



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

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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 (Twinaime 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.

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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). Southeastern 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. vaiensis* 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 Titrand™) 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 ± 0.01 mm) and wet weight (WW ± 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,

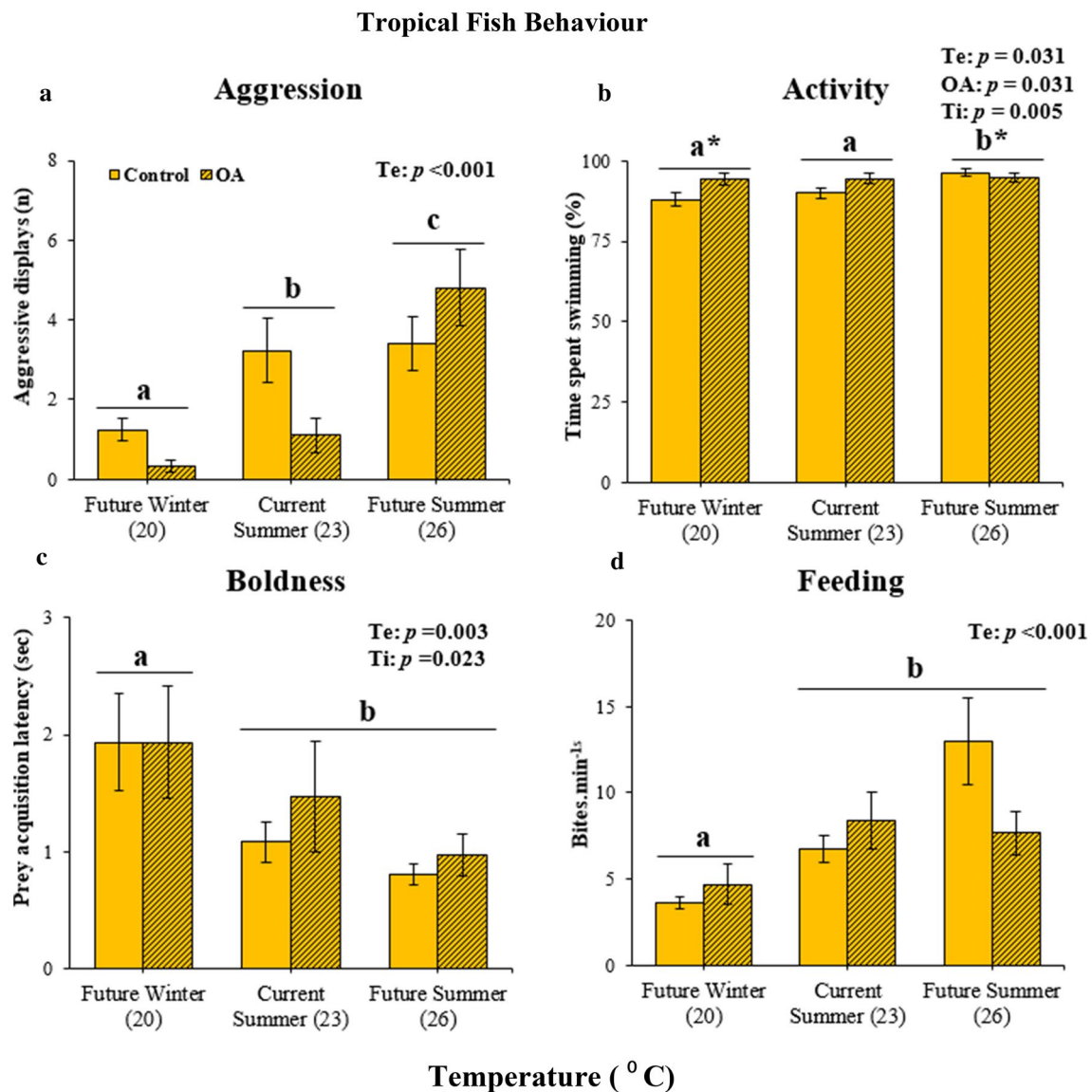


Fig. 1 Aggression (total number of attacks) (a), activity (percentage of time spent swimming) (b), boldness (prey acquisition latency) (c), and feeding (bite rate) (d) responses for tropical fish across temperature (Te) and ocean acidification (OA). Note that decreased prey acquisition latency reflects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

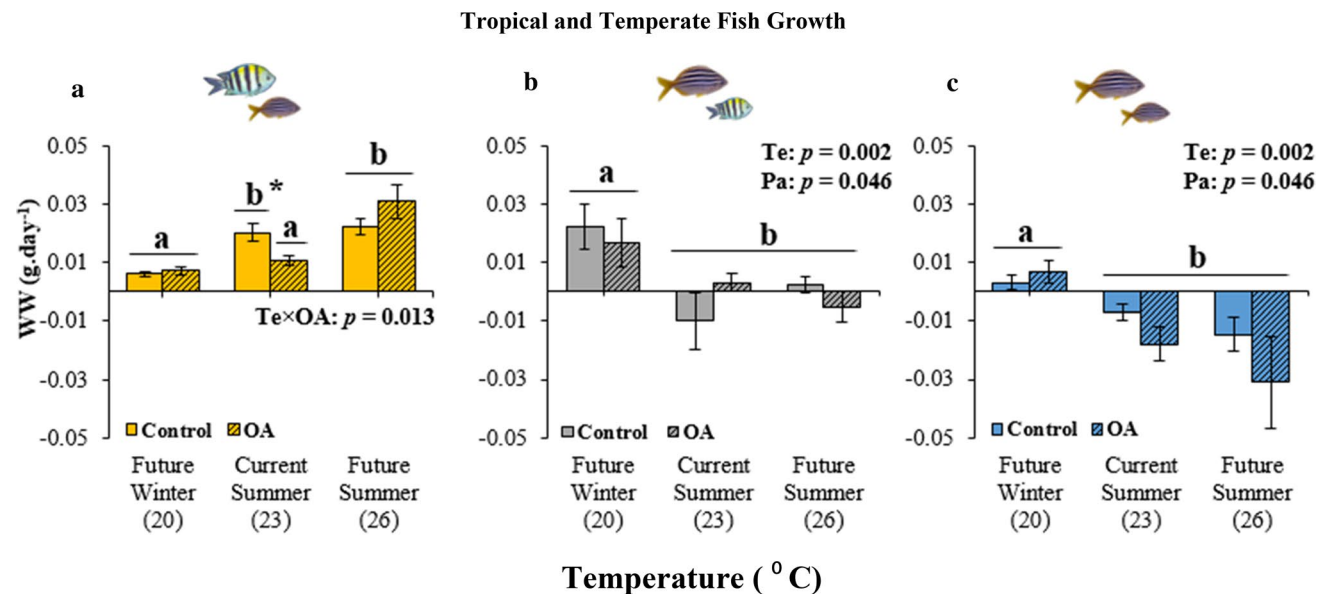


Fig. 2 Growth (wet weight, WW) responses of juvenile tropical fish (a) and temperate fish (b, c) across temperature (Te), ocean acidification (OA) and pairing type (Pa) treatments (as depicted by the fish symbols above the graphs). Ti=time (days). Data for days 19 and 27

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

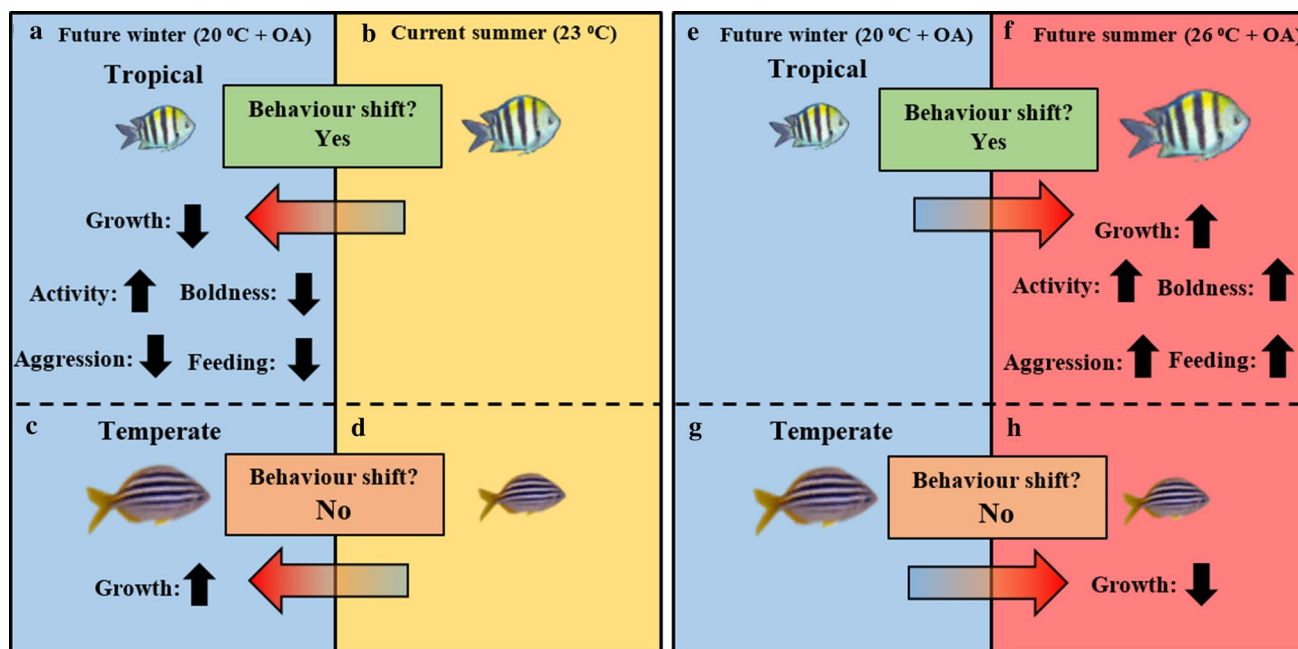


Fig. 3 Conceptual diagram showing how current and projected future natural seasonal temperatures and ocean acidification positively and negatively modify the growth and behaviour of interacting tropical (panels: a, b, e, and f) and temperate fishes (panels: c, d, g, and h) at 34° S, where the ranges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

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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.

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