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Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species

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Abstract

Dispersal is a key element of a species' invasiveness. Although considerable work has addressed how dispersal influences the pattern of spatial spread of invading organisms, few studies investigate whether invasive species are in fact better dispersers than either the species they displace or less successful invaders. Recent work suggests that variation in dispersal may be due to variation in an underlying behavioral trait, boldness. Our study examined the link between dispersal, boldness, and invasiveness by comparing the dispersal characteristics and refuge use of two invasive *Gambusia* species to two congeners in experimental streams. The streams consisted of a series of pools (no flow) connected to a flowing channel. For each species, small groups of females were released at the middle pool, and their movement and activity were recorded over a 1-h period. We found invasive *Gambusia* to be more likely to disperse out of the introductory pool, to disperse sooner, to travel a greater distance in the artificial streams, and thus to exhibit greater dispersal tendencies than their close relatives. Among the invasives, *Gambusia affinis* had a greater dispersal tendency than *G. holbrooki*. We suspect this result indicates variation in the contribution of dispersal to the relative invasiveness of these species. Certain dispersal measures were correlated to time spent out of refuge, although invasive *Gambusia* and their relatives did not differ in the predicted manner. These results argue for the greater incorporation of experimental approaches and analyses of behavioral mechanisms in the study of invasive species.

Introduction

Non-indigenous species are generally defined as invasive only if they are able to spread (i.e., expand their range) beyond their points of initial arrival or introduction (Richardson et al. 2000; Kolar and Lodge 2001). Thus, dispersal ability is generally expected to be a key factor determining invasion success (Ehrlich 1986; Lodge 1993b; Sakai et al. 2001). In particular, the rate of spatial spread of invasions is strongly dependent on the dispersal rates or distances of the invading organisms (Parker and Reichard 1998). Dispersal is also a fundamental component of ecological processes in natural populations, affecting gene flow, population structure, and metapopulation dynamics that have important consequences for species distributions, abundances, and persistence (Endler 1977; Kareiva 1990; Hanski and Gilpin 1991; Tilman 1994).

In aquatic systems, the striking invasion success of some of the best-known invaders has been attributed largely to their dispersal abilities. The classic example comes from the invasion of the Great Lakes by the zebra mussel, *Dreissena polymorpha*. Unlike native bivalves, zebra mussels can disperse quickly and broadly as free-swimming larvae and also as adults that can attach themselves to submerged mobile substrates (Lodge 1993a; Johnson and Carlton 1996). Another example involves common and grass carp, *Cyprinus carpio* and *Ctenopharyngodon idella*, two of the most widespread fish invaders worldwide, whose invasion success, at least in North America, is strongly linked to their ability to disperse rapidly (Moyle 1986). However, beyond these examples little comparative evidence, particularly in vertebrate invaders, exists to show that successful invasive species have greater dispersal tendency, ability, or rates than species that are either not as successful, have failed to spread, or are being displaced by invasives.

The spatial spread of invasions has traditionally been modeled by reaction–diffusion models (e.g., Andow et al. 1990) where dispersal is treated as either a constant or a normally distributed parameter. However, dispersal characteristics are often highly variable among individuals of any one species (Kot et al. 1996), and field data show that dispersal distances are generally leptokurtically distributed (high frequency of values near the mean and tails of distribution – that is, most individuals have intermediate levels of dispersal, whereas few exhibit either very high or very low dispersal) (Okubo 1980; Howe and Westley 1986; Paradis et al. 1998). Indeed, high intraspecific variation in dispersal distances or rates has been documented repeatedly for a variety of taxa (Greenwood and Harvey 1976; Gaines and McClenaghan 1980; Swingland 1983; Bengtsson et al. 1994; O'Riain et al. 1996; Bradford and Taylor 1997). While some of this variation may be attributed to differences among individuals in age, size, condition, or gender, Fraser et al. (2001) argued that this variation might also be the result of variation in an underlying behavioral trait that affects dispersal. Specifically, Fraser et al. (2001) suggested that boldness, defined as the propensity of organisms to move through and explore unfamiliar space (Wilson et al. 1993), might be an important source of intraspecifc variation in dispersal. Whether individuals are bold or shy might determine whether they disperse or remain sedentary, or whether individuals are short- vs long-distance dispersers. Greenberg (1989, 1995) suggested that individual responses to novelty might also be species-specific. So that, species that are bolder might also be better dispersers.

We examined the link between dispersal, boldness, and invasiveness by determining whether dispersal characteristics and refuge use differed between invasive *Gambusia* and their close relatives. If dispersal is a key trait to a species' invasiveness, we expected highly successful invasive species, such as mosquitofish, to be better dispersers than their congeners. Comparisons of closely related species are an insightful approach to the identification of traits conferring invasiveness (Mack 1996; e.g., Rejmanek and Richardson 1996). In two experimental streams, we measured the amount, timing, direction, and endpoint of dispersal of small groups of females of each species. Using a subset of these characteristics, we then calculated a metric that we considered to be representative of each species' dispersal tendency and which we expect to be correlated to field dispersal rates. Finally, we asked whether these dispersal measures could be predicted by a measure of boldness, time spent out of refuge. Boldness should be highly advantageous for species arriving and spreading through novel habitat, such as in an invasion context. We expected invasive *Gambusia* species to be significantly bolder than their congeners.

Gambusia is a genus comprising about 45 species of small, livebearing fishes (Poeciliidae). Most of what we know about this genus comes from the rather extensive study of the two most temperate, most widely distributed and highly invasive species, *G. holbrooki* and *G. affinis*. These two sister species (both known as mosquitofish) have been introduced for mosquito-control purposes worldwide and have spread successfully to over 40 countries (Welcomme 1992; Lever 1996). Their invasion success and impact on native communities have been notable (Lloyd et al. 1986; Courtenay and Meffe 1989; Gamradt and Kats 1996; Webb and Joss 1997; Goodsell and Kats 1999), such that they are considered among the 100 worst invasive species worldwide (ISSG 2000).

In contrast, despite strong similarities in their ecology, morphology, and body size, most of the other species in the genus have restricted geographic distributions, have rarely been introduced, and when they have been introduced have failed to spread. For this study, we focused on two such species, *G. geiseri* and *G. hispaniolae*.

G. geiseri is endemic to two spring habitats (Comal and San Marcos Springs, Texas) in the southwestern US (Hubbs and Springer 1957) and appears to be the sister taxon to the invasive species pair (partial phylogeny by Lydeard et al. 1995). Outside its native range, the success of *G. geiseri* has been very limited. Although this species has been introduced into several habitats similar to its native range (Fuller et al. 1999), *G. geiseri* has not spread and remains localized (C. Hubbs, pers. comm.). *G. hispaniolae* is a Caribbean species (like most *Gambusia*) native to the Neiba Valley and Cul de Sac region of Hispaniola. Although it is the most widespread of the three endemic *Gambusia* species in Hispaniola, it still has a rather restricted range (Burgess and Franz 1989) and has never been translocated. In its native range, even though irrigation canals have increased the connectivity of water bodies in the region, *G. hispaniolae* has not spread whereas other species seem to have (C. Rodriguez, pers. comm.).

Methods

Study organisms

For our study, we focused on adult gravid females that are likely to be the most important stage and gender in an invasion context in this taxon. Previous studies suggest that female invasive *Gambusia* exhibit greater dispersal rates (Robbins et al. 1987; Congdon 1994) and perhaps greater dispersal success (Brown 1987) than either males or juveniles. Females have higher overwintering survival than males (Winkler 1975) – a key trait for invading more temperate habitats. More importantly, female *Gambusia* can retain sperm from multiple mates for several months and even across breeding seasons (Chesser et al. 1984; Zane et al. 1999). Thus, individual females are capable of founding populations in the absence of males without Allele effects or negative genetic founder effects (Chesser et al. 1984; Robbins et al. 1987). Indeed, levels of genetic variation in some newly founded populations are comparable to levels in more established populations (Brown 1985; Scribner et al. 1992; but see Congdon 1995). Additionally, populations founded by females alone vs those founded by both males and females do not differ in either population growth rates or population structure (Resetarits 2000; Resetarits, pers. comm.).

All *Gambusia* used in the experiment were collected from populations within their native range, not their invaded range, in the summer and early fall of 1999. Collections of *G. holbrooki* were made in Leon Hines Lake, Escambia County, Alabama. Collections of *G. affinis* and *G. geiseri* were made in the Comal River and Comal Springs respectively, Comal County, Texas. *G. geiseri* is typically limited to the stenothermal springhead, whereas *G. affinis* is found downstream (*G. affinis* were collected approximately 1.5 km downstream from springhead). *G. hispaniolae* were collected from La Azufrada, a freshwater spring flowing into Lake Enriquillo, Dominican Republic. In order to remove variation due to different experiences in their natural environments, we studied F2 fish that were raised in the laboratory under standardized conditions. All fish were raised under similar densities in 76-l aquaria at 22–26 °C, on a 14-L : 10-D photoperiod and fed *ad libitum* a combination of Tetramin flakes, freshly hatched brine shrimp nauplii, and a calf liver and spinach frozen paste enriched with minerals and vitamins. Males and females were housed together to allow mating to occur. To prevent cannibalism of the young, gravid females were isolated prior to parturition in brood chambers that allow newborns to escape.

Experimental streams

The experiment was conducted in two artificial streams constructed outdoors at the Putah Creek Aquatic Facility at the University of California, Davis, California. Each stream consisted of a series of three pools connected to a large PVC channel via three smaller PVC channels (length of streams is equivalent to approximately 200 body lengths) (Figure 1). All channels were made of semicircular PVC tubing, which made the apparatus open to sunlight and to the observer. In each stream, a $\frac{3}{4}$ HP pump circulated water producing substantial flow in the large channel, but no detectable flow in the three side pools. The large channel (referred to as flowing channel) simulated a flowing stream whereas pools simulated slow moving backwaters typically inhabited by *Gambusia*. Maximum flow velocities at the

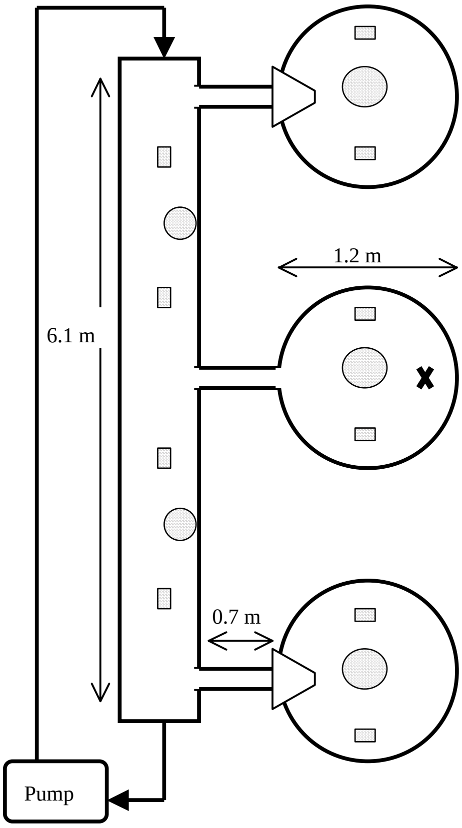

Figure 1. Diagram of one of the artificial streams used in the experiment. Single-pointed arrows indicate direction of water flow, whereas double pointed arrows show dimensions. Shaded areas indicate placement and relative size of refuges. The 'x' shows the location where *Gambusia* groups were released at the start of trials.

upstream outflow averaged 1.19 m/s ($n = 10$, SE = 0.041) for both streams, and decreased away from the outflow, so that average flow velocities were 0.51 m/s ($n = 10$, SE = 0.018).

Each stream was provided with a 2-cm layer of sand as substrate and with refuges (Figure 1) in the form of clumps of *Elodea canadensis* (about 15% of pool area). Due to concerns with pump clogging, refuges in the flowing channel consisted of artificial floating aquarium plants secured to the channel sides. Additional benthic refuges were provided by small pieces of PVC conduit (2 per pool and 4 in the flowing channel, 2.5 cm × 10 cm); however, fish rarely used these. A mesh partition at the end of the flowing channel prevented fish from accessing the pump.

Fish were introduced into the middle of the three pools. From there, they had free access to the flowing channel and the other two pools. Small funnel traps placed in the upstream and downstream pools where each pool met the connecting channel (Figure 1) allowed fish to disperse into pools, but restricted their return back to the channel. These traps facilitated measuring colonization of these pools. No traps were placed in the middle pool.

Dispersal groups and trials

We conducted trials individually by species on groups of three gravid females. We focused on small groups (rather than single individuals or larger schools) for three reasons: (1) individuals in small groups appear less stressed and are more likely to behave naturally than solitary individuals; (2) small groups allowed us to get detailed behavioral data that would be difficult to record for larger schools and (3) research shows that successful dispersal in an invasion front often involves small groups of individuals (Gammon and Maurer 2002). For all variables, we averaged data for the three females to yield a single value per group.

The three, randomly selected females in each group were fed Tetramin flakes *ad libitum* in source tanks and placed in clear, plastic containers (900 ml) with mesh sides the evening before the trial day. Groups were floated in these plastic containers in the middle pool and acclimated for 5 min before being released. Releases were done away from the opening to the flowing stream (Figure 1). Water temperature was recorded just before all releases. We conducted 1-h trials in which fish could either remain in the middle pool or disperse out and move into one of three locations: the flowing channel, the upstream pool, or the downstream pool. Every 10 min, observations noted the location and activity of each female in a group. All observations were made with binoculars from an observation tower from which both streams were visible and fish could be observed undisturbed. Each hour, we conducted a trial with an invasive species in one stream and a congener in the other stream; streams were alternated the following hour. On the last day of the experiment, because we had more invasive females, we ran two additional groups of each invasive species. Overall, over the course of 3 consecutive days in September 2002 we conducted trials on 11 groups of females for each of the 2 invasive species and 9 groups for each of the 2 congeners, giving a total of 40 groups and 120 fish.

Response variables

To examine variation in dispersal among *Gambusia* species, we compared the following variables: (a) whether dispersal occurred; (b) the time elapsed before dispersal occurred; (c) the distance traveled; (d) dispersal endpoint; and (e) dispersal direction. The first three measures describe dispersal *per se*, whereas the latter two describe aspects of habitat use. We expected *G. geiseri* and *G. hispaniolae* to be more likely to colonize pools than *G. affinis* and *G. holbrooki* because of larger refuge area and slower water velocities in pools. We also hypothesized that the congeners, because of potentially lower abilities to cope with high flow, would be more likely to preferentially colonize the downstream pool, i.e., to move with the flow, compared to the invasive *Gambusia*.

Our most basic measure of whether a species was a good disperser or not was the proportion of females in each group that moved out of the middle pool by the end of the 60-min trial. For fish that dispersed, a second dispersal characteristic of interest was the time elapsed before dispersal occurred (referred to as dispersal score). Individuals that dispersed immediately (before the first observation) were assigned a score of 6, individuals that dispersed between the first and second observations were assigned a 5, down to a score of 1 (for individuals that left between the 50- and 60-min observations). The third measure of dispersal was the distance traveled by fish in the flowing channel averaged over three 2-min periods. This type of activity in novel space is typically indicative of exploratory behavior (Walsh and Cummins 1976). In a few instances, fish dispersed quickly from the middle pool into other pools without much exploratory swimming in the channels. Distance traveled for these groups was estimated as the distance between the 2 pools (2.7 m).

These variables are, in principle, independent. In particular, because our dispersal score and distance traveled only considered individuals that actually dispersed, it is possible for a group to have a small proportion of females dispersing and to get a high score for the time elapsed before dispersal or distance traveled if those few dispersers left early and explored a great deal in the flowing channel. It is also possible for a trial to have all individuals disperse quickly (high proportion dispersing and dispersal score) and yet exhibit little movement while in the channel. Thus, if a species exhibits high values for proportion dispersing, dispersal score, and distance traveled, this actually represents three separate measures of high dispersal. To provide an overall measure of the dispersal behavior of groups in our experimental streams, we took the product of these three variables, and we refer to this product as the dispersal tendency.

For dispersers, we calculated the proportion of fish that colonized a new pool vs the proportion that remained in the flowing channel. For fish that entered pools, we also calculated the proportions entering the upstream vs downstream pools. To assess variation in female boldness and its relationship to dispersal, we quantified refuge use of groups before dispersal. Boldness was measured as the proportion of fish out of refuge averaged for all observations where fish remained in the middle pool. For fish that dispersed before the first observation at 10 min, we have no data on their refuge use, and they were excluded from this analysis.

Statistical analyses

Differences among *Gambusia* species in the seven variables describing dispersal and boldness were analyzed with ANOVAs. We looked at the effect of day as a blocking factor and water temperature as a covariate, but these were not significant and were removed from final analyses. In order to better satisfy assumptions of parametric tests, all proportions (the proportion of fish dispersing, the proportion of fish colonizing a pool, the proportion of fish colonizing downstream vs upstream pools, and the proportion of fish out of refuge) were arc-sin square-root transformed. For comparing whether dispersing fish that colonized a pool preferred the upstream vs downstream pool, we looked at the effect of direction and the interaction between direction and species on the proportion of dispersing fish that ended up in pools.

Orthogonal contrasts were used to test for species differences once a significant species effect was found. In three contrasts, we tested whether the two invasive species differed from their two relatives (one-tailed), whether the invasives differed from each other (*G. affinis* vs *G. holbrooki*, two-tailed), and whether the two congeners differed from each other (*G. geiseri* vs *G. hispaniolae*, two-tailed). We tested correlations between dispersal variables and refuge use using Spearman rank coefficients. SYSTAT® Version 10 was used in all analyses.

Results

Dispersal characteristics

Overall, dispersal characteristics varied significantly among the four *Gambusia* species examined. We found a strong effect of species on the proportion of fish that dispersed by the end of the 1-h trial period (Table 1). On average, the 2 invasive *Gambusia* showed greater dispersal than their congeners, with 76% of females dispersing compared to 55% for *G. geiseri* and *G. hispaniolae* combined ($F_{1,36} = 3.2$, $P = 0.04$) (Figure 2a). The greatest dispersal was seen in *G. affinis* (97%), which was even greater that the dispersal seen in its sister species, *G. holbrooki* (55%) ($F_{1,36} = 8.0$, $P = 0.008$). *G. hispaniolae* exhibited the lowest dispersal, only an average of 26% of females dispersing, which was also significantly lower than *G. geiseri*'s dispersal (85%) ($F_{1,36} = 12.5$, $P = 0.001$).

For those fish that dispersed, species differed significantly in the time elapsed before dispersal occurred (Table 1). Invasive *Gambusia* dispersed sooner than their relatives ($F_{1,26} = 5.3$, $P = 0.02$). In particular, *G. affinis* not only dispersed the most but also dispersed first, leaving the introductory tank on average within the first 14 min of the 60-min trial (Figure 2b). In contrast, *G. hispaniolae*, the species with the least dispersal, also took the longest to disperse, with females dispersing on average at minute 38 of the trial period. In this behavior, we found no significant differences when contrasting the 2 invasives species ($F_{1,26} = 2.9$, $P = 0.10$) or the 2 congeners against each other ($F_{1,26} = 3.4$, $P = 0.08$). *G. affinis* tended to disperse sooner than *G. holbrooki*, and *G. geiseri* tended to disperse sooner than *G. hispaniolae*.

The amount of exploratory traveling done by females after leaving the introduction pool also differed significantly among species (Table 1). The invasives, *G. affinis* and *G. holbrooki*, traveled significantly greater distances in the experimental streams than *G. geiseri* and *G. hispaniolae* ($F_{1,25} = 18.4$, $P < 0.0001$). Invasive mosquitofish females covered an average of 3.5 times more ground over a 2-min period than did their relatives (Figure 2c). We found no differences between distances traveled by *G. affinis* and *G. holbrooki* ($F_{1,25} = 0.5$, $P = 0.50$) or by *G. geiseri* and *G. hispaniolae* ($F_{1,25} = 0.01$, $P = 0.99$).

Species differed significantly in overall dispersal tendency (the product of the above three variables, Table 1). As we expected, the overall pattern was for the invasive mosquitofish to exhibit higher dispersal tendency than *G. hispaniolae* and *G. geiseri* ($F_{1,35} = 19.9$, $P < 0.0001$) (Figure 2d). Among the invasives, *G. affinis* exhibited a higher dispersal tendency than *G. holbrooki* ($F_{1,35} = 10.0$, $P = 0.003$). We found no difference between the dispersal tendencies of *G. geiseri* and *G. hispaniolae* ($F_{1,35} = 0.8$, $P = 0.40$).

Table 1. Summary of ANOVA results for all dispersal variables and boldness.

Variable	Effect	df	F	*P*
Proportion of fish dispersing	Species	3, 36	7.9	0.0001
Dispersal score	Species	3, 26	3.6	0.026
Distance traveled	Species	3, 25	7.0	0.001
Dispersal tendency	Species	3, 35	10.3	0.0001
Dispersal location	Species	3, 26	0.5	0.70
Dispersal direction	Species	3, 52	0.4	0.76
	Direction	1, 52	26.8	0.0001
	Species × direction	3, 52	1.7	0.17
Refuge use	Species	3, 28	5.4	0.005

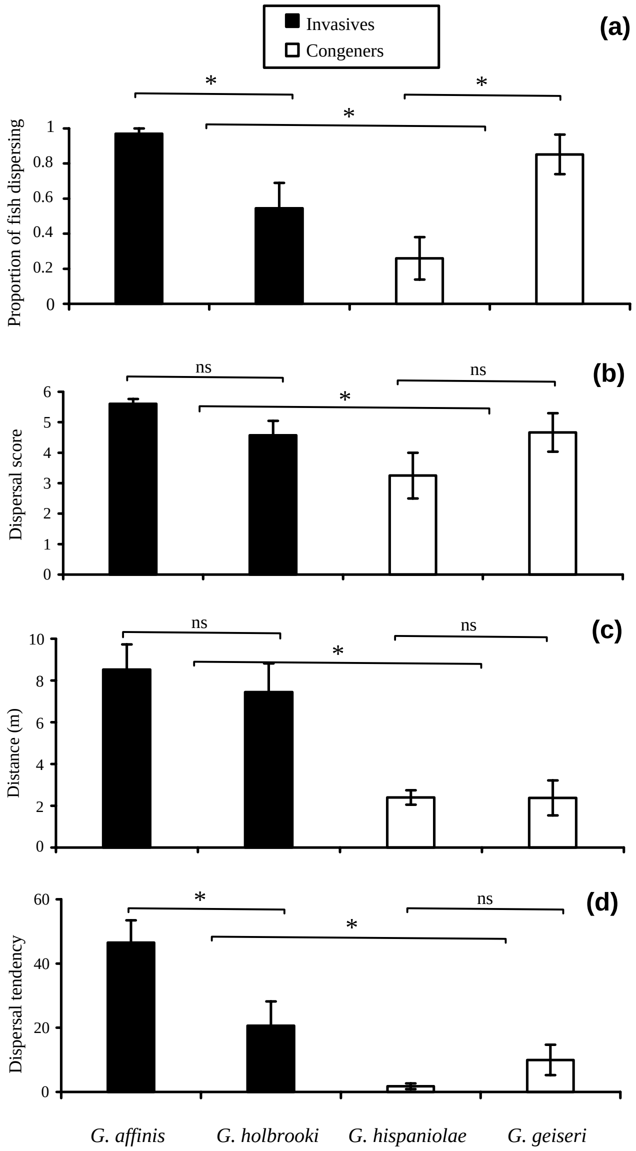

Figure 2. (a) Proportion of fish (out of three females) that dispersed out of the middle pool by the end of 1-h trials; (b) dispersal score for dispersers (the higher the score the sooner in the trial the dispersal event occurred); (c) distance traveled (in meters) by dispersers in the flowing channel over a 2-min period; and (d) dispersal tendency (the product of a, b and c above) for each *Gambusia* species. Bars represent mean values ± 1 standard error (S.E.). Brackets indicate significant differences ($P < 0.05$) between the 2 invasive species and the 2 congeners (longest bracket), between *G. affinis* and *G. holbrooki*, and between *G. geiseri* and *G. hispaniolae*.

With regard to habitat use post-dispersal, we found no species differences in the locations that fish dispersed into after leaving the middle pool (Table 1). On average, 54% of all fish in the experiment colonized either pool (Figure 3), whereas the remaining 46% remained in the flowing channel for the entire trial time. No fish ever returned to the middle pool from the channel. For females that colonized a new pool, we found that invasives and their relatives did not differ in their 'tendency to colonize' upstream vs downstream (Table 1). Instead, all species preferentially colonized the downstream pool, indicating similarities among the species in either their preference for moving with flow (as opposed to against it) or their ability to overcome flow.

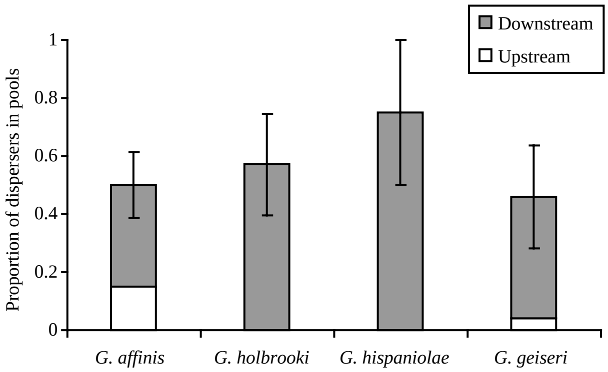

Figure 3. Proportion of dispersing females that colonized pools. Mean proportions are partitioned by whether fish dispersed to the downstream (shaded) or the upstream pool (no shading). Error bars correspond to mean values before partitioning. Proportions of fish dispersing into pools were similar among *Gambusia* species ($P = 0.70$). Dispersal direction also did not differ among the species ($P = 0.76$).

G. affinis and *G. geiseri* were the only species to move into the upstream pool; no *G. holbrooki* or *G. hispaniolae* females ever did (Figure 3).

Boldness and its relationship to dispersal

We detected a strong effect of species identity on boldness, the proportion of females out of refuge while still in the introductory pool (Figure 4). However, when contrasting invasive *Gambusia* with their congeners, we found no significant difference ($F_{1,28} = 1.2$, $P = 0.15$). There was also no difference between refuge use by *G. affinis* and *G. holbrooki* ($F_{1,28} = 1.4$, $P = 0.24$). Instead, time spent out of refuge was lowest for *G. hispaniolae* (38%) and highest for *G. affinis* (83%) and *G. geiseri* (89%) (*G. hispaniolae* vs *G. geiseri*, $F_{1,28} = 12.9$, $P = 0.001$) (Figure 4). Refuge use by *G. holbrooki* was intermediate (69% out of refuge). When examining the Spearman correlation coefficients, we found a positive correlation between boldness and dispersal out of the middle pool (Table 2). There was no relationship detected between refuge use and dispersal score or distance traveled. A weak yet significant positive relationship was detected between boldness and the calculated dispersal tendency scores. Surprisingly, we detected a trend for a negative relationship between boldness and the proportion of fish dispersing to the downstream pool. It seems that the boldest individuals in the experiment (*G. affinis* and *G. geiseri* females) constituted the small number of individuals that dispersed to the upstream pool.

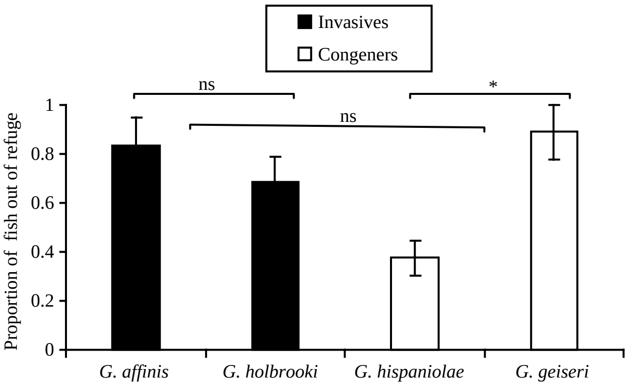

Figure 4. Proportion of females out refuge (mean ± 1 SE) prior to dispersal in the middle pool of streams for each *Gambusia* species. Brackets indicate significant differences ($P < 0.05$) between the two invasive species and the two congeners (longest bracket), between *G. affinis* and *G. holbrooki*, and between *G. geiseri* and *G. hispaniolae*.

Discussion

Recent efforts aimed at gaining a trait-mediated understanding of a species' ability to invade have shown that multiple traits appear to be involved. In particular, life histories and abiotic tolerances are consistent predictors of invasiveness (Duncan et al. 2001; Kolar and Lodge 2002; Marchetti et al. 2004). The results of this study suggest that dispersal, a rarely examined trait, might be another key predictor of invasiveness. The dispersal ability and/or tendency of invading organisms should be an important determinant of the rates of arrival and spread in an invasion. Our comparison of the dispersal characteristics of invasive *Gambusia* to two closely related species found the two invasive species to be better dispersers. In our experimental streams, *G. affinis* and *G. holbrooki* were more likely to disperse out of the introductory pool, dispersed sooner, traveled a greater distance, and thus exhibited higher dispersal tendencies than *G. hispaniolae* and *G. geiseri*. We expect that these differences in dispersal tendencies among *Gambusia* are indicative of differences in dispersal rates in nature and in the potential for invasive spread. Based on our results, *G. affinis* and *G. holbrooki* should have higher dispersal rates and should also be more likely to exhibit the rare long-distance dispersal events known to strongly influence rates of spread (Higgins and Richardson 1999).

The lower dispersal tendency of *G. hispaniolae* and *G. geiseri* compared to mosquitofish might be explained by examining their native distributions. Both species have restricted ranges in spring-associated habitats. Springs constitute geographically isolated habitats typically inhabited by either relict populations of larger distributions or endemic species that evolved in isolation (Hynes 1970). While little is known about the biogeographical history of *G. hispaniolae* or its ancestors, Hubbs and Springer (1957) suggested that the ancestor of *G. geiseri* and other southwestern *Gambusia* was probably widespread, but because of competition with the ancestor of *G. affinis* and drought, became isolated and adapted to spring habitats. If *G. hispaniolae* and *G. geiseri* are unable to persist outside springs, they may be expected to evolve lower dispersal tendencies and perhaps even lower dispersal abilities compared to the wide-ranging and broadly tolerant mosquitofish species.

Our comparative study found evidence of variation in dispersal characteristics among the two invasive sister species. *G. affinis* exhibited a greater dispersal tendency than *G. holbrooki*. *G. affinis* females consistently dispersed out of the middle pool whereas only about half of the *G. holbrooki* females dispersed. *G. affinis* also tended to disperse more quickly than *G. holbrooki*. These results were surprising considering the strong similarities in these species' ecology, physiology, and morphology. In fact, these species are only distinguished from each other by slight differences in the structure of the gonopodium (Rosen and Bailey 1963) and by the number of rays in their dorsal and anal fins (Hubbs and Lagler 1964). Introductions of these species in the US and the rest of the world have not discriminated between them since, until relatively recently, mosquitofish were considered a single species (Wooten et al. 1988). We now know that *G. holbrooki* was introduced to Australia and Mediterranean Europe, whereas *G. affinis* was introduced in the western US, Hawaii, and countries in Africa (Lever 1996; Fuller et al. 1999). Although the relative degree of success of the two species in their invaded ranges is unknown, our dispersal results suggest that these sister taxa might differ in their mechanisms for invasion success. The dispersal results from this experiment suggest that *G. affinis* might be a better invader than *G. holbrooki*, whereas data on other potentially important traits such as fecundity, and maximum population growth rates suggest that *G. holbrooki* is a superior invader (Scribner 1993; J. Schöpf Rehage, unpublished data). The relative invasiveness of these species may thus vary depending on the relative ecological importance of dispersal vs environmental tol-

Table 2. Spearman's ρ correlation coefficients between dispersal characteristics and boldness (proportion of fish out of refuge) for all species combined.

Variable	N	ρ	P
Proportion of fish dispersing	32	0.60	0.0001
Dispersal score	22	0.25	0.26
Distance traveled	21	0.29	0.21
Dispersal tendency	31	0.40	0.024
Dispersal location (into pools)	22	−0.34	0.12
Dispersal direction (downstream)	22	−0.41	0.057

erances and rapid population growth in invaded habitats.

The lower dispersal tendencies shown by *G. hispaniolae* and *G. geiseri* in our artificial streams, along with data on other traits (J. Schöpf Rehage, unpublished data) suggest that these species, have lower invasion potential than mosquitofish. However, we also found some indication of variation between them in their dispersal behavior and potentially their relative non-invasiveness. In particular, *G. geiseri*, the closest relative to the invasive species pair, appears to be a slightly better disperser than *G. hispaniolae*. In spite of similar amounts of exploratory behavior and similar timing of dispersal, more *G. geiseri* females dispersed than did *G. hispaniolae* females. This might have important consequences in an invasion context, since propagule pressure has been shown to be a key factor affecting an invading species' ability to cope with stochastic events, and thus an important determinant of invasion success (Williamson 1999). In addition, the proportion of dispersers and dispersal scores of *G. geiseri* when compared alone to those of mosquitofish were not significantly lower (although exploration distances were), providing further evidence that *G. geiseri* has an intermediate dispersal tendency between mosquitofish and *G. hispaniolae*.

Together these two results suggest that a species' dispersal behavior might be better characterized as a continuum rather than a dichotomy (i.e., high vs low dispersal). The same might be true for characterizing invasiveness (or lack thereof). Even closely related and extremely similar species, upon closer examination, seem to differ in their relative invasiveness. If this is true, quantitative measures of relative invasiveness as a function of species traits would be useful information for policy-makers and natural area managers faced with prioritizing the control of many non-indigenous species.

The study found no differences among species in habitat use. Overall, about half of the fish in the experiment moved into the side pools where flow was absent and refuge area was greater, whereas the other half remained in the flowing portion of the streams. For those fish that moved into the side pools, we found a strong preference across all species for the downstream pool. This might reflect the poor swimming ability of species in this genus in flowing water compared to other fishes. *Gambusia* are fish that prefer slow-moving waters and are not fast swimmers (Casterlin and Reynolds 1977). High flow velocities are a major barrier to dispersal for invasive *Gambusia* (Congdon 1995). For example, invasive *Gambusia* are often flushed out of invaded streams during floods, whereas native fishes are able to persist (Arthington and Lloyd 1989).

Wilson et al. (1993) argued for the existence of a shy–bold continuum in natural animal populations similar to that found in humans. Previous studies have related variation in whether organisms are bold or shy to their learning ability and their antipredator response (Greenberg 1989; Coleman and Wilson 1998; Seferta et al. 2001). Fraser et al. (2001) linked behavioral assays of boldness to dispersal distance in both experimental and natural streams. Our experimental study found a similar positive relationship between some of the dispersal characteristics measured and boldness. Refuge use in the introductory pool was a significant predictor of whether or not the group dispersed out of this pool. The overall dispersal tendency of females was also positively, although not strongly, correlated with the proportion of females out of refuge.

We found species differences in boldness that provided mixed support for our hypothesis of how boldness should relate to invasiveness. Mosquitofish were significantly bolder than *G. hispaniolae*, but not *G. geiseri*. *G. geiseri* appeared as bold as invasive *Gambusia*. This result along with other data on the foraging behavior of *G. geiseri* in response to novel competition (Rehage and Sih, unpublished manuscript) suggests that *G. geiseri* fits the behavioral profile of a successful invader. However, *G. geiseri* is found only in two springs in central Texas and has been unsuccessful at spreading beyond points of introduction where introduced (C. Hubbs, pers. comm.). We suspect that other traits, particularly demographic parameters, and biotic and abiotic tolerances (Rehage and Sih, unpublished manuscript), severely limit its native distribution and its potential invasiveness. This result argues for a need to carefully analyze a range of potentially important traits in order to fully understand their respective contribution to invasion success.

The study of biological invasions has been traditionally descriptive (Kolar and Lodge 2001), especially among vertebrate invaders. Many researchers have called for a larger empirical approach to invasion biology (Vermeij 1996; Parker and Reichard 1998; Williamson 1999). Although experimental streams like the one used here have been useful tools in ecology (Gelwick and Matthews 1993), they have been seldom used to test predictions in invasion biology. Scientists have also pointed out the need for a better understanding of the role of behavioral mechanisms as key predictors of invasion success (Holway and Suarez 1999; e.g. Sol et al. 2002). Whether a species' invasiveness can be predicted from how it responds to novel stimuli is an issue deserving further exploration. Future studies on species invasions would benefit from more experimental manipulations and by further investigation of the potential for behavioral mechanisms to be key traits underlying invasiveness.

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