

# Guppies as heterospecific facilitators: a precursor of exploratory behavior?

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**Abstract** Behavior can explain population-level processes such as dispersal, yet connecting a specific behavioral phenomenon with a larger ecological pattern is often speculative rather than supported by experimental studies. We investigate how exploratory behavior may develop in the killifish, *Rivulus hartii* through association with another taxon, the guppy, *Poecilia reticulata*. We hypothesize that exploratory behavior is enhanced by nearby guppies, which embolden *Rivulus* to move along the river edge, through zones of high predation risk. We tested individual boldness in the presence of both guppies and conspecifics. We also tested for the effect of prior experience with guppies, comparing boldness in *Rivulus* from locations in which it was either allopatric to or sympatric with guppies. Guppies increased boldness in *Rivulus*, equivalent to the effect of conspecifics, and prior experience with guppies also

increased boldness over that of inexperienced *Rivulus*. Sympatric *Rivulus* were shy compared with the allopatric ones when each was tested alone, but this relationship reversed when guppies were present, showing that boldness is a plastic trait that can be influenced by the population of origin. An experimental field-stream test showed that guppies increased movement of *Rivulus* under predation threat, supporting links in a conceptual framework that connects a behavioral phenomenon, exploratory boldness, with a larger ecological pattern, selection of favorable habitats that, in turn, can lead to increased reproduction and fitness relative to non-dispersers.

**Keywords** Bold behavior · Exploratory behavior · Dispersal · Facilitation · Mixed species groups · *Poecilia reticulata* · *Rivulus hartii*

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## Introduction

The ultimate cause of behavioral traits is often uncertain. In a seminal experimental work on the development of schooling behavior in pelagic fish, Williams (1964) hypothesized that schooling in the open ocean should be promoted by the lack of opportunity to hide from predatory attacks except behind another fish, thereby affording some safety. He also pointed out that, once established, other adaptive benefits related to schooling, like enhanced food acquisition, rapidly accrue, but much of his argument was based on logic rather than experiment. Understanding how behavioral traits are established and maintained, and the basis for their between-population differences, including the maintenance of consistent differences between individuals within populations, continues to be a challenge (e.g., Sih et al. 2004).

In contrast to the open ocean environment, freshwater lakes and streams contain a range of habitat structures, and schooling is one of several behaviors that are attributable to reducing predation threat (Pitcher 1986; Alan and Pitcher 1986). Schooling has been reported for many taxa (reviewed by Ward et al. 2002), and predictably, numerous foraging benefits have also been reported, even between distantly related taxa where the foraging activity of one species creates feeding opportunities for a second (reviewed by Matthews 1998). As with schooling of conspecifics, Matthews (1998) also documents heterospecific associations. In addition to anti-predation and foraging benefits, these associations are implicated as a factor in improved species recognition and discrimination (Griffiths and Magurran 1997), which in turn may affect the course of social interactions, such as courtship and mating (Magurran and Ramnarine 2004). Heterospecific associations may also facilitate movement through risky habitats by providing protection from predators, which in turn, may enhance the exploratory behavior of individuals. We further explore these relationships in the context of predator-threatened zones of a stream where we have identified a heterospecific association as a candidate for enhancing exploratory behavior.

In this study, we attempt to connect a behavioral phenomenon with a larger ecological pattern. We begin by asking whether a shoaling taxon, the guppy, *Poecilia reticulata*, facilitates exploratory movement by another non-shoaling species, the killifish, *Rivulus hartii* (Seghers 1973; Gilliam and Fraser 2001). Support for this facilitation hypothesis may suggest a way to link exploratory behavior with longitudinal movement along the river because, while movement away from a home patch is inherently risky, e.g., as in Fraser et al. (2006), facilitated exploratory movement to potentially favorable habitats, e.g., previously depleted patches that currently contain few conspecifics, can be rewarded by increased growth and reproduction. Gilliam and Fraser (2001), for example, found that cobble patches and other possible refuges for *Rivulus*, such as isolated side pools, varied in stability across seasons, and some ephemeral ones had few or no *Rivulus*. These patches could therefore be resource-rich and highly beneficial for a colonizing *Rivulus*.

#### The natural setting and background

In the foothill streams of Trinidad's Northern Range Mountains, early life stages of the killifish, *R. hartii*, are readily found in association with the guppy, *P. reticulata*. Here and below we use the term “association” as a term of convenience. It is noteworthy that these associations do not imply any active, behavioral interactions. In fact, at times, both taxa may simply be feeding on a similar food base.

However, the associations are also inevitably found in a matrix of high-risk versus safe habitats; deep, predator-infested pools are separated by relatively safe refuges, mainly patches of cobble (64 – 256 mm) that are too shallow for predator access and/or provide good hiding places. It is under these stream conditions that heterospecifics may facilitate movement between refuges. All life stages of guppies tend to shoal in predator-threatened zones (Seghers and Magurran 1995), and Croft et al. (2003) found that shoals are strongly size-assorted, with those comprised of smaller size classes swimming within 2 m of shore. We follow Pitcher (1986) and Dugatkin and Godin (1992) and use “shoal” to mean any social group. Large adult females (>25 mm total length), along with actively courting males, can also be found midstream in fast water (Magurran 2005). In contrast, *Rivulus* of all sizes hug the shoreline, and rarely move beyond. Most, and especially adults >30 mm total length (TL), are associated with cobble patches, and adults are infrequently found in the intervening matrix, especially when woody or cobble structure is lacking (Fraser et al. 1995, 1999).

In previous studies, the distribution of distances moved by *Rivulus* was found to be strongly leptokurtic. Most individuals were sedentary (Gilliam and Fraser 2001; Fraser et al. 2006), but a mobile fraction that makes moves well beyond the home patch was always present (Fraser et al. 2001), pointing to the possibility of an underlying behavioral strategy, movers and non-movers, as one explanation for the mobile fraction. We know that individual adult *Rivulus* differ in boldness and exploratory behavior and can be arrayed along a continuum from non-movers to bold explorers (Fraser et al. 2001), but movement and exploratory behavior of early life stages of *Rivulus* remain unknown, and the anecdotal observation that they are often found in river sections exposed to predators, independent of adults, and in association with guppies, suggests that juvenile stages may have fundamentally different habits from the more readily observed and caught adults. The potential association between guppies and juvenile *Rivulus* raises the question of whether guppies may act as facilitators of movement through the habitat matrix by early life stages.

#### Specific tests

Our hypothesis is that the presence of a shoaling taxon causes members of a second, non-shoaling taxon to “feel” safer in predator-threatened habitats, thereby facilitating its movement, dispersal, and habitat selection. Of course, *Rivulus* may also benefit from nearby guppies in other ways as, for example, when the feeding activity of guppies disturbs the bottom sediments to the advantage of *Rivulus*. The challenge of testing the facilitation hypothesis is that it

is not possible to directly observe the behavior and movement of the smaller size classes of *Rivulus* in their natural environment. Instead, we use a suite of behavioral tests to evaluate this hypothesis. First, we test whether another species, guppies, increases the boldness of *Rivulus*. We predicted that boldness would be greater in the presence of guppies than in their absence and that boldness with guppies would be at least as strong as boldness with a conspecific. Because we surmised that previous experience with the heterospecific may be important for the efficacy of facilitating movement, we tested a second hypothesis that boldness is affected by experience by testing the prediction that sympatric *Rivulus* with prior experience of guppies would be bolder than allopatric *Rivulus* with no prior experience. Lastly, we used a field study in an experimental stream facility in Trinidad to directly test for movement of juvenile *Rivulus* from a safe refuge, through open water under predation threat, when guppy shoals were present and absent.

## Methods

### Heterospecific groups in the natural stream

To document our anecdotal observations that groups of guppies of all life stages and immature *Rivulus* of similar sizes occur in loose, heterospecific associations, we sampled sites in the Guanapo and Turure Rivers, with drainages on the south slope of Trinidad's Northern Range Mountains. The Turure River sites were located in lower montane rainforest of the Matura Forest Reserve, between 75 and 200 m elevation where the stream bed is predominantly pebble (4–64 mm) and cobble (64–256 mm) with alternating pools and cobble riffles and with occasional interludes of sand and gravel beaches, grain size <4 mm and 4–32 mm, respectively. The Guanapo sites were all lowland sites at 50 m that contained deep pools and long stretches of predominantly sand and gravel beaches with occasional patches of cobble. All sites were high-risk locations where fish, *Hoplias malabaricus* and *Crenicichla alta*; avian, *Ceryle* sp.; and snake, *Helicops angulatus* and *Hydrops triangularis* predators were observed.

Because we had previously found small *Rivulus* along sandy beaches but not in deep river pools, we seined ten beaches chosen for substrate condition, i.e., sand/gravel, irrespective of taxa present, five in the Turure River and five in the Guanapo River using a seine with 1/16th-inch mesh. We standardized seining distance to 10 m. Each beach was seined several times.

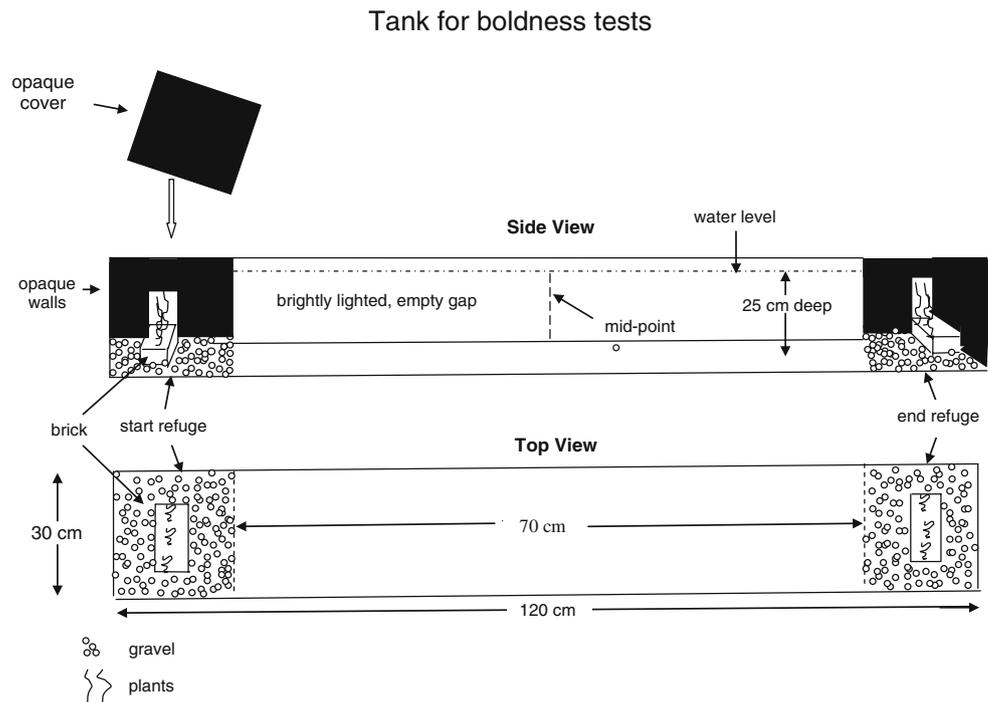
### Experiment 1 Boldness in presence of guppies vs. conspecifics

### Tank assay for boldness

We evaluated whether proximity to guppies caused an increase in the boldness of similarly sized, immature *Rivulus*. To assay for boldness, we followed procedures used previously (Fraser et al. 2001). A long, glass tank (120 cm long × 30 cm deep × 30 cm wide) contained a refuge (25 cm) at either end; the intervening gap was unstructured and brightly lighted by a 75-watt, overhead incandescent light (Fig. 1). The refuges were divided into a front and rearmost section by a red-clay brick that had three attached artificial plants. The floors of the refuges were covered with gravel. The refuges had two sides covered with black plastic, left side and back, and the start refuge had an opaque cover, which created a dimly lighted space. The observer's side was clear, and the observer sat behind a screen, 2 m from the tank, so that all parts of the tank could be viewed simultaneously without disturbing the fish.

*Rivulus* and guppies were obtained by seining in Ramdeen Stream (Dugatkin and Godin 1992), a second-order tributary of the Arima River. They were kept in their respective community tanks for several days prior to testing. *Rivulus* were acclimated overnight (15–21 h) in batches of 12 by confining them to the refuge area of the experimental tank with an opaque, glass partition. After acclimation, fish were transferred to a holding tank where they were fed prior to testing. No food was present in the test tank. Each subject *Rivulus* was tested individually by dipping it from the holding tank with a dip net and releasing it into the far back corner of the refuge. The order of treatments, with or without guppies present, was alternated. In treatments with guppies, 12 guppies of various sizes (range in total length = 25 – 35mm, mean ± SE = 26.5 ± 0.51) were also added outside of the refuge, prior to the test. Guppies immediately formed loose shoals and moved throughout the tank. Similarly, in treatments with conspecifics, six *Rivulus* were added prior to the test. Added *Rivulus* typically remained motionless for several minutes before moving. In either case, whether with guppies or conspecifics, the added fish were continuously within view of the focal *Rivulus*. The focal fish was timed for crossing out of the refuge (>50% of the fish body over a line on side of tank marking limit of refuge), time to cross over to opposite refuge. Once committed to crossing the gap, most *Rivulus* went straight across to the opposite refuge. However, from previous studies, we surmised that a few fish would explore the gap, but then return to the start refuge without entering the opposite refuge. Therefore, we terminated a test when 50% of the fish body had crossed a line drawn on the tank midway between the two refuges. A test was completed when a fish crossed over, or at 20 min if no cross occurred. Fraser et al. (2001) found that every subject, whether alone, with guppies, or with conspecifics,

**Fig. 1** Experimental tank used to test for boldness in *Rivulus*



exhibited some level of “fear and caution” in the refuge. Subjects moved cautiously, stopping and starting, to the front of the brick, usually passing between the end of the brick and the tank wall. Most stopped swimming at the leading edge of the refuge, appearing to look at the empty space before moving out. All moves appeared to be associated with tank exploration as we observed no feeding attempts (no food present). Thus, we believe the assay measured boldness in propensity to explore beyond the refuge.

We calculated a bold index, assumed to correlate with exploratory behavior, by taking the proportion of the total test time (1,200 s) to cross the midway point:

$$\text{bold}_{\text{index}} = 1 - (\text{time to cross(s)}/1200\text{s})$$

Thus, a fish that never crossed received a cross time of 1,200 s and hence a boldness score of zero.

To test for an effect of guppies on *Rivulus* boldness, we tested each fish twice, first in the presence of guppies (*Rivulus*,  $n=32$ ), then the same fish alone with no guppies present, allowing 24 h between successive tests. *Rivulus* size range approximated the sizes that co-occurred with guppies in our beach seines ( $n=32$ , range = 22–41 mm TL, mean  $\pm$  SE = 31.7  $\pm$  0.79). In previous work (Fraser et al. 2001), we found that, within this limited size range, bold<sub>index</sub> scores were affected by neither sex nor total length, and so, we compared the effect of the presence and absence of guppies on *Rivulus* bold<sub>index</sub> scores with paired *t* tests. The

response variable, bold<sub>index</sub>, was bounded at the upper (1.0) and lower (0) ends, and therefore, we transformed it with the arcsine-square root transformation.

To compare the strength of the guppy effect relative to the effect of conspecifics, we tested a second group of *Rivulus* collected in Ramdeen Stream ( $n=20$ , mean  $\pm$  SE = 30.13  $\pm$  0.78 mm TL). We assayed their boldness in the presence of a group of conspecifics using the same protocol as with guppies, and compared the transformed bold<sub>index</sub> with a paired *t* test. We made partial water changes (50%) between tests to limit build up of guppy or *Rivulus* odors.

Table 1 shows that three outcomes in the two-test sequence are possible. A guppy effect is assumed if the subject is less bold in the second test. However, a prior experience effect is indicated in two of the outcomes, a confounding factor. No change in boldness can be interpreted as a no-guppy effect, or that the fish learned in the first test and behaved the same way in the second. Similarly, a subject might be bolder in the second test because the second test added to that learned in the first. Owing to the lack of any negative experience in the first test with guppies, as for example, if a predator threat were present, we surmised that, in the second test, the bold<sub>index</sub> should either remain the same or increase, but not be significantly less than in the first. A possible exception to this reasoning could be a negative effect associated with the transfer of fish by dip net. However, we have not previously observed this effect beyond the brief latency period in which the fish freezes when first placed in the

tank. To help clarify the alternatives in Table 1 and test for a prior experience effect, we followed the first test series with a second series in which *Rivulus*, not previously tested, were tested twice, each time alone. A consistent boldness score here would indicate a lack of an added prior experience effect. The same outcomes and conclusions apply to the conspecific tests (conspecifics used instead of guppies).

#### Experiment 2 Effect of familiarity on boldness

We collected 40 *Rivulus* from above a barrier waterfall in Ramdeen Stream where it was the only fish taxon present and 40 *Rivulus* from below the barrier where they co-occurred with guppies. We split these into two groups; 20 were tested alone and 20 with guppies, using the same procedures as above. As before, we found neither sex nor total length of *Rivulus* to be significant factors in any of the trials. In these tests, however, we tested each fish only once, and used a two-way ANOVA to evaluate the effect of the two factors, *Rivulus* experience (experienced, inexperienced), and guppies (present, absent) on the response variables. As in experiment 1, we arcsine-square root-transformed the  $\text{bold}_{\text{index}}$  response variable. We used the Scheffé test to test for differences between treatments, experience vs no experience and guppies present vs absent.

#### Experiment 3 Effect of guppies on individual movement under predation threat

We used an experimental stream facility in Trinidad to ask whether nearby guppy shoals would facilitate movement between patches by young *Rivulus* under predation threat. The experimental stream, which has concrete walls and floor, lies alongside Ramdeen Stream. Our tests were done

sequentially in a 4-m segment of the stream channel (width=1 m). Water was piped in by gravity flow from an upstream, first-order tributary. Baffles maintained a uniform depth of 2.0–3.0 cm, except in two basins, 10–15 cm deep, sculpted 2 m apart in the floor of the channel. Flow rate through the system was just sufficient to maintain depth. Uniform dry season conditions prevailed during the test period; little rain fell and flow rates through the channel were constant.

We constructed a safe refuge patch at each end of the channel. Each was 0.3 m in longitudinal length by 1.0 m wide (full width of the channel). We screened the refuges with 1.25 cm<sup>2</sup> wire screen to prevent access by the predators. Each refuge had a cobble substrate, leaves, and sticks. The remainder of the stream channel contained no added structure, but the rough concrete walls, baffles, and occasional leaf fall provided some habitat heterogeneity.

We created strong and persistent predation threat by stocking the open channel with two *H. malabaricus*, 150 and 165 mm total length. We provided a shelter for *Hoplias* in each of the two basins by partially covering them with a piece of wood. At dusk, *Hoplias* would typically emerge and assume an ambush posture near their shelters. We never observed them together in the same stream section and usually did not observe them out of their shelters during the day.

*Rivulus* and guppies were collected from the adjacent Ramdeen Stream (*Rivulus*  $n=110$ , TL Range = 20–39mm, mean  $\pm$  SE =  $33.2 \pm 0.44$ mm; guppies,  $n=60$ , TL range = 12 – 36.5mm, mean  $\pm$  SE =  $22.7 \pm 0.70$ mm).

The 2×3 design consisted of two guppy treatments, present or absent, crossed with three densities of *Rivulus*, 4, 8, and 16. A different group of *Rivulus* was used for each treatment combination, and *Rivulus* were released in the natural stream

**Table 1** Alternative outcomes and conclusions of tests for the effect of hetero- and conspecifics on exploratory boldness by *Rivulus*

Effect of	First test	Second test	Comparison of second with first test	Effect indicated by comparison	
Guppies on boldness	R+G	R	No change	Heterospecific	Prior experience
			Less bold	No	Yes
			Bolder	Yes	No
Conspecifics on boldness	R+R	R	No change	No	Yes
			Less bold	Yes	No
			Bolder	No	Yes
Prior experience (consistency)	R	R	No change	No	No
			Less bold	Yes	No <sup>a</sup>
			More bold	No	Yes

In the first test, which tested for the heterospecific effect, *Rivulus* were tested with guppies, followed by *Rivulus* alone. In the second test series, *Rivulus* were tested with conspecifics, followed by *Rivulus* alone. Consistency between tests was tested in the third series by testing *Rivulus* alone in both tests

<sup>a</sup> See [Methods](#) for explanation

at the end of the trial. Guppies ( $n=30$ ) were similarly dipped from a community guppy-tank, but these were returned to the community tank after each trial and reused. All *Rivulus* were acclimated prior to testing by allowing them to explore the experimental stream channel for 48 h before the predator *Hoplias* was stocked. They were stocked into the downstream, start refuge, at one of the three densities at 2000 h. Guppies and the two *Hoplias* were stocked several hours before *Rivulus* to allow time for acclimation.

The stream channel was searched after 12 h, beginning at 0800 h, until all *Rivulus* were recovered, which was the case in all but one trial with guppies absent, density=8, which lost two fish, apparently to predation. Several guppies were also lost from each of the guppy treatments, but the final guppy densities remained in the range of 25–30. Individual guppies, and small and large shoals were well dispersed in the channel, and we also observed them swimming in and out of the refuge sections.

We used net mean distance (in centimeters) moved from the start refuge as the dependent variable. Distances were log-transformed and tested using the general linear model procedure with guppy presence a fixed factor and *Rivulus* density, log-transformed, a covariate.

## Results

### Heterospecific groups in nature

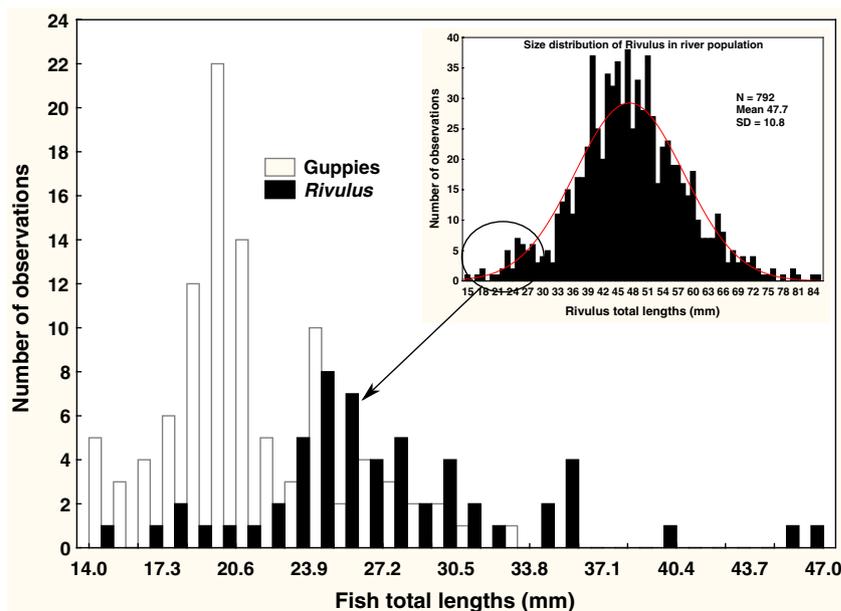
The ten beach seines yielded 99 guppies (mean  $\pm$  SE =  $21.9 \pm 0.28$ ) and 56 *Rivulus* (mean  $\pm$  SE =  $27.3 \pm 0.83$ ). Figure 2 shows that it was predominantly young *Rivulus* that

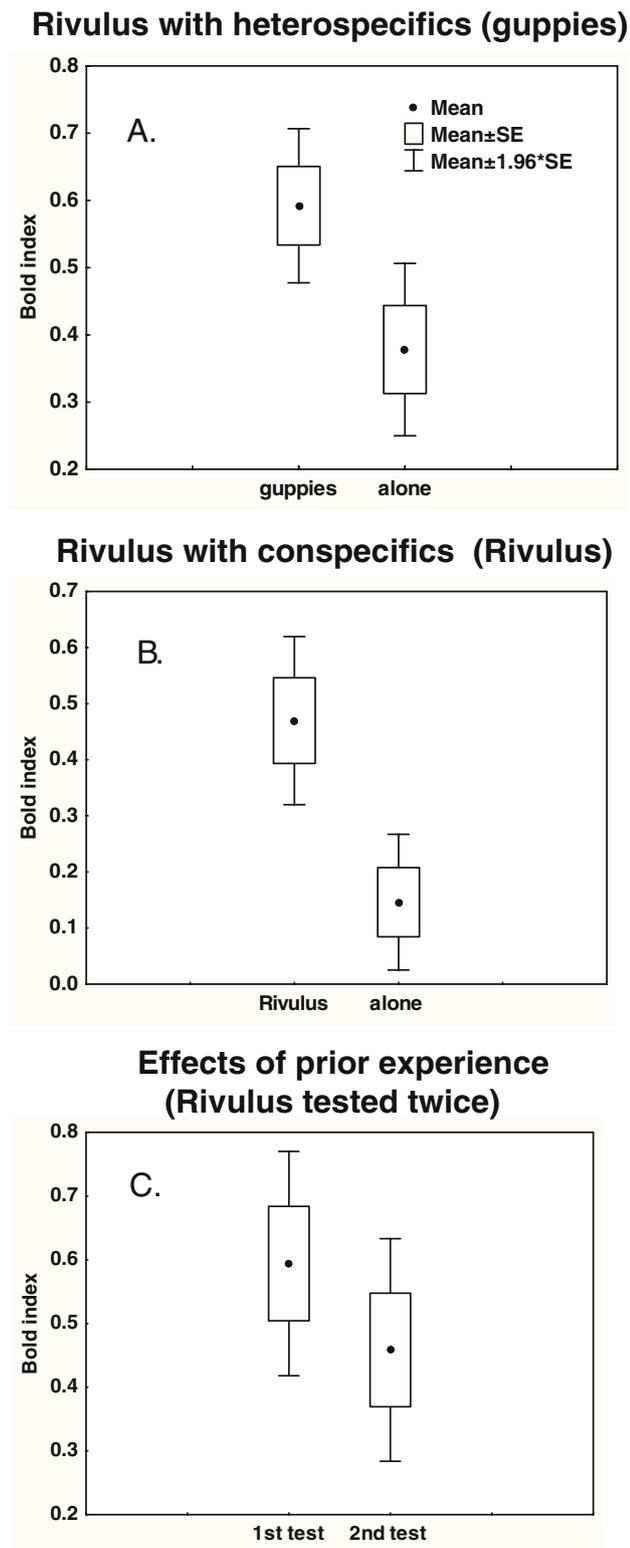
associated with guppies on sandy beaches when compared with the size distribution of the entire local *Rivulus* population shown in the inset. These results imply proximity of the two taxa and suggest loose association, but not necessarily heterospecific shoaling. Guppies were observed swimming in the water column close to shore, while *Rivulus* were closely associated with the bottom, rarely in the water column.

### Experiment 1 Boldness in the presence of guppies vs. conspecifics

*Rivulus* with guppies were significantly bolder in entering and crossing the gap than they were without guppies (Fig. 3a;  $t_{31}=2.70$ ,  $p=0.011$ ). *Rivulus* were less bold when tested alone in the follow-up test, indicating a guppy effect rather than an effect of the prior test experience. Figure 3a, b shows that the relative increase in boldness in the presence of conspecifics was remarkably similar to the increase with guppies (bold<sub>index</sub>  $t_{19}=4.358$ ,  $p<0.001$ ). Following the guppy and conspecific tests, *Rivulus* were tested twice, each time alone, to test whether the boldness score would increase in the second test (prior experience, fish “feel” safer). Figure 3c shows that the boldness score did not change ( $t_{15}=1.452$ ,  $p=0.167$ ; power of test=0.22). Although the power of this test is low, the means show no tendency for increase in the second test (mean<sub>1</sub>=0.59 vs. mean<sub>2</sub>=0.46) indicating that they did not feel safer in the second test. A significant change between the two tests would suggest something related to prior experience, assuming no external effects on the fish between tests. Our general conclusion is that

**Fig. 2** Frequencies of guppies and *Rivulus* in beach seines done in high-risk reaches of the Guanapo and Turure Rivers in Trinidad. Inset: overall size distribution of *Rivulus* in lower Guanapo River, including small, immature *Rivulus* found along edges with guppies. Figure shows that *Rivulus* sizes associated with guppies are a subset of the overall size distribution





**Fig. 3** Tests for the effect of heterospecifics ( $n=32$ ) and conspecifics ( $n=20$ ) on boldness responses in *Rivulus*. Responses of individual *Rivulus* when tested **a** alone and with heterospecifics, **b** alone and with conspecifics. **c** Gives results of prior experience tests in which *Rivulus* was tested alone each time ( $n=13$ ). Boxes give mean±1SE, extensions 95% confidence intervals ( $\approx 2SE$ ) for the mean

the presence of guppies facilitates the expression of exploratory behavior.

#### Experiment 2 Effect of familiarity on boldness

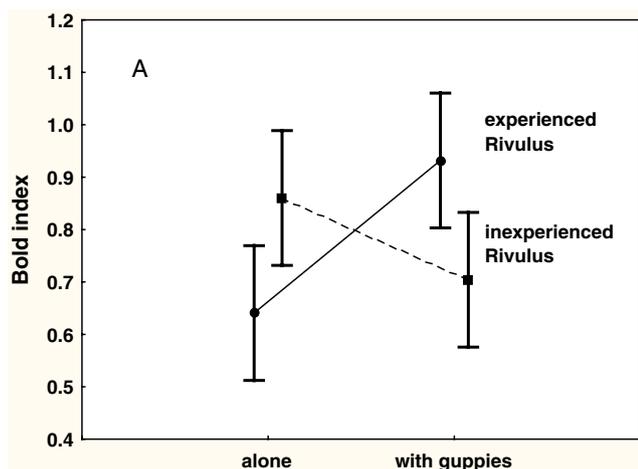
Figure 4 shows that *Rivulus* from lower Ramdeen Stream, with previous experience of guppies, were significantly bolder with guppies, while the inexperienced ones were not (two-way ANOVA: presence of guppies×experience interaction,  $F_{1, 76}=12.0$ ,  $p=0.001$ ; Scheffé post hoc comparisons of experienced *Rivulus* in the presence and absence of guppies,  $p=0.022$ ; inexperienced,  $p=0.409$ ).

#### Experiment 3 Effect of guppies on movement under predation threat

Figure 5 shows a significant overall guppy effect on movement, controlling for *Rivulus* density (guppy effect,  $F_{1, 9}=10.0$ ,  $p=0.01$ ; *Rivulus* density effect,  $F_{1, 9}=0.07$ ,  $p=0.79$ ). The proportion (arcsine-square root-transformed) of *Rivulus* that simply left the refuge, irrespective of distance moved, again indicated a strong positive response to guppies ( $F_{1, 9}=16.60$ ,  $p=0.003$ ). The inset shows that *Rivulus* dispersal was also affected by total density in that individuals dispersed further in the experimental stream as total fish density (*Rivulus*+guppies) increased ( $r^2=0.49$ ,  $p=0.011$ ). We conclude that the main effect on *Rivulus* movement behavior was the guppy effect.

## Discussion

The results of experiments 1 and 2 support the hypothesis that the presence of nearby guppies increases the boldness of young *Rivulus*. When guppies were in the tank, *Rivulus* took significantly less time to leave the refuge and explore or cross the open space between refuges. *Rivulus* appeared to be emboldened by the conspicuous guppy shoals that were in constant motion, frequently forming, disintegrating and reforming. *R. hartii* is a widely distributed, colonizing species in Trinidad, occurring in a wide range of aquatic habitats, such as headwater streams, seepages, rivers, and flood pools. Yet, in an earlier study, Gilliam and Fraser (2001) found only a small percentage of the sampled population, <10%, moved longitudinally along the river, and most of these were larger adults. The results of this study suggest a mechanism by which movement may be facilitated among the smaller size classes of *Rivulus*, <25 mm total length. The small size class, not marked in the 2001 study, may play a significant role in the early dispersal and colonization by this species. It is also important to note that small, immature fish of other taxa are also present in the rivers, often at high densities, e.g., the cichlid fish, *Aequidens pulcher*, and the catfish, *Corydoras aeneus*. These too may facilitate movement by



**Fig. 4** Familiarity tests for the effects of previous experience of *Rivulus* with heterospecific on  $\text{bold}_{\text{index}}$ . Mean  $\text{bold}_{\text{index}} \pm 95\%$  confidence intervals of *Rivulus* from above a barrier in Ramdeen Stream (=no previous experience of guppies, dashed line) and below the barrier (=previous experience, solid line) when tested alone and with guppies present in the tank. Both tests  $n=40$

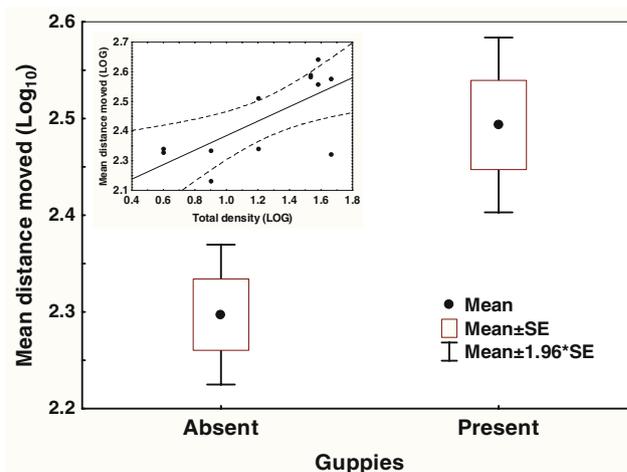
*Rivulus*, although guppies are prime candidates owing to their overall abundance.

Matthews (1998) reviewed studies reporting mixed species associations of fish from lakes and streams and included many observations of his own. For example, of 202 seine hauls that he made along the shores of Lake Texoma, Oklahoma and Texas, 86% contained  $\geq 2$  taxa. Although some have reported evidence for segregation into microhabitats among the taxa collected in seine hauls, e.g., Gorman (1988), Freeman and Grossman (1992), Matthews concluded that the evidence pointed to a limited or lack of any convincing monospecific segregation. Instead, he surmised that the benefits of predator defense and food-finding were likely the causes of real interspecific groupings. The results of our beach seines paralleled those of Matthews, except that ours were made in high-risk areas, i. e., sandy beaches, where mature *Rivulus* were scarce, although they were often abundant in nearby cobble, along with small, immature *Rivulus* (Fraser et al. 1999).

The results of experiment 2, boldness in *Rivulus* that were allopatric vs sympatric with guppies in Ramdeen Stream, suggest that experience and learning may be an important factor in the development of exploratory behavior and facilitated movement in *Rivulus*. *Rivulus* from the area in which they co-occurred with guppies exhibited a striking increase in boldness when guppies were present vs when they were absent, while the boldness of inexperienced *Rivulus* from above the barrier waterfall where there were no guppies showed no significant change between tests. While supporting a prior experience hypothesis, the generality of this pattern remains unknown in that the experiment was not replicated across other streams. Also, it

is important to note that the two collection sites in Ramdeen Stream differed in ways other than in the presence of guppies. The section in which *Rivulus* were sympatric with guppies had other taxa present such as the predator *H. malabaricus*, which, although not a permanent resident, was an occasional visitor. The strength of the behavioral response, e.g., boldness, might be dependent on the degree of threat and on whether it is chronic. Others have found that populations sympatric with predators have a higher probability of developing a suit of antipredator behaviors that ameliorate predation risk than do populations in the absence of strong threat (Dingemans et al. 2007). Brown et al. (2005) found that the Poeciliid *Brachyrhaphis episcopi* exhibited greater exploratory boldness in populations with predators than did those from low-risk habitats.

Can the presence of heterospecifics in the ambient environment promote dispersion? The results of experiment 3 suggest that nearby heterospecifics may enhance dispersal along a river corridor by emboldening *Rivulus* to leave the safety of a refuge in high-risk zones. In experiment 3, subjects crossed a gap whose concrete floor and walls lacked structural features of the natural stream, e.g., woody materials, and where there were no shallow beaches that might have provided temporary escape from the predators. Only the presence of constantly moving shoals of guppies afforded any safety through a dilution effect. While guppies appeared to facilitate the movement of *Rivulus* between refuges, the results of this experiment were peculiar in an important way; nearly all *Rivulus* left the refuge. Were they all bold explorers, something not previously seen? No, rather we posit that it was an unavoidable artifact of using wild-caught *Rivulus* in experimental streams, because all



**Fig. 5** The effect of guppies on movement distance of *Rivulus*, independent of density. Bars represent 95% confidence intervals for the mean. Inset: mean distance moved (in centimeters) in the experimental stream by *Rivulus* relative to total fish density (*Rivulus* + guppies). Dashed lines are the 95% confidence intervals

recently captured *Rivulus* are “lost” when moved to a new environment, as we have previously observed in displacement studies that moved *Rivulus* various distances along the river from their home location (Fraser et al. 2006). Almost all of the displaced fish attempted to home, many successfully. So, it was not surprising that most of the *Rivulus* in experiment 3 moved out of the refuge. However, the activity of the predators may have affected the performance of individuals in their willingness to leave the refuge, or in their reaching the opposite refuge. A *Hoplias* may have blocked or delayed movement if its presence was detected in the immediate vicinity of the refuge, or it may have promoted movement by a *Rivulus* that had already started out of the refuge when the predator was detected. Nonetheless, even when these factors are taken into account, the prevailing effect was that guppies, not predator activity, were the proximal cause of increased movement of *Rivulus* across the gap. If guppies can embolden *Rivulus* to leave the refuge, they may have a similar effect in the field. *Rivulus* that are concentrated in a refuge may experience crowding effects and cannibalism. These factors could encourage young *Rivulus* to move beyond the safety of the patch, especially if that movement is facilitated by the presence of guppies.

Furthermore, by decreasing predator risk relative to no facilitation, heterospecific associations may increase population mobility by affecting the rate of spread and patch recolonizations especially of immature fish, which could develop variable mobilities in response to local conditions. McLaughlin (2001), for example, found that recently emerged brook charr, *Salvelinus fontinalis*, diverged in feeding behavior and degree of mobility, sedentary versus mobile, in response to local competition for spatially separated insect and crustacean prey. Although facilitation is not expected to decrease risk relative to those that stay within the refuge, it may decrease intraspecific competition relative to those that stay. Thus, the accrual of multiple benefits, analogous to the multiple benefits of schooling in pelagic fish, may lead to a relatively mobile fraction within the population, a phenomenon that has been reported for this and other taxa of fish (McLaughlin et al. 1999; Gilliam and Fraser 2001; Rodriguez 2002).

#### A conceptual framework for the acquisition of exploratory boldness

We assume that spawning and early development of *Rivulus* in lowland streams with high predation risk occurs in refuge patches where adult *Rivulus* are concentrated. Various movements within the patch result in a fraction of the small, immature *Rivulus* near the edge of the patch where they become candidates for mixing with nearby guppies. Guppies are highly mobile within stream sections (Croft et al. 2003),

providing constant opportunities for detection by *Rivulus*. Our results suggest that nearby guppies will embolden small *Rivulus* to leave and explore along the river edge, especially the bolder phenotypes with a propensity for exploration. Not all *Rivulus* will move. Shyer phenotypes may stay behind or settle early in near patches, regardless of the patch's quality, but the bolder individuals select new patches and eventually settle where the potential for growth and reproduction are high (habitat selection). Favorable habitat selection could occur, for example, when movement enables individuals to find patches with low conspecific densities, such as those previously depleted by predators. Gilliam and Fraser (2001) found evidence for such a phenomenon, and a fitness consequence for moving, because individual growth rates were significantly and positively correlated with movement along the river. Under these circumstances, exploratory behavior would correlate with increased resource acquisition, increased growth rate, earlier reproductive maturity, and increased fecundity, all of which are correlated with an increase in food availability (Walsh and Reznick 2008). Thus, in a risky habitat where shyness might be the norm, increased exploratory behavior leads to benefits favored by selection and therefore its ultimate establishment and persistence as a selected-for phenomenon.

Clobert et al. (2009) present a conceptual framework that highlights phenotypic and external factors that affect individual variation in the dispersal process, which they divide into departure, transience, and settlement phases. Our framework complements their more inclusive model by adding the concept of facilitation to the list of external factors that affect primarily the departure and transience phases.

The underlying basis for boldness and the degree to which it is phenotypically plastic remain to be experimentally evaluated. It has already been shown that *Rivulus* found above and below the barrier waterfall on Ramdeen Stream have genetic differences in their life histories (Walsh and Reznick 2008), so we know that local adaptation can take place on a fine scale in this species. We also know that phenotypic plasticity may facilitate adaptive evolution in local populations of fish (Rodd et al. 1997; Robinson and Wilson 1996; Ghalambor et al. 2007). But, local populations are established through the dispersal of individuals, and to the extent that movement is voluntary, the facilitation studied here may be one important factor that allows some bold individuals, exhibiting exploratory behavior to reach new habitats favorable for growth and reproduction.

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**Ethical standards** The experiments reported herein comply with the current laws of the USA.

**Conflict of interest** The authors declare that they had no conflicts of interest in the conduct of this research.

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