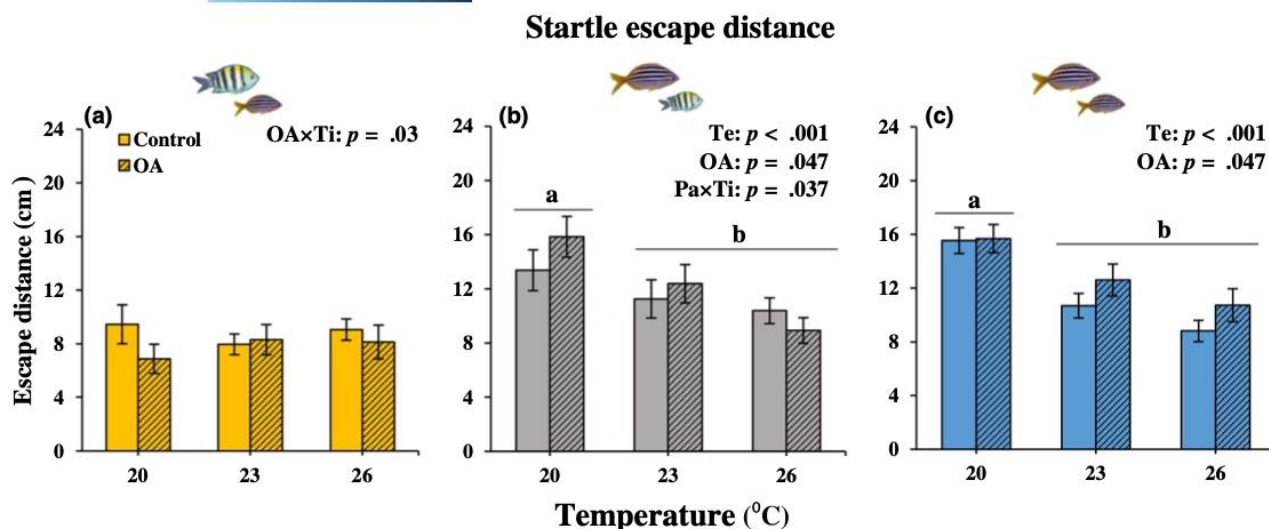


FIGURE 3 Startle escape distance (cm) for tropical (a) and temperate (b, c) fishes across temperature (Te), ocean acidification (OA) and shoaling type (Pa) treatments (the larger fish in (a) and (b) indicate the focal fish species, while the smaller one indicates the co-shoaling fish species). Ti = time (days). Data for days 20 and 27 were pooled for visualization purposes. The different letter above bars indicates significant differences among treatments (Tables S21–S30). Error bars represent standard errors [Colour figure can be viewed at wileyonlinelibrary.com]

performance (Dadda et al., 2010). We assume that the relative lateralization pattern detected in control groups is representative of wild populations of tropical vagrants and temperate co-shoaling fish. Thus, ocean acidification will likely diminish any advantage provided by L_R to gregarious tropical and temperate fish.

Relative lateralization was not repeatable at the individual level yet on average (i.e. across individuals) did not change over time (4 weeks). Our findings suggest fish populations can maintain side bias across extended periods (days to weeks), irrespective of individuals possessing some degree of plasticity in their laterality responses (Stamps et al., 2012). In an ecological context, the predictability of lateralized behaviours can be ecologically detrimental to the fitness of shoaling fishes (Vallortigara & Rogers, 2005). Reduced predictability of predator escape behaviours through variability in individual lateralization responses within a population provides ecological benefits in predator–prey encounters. Plasticity of lateralization might be an artefact of the evolutionary consequences of gregarious species responding to predation due to the costs associated with predictable and strongly lateralized behaviours (Chivers et al., 2016). The plasticity of lateralized behaviour may provide ecological benefits to gregarious species through intra-individual variability (Stamps et al., 2012) and may account for the non-significant individual repeatability we detected. For example, individual fish in a shoal might reduce the predictability of their lateralized behaviours through intra-individual variation while the collective shoal maintains relative side bias over time, supported by our non-significant time effects detected in our L_R results. Plasticity in laterality can enhance species fitness and concurrently maintain ecological advantages attributed to relative laterality (Bibost & Brown, 2014; Bisazza & Dadda, 2005; Dadda et al., 2010). Our findings suggest that L_R and how this is altered by

ocean acidification is repeatable over time despite intra-individual variability within lateralized populations.

Novel shoaling interactions between tropical vagrants and temperate natives can diminish shoal dynamics irrespective of climatic stressors. Here we show that in mixed tropical–temperate species pairs, shoal cohesion and amplitude/oscillation frequency of inter-individual distances increased compared to temperate-only pairs. Slower oscillation frequencies may indicate destabilization of the trade-offs between foraging and predator vigilance observed in single-species shoals (Miller & Gerlai, 2007, 2008). Periodic changes in the inclination of vagrants to match a temperate shoal mate's orientation and vice versa (Partridge, 1981; Partridge & Pitcher, 1980) may also contribute to slower oscillations. Hence, due to the historical unfamiliarity of two species with each other, orientation alignments take longer, leading to a slower information transfer time between mixed-species pair mates, inhibiting the reactivity and responsiveness of the collective mixed-species shoal during critical events. The larger shoaling amplitude observed in mixed-species shoals may be explained by the species (historical) unfamiliarity and account for unfamiliar shoal behaviour compared to the homogenous behaviour characteristic of highly cohesive and polarized single-species shoals (Miller & Gerlai, 2008). Slower oscillation frequency, lower shoal cohesion, greater amplitude and shorter startle escape distance in mixed-species shoals compared to temperate shoals implicates that anti-predator and shoaling performance of temperate species can degrade in novel shoal compositions due to the indirect effect of species range extensions.

A limitation of our study is that we tested the shoaling dynamics of fish pairs. Group size, social and physical factors can alter group dynamics and structure (Krause, 1993; Partridge, 1980). While fish

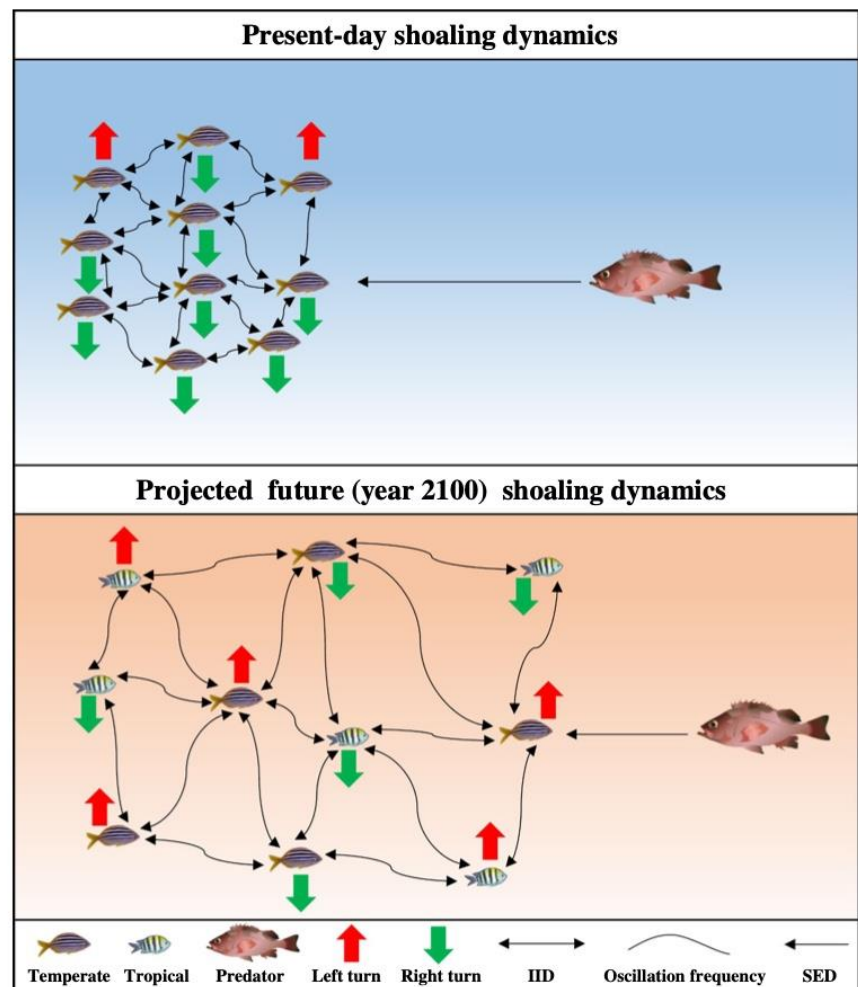
pairs can be considered a shoal (Partridge, 1980), group size and tropical–temperate shoal composition may alter shoal-level responses to climate change (Coni et al., 2021a). To support and generalize our findings, we suggest future climate experiments focus on scaling up from pairs to larger shoals.

Under current climate conditions, novel species interactions could concurrently boost the growth of tropical vagrants (Smith et al., 2018), restrict vagrant foraging performance (Coni et al., 2021a) and reduce the shoaling performance of temperate fish in mixed-species shoals (this study). Under future climate conditions, however, lower temperate and tropical fish fitness may arise through the degradation of L_R , shoaling and anti-predator performance (Figure 4). Ocean warming will provide tropical organisms with thermally suitable ecosystems to invade at high latitudes, while temperate species face physiologically undesirable conditions, likely driving abundance declines at their trailing range edges (Coni et al., 2021a, 2021c). The rate at which temperate population levels decrease or are impacted, however, may be slowed under ocean acidification by destabilizing the shoaling behaviour performance of tropical vagrants and depressing settlement success (reduced competition). We suggest that in future oceans, reduced lateralization, altered shoaling dynamics and

decreased anti-predator performance resulting from co-occurring climate effects may have negative ecological implications on co-shoaling tropical and temperate fish.

Previous tropicalization studies did not account for ocean acidification effects on vagrant range-extension success. Most studies still focus primarily on how ocean warming facilitates tropicalization at higher latitudes, increasing tropical fish abundances and reducing that of temperate fishes. Current climatic conditions and novel species interactions allow for the coexistence of tropical vagrants and co-shoaling temperate fishes (Coni et al., 2021a) at tropical vagrant cold-edge ranges due to the wide trophic niche breadth of tropical vagrants (Kingsbury et al., 2019), their relative dietary generalism (Monaco et al., 2020), physiological benefits accrued from novel tropical–temperate shoaling (Smith et al., 2018) and the mosaic of suitable habitats found in temperate tropicalization hotspots (Coni et al., 2021c). However, the coexistence of interacting gregarious temperate and tropical fishes may be compromised under future climatic conditions. We suggest that a temperature increase of 3–4°C coinciding with ocean acidification and ensuing habitat shifts (caused by ocean acidification; Coni et al., 2021c) at high latitude tropicalization hotspots could simultaneously slow tropical fish range extension and decrease temperate fish abundance, through

FIGURE 4 Conceptual diagram showing how the future combined effect of temperature and CO₂ increases can reduce the inter-individual distance (IID), relative lateralization (L_R) and startle escape distance (SED) of novel shoal types of local temperate and vagrant tropical fishes. Integration of tropical vagrants into residing temperate shoals slows down shoal oscillation frequency (–), increases shoal cohesion amplitude and decreases shoal cohesion compared to temperate-only shoals who exhibit higher shoal cohesion and lower amplitude faster oscillation frequency [Colour figure can be viewed at wileyonlinelibrary.com]



degradation of shoaling performance across novel mixed-species and temperate shoals, in southeastern Australia and possibly in other tropicalization zones as well.

5 | CONCLUSIONS

Ocean warming facilitates the tropicalization of temperate ecosystems, but ocean acidification might partly negate this effect by reducing the lateralization, shoaling dynamics and anti-predator performance of vagrant tropical fishes in novel mixed-species shoals. Resident temperate species face negative implications from novel species interactions and ocean warming on their shoaling and anti-predator performance, while ocean acidification might partly reduce boldness and degrade lateralization of shoaling temperate fishes. We conclude that the degradation of lateralization and shoaling dynamics in novel tropical-temperate shoals by ocean acidification could slow down the rate of tropicalization in some climate change hotspots.

ETHICS STATEMENT

This experiment was conducted according to The University of Adelaide Animal Ethics and University of Technology guidelines and permits: S-2020-13 and 2017-1117, and under New South Wales DPI Scientific Collection Permit: F94/696(A)-9.0.

ACKNOWLEDGEMENTS

IN and DJB were supported by an Australian Research Council (ARC) Discovery Project (DP170101722). We thank the Sydney Institute of Marine Science for their help, guidance and patience during difficult circumstances surrounding COVID-19 when this research was performed. We also thank SIMS staff Amanda, Andrew and Sergio for their help around all facets of the project and Chloe Hayes for logistical support in experimentation.

CONFLICT OF INTEREST

The authors acknowledge that there was no conflict of interest during any part of the creation of this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at <https://doi.org/10.25909/17011619>.

ORCID

Angus Mitchell  <https://orcid.org/0000-0002-2255-9652>

David J. Booth  <https://orcid.org/0000-0002-8256-1412>

Ivan Nagelkerken  <https://orcid.org/0000-0003-4499-3940>

REFERENCES

- Alfonso, S., Gesto, M., & Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, 98(6), 1496–1508. <https://doi.org/10.1111/jfb.14599>
- Anderson, M. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1), 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>
- Beck, H., Feary, D., Fowler, A., Madin, E., & Booth, D. J. (2016). Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Marine Biology*, 163(4), <https://doi.org/10.1007/s00227-016-2844-8>
- Bibost, A., & Brown, C. (2013). Laterality influences schooling position in rainbowfish, *Melanotaenia* spp. *PLoS One*, 8(11), e80907. <https://doi.org/10.1371/journal.pone.0080907>
- Bibost, A., & Brown, C. (2014). Laterality influences cognitive performance in rainbowfish *Melanotaenia duboulayi*. *Animal Cognition*, 17(5), 1045–1051. <https://doi.org/10.1007/s10071-014-0734-3>
- Bisazza, A., & Brown, C. (2011). *Fish cognition and behavior*. C. Brown, K. Laland, & J. Krause (Eds.). 2nd ed. Wiley-Blackwell, Wiley. <https://doi.org/10.1002/9781444342536.ch14>
- Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralised fishes. *Proceedings of the Royal Society B: Biological Sciences*, 272(1573), <https://doi.org/10.1098/rspb.2005.3145>
- Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralisation of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research*, 91(1–2), [https://doi.org/10.1016/S0166-4328\(97\)00114-9](https://doi.org/10.1016/S0166-4328(97)00114-9)
- Booth, D. J., Bond, N., & Macreadie, P. I. (2011). Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research*, 62(9), <https://doi.org/10.1071/MF10270>
- Booth, D. J., Figueira, W. F., Gregson, M. A., Brown, L., & Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: Role of the East Australian Current. *Estuarine Coastal and Shelf Science*, 72(1–2), 102–114. <https://doi.org/10.1016/j.ecss.2006.10.003>
- Booth, D. J., & Sear, J. (2018). Coral expansion in Sydney and associated coral-reef fishes. *Coral Reefs*, 37(4), 995. <https://doi.org/10.1007/s00338-018-1727-5>
- Briffa, M., De La Haye, K., & Munday, P. L. (2012). High CO₂ and marine animal behaviour: Potential mechanisms and ecological consequences. *Marine Pollution Bulletin*, 64(8), 1519–1528. <https://doi.org/10.1016/j.marpolbul.2012.05.032>
- Burrows, M., Schoeman, D., Buckley, L., Moore, P., Poloczanska, E., Brander, K., Brown, C., Bruno, J., Duarte, C., Halpern, B., Holding, J., Kappel, C., Kiessling, W., O'Connor, M., Pandolfi, J., Parmesan, C., Schwing, F., Sydeman, W., & Richardson, A. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science*, 334(6056), 652–655. <https://doi.org/10.1126/science.1210288>
- Caldeira, K., & Wickett, M. E. (2003). Anthropogenic carbon and ocean pH. *Nature*, 425(6956), 365. <https://doi.org/10.1038/425365a>
- Camacho-Cervantes, M., Garcia, C., Ojanguren, A., & Magurran, A. (2014). Exotic invaders gain foraging benefits by shoaling with native fish. *Royal Society Open Science*, 1(3), 140101. <https://doi.org/10.1098/rsos.140101>
- Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, 333(6045), 1024–1026. <https://doi.org/10.1126/science.1206432>
- Chivers, D., McCormick, M., Allan, B., Mitchell, M., Gonçalves, E., Bryshun, R., & Ferrari, M. (2016). At odds with the group: Changes in lateralisation and escape performance reveal conformity and conflict in fish schools. *Proceedings of the Royal Society B: Biological Sciences*, 283(1841), <https://doi.org/10.1098/rspb.2016.1127>
- Colchen, T., Teletchea, F., Fontaine, P., & Pasquet, A. (2016). Temperature modifies activity, inter-individual relationships and group structure in a fish. *Current Zoology*, 63(2), <https://doi.org/10.1093/cz/zow048>
- Coni, E. O. C., Booth, D. J., Ferreira, C., & Nagelkerken, I. (2021b). Behavioural generalism could facilitate coexistence of tropical and temperate fishes under climate change. *Journal of Animal Ecology*. <https://doi.org/10.1111/1365-2656.13599>
- Coni, E. O. C., Booth, D. J., & Nagelkerken, I. (2021a). Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef

- fish. *Coral Reefs*, 40(5), 1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>
- Coni, E. O. C., Nagelkerken, I., Ferreira, C., Connell, S. D., & Booth, D. J. (2021c). Ocean acidification may slow the pace of tropicalisation of temperate fish communities. *Nature Climate Change*, 11(3), <https://doi.org/10.1038/s41558-020-00980-w>
- Dadda, M., & Bisazza, A. (2006). Does brain asymmetry allow efficient performance of simultaneous tasks? *Animal Behaviour*, 72(3), 523–529. <https://doi.org/10.1016/j.anbehav.2005.10.019>
- Dadda, M., Koolhaas, W., & Domenici, P. (2010). Behavioural asymmetry affects escape performance in a teleost fish. *Biology Letters*, 6(3), 414–417. <https://doi.org/10.1098/rsbl.2009.0904>
- Dickson, A., & Millero, F. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A: Oceanographic Research Papers*, 34(10), 1733–1743. [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)
- Domenici, P., Allan, B., Lefrançois, C., & McCormick, M. (2019). The effect of climate change on the escape kinematics and performance of fishes: Implications for future predator–prey interactions. *Conservation Physiology*, 7(1), <https://doi.org/10.1093/conphys/coz078>
- Domenici, P., Allan, B., McCormick, M. I., & Munday, P. L. (2012). Elevated carbon dioxide affects behavioural lateralisation in a coral reef fish. *Biology Letters*, 8(1), <https://doi.org/10.1098/rsbl.2011.0591>
- Domenici, P., Allan, B., Watson, S., McCormick, M. I., & Munday, P. L. (2014). Shifting from right to left: The combined effect of elevated CO₂ and temperature on behavioural lateralization in a coral reef fish. *PLoS One*, 9(1), e87969. <https://doi.org/10.1371/journal.pone.0087969>
- Feary, D. A., Pratchett, M. S., Emslie, M. J., Fowler, A., Figueira, W. F., Luiz, O. J., Nakamura, Y., & Booth, D. J. (2014). Latitudinal shifts in coral reef fishes: Why some species do and others do not shift. *Fish and Fisheries*, 15(4), 593–615. <https://doi.org/10.1111/faf.12036>
- Feely, R. A., Doney, S. C., & Cooley, S. R. (2009). Ocean acidification: Present conditions and future changes in a high-CO₂ world. *Oceanography*, 22(4), 36–47. <https://doi.org/10.5670/oceanog.2009.95>
- Figueira, W. F., Biro, P., Booth, D. J., & Valenzuela Davie, V. C. (2009). Performance of tropical fish recruiting to temperate habitats: Role of ambient temperature and implications of climate change. *Marine Ecology Progress Series*, 384, 231–239. <https://doi.org/10.3354/meps08057>
- Figueira, W. F., & Booth, D. J. (2010). Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Global Change Biology*, 16(2), 506–516. <https://doi.org/10.1111/j.1365-2486.2009.01934.x>
- Figueira, W. F., Curley, B., & Booth, D. J. (2019). Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes? *Marine Biology*, 166(73), <https://doi.org/10.1007/s00227-019-3521-5>
- Hintz, W., & Lonzarich, D. (2018). Maximising foraging success: The roles of group size, predation risk, competition, and ontogeny. *Ecosphere*, 9(10), <https://doi.org/10.1002/ecs2.2456>
- Hoegh-Guldberg, O., & Bruno, J. (2010). The impact of climate change on the world's marine ecosystems. *Science*, 328(5985), 1523–1528. <https://doi.org/10.1126/science.1189930>
- IPCC. (2021). Climate change 2021: The physical science basis. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.) *Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge University Press. In Press.
- Ishimatsu, A., Hayashi, M., Kyoung-Seon, L., Kikawa, T., & Kita, J. (2005). Physiological effects on fishes in a high-CO₂ world. *Journal of Geophysical Research*, 110(C9), <https://doi.org/10.1029/2004JC002564>
- Kingsbury, K., Gillanders, B., Booth, D. J., & Nagelkerken, I. (2019). Trophic niche segregation allows range-extending coral reef fishes to co-exist with temperate species under climate change. *Global Change Biology*, 26(2), 721–733. <https://doi.org/10.1111/gcb.14898>
- Krause, J. (1993). The relationship between foraging and shoal position in mixed shoal of roach (*Rutilus rutilus*) and chub (*Leuciscus leuciscus*): A field study. *Oecologia*, 93(3), <https://doi.org/10.1007/BF00317878>
- Kuiter, R. (2000). *Coastal fishes of southeastern Australia*. Crawford House Press.
- Lemasson, B., Tanner, C., Woodley, C., Threadgill, T., Qarqish, S., & Smith, D. (2018). Motion cues tune social influence in shoaling fish. *Scientific Reports*, 8(1), <https://doi.org/10.1038/s41598-018-27807-1>
- Lloyd, P., Plaganyi, É., Weeks, S., Magno-Canto, M., & Plaganyi, G. (2011). Ocean warming alters species abundance patterns and increases species diversity in an African sub-tropical reef-fish community. *Fisheries Oceanography*, 21(1), 78–94. <https://doi.org/10.1111/j.1365-2419.2011.00610.x>
- Lopes, A., Morais, P., Pimentel, M., Rosa, R., Munday, P. L., Gonçalves, E., & Faria, A. (2016). Behavioural lateralisation and shoaling cohesion of fish larvae altered under ocean acidification. *Marine Biology*, 163(12), <https://doi.org/10.1007/s00227-016-3026-4>
- Lough, J. M. (2008). Shifting climate zones for Australia's tropical marine ecosystems. *Geophysical Research Letters*, 35, <https://doi.org/10.1029/2008GL034634>
- Malavasi, S., Cipolatto, G., Cioni, C., Torricelli, P., Allea, E., Manciocco, A., & Toni, M. (2013). Effects of temperature on the antipredator behaviour and on the cholinergic expression in the European Sea Bass (*Dicentrarchus labrax* L.) Juveniles. *Ethology*, 119, <https://doi.org/10.1111/eth.12100>
- Mathis, A., & Chivers, D. (2003). Overriding the oddity effect in mixed-species aggregations: Group choice by armored and nonarmored prey. *Behavioral Ecology*, 14(3), 334–339. <https://doi.org/10.1093/beheco/14.3.334>
- Mehrbach, C., Culberson, C., Hawley, J., & Pytkowicz, R. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, 18(6), <https://doi.org/10.4319/lo.1973.18.6.0897>
- Miletto Petrazzini, M. E., Sovrano, V. A., Vallortigara, G., & Messina, A. (2020). Brain and behavioral asymmetry: A lesson from fish. *Frontiers in Neuroanatomy*, 14, <https://doi.org/10.3389/fnana.2020.00011>
- Miller, N., & Gerlai, R. (2007). Quantification of shoaling behaviour in zebrafish (*Danio rerio*). *Behavioural Brain Research*, 184(2), 157–166. <https://doi.org/10.1016/j.bbr.2007.07.007>
- Miller, N., & Gerlai, R. (2008). Oscillations in shoal cohesion in zebrafish (*Danio rerio*). *Behavioural Brain Research*, 193(1), 148–151. <https://doi.org/10.1016/j.bbr.2008.05.004>
- Mitchell, A., Nagelkerken, I., & Booth, D. J. (2021). Ocean warming and acidification degrade shoaling performance and lateralisation of novel tropical–temperate fish shoals. The University of Adelaide. Dataset. <https://doi.org/10.25909/17011619>
- Monaco, C., Bradshaw, C., Booth, D. J., Gillanders, B., Schoeman, D., & Nagelkerken, I. (2020). Dietary generalism accelerates arrival and persistence of coral-reef fishes in their novel ranges under climate change. *Global Change Biology*, 26(10), 5564–5573. <https://doi.org/10.1111/gcb.15221>
- Munday, P. L., Dixon, D., Donelson, J., Jones, G., Pratchett, M. S., Devitsina, G., & Døving, K. (2009). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America*, 106(6), 1848–1852. <https://doi.org/10.1073/pnas.0809996106>
- Nadler, L., Killen, S., McCormick, M. I., Watson, S. A., & Munday, P. L. (2016). Effect of elevated carbon dioxide on shoal familiarity and metabolism in a coral reef fish. *Conservation Physiology*, 4(1), cow052. <https://doi.org/10.1093/conphys/cow052>
- Nagelkerken, I., & Munday, P. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: Moving from individual to community-level responses. *Global Change Biology*, 22(3), 974–989. <https://doi.org/10.1111/gcb.13167>

- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, 85(4), <https://doi.org/10.1111/j.1469-185X.2010.00141.x>
- Nakamura, Y., Feary, D., Kanda, M., & Yamaoka, K. (2013). Tropical fishes dominate temperate reef fish communities within western Japan. *PLoS One*, 8(12), e81107. <https://doi.org/10.1371/journal.pone.0081107>
- Nilsson, G., Dixon, D., Domenici, P., McCormick, M. I., Sørensen, C., Watson, S. A., & Munday, P. L. (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change*, 2(3), 201–204. <https://doi.org/10.1038/nclimate1352>
- Pajmans, K., Booth, D. J., & Wong, M. (2020). Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *Journal of Fish Biology*, 96(3), 806–814. <https://doi.org/10.1111/jfb.14277>
- Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918), 37–42. <https://doi.org/10.1038/nature01286>
- Partridge, B. (1980). The effect of school size on the structure and dynamics of minnow schools. *Animal Behaviour*, 28(1), 68–IN3. [https://doi.org/10.1016/S0003-3472\(80\)80009-1](https://doi.org/10.1016/S0003-3472(80)80009-1)
- Partridge, B. (1981). Internal dynamics and the interrelations of fish in schools. *Journal of Comparative Physiology A*, 144(3), 313–325. <https://doi.org/10.1007/BF00612563>
- Partridge, B., & Pitcher, T. (1980). The sensory basis of fish schools: Relative roles of lateral line and vision. *Journal of Comparative Physiology A*, 135(4), 315–325. <https://doi.org/10.1007/BF00657647>
- Pierrot, D., Lewis, E., & Wallace, D. W. R. (2006). MS excel program developed for CO2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center. Oak Ridge National Laboratory; US Department of Energy. Oak Ridge (Ed.), Tennessee.
- Poloczanska, E., Burrows, M., Brown, C., García Molinos, J., Halpern, B., Hoegh-Guldberg, O., Kappel, C., Moore, P., Richardson, A., Schoeman, D., & Sydeman, W. (2016). Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science*, 3, <https://doi.org/10.3389/fmars.2016.00062>
- Ridgway, K. (2007). Long-term trend and decadal variability of the southward penetration of the East Australian Current. *Geophysical Research Letters*, 34(13), L13613. <https://doi.org/10.1029/2007GL030393>
- Ridgway, K., & Hill, K. (2009). The East Australian Current. In E. S. Poloczanska, A. J. Hobday, & A. J. Richardson (Eds), *A marine climate change impacts and adaptation report card for Australia 2009*. Publication 05/09. National Climate Change Adaptation Research Facility.
- Roche, D., Amcoff, M., Morgan, R., Sundin, J., Andreassen, A., Finnøen, M., Lawrence, M., Henderson, E., Norin, T., Speers-Roesch, B., Brown, C., Clark, T., Bshary, R., Leung, B., Jutfelt, F., & Binning, S. (2020). Behavioural lateralisation in a detour test is not repeatable in fishes. *Animal Behaviour*, 167, 55–64. <https://doi.org/10.1016/j.anbehav.2020.06.025>
- Rossi, T., Nagelkerken, I., Pistevidos, J., & Connell, S. D. (2016). Lost at sea: Ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification. *Biology Letters*, 12(1), <https://doi.org/10.1098/rsbl.2015.0937>
- Santiago-Arellano, A., Palomera-Hernandez, V., & Camacho-Cervantes, M. (2021). Con- and heterospecific shoaling makes invasive guppies more risk taking. *Frontiers in Ecology and Evolution*, 9, <https://doi.org/10.3389/fevo.2021.624245>
- Scanes, E., Scanes, P., & Ross, P. (2020). Climate change rapidly warms and acidifies Australian estuaries. *Nature Communications*, 11(1), <https://doi.org/10.1038/s41467-020-15550-z>
- Seidel, D., Fu, Q., Randel, W., & Reichler, T. (2007). Widening of the tropical belt in a changing climate. *Nature Geoscience*, 1(1), 21–24. <https://doi.org/10.1038/ngeo.2007.38>
- Smith, S., Fox, R., Booth, D., & Donelson, J. (2018). Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion frontline. *Global Change Biology*, 24(4), 1663–1672. <https://doi.org/10.1111/gcb.14016>
- Sorte, C., Williams, S., & Carlton, J. (2010). Marine range shifts and species introductions: Comparative spread rates and community impacts. *Global Ecology and Biogeography*, 19(3), 303–316. <https://doi.org/10.1111/j.1466-8238.2009.00519.x>
- Stamps, J., Briffa, M., & Biro, P. (2012). Unpredictable animals: Individual differences in intraindividual variability (IIV). *Animal Behaviour*, 83(6), 1325–1334. <https://doi.org/10.1016/j.anbehav.2012.02.017>
- Stoffel, M., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalised linear mixed-effects models. *Methods in Ecology and Evolution*, 8(11), <https://doi.org/10.1111/2041-210X.12797>
- Turner, G., & Pitcher, T. (1986). Attack abatement: A model for group protection by combined avoidance and dilution. *The American Naturalist*, 128(2), 228–240. <https://doi.org/10.1086/284556>
- Vallortigara, G., & Rogers, L. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences*, 28(4), 575–589. <https://doi.org/10.1017/S0140525X05000105>
- Verges, A., Doropoulos, C., Malcolm, H. A., Skye, M., Garcia-Piz, M., Marzinelli, E. M., Campbell, A. H., Ballesteros, E., Hoey, A. S., Vila-Concejo, A., Bozec, Y. M., & Steinberg, P. D. (2016). Long-term empirical evidence of ocean warming leading to tropicalisation of fish communities, increased herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences of the United States of America*, 113(48), <https://doi.org/10.1073/pnas.1610725113>
- Verges, A., Steinberg, P. D., Hay, M. E., Poore, A. G. B., Campbell, A. H., Ballesteros, E., Heck, K. L., Booth, D. J., Coleman, M. A., Feary, D. A., Figueira, W., Langlois, T., Marzinelli, E. M., Mizerek, T., Mumby, P. J., Nakamura, Y., Roughan, M., van Sebille, E., Sen Gupta, A., ... Wilson, S. K. (2014). The tropicalisation of temperate marine ecosystems: Climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society of B: Biological Sciences*, 281(1789). <https://doi.org/10.1098/rspb.2014.0846>
- Vila Pouca, C., Gervais, C., Reed, J., & Brown, C. (2018). Incubation under climate warming affects behavioral lateralisation in Port Jackson Sharks. *Symmetry*, 10(6), <https://doi.org/10.3390/sym10060184>
- Vinogradov, I., Jennions, M., Neeman, T., & Fox, R. (2021). Repeatability of lateralisation in mosquitofish *Gambusia holbrooki* despite evidence for turn alternation in detour tests. *Animal Cognition*, 24(4), 765–775. <https://doi.org/10.1007/s10071-021-01474-8>
- Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, M., Hoegh-Guldberg, O., & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, 416(6879), 389–395. <https://doi.org/10.1038/416389a>
- Ward, A., Schaerf, T., Burns, A., Lizier, J., Crosato, E., Prokopenko, M., & Webster, M. (2018). Cohesion, order and information flow in the collective motion of mixed-species shoals. *Royal Society Open Science*, 5(12), <https://doi.org/10.1098/rsos.181132>
- Ward, A., Sumpter, D., Couzin, I., Hart, P., & Krause, J. (2008). Quorum decision-making facilitates information transfer in fish shoals. *Proceedings of the National Academy of Sciences of the United States of America*, 105(19), 6948–6953. <https://doi.org/10.1073/pnas.0710344105>
- Warren, D. T., Donelson, J. M., McCormick, M. I., Ferrari, M. C. O., & Munday, P. L. (2016). Duration of exposure to elevated temperature affects competitive interactions in juvenile reef fishes. *PLoS One*, 11(10), e0164505. <https://doi.org/10.1371/journal.pone.0164505>
- Weetman, D., Atkinson, D., & Chubb, J. (1998). Effects of temperature on anti-predator behaviour in the guppy, *Poecilia reticulata*. *Animal Behaviour*, 55(5), 1361–1372. <https://doi.org/10.1006/anbe.1997.0666>

- Wu, L., Cai, W., Zhang, L., Nakamura, H., Timmermann, A., Joyce, T., McPhaden, M., Alexander, M., Qiu, B., Visbeck, M., Chang, P., & Giese, B. (2012). Enhanced warming over the global subtropical western boundary currents. *Nature Climate Change*, 2(3), 161–166. <https://doi.org/10.1038/nclimate1353>
- Yamano, H., Sugihara, K., & Nomura, K. (2011). Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophysical Research Letters*, 38(4), <https://doi.org/10.1029/2010GL046474>

How to cite this article: Mitchell, A., Booth, D. J., & Nagelkerken, I. (2022). Ocean warming and acidification degrade shoaling performance and lateralization of novel tropical–temperate fish shoals. *Global Change Biology*, 28, 1388–1401. <https://doi.org/10.1111/gcb.16022>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Supplementary Information

Table S1: Mean ($\pm$ SE) seawater parameters and treatment levels across the experiment.

Mixed-species pairs					
Pair replicates	Temperature (°C)	pH	Salinity (ppt)	pCO ₂ (μatm)	Total alkalinity (μmol.kg ⁻¹ SW)
10	20.2 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	304 ($\pm$ 8.4)	2104 ($\pm$ 48.6)
10	20.2 ($\pm$ 0.06)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	914 ($\pm$ 11.3)	2135 ($\pm$ 14.0)
10	23.1 ($\pm$ 0.04)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	308 ($\pm$ 3.7)	2155 ($\pm$ 15.3)
10	23.0 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	977 ($\pm$ 16.6)	2214 ($\pm$ 35.5)
10	25.8 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	303 ($\pm$ 2.4)	2165 ($\pm$ 13.0)
10	25.7 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	960 ($\pm$ 4.4)	2186 ($\pm$ 1.6)
Temperate-only pairs					
10	20.1 ($\pm$ 0.05)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	311 ($\pm$ 3.5)	2154 ($\pm$ 14.2)
10	20.3 ($\pm$ 0.06)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	940 ($\pm$ 12.1)	2158 ($\pm$ 12.8)
10	23.1 ($\pm$ 0.04)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	309 ($\pm$ 4.0)	2160 ($\pm$ 22.9)
10	23.0 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	957 ($\pm$ 9.5)	2181 ($\pm$ 20.8)
10	25.8 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	306 ($\pm$ 1.4)	2175 ($\pm$ 10.9)
10	25.9 ($\pm$ 0.04)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	967 ($\pm$ 5.42)	2187 ($\pm$ 3.43)

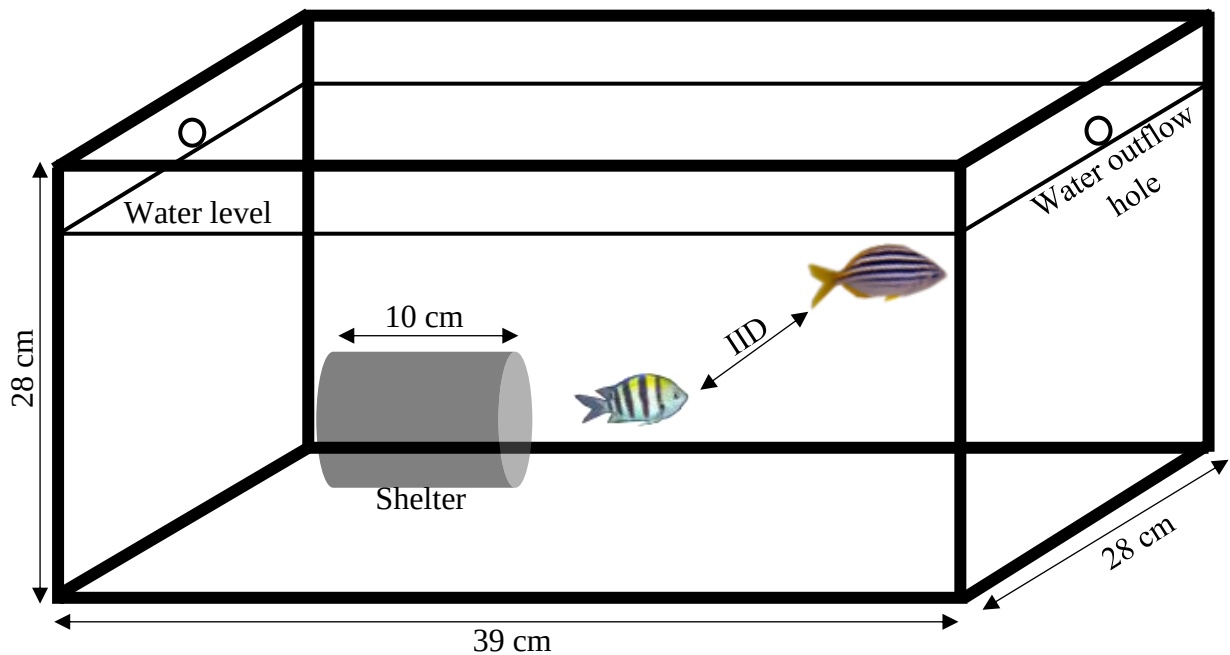


Figure S1: diagram of pairing tank used to house fish pairs during the experimental period (including video recordings of shoaling and startle escape distance).

Table S2: Starting wet weight (WW) and starting standard length (SL) of tropical and temperate fish.

Species	Starting WW (g)	Starting SL (mm)
Tropical vagrant	0.31 (SE $\pm$ 0.04)	18.61 (SE $\pm$ 0.68)
Mixed-spp. temperate fish	1.24 (SE $\pm$ 0.15)	29.34 (SE $\pm$ 1.52)
Non-mixed temperate fish	4.04 (SE $\pm$ 0.2)	53.28 (SE $\pm$ 0.8)

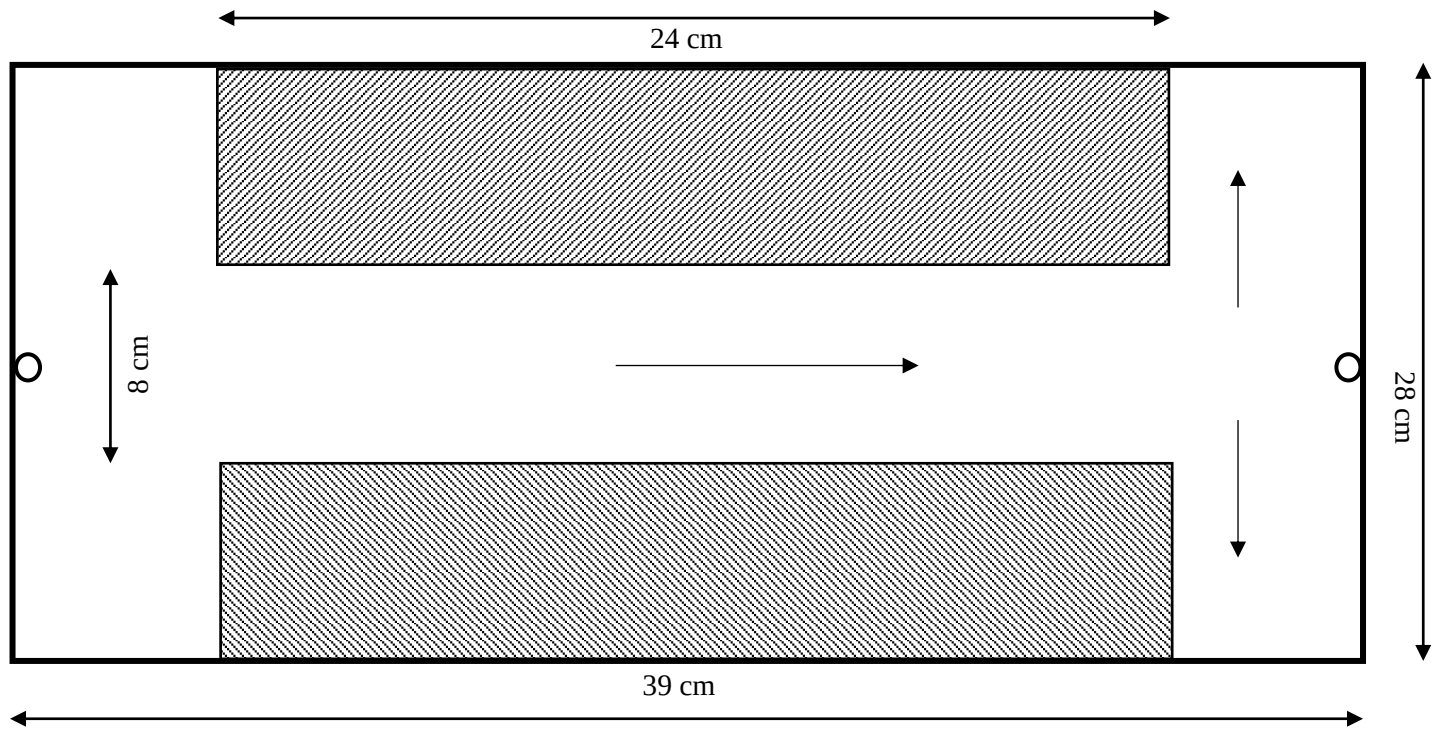


Figure S2: Diagram (top view) of tank used for standardised detour test. Water outflow holes at each end of t-test arena. Opaque barriers placed at each end of the runway below water outflow holes. Note: Not to scale.

Table S3: ANCOVA output of tropical inter-individual distance. Covariate: Relative lateralisation. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Relative lateralisation	1	4.137	4.137	0.466	0.496
Temperature (Te)	2	5.523	2.761	0.390	0.686
Ocean Acidification (OA)	1	9.732	9.732	1.089	0.300
Time (Ti)	1	4.950	4.950	0.557	0.457
Te×OA	2	43.927	21.963	2.303	0.105
Te×Ti	2	7.689	3.844	0.432	0.654
OA×Ti	1	0.935	0.935	0.105	0.742
Fi(Te×OA)	56	497.28	8.880	0.999	0.515
Te×OA×Ti	2	13.464	6.732	0.757	0.483
Res	30	266.760	8.892		
Total	98	854.400			

Table S4: ANCOVA output of tropical flight initiation distance. Covariate: Relative lateralisation. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Relative lateralisation	1	0.932	0.932	1.728	0.202
Temperature (Te)	2	0.972	0.049	0.208	0.816
Ocean Acidification (OA)	1	0.268	0.269	0.545	0.464
Time (Ti)	1	2.734	2.734	5.052	0.030
Te×OA	2	0.831	0.415	0.801	0.456
Te×Ti	2	1.411	0.705	1.303	0.284
OA×Ti	1	3.074	3.074	5.680	0.024
Fi(Te×OA)	52	28.036	0.539	0.995	0.531
Te×OA×Ti	2	0.216	0.108	0.200	0.824
Res	23	12.463	0.542		
Total	87	50.062			

Table S5: ANCOVA output of temperate inter-individual distance. Covariate: Relative lateralisation. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Relative lateralisation	1	3.708	3.708	0.246	0.616
Temperature (Te)	2	117.900	58.949	3.165	0.043
Ocean Acidification (OA)	1	290.420	290.420	15.226	>0.001
Pair Type (Pa)	1	1480.300	1480.300	77.800	>0.001
Time (Ti)	1	10.858	10.858	0.935	0.333
Te×OA	2	32.035	16.017	0.899	0.410
Te×Pa	2	12.852	6.426	0.388	0.675
Te×Ti	2	89.236	44.618	3.782	0.024
OA×Pa	1	60.322	60.322	3.226	0.072
OA×Ti	1	1.424	1.424	0.123	0.725
Pa×Ti	1	3.272	3.272	0.278	0.602
Te×OA×Pa	2	102.920	51.458	2.809	0.063
Te×OA×Ti	2	0.172	0.086	0.007	0.992
Te×Pa×Ti	2	24.510	12.255	1.026	0.362
OA×Pa×Ti	1	9.726	9.726	0.826	0.362
Fi(Pa×Te×OA)	159	2745.200	17.265	1.672	0.006
Te×OA×Pa×Ti	2	22.483	11.241	1.089	0.340
Res	83	857.140	10.327		
Total	266	5864.500			

Table S6: ANCOVA output of temperate flight initiation distance. Covariate: Relative lateralisation. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Relative lateralisation	1	0.015	0.015	0.023	0.879
Temperature (Te)	2	24.235	12.117	16.085	>0.001
Ocean Acidification (OA)	1	1.125	1.125	1.584	0.214
Pair Type (Pa)	1	0.043	0.043	0.157	0.692
Time (Ti)	1	0.122	0.122	0.204	0.650

Te×OA	2	0.161	0.081	0.21	0.812
Te×Pa	2	0.388	0.194	0.354	0.699
Te×Ti	2	3.081	1.541	2.574	0.082
OA×Pa	1	0.006	0.006	0.106	0.743
OA×Ti	1	0.001	0.001	0.002	0.959
Pa×Ti	1	2.627	2.627	4.384	0.041
Te×OA×Pa	2	2.133	1.067	1.522	0.227
Te×OA×Ti	2	1.442	0.721	1.203	0.306
Te×Pa×Ti	2	2.361	1.180	1.967	0.146
OA×Pa×Ti	1	0.536	0.536	0.894	0.347
Fi(Pa×Te×OA)	142	94.464	0.665	1.165	0.260
Te×OA×Pa×Ti	2	0.071	0.035	0.062	0.941
Res	56	31.982	0.571		
Total	222	164.790			

Table S7: MANOVA output of multiple behaviours of tropical fish. Note: **bold** p-values denote significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	158.150	79.076	1.296	0.272
Ocean Acidification (OA)	1	196.870	196.870	3.227	0.041
Time (Ti)	1	166.110	166.110	2.630	0.085
Te×OA	2	324.800	162.400	2.662	0.041
Te×Ti	2	85.329	42.664	0.676	0.588
OA×Ti	1	55.206	55.206	0.874	0.409
Fi(Te×OA)	52	3148.200	60.543	0.959	0.574
Te×OA×Ti	2	42.592	21.296	0.337	0.839
Res	22	1389.300	63.149		
Total	85	5601.00			

Table S8: tropical MANOVA output for resulting pairwise test of term 'Te×OA' for pairs of factor 'Temperature'. t = statistic.

Within level 'Control' of factor 'OA'		
Groups	t	P-value
20, 26	0.389	0.875
20, 23	0.674	0.649
26, 23	1.303	0.192
Within level 'OA' of factor 'OA'		
Groups	t	P-value
20, 26	2.323	0.023
20, 23	0.921	0.470
26, 23	1.327	0.179

Table S9: tropical MANOVA output for resulting pairwise test of term ‘Te×OA’ for pairs of factor ‘OA’. t = statistic.

Within level ‘20’ of factor ‘Temperature’		
Groups	t	P-value
Control, OA	1.918	0.049
Within level ‘26’ of factor ‘Temperature’		
Groups	t	P-value
Control, OA	1.211	0.240
Within level ‘23’ of factor ‘Temperature’		
Groups	t	P-value
Control, OA	1.661	0.057

Table S10: MANOVA output of multiple behaviours of temperate fish. Note: **bold** p-values denote significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square

Source	df	SS	MS	F-value	P-value
Temperature	2	9.392	4.696	0.915	0.408
Ocean Acidification	1	3.333	3.333	0.650	0.476
Pair type	1	19.077	19.077	3.718	0.033
Time	1	2.603	2.603	0.497	0.582
Te×OA	2	10.765	5.383	1.049	0.357
Te×Pa	2	37.782	18.891	3.683	0.014
Te×Ti	2	11.584	5.792	1.106	0.328
OA×Pa	1	0.393	0.393	0.077	0.978
OA×Ti	1	0.566	0.566	0.108	0.944
Pa×Ti	1	7.537	7.537	1.439	0.215
Te×OA×Pa	2	3.376	1.688	0.329	0.873
Te×OA×Ti	2	3.484	1.742	0.333	0.849
Te×Pa×Ti	2	4.156	2.078	0.397	0.789
OA×Pa×Ti	1	6.634	6.634	1.267	0.258
Fi(Pa×Te×OA)	142	724.830	5.104	0.975	0.566
Te×OA×Pa×Ti	2	2.100	1.050	0.201	0.950
Res	55	287.970	5.236		
Total	220	1167.800			

Table S11: temperate MANOVA output for resulting pairwise test of term ‘Te×Pa’ for pairs of factor ‘Temperature’. t = statistic.

Within level ‘Temperate-only’ of factor ‘Pair type’		
Groups	t	P-value
20, 26	2.324	0.013
20, 23	1.172	0.239
26, 23	1.584	0.093
Within level ‘Mixed-species’ of factor ‘Pair type’		
Groups	t	P-value
20, 26	1.744	0.069
20, 23	0.773	0.517
26, 23	1.598	0.095

Table S12: temperate MANOVA output for resulting pairwise test of term ‘Te×Pa’ for pairs of factor ‘Pair type’. t = statistic. Term

Within level ‘20’ of factor ‘Temperature’		
Groups	t	P-value
Temperate-only, Mixed-species	2.127	0.022
Within level ‘26’ of factor ‘Temperature’		
Groups	t	P-value
Temperate-only, Mixed-species	2.320	0.015
Within level ‘23’ of factor ‘Temperature’		
Groups	t	P-value
Temperate-only, Mixed-species	1.408	0.129

Table S13: ANOVA output of relative lateralisation (L_R) of temperate fish. Note: **bold** p-values denote significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Pa	1	0.371	0.371	4.540	0.037
Te	2	0.110	0.055	0.676	0.512
OA	1	0.727	0.727	8.903	0.004
Ti	2	0.114	0.057	0.658	0.518
Pa×Te	2	0.274	0.137	1.677	0.193
Pa×OA	1	0.033	0.033	0.041	0.838
Pa×Te	2	0.039	0.019	0.222	0.798
Te×OA	2	0.374	0.187	2.292	0.110
Te×Ti	4	0.267	0.067	0.771	0.549
OA×Ti	2	0.165	0.083	0.954	0.390
Pa×Te×OA	2	0.072	0.036	0.438	0.638
Pa×Te×Ti	4	0.169	0.042	0.487	0.745
Pa×OA×Ti	2	0.034	0.017	0.195	0.823
Te×OA×Ti	4	0.280	0.070	0.808	0.513
Fi(Pa×Te×OA)	193	15.537	0.081	0.928	0.601
Pa×Te×OA×Ti	4	0.060	0.015	0.017	0.999
Res	268	23.239	0.087		
Total	496	42.533			

Table S14: ANOVA output of absolute lateralisation (L_A) content data of temperate fish. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Pa	1	0.452	0.452	0.377	0.523
Te	2	2.087	1.043	0.871	0.413
OA	1	0.643	0.643	0.537	0.464
Ti	2	2.392	1.196	1.233	0.294
Pa×Te	2	2.529	1.265	1.056	0.354
Pa×OA	1	0.431	0.431	0.360	0.548
Pa×Ti	2	1.514	0.757	0.781	0.463

Te×OA	2	4.406	2.203	1.839	0.164
Te×Ti	4	4.570	1.142	1.178	0.314
OA×Ti	2	0.252	0.126	0.130	0.872
Pa×Te×OA	2	1.451	0.726	0.606	0.554
Pa×Te×Ti	4	3.506	0.877	0.904	0.465
Pa×OA×Ti	2	3.858	1.929	1.989	0.134
Te×OA×Ti	4	1.318	0.330	0.340	0.854
Fi(Pa×Te×OA)	193	240.840	1.248	1.287	0.027
Pa×Te×OA×Ti	4	0.865	0.216	0.223	0.926
Res	268	259.930	0.970		
Total	496	535.440			

Table S15: ANOVA output of relative lateralisation (L_R) content data of tropical fish. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	0.057	0.029	0.061	0.939
OA	1	0.460	0.460	9.858	0.003
Ti	2	0.088	0.044	1.012	0.369
Te×OA	2	0.084	0.042	0.899	0.410
Te×Ti	4	0.113	0.028	0.649	0.632
OA×Ti	2	0.049	0.024	0.561	0.563
Fi(Te×OA)	61	2.868	0.047	1.084	0.359
Te×OA×Ti	4	0.095	0.024	0.546	0.698
Res	98	4.250	0.043		
Total	176	8.0365			

Table S16: ANOVA output of absolute lateralisation (L_A) content data of tropical fish. df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	2.640	1.320	1.252	0.293
OA	1	0.011	0.011	0.011	0.916
Ti	2	1.652	0.826	1.096	0.343
Te×OA	2	0.981	0.491	0.465	0.635
Te×Ti	4	0.770	0.192	0.255	0.902
OA×Ti	2	0.199	0.099	0.132	0.883
Fi(Te×OA)	61	66.174	1.085	1.440	0.056
Te×OA×Ti	4	1.730	0.432	0.574	0.678
Res	98	73.844	0.754		
Total	176	150.650			

Table S17: ANOVA output of average inter-individual distance data of mixed-species and temperate shoals. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	24.552	12.276	0.821	0.452
OA	1	68.306	68.306	4.578	0.036

Pa	1	1221.000	1221.000	81.836	<0.001
Ti	1	5.766	5.766	0.562	0.458
Te×OA	2	18.068	9.034	0.604	0.550
Te×Pa	2	11.520	5.760	0.385	0.684
Te×Ti	2	48.631	24.315	2.370	0.099
OA×Pa	1	20.162	20.162	1.351	0.249
OA×Ti	1	3.440	3.440	0.335	0.575
Pa×Ti	1	3.165	3.165	0.308	0.585
Te×OA×Pa	2	63.724	31.862	2.130	0.119
Te×OA×Ti	2	1.400	0.700	0.068	0.934
Te×Pa×Ti	2	41.388	20.694	2.017	0.147
OA×Pa×Ti	1	0.160	0.160	0.016	0.903
Ta(Te×OA×Pa)	102	1575.200	15.443	1.505	0.037
Te×OA×Pa×Ti	2	11.184	5.592	0.545	0.592
Res	71	728.450	10.260		
Total	196	4163			

Table S18: ANOVA output of average distance amplitude data of mixed-species and temperate shoals. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	0.606	0.303	1.749	0.178
OA	1	0.296	0.296	1.709	0.193
Pa	1	9.372	9.372	54.087	<0.001
Ti	1	0.962	0.962	5.736	0.016
Te×OA	2	0.091	0.046	0.263	0.776
Te×Pa	2	0.437	0.219	1.262	0.291
Te×Ti	2	0.606	0.303	1.807	0.170
OA×Pa	1	0.049	0.049	0.284	0.603
OA×Ti	1	0.134	0.134	0.798	0.373
Pa×Ti	1	0.221	0.221	1.317	0.255
Te×OA×Pa	2	0.436	0.218	1.259	0.289
Te×OA×Ti	2	0.059	0.029	0.175	0.840
Te×Pa×Ti	2	0.666	0.333	1.986	0.146
OA×Pa×Ti	1	0.151	0.151	0.899	0.352
Ta(Te×OA×Pa)	102	17.738	0.174	1.037	0.443
Te×OA×Pa×Ti	2	0.192	0.096	0.574	0.562
Res	71	11.905	0.168		
Total	196	47.164			

Table S19: ANOVA output of oscillation frequency data of mixed-species and temperate shoals. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	1.990	0.995	2.408	0.092
OA	1	0.370	0.370	0.893	0.352
Pa	1	7.982	7.982	19.243	<0.001
Ti	1	1.219	1.219	1.912	0.175
Te×OA	2	0.048	0.024	0.058	0.942

Te×Pa	2	0.866	0.433	1.049	0.352
Te×Ti	2	1.946	0.973	1.526	0.230
OA×Pa	1	0.018	0.018	0.043	0.833
OA×Ti	1	0.648	0.648	1.017	0.317
Pa×Ti	1	0.364	0.364	0.572	0.448
Te×OA×Pa	2	1.000	0.500	1.210	0.300
Te×OA×Ti	2	1.324	0.662	1.039	0.361
Te×Pa×Ti	2	0.382	0.191	0.300	0.743
OA×Pa×Ti	1	0.044	0.044	0.068	0.792
Ta(Te×OA×Pa)	102	39.762	0.390	0.612	0.975
Te×OA×Pa×Ti	2	0.796	0.398	0.624	0.543
Res	71	45.256	0.637		
Total	196	105.640			

Table S20: ANOVA output of temperate flight initiation distance. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Te	2	1.384	0.692	13.560	<0.001
OA	1	0.205	0.205	4.022	0.047
Pa	1	0.031	0.031	0.602	0.438
Ti	1	0.072	0.072	1.783	0.188
Te×OA	2	0.028	0.014	0.270	0.764
Te×Pa	2	0.053	0.027	0.520	0.602
Te×Ti	2	0.076	0.038	0.938	0.394
OA×Pa	1	0.0500	0.050	0.098	0.759
OA×Ti	1	0.039	0.039	0.096	0.755
Pa×Ti	1	0.187	0.187	4.650	0.037
Te×OA×Pa	2	0.154	0.077	1.507	0.227
Te×OA×Ti	2	0.017	0.086	0.213	0.808
Te×Pa×Ti	2	0.035	0.017	0.434	0.647
OA×Pa×Ti	1	0.016	0.016	0.396	0.528
Fi(Te×OA×Pa)	150	7.933	0.053	1.315	0.104
Te×OA×Pa×Ti	2	0.041	0.021	0.051	0.995
Res	71	2.856	0.040		
Total	244	13.74			

Table S21: temperate flight initiation distance ANOVA output for resulting pairwise test of term temperature. Note: **bold** p-value indicates significant p-value ($p < 0.05$). t = t-statistic.

Groups	t	P-value
20, 26	5.274	<0.001
20, 23	4.012	<0.001
26, 23	0.711	0.473

Table S22: temperate flight initiation distance ANOVA output for resulting pairwise test of term 'Pa×Ti' for pairs of the levels of factor Pair type within levels '19' of factor Time. t = statistic.

Groups	t	P-value
Temperate-only, Mixed-spp.	1.29	0.194

Table S23: temperate flight initiation distance ANOVA output for resulting pairwise test of term ‘Pa×Ti’ for pairs of the levels of factor Pair type within levels ‘27’ of factor Time. t = statistic.

Groups	t	P-value
Temperate-only, Mixed-spp.	0.8887	0.369

Table S24: temperate flight initiation distance ANOVA output for resulting pairwise test of term ‘Pa×Ti’ for pairs of factor Time within levels ‘Temperate-only’ of factor Time. t = statistic.

Groups	t	P-value
19, 27	0.7151	0.485

Table S25: temperate flight initiation distance ANOVA output for resulting pairwise test of term ‘Pa×Ti’ for pairs of the levels of factor Time within levels ‘Mixed-spp.’ of factor Time. Note: **bold** p-value indicates significant p-value ($p < 0.05$). t = statistic.

Groups	t	P-value
19, 27	2.163	0.044

Table S26: ANOVA output of tropical flight initiation distance. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
OA	1	0.018	0.018	0.023	0.882
Te	2	0.086	0.043	0.053	0.949
Ti	1	0.128	0.128	1.598	0.219
OA×Te	2	0.136	0.068	0.842	0.454
OA×Ti	1	0.406	0.406	5.071	0.030
Te×Ti	2	0.399	0.199	2.496	0.100
Fi(Te×OA)	53	4.293	0.081	1.011	0.494
OA×Te×Ti	2	0.051	0.025	0.317	0.734
Res	24	1.923	0.080		
Total	88	7.479			

Table S27: tropical flight initiation distance ANOVA output for the resulting pairwise test of term ‘OA×Ti’ for pairs of factor OA within levels ‘19’ of factor Time. t = statistic.

Groups	t	P-value
8.1, 7.7	1.793	0.080

Table S28: tropical flight initiation distance ANOVA output for the resulting pairwise test of term ‘OA×Ti’ for pairs of factor OA within levels ‘27’ of factor Time. t = statistic.

Groups	t	P-value
8.1, 7.7	1.850	0.069

Table S29: tropical flight initiation distance ANOVA output for resulting pairwise test of term ‘OA×Ti’ for pairs of factor Time within levels ‘8.1’ of factor OA. t = statistic.

Groups	t	P-value
19, 27	0.788	0.455

Table S30: tropical flight initiation distance ANOVA output for resulting pairwise test of term ‘OA×Ti’ for pairs of factor Time within levels ‘7.7’ of factor OA. Note: **bold** p-value indicates significant p-value ($p < 0.05$). t = statistic.

Groups	t	P-value
19, 27	2.251	0.040

Table S31: Repeatability of relative lateralisation (L_R) of tropical and temperate fish across pCO_2 , temperature, and pair type treatments. Note: **bold** p-value indicates significant p-value ($p < 0.05$).

Treatment (Species, Temperature (°C), pCO_2)	Repeatability	Standard Error	P-value	Confidence Interval
Tropical, 20 °C, Control	0.172	0.172	0.231	0, 0.565
Tropical, 23 °C, Control	0.057	0.146	0.466	0, 0.498
Tropical, 26 °C, Control	0.380	0.193	0.038	0, 0.701
Tropical, 20 °C, OA	0.214	0.181	0.183	0, 0.609
Tropical, 23 °C, OA	0.000	0.171	1.000	0, 0.417
Tropical, 26 °C, OA	0.000	0.115	1.000	0, 0.394
Mixed- spp. Temperate, 20 °C, Control	0.000	0.135	1.000	0, 0.470
Mixed- spp. Temperate, 23 °C, Control	0.000	0.135	1.000	0, 0.465
Mixed- spp. Temperate, 26 °C, Control	0.000	0.129	1.000	0, 0.436
Mixed- spp. Temperate, 20 °C, OA	0.044	0.142	0.481	0, 0.471
Mixed- spp. Temperate, 23 °C, OA	0.000	0.127	1.000	0, 0.434
Mixed- spp. Temperate, 26 °C, OA	0.184	0.193	0.270	0, 0.634
Temperate-only Temperate, 20 °C, Control	0.000	0.081	1.000	0, 0.281
Temperate-only Temperate, 23 °C, Control	0.119	0.121	0.233	0, 0.406
Temperate-only Temperate, 26 °C, Control	0.126	0.141	0.29	0, 0.477
Temperate-only Temperate, 20 °C, OA	0.000	0.093	0.500	0, 0.307
Temperate-only Temperate, 23 °C, OA	0.000	0.090	1.000	0, 0.308
Temperate-only Temperate, 26 °C, OA	0.227	0.151	0.108	0, 0.522

R code for repeatability tests used on relative lateralisation data (for data see Mitchell et al., 2021):

```
rptGaussian(LR ~ (1 | FishID), grname = c("FishID"), data = latdata, CI = 0.95, nboot
= 1000, npermut = 0, parallel = FALSE, ncores = NULL, ratio = TRUE, adjusted = TRUE,
rptObj = NULL, update = FALSE
```

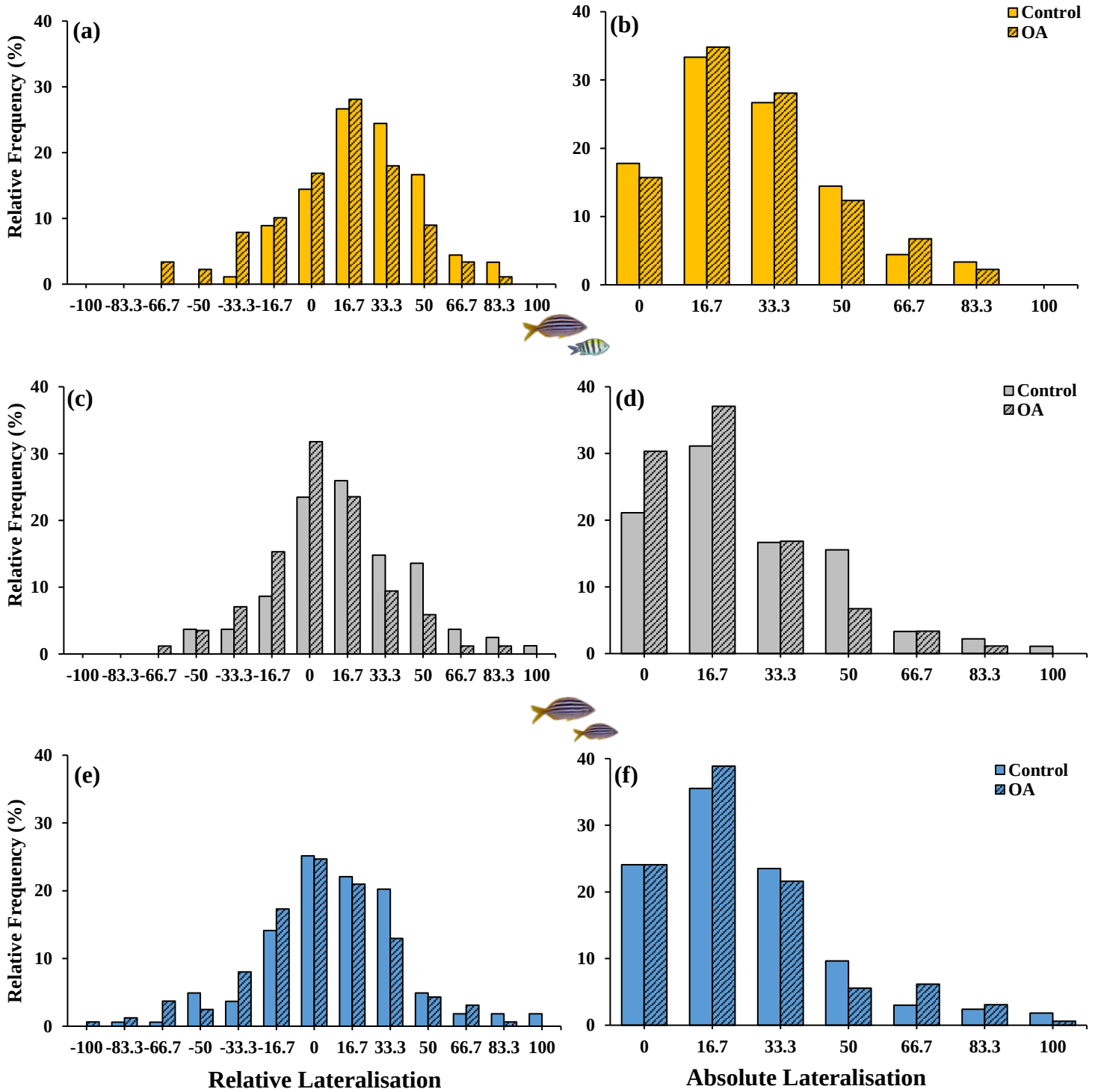


Figure S3: Relative frequency distribution of relative lateralisation and absolute lateralisation across Control/OA $p\text{CO}_2$ treatments for tropical fish (Panels: a, b), and mixed-spp. (Panels: c, d) and temperate-only fish (Panels: e, f). Factor 'Temperature' pooled for visualisation purposes.

Chapter III

Project ocean acidification and seasonal temperature alter the behaviour and growth of a range extending tropical fish

Angus Mitchell¹, Chloe Hayes¹, David J. Booth², Ivan Nagelkerken¹

¹Southern Seas Ecology Laboratories, School of Biological Sciences and The Environment Institute, The University of Adelaide, DX 650 418, Adelaide, SA, 5005, Australia

²School of the Life Sciences, University of Technology Sydney, Ultimo, NSW, 2007, Australia



Statement of Authorship

Title of Paper	Projected ocean acidification and seasonal temperature alter the behaviour and growth of a range extending tropical fish
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Published in Coral Reefs 13/06/2023

Principal Author

Name of Principal Author (Candidate)	Angus Mitchell		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/10/23

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Chloe Hayes		
Contribution to the Paper	Data collection, writing		
Signature		Date	24/02/23

Name of Co-Author	David J. Booth		
Contribution to the Paper	Study design, writing		
Signature		Date	24/02/23

Name of Co-Author	Ivan Nagelkerken		
Contribution to the Paper	Study design, writing		
Signature		Date	24/10/23



REPORT

Projected ocean acidification and seasonal temperature alter the behaviour and growth of a range extending tropical fish

Angus Mitchell¹ · Chloe Hayes¹ · David J. Booth² ·
Ivan Nagelkerken¹

Received: 14 February 2023 / Accepted: 29 May 2023 / Published online: 13 June 2023
© The Author(s) 2023

Abstract Climate-driven invasions of ecosystems by range-extending animals are often mediated by behavioural modifications that increase their chances of establishment in foreign biological communities. This creates novel ecological interactions that can affect the behaviour of native species in recipient ecosystems. However, this question has seldom been addressed in marine systems, in particular with the additive effect of ocean acidification and the mediating effects of seasonal climate variability. Here, we performed a laboratory experiment to evaluate how novel species interactions, ocean acidification, and projected future summer versus winter temperatures could affect the behaviour and growth of a range-extending tropical and co-shoaling temperate fish. Compared to current-day summer temperatures, tropical fish became 4% more active, 90% more aggressive, and increased their growth rates (standard length: +42%) during future summers in their novel poleward ranges, but reduced their aggression by 64%, boldness by 52%, feeding by 45% and growth rates (wet weight: –70%, standard length: –26%) during future winters compared to current-day summer temperatures. Additionally, tropical fish became 3% more active under ocean acidification compared to no ocean acidification. Conversely, temperate fish behaviour was unaffected by climate treatments but their growth rates

were > 200% faster under future winter versus current and future summer temperatures. We conclude that projected future winter conditions in temperate ecosystems may reduce the performance of range-extending fishes in temperate fish communities and slow down tropicalisation of higher latitudes.

Keywords Behavioural flexibility · Climate change · Tropicalisation · Species interactions · Elevated CO₂ · Ocean warming

Introduction

Anthropogenic climate change has facilitated the biogeographic redistribution of species globally (Chen et al. 2011; Pecl et al. 2017). Poleward range shifts act as a mechanism to escape the effects of warming or an opportunity to take advantage of habitats and resources at higher latitudes/altitudes now suitable for invasion (Hoegh-Guldberg and Bruno 2010). Poleward species range shifts have forced invading and local species with overlapping niches to interact for limited resources (Tylianakis et al. 2008; Alexander et al. 2015). However, attempts to understand species redistributions primarily focus on the direct effects of global warming (Figueira and Booth 2010). Such approaches fail to consider that novel species interactions can alter species redistribution outcomes (Alexander et al. 2015). Animals entering novel environments often modify their behaviour through interactions with local species to increase survivorship (Smith et al. 2018; Coni et al. 2021b). Local species can also alter their behaviour to limit the establishment of invading species (Twinn et al. 2022). Novel species interactions can therefore regulate the pace of species range shifts and consequent biodiversity changes in recipient ecosystems (Davis et al.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00338-023-02396-2>.

✉ Ivan Nagelkerken
ivan.nagelkerken@adelaide.edu.au

¹ Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, DX 650 418, Adelaide, SA 5005, Australia

² School of the Life Sciences, University of Technology Sydney, Ultimo, NSW 2007, Australia

1998). Hence, novel species interactions and climate change effects must be considered concurrently to more accurately forecast species range-shift outcomes.

Climate change has facilitated a widening of the tropical belt known as ‘tropicalisation’ (Seidel et al. 2007). Tropicalisation has led to increased dispersal of tropical marine species into sub-tropical and temperate ecosystems (Booth et al. 2011; Vergés et al. 2014). Regions facing tropicalisation (e.g. southeastern Australia (Booth et al. 2011), western Japan (Yamano et al. 2011), East Africa (Lloyd et al. 2011)) have warmed ~3–4 times the rate of global averages (Wu et al. 2012). These regions are commonly termed global warming hotspots (Pecl et al. 2017). In Australia, the East Australian Current has acted as a direct dispersal mechanism into temperate ecosystems for over 150 tropical fish species (Feary et al. 2014; Fowler et al. 2017). For example, range-shifting tropical herbivores modify temperate reef habitats (Vergés et al. 2014, 2016; Pessarrodona et al. 2022), altering marine temperate ecosystem function and structure. Such evidence demonstrates how ocean warming and warm boundary currents drive the tropicalisation of high latitude temperate ecosystems. However, few studies have assessed how interactions between range-shifting and native species may govern the pace of tropicalisation (but see Mitchell et al. 2022a, b).

In ocean warming hotspots, current summer temperatures in recipient marine temperate ecosystems allow range-extending tropical species to persist in high latitude ecosystems for prolonged periods (Booth et al. 2011; Yamano et al. 2011), but lethal winter effects often lead to significant or complete mortality and, as such, limit tropicalisation in temperate ecosystems (e.g. Figueira et al. 2009; Beck et al. 2016). However, future winters will become warmer, allowing most vagrant tropical fish to establish permanently at higher latitudes (Figueira and Booth 2010). To date, most range-extension studies do not explicitly consider how warmer winters will alter the behaviours of competing native and range-extending species in tropicalisation hotspots. Future seasonal temperatures could not only modulate the rate at which tropical species establish in high latitude ecosystems, but also dictate the dynamics between smaller-scale range extensions and contractions across seasons.

Benthic range-extending tropical fishes often form shoals with resident temperate species (Paijmans et al. 2020). Ocean warming is expected to increase shoaling interactions between temperate and tropical fishes in temperate ecosystems (Figueira and Booth 2010; Coni et al. 2021a). Shoaling interactions in novel temperate ecosystems can provide fitness benefits for range-shifting tropical fishes through multiple mechanisms: (1) enhanced learning of novel social cues (Smith et al. 2018), (2) reduced mortality (Mathis and Chivers 2003); (3) increased body size (Smith et al. 2018); (4) greater access to prey (Barlow

1974); and (5) increased foraging efficiency (Camacho-Cervantes et al. 2014). Novel shoaling interactions can also incur costs, including competitive exclusion by co-shoaling resident species (Coni et al. 2021a; 2022) and increased predation threat due to phenotypic oddity (Landeau and Terborgh 1986). Therefore, potential benefits must outweigh potential costs if range-extending tropical fishes are to gain any benefits from novel species interactions. However, ocean warming and acidification can directly alter behaviour and fitness-related traits that determine the costs and benefits derived from novel shoaling interactions (e.g. aggression, boldness, and foraging; Nagelkerken and Munday 2016; Cattano et al. 2018; Goldenberg et al. 2018). This could subsequently modify the fitness benefits and costs of tropical-temperate shoals in a future ocean. Hence, it is necessary to assess how climate change affects novel tropical-temperate shoaling interactions to better predict tropicalisation outcomes.

Ocean acidification can alter the behaviour of tropical and temperate fish species residing in tropicalisation hotspots (Mitchell et al. 2022a, b). However, previous research solely focused on how ocean warming effects can modulate the pace of marine range shifts (Figueira and Booth 2010; but see Coni et al. 2021c). Ocean acidification and warming both affect a wide range of behaviours (e.g. activity, boldness and foraging; Nagelkerken and Munday 2016; Goldenberg et al. 2018; Domenici et al. 2019), and may therefore modify marine range shift outcomes. Ocean warming and acidification can also concurrently affect the fitness of resident and range-extending species, modifying interaction outcomes which shape the initial stages of range shifts (Carmona-Catot et al. 2013; Mitchell et al. 2022a, b). Therefore, ocean acidification and warming effects could determine the true pace of range extensions and ensuing biodiversity shifts in high latitude ecosystems (Coni et al. 2021c). However, the direct effects of ocean acidification on the behaviour and fitness of range-shifting tropical and native temperate species, and their interactions, remain largely unknown.

Here we used a controlled laboratory experiment to test the concurrent effects of projected future summer and winter ocean temperatures, ocean acidification, and novel species interaction on the behaviour and growth of range-extending tropical and co-shoaling native temperate fish species across a 38-day exposure period. Proxies for growth were somatic growth and length increase, while video recordings were used to examine a repertoire of behavioural proxies (aggression, activity, boldness and feeding). We show that seasonal temperatures, ocean acidification, and novel interactions can all alter the behaviour and growth of range-extending tropical fish, and discuss how this could regulate the pace of tropicalisation in high latitude ecosystems.

Materials and methods

Fish collection and acclimation

Above-average rates of ocean warming in high-latitude temperate ecosystems (latitude: 34° S) and strengthening of the East Australian current (Wu et al. 2012) have increased the dispersal of larval-stage tropical fishes from the southern great barrier reef into shallow rocky, temperate ecosystems in southeastern Australian waters (Booth et al. 2007, 2011). One of the most common range-extending tropical fish species, the Indo-Pacific Sergeant (*Abudefduf vaigiensis*), is often observed co-shoaling with temperate species, Australian Mado (*Atypichthys strigatus*) (Smith et al. 2018). South-eastern Australia's cooler conditions have restricted tropical fish from establishing breeding populations as temperatures drop below their thermal minima in winter (~ 17 °C; Booth et al. 2011). However, ocean warming in southeastern Australia is expected to facilitate the successful establishment of breeding tropical populations in the future (Figueira and Booth 2010). *A. vaigiensis* is omnivorous, exhibits a dispersive larval stage, has high site fidelity in the adult stage, and reaches up to 20 cm in length (Kuiter 1993). The co-shoaling temperate species *A. strigatus* grows up to 25 cm in length and is omnivorous (Kuiter 1993).

Tropical range-extending fish obtain fitness benefits by shoaling with temperate residents, growing faster and surviving longer into winter than conspecific tropical shoals in temperate ecosystems (Smith et al. 2018). Hence, we selected two pair types (temperate + temperate and tropical + temperate) to investigate how novel co-shoaling species interactions might alter temperate fish performance in mixed-species shoals (i.e. future conditions) compared

to conspecific temperate fish pairs (i.e. present-day conditions), and how this is modified by the direct project future temperature and ocean acidification effects. Comparisons of the shoaling types can inform us about potential impacts on temperate fish communities in tropicalisation hotspots. As such, and also because of laboratory space limitations, we did not include pairs of tropical species.

Sixty *A. vaigiensis* and 180 *A. strigatus* were collected during 7–24 March 2020, using barrier and hand nets at Little Manly Cove (−33.81° S, 151.29° E) and Freshwater Beach (−33.78° S, 151.29° E) at depths of 0.1–2 m. After capture fish were immediately transported to ambient holding tanks (size: 100 L) at the Sydney Institute of Marine Science (SIMS).

Climate treatments and experimental setup

Flow-through 20-L transparent tanks (IKEA product number: 898.914.70; dimensions: 39 × 28 × 28 cm, height × length × width; $n = 120$) were installed across two adjacent flow-through temperature/pH control rooms at SIMS. Two 0.5 cm (diameter) holes were inserted 4 cm from the top of each tank (lengthways; Fig. S1.) to allow fresh seawater to flow bilaterally out of each tank. Each tank was allocated a tank identifier and a climate treatment (Table 1). We provided 10 cm lengths of PVC piping (diameter: 10 cm) to each tank for shelter (Fig. S1). Tanks were randomly assigned one of the two CO₂ × three temperature treatments ($N = 6$ treatments; Tables 1, 2), with 10 replicate tanks per treatment per fish pairing type (120 tanks in total). The ocean warming and ocean acidification treatments reflected ambient summer temperatures and those forecast during summer and winter for the leading range edge of tropical

Table 1 Mean (± standard error) seawater parameters and treatment levels across experiments

# of replicates of paired tanks	Temperature (°C)	pH	Salinity (ppt)	pCO ₂ (µatm)	Total alkalinity (µmol kg ⁻¹ SW)
<i>Mixed-species pairs</i>					
10	20.2 (±0.06)	8.10 (±0.01)	35.0 (±0.01)	304 (±8.4)	2104 (±48.6)
10	20.2 (±0.06)	7.70 (±0.01)	35.0 (±0.01)	914 (±11.3)	2135 (±14.0)
10	23.1 (±0.04)	8.10 (±0.01)	35.0 (±0.01)	308 (±3.7)	2155 (±15.3)
10	23.0 (±0.03)	7.70 (±0.01)	35.0 (±0.01)	977 (±16.6)	2214 (±35.5)
10	25.8 (±0.06)	8.10 (±0.01)	35.0 (±0.01)	303 (±2.4)	2165 (±13.0)
10	25.7 (±0.03)	7.70 (±0.01)	35.0 (±0.01)	960 (±4.4)	2186 (±1.6)
<i>Temperate-only pairs</i>					
10	20.1 (±0.05)	8.10 (±0.01)	35.0 (±0.01)	311 (±3.5)	2154 (±14.2)
10	20.3 (±0.06)	7.70 (±0.01)	35.0 (±0.01)	940 (±12.1)	2158 (±12.8)
10	23.1 (±0.04)	8.10 (±0.01)	35.0 (±0.01)	309 (±4.0)	2160 (±22.9)
10	23.0 (±0.03)	7.70 (±0.01)	35.0 (±0.01)	957 (±9.5)	2181 (±20.8)
10	25.8 (±0.06)	8.10 (±0.01)	35.0 (±0.01)	306 (±1.4)	2175 (±10.9)
10	25.9 (±0.04)	7.70 (±0.01)	35.0 (±0.01)	967 (±5.42)	2187 (±3.43)
SW seawater					

Table 2 Laboratory experimental temperatures reflecting the average seasonal temperatures at the core range and leading-edge of tropical *A. vaigiensis* in the southern hemisphere (Froese and Pauly 2023)

Range location/Temperature	20 °C	23 °C	26 °C
Tropical fish core range (~19° S)	N/A	Current winter	Future winter
Tropical fish leading edge (~34° S)	Future winter	Current summer	Future summer

fish in southeastern Australian (latitude: ~34° S; Booth et al. 2011) by 2100 under an RCP 8.5 scenario. The current summer temperature (i.e. control, and also reflective of current winter temperatures experienced in the core range of tropical vagrant populations, Figueira and Booth 2010) was 23 °C, the projected future summer temperature was 26 °C, and the projected future winter temperature was 20 °C (IPCC 2021).

On 25/03/2020, collected fish were randomly assigned to pairing tanks: mixed species (1 tropical + 1 temperate fish) and temperate-only pairs (2 temperate fish) in climate-controlled rooms. Temperate-only selected fishes were paired with individuals of similar standard lengths to minimise any competitive advantages that larger fish could develop in obtaining food and shelter. However, mixed-species shoaling fish displayed different body sizes to reflect the current field situation of tropical fish being smaller than their co-shoaling temperate species (Smith et al. 2018; Table S1). Initial wet weight (mean ± SE) was: tropical fish 0.31 ± 0.04 g; mixed-species temperate fish 1.24 ± 0.15 g; temperate-only fish 4.04 ± 0.20 g. The initial standard length was: tropical fish 18.61 ± 0.68 mm; mixed-species temperate fish 29.34 ± 1.52 mm; temperate-only fish 53.28 ± 0.80 mm (Table S1). Initial wet weight and standard length were significantly different ($p < 0.05$) between all tropical and temperate fish across climate treatments, and also between mixed-species paired temperate and single-species paired temperate fish ($p < 0.001$; Tables S2, S3). Fish introduction and acclimation to control and experimental tanks were staggered to prevent fish in control treatments (23 °C, pH 8.1) from gaining an acclimation advantage over fish in treatments that required more acclimation steps (e.g. 26 °C and pH 7.7 or 20 °C and pH 7.7). Fish in the climate treatments were acclimated from ambient temperature conditions (current summer) by decreasing pH at a rate of 0.06 units/day (through gradually increasing the $p\text{CO}_2$ concentration in header tanks) and by raising or decreasing temperatures by 0.5 °C per day to minimise rapid thermal and pH stress responses.

Tanks were maintained at target temperatures and pH levels throughout the experiment (Table 2). Temperature, pH, and salinity were measured daily using an Ohaus starter 300 portable pH metre with probe (IC-ST320)—IC-ST300-G and StarterPen salinity metre—ST20S. Solenoid feedback systems controlled by a computer system maintained the designated temperature within ± 0.25 °C of the tanks' target

temperatures. Ocean acidification treatments were reached by bubbling pure CO_2 into header tanks (one header tank for every four fish tanks). Header tank water flowed into fish tanks at a rate of 150 ml min⁻¹.

Fish were exposed to climate treatments for 19 days before the commencement of behavioural recordings. API™ stress coat solution was added to each tank daily during acclimation and experimental periods. No fish mortality was recorded during the acclimation period. All fish were fed 2.86 g of Ocean Nutrition™ frozen *Artemia* mixed into 60 ml fresh seawater twice daily. Fish pairs were exposed to climate treatments for a maximum length of 40 days.

Water chemistry

Total alkalinity values were estimated by gran titration (Metrohm 888 Titrando™) from 60 ml water samples from all tanks on exposure days 24 and 25. Mean $p\text{CO}_2$ values were calculated using CO2SYS (Pierrot et al. 2006) for Excel with constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) (Table S4).

Behaviour observations

On days 19 and 27 of treatment exposure, between 09:00 and 15:00 h, we filmed the behaviour of all fishes (see Table S4: aggression, activity, bite rate and prey acquisition latency) in 9-min video recordings. During video observations, paired fish (mixed-species or non-mixed pairs) were kept in their pairing tanks (Fig. S1). Tanks were illuminated by LED strip lighting (750 lumens) placed ~20 cm above the water surface outside the tank. White sheeting surrounded the back and sides of fish tanks to minimise any external disturbances to fish during video recordings. We placed GoPro Hero 7 Silver cameras 30 cm away from pairing tanks and commenced recording. Video recordings lasted for 9-min in the absence of any observers and excluded 3-min of acclimation time at the commencement of behavioural observations. Video analysis of behavioural assays was performed on the first 4-min of video recordings, following the 3-min acclimation period, which is considered sufficient to obtain an estimate of selected behaviours (Biro et al. 2010; Coni et al. 2021a). Video analysis was divided into two periods (period 1: general behaviour (3-min duration); period 2: food administration (~1-min duration)). Observers were blinded to the

treatments of each video recording during video analysis until data collection was completed.

Period 1: general behaviour

We measured the behavioural proxies: (1) aggression, (2) activity levels (time spent swimming), and (3) feeding (bite rate) of fishes in both pairing treatments (mixed-species and temperate-only pairs) during the first 3-min period of the video analysis:

- (1) Aggression: we counted the total number of aggressive acts (i.e. flares, chases, lunges, and bites towards opposing fish were quantified as aggressive displays) each fish attempted on the opposing fish in each pairing treatment over the first 3-min of the video recordings.
- (2) Activity levels: we quantified the per cent time spent swimming for each fish in each pairing treatment over the first 3-min of the video recordings using a stopwatch. Time spent swimming was defined as a forward propulsion through the water column through active caudal fin action (see Table S4). Passive movements (e.g. drifting) and resting behaviour, were defined as time not spent swimming.
- (3) Feeding: we quantified the basal total number of bites each fish performed over the first 3-min of video recordings in the absence of frozen *Artemia* and calculated the bite rate as ‘the number of bites.min⁻¹’ (see Table S4).

Period 2: food administration

After 3-min of recording, 1.43 g of Ocean Nutrition™ frozen *Artemia* mixed into 60 ml fresh seawater was administered via a syringe into each tank to elicit a prey acquisition latency response for each fish.

- (4) We measured prey acquisition latency (a proxy for boldness): once *Artemia* was administered into the tanks, we quantified the time (seconds) taken by each fish to initiate a bite response (see Table S4).

Growth measurements

Fish standard length (SL $\pm$ 0.01 mm) and wet weight (WW $\pm$ 0.01 g) were measured at the start and completion of the experimental exposure period for all fish. We then calculated the mean daily SL and WW somatic growth rate for each individual fish. We distinguished between the two temperate paired fish by assuming that a fish with a larger starting WW and SL had a larger ending WW and SL than the smaller temperate fish in the pair.

Statistical analysis

The four behavioural traits were tested separately for each species amongst the twelve treatment combinations using the software package PRIMER v7 (Clarke and Gorley 2015). A four-way MANOVA was performed for tropical fish (Ocean acidification (OA), temperature (Te), time (Ti) and fish ID (Fi)), and a five-way MANOVA (addition of fixed factor: pairing type (Pa)) for temperate fish. Fixed main effect factors were temperature, ocean acidification and pair type. Time was set as a random factor, and fish ID was set as a random repeated measures factor and nested within the main effect factors. The four behaviours (response variables) selected for the MANOVAs were: aggression, activity levels, prey acquisition latency, and feeding. The data were first standardised (to scale the values of the different behavioural traits similarly). The MANOVA datasets were fourth-root transformed and converted into a Euclidean distance matrix. Permutational MANOVAs (9999 permutations) were performed, followed by pairwise post hoc testing to determine among which treatments there were significant differences ($p < 0.05$) in behaviours of each species. Where MANOVAs were significant ($p < 0.05$), SIMPER analysis was performed on standardised behavioural datasets and analysis of variance (ANOVA) was performed on individual behaviours.

Individual behaviours and growth proxies (mean daily somatic length and wet weight growth rate) were tested using permutational ANOVAs (9999 permutations). Data for individual behaviours were fourth root transformed. Pairing type, temperature, and ocean acidification were set as fixed factors and time as a random factor. Fish ID was a random repeated measures factor nested within the main effect factors. Monte-Carlo tests were performed for post hoc tests where unique permutations were less than 500. Lowest order interactions were pooled with residuals in ANOVAs when their p values were $p > 0.25$ (Winer et al. 1991). The permutation method used was a permutation of residuals under a reduced model. A Euclidean distance dissimilarity resemblance matrix was used for all ANOVAs that tested behaviour and growth proxies (Anderson 2001). The raw data used in the statistical analysis are publicly available (Mitchell et al. 2022b).

Results

Tropical fish behaviour and growth

The behavioural repertoire of the tropical fish was modified by temperature (df = 2, F value = 53.606, $p < 0.001$) differing between future winter and current summer ($p = 0.020$), between future and current summer ($p = 0.013$), and between future winter and future summer ($p = 0.006$) temperatures.

Aggression-related behaviours were the primary contributor to temperature-related behavioural changes (future winter vs. future summer: 79%; current summer vs. future summer: 80%; current summer vs. future winter: 75% of the total dissimilarity, respectively; Table S5).

All four behaviours (aggression: +430%, activity levels: +5%, boldness: +54%, and feeding: +148%) were higher for future summer than for future winter temperatures (Fig. 1; $p < 0.012$; Tables S7–10). Aggression (+180%), boldness (+34%), and feeding rates (+82%) were also higher for current summer than for future winter temperatures (aggression: Fig. 1a; $p = 0.028$; boldness: Fig. 1c;

$p = 0.025$; feeding: Fig. 1d; $p = 0.008$; Tables S7–9), whilst tropical fish aggression (Fig. 1a; $p = 0.005$; Table S7) and activity levels (Fig. 1b; $p = 0.025$; Table S10) were 90% and 4% higher, respectively, for future summer than for current summer temperatures.

Activity levels were 3% higher under ocean acidification, irrespective of temperature (Fig. 1b; $df = 1$, F value = 4.502, $p = 0.031$), but all three other behaviours were unaffected by ocean acidification (Fig. 1a, c, d; $p > 0.05$; Tables S7–10).

From day 19–27, tropical fish showed a 27% increase in boldness-related behaviour ($df = 1$, F value = 5.574,

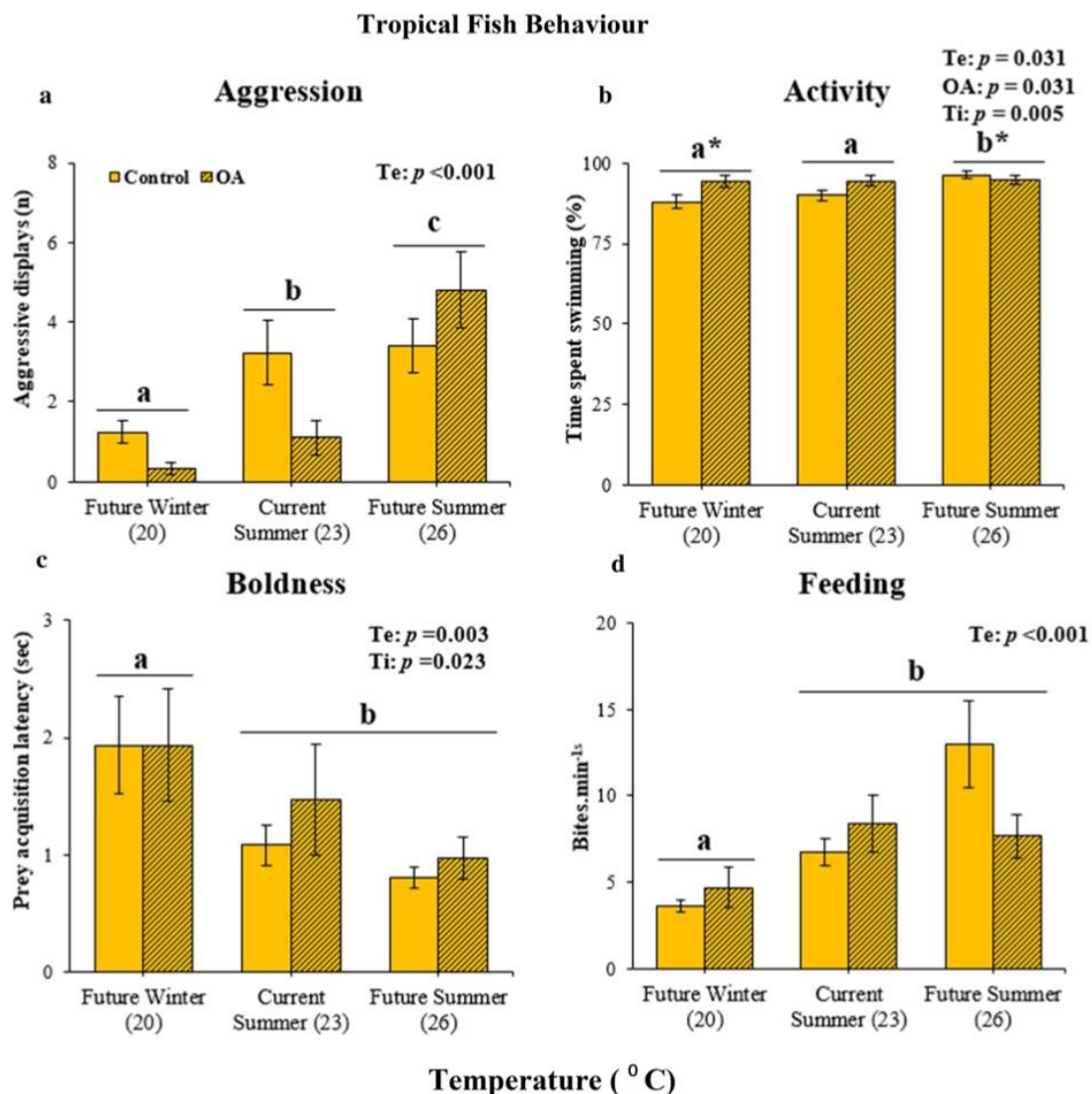


Fig. 1 Aggression (total number of attacks) (a), activity (percentage of time spent swimming) (b), boldness (prey acquisition latency) (c), and feeding (bite rate) (d) responses for tropical fish across temperature (Te) and ocean acidification (OA). Note that decreased prey acquisition latency reflects increased boldness. Ti = time (days). Data

for days 19 and 27 were pooled for visualisation purposes. The different letter above bars indicates significant differences among treatments (Tables S7–10). * denotes significant ocean acidification effect at a specific temperature level. Error bars represent standard error

$p = 0.023$), but decreased activity levels by 5% ($df = 1$, F value = 8.126, $p = 0.005$), irrespective of climate treatments.

Tropical fish somatic growth (SL and WW) was faster at future- (SL: +92%, WW: +302%) and current summer temperatures (SL: +35%, WW: +204%) than at future winter temperature (SL: Fig. S2a; $p < 0.020$; WW: Fig. 2a; $p < 0.002$; Tables S12, 13). At current summer temperature, somatic WW growth decreased 47% under ocean acidification (Fig. 2a; control vs. acidification: $p = 0.030$; Table S12).

Temperate fish behaviour and growth

The behavioural repertoire of the temperate fish did not change either as a result of novel interactions with the tropical fish, or as a result of elevated temperature or ocean acidification (MANOVA: $p > 0.05$, Table S11). Temperate fish growth (SL and WW) for both the mixed-species and temperate-only pairs were elevated under future winter temperatures compared to future- (SL: +481%; WW: +200%) and current (SL: +226%; WW: +250%) summer temperatures (WW: Fig. 2b, c; future winter vs. future summer, $p < 0.001$; future winter vs. current summer, $p < 0.001$; current summer vs. future summer; $p = 0.558$; Table S14; SL: Fig. S3b, c; future winter vs. current summer, $p = 0.002$; future winter vs. future summer, $p = 0.015$; current summer vs. future summer; $p = 0.465$; Table S15). Temperate fish growth was unaffected by ocean acidification ($p > 0.474$; Tables S14, 15). Mixed-species paired temperate fish SL and WW growth was 135% and 148% faster, respectively,

than non-mixed temperate fish (Fig. 2b, c; WW; $p = 0.001$; SL: $p = 0.046$; Tables S14, 15), likely due to size differences (see “Discussion” section).

Discussion

Here we reveal that projected seasonal temperatures and ocean acidification under future climate change could both positively and negatively modify the growth and behaviour of range-extending tropical fish in temperate marine ecosystems. Whilst projected future winter temperatures may allow many tropical vagrants to overwinter in temperate ecosystems (compared to current winter temperatures), future winter conditions will still be a driver of reduced seasonal performance in these fishes at their temperate cold-range limit. Reduced feeding, boldness and aggression-related behaviours during future winters compared to current summer temperatures (at which tropical fish can establish in high latitude ecosystems, Figueira and Booth 2010) may limit access to prey (Biro et al. 2010; Weetman et al. 1998) and increase predation mortality risk (Figueira et al. 2019). Indeed, we found that future winter temperatures decreased their growth relative to current summer temperatures. Our results therefore highlight that seasonal temperatures in temperate ecosystems, which currently modify the pool of arriving tropical vagrant species (Monaco et al. 2021), will continue to regulate the performance of interacting tropical and temperate fishes in high temperatures latitude ecosystems

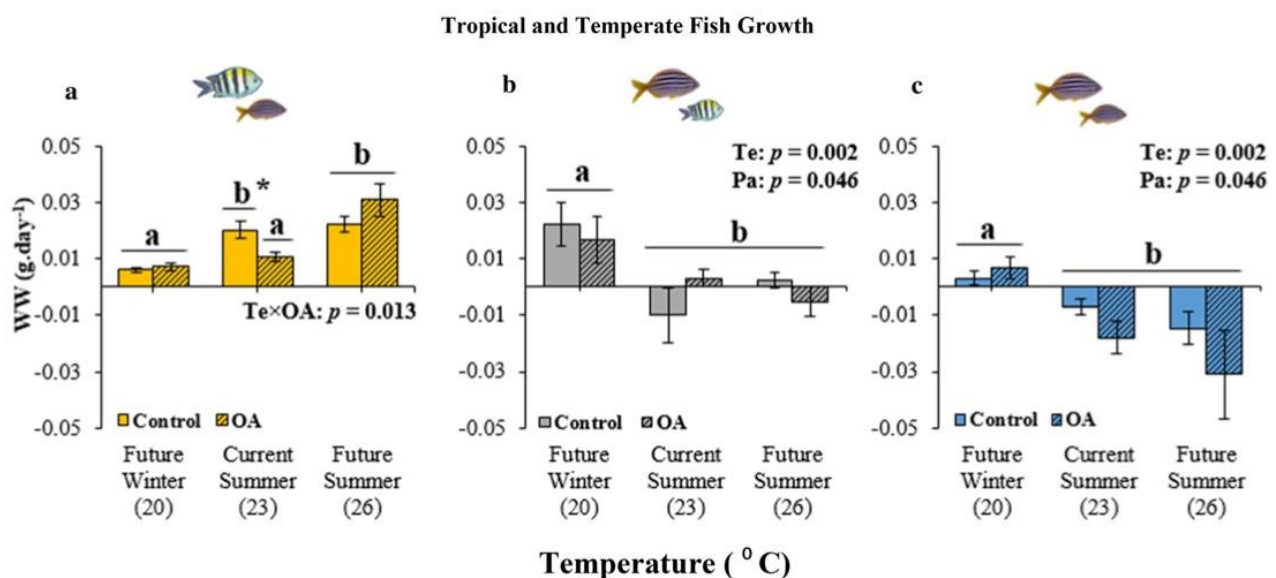


Fig. 2 Growth (wet weight, WW) responses of juvenile tropical fish (a) and temperate fish (b, c) across temperature (Te), ocean acidification (OA) and pairing type (Pa) treatments (as depicted by the fish symbols above the graphs). Ti = time (days). Data for days 19 and 27

were pooled for visualisation purposes. The different letter above bars indicates significant differences among treatments (Tables S12, S15). * denotes significant ocean acidification effect at a specific temperature level. Error bars represent standard errors

under ocean warming (Fig. 3). This finding is further reinforced when considering that future winter temperatures (20 °C) at the cold-range edge of coral reef fishes are still lower than current winter temperatures (23 °C) in their core range (see Table 2). Our data suggest that projected future winter conditions in temperate ecosystems will still reduce the performance of range-extending fishes in temperate fish communities and slow down tropicalisation of higher latitudes.

Temperate fish behaviour was unaffected by the two climate stressors or novel species interactions, but they did show slower growth with increasing warming. Projected future summer temperatures (26 °C) sometimes already occur at the trailing edge (28° S) of the temperate species' historical distribution (Froese and Pauly 2023) and, therefore, they may not need to adjust their behaviour to cope with projected ocean warming in their current core ranges (34° S), but suffer physiologically (e.g. slower growth). We suggest that unaltered behavioural responses and reduced growth during thermally stressful future summers (and autumns) may limit temperate fish performance (Mitchell et al. 2023), and may result in range contractions. This could then positively affect tropical fish abundance or performance during future summers at their leading edge through relief of competition (Coni et al. 2021a) and compromised temperate fish predator avoidance (Figueira et al. 2019). During future winters, however, temperate species would still be at

a higher advantage than tropical species at high latitude ecosystems, exemplifying the reversed performance outcomes of native versus range-extending species as a function of season in a future ocean.

Activity levels of the tropical fish were slightly higher under elevated CO₂, irrespective of temperature. This could put tropical fish at higher risk of predation (Biro et al. 2007; Beck et al. 2016), particularly during cooler months, when tropical fish experience declines in predator escape behaviours and growth in temperate ecosystems (Djurichkovic et al. 2019; Figueira et al. 2019).

Higher growth of temperate fish in mixed-species pairs compared to temperate-only pairs is likely due to size differences between tropical and temperate pair members. The pairing of a larger temperate and a smaller tropical fish in tropical-temperate pairs reflects current size differences observed in high-latitude ecosystems at the current initial stages of range expansion in these species (Smith et al. 2018). Temperate fish in mixed-species shoals may benefit from body-size differences (Ward and Krause 2001) through superior foraging competency (Coni et al. 2021a). However, increasing tropical fish growth rates and consequently increased body sizes in high latitude ecosystems (at their leading edges) versus decreasing temperate fish growth and body size (at their trailing edges) during future summers could eliminate any size-advantages that co-shoaling temperate fish currently obtain from

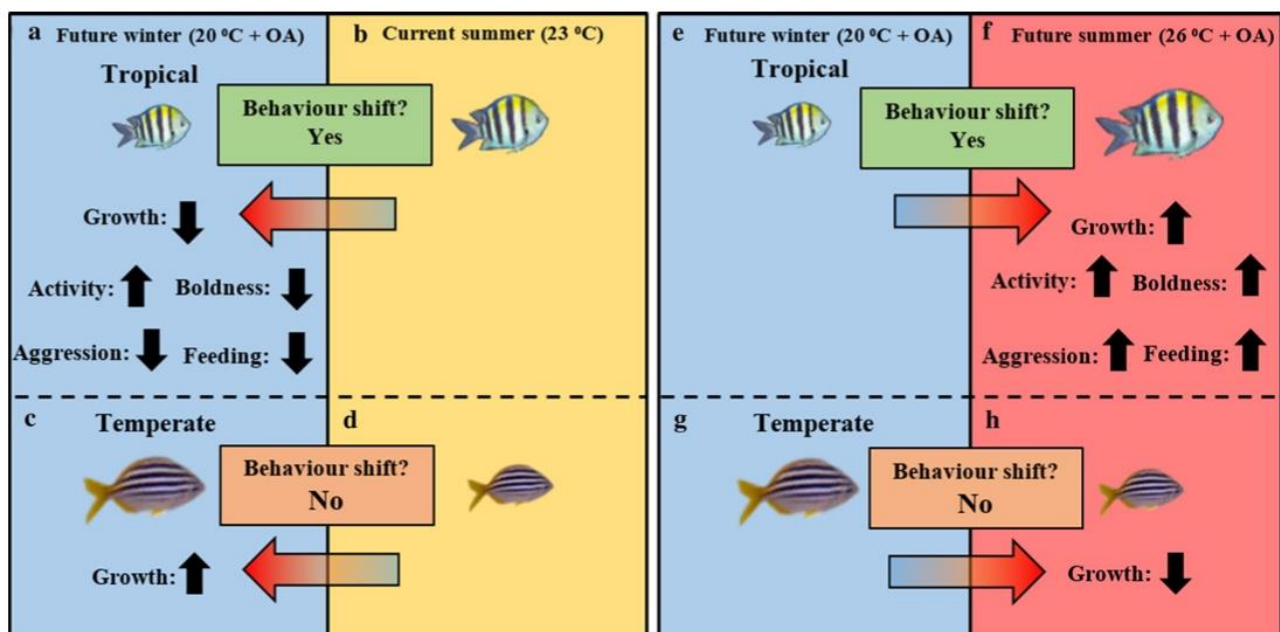


Fig. 3 Conceptual diagram showing how current and projected future natural seasonal temperatures and ocean acidification positively and negatively modify the growth and behaviour of interacting tropical (panels: a, b, e, and f) and temperate fishes (panels: c, d, g, and h) at 34° S, where the ranges of co-shoaling tropical and tem-

perate fishes overlap. Projected future seasonal temperatures strongly regulate the growth and behaviour of range-shifting tropical fish and will likely modify the pace of marine range-shifts in southeastern Australia

tropical-temperate shoaling interactions, although this could be partly regained during future winters.

Shoal size, shoal composition and laboratory settings may alter behavioural responses to competing tropical and temperate fishes residing in temperate ecosystems (Paijmans et al. 2020; Coni et al. 2022). To address these limitations, we suggest future climatic experiments focus on larger shoal size responses to climate change. Additionally, larger shoal size responses could be addressed through the use of natural climatic analogues (see Nagelkerken et al. 2016), and therefore, reveal behavioural responses to concurrent novel interaction and climatic change effects in a natural setting.

Previous studies postulated that ocean warming might promote tropicalisation and the coexistence of tropical and temperate fish in temperate latitudes (Kingsbury et al. 2019; Coni et al. 2021b; 2022). However, such studies did not consider how future winter temperatures may modulate the pace of tropicalisation (but see Mitchell et al. 2023). Here, we highlight how seasonal temperature changes alter the performance of interacting tropical and temperate fishes. These shifts in performance may seasonally modify the establishment and dispersal of range-extending tropical fishes in temperate regions such as south eastern Australia. While future summer temperatures facilitate tropicalisation (increased aggression, activity, and feeding), future winter temperatures may limit these fitness-related behaviours in tropical vagrants. Future winter temperatures comparatively boost the growth of co-shoaling temperate fish compared to current and future summer temperatures, which may further limit the growth of tropical vagrants (during future winters) through competitive interactions. As such, the seasonal rise and fall of ocean temperatures are likely to have alternating positive and negative effects on both tropical vagrants and local temperate species in temperate ecosystems, increasing the likelihood of their coexistence under future climate. While we found limited ocean acidification effects on tropical and temperate fish behaviours, the indirect effects of ocean acidification are likely to have the most significant effect on species responses to tropicalisation (e.g. through habitat shifts, Coni et al. 2021c).

Acknowledgements IN and DJB were supported by an Australian Research Council (ARC) Discovery Project (DP170101722). We thank the Sydney Institute of Marine Science for their help and guidance during the experimental period.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Data availability The data that support the findings of this study are openly available in figshare at <https://doi.org/10.25909/637f2b0fb59a8>

Declarations

Conflict of interest The authors have no conflict of interests to declare.

Ethical approval This experiment was conducted in accordance with The University of Adelaide Animal Ethics and University of Technology guidelines and permits: S-2020-13 and 2017-1117, and under New South Wales DPI Scientific Collection Permit: F94/696(A)-9.0.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alexander J, Diez J, Levine J (2015) Novel competitors shape species' responses to climate change. *Nature* 525(7570):515–518
- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46
- Barlow GW (1974) Extraspecific imposition of social grouping among surgeonfishes (Pisces: Acanthuridae). *J Zool* 174:333–340
- Beck H, Feary D, Fowler A, Madin E, Booth D (2016) Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Mar Biol* 163(4):1–14. <https://doi.org/10.1007/s00227-016-2844-8>
- Biro PA, Post JR, Booth DJ (2007) Mechanisms for climate-induced mortality of fish populations in whole-lake experiments. *Proc Natl Acad Sci USA* 104:9715–9719
- Biro PA, Beckmann C, Stamps J (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society b: Biological Sciences* 277(1678):71–77
- Booth DJ, Figueira WF, Gregson MA, Beretta G (2007) Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuar Coast Shelf Sci* 72(1–2):102–114. <https://doi.org/10.1016/j.ecss.2006.10.003>
- Booth DJ, Bond N, Macreadie PI (2011) Detecting range shifts among Australian fishes in response to climate change. *Mar Freshw Res* 62(9):1027–1042. <https://doi.org/10.1071/MF10270>
- Camacho-Cervantes M, Garcia CM, Ojanguren AF, Magurran AE (2014) Exotic invaders gain foraging benefits by shoaling with native fish. *Royal Society Open Science* 1(3):140101
- Carmona-Catot G, Magellan K, García-Berthou E (2013) Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS ONE* 8(1):e54734. <https://doi.org/10.1371/journal.pone.0054734>
- Cattano C, Claudet J, Domenici P, Milazzo M (2018) Living in a high CO2 world: A global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecol Monogr* 88(3):320–335
- Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. *Science* 333(6045):1024–1026
- Clarke KR, Gorley RN (2015) PRIMER v7: User Manual/Tutorial. PRIMER-E Plymouth.

- Coni EOC, Booth DJ, Nagelkerken I (2021a) Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef fish. *Coral Reefs* 40(5):1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>
- Coni EOC, Booth DJ, Ferreira C, Nagelkerken I (2021b) Behavioural generalism could facilitate coexistence of tropical and temperate fishes under climate change. *J Anim Ecol*. <https://doi.org/10.1111/1365-2656.13599>
- Coni EOC, Nagelkerken I, Ferreira C, Connell SD, Booth DJ (2021c) Ocean acidification may slow the pace of tropicalisation of temperate fish communities. *Nat Clim Chang* 11(3):249–256. <https://doi.org/10.1038/s41558-020-00980-w>
- Coni EOC, Booth DJ, Nagelkerken I (2022) Coral-reef fishes can become more risk-averse at their poleward range limits. *Proceedings of the Royal Society b: Biological Sciences* 289(1971):20212676
- Davis A, Jenkinson L, Lawton J, Shorrocks B, Wood S (1998) Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391(6669):783–786
- Dickson A, Millero F (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A Oceanographic Research Papers* 34(10):1733–1743. [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)
- Djurichkovic L, Donelson J, Fowler A, Feary D, Booth DJ (2019) The effects of water temperature on the juvenile performance of two tropical damselfishes expatriating to temperate reefs. *Sci Rep* 9(1):13937. <https://doi.org/10.1038/s41598-019-50303-z>
- Domenici P, Allan B, Lefrançois C, McCormick M (2019) The effect of climate change on the escape kinematics and performance of fishes: Implications for future predator–prey interactions. *Conservation Physiology* 7(1):coz078. <https://doi.org/10.1093/conphys/coz078>
- Feary DA, Pratchett MS, Emslie MJ, Fowler A, Figueira WF, Luiz OJ, Nakamura Y, Booth DJ (2014) Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish Fish* 15(4):593–615. <https://doi.org/10.1111/faf.12036>
- Figueira WF, Booth DJ (2010) Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Glob Change Biol* 16(2):506–516. <https://doi.org/10.1111/j.1365-2486.2009.01934.x>
- Figueira WF, Biro P, Booth DJ, Valenzuela Davie VC (2009) Performance of tropical fish recruiting to temperate habitats: role of ambient temperature and implications of climate change. *Mar Ecol Prog Ser* 384:231–239. <https://doi.org/10.3354/meps08057>
- Figueira WF, Curley B, Booth DJ (2019) Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes? *Mar Biol* 166(73):1–9. <https://doi.org/10.1007/s00227-019-3521-5>
- Fowler A, Parkinson K, Booth DJ (2017) New poleward observations of 30 tropical reef fishes in temperate southeastern Australia. *Mar Biodivers* 48(4):2249–2254
- Froese R, Pauly D (2023) FishBase. World Wide Web electronic publication. www.fishbase.org
- Goldenberg SU, Nagelkerken I, Marangon E, Bonnet A, Ferreira CM, Connell SD (2018) Ecological complexity buffers the impacts of future climate on marine consumers. *Nat Clim Chang* 8(3):229–233. <https://doi.org/10.1038/s41558-018-0086-0>
- Hoegh-Guldberg O, Bruno J (2010) The impact of climate change on the world's marine ecosystems. *Science* 328(5985):1523–1528. <https://doi.org/10.1126/science.1189930>
- IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P., Zhai, A., Pirani, SL., Connors, C., Péan, S., Berger, N., Caud, Y., Chen, L., Goldfarb, MI., Gomis, M., Huang, K., Leitzell, E., Lonnoy, JBR., Matthews, TK., Maycock, T., Waterfield, O., Yelekçi, R., Yu, B., Zhou (eds.)]. Cambridge University Press. In Press.
- Kingsbury K, Gillanders B, Booth DJ, Nagelkerken I (2019) Trophic niche segregation allows range-extending coral reef fishes to coexist with temperate species under climate change. *Glob Change Biol* 26(2):721–733
- Kuiter RH (1993) Coastal fishes of southeastern Australia. University of Hawaii Press, Honolulu, Hawaii
- Landeau L, Terborgh J (1986) Oddity and the 'confusion effect' in predation. *Anim Behav* 34(5):1372–1380
- Lloyd P, Plaganyi É, Weeks S, Magno-Canto M, Plaganyi G (2011) Ocean warming alters species abundance patterns and increases species diversity in an African sub-tropical reef–fish community. *Fish Oceanogr* 21(1):78–94. <https://doi.org/10.1111/j.1365-2419.2011.00610.x>
- Mathis A, Chivers D (2003) Overriding the oddity effect in mixed-species aggregations: group choice by armored and nonarmored prey. *Behav Ecol* 14(3):334–339
- Mehrbach C, Culbertson C, Hawley J, Pytkowicz R (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18(6):897–907. <https://doi.org/10.4319/lo.1973.18.6.0897>
- Mitchell A, Booth DJ, Nagelkerken I (2022a) Ocean warming and acidification degrade shoaling performance and lateralisation of novel tropical–temperate fish shoals. *Glob Change Biol* 28(4):1388–1401. <https://doi.org/10.1111/gcb.16022>
- Mitchell A, Hayes C, Booth DJ, Nagelkerken I (2023) Ocean acidification and seasonal water temperatures alter the physiology of competing temperate and coral reef fishes. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2023.163684>
- Mitchell A, Hayes C, Booth DJ, Nagelkerken I (2022b) Ocean acidification and seasonal temperature alter the behaviour and fitness of range-extending coral reef fish under future climate. The University of Adelaide. Dataset. https://adelaide.figshare.com/articles/dataset/Ocean_acidification_and_seasonal_temperature_alter_the_behaviour_and_fitness_of_range-extending_coral_reef_fish_under_future_climate/21514683
- Monaco C, Booth DJ, Figueira W, Gillanders B, Schoeman D, Bradshaw C, Nagelkerken I (2021) Natural and anthropogenic climate variability shape assemblages of range-extending coral-reef fishes. *J Biogeogr* 48(5):1063–1075
- Nagelkerken I, Munday P (2016) Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Glob Change Biol* 22(3):974–989. <https://doi.org/10.1111/gcb.13167>
- Nagelkerken I, Russell BD, Gillanders BM, Connell SD (2016) Ocean acidification alters fish populations indirectly through habitat modification. *Nat Clim Chang* 6(1):89–93
- Pajmams K, Booth DJ, Wong M (2020) Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *J Fish Biol* 96(3):806–814
- Pecl G, Araújo M, Bell J, Blanchard J, Bonebrake T, Chen I, Clark T, Colwell R, Danielsen F, Evengård B, Falconi L, Ferrier S, Frusher S, Garcia R, Griffis R, Hobday A, Janion-Scheepers C, Jarzyna M, Jennings S, Lenoir J, Linnetved H, Martin V, McCormack P, McDonald J, Mitchell N, Mustonen T, Pandolfi J, Pettorelli N, Popova E, Robinson S, Scheffers B, Shaw J, Sorte C, Strugnell J, Sunday J, Tuanmu M, Vergés A, Villanueva C, Wernberg T, Wapstra E, Williams S (2017) Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science* 355(6332):eaai9214. <https://doi.org/10.1126/science.aai9214>
- Pessarrodona A, Vergés A, Bosch N, Bell S, Smith S, Sgarlatta M, Wernberg T (2022) Tropicalisation unlocks novel trophic pathways

- and enhances secondary productivity in temperate reefs. *Funct Ecol.* <https://doi.org/10.1111/1365-2435.13990>
- Pierrot D, Lewis E, Wallace DWR (2006) MS excel program developed for CO2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center. Oak Ridge National Laboratory; US Department of Energy. Oak Ridge (Ed.), Tennessee.
- Seidel D, Fu Q, Randel W, Reichler T (2007) Widening of the tropical belt in a changing climate. *Nat Geosci* 1(1):21–24
- Smith S, Fox R, Booth DJ, Donelson J (2018) Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion frontline. *Glob Change Biol* 24(4):1663–1672. <https://doi.org/10.1111/gcb.14016>
- Twine S, Fitzgibbon Q, Hobday A, Carter C, Oellermann M, Pecl G (2022) Resident lobsters dominate food competition with range-shifting lobsters in an ocean warming hotspot. *Mar Ecol Prog Ser* 685:171–181
- Tylianakis J, Didham R, Bascompte J, Wardle D (2008) Global change and species interactions in terrestrial ecosystems. *Ecol Lett* 11(12):1351–1363
- Vergés A, Steinberg PD, Hay ME, Poore AGB, Campbell AH, Ballesteros E, Heck KL, Booth DJ, Coleman MA, Feary DA, Figueira W, Langlois T, Marzinelli EM, Mizerek T, Mumby PJ, Nakamura Y, Roughan M, van Sebille E, Sen Gupta A, Smale DA, Tomas F, Wernberg T, Wilson SK (2014) The tropicalisation of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society of b: Biological Sciences* 281(1789):20140846. <https://doi.org/10.1098/rspb.2014.0846>
- Vergés A, Doropoulos C, Malcolm HA, Skye M, Garcia-Piz M, Marzinelli EM, Campbell AH, Ballesteros E, Hoey AS, Vila-Concejo A, Bozec YM, Steinberg PD (2016) Long-term empirical evidence of ocean warming leading to tropicalisation of fish communities, increased herbivory, and loss of kelp. *Proc Natl Acad Sci USA* 113(48):13791–13796. <https://doi.org/10.1073/pnas.1610725113>
- Ward A, Krause J (2001) Body length assortative shoaling in the European minnow, *Phoxinus phoxinus*. *Anim Behav* 62(4):617–621
- Weetman D, Atkinson D, Chubb J (1998) Effects of temperature on anti-predator behaviour in the guppy, *Poecilia reticulata*. *Anim Behav* 55(5):1361–1372
- Winer BJ, Brown DR, Michels KM (1991) Statistical principles in experimental design, 3rd edn. McGraw-Hill, New York
- Wu L, Cai W, Zhang L, Nakamura H, Timmermann A, Joyce T, McPhaden M, Alexander M, Qiu B, Visbeck M, Chang P, Giese B (2012) Enhanced warming over the global subtropical western boundary currents. *Nat Clim Chang* 2(3):161–166
- Yamano H, Sugihara K, Nomura K (2011) Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophys Res Lett.* <https://doi.org/10.1029/2010GL046474>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information

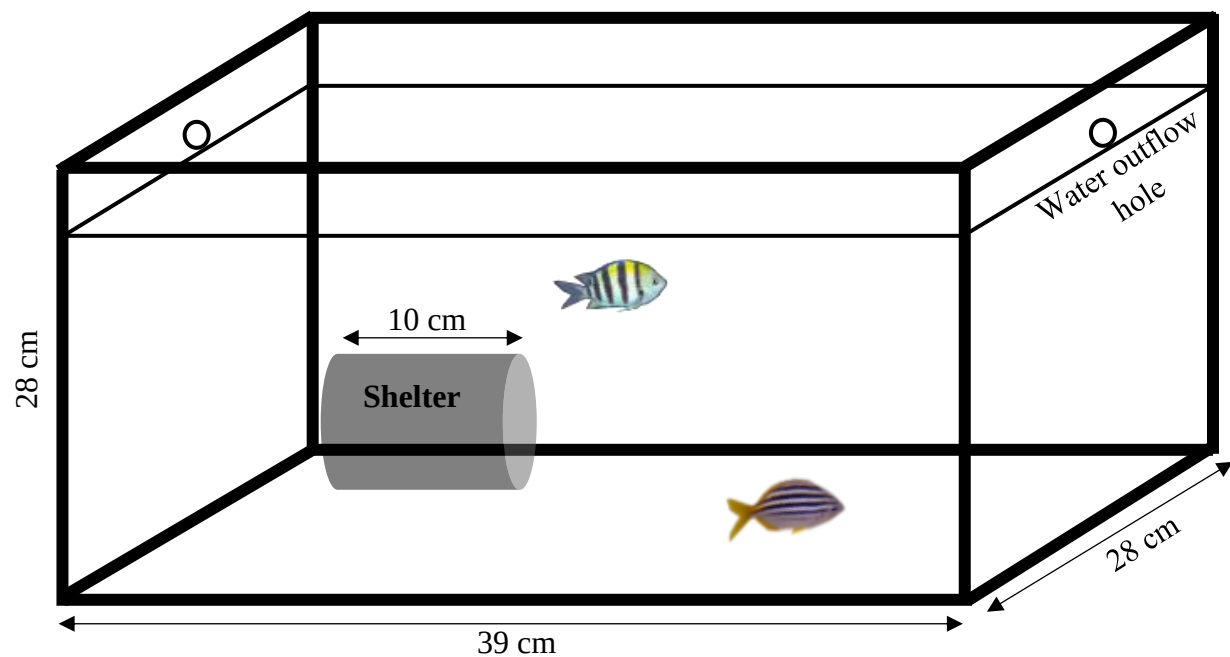


Figure S1: diagram of pairing tank used to house fish pairs during the experimental period (including behaviour video recordings).

Table S1: Starting wet weight (WW) and starting standard length (SL) of tropical and temperate fish.

Affinity	Starting WW (g)	Starting SL (mm)
Tropical	0.31 (SE $\pm$ 0.04)	18.61 (SE $\pm$ 0.68)
Mixed-spp. temperate	1.24 (SE $\pm$ 0.15)	29.34 (SE $\pm$ 1.52)
Non-mixed temperate	4.04 (SE $\pm$ 0.2)	53.28 (SE $\pm$ 0.8)

Table S2: ANOVA output of species starting WW. Note: **bold** *p*-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Species	2	14.742	7.371	328.26	<0.001
Residuals	154	3.458	0.022		
Total	156	18.2			
Pairwise test of term 'Temperature'. <i>t</i> = statistic. Note: bold <i>p</i> -value indicates significant <i>p</i> -value ($p < 0.05$).					
Group		<i>t</i>	<i>p</i> -value		
Non-mixed temperate, Mixed-species temperate		5.612	<0.001		
Non-mixed temperate, Tropical		28.571	<0.001		
Mixed-species temperate, Tropical		14.817	<0.001		

Table S3: ANOVA output of species starting SL. Note: **bold** *p*-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Species	2	12.554	6.277	478.01	<0.001
Residuals	154	2.022	0.013		
Total	156	14.576			
Pairwise test of term 'Temperature'. <i>t</i> = statistic. Note: bold <i>p</i> -value indicates significant <i>p</i> -value ($p < 0.05$).					
Group			<i>t</i>	<i>p</i> -value	
Non-mixed temperate, Mixed-species temperate			5.978	<0.001	
Non-mixed temperate, Tropical			32.782	<0.001	
Mixed-species temperate, Tropical			17.667	<0.001	

Table S4: Mean ($\pm$ SE) seawater parameters and treatment levels across experiments. SW = seawater.

Mixed-species pairs					
# of replicates of paired tanks	Temperature (°C)	pH	Salinity (ppt)	<i>p</i> CO ₂ (μ atm)	Total alkalinity (μ mol.kg ⁻¹ SW)
10	20.2 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	304 ($\pm$ 8.4)	2104 ($\pm$ 48.6)
10	20.2 ($\pm$ 0.06)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	914 ($\pm$ 11.3)	2135 ($\pm$ 14.0)
10	23.1 ($\pm$ 0.04)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	308 ($\pm$ 3.7)	2155 ($\pm$ 15.3)
10	23.0 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	977 ($\pm$ 16.6)	2214 ($\pm$ 35.5)
10	25.8 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	303 ($\pm$ 2.4)	2165 ($\pm$ 13.0)
10	25.7 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	960 ($\pm$ 4.4)	2186 ($\pm$ 1.6)
Temperate-only pairs					
10	20.1 ($\pm$ 0.05)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	311 ($\pm$ 3.5)	2154 ($\pm$ 14.2)
10	20.3 ($\pm$ 0.06)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	940 ($\pm$ 12.1)	2158 ($\pm$ 12.8)
10	23.1 ($\pm$ 0.04)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	309 ($\pm$ 4.0)	2160 ($\pm$ 22.9)
10	23.0 ($\pm$ 0.03)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	957 ($\pm$ 9.5)	2181 ($\pm$ 20.8)
10	25.8 ($\pm$ 0.06)	8.10 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	306 ($\pm$ 1.4)	2175 ($\pm$ 10.9)
10	25.9 ($\pm$ 0.04)	7.70 ($\pm$ 0.01)	35.0 ($\pm$ 0.01)	967 ($\pm$ 5.42)	2187 ($\pm$ 3.43)

Table S5: Ethogram defining behaviours and proxies quantified during video analysis.

Behaviour	Proxy	Definition
Activity	Time spent swimming (%)	Swimming is defined as a forward movement through the water column through caudal fin action.

Boldness	<i>Prey acquisition latency (sec)</i>	Time (seconds) taken for fish to initiate the first bite following the addition of food to the tank.
Aggression	<i>Total number of aggressive acts</i>	(1) Either fish in a pair rapidly approaches opposing fish, initiating a rapid swim response (flee) in the other fish. (2) Fish flaring fins towards opposing fish (3) Either fish in a pair nipping/biting opposing fish
Feeding	<i>Bites.min⁻¹</i>	The number of total bites taken per unit time by the focal fish.

Table S6: SIMPER analysis outputs for tropical and temperate fishes.

SIMPER output of tropical fish behavioural repertoire of individuals exposed to 20 °C. Average squared distance = 0.29						
Behaviour	Av. Abundances	Av. Similarity	Sim/SD	Contribution %	Cumulative %	
Activity	0.988	<0.001	0.45	0.22	0.22	
Boldness	1.04	0.035	0.48	11.98	12.20	
Feeding	0.813	0.04	0.30	13.97	26.17	
Aggression	0.373	0.213	0.57	73.83	100.00	
SIMPER output of tropical fish behavioural repertoire of individuals exposed to 23 °C. Average squared distance = 0.38						
Behaviour	Av. Abundances	Av. Similarity	Sim/SD	Contribution %	Cumulative %	
Activity	0.993	<0.001	0.45	0.12	0.12	
Boldness	0.925	0.032	0.36	8.36	8.48	
Feeding	0.949	0.048	0.28	12.53	21.00	
Aggression	0.621	0.299	0.55	79.00	100.00	
SIMPER output of tropical fish behavioural repertoire of individuals exposed to 26 °C. Average squared distance = 0.14						
Behaviour	Av. Abundances	Av. Similarity	Sim/SD	Contribution %	Cumulative %	
Activity	1	<0.001	0.41	0.13	0.13	
Boldness	0.866	0.018	0.48	12.34	12.47	
Feeding	1.03	0.037	0.44	26.35	38.82	
Aggression	1.06	0.087	0.35	61.18	100.00	
SIMPER output of tropical fish behavioural repertoire between individuals exposed to 20 °C and 23 °C. Average squared distance = 0.74						
20 °C		23 °C				
Behaviour	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Aggression	0.373	0.621	0.559	0.94	75.43	75.43
Feeding	0.813	0.949	0.104	0.48	13.99	89.42
Boldness	1.04	0.925	0.077	0.71	10.43	99.86
Activity	0.988	0.993	0.001	0.68	0.14	100.00

SIMPER output of tropical fish behavioural repertoire between individuals exposed to 26 °C and 23 °C. Average squared distance = 0.71						
	26 °C	23 °C				
Behaviour	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Aggression	0.621	1.06	0.565	0.87	79.97	79.97
Feeding	0.949	1.03	0.090	0.44	12.67	92.64
Boldness	0.925	0.866	0.051	0.51	7.26	99.90
Activity	0.993	1	<0.001	0.63	0.10	100.00
SIMPER output of tropical fish behavioural repertoire between individuals exposed to 26 °C and 20 °C. Average squared distance = 0.96						
	26 °C	20 °C				
Behaviour	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Aggression	0.373	1.06	0.758	1.11	78.68	78.68
Feeding	0.813	1.03	0.124	0.53	12.85	91.53
Boldness	1.04	0.866	0.081	0.70	8.36	99.90
Activity	0.988	1	<0.001	0.61	0.10	100.00

Behavioural repertoire of tropical fish

Table S7: MANOVA output of behavioural repertoire of tropical fish and resulting pairwise tests. Note: **bold** *p*-values denote significant differences ($p < .05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature (Te)	2	8.532	4.266	53.606	<0.001
Ocean acidification (OA)	1	1.085	1.085	10.818	0.064
Time (Ti)	1	0.278	0.278	1.028	0.328
Te×OA	2	1.515	0.757	8.466	0.082
Te×Ti	2	0.159	0.0796	0.294	0.891
OA×Ti	1	0.100	0.100	0.371	0.688
Te×OA×Ti	2	0.179	0.090	0.331	0.866
Fish ID(Te×OA×Ti)	90	24.337	0.270	No test	
Total	101	36.271			
Pairwise test of term 'temperature'. Note: bold <i>p</i> -value indicates significant <i>p</i> -value ($p < 0.05$). t = t-statistic.					
Temperature groups	t		<i>p</i> -value		
20, 23	5.1108		0.02		
20, 26	9.1055		0.006		
23, 26	6.2693		0.013		

Table S8: ANOVA output of tropical fish number of aggressive acts. Note: **bold** *p*-value denotes significant differences ($p < .05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature (Te)	2	11.263	5.631	3738.2	<0.001

Ocean acidification (OA)	1	1.49	1.49	108.09	0.061
Time (Ti)	1	0.219	0.219	0.718	0.388
Te×OA	2	2.122	1.061	13.528	0.073
Te×Ti	2	0.003	0.002	0.005	0.994
OA×Ti	1	0.014	0.014	0.045	0.831
Te×OA×Ti	2	0.157	0.078	0.257	0.78
Fish ID(Te×OA×Ti)	90	27.443	0.305	No test	
Total	101	42.786			
Pairwise test of term 'temperature'. Note: bold <i>p</i> -value indicates significant <i>p</i> -value (<i>p</i> < 0.05). t = t-statistic.					
Temperature groups	t		<i>p</i>-value		
20, 23	21.896		0.028		
20, 26	93.623		0.007		
23, 26	126.85		0.005		

Table S9: ANOVA output of prey acquisition latency for tropical fish and resulting pairwise tests. Note: **bold** *p*-values denote significant differences (*p* < 0.05). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature (Te)	2	0.454	0.227	6.852	0.003
Ocean acidification (OA)	1	0.012	0.011	0.354	0.559
Te×OA	1	0.165	0.165	5.574	0.023
Fish ID(Te×OA)	2	0.011	0.006	0.172	0.837
Pooled	57	1.908	0.033	1.13	0.354
Total	38	1.126	0.03		
Temperature	101	3.783			
Pairwise test of term 'Temperature'. t = statistic. Note: bold <i>p</i> -value indicates significant <i>p</i> -value (<i>p</i> < 0.05)					
Temperature groups	t		<i>p</i>-value		
20, 23	2.369		0.025		
20, 26	3.611		<0.001		
23, 26	1.266		0.217		

Table S10: ANOVA output of tropical fish bite rate and resulting pairwise tests. Note: **bold** *p*-values denote significant differences (*p* < 0.05). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature (Te)	2	1.934	0.967	9.766	<0.001
Ocean acidification (OA)	1	0.233	0.233	2.354	0.124
Te×OA	2	0.293	0.147	1.480	0.238
Fish ID(Te×OA)	57	5.399	0.095	0.663	0.870
Pooled	39	5.570	0.143		
Total	101	13.526			
Pairwise test of term 'Temperature'. t = statistic. Note: bold <i>p</i> -value indicates significant <i>p</i> -value (<i>p</i> < 0.05).					
Temperature groups	t		<i>p</i>-value		
20, 23	2.773		0.008		

20, 26	4.561	<0.001
23, 26	1.559	0.125

Table S11: Tropical fish percentage time spent swimming ANOVA and resulting pairwise tests. Note: **bold** *p*-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature	2	0.026	0.013	3.554	0.031
Ocean acidification	1	0.016	0.016	4.502	0.031
Time	1	0.029	0.029	8.126	0.005
Temperature×Ocean acidification	2	0.018	0.009	2.468	0.089
Pooled	95	0.344	0.004		
Total	101	0.44			
Pairwise test of term 'Temperature'. <i>t</i> = statistic. Note: bold <i>p</i> -value indicates significant <i>p</i> -value ($p < 0.05$).					
Temperature groups		<i>t</i>			<i>p</i> -value
20, 23		0.632			0.535
20, 26		2.563			0.012
23, 26		2.267			0.025

Behavioural repertoire of temperate fish

Table S12: MANOVA output of behavioural repertoire of temperate fish. Note: **bold** *p*-values denote significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>p</i> -value
Temperature (Te)	2	3.839	1.920	1.285	0.251
Ocean acidification (OA)	1	0.635	0.635	0.780	0.498
Pair Type (Pa)	1	4.894	4.894	1.724	0.145
Time (Ti)	1	1.046	1.046	1.102	0.337
Te×OA	2	1.691	0.846	0.974	0.436
Te×Pa	2	2.529	1.265	0.636	0.704
Te×Ti	2	1.951	0.976	1.028	0.40
OA×Pa	1	1.912	1.912	1.089	0.340
OA×Ti	1	0.836	0.836	0.881	0.444
Pa×Ti	1	1.933	1.933	2.037	0.106
Te×OA×Pa	2	2.675	1.338	1.261	0.306
Te×OA×Ti	2	1.326	0.663	0.698	0.640
Te×Pa×Ti	2	4.138	2.069	2.18	0.052
OA×Pa×Ti	1	1.314	1.314	1.384	0.233
Fish ID(Te×OA×Pa)	170	216.88	1.276	1.344	0.014
Te×OA×Pa×Ti	2	1.252	0.626	0.659	0.681
Res	102	96.809	0.949		
Total	295	355.52			

Tropical Fish Fitness

Table S13: Tropical fish wet weight growth ANOVA output and resulting pairwise tests. Note: **bold** p -value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square

Source	df	SS	MS	F-value	p-value
Temperature (Te)	2	0.003	0.002	22.322	<0.001
Ocean acidification (OA)	1	<0.001	<0.001	<0.001	0.978
Te×OA	2	<0.001	<0.001	4.626	0.013
Res	48	0.004			
Total	53	0.008			
'Te×OA' for pairs of the levels of factor Temperature within levels 'Control' of factor OA.					
Temperature groups		t		p-value	
20, 26		5.0854		<0.001	
20, 23		3.9434		0.002	
26, 23		0.52651		0.605	
'Te×OA' for pairs of the levels of factor Temperature within levels 'OA' of factor OA.					
Temperature groups		t		p-value	
20, 26		4.308		<0.001	
20, 23		1.466		0.162	
26, 23		3.528		0.001	
'Te×OA' for pairs of the levels of factor OA within levels '20' of factor Temperature.					
OA groups		t		p-value	
Control, OA		0.602		0.557	
'Te×OA' for pairs of the levels of factor OA within levels '23' of factor Temperature.					
OA groups		t		p-value	
Control, OA		2.412		0.03	
'Te×OA' for pairs of the levels of factor OA within levels '26' of factor Temperature.					
OA groups		t		p-value	
Control, OA		1.402		0.187	

Table S14: ANOVA output of tropical fish somatic length growth ANOVA out and resulting pairwise tests. Note: **bold** p -value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square

Source	df	SS	MS	F-value	p -value
Temperature (Te)	2	0.152	0.076	21.023	<0.001
Ocean acidification (OA)	1	0.002	0.002	0.475	0.496
Te×OA	2	0.023	0.011	3.19	0.051
Res	48	0.173	0.004		
Total	53	0.368			
Pairwise test of term ‘Temperature’. t = statistic. Note: bold p -value indicates significant p -value ($p < 0.05$).					
Temperature groups		t	p -value		
20, 26		6.759	<0.001		
20, 23		2.464	0.020		
26, 23		3.972	<0.001		

Temperate Fish Fitness

Table S15: ANOVA output of temperate fish somatic length growth ANOVA and resulting pairwise tests. Note: **bold** p -value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square. Term ‘ocean acidification’ (OA) pooled during statistical analysis.

Source	df	SS	MS	F-value	p -value
Pair type (Pa)	1	0.02	0.02	3.95	0.046
Temperature (Te)	2	0.078	0.039	7.63	0.002
Pa×Te	2	0.007	0.003	0.65	0.522
Pa×OA	1	<0.001	<0.001	0.026	0.875
Te×OA	2	0.005	0.002	0.443	0.646
Pa×Te×OA	2	0.004	0.002	0.38	0.683
Pooled	92	0.472	0.005		
Total	102	0.588			
Pairwise test of term ‘Temperature’. t = statistic. Note: bold p -value indicates significant p -value ($p < 0.05$).					
Temperature groups	t		p -value		
20, 26	2.58		0.015		
20, 23	3.241		0.002		
26, 23	0.73		0.465		

Table S16: ANOVA output of temperate fish wet weight growth ANOVA and resulting pairwise tests. Note: **bold** p -value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square. Term ‘ocean acidification’ (OA) pooled during statistical analysis.

Source	df	SS	MS	F-value	p -value
Pair type (Pa)	1	0.004	0.004	10.985	0.001
Temperature (Te)	2	0.011	0.005	13.89	<0.001
Ocean acidification (OA)	1	<0.001	<0.001	0.509	0.474
Pa×Te	2	<0.001	<0.001	0.547	0.583
Pa×OA	1	<0.001	<0.001	0.982	0.328
Te×OA	2	<0.001	<0.001	0.625	0.538
Pa×Te×OA	2	<0.001	<0.001	1.197	0.305
Res	91	0.036			
Total	102	0.056			
Pairwise test of term ‘Temperature’. t = statistic. Note: bold p -value indicates significant p -value ($p < 0.05$).					
Temperature groups	t		p -value		
20, 26	4.103		<0.001		
20, 23	4.686		<0.001		
26, 23	0.59		0.558		

Growth (Somatic length)

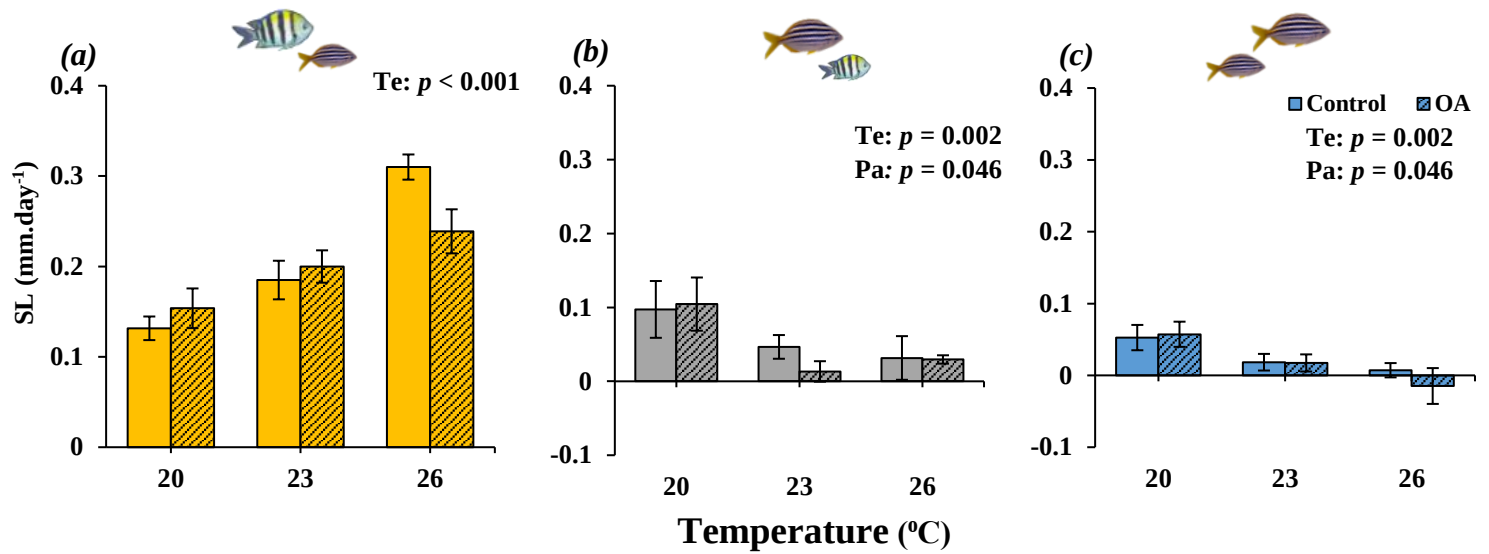


Figure S3: Somatic standard length increase by tropical fish (a) and temperate fish in mixed-species pairs (b) and temperate-only pairs (c) across temperature (Te), ocean acidification (OA), and pair type (Pa) treatments. Data for days 19 and 27 were pooled for visualisation purposes. The different letters above bars indicates significant differences among treatments (Table S14 and S15). Error bars represent standard error.

Chapter IV

Future shock: Ocean acidification and seasonal water temperatures alter the physiology of competing temperate and coral reef fishes

Angus Mitchell¹, Chloe Hayes¹, David J. Booth², Ivan Nagelkerken¹

¹Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, DX 650 418, Adelaide, SA, 5005, Australia

²School of the Life Sciences, University of Technology Sydney, Ultimo, NSW, 2007, Australia



Statement of Authorship

Title of Paper	Future shock: Ocean acidification and seasonal water temperatures alter the physiology of competing temperate and coral reef fishes		
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style		
Publication Details	Published in Science of the Total Environment 20/07/2023		

Principal Author

Name of Principal Author (Candidate)	Angus Mitchell		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/10/23

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Chloe Hayes		
Contribution to the Paper	Data collection, writing		
Signature		Date	24/10/23

Name of Co-Author	David J. Booth		
Contribution to the Paper	Study design, writing		
Signature		Date	24/10/23

Name of Co-Author	Ivan Nagelkerken		
Contribution to the Paper	Study design, writing		
Signature		Date	24/10/23



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

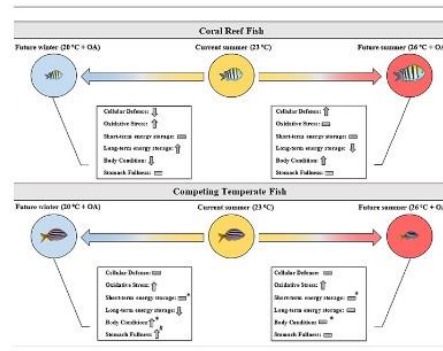
Future shock: Ocean acidification and seasonal water temperatures alter the physiology of competing temperate and coral reef fishes

Angus Mitchell^a, Chloe Hayes^a, David J. Booth^b, Ivan Nagelkerken^{a,*}^a Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, DX 650 418, Adelaide, SA 5005, Australia^b School of the Life Sciences, University of Technology Sydney, Ultimo, NSW 2007, Australia

HIGHLIGHTS

- Temperate and coral reef fishes were exposed to ocean acidification and ocean warming
- Coral reef fish decreased physiological performance in future winters (20 °C + OA)
- Coral reef fish increased lipid energy storage in future winter conditions
- Temperate fish increased oxidative damage in future summers (26 °C + OA)
- Future climate can modify the physiology of temperate and coral fishes seasonally

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Julian Blasco

Keywords:

Climate change
Elevated CO₂
Species range extensions
Ocean warming
Species interactions
Oxidative damage
Tropicalisation

ABSTRACT

Climate change can directly (physiology) and indirectly (novel species interactions) modify species responses to novel environmental conditions during the initial stages of range shifts. Whilst the effects of climate warming on tropical species at their cold-water leading ranges are well-established, it remains unclear how future seasonal temperature changes, ocean acidification, and novel species interactions will alter the physiology of range-shifting tropical and competing temperate fish in recipient ecosystems. Here we used a laboratory experiment to examine how ocean acidification, future summer vs winter temperatures, and novel species interactions could affect the physiology of competing temperate and range-extending coral reef fish to determine potential range extension outcomes. In future winters (20 °C + elevated pCO₂) coral reef fish at their cold-water leading edges showed reduced physiological performance (lower body condition and cellular defence, and higher oxidative damage) compared to present-day summer (23 °C + control pCO₂) and future summer conditions (26 °C + elevated pCO₂). However, they showed a compensatory effect in future winters through increased long-term energy storage. Contrastingly, co-shoaling temperate fish showed higher oxidative damage, and reduced short-term energy storage and cellular defence in future summer than in future winter conditions at their warm-trailing edges. However, temperate fish benefitted from novel shoaling interactions and showed higher body condition and short-term energy storage when shoaling with coral reef fish compared to same-species shoaling. We conclude that whilst during future summers, ocean warming will likely benefit coral reef fishes extending their ranges, future winter conditions may still reduce coral reef fish physiological functioning, and may therefore slow their establishment at higher latitudes. In contrast, temperate fish species benefit from co-shoaling with smaller-sized tropical fishes, but this benefit may dissipate due to their reduced physiological functioning under future summer temperatures and increasing body sizes of co-shoaling tropical species.

* Corresponding author.

E-mail address: ivan.nagelkerken@adelaide.edu.au (I. Nagelkerken).<http://dx.doi.org/10.1016/j.scitotenv.2023.163684>

Received 23 February 2023; Received in revised form 13 April 2023; Accepted 19 April 2023

Available online 25 April 2023

0048-9697/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

1. Introduction

Biogeographic ranges of many species are shifting under climate change (Parmesan and Yohe, 2003; Chen et al., 2011; Pecl et al., 2017). Latitudinal and altitudinal species redistributions are altering ecosystem functionality (Doney et al., 2012), community assemblages (Soler et al., 2022) and the biodiversity of recipient communities (Lloyd et al., 2011; Soler et al., 2022). Biogeographic shifts have forced invaders and local species to interact for resources (Davis et al., 1998; Alexander et al., 2015). These novel interactions can either limit the invasion success of naïve species through competition (Coni et al., 2021a; Twiname et al., 2022) and predation (Beck et al., 2016) or promote establishment through positive novel species interactions (Smith et al., 2018; Pajmians et al., 2020). Therefore, understanding how local and invading species respond to novel biotic interactions, is important to make informed predictions upon range-shift outcomes within invaded ecosystems.

Global warming can modify physiological processes that underpin novel species interactions outcomes (Pörtner and Farrell, 2008; Alfonso et al., 2020). A small temperature increase can enhance the performance of range-extending species at their leading edge (Figueira and Booth, 2010), whilst the same increase can inhibit the physiological function of local species at their trailing range edge (Enzor and Place, 2014). Where range-shifting and resident species ranges overlap, small temperature increases can modify each species' fitness (Killen et al., 2013) and therefore alter biotic interactions that could determine range-shift outcomes (Figueira et al., 2019; Coni et al., 2021a). Thus, the concurrent effects of temperature and novel biotic interactions must be considered, to accurately predict the true pace of species range-extensions.

Marine species are often observed at the forefront of poleward range-extensions (Burrows et al., 2011). Anthropogenic-derived warming, strengthening poleward currents and species traits are the primary drivers of the rapid poleward redistribution of marine organisms (García Molinos et al., 2022). Marine taxa often move into higher latitudes or deeper waters to escape thermally unsuitable conditions at their trailing edges of distribution, or to expand into previously unsuitable ecosystems (Pecl et al., 2017). Physiologically sensitive ectotherms are commonly seen at the forefront of marine range shifts (Gervais et al., 2021). In Australia, over 150 coral reef fish species have been observed moving their distribution poleward into higher-latitude ecosystems (Booth et al., 2007; Feary et al., 2014; Fowler et al., 2018). Range-extending herbivores can reshuffle trophic pathways in recipient communities (Vergés et al., 2014; Pessarrodona et al., 2022), whilst gregarious coral reef fish species form shoals with local temperate reef species (Smith et al., 2018). These range-shifting species are commonly called 'vagrants' (Booth et al., 2011). Shoaling interactions with resident temperate species can provide fitness benefits to tropical vagrants (e.g. increased growth and reduced winter mortality; Smith et al., 2018; increased shoal size, prey access and reduced predation risk; Pajmians et al., 2020). However, little is known about how these novel interactions affect the fitness of resident species or how climate change (e.g. ocean warming and ocean acidification) might alter the outcomes of these novel shoaling interactions in the near future (Coni et al., 2021a; Mitchell et al., 2022).

Ocean acidification is a concurrent environmental driver resulting from anthropogenic carbon dioxide emissions (Doney et al., 2009). Ocean acidification can alter the physiology of marine fishes (Heuer and Grosell, 2014; Heuer et al., 2019) and hence has the potential to also change the pace of species range shifts (Coni et al., 2021b). Past efforts assessing range shift outcomes often focus on ocean warming effects (Figueira and Booth, 2010; but see Coni et al., 2021b). Indeed, species responses to ocean warming could accelerate the pace of marine range-extensions (Figueira and Booth, 2010; Figueira et al., 2019). However, ocean acidification can modify species responses to ocean warming (Nagelkerken and Munday, 2016), and therefore, alter range-extension outcomes in marine ecosystems (Coni et al., 2021b). Ocean acidification can also modify a wide range of physiological parameters (Heuer and Grosell, 2014), and therefore also the fitness of both tropical vagrant and resident temperate fishes (Mitchell et al., 2022). In addition, the interactive effects of ocean

acidification and ocean warming could indirectly slow the pace of marine range extensions into high-latitude ecosystems through habitat regime shifts (Coni et al., 2021b). Therefore, the interaction between ocean acidification and ocean warming must be considered when assessing range shifts outcomes in marine environments.

Species range edges can shift in response to seasonal changes in local conditions (Figueira et al., 2009; Ljungström et al., 2021). Range-shifting species entering novel ecosystems during summer recruitment often fail to survive through winter (Booth et al., 2007), as seasonal shifts in temperature (Figueira et al., 2009), light (Langbehn et al., 2021), energetics (Ljungström et al., 2021) and resources (Figueira and Booth, 2010) can reduce species' fitness and preventing permanent establishment at cold-leading range edges. Species can also attempt to overwinter in novel ecosystems through behavioral modification, such as the expression of risk-averse behaviour (Coni et al., 2022) and physiological responses, such as lipid build-up (Fernandes and McMeans, 2019). Under increasing ocean warming, winter limitations on range-extension success are predicted to dissipate in the near future (Figueira et al., 2009; Figueira and Booth, 2010), which may allow tropical fish to establish in temperate ecosystems permanently. However, it remains unknown how future winter temperatures could modify tropical fish physiology and therefore, their influence their capacity to establish in temperate ecosystems. In contrast, future summer temperatures could be thermally stressful for resident temperate species at their trailing edges, compromising proper physiological function (Birnie-Gauvin et al., 2017), anti-predator behaviour (Figueira et al., 2019) and reducing their ability to compete against invading tropical species (Birnie-Gauvin et al., 2017). Hence, seasonal temperature-driven shifts in the fitness of interacting vagrants and local temperate species may alter competitive interaction outcomes on a seasonal basis.

Here we used a controlled laboratory experiment to assess the multi-stressor effects of future projected summer versus winter ocean temperatures, ocean acidification, and novel species interactions on the physiology of a range-extending coral reef fish and a co-shoaling temperate fish species across a ~40-day exposure period. Our proxies for short-term (hepatosomatic index) and long-term (total lipid content) energetic storage, energetic intake (stomach fullness), oxidative damage (malondialdehyde concentration), cellular defence (total antioxidant capacity), and body condition (Fulton's condition index) were measured at the end of the experimental exposure. This enabled us to test how climate change stressors and novel interactions might alter the physiological performance of co-shoaling coral reef and temperate fish species to infer how future conditions might modify the pace of range-extension by coral reef fish and contractions by temperate fish.

2. Materials & methods

2.1. Experimental setup

Flow-through 20-litre transparent tanks (IKEA product number: 898.914.70; dimensions: 39 × 28 × 28 cm, height × length × width; $n = 120$) were installed across two adjacent flowthrough temperature/pH control rooms at the Sydney Institute of Marine Science in New South Wales, Australia. Two 0.5-cm (diameter) holes were inserted 4-cm from the top of each tank (lengthways; Fig. S1.) to allow water to flow bilaterally out of each tank. Each tank was allocated a tank identifier and an experimental treatment (Fig. S1). We provided PVC piping (10 × 10 cm, length × external diameter) to each tank to act as shelter (Fig. S2). Tanks were randomly assigned one of the two CO₂ × three temperature treatments ($N = 6$ treatments; Table S1). The ocean warming and ocean acidification treatments reflected projected future summer (26 °C and pH 7.7), and winter conditions (20 °C and pH 7.7) for the respective leading range edge of coral reef fish and the trailing edge of co-shoaling temperate fish (latitude: 34° S, IPCC, 2021) by 2100 (Table 1). Current summer temperatures (23 °C and pH 8.1) were selected as the experimental control and are also reflective of

Table 1

Selected experimental temperatures relative to range location average seasonal temperatures within their Great Barrier Reef core range and the leading-edge extents of coral reef fish and the respective trailing edge of temperate fish (coral reef fish: *Abudefduf vaigiensis*, temperate fish: *Atypichthys strigatus*; Froese and Pauly, 2016; Kaschner et al., 2016).

Range locations	Water Temperatures		
	20 °C	23 °C	26 °C
Coral reef fish core range (20° S)	N/A	Current winter	Future winter
Coral reef fish leading edge/ Temperate fish trailing edge (34° S)	Future winter	Current summer	Future summer

current winter temperatures experienced in coral reef fish core breeding population range (Table 1).

The experimental temperature and pH levels of fish tanks were monitored daily (Table S1) using an Ohaus Starter 300 Portable pH Meter with probe (IC-ST320) - IC-ST300-G. Fish tanks were provided with flow-through seawater supplied by a thermocouple solenoid feedback system with constant monitoring and controlled by computer software. Ocean acidification treatments were reached by bubbling pure CO₂ into 100-litre header tanks (one header tank for every four fish tanks) to reflect future values. Header tank water flowed into fish tanks at ~150 mL.min⁻¹.

2.2. Fish acclimation and husbandry

A. vaigiensis is omnivorous, exhibits a dispersive larval stage, has high site fidelity in the adult stage, and reaches up to 20 cm in length. The resident temperate species *A. strigatus* grows up to 25 cm in length, is also omnivorous and is observed shoaling with *A. vaigiensis* in southeastern Australia (Smith et al., 2018).

Sixty *A. vaigiensis* and 180 *A. strigatus* were collected during 7–24 March 2020, using barrier and handnets at Little Manly Cove (−33.806771° S, 151.285644° E) and Freshwater Beach (−33.781688° S, 151.294088° E) at depths of 0.1–2 m. Following collection, fish were transported in aerated 50-litre buckets filled with fresh seawater (~23 °C and pH 8.1) to 23 °C and pH 8.1 holding tanks (volume: 100 l) at the Sydney Institute of Marine Science.

Range-extending coral reef fish obtain fitness benefits by shoaling with temperate residents, growing faster and surviving longer into winter than conspecific tropical shoals in temperate ecosystems (Smith et al., 2018; Pajmans et al., 2020). Hence, we selected temperate + temperate and tropical + temperate fish pairs, respectively, to understand how novel co-shoaling species in temperate ecosystems (i.e. range-extending tropical) might alter temperate fish physiology in mixed-species shoals (i.e. future conditions) compared to conspecific temperate fish pairs (i.e. present-day conditions), and how the project future temperature and ocean acidification effects modify physiology. Comparison of mixed-species and single-species interactions can inform us about potential impacts on temperate fish communities. As a result, and because of space limitations, we did not include pairs of tropical species.

In addition, shoal size and composition can modify novel shoaling interactions and physiological responses of shoal members (Nadler et al., 2016; Pajmans et al., 2020). However, because of space limitations, we did not include shoal size as a treatment in our study.

Fish were randomly assigned to pairing tanks (coral reef fish, *N* = 60, mixed with temperate fish, *N* = 60; temperate-only paired fish, *N* = 120) across environmental-controlled rooms. Temperate-only selected fishes were paired with individuals of similar body sizes to minimise the competitive advantages that larger fish could develop in obtaining food and shelter. However, mixed-species shoaling fish displayed different body sizes, which reflected the current field situation of coral reef fish being smaller than their co-shoaling temperate species (Smith et al., 2018; Table S2). Initial wet weight (mean ± SE) was: coral reef fish 0.31 ± 0.04 g; mixed-species temperate fish 1.24 ± 0.15 g; temperate-

only fish 4.04 ± 0.20 g. The initial standard length was: coral reef fish 18.6 ± 0.7 mm; mixed-species temperate fish 29.3 ± 1.5 mm; temperate-only fish 53.2 ± 0.8 mm. Fish in the environmental treatments were acclimated from 23 °C and pH 8.1 by decreasing pH at a rate of 0.06 units/day (through gradually increasing the pCO₂ concentration in header tanks) and by raising or decreasing temperatures by 0.5 °C per day to minimise rapid thermal and pH stress responses. Fish introduction and acclimation to experimental tanks were staggered to prevent fish in control treatments (23 °C, pH 8.1) from gaining an acclimation advantage over fish in treatments that required more acclimation steps (e.g. 20 °C and pH 7.7). The maximum acclimation period for fish was 6 days.

API™ Stress Coat solution was added to each tank daily during acclimation and experimental periods. No fish mortality was recorded during the acclimation period. All fish were fed in pairing tanks under a restricted feeding regime of 2.86 g of Ocean Nutrition™ frozen Artemia mixed into 60 ml fresh seawater twice daily. Fish were exposed to experimental conditions for a maximum length of 40 days.

After 35–40 days of treatment exposure, fish were euthanised using the *iki jime* technique (Diggles, 2016). We added 2.86 g of Ocean Nutrition™ frozen Artemia to each tank ~8 h before euthanasia protocol commenced to standardise stomach fullness measures. Whole bodies were flash-frozen in liquid nitrogen and stored at −80 °C. Fish samples were transported on dry ice to The University of Adelaide and stored at −80 °C until further processing.

2.3. Water chemistry

Total alkalinity values were estimated by Gran titration (Metrohm 888 Titrando™) from 60 ml water samples from every tank on exposure days 24 and 25. Mean pCO₂ values were calculated using CO2SYS (Pierrot et al., 2006) for Excel with constants from Mehrbach et al. (1973), refitted by Dickson and Millero (1987) (Table S1).

2.4. Physiological proxies

Each fish's cellular, energetic and condition responses were evaluated by assessing different indicators (Table 2).

2.5. TAC and MDA

Muscle tissue (~50 mg) was used to prepare a 10 % tissue homogenate in an ice bath, which was used to assess total protein content (TP), total antioxidant capacity (TAC) and malondialdehyde concentration (MDA, indicative of oxidative damage). The Colorimetric method (per manufacturer's instructions, Elabsience®, catalogue number: E-BC-K168-S) used to measure the protein concentration in the homogenate, and the absorbance (optical density, OD) was measured with a Jenway 6405 spectrophotometer (Cole-Parmer, Staffordshire). Elabsience® assay kits were used to calculate total protein concentration (catalogue number: E-BC-K168-S, measured at 595 nm), TAC (catalogue number: E-BC-K136-S, measured at 520 nm) and MDA (catalogue number: E-BC-K025-S, measured at 532 nm) following the manufacturer's manuals. TAC kits measured the total concentration of

Table 2

List of physiological proxies analysed following the experimental exposure period.

Physiological Proxy	Description
Total antioxidant capacity (TAC)	Indicator of cellular defence (Birnie-Gauvin et al., 2017)
Malondialdehyde (MDA)	Indicator of oxidative damage (Rodriguez-Dominguez et al., 2019)
Fulton's condition index	Indicator of body condition (Izzo et al., 2015)
Hepatosomatic index (HSI)	Indicator of short-term energy storage (Chellappa et al., 1995)
Stomach fullness	Indicator of relative energy acquisition (Booth, 1990)
Total lipid content	Indicator of long-term energy storage (Post and Parkinson, 2001)

antioxidant macromolecules, antioxidant molecules and enzymes in the white muscle tissue of studied fishes. TP, TAC and MDA were calculated as follows:

$$TP \text{ (g/L)} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{standard}} - OD_{\text{blank}}} \times \text{standard conc. (0.563 g/L)}$$

$$TAC \text{ (u/mg protein)} = \frac{OD_{\text{sample}} - OD_{\text{contrast}}}{0.01} \div 30 \times \frac{\text{volume of homogenate}}{\text{protein conc. (mg protein/ml)}}$$

$$MDA \text{ (nmol/mg protein)} = \frac{OD_{\text{sample}} - OD_{\text{contrast}}}{OD_{\text{standard}} - OD_{\text{blank}}} \times \frac{\text{standard conc. (10 nmol/ml)}}{\text{protein conc. (mg protein/ml)}}$$

2.6. Fulton's condition index

Before experimental commencement, all individual fish had their initial wet weight (WW $\pm$ 0.01 g) and standard length (SL $\pm$ 0.01 mm) measured. Final WW and SL were measured after the fish were euthanised. Fulton's condition index was calculated to assess the body condition of each fish. The initial and final Fulton's condition index was calculated as follows:

$$\text{Fulton's condition index} = \frac{WW \text{ (g)}}{SL \text{ (mm)}^3} \times 100$$

The final Fulton's condition index was subtracted by the initial Fulton's condition index to calculate a treatment's effect (e.g., increase or decrease in body condition under ocean warming) on body condition.

2.7. Hepatosomatic index

Livers were dissected from the frozen fishes, fixed in 100 % ethanol (~24 h), and then weighed to the nearest 0.0001 g. The hepatosomatic index for each fish was calculated as liver weight relative to total fish wet weight and expressed as a percentage (Chellappa et al., 1995).

$$\text{Hepatosomatic index} = \frac{\text{weight of liver (g)}}{\text{wet weight of fish (g)}} \times 100$$

2.8. Stomach fullness

We added 2.86 g of Ocean Nutrition™ frozen Artemia to each tank ~8 h before euthanasia protocol commenced to standardise stomach fullness measures. Fish were then euthanised using the *iki jime* technique (Digges, 2016) and stomachs were dissected from each fish. Stomach contents were dried in a drying oven at 60 °C for 24 h. Dried stomach contents were weighed to the nearest 0.0001 g. Stomach fullness was calculated as follows:

$$\text{Fullness index} = \frac{\text{dry weight of stomach contents (g)}}{\text{wet weight of fish (g)}} \times 100$$

2.9. Total lipid content

Muscle tissue (0.05 g for *A. vaigiensis* and 0.40 g for *A. strigatus*) was used to determine lipid content using Bligh and Dyer lipid extraction (Bligh and Dyer, 1959). Extracted lipids were dried, weighed and expressed as a percentage of muscle tissue weight.

$$\text{Total lipid percent} = \frac{\text{weight of dried lipid (g)}}{\text{weight of muscle tissue (g)}} \times 100$$

2.10. Statistical analysis

We first investigated individual temperate fish physiological proxies using linear regression models. We used R (R Core Team, 2021) and the 'lme4' package (Bates et al., 2015) for linear regression model analysis (LM). To disentangle potential body-size difference effects between mixed-species and single-species shoaling temperate fish, initial wet weight (WW) was included as a variable for temperate fish analyses. Dependent variables were square root transformed to achieve normality.

Linear regression analyses, including covariate initial 'wet weight', did not show significant relationships ($p < 0.05$) between factors (independent) and physiological proxies (dependent); hence, we chose not to include starting WW as an explanatory variable in our statistical analysis.

The effect of pair type (temperate-only vs. mixed-species) and temperature (23 °C vs. 26 °C), ocean acidification (control vs ocean acidification), and their interactions on cellular defence (TAC), oxidative damage (MDA), Fulton's body condition (k), short-term (HSI) and long-term energy storage (total lipid content) were analysed using a permutational ANOVA with 9999 permutation in statistical software package Primer v.7 (Clarke and Gorley, 2015). ANOVAs were performed on Euclidian resemblance matrices. Where significance ($p < 0.05$) of the main effect (explanatory variables) were found pairwise tests were used to compare the respective means (Anderson, 2008).

3. Results

Physiological responses of coral reef fish to temperature and ocean acidification.

Oxidative damage (MDA) and cellular defence (TAC) significantly increased, whilst body condition (Fulton's condition index) significantly decreased at future winter (20 °C) compared to current summer temperatures (26 °C) (Figs. 1A, D, 2D; $p < 0.039$; Tables 3, S3–5). Cellular defence (TAC) and body condition significantly increased, whilst long-term energy storage (total lipid content) significantly decreased at future summer (26 °C) compared to future winter temperatures (20 °C) (Figs. 1A, 2A, D; $p < 0.041$; Tables S4–S6).

Under ocean acidification, oxidative damage (MDA) significantly increased, whilst cellular defence (TAC) significantly decreased for future winter temperatures (20 °C) compared to current (23 °C) and future summer temperatures (26 °C) (Fig. 1A, B; $p < 0.048$; Tables S3–4). At future winter temperatures (20 °C), oxidative damage (MDA) significantly increased, whilst cellular defence (TAC) significantly decreased under ocean acidification compared to control pCO_2 (Fig. 1A, B; $p < 0.001$; Tables 3, S3–4). Long-term energy storage and body condition remain unaffected by ocean acidification (Fig. 2A, D; $p > 0.05$; Tables 3, S5–6). Stomach fullness and short-term energy storage (HSI) remained unaffected by experimental treatments (Figs. 1G; S3A; $p > 0.05$; Tables 3, S7–8).

3.1. Physiological responses of temperate fish to temperature, ocean acidification, and novel shoaling

Oxidative damage (MDA) and long-term energy storage (total lipid content) significantly increase, whilst cellular defence (TAC) and short-term energy storage (HSI) significantly decreased at future summer (26 °C) compared to future winter temperatures (20 °C) (Figs. 1B, C, E, F, H, I, 2A; $p < 0.042$; Tables 4, S9–10, S12, S14). Body condition (Fulton's condition index) significantly increased at future winter (20 °C) than current summer temperature (23 °C) (Fig. 2E, F; $p = 0.011$; Tables 4, S13).

Oxidative damage (MDA) significantly increased for ocean acidification than control pCO_2 , but only at future winter temperatures (20 °C) (Fig. 1E, F; $p < 0.001$; Tables 4, S10).

Short-term energy storage (HSI) and body condition significantly increased in mixed-species pairs than in single-species pairs (Fig. 1H, I; $p < 0.001$; Table S12; Fig. 2E, F; $p < 0.001$; Tables 4, S13). Long-term energy storage significantly increased in mixed-species pairs compared to single-species pairs, but only under control pCO_2 levels (Fig. 2B, C; $p < 0.001$;

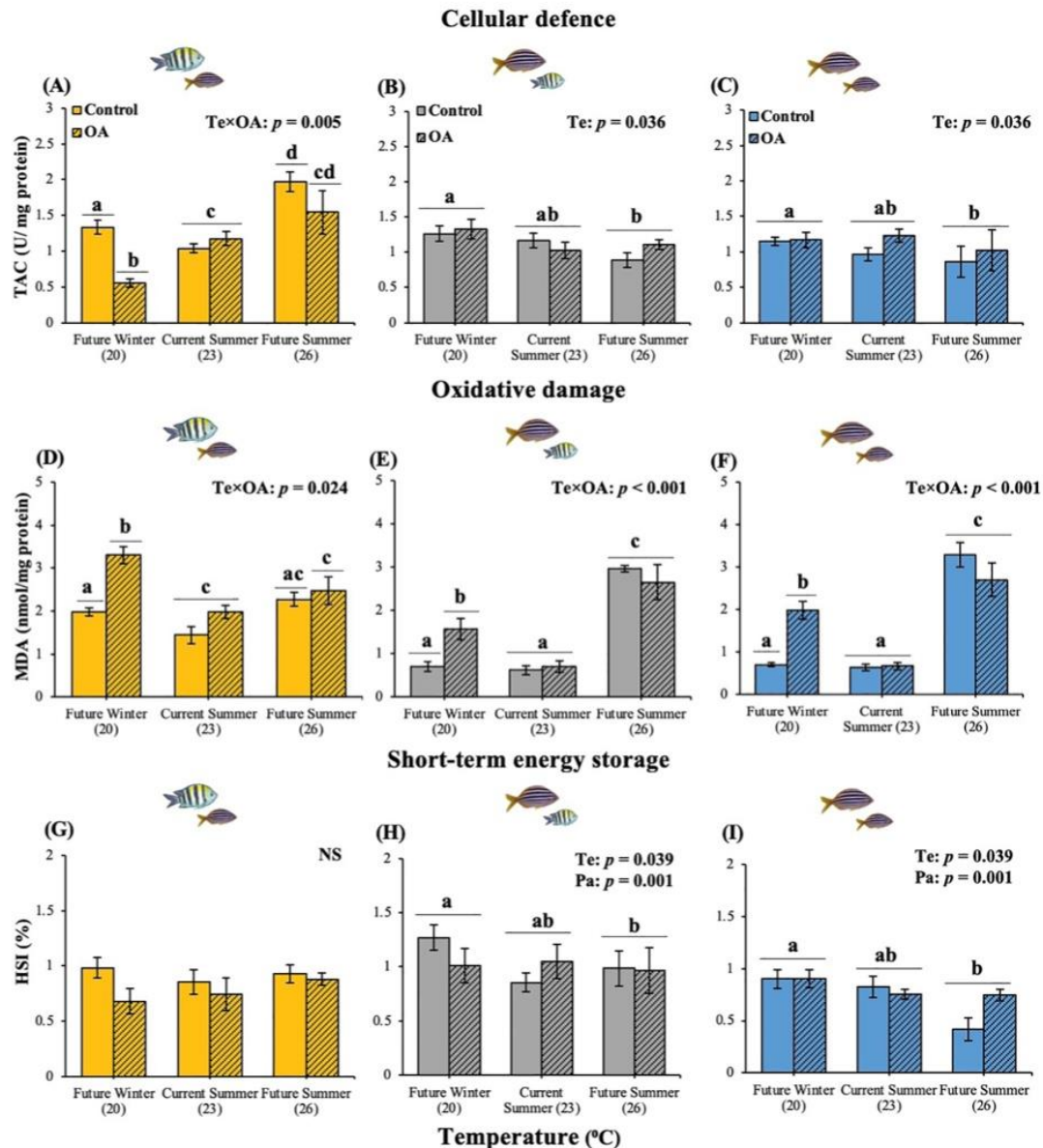


Fig. 1. Mean differences ($\pm$ SE) of muscle tissue Total Antioxidant Capacity (Cellular defence; A, B, C), Malondialdehyde concentration (MDA, oxidative damage; D, E, F), and hepatosomatic index (HSI, short-term energy storage; G, H, I) for coral reef fish (yellow), temperate fish paired with a tropical fish (grey), and temperate fish in temperate-only pairs (blue). Different letters above bars indicate significant differences between experimental treatments ($p < 0.05$; Table S). NS = not significant ($p > 0.05$).

Tables 4, S14). Stomach fullness significantly increased in mixed-species pairs than in single-species pairs, but only at current summer temperatures (23 °C) (Fig. S3B, C; $p = 0.014$; Tables 4, S11).

4. Discussion

Here we show that ocean acidification and future winter temperatures projected for temperate ecosystems could negatively modify the physiological function of range-extending coral reef fishes. Reduced cellular defence and body condition, and increased oxidative damage during future winters compared to current summer temperatures (at which coral reef fish currently establish in higher latitude ecosystems, Figueira and Booth, 2010) may seasonally limit coral reef fish persistence in temperate ecosystems. This effect appears to be exacerbated by ocean acidification. Increased oxidative damage during future winters could degrade cellular integrity and negatively modify cellular processes and physiological traits (e.g., growth and digestion: Costantini, 2019) which underpin successful range-

extensions into temperate ecosystems. Indeed, the overall effect of ocean warming will likely benefit range-extending coral reef fishes (compared to current-day seasonal temperatures; Figueira et al., 2009; Figueira and Booth, 2010). However, whilst previous models suggest future summer temperatures will accelerate coral reef fish establishment at temperate latitudes (Figueira and Booth, 2010), our findings emphasise that future winter conditions (ocean acidification and cold waters at 20 °C) are likely to reduce coral reef fish physiological function (Fig. 3) and will continue to seasonally modify their performance at temperate latitudes. We conclude that whilst ongoing ocean warming will increasingly relax temperature stress during summers, future winter conditions will still limit the physiological performance of coral reef fishes, and therefore slow down their permanent establishment or performance in temperate ecosystems.

Future winter conditions may negatively impact coral reef fish physiological functions, but their overwintering capacity in temperate ecosystems appears to be increased through physiological trade-offs. Increased long-term energy storage, maintenance of stomach fullness and short-term

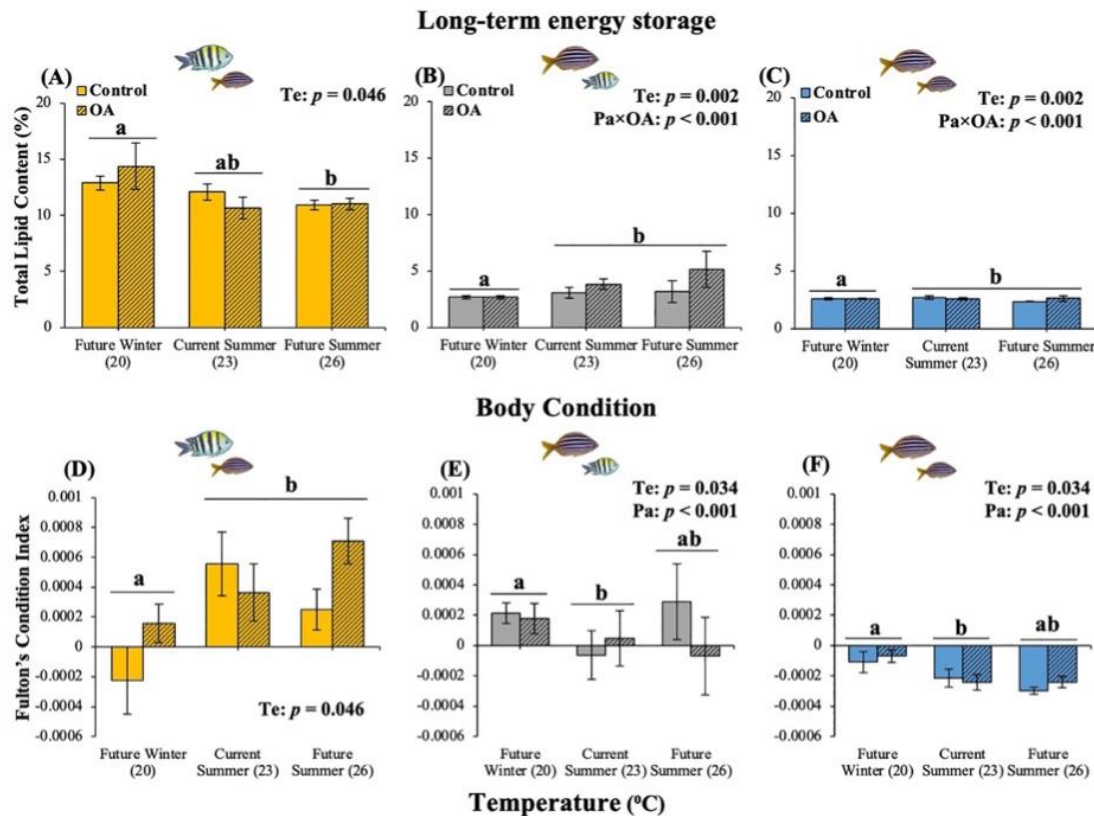


Fig. 2. Mean differences ($\pm$ SE) of muscle tissue total lipid content (long term energy storage; A, B, C), Fulton's condition index (body condition; D, E, F) for coral reef fish (yellow), temperate fish paired with a tropical fish (grey), and temperate fish in temperate-only pairs (blue). Different letters above bars indicate significant differences between experimental treatments ($p < 0.05$; Table S). NS = not significant ($p > 0.05$).

energy reserves, but reductions in body condition, suggests that coral reef fish prioritise long-term energy storage over body condition, under future winter conditions. Seasonal energy partitioning through the accumulation of long-term energy reserves in future winters may also aid future summer growth (Booth and Keast, 1986), resulting in increased summer body condition and overwinter survival (Fernandes and McMeans, 2019) of coral reef fishes in temperate ecosystems. Maintenance of stomach fullness by tropical fishes across temperature treatments, could occur through reduced metabolic rate that coral reef fish experience at cooler temperatures (Djurichkovic et al., 2019), and may therefore slow digestion, causing the stomach to stay full for longer (Booth, 1990). We suggest that under future winter conditions, coral reef fish show physiological plasticity by trading-off body condition in favour of long-term energy storage, which could increase their capacity to overwinter in temperate ecosystems.

Temperate fish physiology was degraded by future summer temperatures (compared to future winter temperatures), irrespective of ocean acidification. Decreases in cellular defence, body condition and short-term energy storage, and increased cellular stress under future summer conditions may reduce temperate fish physiological function and reduce their abundances and performance at their warm-range trailing edge (Fig. 3). Declines in temperate fish physiological function coincides with increased coral reef fish physiological function (this study), fitness (Djurichkovic et al., 2019; Mitchell et al., 2022) and reduced performance (Figueira et al., 2019) under future summer temperatures, which could seasonally improve coral reef fish performance in temperate ecosystems. However, future winter conditions will be advantageous to temperate and detrimental to coral reef species at higher latitudes, leading to a reversal in the physiological performance of competing range-extending and local species as a function future seasonal conditions. We suggest that the opposing physiological performance of the temperate and coral reef species during future

summers vs future winters are likely to seasonally modify the abundance of both species at their overlapping leading (tropical) and trailing (temperate) range edges in temperate ecosystems.

Novel species interactions may temper negative ocean warming effects on temperate fish physiological performance under current ocean warming. Increases in body condition and short-term energy storage of the temperate fish paired with a tropical fish (compared to single-species temperate pairs) could enhance their current performance at their warm-range trailing edges. In mixed-species pairs, body size differences between temperate and coral reef fish (i.e., current temperate summers, with temperate fish generally being larger than coral reef vagrants; Coni et al., 2021a) may explain physiological differences between mixed- and single-species paired temperate fish (Ward et al., 2006). Larger temperate fish often outcompete smaller-bodied coral reef fish in present-day temperate ecosystems (Coni et al., 2021a). Therefore, superior foraging competency by larger-bodied temperate fish may explain why temperate fish in mixed-species pairs show higher body conditions, short-term energy storage and stomach fullness (but only in present-day summer conditions) compared to single-species paired temperate fish. We suggest that mixed-species shoaling could enhance temperate fish physiology by reducing intra-shoal competition and lessen ocean warming impacts on temperate species residing at their warm-range edges.

5. Conclusion

We conclude that whilst ocean warming will likely benefit coral reef fishes extending their ranges into temperate ecosystems, future winter conditions may still reduce coral reef fish physiological function, and may therefore, slow their establishment at higher latitudes. In

Table 3

Summary of analysis using ANOVA testing the effects of seasonal water temperatures ("temperature"), ocean acidification and their interaction ("Te × OA") on physiological responses of coral reef fish (*A. vaigiensis*). Note: **bold** denotes significant differences ($p < 0.05$).

Coral reef fish				
Physiological response	df	SS	F-value	p-value
Cellular defence				
Temperature (Te)	2	6.295	17.764	<0.001
Ocean acidification (OA)	1	1.667	9.410	0.003
Te × OA	2	1.979	5.585	0.005
Residuals	48	5.179		
Oxidative damage				
Temperature (Te)	2	8.467	11.684	<0.001
Ocean acidification (OA)	1	6.348	17.521	<0.001
Te × OA	2	2.797	3.860	0.024
Residuals	48	17.392		
Short-term Energy Storage				
Temperature (Te)	2	0.102	0.452	0.639
Ocean acidification (OA)	1	0.300	2.666	0.114
Te × OA	2	0.141	0.626	0.531
Residuals	46	5.179		
Long-term energy storage				
Temperature (Te)	2	72.009	3.154	0.046
Ocean acidification (OA)	1	2.892	0.253	0.624
Te × OA	2	8.464	0.371	0.695
Residuals	48	547.88		
Body condition				
Temperature (Te)	2	<0.001	4.517	0.015
Ocean acidification (OA)	1	<0.001	1.892	0.177
Te × OA	2	<0.001	1.743	0.185
Residuals	48	<0.001		
Stomach fullness				
Temperature (Te)	2	0.102	4.517	0.015
Ocean acidification (OA)	1	0.300	1.892	0.177
Te × OA	2	0.141	1.743	0.185
Residuals	46	5.179		

comparison, competing temperate fish species gain benefits from co-shoaling with smaller-sized coral reef fish, but this benefit may dissipate due to increased coral reef fish body size and decreased physiological function under future summer temperatures.

CRediT authorship contribution statement

AM, DB, IN conceived the study. AM and CH were responsible for experimental set-up, fish husbandry and physiological analysis. AM performed data analysis. AM wrote the manuscript with help from all authors. All authors read and approved the final manuscript.

Ethics statement

This experiment was conducted according to The University of Adelaide Animal Ethics and University of Technology guidelines and permits: S-2020-13 and 2017-1117, and under New South Wales DPI Scientific Collection Permit: F94/696(A)-9.0.

Data availability

The data that support the findings of this study are publicly available in Figshare at <https://doi.org/10.25909/22144460>.

Declaration of competing interest

The authors declare no competing interest.

Table 4

Summary of analysis using ANOVA testing the effects of seasonal water temperatures ("temperature"), ocean acidification, pair type, and their interactions on physiological responses of coral reef fish (*A. strigatus*). Note: **bold** denotes significant differences ($p < 0.05$).

Temperate fish				
Physiological response	df	SS	F-value	p-value
Cellular defence				
Temperature (Te)	2	0.215	3.475	0.036
Ocean acidification (OA)	1	0.030	0.968	0.322
Pair type (Pa)	1	0.035	1.125	0.293
Te × OA	2	0.015	0.243	0.778
Te × Pa	2	0.018	0.284	0.748
OA × Pa	1	0.049	0.157	0.687
Te × OA × Pa	2	0.071	1.141	0.324
Residual	91	2.816		
Oxidative damage				
Temperature (Te)	2	50.638	78.341	<0.001
Ocean acidification (OA)	1	0.934	2.890	0.095
Pair type (Pa)	1	0.312	0.966	0.326
Te × OA	2	8.576	13.267	<0.001
Te × Pa	2	0.228	0.353	0.704
OA × Pa	1	0.003	0.010	0.917
Te × OA × Pa	2	0.442	0.684	0.502
Residual	91	29.411		
Short-term energy storage				
Temperature (Te)	2	0.764	3.358	0.042
Ocean acidification (OA)	1	0.015	0.134	0.710
Pair type (Pa)	1	1.260	11.075	<0.001
Te × OA	2	0.279	1.226	0.296
Te × Pa	2	0.131	0.577	0.562
OA × Pa	1	0.058	0.510	0.477
Te × OA × Pa	2	0.392	1.725	0.187
Residuals	84	9.558		
Long-term energy storage				
Temperature (Te)	2	10.588	6.947	0.002
Ocean acidification (OA)	1	2.015	2.645	0.112
Pair type (Pa)	1	9.36	12.282	0.002
Te × OA	2	0.663	0.435	0.637
Te × Pa	2	3.353	2.2	0.128
OA × Pa	1	5.584	7.327	0.011
Te × OA × Pa	2	4.852	3.183	0.051
Residuals	91	69.351		
Body condition				
Temperature (Te)	2	<0.001	3.458	0.034
Ocean acidification (OA)	1	<0.001	0.270	0.601
Pair type (Pa)	1	<0.001	18.167	<0.001
Te × OA	2	<0.001	0.537	0.588
Te × Pa	2	<0.001	0.3677	0.698
OA × Pa	1	<0.001	0.736	0.39
Te × OA × Pa	2	<0.001	1.088	0.346
Residuals	91	<0.001	3.458	0.034
Stomach fullness				
Temperature (Te)	2	0.038	0.389	0.683
Ocean acidification (OA)	1	0.149	1.531	0.221
Pair type (Pa)	1	0.009	0.092	0.767
Te × OA	2	0.473	2.434	0.091
Te × Pa	2	0.742	3.820	0.023
OA × Pa	1	0.035	0.355	0.552
Te × OA × Pa	2	0.041	0.212	0.805
Residuals	91	8.841		

Acknowledgements

IN and DJB were supported by an Australian Research Council (ARC) Discovery Project (DP170101722). We thank the Sydney Institute of Marine Science (SIMS) for their help and guidance throughout the experimental period.

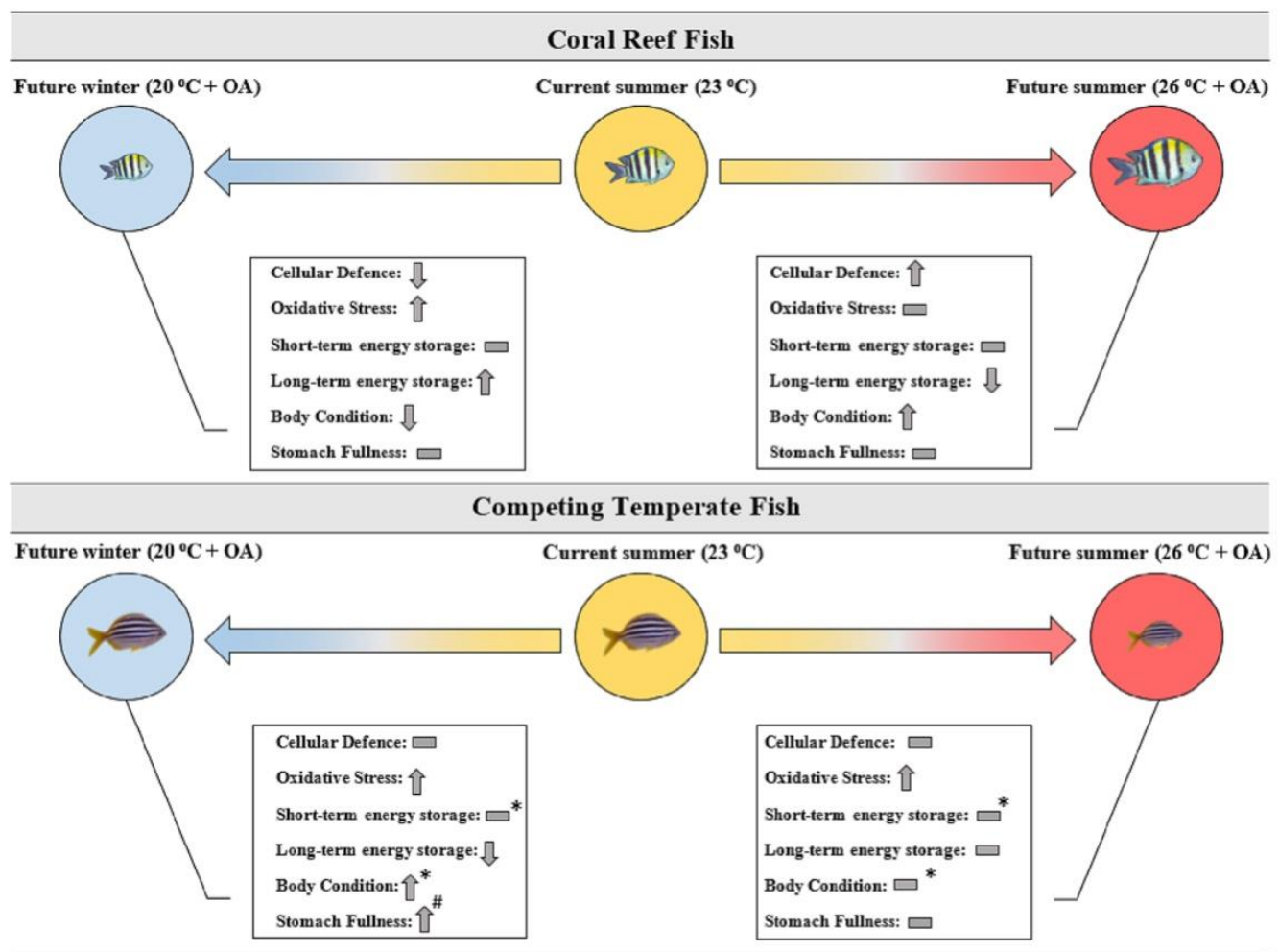


Fig. 3. Schematic diagram showing how projected future winter and summer water temperatures and ocean acidification modify the physiological function of interacting coral reef fish (top panel) and competing temperate fish (bottom panel) compared to current summer conditions (23 °C and pH 8.1) at 34° S, where the distributions of co-shoaling coral reef and temperate fishes overlap. Arrows indicate significant change (comparisons between future summer and future winter vs current summer conditions; $p < 0.05$) in a proxy. ‘—’ indicates proxy remained unaffected ($p > 0.05$). * indicates significantly higher response in mixed-spp. paired temperate fish compared to single-spp. pairs ($p < 0.05$). # denotes significant increase in stomach fullness in temperate fish, but only in single-species pairs ($p < 0.05$).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.163684>.

References

- Alexander, J., Diez, J., Levine, J., 2015. Novel competitors shape species' responses to climate change. *Nature* 525 (7570), 515–518. <https://doi.org/10.1038/nature14952>.
- Alfonso, S., Gestó, M., Sadoul, B., 2020. Temperature increase and its effects on fish stress physiology in the context of global warming. *J. Fish Biol.* 98 (6), 1496–1508. <https://doi.org/10.1111/jfb.14599>.
- Anderson, M.J., 2008. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beck, H., Feary, D., Fowler, A., Madin, E., Booth, D., 2016. Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Mar. Biol.* 163 (4). <https://doi.org/10.1007/s00227-016-2844-8>.
- Birmie-Gauvin, K., Costantini, D., Cooke, S., Willmore, W., 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish Fish.* 18 (5), 928–942. <https://doi.org/10.1111/faf.12215>.
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37 (8), 911–917. <https://doi.org/10.1139/o59-099>.
- Booth, D.J., 1990. Effect of water temperature on stomach evacuation rates and estimates of daily food intake of bluegill sunfish (*Lepomis macrochirus Rafinesque*) can. *J. Zool.* 68, 591–595. <https://doi.org/10.1139/z90-084>.
- Booth, D.J., Keast, J.A., 1986. Growth energy partitioning by juvenile bluegill sunfish. *Lepomis macrochirus rafinesque*. *J. Fish Biol.* 28 (1), 37–45. <https://doi.org/10.1111/j.1095-8649.1986.tb05139.x>.
- Booth, D.J., Figueira, W.F., Gregson, M.A., Beretta, G., 2007. Occurrence of tropical fishes in temperate southeastern Australia: role of the east Australian current. *Estuar. Coast. Shelf Sci.* 72 (1–2), 102–114. <https://doi.org/10.1016/j.ecss.2006.10.003>.
- Booth, D.J., Bond, N., Macreadie, P.I., 2011. Detecting range shifts among Australian fishes in response to climate change. *Mar. Freshw. Res.* 62 (9), 1027–1042.
- Burrows, M., Schoeman, D., Buckley, L., Moore, P., Poloczanska, E., Brandner, K., Brown, C., Bruno, J., Duarte, C., Halpern, B., Holding, J., Kappel, C., Kiessling, W., O'Connor, M., Pandolfi, J., Parmesan, C., Schwing, F., Sydeman, W., Richardson, A., 2011. The pace of shifting climate in marine and terrestrial ecosystems. *Science* 334 (6056), 652–655. <https://doi.org/10.1126/science.1210288>.
- Chellappa, S., Huntingford, F., Strang, R., Thomson, R., 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *J. Fish Biol.* 47 (5), 775–787. <https://doi.org/10.1111/j.1095-8649.1995.tb06002.x>.
- Chen, I.C., Hill, J.K., Ohlemüller, R., Roy, D.B., Thomas, C.D., 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333 (6045), 1024–1026. <https://doi.org/10.1126/science.1206432>.
- Clarke, K.R., Gorley, R.N., 2015. *Getting Started With PRIMER v7. PRIMER-E. Plymouth Marine Laboratory, Plymouth.*
- Coni, E.O.C., Booth, D.J., Nagelkerken, I., 2021a. Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef fish. *Coral Reefs* 40 (5), 1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>.
- Coni, E.O.C., Nagelkerken, I., Ferreira, C., Connell, S.D., Booth, D.J., 2021b. Ocean acidification may slow the pace of tropicalisation of temperate fish communities. *Nat. Clim. Chang.* 11 (3), 249–256. <https://doi.org/10.1038/s41558-020-00980-w>.

- Coni, E.O.C., Booth, D.J., Nagelkerken, I., 2022. Coral-reef fishes can become more risk-averse at their poleward range limits. *Proc. R. Soc. B Biol. Sci.* 289, 20212676. <https://doi.org/10.1098/rspb.2021.2676>.
- Costantini, D., 2019. Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. *J. Exp. Biol.* 222 (13), jeb194688. <https://doi.org/10.1242/jeb.194688>.
- Davis, A., Jenkinson, L., Lawton, J., Shorrocks, B., Wood, S., 1998. Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391 (6669), 783786. <https://doi.org/10.1038/35842>.
- Dickson, A., Millero, F., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res. Part A* 34 (10). [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5).
- Diggles, B.K., 2016. Development of resources to promote best practice in the humane dispatch of finfish caught by recreational fishers. *Fish. Manag. Ecol.* 23 (3–4), 200–207. <https://doi.org/10.1111/fme.12127>.
- Djurichkovic, L., Donelson, J., Fowler, A., Feary, D., Booth, D.J., 2019. The effects of water temperature on the juvenile performance of two tropical damselfishes expatriating to temperate reefs. *Sci. Rep.* 9 (1). <https://doi.org/10.1038/s41598-019-50303-z>.
- Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO₂ problem. *Climate change impacts on marine ecosystems*. *Annu. Rev. Mar. Sci.* 1, 169–192. <https://doi.org/10.1146/annurev-marine.010908.163834>.
- Doney, S., Ruckelshaus, M., Emmett Duffy, J., Barry, J., Chan, F., English, C., Galindo, H., Grebmeier, J., Hollowed, A., Knowlton, N., Polovina, J., Rabalais, N., Sydeman, W., Talley, L., 2012. Climate change impacts on marine ecosystems. *Annu. Rev. Mar. Sci.* 4 (1), 11–37. <https://doi.org/10.1146/annurev-marine-041911-111611>.
- Enzor, L.A., Place, S.P., 2014. Is warmer better? Decreased oxidative damage in notothenioid fish after long-term acclimation to multiple stressors. *J. Exp. Biol.* 217 (18), 3301–3310. <https://doi.org/10.1242/jeb.108431>.
- Feary, D.A., Pratchett, M.S., Emslie, M.J., Fowler, A., Figueira, W.F., Luiz, O.J., Nakamura, Y., Booth, D.J., 2014. Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish. Fish.* 15 (4). <https://doi.org/10.1111/faf.12036>.
- Fernandes, T., McMeans, B., 2019. Coping with the cold: energy storage strategies for surviving winter in freshwater fish. *Ecography* 42 (12), 2037–2052. <https://doi.org/10.1111/ecog.04386>.
- Figueira, W.F., Booth, D.J., 2010. Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Glob. Chang. Biol.* 16 (2). <https://doi.org/10.1111/j.1365-2486.2009.01934.x>.
- Figueira, W.F., Biro, P., Booth, D.J., Valenzuela Davie, V.C., 2009. Performance of tropical fish recruiting to temperate habitats: role of ambient temperature and implications of climate change. *Mar. Ecol. Prog. Ser.* 384. <https://doi.org/10.3354/meps08057>.
- Figueira, W.F., Curley, B., Booth, D.J., 2019. Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes? *Mar. Biol.* 166 (73). <https://doi.org/10.1007/s00227-019-3521-5>.
- Fowler, A., Parkinson, K., Booth, D.J., 2018. New poleward observations of 30 tropical reef fishes in temperate southeastern Australia. *Mar. Biodivers.* 48 (4), 2249–2254. <https://doi.org/10.1007/s12526-017-0748-6>.
- Froese, R., Pauly, D., 2016. FishBase. <http://www.fishbase.org>.
- García Molinos, J., Hunt, H.L., Green, M.E., Champion, C., Hartog, J.R., Pecl, G.T., 2022. Climate, currents and species traits contribute to early stages of marine species redistribution. *Commun. Biol.* 5 (1), 1329. <https://doi.org/10.1038/s42003-022-04273-0>.
- Gervais, C., Champion, C., Pecl, G., 2021. Species on the move around the Australian coastline: a continental-scale review of climate-driven species redistribution in marine systems. *Glob. Chang. Biol.* 27 (14), 3200–3217. <https://doi.org/10.1111/gcb.15634>.
- Heuer, R., Grosell, M., 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Phys. Regul. Integr. Comp. Phys.* 307 (9), R1061–R1084. <https://doi.org/10.1152/ajpregu.00064.2014>.
- Heuer, R., Hamilton, T., Nilsson, G., 2019. The physiology of behavioral impacts of high CO₂. *Fish. Physiol.*, 161–194. <https://doi.org/10.1016/bs.fp.2019.08.002>.
- IPCC, 2021. Climate change 2021: the physical science basis. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.L., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press <https://doi.org/10.1017/9781009157896> In Press.
- Izzo, C., Doubleday, Z., Schultz, A., Woodcock, S., Gillanders, B., 2015. Contribution of water chemistry and fish condition to otolith chemistry: comparisons across salinity environments. *J. Fish Biol.* 86 (6), 1680–1698. <https://doi.org/10.1111/jfb.12672>.
- Kaschner, K., Kesner-Reyes, K., Garilao, C., Rius-Barile, J., Rees, T., Froese, R., 2016. AquaMaps: predicted range maps for aquatic species. World Wide Web electronic publication Version 08/2016 www.aquamaps.org.
- Killen, S., Marras, S., Metcalfe, N., McKenzie, D., Domenici, P., 2013. Environmental stressors alter relationships between physiology and behaviour. *Trends Ecol. Evol.* 28 (11), 651–658. <https://doi.org/10.1016/j.tree.2013.05.005>.
- Langbehn, T., Aksnes, D., Kaartvedt, S., Fiksen, Ø., Ljungström, G., Jørgensen, C., Bates, A., 2021. Poleward distribution of mesopelagic fishes is constrained by seasonality in light. *Glob. Ecol. Biogeogr.* 31 (3), 546–561. <https://doi.org/10.1111/geb.13446>.
- Ljungström, G., Langbehn, T., Jørgensen, C., 2021. Light and energetics at seasonal extremes limit poleward range shifts. *Nat. Clim. Chang.* 11 (6), 530–536. <https://doi.org/10.1038/s41558-021-01045-2>.
- Lloyd, P., Plaganyi, É., Weeks, S., Magno-Canto, M., Plaganyi, G., 2011. Ocean warming alters species abundance patterns and increases species diversity in an African sub-tropical reef-fish community. *Fish. Oceanogr.* 21 (1). <https://doi.org/10.1111/j.1365-2419.2011.00610.x>.
- Mehrbach, C., Culbertson, C., Hawley, J., Pytkowicz, R., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18 (6). <https://doi.org/10.4319/lo.1973.18.6.0897>.
- Mitchell, A., Booth, D.J., Nagelkerken, I., 2022. Ocean warming and acidification degrade shoaling performance and lateralisation of novel tropical-temperate fish shoals. *Glob. Chang. Biol.* 28 (4), 1388–1401. <https://doi.org/10.1111/gcb.16022>.
- Nadler, L.E., Killen, S.S., McClure, E.C., Munday, P.L., McCormick, M.I., 2016. Shoaling reduces metabolic rate in a gregarious coral reef fish species. *J. Exp. Biol.* 219 (18), 2802–2805. <https://doi.org/10.1242/jeb.139493>.
- Nagelkerken, I., Munday, P.L., 2016. Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Glob. Chang. Biol.* 22 (3), 974–984. <https://doi.org/10.1111/gcb.13167>.
- Pajmians, K., Booth, D., Wong, M., 2020. Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *J. Fish Biol.* 96 (3), 806–814. <https://doi.org/10.1111/jfb.12477>.
- Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421 (6918). <https://doi.org/10.1038/nature01286>.
- Pecl, G., Araújo, M., Bell, J., Blanchard, J., Bonebrake, T., Chen, I., Clark, T., Colwell, R., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R., Griffiths, R., Hobday, A., Janion-Scheepers, C., Jarzyna, M., Jennings, S., Lenoir, J., Linnetved, H., Martin, V., McCormack, P., McDonald, J., Mitchell, N., Mustonen, T., Pandolfi, J., Pettolelli, N., Popova, E., Robinson, S., Scheffers, B., Shaw, J., Sorte, C., Strugnell, J., Sunday, J., Tuanmu, M., Vergés, A., Villanueva, C., Wernberg, T., Wapstra, E., Williams, S., 2017. Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. *Science* 355 (6332). <https://doi.org/10.1126/science.1249214>.
- Pessarrodona, A., Vergés, A., Bosch, N., Bell, S., Smith, S., Sgarlatta, M., Wernberg, T., 2022. Tropicalisation unlocks novel trophic pathways and enhances secondary productivity in temperate reefs. *Funct. Ecol.* <https://doi.org/10.1111/1365-2435.13990>.
- Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel Program Developed for CO₂ System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center. Oak Ridge National Laboratory; US Department of Energy. Oak Ridge, Tennessee.
- Pörtner, H., Farrell, A., 2008. Physiology and climate change. *Science* 322 (5902), 690–692. <https://doi.org/10.1126/science.1163156>.
- Post, J., Parkinson, E., 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82, 1040–1051. <https://doi.org/10.2307/2679901>.
- R Core Team, 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rodriguez-Dominguez, A., Connell, S., Leung, J., Nagelkerken, I., 2019. Adaptive responses of fishes to climate change: feedback between physiology and behaviour. *Sci. Total Environ.* 692, 1242–1249. <https://doi.org/10.1016/j.scitotenv.2019.07.226>.
- Smith, S., Fox, R., Booth, D.J., Donelson, J., 2018. Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion front-line. *Glob. Chang. Biol.* 24 (4). <https://doi.org/10.1111/gcb.14016>.
- Soler, G.A., Edgar, G.J., Barrett, N.S., Stuart-Smith, R.D., Oh, E., Cooper, A., Ridgway, K.R., Ling, S.D., 2022. Warming signals in temperate reef communities following more than a decade of ecological stability. *Proc. R. Soc. B* 289 (1989), 20221649. <https://doi.org/10.1098/rspb.2022.1649>.
- Twinn, S., Fitzgibbon, Q., Hobday, A., Carter, C., Oellermann, M., Pecl, G., 2022. Resident lobsters dominate food competition with range-shifting lobsters in an ocean warming hotspot. *Mar. Ecol. Prog. Ser.* 685, 171–181. <https://doi.org/10.3354/meps13984>.
- Vergés, A., Steinberg, P.D., Hay, M.E., Poore, A.G.B., Campbell, A.H., Ballesteros, E., Heck, K.L., Booth, D.J., Coleman, M.A., Feary, D.A., Figueira, W., Langlois, T., Marzinelli, E.M., Mizerek, T., Mumby, P.J., Nakamura, Y., Roughan, M., van Sebille, E., Sen Gupta, A., Smale, D.A., Tomas, F., Wernberg, T., Wilson, S.K., 2014. The tropicalisation of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proc. R. Soc. B Biol. Sci.* 281 (1789). <https://doi.org/10.1098/rspb.2014.0846>.
- Ward, A., Webster, M., Hart, P., 2006. Intraspecific food competition in fishes. *Fish. Fish.* 7 (4), 231–261. <https://doi.org/10.1111/j.1467-2979.2006.00224.x>.

Supplementary Information

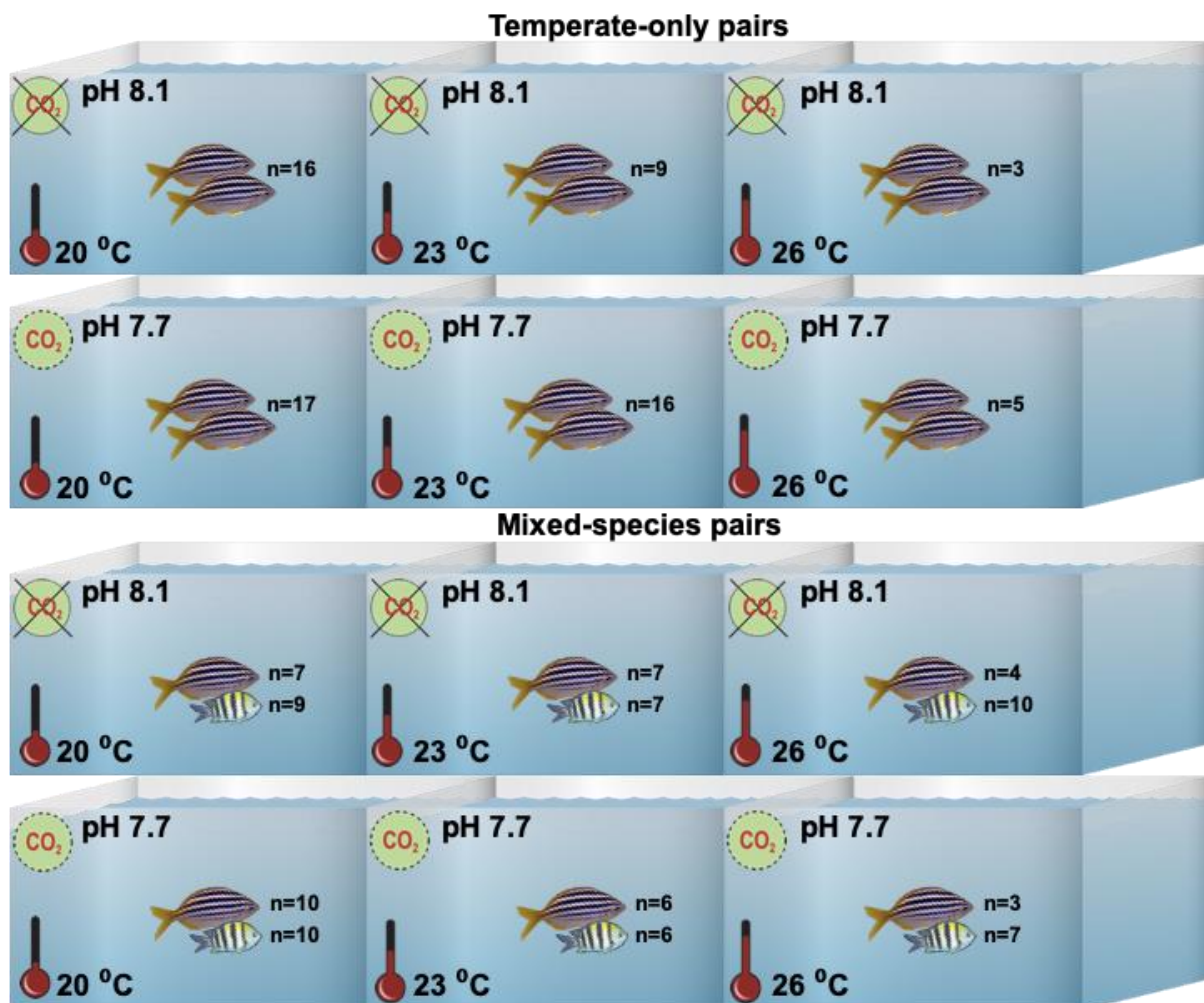


Figure S1: visualisation of $p\text{CO}_2$, temperature, and pairing treatment combinations for experimental. N (number of samples) = number of fish collected on the final day of experimental exposure for physiological analysis.

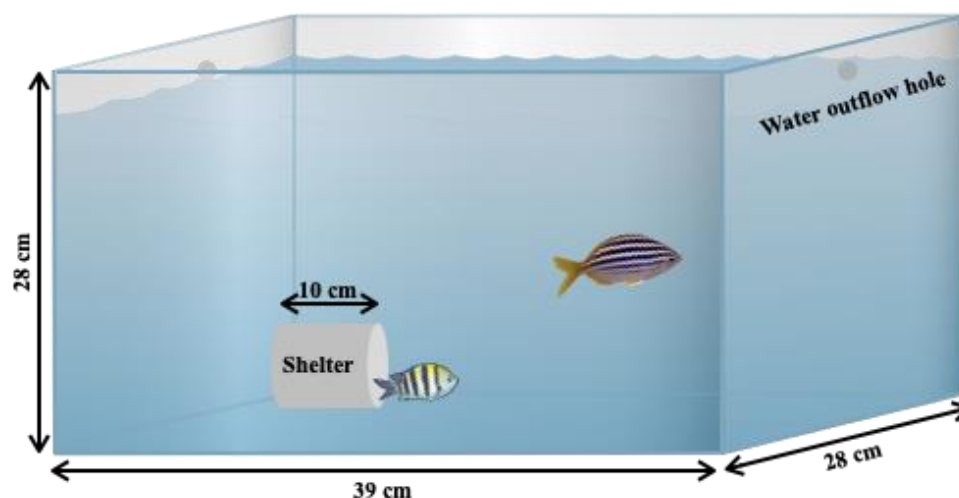


Figure S2: Schematic diagram of pairing tanks used during the experimental period. Figure not to scale, only for visualisation purposes.

Stomach fullness

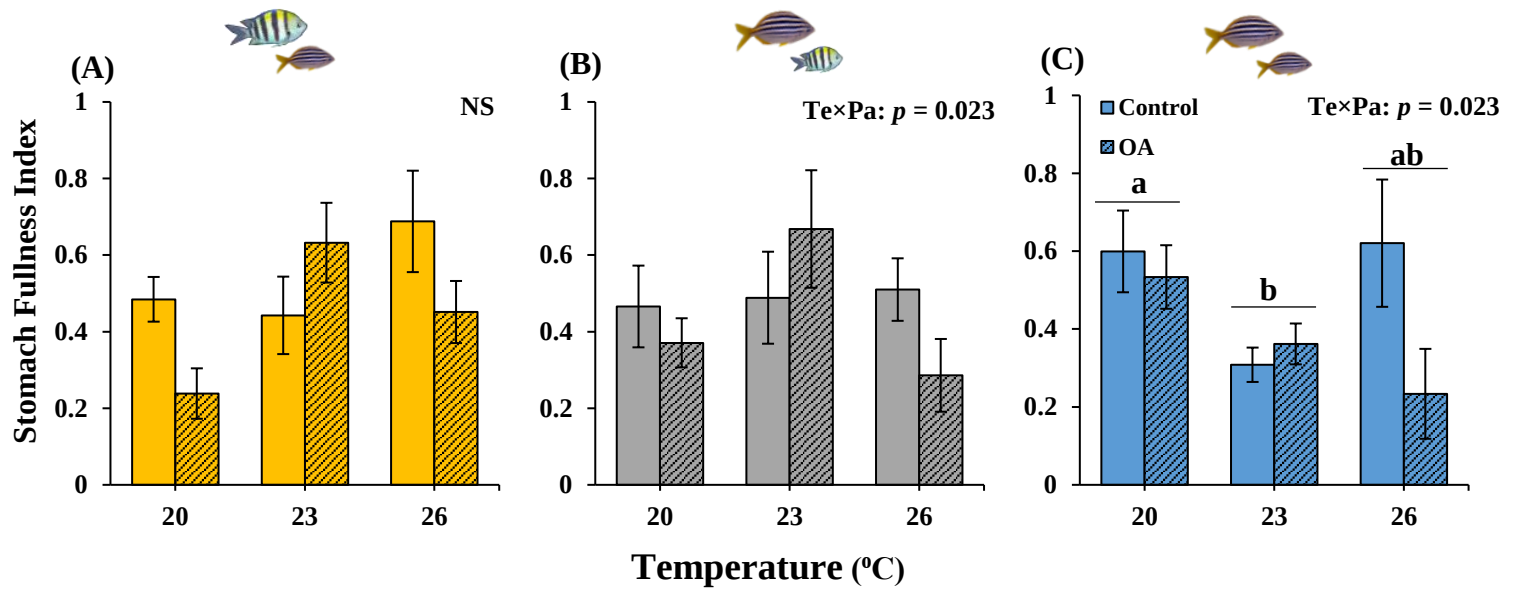


Figure S3: Mean differences ($\pm$ SE) of stomach fullness (A, B, C) for coral reef fish (yellow), temperate fish paired with a tropical fish (grey), and temperate fish in temperate-only pairs (blue). The different letter above bars indicates significant differences among treatments; NS = not significant. * denotes a significant ocean acidification effect for a specific temperature treatment (e.g. future winter temperature). Error bars represent standard error.

Table S1: Mean ($\pm$ SE) seawater parameters and treatment levels across the experiment.

Mixed-species tanks					
OA Treatment	Temperature ($^{\circ}$ C)	pH	Salinity (ppt)	$p\text{CO}_2$ (μatm)	Total alkalinity ($\mu\text{mol.kg}^{-1}\text{SW}$)
Control	20.2 ($\pm$ 0.06)	8.1 ($\pm$ 0.002)	35.0 ($\pm$ 0.007)	304 ($\pm$ 8.4)	2104 ($\pm$ 48.6)
OA	20.2 ($\pm$ 0.06)	7.7 ($\pm$ 0.003)	35.0 ($\pm$ 0.008)	914 ($\pm$ 11.3)	2135 ($\pm$ 14.0)
Control	23.1 ($\pm$ 0.04)	8.1 ($\pm$ 0.001)	35.0 ($\pm$ 0.008)	308 ($\pm$ 3.7)	2155 ($\pm$ 15.3)
OA	23.0 ($\pm$ 0.03)	7.7 ($\pm$ 0.002)	35.0 ($\pm$ 0.006)	977 ($\pm$ 16.6)	2214 ($\pm$ 35.5)
Control	25.8 ($\pm$ 0.06)	8.1 ($\pm$ 0.001)	35.0 ($\pm$ 0.008)	303 ($\pm$ 2.4)	2165 ($\pm$ 13.0)
OA	25.7 ($\pm$ 0.03)	7.7 ($\pm$ 0.001)	35.0 ($\pm$ 0.007)	960 ($\pm$ 4.4)	2186 ($\pm$ 1.6)
Conspecific temperate tanks					
Control	20.1 ($\pm$ 0.05)	8.1 ($\pm$ 0.002)	35.0 ($\pm$ 0.008)	311 ($\pm$ 3.5)	2154 ($\pm$ 14.2)
OA	20.3 ($\pm$ 0.06)	7.7 ($\pm$ 0.003)	35.0 ($\pm$ 0.006)	940 ($\pm$ 12.1)	2158 ($\pm$ 12.8)
Control	23.1 ($\pm$ 0.04)	8.1 ($\pm$ 0.001)	35.0 ($\pm$ 0.007)	309 ($\pm$ 4.0)	2160 ($\pm$ 22.9)
OA	23.0 ($\pm$ 0.03)	7.7 ($\pm$ 0.001)	35.0 ($\pm$ 0.009)	957 ($\pm$ 9.5)	2181 ($\pm$ 20.8)
Control	25.8 ($\pm$ 0.06)	8.1 ($\pm$ 0.001)	35.0 ($\pm$ 0.01)	306 ($\pm$ 1.4)	2175 ($\pm$ 10.9)
OA	25.9 ($\pm$ 0.04)	7.7 ($\pm$ 0.001)	35.0 ($\pm$ 0.006)	967 ($\pm$ 5.42)	2187 ($\pm$ 3.43)

Table S2: Starting wet weight (WW) and standard length (SL) of tropical and temperate fish.

Species	Starting WW (g)	Starting SL (mm)
Tropical vagrant	0.31 (SE $\pm$ 0.04)	18.61 (SE $\pm$ 0.68)
Mixed-spp. temperate fish	1.24 (SE $\pm$ 0.15)	29.34 (SE $\pm$ 1.52)
Non-mixed temperate fish	4.04 (SE $\pm$ 0.2)	53.28 (SE $\pm$ 0.8)

Table S3: MANOVA output of physiological repertoire of coral reef fish. Note: **bold** p -values denote significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	811.63	405.81	4.807	0.003
Ocean acidification (OA)	1	285.03	285.03	3.377	0.045
Te $\times$ OA	2	312.68	156.34	1.852	0.131
Residual	48	4051.9	84.414		
Total	53	5551.7			
Pairwise test of term 'Temperature'					
Groups	t				P-value
20, 23	1.474				0.131
20, 26	2.792				0.002
23, 26	2.318				0.010

Table S4: coral reef fish total antioxidant capacity. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	6.295	3.148	17.764	<0.001
Ocean acidification (OA)	1	1.667	1.667	9.410	0.003
Te×OA	2	1.979	0.990	5.585	0.005
Residual	48	5.179	0.177		
Total	53	19.162			
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘Control’ of factor Ocean acidification.					
Groups		t		P-value	
20, 23		2.510		0.025	
20, 26		3.527		0.004	
23, 26		5.913		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘OA’ of factor Ocean acidification.					
Groups		t		P-value	
20, 23		5.098		<0.001	
20, 26		3.368		<0.001	
23, 26		1.201		0.269	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘20’ of factor Temperature.					
Groups		t		P-value	
Control, OA		6.453		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘23’ of factor Temperature					
Groups		t		P-value	
Control, OA		1.166		0.251	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘26’ of factor Temperature.					
Groups		t		P-value	
Control, OA		1.326		0.209	

Table S5: coral reef fish Malondialdehyde concentration. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	8.467	4.234	11.684	<0.001
Ocean acidification (OA)	1	6.348	6.348	17.521	<0.001
Te×OA	2	2.797	1.399	3.860	0.024
Residual	48	17.392	0.362		
Total	53	35.455			
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘Control’ of factor Ocean acidification.					
Groups	t			P-value	
20, 23	2.252			0.039	
20, 26	1.367			0.181	
23, 26	3.031			0.006	
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘OA’ of factor Ocean acidification.					
Groups	t			P-value	
20, 23	5.050			<0.001	
20, 26	2.142			0.048	
23, 26	1.387			0.189	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘20’ of factor Temperature.					
Groups	t			P-value	
Control, OA	5.767			<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘23’ of factor Temperature.					
Groups	t			P-value	
Control, OA	2.025			0.059	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘26’ of factor Temperature.					
Groups	t			P-value	
Control, OA	0.594			0.557	

Table S6: coral reef fish Fulton's condition index ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	<0.001	<0.001	4.517	0.015
Ocean acidification (OA)	1	<0.001	<0.001	1.892	0.177
Te×OA	2	<0.001	<0.001	1.743	0.185
Residuals	48	<0.001	<0.001		
Total	53	<0.001			
Pairwise of term 'Temperature'					
Groups	t		P-value		
20, 23	2.386		0.025		
20, 26	2.873		0.007		
23, 26	0.105		0.919		

Table S7: coral reef fish total lipid content ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	72.009	36.004	3.154	0.046
Ocean acidification (OA)	1	2.892	2.892	0.253	0.624
Te×OA	2	8.464	4.232	0.371	0.695
Residuals	48	547.88	11.414		
Total	53	632.49			
Pairwise of term 'Temperature'					
Groups	t		P-value		
20, 23	1.7186		0.089		
20, 26	2.145		0.041		
23, 26	0.52802		0.605		

Table S8: coral reef fish stomach fullness ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity.

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	0.444	0.222	2.316	0.108
Ocean acidification (OA)	1	0.126	0.127	1.323	0.158
Te×OA	2	0.569	0.284	2.965	0.056
Residuals	48	4.606	0.096		
Total	53	5.782			

Table S9: coral reef fish hepatosomatic index ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity.

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	0.102	0.051	0.452	0.639
Ocean acidification (OA)	1	0.300	0.300	2.666	0.114
Te×OA	2	0.141	0.070	0.626	0.531
Residuals	46	5.179	0.113		
Total	51	5.709			

Table S10: MANOVA output of physiological repertoire of temperate fish. df = degrees of freedom, SS = sum of squares, MS = mean square. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	7670.4	3835.2	26.369	<0.001
Ocean acidification (OA)	1	62.142	62.142	0.427	0.777
Pair type (Pa)	1	488.27	488.27	3.357	0.018
Te×OA	2	1973.1	986.54	6.783	<0.001
Te×Pa	2	382.2	191.1	1.314	0.230
OA×Pa	1	301.42	301.42	2.073	0.090
Te×OA×Pa	2	279.3	139.65	0.960	0.443
Residual	91	13235	145.44		
Total	102	25108			
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘Control’ of factor Ocean Acidification.					
Groups		t		P-value	
20, 23		1.633		0.031	
20, 26		6.438		<0.001	
23, 26		5.945		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘OA’ of factor Ocean Acidification					
Groups		t		P-value	
20, 23		4.04		<0.001	
20, 26		1.746		0.035	
23, 26		3.825		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘20’ of factor Temperature.					
Groups		t		P-value	
Control, OA		3.771		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘23’ of factor Temperature.					
Groups		t		P-value	
Control, OA		0.56		0.857	
Pairwise test of term ‘Te×OA’ for pairs of factor Ocean acidification levels within level ‘26’ of factor Temperature.					
Groups		t		P-value	
Control, OA		1.348		0.174	

Table S11: total antioxidant capacity for temperate fish. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	0.215	0.108	3.475	0.036
Ocean acidification (OA)	1	0.030	0.030	0.968	0.322
Pair type (Pa)	1	0.035	0.035	1.125	0.293
Te×OA	2	0.015	0.008	0.243	0.778
Te×Pa	2	0.018	0.009	0.284	0.748
OA×Pa	1	0.049	0.005	0.157	0.687
Te×OA×Pa	2	0.071	0.035	1.141	0.324
Residual	91	2.816	0.031		
Total	102	3.199			
Pairwise test of term 'temperature'					
Groups	t		P-value		
20, 26	2.409		0.022		
20, 23	1.623		0.111		
26, 23	1.264		0.213		

Table S12: temperate Malondialdehyde concentration. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	50.638	25.319	78.341	<0.001
Ocean acidification (OA)	1	0.934	0.934	2.890	0.095
Pair type (Pa)	1	0.312	0.312	0.966	0.326
Te×OA	2	8.576	4.288	13.267	<0.001
Te×Pa	2	0.228	0.114	0.353	0.704
OA×Pa	1	0.003	0.003	0.010	0.917
Te×OA×Pa	2	0.442	0.221	0.684	0.502
Residual	91	29.411	0.323		
Total	102	100			
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘Control’ of factor Ocean Acidification.					
Groups		t		P-value	
20, 26		18.994		<0.001	
20, 23		0.859		0.392	
26, 23		17.818		<0.001	
Pairwise test of term ‘Te×OA’ for pairs of factor Temperature levels within level ‘OA’ of factor Ocean acidification.					
Groups		t		P-value	
20, 26		2.441		0.020	
20, 23		5.056		<0.001	
26, 23		8.289		<0.001	
Pairwise test of term 'Te×OA' for pairs of factor Ocean acidification levels within level ‘20’ of factor Temperature					
Groups		t		P-value	

Control, OA	5.523	<0.001
Pairwise test of term 'Te×OA' for pairs of factor Ocean acidification levels within level '23' of factor Temperature.		
Groups	t	P-value
Control, OA	0.491	0.627
Pairwise test of term 'Te×OA' for pairs of factor Ocean acidification levels within level '26' of factor Temperature.		
Groups	t	P-value
Control, OA	1.129	0.291

Table S13: temperate stomach fullness ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** indicates significant p-value ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	0.038	0.038	0.389	0.683
Ocean acidification (OA)	1	0.149	0.149	1.531	0.221
Pair type (Pa)	1	0.009	0.009	0.092	0.767
Te×OA	2	0.473	0.237	2.434	0.091
Te×Pa	2	0.742	0.371	3.820	0.023
OA×Pa	1	0.035	0.035	0.355	0.552
Te×OA×Pa	2	0.041	0.021	0.212	0.805
Residuals	91	8.841	0.097		
Total	102	10.380			
Pairwise test of term ‘Te×Pa’ for pairs of factor Temperature levels within level ‘Temperate-only’ of factor Pair type.					
Groups		t		P-value	
20, 26		0.913		0.371	
20, 23		2.658		0.013	
26, 23		0.984		0.336	
Pairwise test of term ‘Te×Pa’ for pairs of factor Temperature levels within level ‘Mixed-species’ of factor Pair type.					
Groups		t		P-value	
20, 26		0.180		0.860	
20, 23		1.386		0.176	
26, 23		1.157		0.262	
Pairwise test of term ‘Te×Pa’ for pairs of factor Pair type levels within level ‘20’ of factor Temperature.					
Groups		t		P-value	
Temperate-only, Mixed-species		1.428		0.161	
Pairwise test of term ‘Te×Pa’ for pairs of factor Pair type levels within level ‘23’ of factor Temperature.					
Groups		t		P-value	
Temperate-only, Mixed-species		2.626		0.014	
Pairwise test of term ‘Te×Pa’ for pairs of factor Pair type levels within level ‘26’ of factor Temperature.					
Groups		t		P-value	

Temperate-only, Mixed-species	0.210	0.826
-------------------------------	-------	-------

Table S14: temperate hepatosomatic index ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** indicates significant p -value ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	0.764	0.382	3.358	0.042
Ocean acidification (OA)	1	0.015	0.015	0.134	0.710
Pair type (Pa)	1	1.260	1.260	11.075	<0.001
Te×OA	2	0.279	0.139	1.226	0.296
Te×Pa	2	0.131	0.066	0.577	0.562
OA×Pa	1	0.058	0.058	0.510	0.477
Te×OA×Pa	2	0.392	0.196	1.725	0.187
Residuals	84	9.558	0.114		
Total	95	12.02			
Pairwise test of term 'Temperature'					
Groups	t		P-value		
20, 26	2.118		0.042		
20, 23	1.861		0.071		
26, 23	0.994		0.324		

Table S15: temperate fish Fulton's condition index ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	<0.001	<0.001	3.458	0.034
Ocean acidification (OA)	1	<0.001	<0.001	0.270	0.601
Pair type (Pa)	1	<0.001	<0.001	18.167	<0.001
Te×OA	2	<0.001	<0.001	0.537	0.588
Te×Pa	2	<0.001	<0.001	0.3677	0.698
OA×Pa	1	<0.001	<0.001	0.736	0.39
Te×OA×Pa	2	<0.001	<0.001	1.088	0.346
Residuals	91	<0.001	<0.001		
Total	102	<0.001			
Pairwise of term 'Temperature'					
Groups	t		P-value		
20, 23	2.656		0.011		
20, 26	1.549		0.125		
23, 26	0.364		0.717		

Table S16: temperate fish total lipid content ANOVA output. Permutation method: Permutation of residuals under a reduced model. The used Resemblance matrix used Euclidean Distance dissimilarity. Note: **bold** denotes significant differences ($p < 0.05$).

Source	df	SS	MS	F-value	P-value
Temperature (Te)	2	10.588	5.294	6.947	0.002
Ocean acidification (OA)	1	2.015	2.015	2.645	0.112
Pair type (Pa)	1	9.36	9.36	12.282	0.002
Te×OA	2	0.663	0.331	0.435	0.637
Te×Pa	2	3.353	1.677	2.2	0.128
OA×Pa	1	5.584	5.584	7.327	0.011
Te×OA×Pa	2	4.852	2.426	3.183	0.051
Residuals	91	69.351	0.762		
Total	102	95.392			
Pairwise of term ‘Temperature’					
Groups		t		P-value	
20, 23		2.841		0.003	
20, 26		3.431		0.004	
23, 26		1.639		0.114	
Pairwise test of term 'OA×Pa' for pairs of factor OA within level ‘Temperate-only’ of factor Pair type.					
Groups		t		P-value	
Control, OA		Negative			
Pairwise test of term 'OA×Pa' for pairs of factor OA within level ‘Mixed-species’ of factor Pair type.					
Groups		t		P-value	
Control, OA		1.9		0.07	
Pairwise test of term 'OA×Pa' for pairs of factor Pair type within level ‘Control’ of factor Ocean acidification.					
Groups		t		P-value	
Temperate-only, Mixed-species		4.662		< 0.001	
Pairwise test of term 'OA×Pa' for pairs of factor Pair type within level ‘OA’ of factor Ocean acidification.					
Groups		t		P-value	
Temperate-only, Mixed-species		Negative			

Chapter V

Tropical fishes benefit more from novel than familiar species interactions at their cold-range edges

Angus Mitchell¹, Chloe Hayes¹, David J. Booth², Ivan Nagelkerken¹

¹Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, DX 650 418, Adelaide, SA, 5005, Australia

²School of the Life Sciences, University of Technology Sydney, Ultimo, NSW, 2007, Australia



Statement of Authorship

Title of Paper	Tropical fishes benefit more from novel than familiar species interactions at their cold-range edges		
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style		
Publication Details	In preparation for journal submission.		

Principal Author

Name of Principal Author (Candidate)	Angus Mitchell		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/10/23

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Chloe Hayes		
Contribution to the Paper	Data collection, writing		
Signature		Date	24/10/23

Name of Co-Author	David J. Booth		
Contribution to the Paper	Study design, writing		
Signature		Date	24/10/23

Name of Co-Author	Ivan Nagelkerken		
Contribution to the Paper	Study design, writing		
Signature		Date	24/10/23

Abstract

Animals that extend their biogeographic ranges in response to global warming often interact with local species for limited resources. Whether these novel species interactions facilitate or inhibit range extension success in novel environments remains largely unknown. Here, we evaluate in the wild how range-extending coral reef fishes modify their behaviours (shoaling dynamics, foraging, and anti-predator) at their cold-water ranges to overcome biotic resistance by temperate species and adjust to novel species interactions resulting from climate change. We study range-extending tropical as well as their co-shoaling temperate fishes across a 2,000-km latitudinal gradient from tropical to temperate rocky reefs in a global warming hotspot. In their novel temperate ranges, tropical fishes showed increased anti-predator behaviours (increased shoal cohesion and shoal alignment, increased flight initiation distance, and increased lateralisation) and decreased bite rates compared to their native tropical and subtropical ranges. However, tropical fish in novel mixed-species shoals had higher bite rates compared to tropical-only shoals. In contrast, temperate fish behaviours and shoaling dynamics were unaffected by novel shoaling interactions across their subtropical and temperate ranges studied. Our findings reveal that tropical fish trade-off foraging efficiency for predator vigilance in their new and unfamiliar warm- and cold-temperate ranges, independent of novel shoaling interactions. However, novel mixed-species shoaling can increase the foraging efficiency of tropical fish, which may be a mechanism (phenotypic plasticity) that allows them to perform better at their novel cold-temperate range edges. Since these novel species interactions can enhance species fitness of tropical fishes, we conclude that behavioural interactions between invading and local species can accelerate successful range-extensions of some species into novel climatic environments.

Introduction

Species redistributions are rapidly increasing under climate change (Burrows et al., 2011; Chen et al., 2011; Pecl et al., 2017). Global warming is driving species distributions poleward to escape thermally unsuitable environments, allowing organisms to invade novel higher-latitude ecosystems that have become thermally accessible due to anthropogenic warming. Climate-induced range shifts have facilitated novel interactions between local and invading species and often determine range-shift and biodiversity outcomes in recipient ecosystems (Alexander et al., 2015). Range extension success is often limited through environmental novelty posed by new ecosystems (e.g. lethal winter temperatures, and novel predators, habitat, and prey availability; Pinksy et al., 2013) or by local species which can outcompete invaders for resources (Twine et al. 2022). Hence, invading species must overcome dynamic biotic resistance to establish at higher latitudes (Tekwa et al., 2022). Local conditions can alter range-shift outcomes, yet, range-shifting species can counter novel biotic resistance by modifying their behaviour to adapt to local conditions (Smith et al., 2018, Coni et al., 2022). Nevertheless, how range-shifting species alter their behaviour to overcome biotic resistance remains largely untested, particularly for schooling species which can show strong interactions with other individuals to determine schooling dynamics.

Range shifting species that show high phenotypic plasticity have a greater chance of establishing in new environments than species exhibiting limited plasticity (Donelson et al., 2019). Behavioural plasticity can allow range-shifting species to modify behaviour and increase persistence at their novel range edges (Coni et al., 2021; Smith et al., 2018). Species of recipient communities must also possess some degree of behavioural plasticity to compete against or limit the establishment of novel range-extending competitors (Twine et al., 2022). Behavioural plasticity can also allow resident species to adjust their behaviour at their range

trailing edge, where the introduction of novel competitors is likely to occur (Twinn et al., 2022). Hence, understanding whether behavioural shifts mediated by range shifts are beneficial to either resident or range shifting species is necessary to assess if species can adapt to novel environmental climatic conditions.

Tropical fishes are the fastest poleward range-extending organisms on Earth (Burrows et al., 2011; Chen et al., 2011). Ocean warming and strengthening poleward boundary currents have facilitated the rapid poleward redistribution of tropical fishes into sub-tropical and temperate marine ecosystems globally (Booth et al., 2011; Yamano et al., 2011; Vergés et al., 2014). In Australia, recipient temperate ecosystems experience above-average warming rates (approx. 3-4× above the global average, Lough et al., 2008), and the strengthening of the East Australian Current (EAC; Suthers et al., 2011) has facilitated dispersal of >150 tropical fish species into southeastern Australian marine ecosystems (Booth et al., 2011; Feary et al., 2014). Tropical herbivores entering temperate marine ecosystems change ecosystem function by overgrazing kelp forests, turning them into turf-dominated habitats (Vergés et al., 2014), and unlocking novel trophic pathways (Pessarrodona et al., 2022). These examples highlight how invading tropical fishes could severely modify temperate ecosystems. Yet, whether and how biotic interactions amongst range-extending and local fishes mediate range-shift outcomes remains largely unknown.

Lethal winter temperatures in high latitude regions can limit the range extension success of species entering from warmer lower latitudes (Figueira et al., 2009). Winter sea surface temperatures can be detrimental to warm-adapted species through physiological (e.g., reduced growth; Figueira et al., 2009) and behavioural impacts (e.g., decreased feeding, Coni et al., 2021; slower predator escape response, Djurichkovic et al., 2019; Figueira et al., 2019). Novel species interactions can enhance tropical fish fitness and temper lethal winter temperature effects (Smith et al., 2018). However, when combined with the unknown risks posed by

temperate ecosystems, sub-optimal winter temperatures can degrade the fitness of range-shifting species (Beck et al., 2016), slowing down tropical fish range-shifts at their cold range limits.

Predation risk and competition levels in novel environments are often unknown and unpredictable to range-shifting species (Brown et al., 2013; 2022). Range-shifting species can escape historical predators and competitors under climate change but often gain new ones at their poleward range edges (Beck et al., 2016; Figueira et al., 2019). Thus, vulnerability to novel predators and competitors in new environments can increase due to a lack of eco-evolutionary experiences shared between invading and local species (Saul & Jeschke, 2015). Invading species which ignore or fail to identify risks can experience increased predation (Beck et al., 2016). Hence, to reduce the risk of novel biotic factors (e.g. predators and competitors), tropical fishes can learn environmental and social cues of their new ranges through social interactions with local temperate species (Smith et al., 2018). Social learning can inform range-shifting species about novel predators and enhance foraging opportunities (e.g. through olfactory and visual cues; Hoare & Krause, 2003; Ward et al., 2011; Paijmans et al., 2020). Shoaling behaviour enhances anti-predator performance in general, but also in novel environmental contexts (i.e. confusion and dilution effects, Pitcher, 1983; Paijmans et al., 2020). Shoaling species also have advantages over solitary species when entering novel environments, as shoaling behaviour can increase intra-shoal information transfer, reduce individual predation risk, and improve social learning (Pitcher, 1983; Smith et al., 2018). Shoaling tropical fishes are commonly observed at the forefront of range extensions in temperate southeastern Australia (Booth et al., 2007; Booth et al., 2011). Gregarious tropical damselfish species (genus: *Abudefduf*) are the most abundant tropical fishes observed entering southeastern Australian temperate ecosystems and often form shoals with morphologically similar local temperate species (Smith et al., 2018). Such evidence suggests that shoaling

behaviour and novel interactions might benefit invasions. However, it remains largely unclear how co-shoaling coral reef and local temperate fish alter their behaviour, and to what degree they benefit, or not, from novel shoaling interactions.

Shifts in foraging and anti-predator behaviour (e.g. predator vigilance) are common in animals exposed to novel environmental contexts (Crane & Ferrari, 2017). Behavioural trade-offs can arise from elevated competition and uncertain risk or foraging opportunities (Brown et al., 2022), all of which are present in novel temperate ecosystems for range-extending tropical fishes (Coni et al., 2021). Risk-averse behaviour, which range-extending tropical fish often display in temperate ecosystems, can either slow establishment by reducing foraging opportunities (Coni et al., 2021) or provide fitness-enhancing benefits by minimizing predation risk (Ferrari et al., 2015). Trade-offs in behaviour can be expressed through modifications in shoaling (Bisazza & Dadda, 2005), anti-predator (Coni et al., 2022) and foraging behaviour (Coni et al., 2021). Studies assessing behavioural responses of range-shifting tropical fishes focus on individual-level responses to single-stressors (i.e. temperature, Beck et al., 2016; Djurichkovic et al., 2019). However, the true complexity and uncertainty of novel cold-temperate range edges could facilitate differences in behavioural responses that shoal-forming tropical fish experience under isolated climatic stressors. Therefore, shoal-level responses of range-shifting tropical fishes exposed to novel environments may reveal how behavioural modifications modulate species range-shifts.

Here we investigate how range-extending tropical and co-shoaling temperate fishes modify their foraging and predator vigilance along a 2,000-km latitudinal gradient (latitude: 19.1°–36.9° S) stretching from the tropical species core range into their novel leading edges in rapidly-warming southeastern Australian temperate ecosystems. Towards the cold-range edge of tropical fishes, temperate ecosystems are novel environments, with novel competitors, habitats, predators, and lethal winter temperatures, potentially preventing the permanent

establishment of tropical fishes. In response to cold-stress and environmental novelty posed by temperate ecosystems to range-extending tropical fish, we hypothesize (1): that range-extending tropical fish display increased anti-predator behaviours at group and individual levels (e.g. increased shoal cohesion, flight initiation distance), in their cold-temperate ranges compared to their historical subtropical and tropical ranges, because temperatures and environmental novelty posed in cold-temperate ecosystems increase risk averse behaviour in range-extending tropical fishes (Coni et al., 2022). Additionally, we hypothesize (2): that mixed-species shoaling (tropical-temperate shoals) benefits tropical fish by increasing foraging efficiency (bite rate), compared to tropical fish in tropical-only shoals at their cold-temperate ranges, because mixed-species shoaling increases prey access and social learning in range-extending tropical fishes (Smith et al., 2018; Paijmans et al., 2020).

Materials and methods

Range zones and locations

Range zones (Fig. S1) were selected along a 2,000 km latitudinal gradient encapsulating the historical breeding region of range-extending tropical fish: (1) tropical zone: 19.1–24.9° S; Froese & Pauly, 2016), (2) subtropical zone (latitude: 28.2– 30.9° S) including locations within the most southern breeding population of the model tropical species (Booth et al., 2007), (3) warm-temperate zone (latitude: 33.8° S) where tropical fish have been observed overwintering but not producing breeding populations (Booth et al., 2011), and (4) cold-temperate zone (36.2–36.9° S), where juvenile tropical fish reside during summer months but fail to overwinter (Booth et al., 2007; 2011). We collected *in situ* data at depths of 0.25–3 metres during peak tropical recruitment in temperate ecosystems (Booth et al., 2007; 2011), moving from cold-

temperate to tropical range locations. In the cold- and warm-temperate regions, we surveyed sites characterised by mosaic habitat structures (kelp, turf, rocky barrens and oyster reefs) between 15/04-10/05 (cold-temperate region) and 10/05-20/05/2021 (warm-temperate region). In the subtropical region, we surveyed sites characterised by oyster reefs, turfs, soft corals and rocky barrens between 21/05-18/06/2021 and 08/07-12/07/2021. In the tropical region, we surveyed sites dominated by rocky barrens and turfs, with soft and branching corals also present, between 24-25/04/2021 and 20/06-04/07/2021.

Fish collection for lateralization tests

We selected one model species for each temperature affinity (tropical vs temperate): (1) the most commonly observed range-extending tropical fish *Abudefduf vaigiensis* (Booth et al., 2011) and the morphologically similar co-shoaling temperate species *Atypichthys strigatus* (Smith et al., 2018). The temperate species occurs in the subtropical zone (Southern Queensland) and on temperate (southern New South Wales) Australian reefs. These two species have coexisted for longer periods at our subtropical locations (during all seasons; Coni et al., 2021) than in the warm and cold temperate zones. The temperate species is absent from the tropical zone, whilst both species only co-occur in the cold temperate zone during the summer and autumn months (Fig. S1; Booth et al., 2011).

A. vaigiensis (mean $\pm$ SE wet weight: 1.05 ± 0.14 g; length: 23.8 ± 0.8 mm) and *A. strigatus* (mean wet weight: 2.98 ± 0.24 g, length: 40.1 ± 1.5 mm) were collected using hand and seine nets between April 15 and July 12, 2021. Fish were collected to infer how range zone (tropical, subtropical, warm-temperate or cold-temperate) and shoal type (tropical-only, mixed-species, or temperate-only) affected lateralization (a measure of cognition and anti-predator behaviour; Bisazza & Dadda, 2005). Fish were collected using 9:1 ethanol:clove oil spray, and

hand nets. Collected fish were placed into a 50-litre plastic bucket filled with fresh seawater and an aerator attached to the bucket.

Lateralization test

Motor lateralization was evaluated in the field using a standard detour test (T-test; Bisazza et al., 1998; Brown et al., 2007). The arena used in this study consists of a two-way T-shape runway that allows scoring the direction of each individual's turn (e.g. left or right) over twelve consecutive trials (Fig. S2). The arena consisted of a transparent plastic tank (IKEA product number: 198.856.46; 39×28×14 cm, length×width×height) with a runway in the middle (20×6 cm, length×width). At both ends of the runway, white acrylic barriers (12×12×1 cm) were positioned perpendicular to the runway. The runway was created by placing acrylic inserts onto each side of the arena to form the runway (20×6×15 cm). The water depth in the arena was 12 cm. The arena was placed on a shaded, level surface at the collection site.

At the start of each trial, a single fish was introduced from the 50-litre bucket into the arena and left to acclimate to the novel arena environment for 2 minutes. We conducted 12 consecutive turning trials for each individual. During each trial, we chased the focal fish with a small handheld net along the runway to force a left or right turning choice at the end of the runway (see Mitchell et al., 2022). The observer was always positioned at the same end of the arena to minimize side bias. After each trial, the experimental arena was emptied and refilled with fresh seawater until all collected fish were scored.

Mean relative lateralization index (L_R) was calculated from the 12 turning decisions (per fish) and used to identify the strength of population-level laterality and turn preference (e.g. bias in left or right turns). For the L_R index, individuals were classified between the extreme values of '+100' (fish that turned right on all 12 turning decisions) and '-100' (fish that turned left on all 12 turning decisions). An individual L_R close to zero indicates that a fish

is neither left- nor right-biased in its turning tendency (Bisazza et al., 1998). Because individuals could be left-biased (all negative scores) or right-biased (all positive scores), the average L_R across replicate fish within a range zone could hypothetically be zero (i.e. even number of entirely left- vs right-biased fish). This would not reveal the presence of a strong lateralization response that is independent of the turning direction. Therefore, the absolute lateralization index (L_A) was also calculated (Bisazza et al., 1998). L_A , in contrast to L_R , evaluates individual lateralization strength irrespective of the direction of their turns. The L_A index corresponds to the absolute value of L_R , thus ranging from 0 (an individual that turned in equal proportion to the right and the left) to 100 (an individual that turned right or left on all 12 trials).

Lateralization was tested for 150 temperate and 169 tropical fish from 12–4 pm between April 15–July 12, 2021 (Table S1).

Shoaling dynamics *in situ*

The most commonly observed tropical genus of vagrant tropical fishes (*Abudefduf*; Feary et al., 2014) entering temperate ecosystems often form mixed-species shoals with temperate species (including *Atypichthys strigatus*, *Microcanthus strigatus*, *Scorpius lineolate*, *Trachinops taeniatus*; Smith et al., 2018; Paijmans et al., 2020). Range-extending tropical fishes can obtain fitness benefits by shoaling with resident temperate species, growing faster and surviving longer into winter than tropical-only shoaling fish (Smith et al., 2018). It is unknown how tropical-temperate shoaling interactions alter the shoaling dynamics of temperate species and if changes in shoaling dynamics alter temperate fish performance.

To quantify *in situ* shoaling dynamics, GoPro Hero 7 Silver cameras were placed in front of juvenile fish shoals (Mean shoal size = 12.11 fish per shoal, SE $\pm$ 1.1 fish per shoal; shoal types: tropical, mixed-species or temperate; Fig. S4) at 0.5 to 2 metres away from shoals,

which encapsulated the entire shoal, recording for 5–10 minutes (recording settings: 30 frames per second). Tropical shoals consisted of *A. vaigiensis* and other range-shifting tropical species (Feary et al., 2014), temperate shoals consisted of *A. strigatus* and other co-shoaling temperate species (Paijmans et al., 2022) and mixed-species shoals comprised of both *A. vaigiensis* and *A. strigatus*, as well as tropical and temperate shoaling species. We targeted juvenile fish shoals in videos (fish total length: 0–5 cm). We recorded videos at depths of 0.25–3 metres across the range locations sampled (19.1° S – 36.9° S; Fig. S1). Shoals were recorded with naturally occurring rocky/turf substrate situated behind shoals to reduce depth perception skew. The first and last 1.5 minutes of video recordings were discarded during video shoal analysis. A ten-still frame sequence was randomly selected and analysed on a monitor screen using software VLC media player (one still frame every 10 seconds).

Shoaling dynamic metrics are often used to assess shoaling performance in gregarious fish species (Delcourt & Poncin, 2012). Here we use three commonly used shoaling metrics to determine how shoal type and range zone affect the shoaling dynamics of tropical, temperate and mixed-species shoals (Delcourt & Poncin, 2012; Mitchell et al., 2022).

- (1) Inter-individual distance (IID): a measure of shoal cohesion (Lopes et al., 2016), was calculated as follows. A focal fish from each shoaling video was randomly selected in each frame (n=10 frames, per shoaling sequence). The on-screen body length of the focal fish and up to four nearest neighbouring fish were measured using a transparent grid ruler on a screen monitor along a transverse plane to calculate the mean on-screen shoal body length (cm). The mean on-screen shoal body length distance between the focal fish and up to four nearest neighbouring fish was then measured with a transparent grid ruler, and the four mean shoal body length distance between shoal members was quantified. The average of the four measurements is defined as the IID of this frame. The IID was divided by the frame's on-screen mean shoal body length. As fish were not the same size and

recordings were not taken at precisely the same distance from each individual, IID was divided by mean on-screen shoal body length to minimize depth perception skew. The mean IID was then calculated for all ten frames for each shoaling video.

- (2) Distance amplitude variability (DAV): from the ten repeated measurements of shoaling distance, the standard deviation of IID was calculated for each shoal. The mean and standard deviation for each shoal type (mixed, tropical or temperate) was calculated for each range location to determine shoal distance amplitude variability (Mitchell et al., 2022).
- (3) Shoal alignment (a metric of shoal vigilance; Couzin et al., 2002): from the still frame sequences for each shoal, we quantified the head orientation of shoal members across a transverse plane to determine shoal coordination. We counted the number of fish orientated in the same direction along a transverse plane ($< 20^\circ$ directional variation) as being in alignment. The majority of fish oriented in the same direction determined the percentage of shoal alignment. When shoals were aggregated but formed disordered groups (*i.e.* swarm state; no fish aligned with another fish in the shoal; Couzin et al., 2002), a shoal alignment score of zero was given. Shoal alignment was calculated as follows:

$$Shoal\ alignment = \left(\frac{n. aligned\ fish}{total\ n. fish} \right) \times 100$$

In shoaling videos, across each frame ($n = 10$ frames per shoaling sequence), we also quantified the mean number of tropical and temperate fish, total species number, and the total number of individuals in each shoal.

Mixed-species shoaling metrics

Fish within mixed-species shoals often form intra-species subgroups (Paijmans et al., 2021), opting to shoal more cohesively with same-species members within mixed-species shoals. To test for intra-species groups within mixed shoals, we used mixed-species shoal videos to measure the: (1) conspecific inter-individual distance of both tropical (*A. vaigiensis*) and temperate fish (*A. strigatus*). For each shoal, we measured the body length distance of two randomly selected focal fish (one tropical and one temperate fish) to up to four nearest neighbouring conspecifics (fish of the same species) of either species, in every frame ($n = 10$). We also quantified: (2) heterospecific inter-individual distance (body lengths) by measuring the body length distance of the tropical and temperate randomly fish' to $\leq$ four nearest heterospecific (shoal member of different species) shoal members. Conspecific- and heterospecific IID was then calculated by dividing the mean inter-individual distances by the focal fish body length for each video frame. The average of the ten still frame sequences was then calculated to obtain the mean conspecific and heterospecific IID and distance amplitude variability (DAV) for temperate and tropical fish within mixed-species shoals.

Flight initiation distance *in situ*

We defined the flight initiation distance “as the distance at which a fish initiates an escape response towards an artificial threat”. We tested the flight initiation distance of 53 tropical fish (species: *A. vaigiensis*; tropical zone 10 fish; subtropical zone 17 fish; warm temperate 8 fish; cold temperate 18 fish) and 55 temperate fish (species: *A. strigatus*; warm temperate 21 fish; cold temperate 34 fish; Table S1).

An artificial threat-eliciting stimulus was used to mimic a predator attack. The predator threat was created using a cubical PVC frame connected to a 60 cm iron rod, which supported a 30 cm metal ruler at its most distal end (Coni et al., 2022; following methods described in

Nagelkerken et al., 2017). A GoPro Hero Silver 7 camera was fixed to the cubical frame and positioned towards the ruler. Once a focal fish was found, the snorkeler slowly approached the fish and moved the end of the ruler from above the fish towards its head at a constant speed while the camera was recording its escape behaviour (recording at 30 frames per second). The approach by the snorkeler towards the focal fish always started from approximately 3 metres across all range locations. Flight initiation distances were calibrated according to the on-screen size of the attached metal ruler and quantified on the software VLC media player.

Bite rates *in situ*

In each shoaling recording, we quantified the number of bites taken by a randomly selected single focal fish for one minute. To avoid pseudoreplication, we only selected one focal fish per shoaling recording. The number of bites taken by the focal fish was converted into a rate of bites*sec⁻¹. We quantified the bite rate for one tropical (*A. vaigiensis*) and one temperate (*A. strigatus*) focal fish in mixed-species shoals. In case a fish's bite rate could not be quantified (e.g. poor visibility), a 'not applicable' score was given (10.3% of video samples).

Statistical analysis

All analyses (except linear mixed-effect models) were performed on fourth-root transformed data and used Euclidian resemblance matrices using Primer version 7 (Clarke & Gorley, 2015). Permutations were set to 9,999 for all analyses. For covariate analysis, we tested for homogeneity of slopes by including interactions between covariates and main factors in the initial tests. Interactions between the categorical factor (range zone) and the covariates were removed from the final models (MANCOVA/ ANCOVA) because their initial inclusion did not show a significant effect (i.e. meaning that the effect of range zone on selected behaviours or shoaling dynamics is independent of shoal composition and shoal size), and maintaining

them in the final model could lead to misinterpretation of the results (Engqvist 2005; Beck & Bliwise 2014). When the number of permutations was < 250 , Monte Carlo tests were performed and *post-hoc* pooling of factor: range location was performed to enable a more robust test of the main effect (only where range location P -value was > 0.25 ; Winer et al., 1991). For significant effects ($P < 0.05$), pairwise tests were used to compare the respective means (Anderson, 2001).

Shoaling dynamics (inter-individual distance, distance amplitude variability and shoal alignment) were tested separately for each shoal type in a two-way multivariate analysis of covariance (MANCOVA; covariate: shoal size; fixed factor: range zone; random factor: range location (nested within range zone)). Permutational MANCOVAs were performed, followed by pairwise post hoc tests (significance accepted at $P < 0.05$; Anderson, 2001). Univariate permutational analysis of covariance (ANCOVA) was then performed for each shoaling dynamic (IID, DAV, and shoal alignment).

For the shoaling dynamics, which showed a significant effect of the covariate (shoal size) in the ANCOVAs, we calculated an adjusted R^2 value to evaluate the strength of the covariate-shoaling dynamics relationship for each shoal type (tropical, temperate or mixed-species). The R^2 can indicate to what extent (expressed in %) the variance of the covariate (shoal size) explains the variance of dependent variables (shoaling dynamics) (Miles, 2014).

Bite rate was tested separately for the tropical and temperate fish using a three-way analysis of variance (ANOVA). Range location was set as a random factor and nested within the fixed factor 'range zone'. Shoal type (mixed- or single-species) was set as a fixed factor.

Flight initiation distance was tested separately for the tropical and temperate fishes in a two-way ANOVA. Range location was set as a random factor and nested within the fixed factor 'range zone'.

Lateralization (L_R and L_A) was tested separately for tropical and temperate fishes in a three-way ANOVA. Range location (nested in ‘range zone’) and shoal type (nested in ‘range location’) were set as random factors.

Mixed-species shoal behaviours were tested in a three-way univariate permutation (ANCOVA; fixed factors: neighbour type and range zone; random factor: range location, nested within fixed factor ‘range zone’; covariate: shoal composition (percentage tropical fish in shoal)). Permutational ANCOVAs were performed, followed by pairwise post hoc tests (significance accepted at $P < 0.05$; Anderson, 2001).

Linear mixed-effects models

We investigated the effect of shoal composition (percentage of tropical fish in mixed-species shoal) on IID and DAV of tropical and temperate fish in mixed-species shoaling using linear mixed-effect models (LMM). Main effects: ‘range zone’, ‘neighbour type (conspecific or heterospecific)’ and ‘% tropical fish’ were fixed effects, with ‘range location’ set as a random factor, nested in ‘range zone’. We used R (R Core Team, 2021) and the ‘lme4’ package (Bates et al., 2015) for linear mixed-effect model analysis (LMM). Interactions between ‘range zone’ and ‘neighbour type’, and between ‘% tropical fish’ and ‘neighbour type’ were included in final models. Significance tests for LMMs were performed by Wald F tests of the whole model with Kenward-Roger approximation for degrees of freedom using the “ANOVA” function in package ‘Car’ (Fox et al., 2016). No obvious deviations from homoscedasticity were detected by visually inspecting the residual plots. Conditional and marginal R^2 values were obtained using function ‘r.squaredGLMM’ in package ‘MuMIn’ (Nakagawa & Schielzeth, 2013). IID and DAV for tropical and temperate fish were cube transformed to account for non-normal distributions. Models were fitted using restricted maximum-likelihood estimates (REML).

Results

Tropical fish behaviour and shoaling dynamics

The shoaling dynamics of tropical-only shoals differed significantly between subtropical and cold-temperate zones ($P < 0.001$; Table S2). The shoaling dynamics of tropical shoals also significantly differed among range locations within the tropical (19.1° S vs 24.8° S ; $P = 0.024$; Table S2) and subtropical range zones (28.2° S vs 30.9° S ; $P = 0.005$; 28.2° S vs 30.4° S ; $P = 0.004$; Table S2). The shoaling dynamics of tropical-only shoals were unaffected by shoal size ($P = 0.586$; Table S2).

Tropical shoals had reduced inter-individual distance (increased cohesion) at their warm- and cold-temperate zone than their subtropical zone (but not their tropical range zone; Fig. 1A; $P < 0.049$; Table S3). Tropical shoals were more aligned at their cold-temperate range than subtropical and tropical zones (Fig. 1D; $P < 0.045$; Table S5). In contrast, tropical shoal distance amplitude variability remained unaffected by range zone (Fig. 1G; $P > 0.05$; Table S4).

Bite rate of the focal tropical fish was lower in warm- and cold-temperate zones than in subtropical and tropical zones (Fig. 2A, B; $P < 0.009$; Table S14). The focal tropical fish in mixed-species shoals had higher bite rates than conspecifics in tropical-only shoals (Fig. 2A, C; $P = 0.023$; Table S14). In their cold-temperate range zone, the focal tropical fish had higher population-level lateralization (L_R) than in subtropical and tropical zones, respectively (Fig. 3A; $P < 0.046$; Table S16), irrespective of shoal type (Fig. 3A; $P > 0.05$; Table S16). Individual lateralization (L_A) remained unaffected by range zone and shoal type (Fig. 3C; $P > 0.05$; Table S17). The focal tropical fish had larger flight initiation distances in the warm- and cold-temperate zones than in subtropical and tropical zones (albeit no difference between warm-temperate and tropical zones, $P > 0.05$; Fig. 3E; $P < 0.012$; Table S20).

Temperate fish behaviour and shoaling dynamics

Temperate-only shoal dynamics changed with increasing mean shoal size ($P = 0.022$; Table S6) but were unaffected by range zone ($P > 0.05$; Table S6). None of the focal temperate fish behaviours (bite rate, L_R , L_A , and flight initiation distance) or shoaling dynamics differed among range zones (Figs. 1C, F, I, 2B, D, 3B, D, F; $P > 0.05$; Table S7-9, S15, S18-19, S21). The three shoaling dynamics, however, showed negative relationships with mean shoal size (inter-individual distance: Figs. 1C, S3A; $R^2 = 0.346$; $P < 0.001$; shoal alignment: Figs. 1F, S3C; $R^2 = 0.178$; $P = 0.006$; distance amplitude variability: Figs. 3I, S3B; $R^2 = .253$; $P = 0.02$; Table S7-9).

Mixed-species fish behaviours and shoaling dynamics

Mixed-species shoaling dynamics differed between warm temperate vs subtropical zones ($P = 0.036$; Table S10) and cold-temperate vs subtropical zones ($P = 0.002$; Table S10). Inter-individual distance and distance amplitude variability decreased as mean shoal size increased in mixed-species shoals (Inter-individual distance: Fig. 1B, S3A; $R^2 = 0.279$; $P = 0.002$; distance amplitude variability: Fig. 1H, S3B; $R^2 = 0.192$; $P = 0.001$; Table S11-12). Mixed-species shoal alignment was unaffected across range zones (Fig. 1E; $P > 0.05$; Table S13).

In mixed-species shoals, both the focal tropical and temperate fish were more cohesive with their own species than with others, irrespective of range zone (tropical fish: Fig. S5A; $F_{105.01} = 56.92$, $P < 0.001$; Table S22-23; temperate fish: Fig. S5B; $F_{106.02} = 77.43$ $P < 0.001$; Marginal $R^2 = 0.277$, Conditional $R^2 = 0.661$; Table S24). The focal tropical fish became less cohesive (increased inter-individual distance) and showed increased distance amplitude variability with shoal neighbours as the proportion of tropical fish in the mixed-species shoals increased, irrespective of range zone (Fig. S5A; Inter-individual distance: $F_{106.55} = 15.18$, $P <$

0.001; Marginal $R^2 = 0.328$, Conditional $R^2 = 0.594$; Fig. S5C; distance amplitude variability: $F_{106.89} = 10.46$, $P = 0.002$; Marginal $R^2 = 0.259$, Conditional $R^2 = 0.367$; Table S22-24). Distance amplitude variability was significantly higher between tropical and temperate neighbours than tropical and other tropical neighbours in mixed-species shoals (Fig. S5C; $F_{105.09} = 25.66$, $P < 0.001$; Table S25). Distance amplitude variability between temperate conspecifics increased as the proportion of tropical fish in mixed-species shoals increased, irrespective of range zone (Fig. S5D; $F_{106.07} = 6.24$, $P = 0.014$; Marginal $R^2 = 0.220$, Conditional $R^2 = 0.507$; Table S28-29).

Shoaling Dynamics

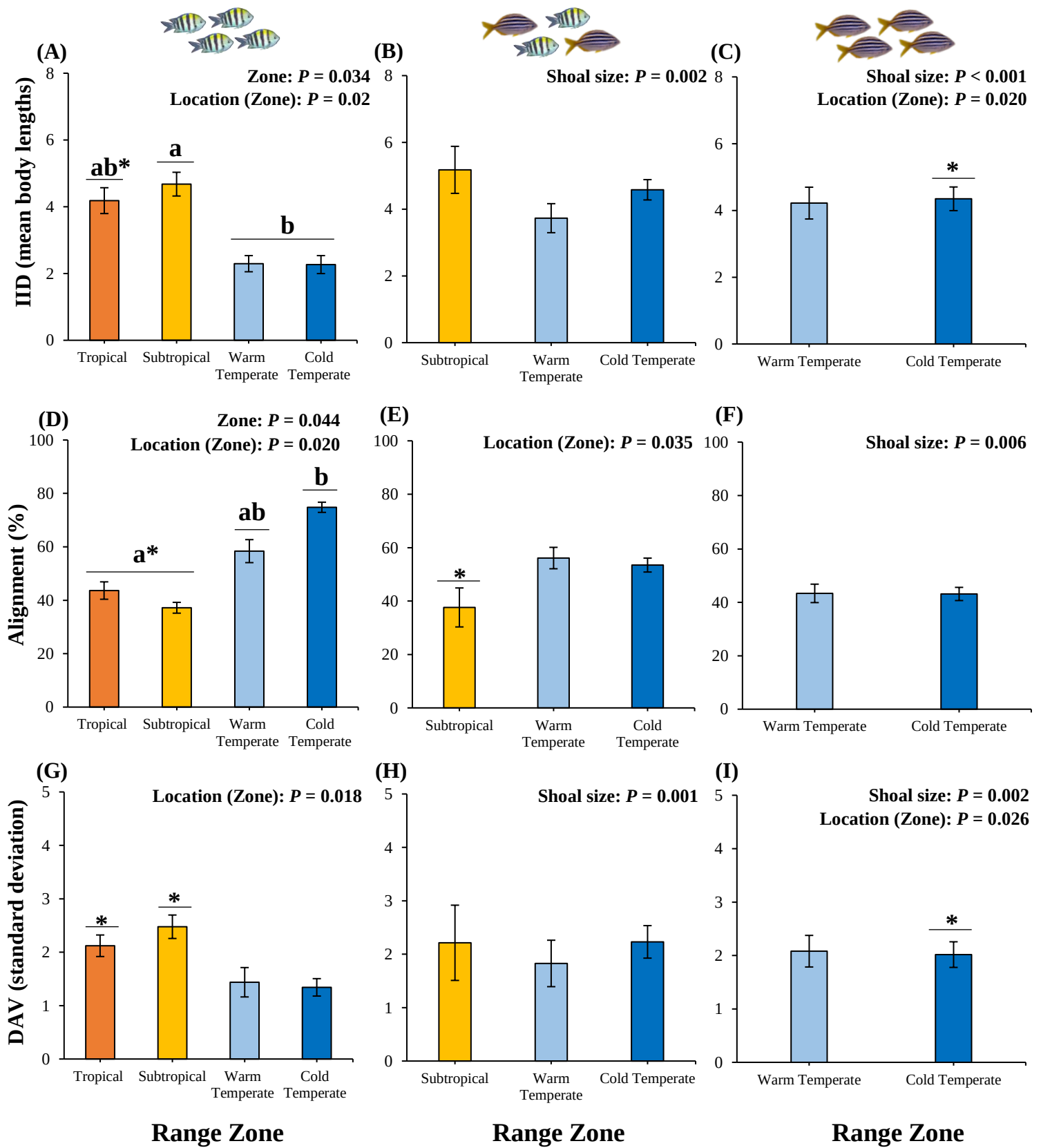


Figure 1: In situ mean inter-individual distance (cohesion, mean body length; panels: A, B, C), mean shoal alignment (%; panels: G, H, I), and mean distance amplitude of inter-individual distance (standard deviation; panels: D, E, F) across tropical ((A), (D), and (G)), mixed-species

((B), (E), and (H)), and temperate shoals ((C), (F), and (I)). Different letters above bars indicate significant differences among range zones ($P < 0.05$; Table S). Error bars represent standard error. * denotes significant difference ($P < 0.05$) among range locations within range zones.

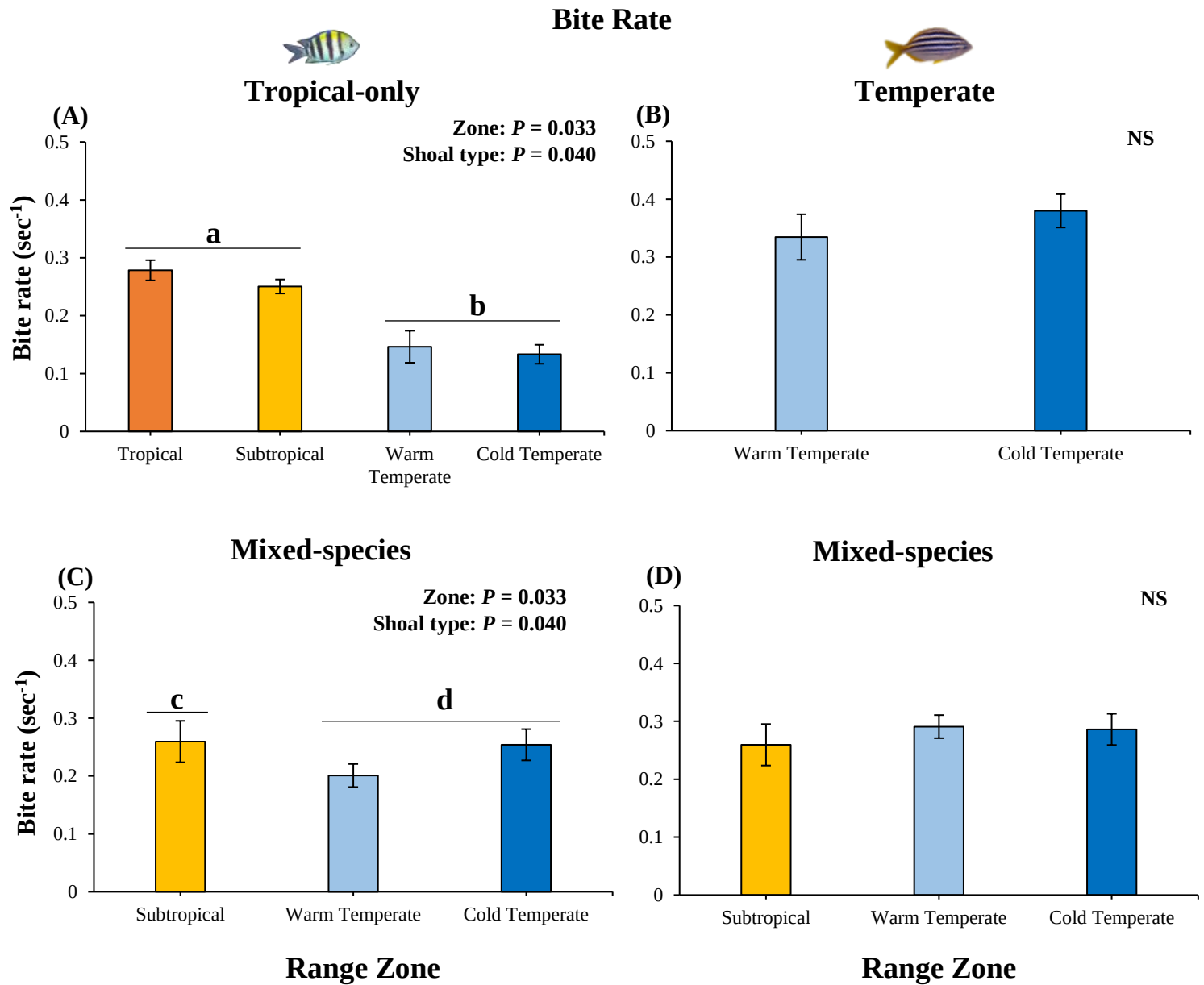
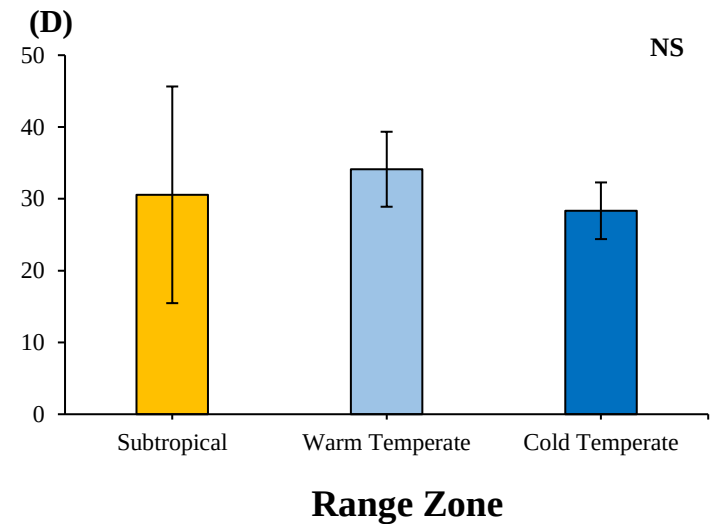
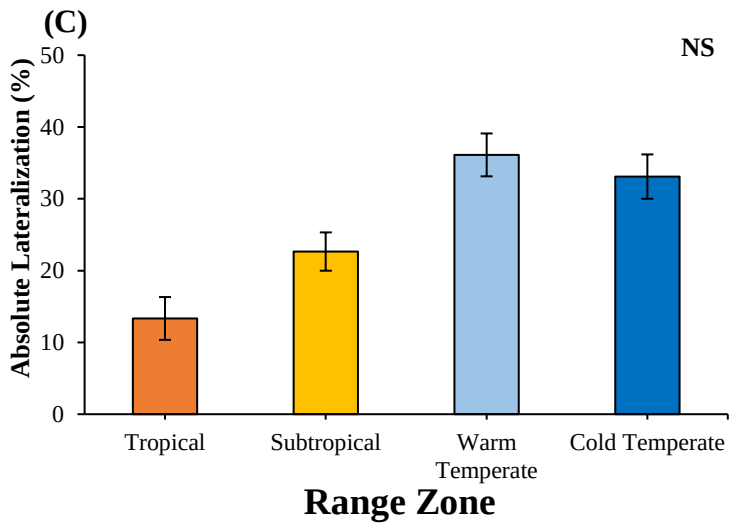
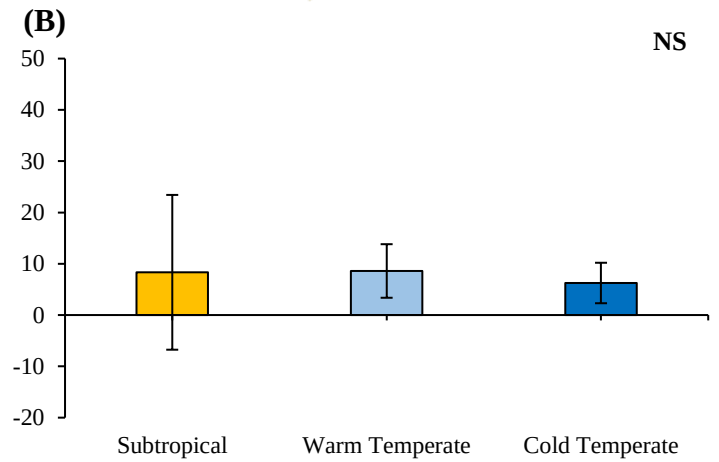
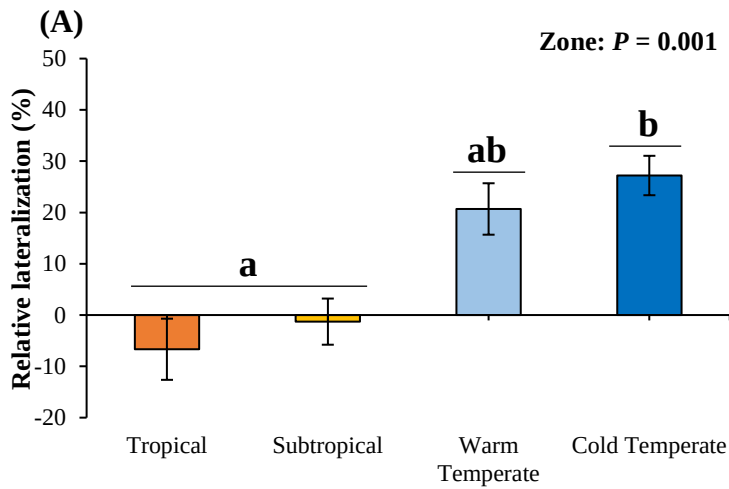


Figure 2: In situ bite rate (bites/sec⁻¹) of the focal tropical fish in tropical-only (A) and mixed-species shoals (C) and the focal temperate fish in temperate-only (B) and mixed-species shoals (D) across range locations. Different letters above bars indicate significant differences among range zones ($P < 0.05$; Table S). Error bars represent standard errors. NS = not significant ($P > 0.05$).

Motor Lateralization



Flight Initiation Distance

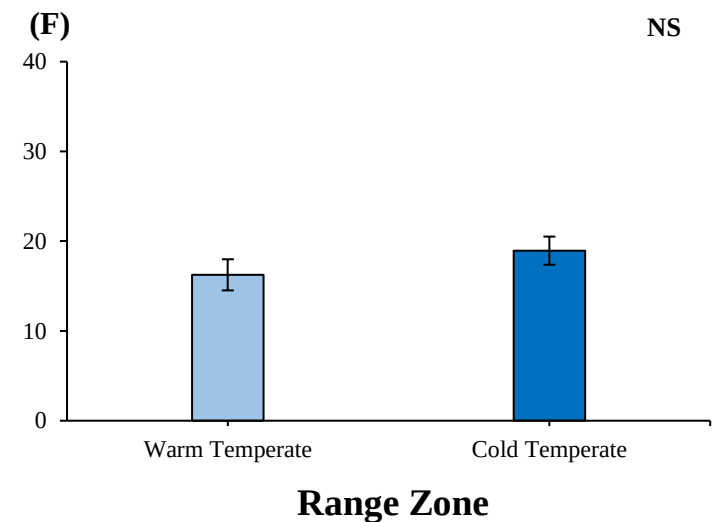
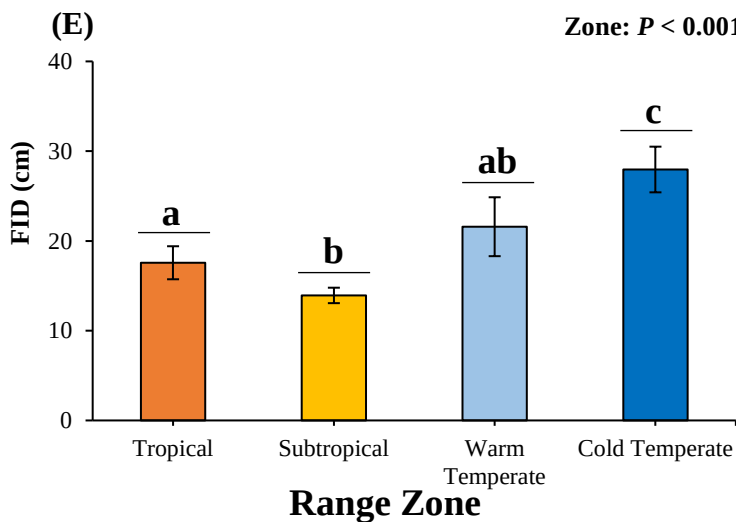


Figure 3: Relative lateralization (L_R ; a measure of population-level lateralization) and absolute lateralization (L_A ; a measure of individual lateralization) responses across range locations of

A. vaigiensis (tropical vagrant; (A) and (C)) and co-shoaling temperate species *A. strigatus* ((B) and (D)). Flight initiation distance (cm) responses for the tropical (E) and the temperate (F) focal fish species across range locations. Key displays range location latitude (° S) within respective range zone. Note: factor 'shoal type' was pooled for visualization. Error bars represent standard errors. NS = not significant ($P > 0.05$). Note: *A. strigatus* were not present at range locations 30.4° S and 30.9° S.

Discussion

Here we reveal that novel species interactions (tropical-temperate species co-shoaling) resulting from climate-driven range extensions can affect a wide suite of behaviours with ensuing benefits for tropical fishes. Firstly, bites rate of range-extending tropical fish was lower in their cold- and warm-temperate ranges compared to their subtropical and temperate ranges. However, their increased bite rate in novel mixed-species shoals than tropical-only shoals, suggests that mixed-species shoaling can enhance foraging efficiency across their novel temperate ranges, and allows tropical fish to obtain larger body sizes compared to tropical-only shoals (Smith et al., 2018). Indeed, tropical fish can be outcompeted by local competitors in temperate ecosystems (Coni et al., 2021). Yet, the formation of same-species subgroups within mixed-species shoals may allow tropical fish to reduce inter-species competition and minimize predation-mortality risk (e.g., novel predator detection; Paijmans et al., 2020; 2021; dilution and confusion effects; Landeau & Terborgh, 1986), increase prey access (Paijmans et al., 2020) and enhance social cue learning from temperate shoal mates (Smith et al., 2018). These benefits may allow range-extending tropical fish to increase foraging (this study) and extend their persistence at their cold-temperate range limits (Smith et al., 2018). Thus, mixed-species shoaling may enhance tropical fish fitness in novel temperate environments and facilitate their range extensions. Our findings reveal a potential underlying mechanism of how mixed-species

shoaling can boost the performance of range-extending tropical fish compared to single-species tropical shoals in novel temperate ecosystems. We conclude that novel species interactions may strengthen range-extension success by increasing the foraging efficiency of range-extending tropical fish in novel temperate environments.

Whilst novel mixed-species shoaling provides foraging benefits in tropical-only shoals, tropical fish trade-off foraging efficiency for collective predator vigilance at their warm- and cold-temperate range limit compared to their native sub-tropical and tropical ranges. Increased shoaling cohesion, shoal alignment, population-level lateralization and flight initiation distance, but reductions in bite rate at their cold-range limit in tropical-only shoals, suggests that tropical fish increase anti-predator behaviour and reduce foraging compared to their subtropical and tropical native ranges. Shifts in behavioural trade-offs can prove costly (e.g. reduced foraging efficiency (this study) and energy (Lima & Stuey, 2005)), but this might be outweighed by enhanced survival (Ferrari et al., 2015), in particular for inexperienced juvenile shoaling tropical fish in novel contexts (e.g. minimize novel predation risk; Coni et al., 2022). Hence, shifts in trade-offs between foraging and shoal-level predator vigilance may be a plastic behavioural response that tropical fishes express in response to the environmental uncertainty experienced at their novel poleward range limits. In addition to trade-offs, increased population-level lateralization, flight initiation distance, and shoaling performance by tropical fish may act as a compensatory mechanism, whereby tropical fish increase anti-predator behaviour to compensate for reductions in burst swim speed (Djurichkovic et al., 2019), growth (Figueira et al., 2009) and foraging behaviour experienced under cold temperatures in temperate ecosystems (Coni et al., 2021). Our findings suggest that elevated population-level and risk-averse shoaling dynamics expressed by tropical fish may boost anti-predator performance (e.g., flight initiation distance) but at the cost of foraging efficiency at their novel warm- and cold- temperate range limits. We conclude that range-extending tropical fish trade-

off foraging efficiency for predator vigilance in their warm- and cold-temperate range zones in response to the uncertain risk posed by these novel temperate ecosystems.

Shoal size was also a driver of shoaling dynamics for both species. Increased shoal cohesion (IID) and reduced distance amplitude variability coincided with increasing shoal size in temperate-only and mixed-species shoals. Larger shoals can benefit from increased decision-making accuracy (Krause et al., 2010), foraging efficiency (Pitcher & Magurran, 1983; Hintz & Lonzarich, 2018) and anti-predator behaviour (e.g. rapid information transfer, Ward et al., 2011; increased predator vigilance, dilution and confusion effects; Landeau & Terborgh, 1986). Thus, while increasing shoal size can reduce individual predator vigilance (reduced alignment) and increase detection by predators (Hintz & Lonzarich, 2018), fish residing in larger temperate and mixed-species shoals may display improved shoaling performance (e.g. increased cohesion) compared to smaller-sized shoals.

Shoal composition mediated the dynamics of novel species shoals. An increased proportion of tropical fish in mixed-species shoals coincided with reduced cohesion and increased distance amplitude variability between mixed-species shoal members. An increase of tropical fish in mixed-species shoals under future warming may degrade current benefits (e.g. reduced mortality and increased growth benefits: Smith et al., 2018; Paijmans et al., 2020) obtained through mixed-species shoaling behaviour in temperate range locations. Reductions in cohesion and increased cohesion variability, as the proportion of tropical fish in mixed-species increases, can slow information transfer between mixed-species shoal members, reducing the responsiveness of individuals during critical fast events (Ward et al., 2018). We suggest mixed-species shoaling benefits may only assist range-extending tropical fishes during the initial stages of range extensions but weaken when shoals become more tropicalized under increasing warming.

The bite rate, lateralization and flight initiation distance of the temperate fish were unaffected by mixed-shoaling interactions across subtropical and temperate ranges. The formation of temperate intra-species groups in mixed-species shoals, may have minimised species interactions between tropical and temperate fishes, which could influence temperate fish behaviour. In addition, unchanged behaviours at temperate fish' trailing edge compared to their core range may simply show a lack of novelty posed by their trailing edge range, where temperate fish have coexisted with tropical fishes for some time (Coni et al., 2022).

Conclusions

We reveal that range-extending tropical fish trade-off foraging efficiency for predator vigilance at their warm- and cold temperate range zones compared to their (sub)tropical range extent (i.e. increased population-level lateralization, flight initiation distance and shoaling dynamics), independent of novel shoaling interactions. However, novel mixed-species shoaling increases the foraging efficiency of tropical fish, which may allow them to persist longer or perform better at their cold-temperate range edge. Co-shoaling temperate fish behaviour remained unaffected along their studied range or due to novel shoaling interactions. We suggest that novel species interactions (mixed-species shoaling) provide greater benefit than familiar interactions and behavioural trade-offs expressed by tropical fish at their cold-range edges, and, therefore, may increase range-extension success by enhancing range-extending fish foraging behaviour in novel temperate environments.

Acknowledgements

IN and DJB were supported by an Australian Research Council (ARC) Discovery Project (DP170101722). We thank Erin Pichler and Steven Delean for their statistical support.

Ethics Statement

This experiment was conducted according to The University of Adelaide Animal Ethics guidelines and permit: S-2020-13, GBRMPA permit: G21/45557.1, and under New South Wales DPI Collection Permit: F94/696(A)-9.0.

Conflict of Interests

The authors acknowledge there was no conflict of interest during any part of the creation of this research.

References

- Alexander, J., Diez, J., Levine, J. (2015). Novel competitors shape species' responses to climate change. *Nature*. 525(7570). pp. 515–518. <https://doi.org/10.1038/nature14952>.
- Anderson, MJ. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*. 26. pp. 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.
- Bates, D., Maechler, M., Bolker, B. (2011) lme4: linear mixed-effects models. R package, version 0.999375-42. <http://CRAN.R-project.org/package=lme4>.
- Beck, H., Feary, D., Fowler, A., Madin, E., Booth, DJ. (2016). Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Marine Biology*. 163(4). 70. <https://doi.org/10.1007/s00227-016-2844-8>.
- Beck, CW., Bliwise, NG. (2014). Interactions are critical. *Like Sciences Education*. 13. pp. 371–372. <https://doi.org/10.1187/cbe.14-05-0086>.
- Bisazza, A., Facchin, L., Pignatti, R., Vallortigara, G. (1998). Lateralisation of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research*. 91(1-2). [https://doi.org/10.1016/s0166-4328\(97\)00114-9](https://doi.org/10.1016/s0166-4328(97)00114-9).
- Bisazza, A., Dadda, M. (2005). Enhanced schooling performance in lateralised fishes. *Proceedings of the Royal Society B: Biological Sciences*. 272(1573). <https://doi.org/10.1098/rspb.2005.3145>.
- Booth, DJ., Bond, N., Macreadie, PI. (2011). Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research*. 62(9). <https://doi.org/10.1071/MF10270>.
- Booth, DJ., Figueira, WF., Gregson, MA., Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine Coastal and Shelf Science*. 72(1-2). <https://doi.org/10.1016/j.ecss.2006.10.003>.
- Brown, GE., Crane, A., Demers, E., Chivers, DP., Ferrari, MC. (2022). Uncertain foraging opportunities and predation risk exert additive effects on induced neophobia in cichlids. *Animal Behaviour*. 186. pp. 21–28. <https://doi.org/10.1016/j.anbehav.2022.01.013>.
- Brown, GE., Ferrari, MC, Elvidge CK, Ramnarine I, Chivers, DP. (2013). Phenotypically plastic neophobia: a response to variable predation risk. *Proceedings of the Royal Society B: Biological Sciences*. 280:20122712. <https://doi.org/10.1098/rspb.2012.2712>.
- Brown, C., Western, J., Braithwaite, V. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour*. 74(2). pp. 231–238. <https://doi.org/10.1016/j.anbehav.2006.08.014>.
- Burrows, M., Schoeman, D., Buckley, L., Moore, P., Poloczanska, E., Brander, K., Brown, C., Bruno, J., Duarte, C., Halpern, B., Holding, J., Kappel, C., Kiessling, W., O'Connor, M., Pandolfi, J., Parmesan, C., Schwing, F., Sydeman, W., Richardson, A. (2011). The Pace of Shifting Climate in Marine and Terrestrial Ecosystems. *Science*. 334(6056). pp. 652–655. DOI: 10.1126/science.1210288.
- Chen, IC., Hill, JK., Ohlemüller, R., Roy, DB., Thomas, CD. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*. 333(6045). pp. 1024–1026. DOI: 10.1126/science.1206432.
- Clarke, KR, Gorley, RN. (2015). PRIMER v7: User Manual/Tutorial. PRIMER-E Plymouth.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2021). Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef fish. *Coral Reefs*. 40(5). pp. 1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2022). Coral-reef fishes can become more risk-averse at their poleward range limits. *Proceedings of the Royal Society B: Biological Sciences*. 289(1971). 20212676. <https://doi.org/10.1098/rspb.2021.2676>.

- Couzin, I., Krause, J., James, R., Ruxton, G., Franks, N. (2002). Collective Memory and Spatial Sorting in Animal Groups. *Journal of Theoretical Biology*. 218(1). pp. 1–11. <https://doi.org/10.1006/jtbi.2002.3065>
- Crane, A., Ferrari, MC. (2017). Patterns of predator neophobia: a meta-analytic review. *Proceedings of the Royal Society B: Biological Sciences*. 284(1861). 20170583. <https://doi.org/10.1098/rspb.2017.0583>.
- Delcourt, J., Poncin, P. (2012). Shoals and schools: back to the heuristic definitions and quantitative references. *Reviews in Fish Biology and Fisheries*. 22(3). pp. 595–619. <https://doi.org/10.1007/s11160-012-9260-z>.
- Djurichkovic, L., Donelson, J., Fowler, A., Feary, D., Booth, DJ. (2019). The effects of water temperature on the juvenile performance of two tropical damselfishes expatriating to temperate reefs. *Scientific Reports*. 9(1). 13937. <https://doi.org/10.1038/s41598-019-50303-z>.
- Donelson, JM., Sunday, JM., Figueira, WF., Gaitán-Espitia, JD, Hobday, AJ., Johnson, CR., Leis, JM., Ling, SD., Marshall, D, Pandolfi, JM., Pecl GT, Rodgers, GG., Booth, DJ., Munday PL. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Phil. Trans. R. Soc. B*3742018018620180186. <http://doi.org/10.1098/rstb.2018.0186>.
- Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*. 70. pp. 967–971. <https://doi.org/10.1016/j.anbehav.2005.01.016>.
- Feary, DA., Pratchett, MS., Emslie, MJ., Fowler, A., Figueira, WF., Luiz, OJ., Nakamura, Y., Booth, DJ. (2014). Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*. 15(4). pp. 593–615. <https://doi.org/10.1111/faf.12036>.
- Ferrari, MCO., McCormick, MI., Meekan, MG., Chivers, DP. (2015). Background level of risk and the survival of predator-naïve prey: can neophobia compensate for predator naivety in juvenile coral reef fishes? *Proceedings of the Royal Society B: Biological Sciences*. 20142197. <https://doi.org/10.1098/rspb.2014.2197>.
- Figueira, WF., Biro, P., Booth, DJ., Valenzuela Davie, VC. (2009). Performance of tropical fish recruiting to temperate habitats: role of ambient temperature and implications of climate change. *Marine Ecology Progress Series*. 384. pp. 231–239. <https://doi.org/10.3354/meps08057>.
- Figueira, WF., Curley, B., Booth, DJ. (2019). Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes?. *Marine Biology*. 166. 73. <https://doi.org/10.1007/s00227-019-3521-5>.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., et al. (2016). Package ‘Car.’ Available online at: <http://cran-r.project.org/web/packages/car/car.pdf>
- Froese, R., Pauly, D. (2016) FishBase. <http://www.fishbase.org>.
- Hintz, W., Lonzarich, D. (2018). Maximizing foraging success: the roles of group size, predation risk, competition, and ontogeny. *Ecosphere*. 9(10). e02456. <https://doi.org/10.1002/ecs2.2456>.
- Hoare, DJ., Krause, J. (2003). Social organisation, shoal structure and information transfer. *Fish and Fisheries*. 4. pp. 269–279. <https://doi.org/10.1046/j.1467-2979.2003.00130.x>.
- Krause, J., Ruxton, G., Krause, S. (2010). Swarm intelligence in animals and humans. *Trends in Ecology & Evolution*. 25(1). pp. 28–34. <https://doi.org/10.1016/j.tree.2009.06.016>.
- Landeau, L., Terborgh, J. (1986). Oddity and the ‘confusion effect’ in predation. *Animal Behaviour*. 34(5). pp. 1372–1380. [https://doi.org/10.1016/S0003-3472\(86\)80208-1](https://doi.org/10.1016/S0003-3472(86)80208-1).

- Lima, S.L., Steury, T.D. (2005). Perception of predation risk: the foundation of nonlethal predator–prey interactions. *Ecology of predator–prey interactions* (eds, Barbosa P & Castellanos I). pp. 166–188. Oxford, UK: Oxford University Press
- Lopes, A., Morais, P., Pimentel, M., Rosa, R., Munday, P.L., Gonçalves, E., Faria, A. (2016). Behavioural lateralization and shoaling cohesion of fish larvae altered under ocean acidification. *Marine Biology*. 163(12). pp. 1–10. <https://doi.org/10.1007/s00227-016-3026-4>.
- Lough, J. M. (2008). Shifting climate zones for Australia's tropical marine ecosystems. *Geophysical Research Letters*. 35. <https://doi.org/10.1029/2008GL034634>.
- Miles, J. (2014). R Square, Adjusted R Square. Wiley Stats Ref: Statistics Reference Online: John Wiley and Sons, Ltd.
- Mitchell, A., Booth, D.J., Nagelkerken, I. (2022). Ocean warming and acidification degrade shoaling performance and lateralization of novel tropical–temperate fish shoals. *Global Change Biology*. 28(4). pp. 1388–1401. <https://doi.org/10.1111/gcb.16022>.
- Nagelkerken I, Goldenberg SU, Ferreira CM, Russell BD, Connell, SD. (2017). Species interactions drive fish biodiversity loss in a high-CO₂ world. *Current Biology*. 27. pp. 2177–2184. <https://doi.org/10.1016/j.cub.2017.06.023>.
- Nakagawa, S., Schielzeth, H. (2013). A general and simple method for obtaining r^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*. 4. pp. 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>.
- Paijmans, K., Booth, D.J., Wong, M.Y. (2020). Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *Journal of Fish Biology*. 96(3). pp. 806–814. <https://doi.org/10.1111/jfb.14277>.
- Paijmans, K.C., Booth, D.J., Wong, M.Y. (2021). Single-species subgroups form within mixed-species shoals of tropical and temperate fishes. *Environmental Biology of Fishes*. 105(42). pp. 1–14 <https://doi.org/10.1007/s10641-021-01188-3>.
- Pecl, G., Araújo, M., Bell, J., Blanchard, J., Bonebrake, T., Chen, I., Clark, T., Colwell, R., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R., Griffis, R., Hobday, A., Janion-Scheepers, C., Jarzyna, M., Jennings, S., Lenoir, J., Linnetved, H., Martin, V., McCormack, P., McDonald, J., Mitchell, N., Mustonen, T., Pandolfi, J., Pettorelli, N., Popova, E., Robinson, S., Scheffers, B., Shaw, J., Sorte, C., Strugnell, J., Sunday, J., Tuanmu, M., Vergés, A., Villanueva, C., Wernberg, T., Wapstra, E. and Williams, S. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*. 355(6332). <https://doi.org/10.1126/science.aai9214>.
- Pessarrodona, A., Vergés, A., Bosch, N., Bell, S., Smith, S., Sgarlatta, M., Wernberg, T. (2022). Tropicalization unlocks novel trophic pathways and enhances secondary productivity in temperate reefs. *Functional Ecology*. 36. pp. 659–673. <https://doi.org/10.1111/1365-2435.13990>.
- Pinsky, M., Worm, B., Fogarty, M., Sarmiento, J., Levin, S. (2013). Marine taxa track local climate velocities. *Science*. 341(6151). pp. 1239–1242. <https://doi.org/10.1126/science.1239352>.
- Pitcher, T., Magurran, A. (1983). Shoal size, patch profitability and information exchange in foraging goldfish. *Animal Behaviour*. 31(2). pp. 546–555. [https://doi.org/10.1016/S0003-3472\(83\)80078-5](https://doi.org/10.1016/S0003-3472(83)80078-5).
- R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Saul, W.C., Jeschke, J.M. (2015). Eco-evolutionary experience in novel species interactions. *Ecology Letters*. 18(3). pp. 236–245. <https://doi.org/10.1111/ele.12408>.

- Smith, S., Fox, R., Booth, DJ., Donelson, J. (2018). Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion frontline. *Global Change Biology*. 24(4). pp. 1663–1672. <https://doi.org/10.1111/gcb.14016>.
- Suthers, I., Young, J., Baird, M., Roughan, M., Everett, J., Brassington, G., Byrne, M., Condie, S., Hartog, J., Hassler, C., Hobday, A., Holbrook, N., Malcolm, H., Oke, P., Thompson, P., Ridgway, K. (2011). The strengthening East Australian Current, its eddies and biological effects — an introduction and overview. *Deep Sea Research Part II: Topical Studies in Oceanography*. 58(5). pp. 538–546. <https://doi.org/10.1016/j.dsr2.2010.09.029>.
- Tekwa, E., Watson, J., Pinsky, M. (2022). Body size and food–web interactions mediate species range shifts under warming. *Proceedings of the Royal Society B: Biological Sciences*. 289(172). 20212755. <https://doi.org/10.1098/rspb.2021.2755>.
- Twiname, S., Fitzgibbon, Q., Hobday, A., Carter, C., Oellermann, M., Pecl, G. (2022). Resident lobsters dominate food competition with range-shifting lobsters in an ocean warming hotspot. *Marine Ecology Progress Series*. 685. pp. 171–181. <https://doi.org/10.3354/meps13984>.
- Ward, AJW., Herbert-Read, JE., Sumpter, DJT., Krause, J. (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proceedings of the National Academy of Sciences*. 108(6). pp. 2312–2315. <https://doi.org/10.1073/pnas.1007102108>.
- Ward, AJW., Schaerf, TM., Burns, ALJ., Lizier, JT., Crosato, E., Prokopenko, M., Webster, M.M. (2018). Cohesion, order and information flow in the collective motion of mixed-species shoals. *Royal Society open science*. 5. 181132. <http://doi.org/10.1098/rsos.181132>.
- Winer, BJ, Brown, DR, Michels, KM. (1991). *Statistical principles in experimental design*. 3rd edn. McGraw-Hill, New York.
- Vergés, A., Steinberg, PD., Hay, ME., Poore, AGB., Campbell, AH., Ballesteros, E., Heck, KL., Booth, DJ., Coleman, MA., Feary, DA., Figueira, W., Langlois, T., Marzinelli, EM., Mizerek, T., Mumby, PJ., Nakamura, Y., Roughan, M., van Sebille, E., Sen Gupta, A., Smale, DA., Tomas, F., Wernberg, T., Wilson, SK. (2014). The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society of B: Biological Sciences*. 281(1789). 20140846. <https://doi.org/10.1098/rspb.2014.0846>.
- Yamano, H., Sugihara, K., Nomura, K. (2011). Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophysical Research Letters*. 38(4). L04601. <https://doi.org/10.1029/2010GL046474>.

Supplementary Information

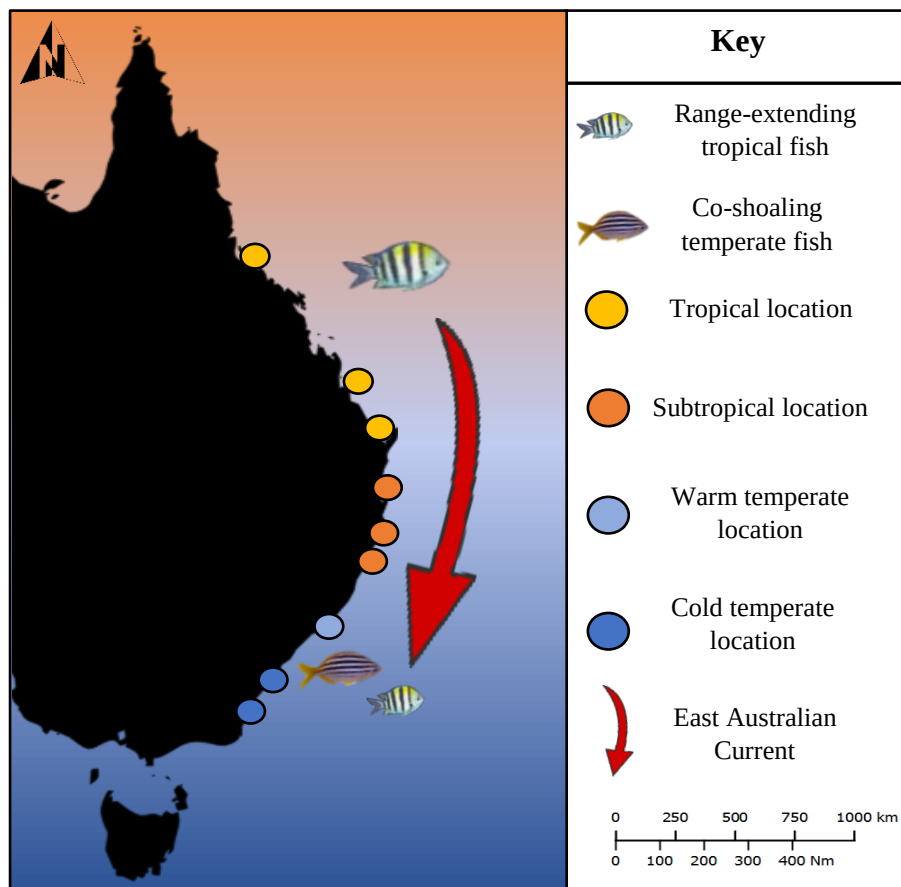


Figure S1: Biogeographic range locations of range-extending tropical fish that were studied along the southeastern coast of Australia. The East Australian Current transports dispersive larvae of coral reef tropical fish into cold-temperate range locations during the Austral summer and autumn (Booth et al., 2007). Juvenile tropical fish currently fail to permanently establish in temperate locations but can reduce novel environmental effects by shoaling with local temperate fishes (Figueira et al., 2009; Booth et al., 2011; Smith et al., 2018). Note: distance scale bar approximated.

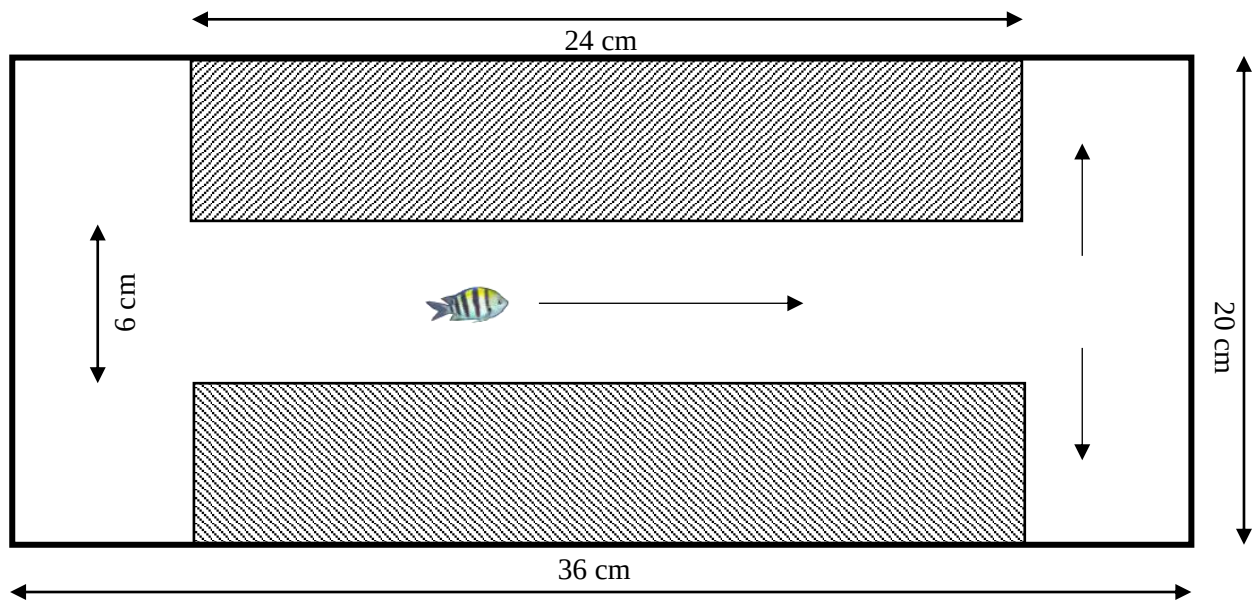


Figure S2: Diagram (top view) of tank used for standardised detour test for focal tropical (*A. vaigiensis*) and focal temperate (*A. strigatus*) species. Not to scale.

Table S1: Replication for the various behaviours assessed across range zones. *N/A* = not applicable.

Proxy	Range zone			
Lateralization (L_R and L_A)	Tropical	Subtropical	Warm Temperate	Cold Temperate
<i>Tropical</i>	5	39	54	68
<i>Temperate</i>	<i>N/A</i>	6	64	80
Shoaling dynamics	Tropical	Subtropical	Warm Temperate	Cold Temperate
<i>Tropical</i>	27	41	9	23
<i>Temperate</i>	<i>N/A</i>	<i>N/A</i>	14	31
<i>Mixed-species</i>	<i>N/A</i>	8	21	34
Flight initiation distance	Tropical	Subtropical	Warm Temperate	Cold Temperate
<i>Tropical</i>	10	17	8	18
<i>Temperate</i>	<i>N/A</i>	<i>N/A</i>	21	34

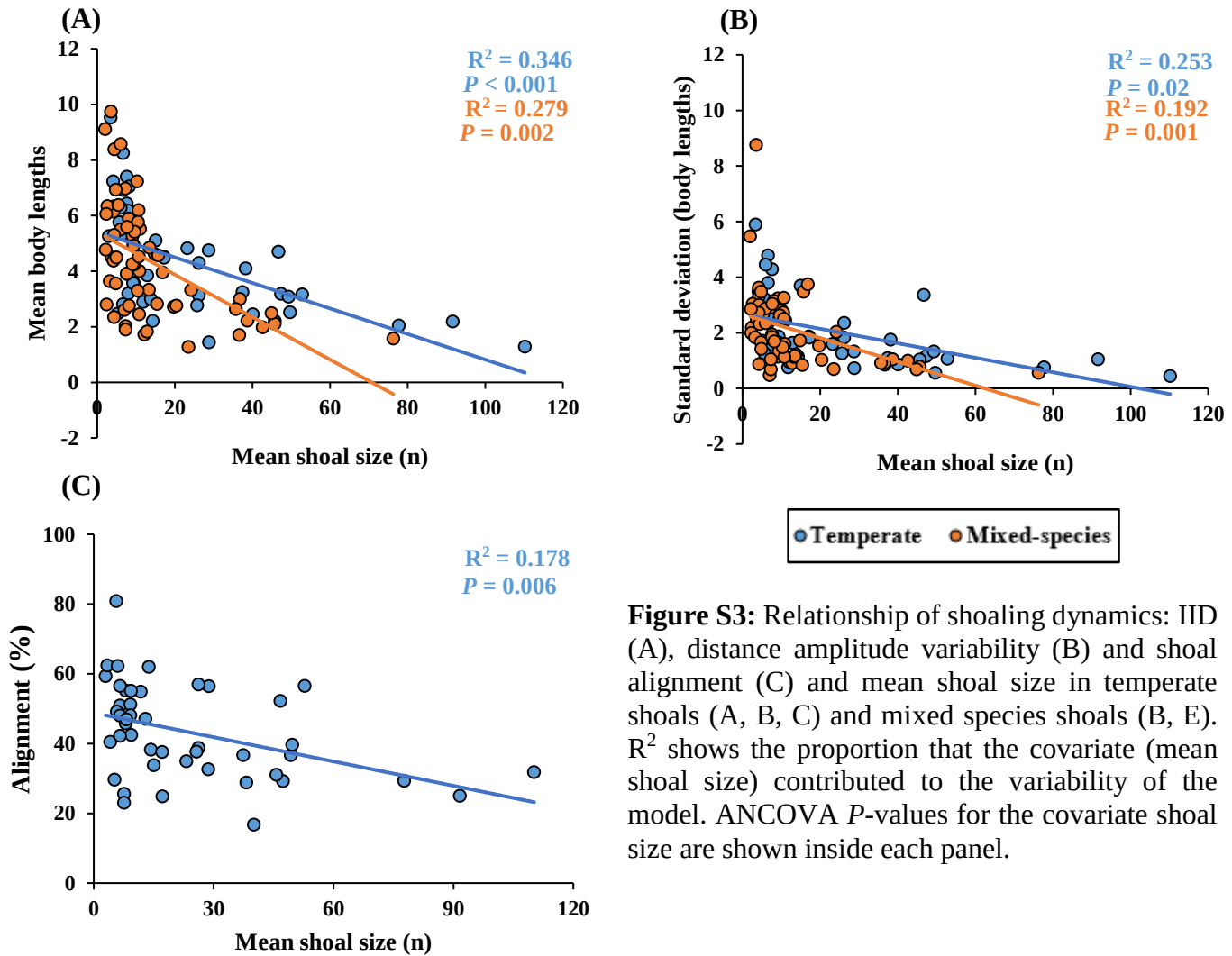


Figure S3: Relationship of shoaling dynamics: IID (A), distance amplitude variability (B) and shoal alignment (C) and mean shoal size in temperate shoals (A, B, C) and mixed species shoals (B, E). R^2 shows the proportion that the covariate (mean shoal size) contributed to the variability of the model. ANCOVA P -values for the covariate shoal size are shown inside each panel.

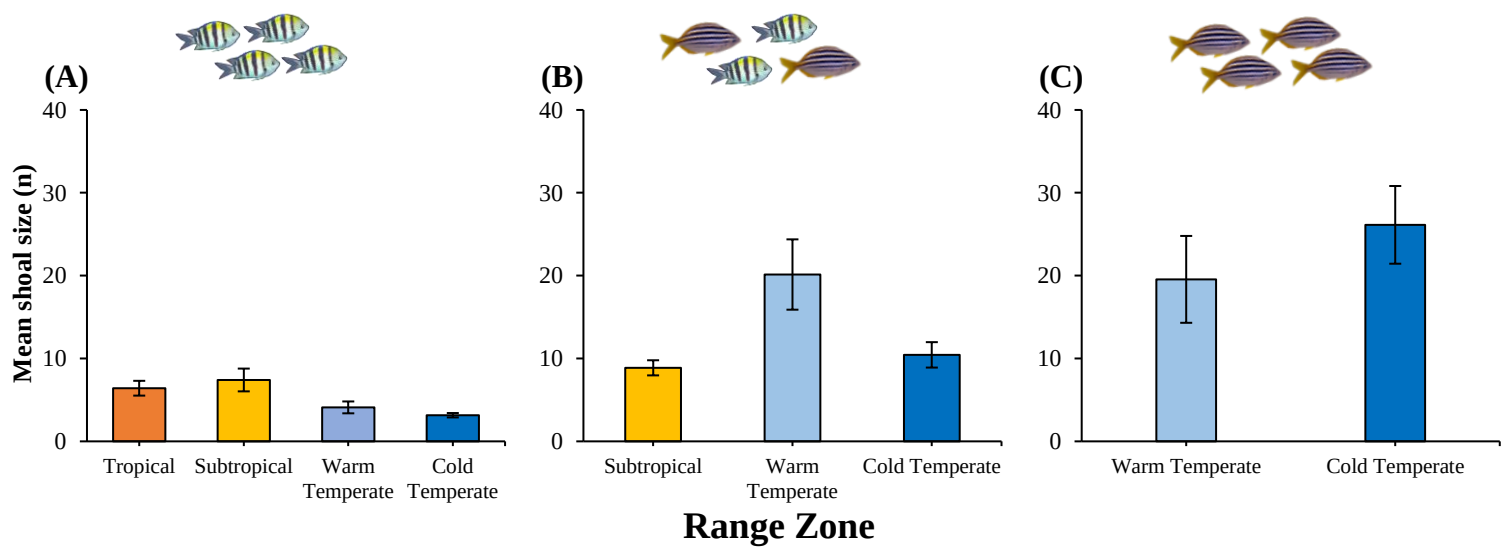


Figure S4: Mean shoal size ($\pm$ SE) of tropical (A), mixed-species (B), and temperate (C) shoals across range zones. Error bars denote standard error.

Mixed-species shoal IID and DAV

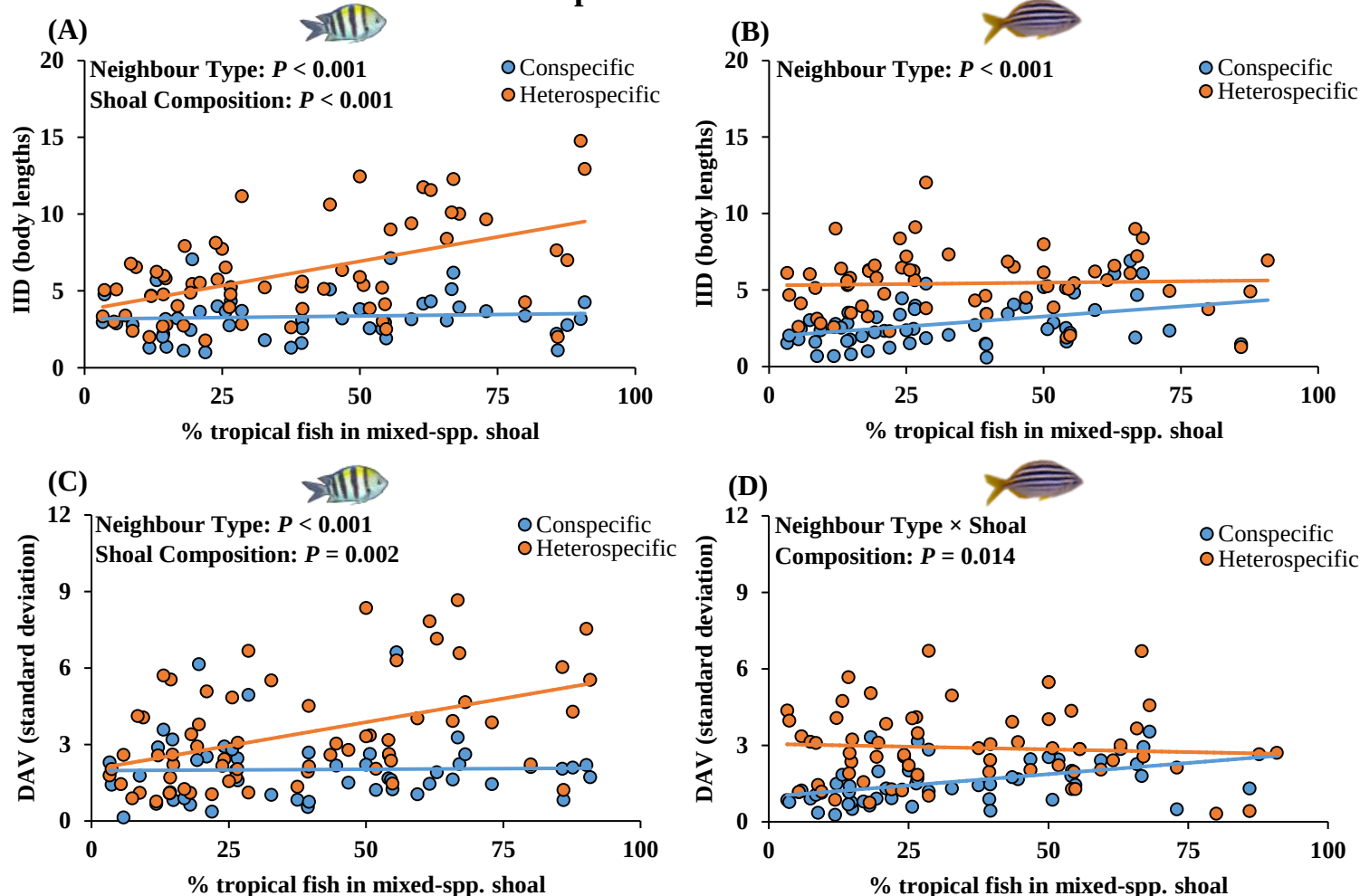


Figure S5: Results of linear mixed-effect models for conspecific and heterospecific IID (A, B) and DAV (C, D) within mixed-species shoals across range zones for tropical (A, C) and temperate fish (B, D). For tropical IID and DAV values conspecific neighbour type refers to focal tropical species neighbours, while heterospecific refers to temperate fish neighbours. Vice versa, temperate conspecific refers to *A. strigatus* shoal neighbours, and heterospecific neighbours refer to tropical shoal neighbours in mixed-species shoals. Corresponding model outputs are in Tables S22-29.

Table S2: MANCOVA output of tropical shoaling dynamics and resulting pairwise tests. Note: **bold** p -value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	8.290	8.290	0.276	0.586
Range zone	3	1382.200	460.730	5.125	0.049
Range location	5	380.860	76.172	5.451	<0.001
Residuals	90	1257.700	13.975		
Total	99	3029.100			
Pairwise test of term 'Range Zone'. t = statistic. Note: bold P -value indicates significant P -value ($P < 0.05$).					
Range Zone			t	P -value	
Tropical Zone, Subtropical Zone			0.739	0.471	

Tropical Zone, Warm Temperate Zone	1.458	0.283
Tropical Zone, Cold Temperate Zone	2.622	0.084
Subtropical Zone, Warm Temperate Zone	2.119	0.164
Subtropical Zone, Cold Temperate Zone	3.475	0.044
Warm Temperate Zone, Cold Temperate Zone	1.124	0.457
Pairwise test of term 'Range location' within level 'Tropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).		
Range location	t	P-value
19.1° S, 23.4° S	2.614	0.008
19.1° S, 24.8° S	2.467	0.024
23.4° S, 24.8° S	0.795	0.480
Pairwise test of term 'Range location' within level 'Subtropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).		
Range location	t	P-value
28.2° S, 30.4° S	3.195	0.005
28.2° S, 30.9° S	3.150	0.004
30.4° S, 30.9° S	1.433	0.174
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).		
Range location	t	P-value
36.2° S, 36.9° S	1.249	0.225

Table S3: ANCOVA output of tropical shoal inter-individual distance and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.002	0.002	0.071	0.794
Range zone	3	1.221	0.407	6.134	0.034
Range location	5	0.282	0.056	2.855	0.020
Residuals	90	1.778	0.020		
Total	99	3.283			
Pairwise test of term ‘Range Zone’. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range zone		t		P-value	
Tropical Zone, Subtropical Zone		0.758		0.451	
Tropical Zone, Warm Temperate Zone		1.622		0.249	
Tropical Zone, Cold Temperate Zone		2.308		0.111	
Subtropical Zone, Warm Temperate Zone		3.580		0.049	
Subtropical Zone, Cold Temperate Zone		4.552		0.020	
Warm Temperate Zone, Cold Temperate Zone		0.491		0.895	
Pairwise test of term ‘Range location’ within level ‘Tropical Zone’ of factor ‘Range Zone’. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	

19.1° S, 23.4° S	2.864	0.010
19.1° S, 24.8° S	1.008	0.329
23.4° S, 24.8° S	1.673	0.120
Pairwise test of term 'Range location' within level 'Subtropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).		
Range location	t	P-value
28.2° S, 30.4° S	0.827	0.425
28.2° S, 30.9° S	1.602	0.120
30.4° S, 30.9° S	1.038	0.312
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).		
Range location	t	P-value
36.2° S, 36.9° S	1.319	0.198

Table S4: ANCOVA output of tropical shoal amplitude variability and resulting pairwise tests. Note: **bold** p-value denotes significant differences ($p < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.057	0.056	1.785	0.189
Range zone	3	0.663	0.221	3.069	0.112
Range location	5	0.309	0.062	2.907	0.018
Residuals	90	1.914	0.021		
Total	99	2.944			
Pairwise test of term 'Range location' within level 'Tropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
19.1° S, 23.4° S		2.674		0.016	
19.1° S, 24.8° S		0.676		0.506	
23.4° S, 24.8° S		2.051		0.059	
Pairwise test of term 'Range location' within level 'Subtropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
28.2° S, 30.4° S		3.074		0.008	
28.2° S, 30.9° S		1.686		0.103	
30.4° S, 30.9° S		0.145		0.888	
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
36.2, 36.9		1.254		0.230	

Table S5: ANCOVA output of tropical shoal alignment and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.270	0.270	2.993	0.075
Range zone	3	3.826	1.275	5.048	0.044

Range location	5	1.071	0.214	4.555	0.002
Residuals	90	4.234	0.047		
Total	99	9.400			
Pairwise test of term 'Range Zone'. t = statistic. Note: bold p-value indicates significant P-value ($P < 0.05$).					
Range zone		t		P-value	
Tropical Zone, Subtropical Zone		0.734		0.459	
Tropical Zone, Warm Temperate Zone		1.223		0.347	
Tropical Zone, Cold Temperate Zone		3.227		0.045	
Subtropical Zone, Warm Temperate Zone		1.706		0.229	
Subtropical Zone, Cold Temperate Zone		3.610		0.036	
Warm Temperate Zone, Cold Temperate Zone		7.229		0.093	
Pairwise test of term 'Range location' within level 'Tropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
19.1° S, 23.4° S		1.455		0.149	
19.1° S, 24.8° S		2.520		0.022	
23.4° S, 24.8° S		0.553		0.628	
Pairwise test of term 'Range location' within level 'Subtropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
28.2° S, 30.4° S		3.106		0.001	
28.2° S, 30.9° S		3.089		0.006	
30.4° S, 30.9° S		1.582		0.131	
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location		t		P-value	
36.2° S, 36.9° S		0.302		0.763	

Table S6: MANCOVA output of temperate shoaling dynamics and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	64.498	64.498	5.901	0.022
Range zone	1	1.112	1.112	0.156	0.944
Range location	1	13.909	13.909	1.392	0.243
Residuals	41	409.700	9.993		
Total	44	489.22			

Table S7: ANCOVA output of temperate shoal inter-individual distance and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.461	0.461	15.518	<0.001
Range zone	1	0.001	0.001	0.128	0.841

Range location	1	0.080	0.080	5.756	0.021
Residuals	41	0.569	0.013		
Total	44	1.120			
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range location			t	P-value	
36.2° S, 36.9° S			2.379	0.023	

Table S8: ANCOVA output of temperate shoal amplitude variability and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.465	0.465	12.133	0.002
Range zone	1	<0.001	<0.001	0.024	1.000
Range location	1	0.101	0.101	5.446	0.027
Residuals	41	0.761	0.019		
Total	44	1.328			
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold p-value indicates significant P-value ($P < 0.05$).					
Range location			t	P-value	
36.2° S, 36.9° S			2.265	0.031	

Table S9: ANCOVA output of temperate shoal alignment and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.329	0.329	9.046	0.006
Range zone	1	0.003	0.003	0.181	0.944
Range location	1	0.039	0.039	1.096	0.302
Residuals	41	1.461	0.036		
Total	44	1.833			

Table S10: MANCOVA output of mixed-species shoaling dynamics and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	324.840	324.840	13.549	0.001
Range zone	2	236.880	118.440	41.986	0.013
Range location	2	5.178	2.589	0.083	0.928
Residuals	57	1768.100	31.020		
Total	62	2335			
Pairwise test of term 'Range Zone'. t = statistic. Note: bold p-value indicates significant P-value ($P < 0.05$).					
Range zone			t	P-value	
Subtropical Zone, Warm Temperate Zone			6.942	0.036	
Subtropical Zone, Cold Temperate Zone			3.443	0.002	

Warm Temperate Zone, Cold Temperate Zone	2.790	0.160
--	-------	-------

Table S11: ANCOVA output of mixed-species shoal inter-individual distance and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.578	0.578	20.750	<0.001
Range zone	2	0.020	0.010	0.190	0.890
Range location	2	0.115	0.057	3.167	0.054
Residuals	57	1.033	0.018		
Total	62	1.745			

Table S12: ANCOVA output of mixed-species shoal amplitude variability and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.555	0.555	26.489	<0.001
Range zone	2	0.002	0.001	0.235	0.970
Range location	2	0.031	0.015	0.677	0.512
Residuals	57	1.298	0.023		
Total	62	1.887			

Table S13: ANCOVA output of mixed-species shoal alignment and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Shoal size	1	0.026	0.026	0.305	0.577
Range zone	2	0.631	0.315	1.534	0.397
Range location	2	0.373	0.187	3.470	0.035
Residuals	57	3.064	0.054		
Total	62	4.095			
Pairwise test of term 'Range location' within level 'Subtropical Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($p < 0.05$).					
Range location		t		P-value	
28.2° S, 30.4° S		1.626		0.174	
Pairwise test of term 'Range location' within level 'Cold Temperate Zone' of factor 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($p < 0.05$).					
Range location		t		P-value	
36.2° S, 36.9° S		0.659		0.545	

Table S14: ANOVA output of tropical fish bite rate and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
--------	----	----	----	---------	---------

Range zone	3	0.189	0.063	5.489	0.033
Shoal type	1	0.052	0.052	22.825	0.040
Range location	5	0.052	0.010	1.187	0.311
Range zone × Shoal type	2	0.044	0.022	9.849	0.092
Range location × Shoal type	2	0.004	0.002	0.230	0.763
Residuals	137	1.199	0.009		
Total	150	1.619			
Pairwise test of term ‘Range zone’. t = statistic. Note: bold <i>P</i> -value indicates significant <i>P</i> -value (<i>P</i> < 0.05).					
Range zone		t	P-value		
Tropical Zone, Subtropical Zone		1.226	0.225		
Tropical Zone, Warm Temperate Zone		2.737	0.009		
Tropical Zone, Cold Temperate Zone		5.441	<0.001		
Subtropical Zone, Warm Temperate Zone		2.962	0.005		
Subtropical Zone, Cold Temperate Zone		3.256	0.002		
Warm Temperate Zone, Cold Temperate Zone		0.668	0.515		
Pairwise test of term ‘Shoal type. t = statistic. Note: bold <i>P</i> -value indicates significant <i>P</i> -value (<i>P</i> < 0.05).					
Shoal type		t	P-value		
Tropical-only, Mixed-species		2.336	0.023		

Table S15: ANOVA output of temperate fish bite rate and resulting pairwise tests. df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Range zone	2	0.003	0.002	0.200	0.959
Shoal type	1	0.039	0.039	0.985	0.495
Range location	2	0.029	0.014	1.624	0.206
Range zone × Shoal type	1	0.008	0.008	0.239	0.706
Range location × Shoal type	1	0.035	0.035	3.971	0.056
Residuals	86	0.762	0.009		
Total	93	0.896			

Table S16: ANOVA output of tropical fish relative lateralization and resulting pairwise tests. Note: **bold** P-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	P-value
Range zone	3	1.255	0.418	17.165	0.010
Range location (RZ)	5	0.057	0.011	0.331	0.807
Shoal type (RL)	3	0.040	0.013	0.203	0.897
Residuals	157	10.278	0.065		
Total	168	11.654			
Pairwise test of term 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($P < 0.05$).					
Range zone		t	P-value		
Cold Temperate Zone, Warm Temperate Zone		1.964	0.046		

Cold Temperate Zone, Subtropical Zone	13.670	<0.001
Cold Temperate Zone, Tropical Zone	2.861	0.005
Warm Temperate Zone, Subtropical Zone	1.438	0.370
Warm Temperate Zone, Tropical Zone	1.249	0.264
Subtropical Zone, Tropical Zone	0.186	0.853

Table S17: ANOVA output of tropical fish absolute lateralization and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>P</i> -value
Range zone	3	2.878	0.959	1.255	0.404
Range location (RZ)	5	3.737	0.747	1.442	0.426
Shoal type (RL)	3	0.999	0.333	0.423	0.742
Residuals	157	123.760	0.788		
Total	168	132.090			

Table S18: ANOVA output of temperate fish relative lateralization. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>P</i> -value
Range zone	2	0.003	0.002	0.107	0.879
Range location (RZ)	1	0.117	0.117	2.073	0.247
Shoal type (RL)	3	0.064	0.021	0.194	0.898
Residuals	143	15.652	0.109		
Total	149	16.046			

Table S19: ANOVA output of temperate fish absolute lateralization. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square.

Source	df	SS	MS	F-value	<i>P</i> -value
Range zone	2	0.423	0.212	0.273	0.805
Range location (RZ)	1	1.084	1.084	2.485	0.223
Shoal type (RL)	3	0.366	0.122	0.135	0.938
Residuals	143	129.700	0.907		
Total	149	133.860			

Table S20: ANOVA output of tropical fish flight initiation distance and resulting pairwise tests. Note: **bold** *P*-value denotes significant differences ($P < 0.05$). df = degrees of freedom, SS = sum of squares, MS = mean square. Random nested factor Range Location was pooled as in final model were $P > 0.25$.

Source	df	SS	MS	F-value	<i>P</i> -value
Range Zone	3	1830.500	610.170	8.963	<0.001
Pooled	49	3335.900	68.080		
Total	52	5166.400			

Pairwise test of term 'Range Zone'. t = statistic. Note: bold P-value indicates significant P-value ($p < 0.05$).		
Range zone	t	P-value
Cold Temperate Zone, Warm Temperate Zone	1.393	0.175
Cold Temperate Zone, Subtropical Zone	4.959	<0.001
Cold Temperate Zone, Tropical Zone	2.723	0.012
Warm Temperate Zone, Subtropical Zone	2.849	0.006
Warm Temperate Zone, Tropical Zone	1.056	0.312
Subtropical Zone, Tropical Zone	1.944	0.062

Table S21: ANOVA output of temperate fish flight initiation distance. df = degrees of freedom, SS = sum of squares, MS = mean square. Random nested factor Range Location was pooled as in final model were $P > 0.25$.

Source	df	SS	MS	F-value	P-value
Range Zone	1	0.074	0.074	1.280	0.260
Pooled	53	3.074	0.058		
Total	54	3.148			

Table S22: Mixed-species shoal tropical fish IID LMM estimates for fixed and random effects. LMM fit by REML. T-tests use Kenward-Roger's method. Note: **bold** P-value denotes significant effect ($P < 0.05$). The p-value for random effects denotes Ranova test. Marginal $R^2 = 0.328$. Conditional $R^2 = 0.594$.

Fixed effects	Estimate	Std. Error	95% CI	df	t-value	P-value
(Intercept)	1.48	0.10	1.27 – 1.68	2.95	14.21	<0.001
Shoal composition	0.39	0.10	0.19 – 0.59	106.55	3.79	<0.001
Range zone 1 (Subtropical)	0.03	0.12	-0.21 – 0.28	1.89	0.28	0.808
Range zone 2 (Warm temperate)	0.05	0.13	-0.20 – 0.30	2.14	0.43	0.709
Neighbour type (Heterospecific)	-0.11	0.05	-0.22 – -0.01	105.02	-2.16	0.033
Shoal composition × Neighbour type	-0.18	0.10	-0.38 – 0.02	105.02	-1.82	0.072
Range zone 1 × Neighbour type	0.04	0.04	-0.03 – 0.11	105.01	1.19	0.235
Range zone 2 × Neighbour type	-0.06	0.05	-0.15 – 0.03	105.01	-1.35	0.179
Random effects/ Group	Name	Variance		Std. dev		P-value
Range location	(Intercept)	0.036		0.19		<0.001
Residual		0.054		0.23		

Table S23: Mixed-species shoal tropical fish IID Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df). Neighbour type refers to either conspecific tropical or heterospecific temperate neighbour within mixed-species shoals. Note: **bold** *P*-value denotes significant effect ($P < 0.05$).

Group	F	df	df.res	<i>P</i> -value
Shoal composition	15.18	1	106.55	<0.001
Range zone	0.12	2	1.91	0.89
Neighbour type	56.92	1	105.01	<0.001
Shoal composition × Neighbour type	3.30	1	105.02	0.072
Range zone × Neighbour type	0.98	2	105.01	0.381

Table S24: Mixed-species shoal tropical fish DAV LMM estimates for fixed and random effects. LMM fit by REML. T-tests use Kenward-Roger's method. Note: **bold** *P*-value denotes significant effect ($P < 0.05$). The *p*-value for random effects denotes Ranova test. Marginal $R^2 = 0.259$. Conditional $R^2 = 0.367$.

Fixed effects	Estimate	Std. Error	95% CI	df	t-value	<i>P</i> -value
(Intercept)	1.20	0.08	1.04 – 1.35	5.49	15.37	<0.001
Shoal composition	0.36	0.11	0.14 – 0.58	106.89	3.18	0.002
Range zone 1 (Subtropical)	0.03	0.8	-0.12 – 0.18	1.81	0.38	0.741
Range zone 2 (Warm temperate)	0.04	0.08	-0.13 – 0.21	2.58	0.46	0.685
Neighbour type (Heterospecific)	-0.05	0.06	-0.16 – 0.07	105.09	-0.815	0.417
Shoal composition × Neighbour type	-0.20	0.11	-0.42 – 0.02	105.09	-1.83	0.070
Range zone 1 × Neighbour type	0.01	0.04	-0.07 – 0.09	105.07	0.28	0.784
Range zone 2 × Neighbour type	-0.01	0.05	-0.11 – 0.09	105.07	-0.25	0.805
Random effects/ Group	Name	Variance	Std. dev	<i>P</i> -value		
Range location	(Intercept)	0.036	0.189	0.35		
Residual		0.054	0.233			

Table S25: Mixed-species shoal tropical fish DAV Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df). Note: **bold** *P*-value denotes significant effect ($P < 0.05$).

Group	F	df	Df.res	<i>P</i> -value
Shoal composition	10.46	1	106.89	0.002
Range zone	0.18	2	1.75	0.850

Neighbour type	25.66	1	105.09	<0.001
Shoal composition × Neighbour type	3.34	1	105.09	0.070
Range zone × Neighbour type	0.04	2	105.08	0.960

Table S26: Mixed-species shoal temperate fish IID LMM estimates for fixed and random effects. LMM fit by REML. T-tests use Kenward-Roger's method. Note: **bold** *P*-value denotes significant effect ($P < 0.05$). The *P*-value for random effects denotes Ranova test. Marginal $R^2 = 0.277$. Conditional $R^2 = 0.661$.

Fixed effects	Estimate	Std. Error	95% CI	df	t-value	P-value
(Intercept)	1.26	0.17	0.91 – 1.60	2.1 1	7.21	0.016
Shoal composition	0.42	0.16	0.10 – 0.74	106 .46	2.61	0.011
Range zone 1 (Subtropical)	0.01	0.26	-0.50 – 0.53	2.5 6	0.46	0.967
Range zone 2 (Warm temperate)	-0.11	0.29	-0.69 – 0.47	1.8 7	-0.38	0.741
Neighbour type (Heterospecific)	0.48	0.08	0.32 – 0.63	106 .02	6.14	<0.001
Shoal composition × Neighbour type	-0.37	0.21	-0.77 – 0.04	106 .04	-1.77	0.080
Range zone 1 × Neighbour type	-0.01	0.15	-0.31 – 0.29	106 .02	-0.06	0.955
Range zone 2 × Neighbour type	0.024	0.09	-0.16 – 0.21	106 .01	0.27	0.790
Random effects/ Group	Name	Variance		Std. dev		P-value
Range location	(Intercept)	0.054		0.23		0.011
Residual		0.048		0.22		

Table S27: Mixed-species shoal temperate fish IID Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df). Note: **bold** *P*-value denotes significant effect ($P < 0.05$).

Group	F	df	Df.res	P-value
Shoal composition	3.79	1	106.71	0.054
Range zone	0.06	2	1.92	0.946
Neighbour type	77.43	1	106.02	<0.001
Shoal composition × Neighbour type	3.12	1	106.04	0.080
Range zone × Neighbour type	0.05	2	106.01	0.955

Table S28: Mixed-species shoal temperate fish DAV LMM estimates for fixed and random effects. LMM fit by REML. T-tests use Kenward-Roger's method. Note: **bold** *P*-value denotes significant effect ($P < 0.05$). The *P*-value for random effects denotes Ranova test. Marginal $R^2 = 0.220$. Conditional $R^2 = 0.507$.

Fixed effects	Estimate	Std. Error	95% CI	df	t-value	<i>P</i> -value
(Intercept)	1.20	0.10	1.01 – 1.39	2.95	12.51	0.001
Shoal composition	0.15	0.11	-0.06 – 0.36	107.13	1.42	0.159
Zone 1 (Subtropical)	0.01	0.11	-0.21 – 0.23	1.80	0.06	0.961
Zone 2 (Warm temperate)	0.02	0.12	-0.21 – 0.26	2.23	0.20	0.857
Neighbour type (Heterospecific)	-0.21	0.05	-0.31 – -0.11	106.05	-4.22	<0.001
Shoal composition × Neighbour type	0.26	0.11	0.05 – 0.47	106.07	2.50	0.014
Range zone 1 × Neighbour type	0.002	0.03	-0.06 – 0.07	106.03	0.09	0.930
Range zone 2 × Neighbour type	0.03	0.05	-0.07 – 0.12	106.04	0.51	0.610
Random effects/ Group	Name	Variance		Std. dev		<i>P</i> -value
Site	(Intercept)	0.029		0.170		0.082
Residual		0.050		0.223		

Table S29: Mixed-species shoal temperate fish DAV Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df). Note: **bold** *P*-value denotes significant effect ($P < 0.05$).

Group	F	df	df.res	<i>P</i> -value
Shoal composition	0.90	1	107.10	0.344
Range zone	0.02	2	1.86	0.982
Neighbour type	38.82	1	106.04	<0.001
Shoal composition × Neighbour type	6.24	1	106.07	0.014
Range zone × Neighbour type	0.35	2	106.04	0.704

Chapter VI

General Discussion



General Discussion

My thesis advances our understanding of the mechanisms driving tropicalisation in temperate fish communities under a changing climate. By analysing both the direct effects of ocean warming and acidification and the indirect effects of novel species interactions, I reveal the complex interplay between these factors on range-extension outcomes (Table 1). In addition, I highlight the capacity of local temperate fish communities to respond to tropical fish range extensions under current and future climatic scenarios. My research demonstrates the importance of novel species interactions in predicting future fish assemblages in warming marine ecosystems. The capacity of tropical and temperate species to adjust their behavioural and physiological responses plays a key role in these interactions (Nagelkerken & Munday, 2016) and currently enables for their coexistence at temperate latitudes (Kingsbury et al., 2020; Coni et al., 2021a; 2021b). However, as climate change shifts environmental conditions within temperate ecosystems, tropical and temperate species must adapt their responses to persist at their respective leading and trailing range edges (Donelson et al., 2019). By investigating how novel shoaling interactions and the behavioural and physiological responses underpinning these interactions are affected by current and future climatic conditions, I have provided novel insights into how the performance of range-extending tropical and local temperate fishes may change in a future ocean (Fig. 1). Overall, my findings have significant implications for our understanding of the impacts of climate change on the tropicalisation of temperate marine ecosystems, and the capacity of tropical and temperate fishes to adapt to changing environments.

Table 1: Key findings of my PhD research which how ocean acidification, warming and novel species interactions modify range-shift outcomes in southeastern Australian marine ecosystems.

Key Findings
The shoaling dynamics and lateralisation of co-shoaling tropical and temperate fishes are degraded due to the direct effects of ocean acidification and may slow down tropical species range extensions (Chapter 2)
Future seasonal temperatures seasonally shift the behaviour (but only in the tropical fish), and fitness and physiological performance of co-shoaling tropical and temperate fishes, which could seasonally modify range-shift outcomes (Chapters 3 and 4)
Tropical fishes benefit more from novel species interactions (mixed-species shoaling) than conspecific interactions at their cold-range edges through increased foraging efficiency (Chapter 5). However, future climatic conditions (ocean warming and acidification) may degrade novel shoaling interactions and slow range extensions in a future ocean (Chapter 2).
Temperate fish behaviour remained unaffected by novel species interactions across their subtropical and temperate ranges (Chapter 5) and under future climatic conditions (Chapter 3)

Future Seasonal Effects on Tropical and Temperate Species Performance

Under current climatic conditions, novel shoaling interactions concurrently enhance tropical fish fitness (Chapter 5; Smith et al., 2018; Paijmans et al., 2020; but see Coni et al., 2021a). Other research has postulated that tropical and temperate fishes will likely coexist at

temperate latitudes under ocean warming (Coni et al., 2021b; Kingsbury et al., 2019). However, my findings reveal contrasting seasonal shifts in the fitness and physiological performance of co-shoaling tropical and temperate fishes (Chapters 3 and 4). In general, I found that future seasonal temperatures drive both positive and negative shifts in the behavioural (Chapter 3) and physiological (Chapter 4) performance of co-shoaling tropical and temperate fishes. Future summer conditions concurrently facilitate declines in temperate fish fitness and physiological function while enhancing the tropical species' behavioural and physiological performance. In contrast, future winter conditions may be advantageous to temperate than tropical species at high latitude ecosystems, facilitating a reversal in the overall performance of competing range-extending and local species as a function of future seasonal conditions. Ocean acidification also amplified oxidative stress responses to future winter conditions in competing tropical and temperate fishes (Chapter 4). Thus, opposing responses to changes in seasonal conditions could modify competitive (Coni et al., 2021a), shoaling (Smith et al., 2018; Paijmans et al., 2020) and predator-prey (Beck et al., 2016; Figueira et al., 2019) interactions which currently mediate tropical-temperate species coexistence. I conclude that seasonal performance shifts could temporally modify both tropical and temperate species respective leading and trailing range edges across temporal and spatial scales under a future climate.

Range-Shift Outcomes under Ocean acidification

Ocean warming may facilitate the tropicalisation of temperate fish communities, but ocean acidification may directly slow the establishment of tropical fish at higher latitudes. Reductions in shoaling performance and lateralisation are likely to degrade the fitness of competing tropical and temperate fishes and could, therefore, negatively modify range-shift outcomes (Chapter 2). Tropical fishes benefit from interactions with local temperate species

(Smith et al., 2018; Paijmans et al., 2020; Chapter 5). However, ocean acidification negatively impacts the shoaling behaviours of co-shoaling tropical and temperate fishes and may therefore temper any benefits accrued from tropical-temperate shoaling opportunities. Previous research has shown that ocean acidification can also indirectly slow tropicalisation through habitat shifts, which facilitate the emergence of unsuitable turf habitats (Coni et al., 2021c). Ocean acidification's indirect effects may ultimately simplify (Agostini et al., 2021) and reshuffle (Nagelkerken & Connell, 2022) temperate ecosystems undergoing range shifts. In addition, I reveal that direct ocean acidification effects may also slow tropicalisation and can have fitness-limiting effects on local temperate fishes. I conclude that ocean acidification can alter the novel species interactions that mediate species range-shift outcomes and slow down the pace of tropicalisation in southeastern Australia.

Temperate Fish Performance under a Future Climate

Temperate fish behaviour remained unaffected by novel species interactions across their subtropical and temperate ranges (Chapter 5), and under future climatic conditions (Chapter 3). In contrast, their fitness and physiological performance declined in response to future summer conditions (ocean acidification and warming; Chapter 3 and 4). Reduced fitness and physiological function coinciding with no change in behaviour suggests low behavioural plasticity in their responses to future climatic conditions and novel species interactions, which may limit their ability to adapt to warming conditions at their trailing edges (Donelson et al., 2019). In addition, as climate change ramps up, the narrowing of their behavioural plasticity (Rodriguez-Dominguez et al., 2022) could reduce their capacity to respond to arriving low-latitude competitors (Alexander et al., 2015) and predators (Jones et al., 2020; Shepard et al., 2021), accelerating temperate species declines at their trailing range edges. Thus, while

temperate and tropical fishes may coexist at the temperate species' current warm-trailing edges (Coni et al., 2021b), their ability to compete against co-shoaling tropical fishes may be limited by future climate.

Tropical Fish Species Responses to Novel Temperate Environments

Using a latitudinal climate gradient allowed me to reveal behavioural shifts of range-extending tropical fish entering novel environments. Specifically, I reveal that range-extending tropical fish trade-off foraging efficiency for predator vigilance at their warm- and cold-temperate ranges compared to their (sub)tropical ranges (i.e. increased population-level lateralisation, flight initiation distance and shoaling dynamics), independent of novel shoaling interactions. However, novel mixed-species shoaling increases the foraging efficiency of tropical fish, which may allow them to persist longer or perform better at their cold-temperate range edges (Chapter 5). My findings highlight that shifts in trade-offs between foraging and predator vigilance may enhance survival during the initial stages of range extensions. However, diminished foraging could limit tropical fish survivorship and prevent overwintering in temperate ecosystems. Since not all behavioural trade-offs are adaptive, intra-individual variability and plasticity in their responses to novel conditions (Donelson et al., 2019) and positive species interactions could enhance the likelihood of long-term establishment in temperate ecosystems (e.g. shoaling with local species; Paijmans et al., 2020). For example, I found that by shoaling with temperate species, tropical fish increased foraging behaviour, which may allow them to persist longer into winter than their tropical-only shoaling counterparts (Smith et al., 2018; Paijmans et al., 2020). Increased foraging behaviour in mixed-

species shoals by tropical fish compared to tropical-only shoals may also be driven by trophic niche segregation (Kingsbury et al., 2019), formation of sub-shoal intraspecies groups, may limit the role of competition in tropical fish establishment at temperate latitudes. The alleviation of cold stress under ocean warming and functional niche availability (Miller et al., 2023) in temperate fish communities may determine the establishment success of tropical fish at higher latitudes in a future ocean. Nonetheless the adjustment of range-shifting species behaviour through novel tropical-temperate shoaling demonstrates the high behavioural plasticity and, therefore, the capacity of the tropical fishes studied to establish breeding populations in high-latitude temperate communities in the near future.

Use of a Shared Experimental Framework

It is important to acknowledge that Chapters 2, 3, and 4 share a common experimental framework involving the same set of fish subjects. This design was chosen to explore three distinct research questions testing the effects of ocean warming and acidification on tropical-temperate fish interactions, behaviour, and physiology. While this shared experimental approach could raise concerns about potential confounding effects, several measures were taken to address these considerations.

Notably, the startle escape response tests were conducted immediately after passive behavioural videos reported in Chapters 2 and 3, and lateralisation tests were finalised 24-hours before video trials commenced. This rest duration is adequate in alleviating potential stress responses in tropical and temperate fishes across temperatures tested (Pankhurst, 2011). Fish were also euthanised 8-13 days after behavioural trials to minimise the potential impacts of behavioural trials on fish cellular response, which are impacted by abiotic factors (e.g. temperature and ocean acidification) over minute-level time frames (Nagelkerken et al., 2023).

The experimental protocols followed were designed to minimise the potential for carry-over effects of lateralization tests upon the behavioural and physiological responses reported in the subsequent chapters. Indeed, lateralization tests and startle escape tests (Chapter 2) may have introduced some level of influence on subsequent behaviour responses detected in Chapter 3. Nonetheless, Chapter 2 and 3 findings are consistent with previous studies which tested behavioural responses under ocean warming and acidification in tropical and temperate fishes (Clements & Hunt, 2015; Nagelkerken & Munday, 2016), which suggests that any differences in fish behaviour should be primarily attributed to the specific experimental treatments applied in Chapters 2, 3, and 4. Furthermore, API Stress Coat™ was added tanks daily to minimise handling stress and shown to reduce stress responses in fishes produced by handling and transport (Vanderzwalmen et al., 2019). Each chapter's analysis and interpretation were conducted with the awareness of the shared experimental design. Transparently addressing this experimental sequence aims to ensure the robustness and credibility of the findings across Chapters 2, 3, and 4.

To further address potential confounding effects of the lateralization and startle escape response tests, I recommend that future research could explore each component of the experimental sequence in isolation (e.g., running separate experiments for each research question) to minimise the potential impact of lateralisation and startle escape response tests on fish behaviour and physiology. This approach would involve conducting lateralization and startle escape response tests on a separate set of fishes to the other experiments to understand each test's individual impact on fish behaviour and physiology compared to ocean acidification and warming effects alone. Such investigations may disentangle the distinct contributions of the lateralization and startle escape responses tests from other factors in the observed behaviours.

Future Directions

In my thesis, I studied novel species interactions and the underpinning behavioural and physiological responses of the most common range-extending tropical and co-shoaling temperate fishes in southeastern temperate ecosystems. I used a climate-manipulated laboratory experiment and a latitudinal climate gradient to study how the physiological, behaviour and novel species interactions (novel tropical-temperate shoals) responses may either facilitate or negate the tropicalisation of temperate fish communities of southeastern Australia in the near future.

However, further work is required to extend upon the findings of my thesis. Some suggestions to improve our understanding of the mechanisms underpinning the future outcomes of species range shifts may include:

- (1) Complex mesocosm experiments testing how ecosystem complexity (e.g. changes in biodiversity and habitat types) and trophic interactions (e.g. increased vs decreased trophic flow) alter range shifts outcomes under future climatic conditions. Complex food web interactions (e.g. food webs with multiple trophic pathways compared) can limit range-extension success under present-day climatic conditions (Tekwa et al., 2022). Since ocean warming and acidification can modify the food web and trophic interactions and reshuffle ecological communities (Goldenberg et al., 2018; Ullah et al., 2018), understanding how climate change mediates shifts in trophic interactions of high-latitude ecosystems could modify range-shift outcomes at an ecological community scale. Additionally, these experiments could examine the role of food web complexity, such as multiple vs single food web pathways, in buffering or resisting climate change-mediated range shifts in high-latitude communities. This type of research could offer a novel opportunity to further our understanding of how more

complex communities may be able to adapt to changing climatic conditions and reveal potential future range-shift outcomes.

(2) I investigated the combined effects of ocean acidification, ocean warming and novel species interactions on range-shift outcomes in a laboratory setting. In contrast, the interpretation of laboratory experiments is limited as other abiotic and biotic factors might alter or enhance climate change effects in natural environmental settings. Hence, natural analogues incorporating naturally occurring abiotic and biotic factors could reveal future range-shift outcomes in climate change hotspots. For example, the southeastern coast of Japan is undergoing similar marine species range shifts to that of southeastern Australia (Booth et al., 2011; Nakamura et al., 2013). In addition, volcanic CO₂ vents in eastern Japan offer a novel way to combine ocean warming and acidification effects in natural ecological settings, which laboratory and mesocosm experiments cannot test. By combining latitudinal climate gradients and natural CO₂ vents in Japan, where species range extensions overlap with naturally occurring CO₂ vents, future *in situ* research could reveal how changes in ecological communities under future climate scenarios mediate species range-extension outcomes.

(3) Parasitism may modify range-extension outcomes as range-extending species may encounter new parasites at their leading range edges (Carlson et al., 2017). Research has shown that highly cohesive shoals often experience higher incidences of parasitism than less cohesive shoals (Walsman et al., 2022), and that tropical fish, which become more cohesive in their novel range extent, may be more susceptible to at their novel cold-range limits. Additionally, ocean warming can also speed up the lifecycle of copepod parasites, allowing them to proliferate more freely in a future ocean (Godwin

et al., 2020), and disrupt the shoaling of range-extending fishes (Demandt et al., 2020). Therefore, future research should investigate ecologically relevant levels of parasitism upon range-extending and resident species to better understand the implications of parasitism on range-extension outcomes.

(4) Alternatively, temperate fish communities may be negatively affected by range-extending parasites and pathogens that they have not evolved to coexist with. Warm-adapted parasites and pathogens could increase disease and mortality among temperate fish populations, potentially leading to declines in population size and range reductions (Cable et al., 2017). New pathogens could disrupt existing ecological interactions and trophic relationships within temperate fish communities, causing changes in community structure and function (Dunn et al., 2012). Furthermore, range-extending pathogens and parasites may also have indirect effects on temperate fish communities (Allan et al., 2020). For example, parasites may increase predation pressure on temperate fish populations by altering the behaviour or susceptibility of the host temperate fish (Allan et al., 2020; Demandt et al., 2020), and therefore reduce predation pressure on competing tropical fishes. Thus, the arrival of new pathogens in temperate ecosystems could modify the ability of temperate fishes to compete against range-extending tropical fishes. In turn, future research should investigate how range-extending parasites and pathogens may directly and indirectly modify the pace of tropical fish range-extensions into temperate ecosystems, under climate change.

(5) Most studies assessing range shifts consider worse-case climate scenarios. Shifts in political stances and policy on climate change should be incorporated into models and experiments analysing species range shifts. Therefore, future research should assess

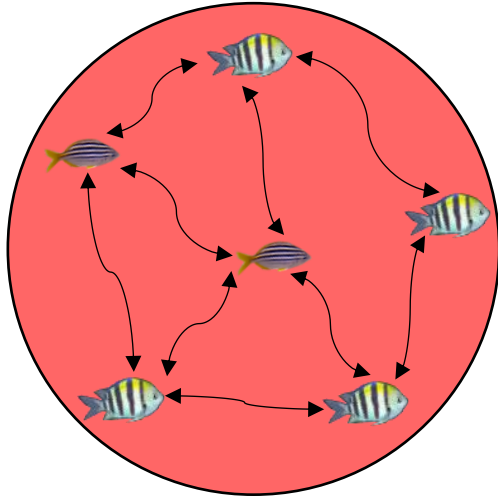
multiple future climatic scenarios (e.g., best, moderate and worst-case scenarios) to better understand differential future species range-shift outcomes.

General Conclusion

My thesis advances our understanding of the mechanisms driving tropicalisation in temperate fish communities under a changing climate. By examining the combined effects of ocean warming, acidification, and novel species interactions, I reveal the complex interplay between these factors on range-extension outcomes. While ocean warming may be the primary driver of marine range shifts, when climatic stressors and species interactions are combined, tropicalisation outcomes can be modified. My findings demonstrate that future seasonal temperatures and ocean acidification can counter the positive effects of ocean warming and novel species interactions on tropical fish range-extensions. I reveal that ocean acidification and future winter conditions might slow tropicalisation through reduced shoaling performance of novel tropical-temperate fish shoals and declines in tropical fish behaviour, fitness and physiological function in temperate ecosystems. In contrast, temperate fish behaviour remained unaffected by future climatic conditions but experienced increased growth and physiological performance under future winter conditions compared to current and future summer conditions, which could seasonally modify tropical-temperate interactions. I further reveal that novel species interactions can enhance tropical fish foraging, which may allow them to perform better at their cold-temperate range edges. Therefore, I suggest that although present-day winter temperatures and novel temperate environmental conditions may slow tropicalisation in temperate ecosystems, the overall effect of ocean warming and positive tropical-temperate interactions will facilitate the tropicalisation of temperate marine ecosystems. However, in a future ocean, winter temperatures and ocean acidification could reduce positive novel species

interactions and warming effects on tropicalisation, therefore limiting tropical fish performance at temperate latitudes. Overall, my research has significant implications for understanding the impacts of climate change on the tropicalisation of temperate fish communities and the capacity of species to adapt to changing environments.

Future Summer (26 °C and pH 7.7)



Tropical Fish Responses

Tropical fish fitness, physiology and behavioural performance are higher compared to future winter conditions (Chapters 3 and 4)

Temperate Fish Responses

Temperate fish behaviour, fitness and physiological function is degraded under current- and future summer conditions compared to future winter conditions (Chapters 3 and 4)

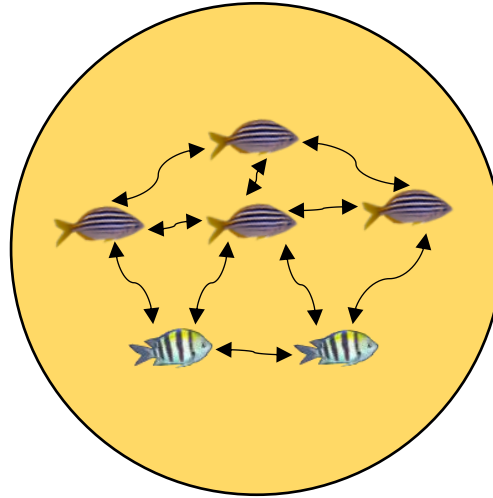
Shoal Level Responses

Under ocean warming, mixed-spp. shoals may experience increased proportion of tropical members which may facilitate decline in benefits tropical fish obtain from mixed species shoaling (Chapter 5)

Effects on Range-Extensions

Accelerates tropical fish range extensions in temperate ecosystems by enhancing physiology, behaviour and fitness (Chapter 3 and 4)

Present-Day Summer (23 °C and pH 8.1)



Tropical Fish Responses

Tropical fish trade-off foraging efficiency in favour of anti-predator behaviour (Chapter 5)
Novel shoaling interactions benefit tropical fish more than familiar interactions (Chapter 5)

Temperate Fish Responses

Novel interactions do not alter temperate fish behaviour (Chapter 3)

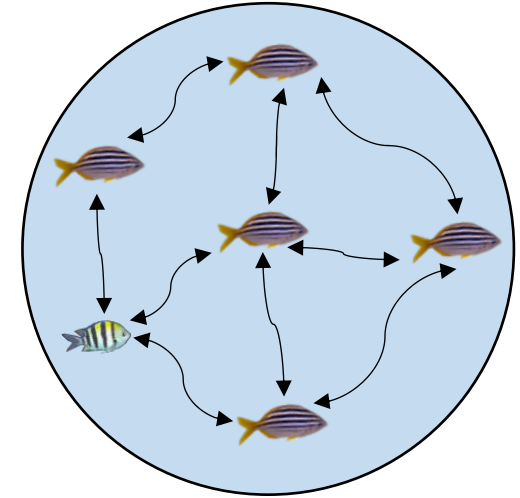
Shoal Level Responses

Tropical fish form more cohesive and aligned single-spp. shoals (Chapter 5)
Formation of intra-specific groups within mixed-spp. shoals (Chapter 5)
Temperate single-spp. shoals exhibit higher shoal cohesion than mixed-spp. shoals (across all treatments; Chapter 2)

Effects on Range Extensions

Promotes tropical fish range extensions in temperate ecosystems (Booth et al., 2007; 2011)

Future Winter (20 °C and pH 7.7)



Tropical Fish Responses

Future winter degrade the physiological function (Chapter 4), fitness and behaviour (Chapter 3) of range-extending tropical fish
At future winter temperatures, ocean acidification generated oxidative stress condition in tropical fish (Chapter 4)

Temperate Fish Responses

Temperate fish behaviour, fitness and physiological function is higher compared to current- and future summer conditions (Chapters 3 and 4)

Effects on Range Extensions

Slows down tropical fish range extensions into temperate ecosystems (Chapters 2 and 3)

Direct Responses to Ocean Acidification

Degraded lateralisation and shoaling dynamics experienced fishes in temperate-only and mixed-species shoals under ocean acidification (Chapter 2)
Tropical fishes experience elevated activity levels under ocean acidification (Chapter 3)

Effects on Range Extensions

Slows down tropical fish range extension in temperate ecosystems by degrading shoaling performance (Chapter 2)

Figure 1: Conceptual diagram which shows how my PhD research advances our understanding of the tropicalisation of southeastern Australian temperate fish communities under novel species interactions, and current and future climatic conditions (ocean acidification and warming). Novel tropical-temperate shoaling interactions could be modified under future climatic conditions, which could alter the pace of tropicalisation into southeastern Australian temperate ecosystems.

References

- Agostini, S., Harvey, BP., Milazzo, M., Wada, S., K. Kon, K., Floc'h, N., Komatsu, K., Kuroyama, M., Hall-Spencer, JM. (2021). Simplification, not “tropicalisation”, of temperate marine ecosystems under ocean warming and acidification. *Global Change Biology*. 27. pp. 4771–4784. <https://doi.org/10.1111/gcb.15749>.
- Alexander, J., Diez, J., Levine, J. (2015). Novel competitors shape species' responses to climate change. *Nature*. 525(7570). pp. 515–518. <https://doi.org/10.1038/nature14952>.
- Allan, BJ., Illing, B., Fakan, EP., Narvaez, P., Grutter, AS., Sikkell, PC., McClure, EC., Rummer, JL., McCormick, MI. (2020). Parasite infection directly impacts escape response and stress levels in fish. *Journal of experimental biology*. 223(16). jeb230904. <https://doi.org/10.1242/jeb.230904>.
- Baumann, H., Talmage, SC., Gobler CJ., (2012). Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change*. 2. pp. 38–41. <https://doi.org/10.1038/nclimate1291>.
- Beck, H., Feary, D., Fowler, A., Madin, E., Booth, DJ. (2016). Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish. *Marine Biology*. 163(4). <https://doi.org/10.1007/s00227-016-2844-8>.
- Booth, DJ., Bond, N., Macreadie, PI. (2011). Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research*. 62(9). <https://doi.org/10.1071/MF10270>.
- Booth, DJ., Figueira, WF., Gregson, MA., Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine Coastal and Shelf Science*. 72(1–2). <https://doi.org/10.1016/j.ecss.2006.10.003>.
- Cable, J., Barber, I., Boag, B., Ellison, AR., Morgan, ER., Murray, K., Pascoe, EL., Sait, S. M., Wilson, AJ., Booth, M. (2017). Global change, parasite transmission and disease control: lessons from ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372(1714). 20160088. <https://doi.org/10.1098/rstb.2016.0088>.
- Carlson, CJ., Burgio, KR., Dougherty, ER., Phillips, AJ., Bueno, VM., Clements, CF., Castaldo, G., Dallas, TA., Cizauskas, CA., Cumming, GS., Doña, J., Harris, NC., Jovani, R., Mironov, S., Muellerklein, OC., Proctor, HC., Getz, WM. (2017). Parasite biodiversity faces extinction and redistribution in a changing climate. *Science advances*. 3(9). pp. e1602422. <https://doi.org/10.1126/sciadv.1602422>.
- Clements, JC., Hunt, HL. (2015). Marine animal behaviour in a high CO₂ ocean. *Marine Ecology Progress Series*. 536. pp. 259–279. <https://doi.org/10.3354/meps11426>.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2021). Novel species interactions and environmental conditions reduce foraging competency at the temperate range edge of a range-extending coral reef fish. *Coral Reefs*. 40(5). pp. 1525–1536. <https://doi.org/10.1007/s00338-021-02150-6>.
- Coni, EOC., Booth, DJ., Ferreira, C., Nagelkerken, I. (2021b). Behavioural generalism could facilitate coexistence of tropical and temperate fishes under climate change. *Journal of Animal Ecology*. 91(1). pp. 86 – 100. <https://doi.org/10.1111/1365-2656.13599>.
- Coni, EOC., Nagelkerken, I., Ferreira, C., Connell, SD., Booth, DJ. (2021c). Ocean acidification may slow the pace of tropicalisation of temperate fish communities. *Nature Climate Change*. 11(3). pp. 249–256. <https://doi.org/10.1038/s41558-020-00980-w>.
- Coni, EOC., Booth, DJ., Nagelkerken, I. (2022). Coral-reef fishes can become more risk-averse at their poleward range limits. *Proceedings of the Royal Society B: Biological Sciences*, 289(1971). 20212676. <https://doi.org/10.1098/rspb.2021.2676>.

- Demandt, N., Praetz, M., Kurvers, RHJM., Krause, J., Kurtz, J., Scharsack, JP. (2020). Parasite infection disrupts escape behaviours in Fish Shoals. *Proceedings of the Royal Society B: Biological Sciences*. 287(1938). 20201158. <https://doi.org/10.1098/rspb.2020.1158>.
- Djurichkovic, L., Donelson, J., Fowler, A., Feary, D., Booth, DJ. (2019). The effects of water temperature on the juvenile performance of two tropical damselfishes expatriating to temperate reefs. *Scientific Reports*. 9(1). 13937. <https://doi.org/10.1038/s41598-019-50303-z>.
- Donelson, JM., Sunday, JM., Figueira, WF., Gaitán-Espitia, JD, Hobday, AJ., Johnson, CR., Leis, JM., Ling, SD., Marshall, D, Pandolfi, JM., Pecl GT, Rodgers, GG., Booth, DJ., Munday PL. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philosophical Transactions of the Royal Society B*. B3742018018620180186. <http://doi.org/10.1098/rstb.2018.0186>.
- Dunn, AM., Torchin, ME., Hatcher, MJ., Kotanen, PM., Blumenthal, DM., Byers, JE., Coon, CA., Frankel, VM., Holt, RD., Hufbauer, RA., Kanarek, AR. (2012). Indirect effects of parasites in invasions. *Functional Ecology*. 26(6). pp. 1262–1274. <https://doi.org/10.1111/j.1365-2435.2012.02041.x>.
- Feary, DA., Pratchett, MS., Emslie, MJ., Fowler, A., Figueira, WF., Luiz, OJ., Nakamura, Y., Booth, DJ. (2014). Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*. 15(4). pp. 593–615. <https://doi.org/10.1111/faf.12036>.
- Figueira, WF., Biro, P., Booth, DJ., Valenzuela Davie, VC. (2009). Performance of tropical fish recruiting to temperate habitats: role of ambient temperature and implications of climate change. *Marine Ecology Progress Series*. 384. pp. 231–239. <https://doi.org/10.3354/meps08057>.
- Figueira, WF., Booth, DJ. (2010). Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Global Change Biology*. 16(2). pp. 06–516. <https://doi.org/10.1111/j.1365-2486.2009.01934.x>.
- Figueira, WF., Curley, B., Booth, DJ. (2019). Can temperature-dependent predation rates regulate range expansion potential of tropical vagrant fishes?. *Marine Biology*. 166. 73. <https://doi.org/10.1007/s00227-019-3521-5>.
- Godwin, SC., Fast, MD., Kuparinen, A., Medcalf, KE., & Hutchings, JA. (2020). Increasing temperatures accentuate negative fitness consequences of a marine parasite. *Scientific Reports*. 10(1). 18467. <https://doi.org/10.1038/s41598-020-74948-3>.
- Goldenberg, SU., Nagelkerken, I., Marangon, E., Bonnet, A., Ferreira, CM., Connell, SD. (2018). Ecological complexity buffers the impacts of future climate on marine consumers. *Nature Climate Change*. 8. pp. 229–233. <https://doi.org/10.1038/s41558-018-0086-0>.
- Jones, NT., Symons, CC., Cavalheri, H., Pedroza-Ramos, A., Shurin, JB. (2020) Predators drive community reorganisation during experimental range shifts. *Journal of Animal Ecology*. 89. pp. 2378–2388. <https://doi.org/10.1111/1365-2656.13289>.
- Kingsbury, K., Gillanders, B., Booth, DJ., Nagelkerken, I. (2019). Trophic niche segregation allows range-extending coral reef fishes to coexist with temperate species under climate change. *Global Change Biology*. 26(2). pp. 721–733. <https://doi.org/10.1111/gcb.14898>.
- Lopes, A., Morais, P., Pimentel, M., Rosa, R., Munday, PL., Gonçalves, E., Faria, A. (2016). Behavioural lateralization and shoaling cohesion of fish larvae altered under ocean acidification. *Marine Biology*. 163(12). pp. 1–10. <https://doi.org/10.1007/s00227-016-3026-4>.
- Miller, MG., Reimer, JD., Sommer, B., Cook, KM., Pandolfi, JM., Obuchi, M., Beger, M., (2023). Temperate functional niche availability not resident-invader competition

- shapes tropicalisation in reef fishes. *Nature Communications*. 14(1). 2181. <https://doi.org/10.1038/s41467-023-37550-5>.
- Monaco, C., Booth, DJ., Figueira, W., Gillanders, B., Schoeman, D., Bradshaw, C., Nagelkerken, I. (2021). Natural and anthropogenic climate variability shape assemblages of range-extending coral-reef fishes. *Journal of Biogeography*. 48(5). pp. 1063–1075. <https://doi.org/10.1111/jbi.14058>.
- Nagelkerken, I., Munday, PL. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Global Change Biology*. 22(3). pp. 974 – 989. <https://doi.org/10.1111/gcb.13167>.
- Nagelkerken I., Connell, SD. (2022). Ocean acidification drives global reshuffling of ecological communities. *Global Change Biology*. 28(23). pp. 7038–7048. <https://doi.org/10.1111/gcb.1641>.
- Nagelkerken, I., Allan, BJ., Booth, DJ., Donelson, JM., Edgar, GJ., Ravasi, T., Rummer, JL., Vergés, A., Mellin, C. (2023). The effects of climate change on the ecology of fishes. *PLOS Climate*. 2(8). e0000258. <https://doi.org/10.1371/journal.pclm.0000258>.
- Nakamura, Y., Feary, D., Kanda, M., Yamaoka, K. (2013). Tropical fishes dominate temperate reef fish communities within western Japan. *PLoS One*. 8(12). e81107. <https://doi.org/10.1371/journal.pone.0081107>.
- Pankhurst, NW. (2011). The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology*. 170(2). pp. 265–275. <https://doi.org/10.1016/j.ygcen.2010.07.017>.
- Paijmans, K., Booth, DJ., Wong, MY. (2020). Predation avoidance and foraging efficiency contribute to mixed-species shoaling by tropical and temperate fishes. *Journal of Fish Biology*. 96(3). pp. 806–814. <https://doi.org/10.1111/jfb.14277>.
- Paijmans, KC., Booth, DJ., Wong, MY. (2021). Single-species subgroups form within mixed-species shoals of tropical and temperate fishes. *Environmental Biology of Fishes*. 105(42). pp. 1–14 <https://doi.org/10.1007/s10641-021-01188-3>.
- Rodriguez-Dominguez, A., Connell, SD., Coni, EOC., Sasaki, M., Booth, DJ., Nagelkerken, I. (2022). Phenotypic responses in fish behaviour narrow as climate ramps up. *Climatic Change*. 171. 19. <https://doi.org/10.1007/s10584-022-03341-y>.
- Shepard, ID., Wissinger, SA., Wood, ZT., Greig, HS. (2022). Predators balance consequences of climate-change induced habitat shifts for range-shifting and resident species. *Journal of Animal Ecology*. 91. pp. 334–344. <https://doi.org/10.1111/1365-2656.13631>.
- Smith, S., Fox, R., Booth, DJ., Donelson, J. (2018). Stick with your own kind, or hang with the locals? Implications of shoaling strategy for tropical reef fish on a range-expansion frontline. *Global Change Biology*. 24(4). pp. 1663–1672. <https://doi.org/10.1111/gcb.14016>.
- Tekwa, E., Watson, J., Pinsky, M. (2022). Body size and food–web interactions mediate species range shifts under warming. *Proceedings of the Royal Society B: Biological Sciences*. 289(1972). 20212755. <https://doi.org/10.1098/rspb.2021.2755>.
- Twiname, S., Fitzgibbon, Q., Hobday, A., Carter, C., Oellermann, M., Pecl, G. (2022). Resident lobsters dominate food competition with range-shifting lobsters in an ocean warming hotspot. *Marine Ecology Progress Series*. 685. pp. 171–181. <https://doi.org/10.3354/meps13984>.
- Ullah H., Nagelkerken I., Goldenberg, SU., Fordham, DA. (2018) Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. *PLOS Biology*. 16(1). e2003446. <https://doi.org/10.1371/journal.pbio.2003446>.

- Vanderzwalm, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D. and Sloman, K.A., 2019. The use of feed and water additives for live fish transport. *Reviews in Aquaculture*. 11(1). pp. 263–278. <https://doi.org/10.1111/raq.12239>.
- Walsman, J.C., Janecka, M.J., Clark, D.R., Kramp, R.D., Rovenolt, F., Patrick, R., Stephenson, J.F. (2022). Shoaling guppies evade predation but have deadlier parasites. *Nature Ecology & Evolution*. 6(7). pp. 945–954. <https://doi.org/10.1038/s41559-022-01772-5>.