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Trade-offs between salinity preference and antipredator behaviour in the euryhaline sailfin molly *Poecilia latipinna*

S. M. TIETZE[†] and G. W. GERALD^{*}

Department of Biology, Nebraska Wesleyan University, Lincoln, NE 68505, U.S.A

Abstract

Salinity preference and responses to predatory chemical cues were examined both separately and simultaneously in freshwater (FW) and saltwater (SW)-acclimated sailfin mollies *Poecilia latipinna*, a euryhaline species. It was hypothesized that *P. latipinna* would prefer FW over SW, move away from chemical cues from a crayfish predator, and favour predator avoidance over osmoregulation when presented with both demands. Both FW and SW-acclimated *P. latipinna* preferred FW and actively avoided predator cues. When presented with FW plus predator cues *v.* SW with no cues, *P. latipinna* were more often found in FW plus predator cues. These results raise questions pertaining to the potential osmoregulatory stress of salinity transitions in euryhaline fishes relative to the potential fitness benefits and whether euryhalinity is utilized for predator avoidance. This study sheds light on the potential benefits and consequences of being salt tolerant or intolerant and complicates the understanding of the selection pressures that have favoured the different osmoregulatory mechanisms among fishes.

Keywords

chemical cues; euryhalinity; movement; osmoregulation; predator avoidance

INTRODUCTION

The ability to withstand a wide range of salinities during at least some phase of their life cycle, termed euryhaline, is rare among fishes (Moyle & Cech, 2004). The majority of freshwater (FW) and saltwater (SW) fishes cannot easily move between salinities due to the osmotic stresses imposed on body-fluid homeostasis by disparate haloenvironments relative to the uniform salinity of fish body fluids. Saltwater fishes must drink water and eliminate excess salts, while freshwater fishes do not drink and must absorb salts from the surrounding water (Evans, 1993). The physiological mechanisms involved in ion and water regulation by both FW and SW fishes have been well studied (Hickman, 1968; Shehadeh & Gordon, 1969; Marshall, 2002; Ando *et al.*, 2003; Evans, 2008; Yang *et al.*, 2009; Cerda & Finn, 2010). For euryhaline fishes, modification of ion transport processes through changes in the activity of sodium–potassium pumps in the gills, intestines and kidney, and plasticity of expression of aquaporin and ion-transport proteins are presumably energetically costly to cope with the

^{*}Author to whom correspondence should be addressed: Tel. +1 402 465 2453; ggerald@nebrwesleyan.edu.

[†]Present address: Department of Biology, Georgia Southern University, Statesboro, GA, 30458, U.S.A.

demands of being euryhaline (Tipsmark *et al.*, 2002; Evans *et al.*, 2005; Cerda & Finn, 2010; Hwang *et al.*, 2011; Jung *et al.*, 2012). The rarity of euryhalinity among fishes (2% of ray-finned fishes are found in both FW and SW) is probably due to the high physiological and fitness costs of transitioning back and forth among salinities (Schultz & McCormick, 2013). The exact osmoregulatory costs of euryhalinity, however, are difficult to quantify because of interactions with changes in activity levels, growth rates and potential compensatory mechanisms experienced by fishes at different salinities (Febry & Lutz, 1987; Morgan & Iwama, 1991; Nordlie *et al.*, 1991; Haney & Nordlie, 1997; Beouf & Payan, 2001; Kidder *et al.*, 2006).

Despite the costs, the ability to tolerate and utilize a wide range of salinities has evolved many times in both cartilaginous and bony fishes (Schultz & McCormick, 2013) and has ecological and evolutionary implications associated with the exploitation of habitats unused by stenohaline species. Utilization of different salinities can enhance feeding opportunities, reduce competition, permit the use of disparate areas for reproduction and provide a refuge from predators (Palkovacs & Post, 2009; Betancur-R *et al.*, 2012; Schultz & McCormick, 2013). It has been suggested that the aforementioned reasons defined a new niche available that led to repeated invasions into new halohabitats, leading to the subsequent radiation of fishes (Bamber & Henderson, 1988; Lee & Bell, 1999; Betancur-R, 2010; Whitehead, 2010). A major advantage euryhaline species should have over stenohaline species is the ability to move from one salinity to the next when the benefits of moving outweigh the osmoregulatory and fitness costs. It might predict that salinity movements are influenced by biotic factors, such as food availability and the presence of competitors or predators. This means that euryhaline species should be capable of finding a balance between the potentially conflicting needs of satisfying biotic demands (*e.g.* finding food, avoiding competitors and predators) and mitigating osmoregulatory costs.

One of the most important biotic demands that forces animals to modify optimal behaviours is predation (Lima & Dill, 1990). It is well known that species modify foraging behaviours (Sih, 1982), reproductive strategies (Endler, 1987) and patterns of habitat usage (Schlosser, 1987) as a result of the threat of predation (Lima & Dill, 1990). Altering optimal behaviours in favour of antipredator behaviours should be selected for because predation presents an immediate threat to fitness. Few studies, however, have examined how fishes balance the contradictory demands of minimizing physiological stresses and avoiding predators. The presence of a predator has been shown to reduce air breathing in some fishes, which presumably results in a physiological cost (Kramer *et al.*, 1983; Lima & Dill, 1990). Similarly, some fishes have been found to utilize hypoxic areas more often as a refuge from predators (Chapman *et al.*, 1996; Anjos *et al.*, 2008). It has also been hypothesized that many FW and SW fishes use brackish estuaries as refugia from predators while coping with the energetic challenges of osmoregulation (Moyle & Cech, 2004; Heupel *et al.*, 2007). As far as is known, however, no study has directly tested the hypothesis that the need to avoid predators will outweigh the physiological costs of an immediate salinity transition in euryhaline fishes.

The aim of this study was to determine if the euryhaline, sailfin molly *Poecilia latipinna* (LeSueur 1821) will utilize their ability to tolerate a wide range of salinities for predator

avoidance. Both the salinity preference and predator avoidance behaviours of both FW and SW-acclimated *P. latipinna* were assessed and then tested to see how the fish dealt with the conflicting trade-off. More specifically, it was hypothesized that *P. latipinna* acclimated to different salinities would prefer their acclimated salinity and would be able to detect and avoid water containing chemical cues from a potential predator. It was also hypothesized that *P. latipinna* would choose to avoid predator cues over optimal salinities when the choices conflicted. If so, this would provide empirical evidence of an additional fitness benefit for euryhalinity.

MATERIALS AND METHODS

STUDY ANIMALS

Poecilia latipinna is a euryhaline species inhabiting ponds, lakes, swamps, the backwaters of freshwater streams, salt marshes, estuaries and freshwater tributaries throughout coastal areas of the western Atlantic Ocean, from North Carolina to Florida and areas of the Gulf of Mexico from Florida to the Yucatan Peninsula (Burgess, 1980; Page & Burr, 1991; Nordlie *et al.*, 1992). In addition to regularly being found in brackish waters, it is not uncommon for them to be observed off the coast of the Gulf of Mexico in full sea water (Smith, 1997), but they are most commonly found in salinities < 7 (Ross, 2001; Hendrickson & Cohen, 2012). This species is also capable of tolerating hyper-saline salinities by maintaining plasma osmolarity regardless of salinity (Nordlie *et al.*, 1992; Gonzalez *et al.*, 2005). Adult *P. latipinna* were acquired from a commercial supplier (www.liveaquaria.com) and included a mixture of wild-caught and captive-born individuals originally collected from primarily brackish estuaries in northern Florida and maintained at brackish salinities (range 3–10) until shipment. It is unknown whether or not any of the individuals used in the study were able to move among different salinities in their native habitats or if they were restricted to one salinity. As far as is known, information regarding how often various populations of *P. latipinna* move back and forth among salinities in nature is scarce. In the laboratory, *P. latipinna* were kept together in two 170 l aquaria for at least 6 weeks before the beginning of trials. Several species have been shown to exhibit plasticity in physiology and gene expression to permit acclimation to changes in salinity in as little as 2 weeks when transferred from FW to SW or *vice versa* (Marshall *et al.*, 1999; Jung *et al.*, 2012). Half of the acquired *P. latipinna* were kept in fresh water (salinity < 5), while the other half were kept in full sea water (salinity $c. 28$) *via* the addition of commercial sea salt (Instant Ocean; www.instantocean.com). Both aquaria were kept at 27° C, range $\pm 2^\circ$ C with a 12L:12D photoperiod and had daily 10% water changes. *Poecilia latipinna* were fed commercial TetraMin (www.tetra-fish.com) fish flakes daily. Each individual *P. latipinna* was tested only once for each of the three experiments and not reused.

EXPERIMENTAL APPARATUS AND PROCEDURES

A custom-made shuttle-box system (designed by Loligo systems; www.loligosystems.com) was used in three two-choice experiments. This shuttle-box system consisted of two circular compartments (29.6 cm diameter $\times$ 14.9 cm depth) connected by a bridge (7.5 cm long $\times$ 4.8 cm width) that permitted *P. latipinna* to voluntarily move between the two compartments (Fig. 1). Each compartment was connected to two large buffer tanks (50 $\times$ 20 $\times$ 20 cm) *via* a

water pump and plastic tubing. The buffer tanks were used to recirculate the water separately from each compartment (Fig. 1). Water was pumped from the buffer tanks to the compartments and back, which resulted in water circulation that minimised water mixing between compartments and resulted in a water depth of *c.* 11 cm. A partition was placed in the bridge until the beginning of trials. Preliminary data showed that salinity remained mostly unchanged throughout the duration of each trial (30 min). A change of only 2 was found over 30 min with a starting salinity gradient of 28 *v.* 1. In addition, the diffusion rate of 5 ml of methylene blue added to one compartment was assessed. Though not quantified, methylene blue was not observed in the opposite compartment after 30 min. Speed of water flow was controlled by a rheostat and minimised to reduce turbulence (which might impede movement) while maintaining water flow between the compartments and buffer tanks. The entire apparatus was positioned so that it received only artificial room lighting and each compartment received the same amount of light.

Individuals were placed, one at a time, in the middle of the apparatus (bridge) and allowed to move into one compartment or the other. During each trial, individuals could move freely back and forth between the two compartments. Individuals were videotaped with a camera (Sony Digital8 DCR-TRV 460; www.sony.com) mounted above the apparatus for 30 min. Time spent in each compartment (and the connecting bridge), the final selection (*i.e.* the compartment the *P. latipinna* was in at the end of the trial) and activity (measured as the number of times individuals crossed from one compartment to the other) were quantified from video analyses. The entire apparatus was drained, rinsed and re-filled following each trial. This setup was used for all three of the following experiments.

SALINITY PREFERENCE

First, preference for either FW or SW in both FW and SW-acclimated *P. latipinna* was assessed. One side of the apparatus was filled with fresh water (salinity *c.* 1), while the other was filled with salt water (salinity *c.* 28). Thirty-five individuals (21 FW-acclimated and 14 SW-acclimated) were tested individually to determine their salinity preference. Salinity preference was determined by measuring time spent on each side (FW *v.* SW). The number of times individuals crossed from one side to the other and final selected compartment was also recorded.

PREDATOR AVOIDANCE

Twenty-one FW-acclimated individuals were tested to see if they could detect and avoid chemical cues from a crayfish *Procambarus clarkii* predator that is found sympatrically within the natural range of *P. latipinna* (Hobbs, 1974). *Procambarus clarkii* were acquired from a commercial supplier (Carolina Biological; www.carolina.com) and kept in separate tanks in fresh water (salinity *c.* 1) and fed individual *P. latipinna* before being used in preference trials, as chemical cues from both the predator itself (especially those that have recently fed on the prey animal) and conspecific alarm cues have been shown to elicit antipredator responses in many aquatic animals (Lima & Dill, 1990). *Poecilia latipinna* were quickly euthanized by a blow to the head before being fed to *P. clarkii*. Approximately 100 ml of water from the tank holding *P. clarkii* was mixed with new fresh water (salinity *c.* 1) and used in one compartment of the shuttle-box, while fresh water (salinity *c.* 1) containing

no chemical cues was added to the other. Additionally, an individual *P. clarkii* was placed in the buffer tank (side with the chemical cues) for the duration of each trial. *Procambarus clarkii* were not visible to *P. latipinna* at any point during the trial. Time spent (*i.e.* amount of time spent in the compartment containing cues *v.* no cues), number of crosses and final compartment selection was then analysed from video analyses as described above.

SALINITY PREFERENCE X PREDATOR AVOIDANCE TRADE-OFF

Following the testing of salinity preference and predator avoidance, both variables were combined to determine how *P. latipinna* would choose between two contradictory demands. As before, one side of the apparatus was filled with fresh water (salinity *c.* 1), while the other was filled with salt water (salinity *c.* 28). A sample of 100 ml of water from the *P. clarkii* holding tank, however, and an adult *P. clarkii* were added to the buffer tank on the side of the apparatus that contained the previously determined preferred salinity. There were two possible combinations of salinities and cues that could be used depending on the outcome of the salinity preference experiment (Table I). The goal was to pair the preferred salinity with predatory chemical cues (assuming cues were detected and avoided) and the non-preferred salinity with no cues (Table I). This permitted the examination of whether *P. latipinna* would switch their salinity preference in response to the presence of a predator. For both potential combinations, it was hypothesized that *P. latipinna* would move away from the cues regardless of the salinity because of the immediate risk of death imposed by the chemical cues. As in the first experiment (salinity preference), 35 individuals (21 FW-acclimated and 14 SW-acclimated) were tested individually and salinity selection was determined by quantifying time spent on each side (non-preferred salinity + no predator *v.* preferred salinity + predator) and final compartment selection. As above, the number of crosses from one side to the other was also recorded to quantify activity.

STATISTICAL ANALYSES

For each of the three experiments, χ^2 goodness-of-fit tests were used to assess preferences among the three compartments (two experimental compartments and the small connecting bridge in the middle) using the time spent in each compartment. Expected values for the analyses reflected the volume of each of the three compartments. Contingency tables were used to test for differences in the final compartment selection between FW and SW-acclimated *P. latipinna* and to determine if there was a preference for one compartment at the end of individual trials. Mann-Whitney *U*-tests were used to test for preference differences in the time spent on the preferred sides between FW and SW-acclimated individuals for the two salinity-preference experiments. Moreover, ANOVAs were used to see if the intensity of preference differed among the three experiments for both time spent and activity.

RESULTS

SALINITY PREFERENCE

Both FW and SW-acclimated *P. latipinna* spent more time in the compartment containing fresh water [Fig. 2(a)] as compared with time spent in the bridge connecting the salinities and the compartment containing salt water (χ^2 -tests, FW: $\chi^2 = 1446.4$, d.f. = 2, $P < 0.001$; SW: $\chi^2 = 640.3$, d.f. = 2, $P < 0.001$). There was no difference in time spent on the FW side

between FW and SW-acclimated individuals (Mann-Whitney U -test, $U_{21,14} = 49$, $P > 0.05$). Individuals crossed from one side of the apparatus to the other on mean $\pm$ S.D. of 8.3 ± 1.8 times over the course of a trial, thereby indicating that *P. latipinna* were actively sampling both salinities. Also, at the end of each trial, the final compartment selection (FW v. SW side) was not significantly different between acclimation groups ($\chi^2 = 0.005$, d.f. = 1, $P > 0.05$).

PREDATOR AVOIDANCE

Poecilia latipinna actively avoided the side of the apparatus that contained *P. clarkii* chemical cues and conspecific alarm cues by spending less time in that compartment compared with the bridge and the side containing no chemical cues [$\chi^2 = 4011.3$, d.f. = 2, $P < 0.001$; Fig. 2(b)]. *Poecilia latipinna* had mean $\pm$ S.D. 6.8 ± 1.4 crosses from one side of the apparatus to the other. At the end of the trials, individuals were most often found on the side with no chemical cues (14/21 individuals), even though this trend was not significant ($\chi^2 = 2.33$, d.f. = 1, $P > 0.05$).

SALINITY PREFERENCE X PREDATOR AVOIDANCE TRADE-OFF

Since *P. latipinna* significantly preferred FW over SW and actively avoided chemical cues originating from *P. clarkii*, these factors were combined to present the two non-preferred options from the two previous experiments. One side of the apparatus contained FW with a *P. clarkii* and chemical cues (FW + predator), while the other side contained SW with no predator or chemical cues (SW only). Surprisingly, both FW and SW-acclimated individuals preferred the FW + predator side over the SW side and the bridge [$\chi^2 = 8990.75$, d.f. = 2, $P < 0.001$; Fig. 2(c)]. There was no difference in time spent on the FW + predator side between FW and SW-acclimated fish (Mann-Whitney U -test, $U_{21,14} = 58$, $P > 0.05$).

Poecilia latipinna crossed back and forth mean $\pm$ S.D. of 7.5 ± 2.3 times to sample both test conditions. Moreover, at the end of the trials, more individuals were found to be on the FW + predator side rather than on the side with SW only (24/35 individuals; $\chi^2 = 5.83$, d.f. = 1, $P > 0.05$). Across the three experiments, no difference was found in the time spent in the preferred compartment ($F_{2,60} = 1.93$, $P > 0.05$) or in activity ($F_{2,60} = 1.12$, $P > 0.05$).

DISCUSSION

SALINITY PREFERENCE

As expected, *P. latipinna* preferred fresh water over salt water. This is not surprising since *P. latipinna* is found in mostly FW habitats, with some populations occupying brackish waters with only occasional forays into SW (Page & Burr, 1991; Nordlie *et al.*, 1992; Smith, 1997). Despite *P. latipinna* being a euryhaline species widely known for being able to tolerate salinities ranging up to hypersaline (Nordlie *et al.*, 1992), the behavioural avoidance of SW suggests that there is a significant physiological stress associated with osmoregulation in salinities above that of FW. Avoidance of SW by individuals acclimated to SW for 6 weeks strengthens the idea that there are substantial costs to being in high salinities. It should be noted, however, that salinity preference in some euryhaline species has been shown to be unaffected by prior acclimation to either FW or SW (Fritz & Garside, 1974; Meador & Kelso, 1989). *Poecilia latipinna* display a higher rate of O₂ consumption and a higher rate of

Na⁺–K⁺ ATPase activities when exposed to both SW and hypersaline waters (Gonzalez *et al.*, 2005). Additionally, Yang *et al.* (2009) found that SW-acclimated *P. latipinna* exhibit higher plasma glucose levels and increased expression of branchial heat-shock protein 90 while maintaining homeostasis, showing that SW is stressful to this species. Though *P. latipinna* is capable of tolerating and inhabiting SW habitats, they probably prefer FW when given a choice in order to reduce the stress associated with osmoregulation in higher salinities.

PREDATORY AVOIDANCE

In experiment 2, *P. latipinna* was able to detect and avoid water containing chemical cues from *P. clarkii* and alarm cues from injured conspecifics. These results were expected as many aquatic animals are well known to detect and exhibit antipredator behaviours in response to both the chemical cues from predators and injured conspecifics (Chivers & Smith, 1998; Kats & Dill, 1998; Ferrari *et al.*, 2010). Although the type of predator and relative concentration of cues from both predators and conspecifics (both singly and in combination) has been shown to influence the antipredator response in prey animals (Brown *et al.*, 2001; Golub & Brown, 2003; Lonnstedt & McCormick, 2011), the combination of *P. clarkii* cues and alarm cues released by feeding *P. latipinna* to *P. clarkii* prior to trials was sufficient to elicit avoidance behaviours and could be used to explore the question of how *P. latipinna* balance the conflicting demands of antipredator behaviour and osmoregulation.

SALINITY PREFERENCE X PREDATOR AVOIDANCE TRADE-OFF

During the final phase of this study that involved *P. latipinna* choosing to either avoid a non-preferred salinity or avoid chemical cues of a potential predator, it was hypothesized that *P. latipinna* would utilize their euryhalinity (by moving from FW to SW) to move away from the predatory cues. Avoiding predation should be a demand that outweighs the osmoregulatory stresses of moving to SW in such a widely salinity-tolerant species. *Poecilia latipinna* significantly chose to stay in FW containing predatory cues over SW with no cues. This result is surprising since the threat of predation should take priority due to the potential for fitness reduction if killed as compared with any other setback (*i.e.* failure to find food, mates or optimal habitat) (Lima & Dill, 1990). Predation has been strongly suggested to influence the evolutionary histories of many animals, resulting in physiological and behavioural strategies that reflect a compromise between reducing predation risk and maximizing the ability to find suitable food, mates and habitat (Mayr, 1963; Sih, 1987; Lima & Dill, 1990) and, thereby, having a large influence on the ecology of many prey animals (Holt & Barfield, 2009; Holt *et al.*, 2011).

A number of other studies have found that animals will alter their activity patterns (Templeton & Shriner, 2004), feeding strategies (Lima & Dill, 1990) and patterns of microhabitat usage (Schlosser, 1987; Anjos *et al.*, 2008), while often foregoing opportunities for feeding, mating and thermoregulation, in favour of antipredator behaviours (Rodd & Reznick, 1997; Gerald & Spezzano, 2005). This makes the finding that *P. latipinna* will select an optimal salinity (osmoregulation) over predator avoidance somewhat puzzling. Although there are significant physiological costs associated with osmoregulation that have been previously estimated to be a small percentage of the total energy budget (McCormick

et al., 1989), the threat of predation to lifetime fitness should far outweigh moving into a sub-optimal salinity for a short period of time. This is more likely for a species well known to be very tolerant of a wide range of salinities (Nordlie *et al.*, 1992) and often found in brackish water and SW in nature. Moreover, the fact that SW-acclimated individuals preferred FW + predator over SW only, suggests that the osmoregulatory requirements of living at high salinity and the subsequent effect on lifetime fitness might be more costly than previously suggested relative to other biological demands (*e.g.* feeding and thermoregulation). Without information on the fitness of euryhaline individuals that frequently switch salinities *v.* those that do not, it will be difficult to quantify the fitness costs related to the acute physiological costs of transitioning among salinities.

Another variable that might explain the lack of predator avoidance behaviour in favour of osmoregulation could be the influence of salinity on swimming performance. Several studies have found decreases in activity levels and performance at salinities at the high end of the salinity tolerance range in various euryhaline species (Swanson, 1998; Edeline *et al.*, 2005; Serrano *et al.*, 2010). Changes in performance capabilities caused by abiotic factors (such as temperature) have been shown to change the behavioural strategies used during encounters with predators (Hertz *et al.*, 1982; Gerald, 2008). Although no evidence of this was found, the reduction in performance ability in SW could have resulted in a shift from a more active, fleeing behavioural strategy to a more stationary, freezing or fighting behavioural strategy to balance the trade-off between osmoregulation and predator avoidance. Alternatively, *P. latipinna* might have simply selected the salinity that permitted maximal performance to allow the highest chance of escape from the predator regardless of where the highest concentration of cues was located. It is also possible that the predator cues were not threatening enough to elicit movement into SW. Though visual or mechanical predatory cues were not used in the present study, it is possible that these cues could enhance the perceived threat to influence salinity selection. Avoidance of cues in the FW-only experiment, however, suggests that the chemical cues used were a threatening stimulus. Future studies would be needed to determine the proximal causes of *P. latipinna* choosing osmoregulation over predator avoidance.

This study raises questions concerning the costs of euryhalinity and why some species utilize multiple salinities in nature if predation risk alone may not be enough to elicit movement into a non-preferred salinity. Though many of the costs related to the physiological mechanisms of switching salinities have been empirically quantified (Yang *et al.*, 2009), perhaps a combination of multiple factors (*e.g.* predation risk, food availability and temperature) is needed to overcome the osmoregulatory costs of moving from one salinity to another. Bucking *et al.* (2012) concluded that the euryhaline killifish *Fundulus heteroclitus* (L. 1766) would alter their salinity preference to enhance digestion to maintain calcium homeostasis. Also, Serrano *et al.* (2010) found inconsistencies between salinity preferences measured in the field and in the laboratory. They suggested that the need to use higher salinities for reproductive purposes outweighed the osmoregulatory costs (Serrano *et al.*, 2010). These studies, along with the present study, highlight the need to consider both ecological needs and osmoregulatory costs when attempting to explain how euryhalinity and stenohalinity evolved and are maintained in the various lineages of fishes.

The finding that SW-acclimated *P. latipinna* significantly selected the side with FW + predator cues raises additional questions about how ecologically relevant are the up regulation of various physiological, cellular and biochemical mechanisms when acclimated to non-preferred salinities in controlled conditions (Yang *et al.*, 2009). Though plasticity mechanisms related to acclimation might mitigate osmoregulatory costs, it may not be enough to offset the costs for some fishes to move into a non-preferred salinity when presented with an immediate threat (*e.g.* aggressive competitor or predator). This study raises additional questions related to how exposure to different salinities early during development might influence salinity preferences as adults since all individuals used in this study probably had previously experienced similar salinities (3–10) since birth. More studies that examine how fishes make decisions when presented with various conflicting demands (both singly and in combination) are needed. These studies might also shed more light on salinity utilization patterns and the multiple evolutionary salinity transitions (both FW → SW and SW → FW); along with the numerous movements towards and away from euryhalinity during the diversification of fishes (Schultz & McCormick, 2013).

The ability to be euryhaline requires plasticity in a number of physiological and biochemical mechanisms that have been shown to be energetically costly. Presumably, the relative rarity of extant euryhaline species among fishes reflects these energetic costs. Fitness costs of euryhalinity, however, are difficult to quantify and require a consideration of both physiological costs and ecological demands to establish the actual costs of euryhalinity. These results suggest that the physiological costs of moving from FW to SW are much greater than previously thought and outweigh predator avoidance in *P. latipinna*. It might require many ecological benefits simultaneously (*e.g.* predator avoidance, food availability and optimal sites for reproduction) to overcome both the acute and chronic osmoregulatory costs of switching to a non-preferred salinity. Future studies that examine salinity preferences in euryhaline fishes should consider multiple ecological factors when exploring ultimate questions related to salinity selection and utilization.

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FIG. 1.

Schematic of the shuttle-box choice system used to assess salinity selection and predator avoidance behavior in *Poecilia latipinna*. Individuals were able to swim back and forth between the two compartments (each 29.6 cm diameter 14.9 cm deep) via a 7.5 cm long connector. Arrows in the compartments represent the direction of water circulation created by water pumps that pumped water toward a buffer tank associated with each compartment. Opposite directions of water circulation between the compartments minimized mixing.

FIG. 2.

Percentage of the total time spent in each of two shuttle-box choice compartments containing different salinities and chemical cues during three separate choice experiments by *Poecilia latipinna*. (a) Time spent in compartments with fresh water (FW) and salt water (SW) for both FW and SW-acclimated *P. latipinna*. (b) Time spent in compartments containing fresh water (FW) with predator (*Procambarus clarkii*) cues and conspecific alarm cues v. FW with no predator cues. (c) Time spent in a compartment containing fresh water with predator (*P. clarkii*) cues + conspecific alarm cues that signal the presence of a predator (FW + pred) relative to a compartment containing salt water with no cues (SW only) for both FW and SW-acclimated *P. latipinna*. Data on time spent in the bridge portion of the apparatus were not included because there was no significant preference or avoidance of this area and no significant differences between acclimation groups. ■, FW-acclimated *P. latipinna*; ▒, SW-acclimated *P. latipinna*. *, $P < 0.05$.

Table I

Two potential pairings for the salinity preference $\times$ predator avoidance trade-off experiment. Chemical cues were combined with the preferred salinity [fresh water (FW) or salt water SW)] of *Poecilia latipinna* determined from the salinity preference experiment. One side of the two-choice test apparatus contained the preferred salinity + chemical cues, while the other side contained the non-preferred salinity with no cues

Salinity preference	Salinity-cue Combinations
FW	FW + cue v. SW + no cue
SW	SW + cue v. FW + no cue