

LETTER

A genetically based ecological trade-off contributes to setting a geographic range limit

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Abstract

Understanding the ecological factors that shape geographic range limits and the evolutionary constraints that prevent populations from adaptively evolving beyond these limits is an unresolved question. Here, we investigated why the euryhaline fish, *Poecila reticulata*, is confined to freshwater within its native range, despite being tolerant of brackish water. We hypothesised that competitive interactions with a close relative, *Poecilia picta*, in brackish water prevents *P. reticulata* from colonising brackish water. Using a combination of field transplant, common garden breeding, and laboratory behaviour experiments, we find support for this hypothesis, as *P. reticulata* are behaviourally subordinate and have lower survival in brackish water with *P. picta*. We also found a negative genetic correlation between *P. reticulata* growth in brackish water versus freshwater in the presence of *P. picta*, suggesting a genetically based trade-off between salinity tolerance and competitive ability could constrain adaptive evolution at the range limit.

KEYWORDS

distribution limits, euryhaline, fish, interference competition, realised niche, salinity, translocation experiment/field transplants

INTRODUCTION

A fundamental goal of ecology and evolution is to understand the mechanisms that determine the geographic distributions of organisms. The current limits to species' distributions can largely be explained via proximate hypotheses that test species' eco-physiological responses to abiotic gradients and/or biotic interactions (Gaston, 2009; Hargreaves et al., 2014; Holt, 2003; Louthan et al., 2015; Sexton et al., 2009; Willi & Van Buskirk, 2019). However, understanding the ultimate mechanisms preventing

populations from *adaptively evolving* and expanding to environments beyond their current range requires an evolutionary explanation with a different set of hypotheses (Angert et al., 2020; Futuyma, 1998; Willi & Van Buskirk, 2019). Evolutionary hypotheses for the causes of geographic range limits fall under the broader problem of 'evolvability', which seeks to understand the constraints on adaptive evolution (Futuyma, 2010; Hansen & Houle, 2008; Louthan et al., 2015). Specifically, given enough time and the absence of physical barriers to dispersal, or selection regimes that would prohibit evolution, what prevents populations from evolving to new environments and expanding their geographic range? In this context, the evolutionary mechanisms underlying

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range limits are examples of the limits of adaptation in nature and thus provide ‘testing grounds’ to better understand the adaptive evolutionary process (Sexton et al., 2009).

Evolutionary theory suggests the same factors that can constrain adaptation, such as a lack of genetic variation (e.g. Blows & Hoffmann, 2005), the introduction of maladaptive alleles through gene flow (e.g. gene swamping; Tigano & Friesen, 2016), abrupt shifts in the strength and form of selection across space (e.g. Anderson et al., 2015) and the presence of genetically based trade-offs between traits (Hoffmann & Blows, 1994; Martin, 2015; Roff & Fairbairn, 2007) contribute to observed range limits (Gaston, 2009; Kawecki, 2008; Willi & Van Buskirk, 2019). These factors are not mutually exclusive, but conclusive support for any of these evolutionary mechanisms as a general explanation for setting range limits remains elusive (Angert et al., 2020). For example, high levels of additive genetic variation are commonly found for most traits, and few studies have found evidence for a lack of genetic variation setting range limits (e.g. Willi et al., 2006; Eckert et al., 2008; Gould et al., 2014; but see Kellermann et al., 2006). Similarly, there is a paucity of empirical evidence for the role of gene flow introducing maladaptive alleles to populations at the range edge and thus preventing adaptive evolution at a population's range limit (e.g. Fitzpatrick et al., 2015; Dennenmoser et al., 2017; but see Pedersen et al., 2017; Sexton et al., 2009).

Less attention has been given to the role negative genetic correlations and pleiotropy might play in generating fitness trade-offs that prevent adaptive evolution at the range edge (Duffy et al., 2006; Hoffmann & Blows, 1994; Mauro & Ghalambor, 2020; Sgrò & Hoffmann, 2004; Tiffin et al., 2013), despite a large body of research demonstrating that such fitness trade-offs are common across environmental gradients (Kneitel & Chase, 2004; Martin, 2015). For example, trade-offs between traits that deal with biotic and abiotic challenges have been described for a wide range of taxa including heat tolerance and competition in copepods, fish, birds, and mammals (Chappell 1978; Fausch et al. 1994; Gross and Price 2000; Martin, 2015; Willett, 2010), salinity tolerance and competition in fish and plants (Greiner et al. 2001; Alcaraz et al., 2008), metabolic plasticity and bacterial defense in beetles (Cioffi et al., 2016) and desiccation tolerance and competition in barnacles (Connell, 1961). However, the genetic basis of ecological trade-offs is unknown for all but a few species, preventing evolutionary insights into their importance as a constraint on the evolution of range limits (Anderson et al., 2013; Olsen et al., 2019). Here, we test if an ecological trade-off between salinity tolerance and interspecific competition determines the range boundary of the Trinidadian guppy (*Poecilia reticulata*) and if so, whether there is a genetic basis to the trade-off.

Study system

The Trinidadian guppy is a euryhaline fish native to the Caribbean, Central America, and South America and is a model for studying evolution in nature (e.g. Reznick & Travis, 2019). Guppies are a prime example of rapid adaptation following the colonization of new environments within their native range (Reznick et al., 1997) and have been introduced and become established throughout the world (Deacon et al., 2011). However, in their native Trinidad, their distributions exhibit an abrupt limit at the freshwater-brackish water boundary across all lowland rivers (Torres-Dowdall et al., 2013; Figure 1). This is a dramatic observation because the freshwater boundary point fluctuates daily with the tides and seasonally with the wet/dry season and yet *P. reticulata* is never found in conditions of even 1 psu (practical salinity unit), suggesting that this species behaviorally avoids brackish water (Torres-Dowdall et al., 2013). This occurs despite numerous studies showing Trinidadian guppies can tolerate, reproduce in, and evolve improved tolerance to brackish water in the lab (Gibson & Hirst, 1955; Shikano et al., 2001; Shikano & Fujio, 1998) and persist in brackish water parts in their non-native range (Courtenay et al., 1974). Such results suggest that brackish water alone does not pose an abrupt and strong selection gradient that would prohibit guppies from colonizing and establishing within brackish waters. Yet, the freshwater boundary point results in an abrupt shift from high population density to none, despite the salinity gradient being gradual and there being little change in abiotic features like dissolved oxygen and pH, or biotic features like predation (Magurran, 2005; Torres-Dowdall et al., 2013). Collectively, this suggests that the range of *P. reticulata* is not limited because of dispersal, low population size, predation, or abiotic factors other than salinity. The only factor that is consistently associated with *P. reticulata*'s range limit in addition to salinity is the presence of *Poecilia picta*. *P. picta* is a closely related and similar sized euryhaline fish found in Trinidad. It is found primarily in brackish waters, but its distribution extends short distances into lowland freshwater sections of rivers where it coexists with *P. reticulata*, in a pattern repeated across most rivers in Trinidad (Figure 1; Torres-Dowdall et al., 2013). Due to the nature of *P. reticulata*'s distribution pattern, we hypothesized that *P. reticulata* avoids brackish water because *P. picta* is competitively dominant under saline conditions. We predicted that this relationship arises because osmoregulation is energetically demanding (e.g. Boeuf & Payan, 2001; Tseng & Hwang, 2008) and pleiotropically linked to aggression and competitive ability (Mauro & Ghalambor, 2020) such that the energetic demands and pleiotropic nature of osmoregulation would decrease aggression in saline conditions. Thus, we predicted that the range limit is set by an ecological trade-off between salinity tolerance and competitive ability that is evolutionarily constrained.

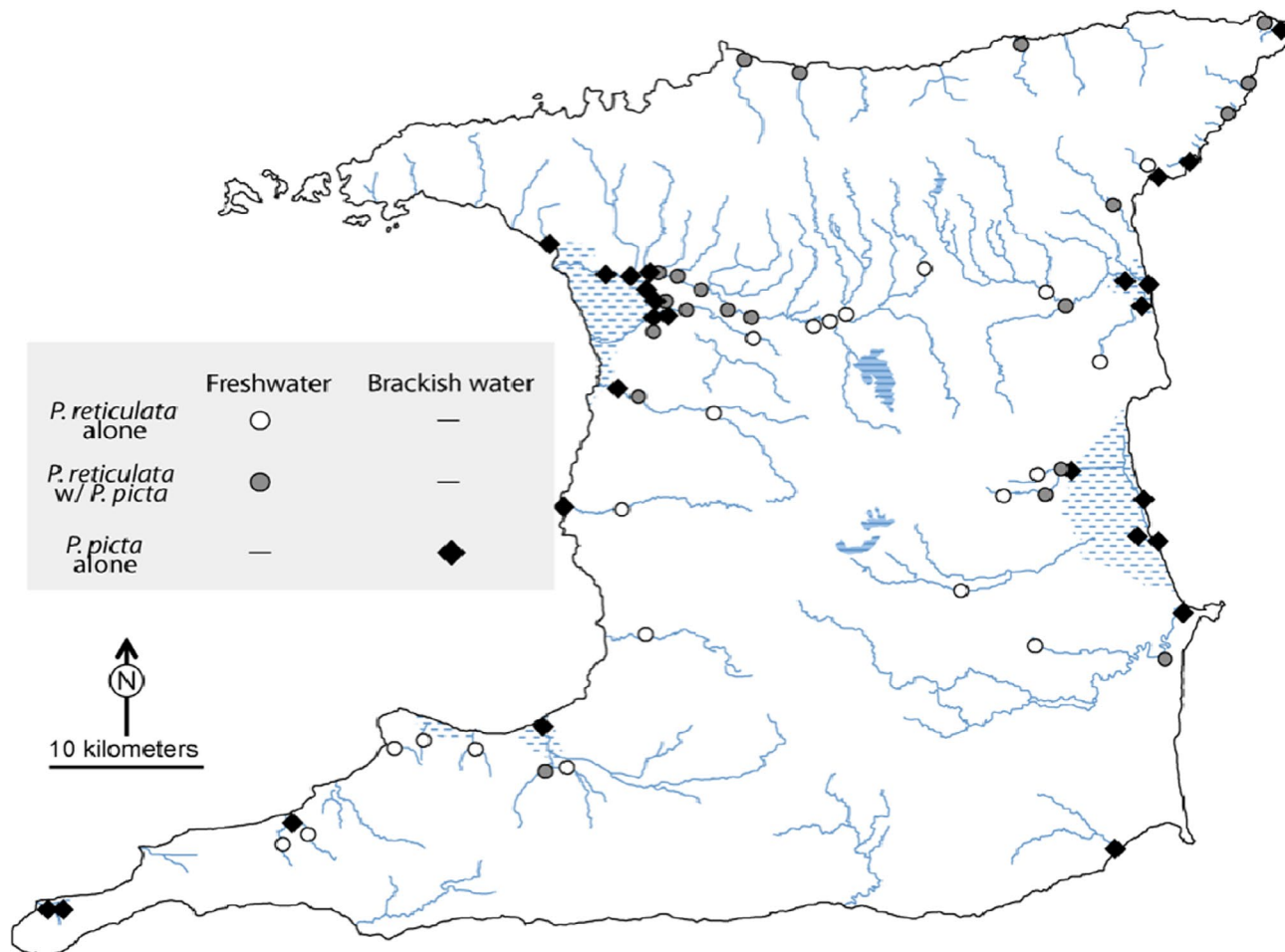


FIGURE 1 The distribution of *P. reticulata* and *P. picta* in the coastal rivers along the eastern and western coasts of Trinidad (adapted from Torres-Dowdall et al., 2013). Open circles represent freshwater sites where only *P. reticulata* has been found, grey circles represent freshwater sites where both species have been found together, and black diamonds represent brackish sites where only *P. picta* have been found. In all the rivers sampled, *P. reticulata* and *P. picta* only overlap in freshwater regions of rivers near the freshwater/brackish water boundary and *P. reticulata* is never found in brackish water

Here we tested several of the ecological/evolutionary assumptions and predictions of the general hypothesis that salinity and interspecific competition set the range limit for *P. reticulata*. We (1) used a binary choice experiment using freshwater and brackish water to test if *P. reticulata* exhibits behavioural avoidance of brackish water, (2) conducted a laboratory growth experiment to test if salinity exposure increases energetic costs by manipulating food levels, (3) conducted a breeding experiment in which full-sibling family lines were split and raised in either stressful salinity conditions or stressful competitive conditions to test if there is a genetic basis to the trade-off between salinity tolerance and competitive ability, (4) carried out a field transplant experiment to investigate the effects of salinity and competition on survival in nature, and (5) used a lab experiment to investigate if the mechanism by which salinity and interspecific competition reduce fitness is through changes in behavioural dominance. The results of these experiments build upon each other and collectively provide

insight into the ecological and evolutionary mechanisms underlying the range limit.

MATERIALS AND METHODS

Behavioural avoidance of brackish water in *Poecilia reticulata*

To test if *P. reticulata*'s absence in brackish water is due to behavioural avoidance, we measured the salinity preference (brackish water vs. freshwater) of 33 wild-caught *P. reticulata* (collected from the Caroni River in March 2011) using a 'Y-maze' binary choice test (Barnett, 1977). This experiment gave fish the option to swim either toward brackish water or freshwater collected from the river from which the fish were caught. The largest difference between these water sources was salinity (0–0.23 psu vs. 17–23 psu). Variation in other physicochemical variables was minimal (freshwater: temperature = 28.2°C,

dissolved oxygen = 42%, pH = 7.75; brackish water: temperature = 29.6°C, dissolved oxygen = 51%, pH = 7.19). Each trial consisted of placing an individual in freshwater in the base arm of the Y-maze for a 10-min acclimation period. After this period, water was simultaneously released into the two response arms, one with freshwater and the other with brackish water (side selected randomly), and a drain in the base arm maintained a linear flow from the response to the base arm (taking advantage of the natural tendency of fish to swim against currents). We considered a preference to have been made when a fish left the base arm and stayed in the selected arm for at least two minutes (see schematic in Figure S1). We used a Pearson's χ^2 test to determine salinity preference.

Effect of salinity and food on juvenile growth rate

To test the assumption that exposure to brackish water results in elevated energetic costs associated with osmoregulation, we conducted a common garden breeding experiment to test how salinity and amount of food influenced growth rate. We collected 20 gravid female *P. reticulata* from the Caroni River in 2009 and transported them to Colorado State University where they were individually housed in 10-litre tanks and kept on a 12:12 h light cycle at $25 \pm 1^\circ\text{C}$. We propagated fish for two generations in the lab to minimise maternal and environmental effects (Reznick & Endler, 1982). Second generation (G2) family lines were generated by randomly crossing lab-born fish within each generation. First generation (G1) litters were split into the two salinity levels used in this study (0 psu and 20 psu), thus experimental G2 fish were born at the salinity level in which they were tested. Within 24 h post-parturition, G2 siblings were weighed and assigned to one of three food levels. The middle food level was equivalent to that used by Reznick (1982) to achieve 85%–90% maximum growth rate (10 mg of a paste consisting of tropical fish flakes and water given daily). The other food levels consisted of either twice as much or half as much food as the middle food level. Thus, there were six possible treatments resulting from the combination of two salinity levels (0 psu and 20 psu) and three food levels (low, medium, high). Overall, the experiment included 28 families and 130 total fish (1–13 fish per family; average of 4.6 fish per family). To measure specific growth rate (SGR) we weighed fish at birth and then 28 days later and then used the equation: $\text{SGR} = (\text{LN}(\text{Mass}_{\text{week-4}}/\text{Mass}_{\text{birth}})) \times \text{days}^{-1} \times 100$ (Lugert et al., 2016). We analysed the data using a mixed model ANOVA with SGR as the response, salinity and food levels and their interaction as fixed effects, and family as a random effect using the package lme4 (Douglas et al., 2015). Least-squared means contrasts between treatments were two-tailed with a Tukey adjustment for

multiple tests using the lsmeans package (Lenth, 2016). Analyses were done in R (R Core Team, 2019).

Genetic basis of trade-off between salinity tolerance and competitive ability

To test if there is a genetically based trade-off between salinity tolerance and competitive ability with *P. picta*, we measured *P. reticulata* juvenile growth rate in G2s utilising a split-sibling design. These fish were from the same stock and raised in the same manner as those from the food level experiment. All *P. picta* used in this study were G2s bred from wild-caught fish collected at the same time and from the same location as the *P. reticulata* and raised in the same manner. We measured growth as fish developed in one of two environments: a salinity challenge environment consisting of *P. reticulata* raised by themselves at our low food level in 20 psu and a competitive challenge environment consisting of a single *P. reticulata* raised in freshwater with a single G2 *P. picta* competitor at a high food level. We used a low food level for the salinity treatment because the results of the food level experiment showed that the effects of salinity on growth rate are most pronounced in a low food environment (Figure 2). We used a high food level in the competition treatment to ensure that the challenge was solely due to interference competition and not simply food limitation. The goal of this experiment was not to test how the two treatments affected growth rate, but rather to test for a genetically based trade-off between the treatments by measuring a change in the rank order of the families across treatments (Conner & Hartl, 2004). In total, 10 family lines and 47 *P. reticulata* were used (1–5 fish per family in each treatment). We used a Pearson correlation rank test to test for a genetically based trade-off between the treatments. Additionally, because we were interested in how the family means differed between the treatments, we used an ANOVA in R Core Team (2019) using the package lme4 (Douglas et al., 2015) to test for the effects of family line (fixed effect), treatment (fixed effect), and their interaction (fixed effect) on growth rate.

Testing the effects of salinity and competition using a field enclosure translocation

To test if a trade-off between salinity tolerance and competitive ability reduces *P. reticulata* fitness beyond their freshwater distribution in nature, we conducted a short-term transplant experiment in Trinidad. We set up experimental enclosures at a brackish site (where only *P. picta* naturally occurs) on the Madame Espagnol River (salinity 15–20 psu) and at a freshwater site (where only *P. reticulata* naturally occurs) on the nearby Guayamare River (salinity <0.5 psu). Within each site, we compared the survival rate over a one-week period. At each site,

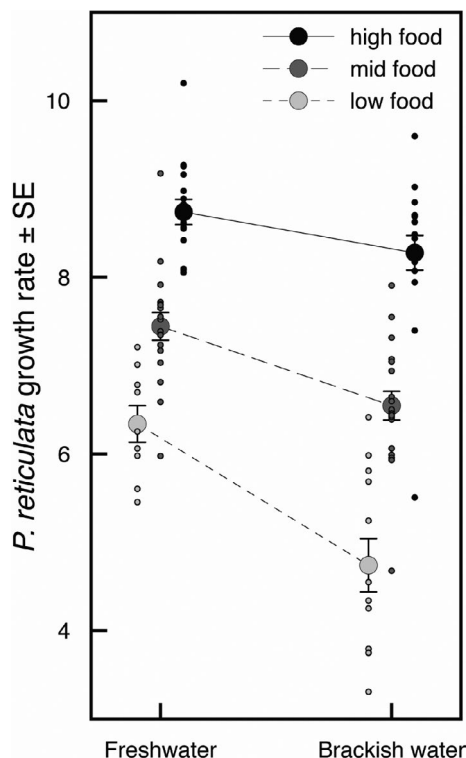


FIGURE 2 Salinity and lower food levels have a negative effect on the growth rate of *P. reticulata*. The negative effects of salinity are only evident at the lower food levels as no difference in growth rate was observed between fish reared in brackish and freshwater at the highest food level. There were, however, significant differences between the salinity treatments at the other food levels (least-squares mean differences with Tukey HSD adjustment). Means \pm SEs are displayed and individual points represent family means

we had two treatments: one treatment simulated an established population subject only to intraspecific interactions (100% conspecifics; *P. reticulata*), and the other simulated an invasion by *P. reticulata* into a habitat occupied by *P. picta* and the ensuing interspecific interactions (25% conspecific *P. reticulata*, 75% heterospecific *P. picta*). These two treatments were replicated six times per salinity/site ($2 \times 2 \times 6 = 24$ enclosures). We placed a total of eight fish in each mesh enclosure (6.5 litres; diameter = 18 cm, height = 25 cm, water able to flow through enclosures), approximately the natural density at these sites (personal observations). The fish in the experiment were *P. reticulata* and *P. picta* collected from a freshwater site along the Guayamare River and *P. picta* collected from a brackish water site at the Madame Espagnol River (15–20 psu). Prior to being placed in the enclosures, fish were individually marked using elastomer implants (Northwest Marine Technologies, Inc.). Fish were randomly assigned to one competition/salinity treatment. *P. reticulata* that were assigned to brackish treatments were acclimatised to the target salinity by increasing the salinity of their laboratory tank by 3 psu per day until reaching a salinity of 15 psu. All *P. picta* were housed in their home salinity before the experiment (0 or 15 psu). All fish were held in laboratory tanks for

7–8 days between being captured and starting the experiment to ensure they were healthy before being placed in enclosures.

The effect of competition and salinity on survival was analysed using a generalised linear model with competition, salinity, and their interaction as fixed effects and log-transformed mass as a covariate. The analysis was done in R (R Core Team, 2019) using the `glm` function with a logit link function from the `lme4` package (Douglas et al., 2015).

Effect of salinity on behavioural dominance

To test the mechanism by which salinity alters competitive ability, we tested if salinity altered dominance relationships between adult male *P. reticulata* and *P. picta*. Differences in aggressive behaviour are a way to assess social dominance in fish (Gilmour et al., 2005), hence we analysed the difference in aggressive behaviours between *P. reticulata*/*P. picta* pairs in freshwater tanks (0 psu, $n = 7$) and brackish water tanks (15 psu, $n = 6$) over the course of a week. The *P. reticulata* used in this experiment were G2 descendants of fish taken from the same portion of the Caroni River as in previous experiments but in June 2016. The wild-caught fish were transported to Colorado State University and housed in 1.5L tanks on large recirculating systems (Ghalambor et al., 2015) but were otherwise bred and raised in the same manner as the other fish. To serve as competitors to *P. reticulata* in the 15 psu treatment, we used G2 *P. picta* that were descendants of fish taken from a brackish portion of the Caroni River that varied from 25 to 35 psu salinity in June 2016. These fish were kept at a salinity of 30 psu but were otherwise housed and bred in the same fashion as other fish. To serve as competitors to *P. reticulata* in 0 psu, we used *P. picta* taken from the same freshwater site as the *P. reticulata*. They were bred and housed in the same manner as described for *P. reticulata*. However, the fish used were either wild caught, G1, or G2 individuals. We did not detect behavioural differences between freshwater *P. picta* of different generations, but we did not have proper replication to formally test this potentially confounding variable. However, previous behavioural studies on *P. reticulata* from Trinidad found no difference in behaviour due to generation in the lab (Gorlick, 1976; Seghers & Magurran, 1991).

Our experiment began by transferring fish to 10-gallon experimental tanks from laboratory tanks. Therefore, both the *P. reticulata* and the *P. picta* in the 15 psu experimental tanks experienced an abrupt salinity transfer of 15 psu to begin the experiment (+15 psu for the *P. reticulata* and –15 psu for *P. picta*; the *P. reticulata* and *P. picta* in 0 psu competition tanks did not experience a salinity transfer because they were housed in 0 psu). During the study, fish were fed food equivalent to the ‘low’ amount described in the juvenile growth rate experiment. Food was provided to

each pair by placing it on a single side of a small dice-sized square. This localised access to food and encouraged interaction between the fish (Dale Broder; personal communication). We observed each pair of fish for a 5-minute period directly after feeding on days 1, 2, 3, 4, 7 and recorded aggressive behaviours. Specifically, we recorded chases, nips, and guarding/monopolising the food source and defined these behaviours as they were defined in Seghers and Magurran (1991) (ethogram in Supporting Information). We analysed the difference in total aggressive behaviours between all *P. reticulata*/*P. picta* pairs using a repeated measures mixed model ANOVA with salinity as a fixed factor and fish ID as a random factor using the package lme4 (Douglas et al., 2015) in R (R Core Team, 2019).

RESULTS

P. reticulata behaviourally avoid brackish water in Y-maze experiment

Wild-caught *P. reticulata* in the Y-maze experiment exhibited a significant preference for freshwater and avoidance of brackish water (Probability_(choosing freshwater) = 0.76, standard deviation = 0.07, Pearson's $\chi^2_{1,N=33} = 8.77$, $p = 0.03$), independent of the sex of the fish tested ($\chi^2_{1,N=33} = 0.02$, $p = 0.9$).

Salinity impacts growth rate, but not at high food levels

Lab-raised *P. reticulata* had a reduced growth rate in brackish water compared to freshwater (Salinity: $F_{1,122} = 36.8$, $p < 0.0001$; Figure 2; Table S1). Growth rate decreased as food level decreased, but the change in growth rate depended on the salinity (Food level: $F_{2,118} = 118.2$, $p < 0.0001$; Food level \times Salinity: $F_{2,115} = 4.5$, $p = 0.013$; Table S1) as individuals in brackish water exhibited a greater decrease in growth rate as food level decreased compared to individuals in freshwater (Figure 2). At the highest food level, salinity had no significant effect on growth rate (lsmeans test: $t_{121} = -1.671$, $p = 0.097$; Table S2).

A negative genetic correlation between growth under salinity stress and growth under competitive stress

Lab-raised *P. reticulata* grew faster in the competitive challenge treatment (with *P. picta* in freshwater) than in the salinity challenge treatment (Figure 3; $F_{1,25} = 18.1$, $p < 0.001$; Table S3). The effects of family line ($F_{10,25} = 2.4$, $p = 0.0403$; Table S3) and the interaction between family and treatment ($F_{10,25} = 4.7$, $p < 0.001$; Table S3) were also significant. Importantly, there was a strong trend for a change in the rank order of families between the treatments in which

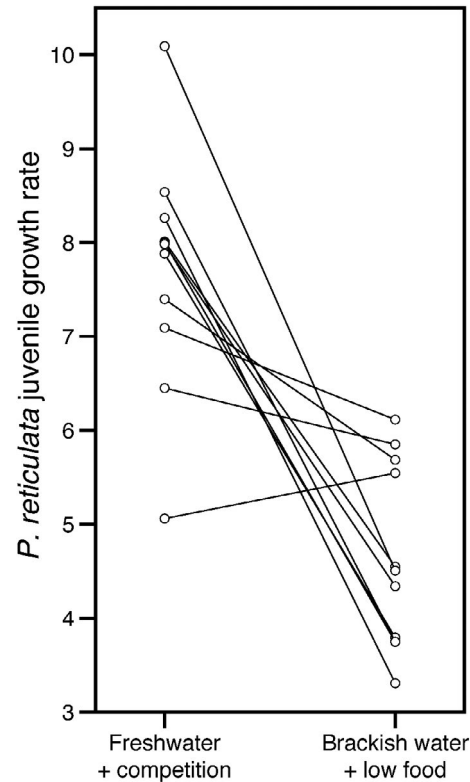


FIGURE 3 Juvenile growth rate of *P. reticulata* family lines with siblings split into either a salinity tolerance (brackish water + low food alone) or a competition (freshwater + competition with *P. picta*) treatment. Ends of lines represent family mean growth rates and lines connect family means across treatments. Fish grew significantly better in the competition treatment than the salinity treatment and families that grew relatively better in one treatment tended to grow significantly worse in the other treatment

family lines that grew best in the salinity treatment tended to have lower growth rate in the competitive treatment ($\rho = -0.64$, $p = 0.054$; Figure 3; Table S4).

Salinity and interspecific interactions affect survival in nature

Survival of wild-caught *P. reticulata* in the field transplant experiment was significantly decreased by salinity ($\chi^2_{1,N=192} = 8.2$, $df = 1$, $p = 0.004$), and there was a significant interaction between salinity and competition type ($\chi^2_{1,N=192} = 9.6$, $p = 0.002$) (Table S5). The significant interaction occurred because *P. reticulata* survival was reduced in brackish water compared to freshwater under the interspecific competition treatment, but not under the intraspecific competition treatment (Table S6; Figure 4).

Salinity alters interspecific dominance relationships

Among lab-raised fish, aggressive behaviours were similar between the two species in freshwater, but *P. picta*

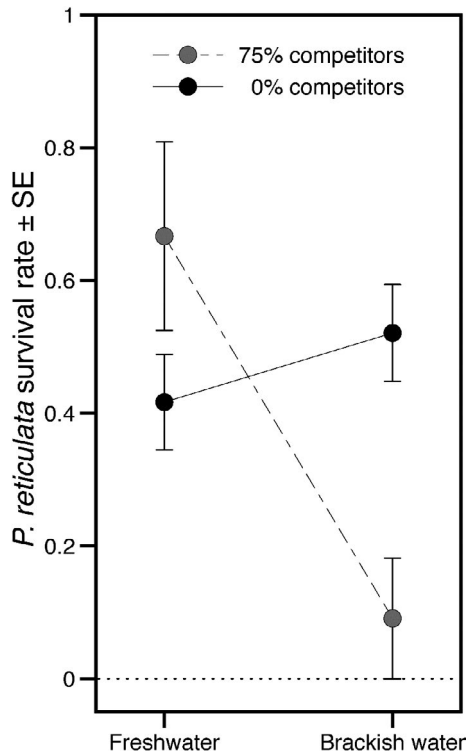


FIGURE 4 Survival rate over the course of one week for *P. reticulata* that were placed in enclosures in rivers in Trinidad. The enclosures were either in freshwater or brackish water sites and either contained only other *P. reticulata* (0% interspecific competitors; black, solid line) or 75% *P. picta*/25% *P. reticulata* (75% interspecific competitors; grey, dashed line). Means \pm SEs are displayed

exhibited significantly more aggressive behaviours than its *P. reticulata* competitor in brackish water ($F_{1,14} = 5.7$, $p = 0.031$; Figure 5; Table S7). This asymmetry in aggressive behaviours across salinity is largely driven by *P. picta* increasing aggressive behaviour in brackish water while *P. reticulata* aggression remained relatively constant across salinities (Figure S2; Table S8) This pattern was not correlated with differences in body mass between pairs of fish (Figure S3).

DISCUSSION

What factors shape range limits and what prevents populations from adapting and expanding their ranges? Answering these questions has proven exceedingly difficult because it not only requires understanding the ecological and physiological mechanisms determining contemporary distributions (Gaston, 2009; Sexton et al., 2009; ; Willi & Van Buskirk, 2019), but also an understanding of the factors that constrain adaptive evolution (Angert et al., 2020; Hoffmann & Blows, 1994). Here, we asked the general question: why is the distributional limit of *P. reticulata* associated with freshwater when it is euryhaline and tolerant of brackish water? Using a

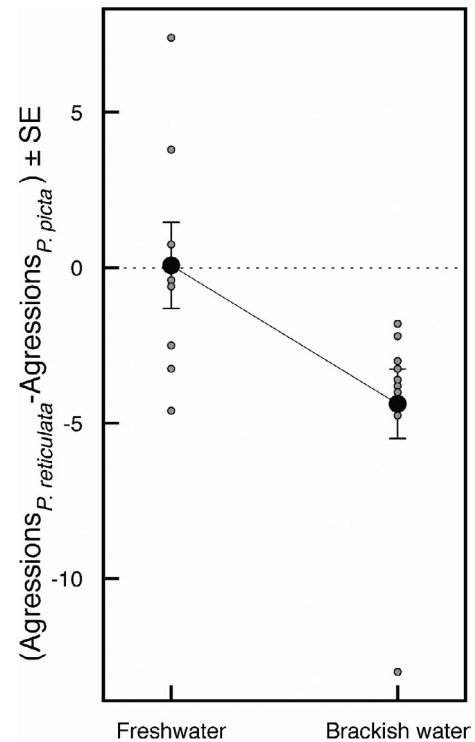


FIGURE 5 The difference in aggressive behaviours between a *P. reticulata* and a *P. picta* in either a freshwater or brackish water competition tank. Hence, positive values indicate that *P. reticulata* was dominant and negative values indicate that *P. reticulata* was subordinate. *P. reticulata* was neither dominant nor subordinate to *P. picta* in freshwater but was subordinate to *P. picta* in brackish water. Means \pm SEs are displayed and individual points represent values from each competition tank

combination of field and lab experiments, we find support for the hypothesis that *P. reticulata* avoids brackish water because it becomes behaviourally subordinate to its close relative *P. picta* (Figure 5) and suffers higher mortality (Figure 4) in brackish water. Because food limitation reduces salinity tolerance (Figure 2), interference competition could be the mechanism underlying higher mortality in brackish water. Furthermore, we find preliminary evidence that increased salinity tolerance in *P. reticulata* exhibits a negative genetic correlation with competitive ability with *P. picta* in freshwater, suggesting the evolution of increased salinity tolerance would come at the expense of competitive ability.

Behavioural and eco-physiological mechanisms shaping the range limit

The behavioural avoidance of brackish water by *P. reticulata* appears to be driven by a predictable decrease in fitness due to the combined effects of elevated osmoregulatory costs that are augmented by interspecific competition with *P. picta*. Theory predicts behavioural avoidance and reduced dispersal beyond the range limit should evolve when there is a predictable fitness cost to the

avoided environment (Duckworth, 2009; Holt, 2003), and our Y-maze experiment provides empirical support for such behavioural avoidance. We also find *P. reticulata* fitness declines in brackish water, but the mechanisms appear to be dependent on how competitive interactions with *P. picta* increase the costs of osmoregulation. As a euryhaline fish, *P. reticulata* is tolerant of moderate levels of salinity, but osmoregulation in brackish water increases energetic demands as evident by the effects of food limitation on juvenile growth (Figure 2). This is consistent with previous work on the energetics of osmoregulation (Boeuf & Payan, 2001; Tseng & Hwang, 2008). Thus, elevated osmoregulatory costs only manifest themselves when access to food is limited. However, in the lowland rivers of Trinidad where freshwater transitions to brackish water, there are no changes suggestive of drastically reduced food availability (Magurran, 2005). Hence, food limitation in nature would likely arise through interference competition (as opposed to exploitative competition) with *P. picta*, as is observed in many contexts where closely related species replace each other along environmental gradients (Martin, 2015).

Salinity often predicts aquatic species distributions and community composition (e.g. Kefford et al., 2004, 2012), but the degree to which salinity acts in conjunction with biotic factors, such as interspecific competition, to shape distributions remains largely unexplored. We found two lines of evidence suggesting asymmetric interspecific competition favours *P. picta* over *P. reticulata* in brackish water. First, in our field enclosure experiments we found that *P. reticulata* survival was significantly reduced in brackish water in the presence of *P. picta*, but not in the presence of *P. reticulata* (Figure 4). This reinforces previous studies on *P. reticulata* that have demonstrated salinity alone is not a sufficiently strong enough selection pressure to prohibit establishment within brackish water (see introduction). Second, in laboratory competition experiments between *P. reticulata* and *P. picta* we found *P. reticulata* exhibited fewer aggressive behaviours than *P. picta* in brackish water, but a similar number of aggressive behaviours in freshwater (Figure 5), suggesting elevated *P. picta* aggression in brackish water is a mechanism that contributes to *P. reticulata* absence in brackish water. Alcaraz et al. (2008) also found that salinity mediated competitive interactions between an invasive mosquitofish (*Gambusia holbrooki*) and a native cyprinodont (*Aphanius fasciatus*). The trade-off between the ability to tolerate salinity and maintain aggressive behaviours among euryhaline fish may be a general pattern, as the same hormones involved in osmoregulation have pleiotropic effects on the pathways that control aggression (Mauro & Ghilambor, 2020). These pleiotropic connections are also observed when fish become subordinate within social hierarchies and have greater difficulty in regulating ions (Gilmour et al., 2005; Jeffrey et al., 2014).

More generally, these results add to a growing number of experimental studies that have found biotic interactions to be context dependent across abiotic environments and may generally shape range limits (e.g. Benning et al., 2019; Benning & Moeller, 2019; HilleRisLambers et al., 2013; Louthan et al., 2015).

Potential evolutionary constraints on niche expansion

Trade-offs between different functions underlie many ecological theories explaining patterns of species diversity (Thomas Clark et al. 2018; Jessup & Bohanna, 2008; Martin, 2015), yet the evolutionary basis of such trade-offs remains an open question (Anderson et al., 2013). By comparing growth rates of full-siblings, we found that the families that had higher growth under a salinity challenge tended to have lower growth when competing with *P. picta* in fresh water (Figure 3). Such results suggest that evolving increased salinity tolerance could come at the expense of any competitive advantage *P. reticulata* has in freshwater. Given that *P. reticulata* is subordinate to *P. picta* in brackish water but not in freshwater (Figure 5), *P. reticulata*'s competitive ability in freshwater could be eroded by a correlated response to selection for improved salinity tolerance. While such conclusions should be tempered by the recognition that our experiments were limited to a single *P. reticulata* population with low levels of replication within family lines, we hope this approach inspires other studies to examine the genetic basis of similar ecological trade-offs. If pleiotropic effects are common, they may frequently constrain the evolution required for populations to expand their ranges (e.g. Duffy et al., 2006).

Other non-mutually exclusive mechanisms could also constrain evolution to brackish conditions and expansion of *P. reticulata* into brackish waters on Trinidad. Based on extensive sampling over time and space (Magurran, 2005; Torres-Dowdall, 2013), it is unlikely *P. reticulata* is dispersal limited at the freshwater/brackish water range edge. Rather our results suggest that *P. reticulata* is instead range limited due to a lack of adaptation to the environment beyond its current range (Hargreaves et al., 2014). A common hypothesis for lack of adaptive evolution is low levels of genetic variation (Blows & Hoffmann, 2005). Yet this is unlikely in this case because we found considerable among family variation in salinity tolerance (Figure 3), suggesting the presence of abundant genetic variation. This is especially important in light of theoretical work which suggests species require enough genetic variation to counteract genetic drift in order to expand their range into new environments (e.g. Polechová, 2018). Further, gene flow from large central populations could prevent the fixation of adaptive alleles in the population at the range limit (Gaston, 2009; Lenormand, 2002; Tigano & Friesen, 2016). It seems

likely that all gene flow into *P. reticulata*'s freshwater edge populations is coming from populations that rarely experience brackish water (Figure 1). This creates a scenario in which this type of gene swamping could be prominent in our system. However, gene swamping is more likely when the population providing the 'maladaptive migrants' is larger than the recipient population (Gaston, 2009). There is an overall lack of evidence that edge populations are smaller than central ones (Dallas et al., 2017) and *P. reticulata* in Trinidad are known to have large populations near their freshwater range limit (Magurran, 2005). Additionally, adaptive divergence has been observed to evolve among *P. reticulata* populations experiencing substantial levels of gene flow over short spatial distances (Fitzpatrick et al., 2015; Torres-Dowdall et al., 2018). Nevertheless, given the lack of any barriers to the movement, we cannot discount a role for gene swamping in constraining evolutionary change at the range margin.

CONCLUSIONS

Fitness trade-offs between the ability to cope with biotic and abiotic challenges have frequently been found to explain species turnover and community composition across environmental gradients (Martin, 2015). Negative genetic correlations and trade-offs between traits have also been commonly invoked as potential evolutionary constraints (e.g. Hughes & Leips, 2018). Here we combine these perspectives to better understand the potential ecological and evolutionary determinants constraining local distribution limits. We find support for the hypothesis that the range limit of *P. reticulata* on Trinidad is shaped by behavioural avoidance of brackish water because of the combined effects of elevated osmoregulatory costs and interspecific competition. Furthermore, we found evidence consistent with salinity tolerance and competitive ability exhibiting a negative genetic correlation across family lines, suggesting *P. reticulata* populations may be constrained to evolve beyond their current range limit. Thus, ecological and evolutionary mechanisms may jointly shape *P. reticulata* distributional limits.

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AUTHORSHIP

A.A.M, J.T-D, C.A.M and C.K.G designed experiments; A.A.M, C.A.M and J.T-D collected fish & bred fish; J.T-D collected common garden and transplant data, A.A.M collected behaviour data, J.T-D and A.A.M performed analyses, A.A.M wrote the first draft of the manuscript and all other authors contributed substantially to revisions. A.A.M and J.T-D should be credited as co-first authors and be listed in the order presented above.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All authors confirm that upon the acceptance of this manuscript, data supporting the results will be archived on Dryad and the data DOI will be included at the end of the article.

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