

Ex situ evaluation of impacts of invasive mosquitofish on the imperiled Barrens topminnow

Malabika Laha · Hayden T. Mattingly

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Abstract Central to the protection of native species is an understanding of impacts of actual or potential invasive species and also the mechanisms through which those impacts are mediated. The introduction and spread of western mosquitofish, *Gambusia affinis*, into spring systems of the Barrens Plateau region of middle Tennessee is a concern for native species such as the Barrens topminnow, *Fundulus julisia*. We investigated whether mosquitofish might act as predators on early life stages of topminnows as well as affect the physiological well being of adults through aggressive interactions. A short-term, 24-h laboratory study with mosquitofish and topminnows demonstrated the vulnerability of young topminnow life stages to large mosquitofish predation and aggression. Survival of topminnow young, <16 mm total length (TL), was 0% and was attributed to predation by mosquitofish. Survival of juveniles, 20–30 mm TL, was 25%; juveniles mostly succumbed (post 24-h) to injuries inflicted by large mosquitofish. Adult topminnow survival was 100% but adults faced injury risk, primarily during the initial stages of their interaction with large mosquitofish. A long-term, 60-day laboratory study with syntopic and allotopic populations

of adult topminnows and mosquitofish failed to detect any negative impacts on topminnows due to coexistence. Survival, growth, and fecundity of adult topminnows syntopic with mosquitofish were not different from the allotopic population, although injury risk in the form of fin damage was greater syntopically. Thus, predation and aggression towards young topminnows may be the primary mechanisms by which western mosquitofish jeopardize the persistence of native Barrens topminnows in the wild. Our results reemphasize the danger to native aquatic biodiversity of unregulated introductions of *Gambusia* species.

Keywords *Fundulus* · *Gambusia* · Invasive species · Aggressive behavior · Fin damage index

Introduction

Increase in transport and commerce during the past few centuries has accelerated the pace of introduction of organisms outside their native range (Mack et al. 2000). Adverse effects of many of these introductions on native species, communities, and ecosystems have long been recognized (Elton 1958). Impacts of invasive species are often mediated through competition, predation, introduction of diseases, and hybridization. Identifying the impact of an invasive species, and the mechanism by which it is mediated, is the

M. Laha · H. T. Mattingly (✉)
Department of Biology, Tennessee Technological
University, Box 5063, Cookeville, TN 38505, USA
e-mail: hmattingly@tntech.edu

basis of formulating effective management decisions. However, it is often difficult to detect the exact mechanism of impact.

Here we report an *ex situ* investigation of impacts of non-native western mosquitofish, *Gambusia affinis*, on the well being of an imperiled spring-dwelling fish, the Barrens topminnow, *Fundulus julisia*. The topminnow is endemic to the Barrens Plateau region of middle Tennessee, residing in a few springs and spring-influenced first- or second-order streams of the Caney Fork, Duck, and Elk river systems (Williams and Etnier 1982; Rakes 1989; Johnson and Bettoli 2003). At the time of its description in the early 1980s, there were 14 known populations of Barrens topminnow spread over its range. The number of known wild populations in 2000 had dwindled to four. A reintroduction program was initiated in 2001 to attempt establishment of hatchery-bred topminnows within the species' historic range (Johnson 2004; Goldsworthy 2005).

Concurrent with topminnow declines, the range of western mosquitofish on the Barrens Plateau has been expanding. Mosquitofish invasion of this region has apparently occurred over the past 60 years. They were not found in the Duck River system in 1940s (Etnier and Starnes 2001) and were unknown from the Barrens Fork River system even in early 1980s. Recent surveys, however, have reported high numbers of mosquitofish in many streams throughout the Barrens Plateau (Goldsworthy 2005).

The expanding range and number of mosquitofish in Barrens topminnow habitat is viewed with great concern. For example, mosquitofish apparently replaced a robust population of Barrens topminnow in a McMahan Creek tributary between October 1993 and August 1995 (P.L. Rakes, Conservation Fisheries, Inc., personal communication). If mosquitofish indeed replace topminnows in the Barrens region, the mechanism of species replacement remains unknown. Further, restored Barrens topminnow populations should display higher viability if the species-replacement mechanism could be discovered.

Introduction of a non-native predator may alter community composition, species abundance, and inhibit persistence of native species (e.g., Hurlbert et al. 1972; Minckley 1973; Kitchell et al.

1997). Most negative impacts of introduced poeciliids like mosquitofish indeed seem to involve predation on larvae, juveniles or small adults of other fishes (Courtenay and Meffe 1989). The strong, firmly attached conical teeth of mosquitofish are appropriate for capture of motile prey such as aquatic insects, crustaceans and small fish (Schoenherr 1981). Also, because of the small size of mosquitofish, they can gain access to small and shallow pools that are generally avoided by large predators. Thus, young Barrens topminnows may be vulnerable to mosquitofish predation.

Changes in abundance of native species after introductions of exotic fishes have been commonly accepted as evidence of ongoing competition (Matthews 1998). Many studies have documented that invasive species showed superior competitive ability manifested either through exploitation (Petren and Case 1996; Byers 2000) or interference (Usio et al. 2001) or both (Holway 1999). Arthington and Lloyd (1989) reported a shift in native species' diets after the introduction of mosquitofish in the stream systems of Australia. There is likely some degree of overlap in diet and habitat requirements of Barrens topminnow and mosquitofish. However, considering the aggressive nature of mosquitofish (Courtenay and Meffe 1989), interference competition may be more problematic than superior exploitation of food resources. Mosquitofish may chase away others to secure favorable spatial positions.

Alternatively, such aggression may simply be a behavioral trait of mosquitofish and may not be directly linked to competition for resources. Mosquitofish are known to attack native fish and inflict caudal fin damage, leaving fish susceptible to diseases. Such aggression could cause topminnows to cease feeding in presence of mosquitofish and retreat to cover, which may ultimately affect their survival, growth and reproductive potential as concluded by Schoenherr (1981) for the Sonoran topminnow, *Poeciliopsis occidentalis sonoriensis*. He documented four separate instances in which mosquitofish eliminated the Sonoran topminnow from its native habitat.

We undertook a laboratory study of behavioral interactions between mosquitofish and Barrens topminnows in an attempt to elucidate mechanisms for the decline of topminnows where

mosquitofish are present. Specifically, we (1) documented aggressive behaviors of both species and determined vulnerability of different size classes of Barrens topminnow to mosquitofish aggression and predation over a 24-h period, and (2) measured direct and indirect impacts imparted to adult Barrens topminnows when coexisting with mosquitofish for 60 days.

Methods

Short-term behavioral study

We adopted a modification of the methods of Barrier and Hicks (1994) to document aggressive behaviors of mosquitofish and Barrens topminnows and to identify vulnerable topminnow size classes. Three topminnow size classes were used: young [12–16 mm total length (TL)], juvenile (20–30 mm TL) and adult (>45 mm TL). Two size classes were used for mosquitofish: small (17–22 mm TL) and large (30–35 mm TL). Because all the large mosquitofish were females, we used only adult female topminnows to be consistent with the sex of the adults. Small mosquitofish and the two smallest topminnow size classes were not sexed.

Each size class of a species was tested twice with each size class of the other species, yielding six treatment combinations with two replicates for each combination. Thirty-eight-l glass tanks served as experimental units without any substrate. A single sponge filter attached to an aerator was kept in each tank, which provided the only form of cover. All experiments were conducted at room temperature (19.5–20.5°C), during daylight conditions, at a pH of 7.8 and dissolved oxygen concentration of 7.5 mg l⁻¹.

For each replicate, we released four fish of each species in the tanks 3–4 h before commencement of filming to briefly acclimatize the fish to the experimental tanks. A plastic screen kept the two species separated. Feeding was stopped 8 h prior to the commencement and during experiment. At 0 h, the plastic screen was lifted and fishes were filmed for a 20 min period, using a Canon XL1S video camera which was

positioned at right angle to the longest side of the tanks. After 24 h, a second 20-min filming was done. Between the two filming periods, fishes were allowed to interact under a 13-h light, 11-h dark photoperiod.

The resulting video footage was reviewed and interspecific aggressive movements initiated by both species were recorded and analyzed using observational software ODLog (Macropod Software). The initiator, the target, the movement type and the duration of each movement were noted. We categorized aggressive movements as intention movements, chases, or nips, defined as follows: intention movements (Hartman 1965) occurred when a fish only turned its head quickly towards another or made a short plunge towards it for a duration of <0.5 s; chases were any active pursuit lasting >0.5 s in which an individual chasing another was separated by a distance of ≤10 cm; and nips were actual physical contact between two individuals in the form of biting, pushing, or other contact. For judging the distance between two fish, we marked the sides of aquariums horizontally and vertically at intervals of 5 cm.

For analyses, total aggressive movements were considered to be the sum of intention movements, chases, and nips [movement tallies by separate categories are reported in Laha (2004)]. The total aggressive movements initiated by the two species were compared to determine the more aggressive species. Moreover, we determined the more aggressive size-class of that species by comparing the aggressive movements initiated by each size class. The most affected size class of the targeted species was also established by comparing the total aggressive movements directed towards them by the aggressive species. The aggressive behavior of both size classes of the more aggressive species was compared over time. Comparisons between groups were made using unpaired *t*-tests performed with statistical software SAS 8.2 (SAS Institute 1997) and were considered significant at $P \leq 0.05$. Data from trials with Barrens topminnow young were excluded from overall statistical analysis because young were preyed upon in trials with large mosquitofish, thereby generating incomplete behavioral data.

Long-term coexistence study

We measured survival, fin damage, growth, reproductive effort and fecundity of syntopic and allotopic populations of Barrens topminnow under laboratory conditions for 60 days to test the hypothesis of physiological harm being imparted by aggressive mosquitofish to topminnows. Two arrangements were used, one with experimental and conspecific control treatments, and the second with experimental, conspecific control, and an additional treatment with flame chubs, *Hemitremia flammea*, which acted as a hetero-specific control (Table 1). Flame chubs were chosen because they are often syntopic with the topminnows in their natural habitats (Johnson 2004) and are not known to have any adverse impacts on topminnow persistence. All experimental tanks were provided with substrate of gravel and crushed corals. Flat rocks, PVC pipes and artificial floating woolen-yarn mops provided moderate cover.

Fishes were acclimatized separately in the experimental tanks for approximately one month before the start of the experiment. A day before the experiments began, all topminnows were anesthetized with 60 mg l⁻¹ clove oil, sexed and measured for their total and standard lengths, and wet weights. However, some of the young adult

individuals had not developed breeding colors and could not be sexed. They were haphazardly assigned to treatment tanks, maintaining a visually determined ratio of 1.5 female:1 male (however, post-experimental analysis revealed the actual sex ratio of 1–1.5 female:1 male). The relationship between total and standard length of Barrens topminnow was established with linear regression to facilitate accurate length measurement in individuals with damaged fins. Mosquitofish and flame chubs were not individually measured but the lengths of the smallest and largest fish were noted (Table 1). The sex ratio used for these two species was approximately 1.5–2 female:1 male.

All fish were fed ad lib twice daily with a diet of frozen bloodworms, *Chironomus plumosus*, in the morning and commercial flake food (Tetramin) in the evening. The bloodworms were substituted with frozen brine shrimp, *Artemia salina*, once a week. Fish were fed in excess to deter competition for food. The tanks were cleaned and 25%–50% water was changed at least once a week with care taken to minimize disturbance to the fish. Any dead fish were removed and preserved in 10% formalin.

At the end of the experiment, topminnows were euthanized with a dose of 175 mg l⁻¹ MS-222. They were measured for total length and wet

Table 1 Experimental characteristics of the 60-day, long-term coexistence study with *Fundulus julisia* (*Fund*), *Gambusia affinis* (*Gamb*), and *Hemitremia flammea* (*Hemi*)

Characteristic	First arrangement	Second arrangement
Treatments (Species and number of individuals per replicate)	1. Conspecific control (<i>Fund</i> 24) 2. Experimental (<i>Fund</i> 12 + <i>Gamb</i> 12)	1. Conspecific control (<i>Fund</i> 14) 2. Heterospecific control (<i>Fund</i> 7 + <i>Hemi</i> 7) 3. Experimental (<i>Fund</i> 7 + <i>Gamb</i> 7)
Replicates per treatment	Three	Three
Initial length of <i>Fund</i> (mean ± SD; mm SL)	34.4 ± 4.9	37.0 ± 4.2
Initial length range of <i>Hemi</i> (mm SL)		38.0–49.6
Initial length range of <i>Gamb</i> males (mm SL)	21.3–24.2	22.0–25.1
Initial length range of <i>Gamb</i> females (mm SL)	26.0–32.6	27.6–33.1
Experimental facility type	Structured-foam troughs	Glass aquaria
Dimensions (cm)	132 × 79 × 30	76 × 30 × 46
Water volume (l)	189	110
Water depth (cm)	26	38
Water surface area (cm ²)	10,400	2,324
Fish density	1 fish per 7.7 l	1 fish per 7.6 l
Temperature (°C)	19–21	19–21
Photoperiod	14 h light, 10 h dark	14 h light, 10 h dark

weight, and the specific growth rates (SGR) were calculated for each replicate by the following formula (e.g., Wurtsbaugh and Cech 1983):

$$\text{SGR} = \frac{\ln(W_f) - \ln(W_i)}{\text{Number of days}} \times 100,$$

where W_i = initial wet body weight and W_f = final wet body weight.

We next assigned ranks to each fin of each topminnow based on extent of damage (no damage was seen in the pelvic and pectoral fins of any fish; these two fins were excluded from analyses). The following ranks were assigned to each fin: rank 0 = no damage; rank 1 = 1%–10% of total fin area damaged; rank 2 = 11%–25% damage; and rank 3 = >25% damage. To measure the severity of mosquitofish aggression, we developed and calculated a fin damage index (FDI) for each replicate using the following formula:

$$\text{FDI} = \frac{\sum_{i=1}^n \sum_{j=1}^3 R_{ij}}{n},$$

where R = ranks assigned to each fin, i = individual topminnows, j = number of fins (anal, dorsal and caudal), and n = number of topminnows in each replicate. For example, if in a given replicate, 7 topminnows survived the 60-day period and each fish received a rank 1 score for caudal fin damage and one individual also had a rank 2 damage of its anal fin, then for this replicate the $\text{FDI} = [(1 + 1 + 1 + 1 + 1 + 1 + (1 + 2))/7] = 1.286$.

The gonads of both males and females were dissected out, weighed and preserved in 10% formalin. Gonadosomatic index (GSI) was calculated as a measure of reproductive effort of a fish by the following formula,

$$\text{GSI} = \frac{\text{Wet weight of gonad}}{\text{Total body wet weight}} \times 100.$$

Only female GSI was used for statistical analysis. We later examined the preserved gonads of the females for total number of eggs and their stages of maturity. The ovaries were teased apart and the eggs were categorized as immature,

maturing, or ripe based on diameter, transparency, and location of oil droplets. For certain analyses, counts of maturing and ripe eggs were pooled into a single category termed mature eggs, which also served as an index of fecundity. Immature eggs were very small, 0.5–1.0 mm, opaque and lacked visible oil droplets. Maturing eggs measured about 1.2–2.0 mm and were slightly transparent with oil droplets evenly spaced out across the inner surface of the egg. Ripe eggs were slightly larger than the maturing eggs, 2.0–2.7 mm, but were more transparent and oil droplets were concentrated at one pole.

A similar classification was used by Blanchard (1996) for categorizing eggs of blackspotted topminnow, *Fundulus olivaceus*, and broadstripe topminnow, *F. euryzonas*. We counted the number of eggs in each category and measured diameters using an ocular micrometer (40×) in a compound microscope.

Survival, SGR, FDI, GSI, and the number of mature eggs in females between control and experimental groups were compared using one-way ANOVAs for each experimental arrangement. All percentage data were arcsine square-root transformed. Due to high variation in GSI of females, coefficient of variation (CV) was calculated for each replicate and also compared among treatments with ANOVA. Statistical analyses were performed with SAS version 8.2 (SAS Institute 1997) and were considered significant at $P \leq 0.05$.

Sources of fish

All topminnows used in both studies were hatchery-bred from the type locality population stock. Adult topminnows belonged to the 2002-year class and juvenile and young topminnows were from 2003-year class. Topminnows for the short-term study were provided by Conservation Fisheries, Inc., Knoxville, TN, and those for the long-term study were obtained from Dale Hollow National Fish Hatchery, TN. Western mosquitofish and flame chubs were obtained from sites on Hickory Creek, Coffee County, TN (N 35°30', W 85°52' and N 35°30', W 85°53'), and were allowed to habituate to laboratory conditions for ≥ 3 months before being used for any experiment.

Results

Short-term behavioral study

Gambusia affinis was the more aggressive species across all size-classes and over two time-periods ($t = -3.0$; $df = 30$; $P = 0.005$). The total number of aggressive movements initiated by mosquitofish was greater than eight times than those initiated by Barrens topminnows. Both small and large mosquitofish were equally aggressive ($t = 1.62$; $df = 14$; $P = 0.128$). However, individual variation in aggressive behavior was noticeable for all size classes of both species (Fig. 1).

Between adult and juvenile topminnows, the juveniles tended to be the most targeted size-class ($t = -2.0$; $df = 14$; $P = 0.065$). They were chased and nipped by both large and small mosquitofish, but large mosquitofish inflicted severe fin damage. After the end of the first 20-min trial period, most juveniles lost their locomotor abilities, which incapacitated them to escape subsequent mosquitofish aggression. During the 24-h coexistence, seven out of eight juveniles had lost their fins completely to large mosquitofish aggression and were observed hovering near the water's surface. Although large mosquitofish could not ingest the juvenile whole, in a single case the head and caudal portion of a juvenile was eaten away. Five other juveniles died within 7 days of the post-experimental period, bringing the total mortality to six (i.e., experiment-wide 75% mortality). Duration of most interspecific chases by large mosquitofish was 0.5–2 s, and most chases did not exceed 5 s.

Aggression of small mosquitofish did not differ between the two filming periods at 0 and 24 h ($t = -0.18$; $df = 6$; $P = 0.865$). However, aggression of large mosquitofish decreased considerably over time ($t = 2.73$; $df = 6$; $P = 0.034$).

The greatest impact of mosquitofish aggression was observed in trials with topminnow young. In small-mosquitofish trials, the topminnow young were chased intermittently during 0 and 24 h (Fig. 1). In trials with large mosquitofish and topminnow young at 0 h, chases by large mosquitofish were limited mostly to the first few minutes of interaction as young retreated behind the filters and were not seen in the water column.

Predation was not observed during this 0-h period, but none of the young were retrieved in either replicates after 24 h. Stomach contents of large mosquitofish were checked immediately after completion of trials. Fish vertebral bones were retrieved from 50% of the large mosquitofish of each replicate, which confirmed predation of the topminnow young by the large mosquitofish. Chances of mosquitofish cannibalizing their own young before, during, or after the trial were minimal because these mosquitofish were held in isolation from their young prior to the experiment and no females were heavily gravid.

Long-term coexistence study

Survival of topminnow adults at the end of the 60-day experimental period was similar between experimental and control groups in both arrangements (First arrangement: $F = 4.0$; $df = 1, 4$; $P = 0.11$; Second arrangement: $F = 0.57$; $df = 2, 6$; $P = 0.59$). Overall 60-day survival of adult Barrens topminnow was 95%, with no topminnow deaths in conspecific controls, one death in the heterospecific (flame chub) controls and three deaths in experimental treatments with mosquitofish. Three mosquitofish were also lost when they jumped out of the experimental facilities. No other mortality was observed during the experimentation.

Topminnow fin damage was significantly greater in experimental treatments than controls in both arrangements (Fig. 2). In the first arrangement, 58% of the topminnows in experimental treatments showed some degree of fin damage compared to 21% in control treatments. The extent of fin damage as measured by FDI was greater in topminnows that were present with mosquitofish ($F = 25.12$; $df = 1, 4$; $P = 0.007$). In the second arrangement, 90% of the topminnows with mosquitofish had some degree of fin damage compared to 40%–45% in the conspecific and heterospecific controls. FDI of the topminnows in the experimental group was also different from the controls groups ($F = 9.77$; $df = 2, 6$; $P = 0.013$). However, extent of fin damage in topminnows with mosquitofish in the second arrangement was less at the end of 60-day experimental period than it was during the first week of starting the experiment. This inference is largely

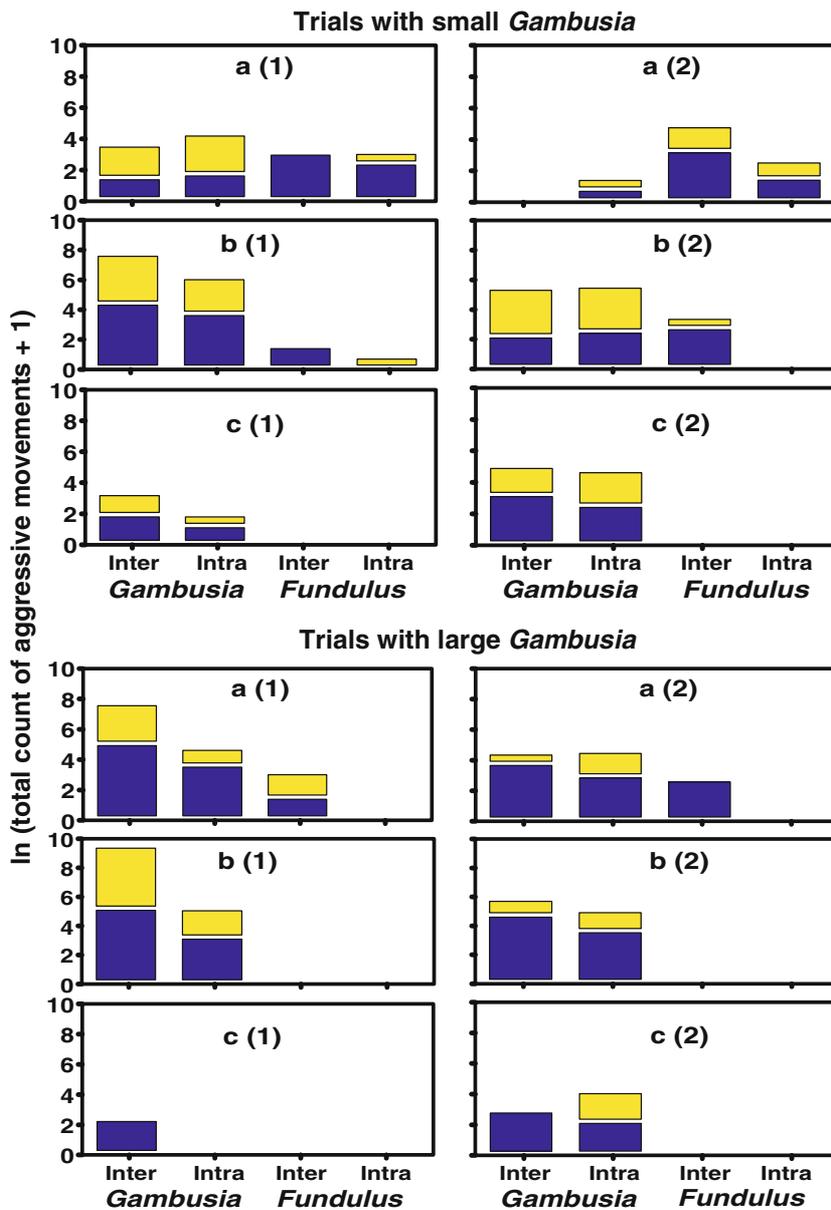


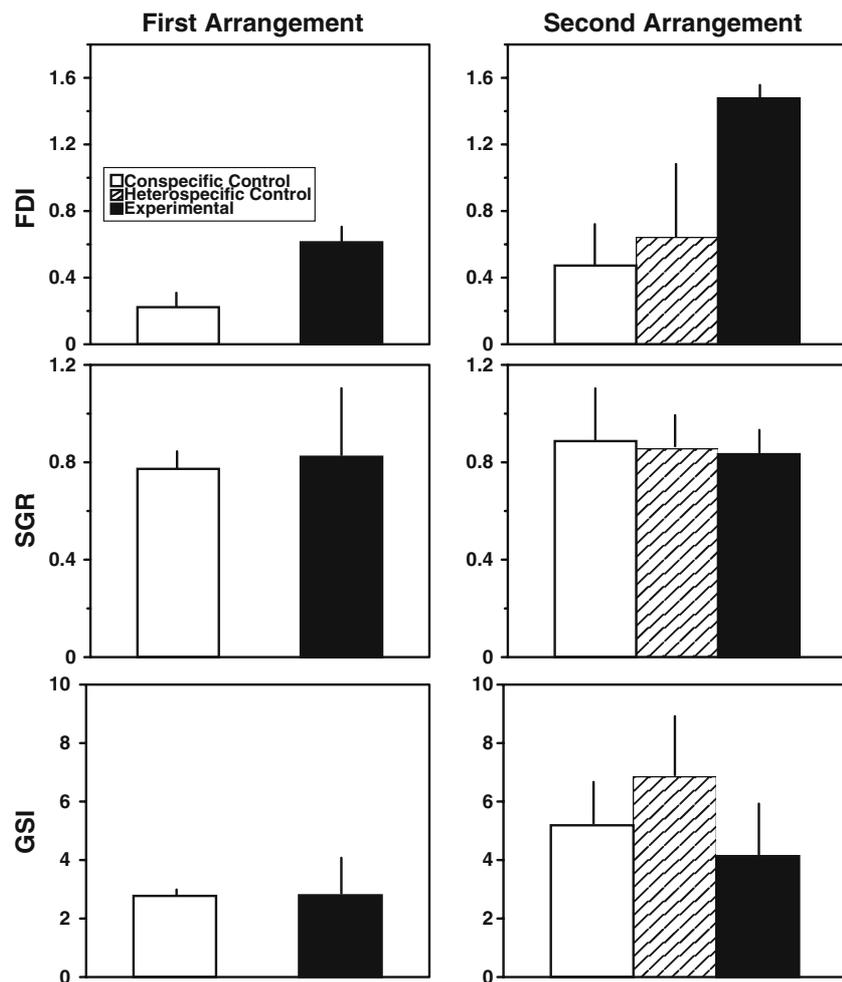
Fig. 1 Aggressive interactions among small (top panels) and large (bottom panels) *Gambusia affinis* matched with (a) adult, (b) juvenile, and (c) young *Fundulus julisia*. (1) and (2) represent the two replicates of each treatment. In each plate the first two bars from left indicate aggressive movements initiated by mosquitofish with the first bar and second bar representing inter- and intraspecific move-

ments, respectively. The remaining two bars represent movements initiated by Barrens topminnows with the third bar representing inter- and the rightmost bar representing intraspecific movements. The darker stack below represents movements at 0 h and the lighter stack above represents movements at 24 h

anecdotal as fin damage was measured only once at the end of experimental period. Most 60-day damages were limited to <10% of the total area of anal, caudal or dorsal fin and seldom did a fish exhibit damage to more than two of its fins.

The growth rate of adult topminnows in both arrangements was similar among the treatments (First arrangement: $F = 0.03$, $df = 1, 4$, $P = 0.89$; Second arrangement: $F = 0.85$, $df = 2, 6$, $P = 0.47$; Fig. 2). The mean (\pm SD) SGR in the first

Fig. 2 Mean FDI, SGR, and GSI of *Fundulus julisia* after 60 days in conspecific control (with only *F. julisia*; open bars), heterospecific control (with *Hemitemia flammea*; hatched bars), and experimental (with *Gambusia affinis*; solid bars) treatments of two arrangements. See text and Table 1 for details of experimental arrangements. Error bars are 1 SD



arrangement was $0.78 \pm 0.18\%$ per day ($n = 6$) and $0.86 \pm 0.14\%$ per day ($n = 9$) in the second arrangement. Likewise, there was no detectable effect of mosquitofish on the reproductive effort of topminnows as measured by female GSI in either arrangement (First arrangement: $F = 0.02$, $df = 1, 4$, $P = 0.88$; Second arrangement: $F = 1.75$; $df = 2, 6$, $P = 0.25$; Fig. 2). Individual GSI ranged 0.9–7.1 in the first arrangement and 1.2–12.0 in the second arrangement. There was also no difference in CV of female GSI between control and experimental units (First arrangement: $F = 0.42$, $df = 1, 4$, $P = 0.55$; Second arrangement: $F = 1.21$, $df = 2, 6$, $P = 0.36$). In the first arrangement CVs were 56.9% (control) and 47.5% (experimental), whereas CVs in the second arrangement were 41.6% (conspecific control), 39.8% (heterospecific control), and 49.8% (experimental).

Topminnow ovaries yielded eggs at three stages of maturity. Data from 60 adult females (38–61 mm SL) yielded an average of 102 (range 16–288) immature eggs, 6 (0–47) maturing eggs and 2 (0–10) ripe eggs. However, topminnows showed individual variation in the total yield of mature eggs and effect of treatment was not established (First arrangement: $F = 0.29$, $df = 1, 4$, $P = 0.62$; Second arrangement: $F = 0.57$, $df = 2, 6$, $P = 0.59$).

Discussion

Aggressive behavior of small and large mosquitofish towards different size classes of Barrens topminnow was well established through our study. Such aggression negatively affected the

survival of young life stages (<30 mm TL) of Barrens topminnow either by direct predation or by fatally injuring them. Further, fin injuries that may not lead to mortality can still impact the juveniles by increasing their vulnerability to secondary infections and by affecting their motility, thereby increasing the chances of subsequent attack and predation as also observed in this study. Similar aggressive behavior of small and large mosquitofish was observed during behavioral interactions with black mudfish, *Neochanna diversus* (Barrier and Hicks 1994), although the number of interspecific aggressive interactions was much more in our study. Large mosquitofish preyed on the mudfish young shortly after they were released into the tank. In our study, however, predation by large mosquitofish was not documented within 20 min of young introduction, although they made attempts to capture the young. The sponge filters in our tanks may have provided temporary shelter to the young.

It could be argued that lack of other food source during our short-term experiments was responsible for topminnow predation by mosquitofish. We avoided feeding during the experiments to reduce the confounding effects of food in fish behavioral interactions. However, aquarium studies have repeatedly confirmed the aggressive and voracious predatory behavior of mosquitofish towards small fish and anuran larvae (Johnson 1976; Meffe 1985; Barrier and Hicks 1994; Baber and Babbitt 2004). Moreover, recent field studies have indicated recruitment failure of reintroduced Barrens topminnows in sites with mosquitofish presence (Goldsworthy 2005).

Survival of adult topminnows was not affected in spite of aggression by mosquitofish. However, injuries in the form of fin damage were observed. Mosquitofish aggression towards the adult topminnows was highest during initial stages of interaction and decreased over time. This was observed in both short-term and long-term studies, where number of aggressive movements initiated by mosquitofish decreased over a 24-h period and where extent of fin damage appeared to attenuate over the course of 60 days. Meffe (1985) also reported that mosquitofish became aggressive as soon as the Sonoran topminnows and mosquitofish were placed together and most

topminnows were attacked within an hour of coexistence. In contrast, Barrier and Hicks (1994) did not document any mosquitofish aggression towards adult mudfish.

The general well being of adult Barrens topminnows was not compromised due to the presence of mosquitofish as observed from this study. Fitness correlates such as topminnow growth, reproductive effort, and fecundity were not affected as a result of coexistence with mosquitofish. The topminnows fed and moved freely despite the presence of mosquitofish and did not retreat behind covers. In the field, adult topminnows were equally robust in presence or absence of mosquitofish (Goldsworthy 2005). Meffe (1985) observed that Sonoran topminnow feeding was not impaired in laboratory sympatric populations, but they were lighter in somatic weight and produced fewer embryos and mature ova. However, no significant differences in body and ovary weight of allopatric and sympatric populations could be detected in a field mesocosm study. Thus, Meffe (1985) suggested that physiological stress responses in adult Sonoran topminnows play a minor role in species replacement.

Field observations by Galat and Robertson (1992) of Sonoran topminnows and mosquitofish showed that sympatric topminnow populations had higher growth and fecundity than allopatric populations. They suggested that size-selective predation by mosquitofish removed smaller topminnows, resulting in larger average size of remaining topminnows where they coexisted with mosquitofish. No such pattern was discernible from our coexistence study, although its duration was only two months.

Mosquitofish are known to prey on diverse items under different circumstances (Hurlbert and Mulla 1981; Meffe 1985; Arthington 1989; Goodsell and Kats 1999; Margaritora et al. 2001). Barrens topminnows are also opportunistic carnivores (Rakes 1989). Habitat-wise, both fish prefer small, shallow bodies of water with vegetation and low-flow conditions (Miura et al. 1979; Rakes 1989; Johnson and Bettoli 2003). These observations suggest that the two fish might compete for resources and evidence of such competition should be expressed in growth, reproduction and survival of the competing

species. Our experiments were not designed to fully elucidate effects of competition for food and space between the two species because we used ad lib feeding and low mosquitofish densities. Experiments using higher mosquitofish densities and limiting resources could provide better insight into potential competitive interactions between the two species.

Ex situ experiments in artificial settings suffer from the inherent inability to represent all dimensions of the natural environment. For example, Barrens topminnows in our study were hatchery-bred, whereas mosquitofish were collected from the wild. Field-captured topminnows possibly could be more successful in withstanding mosquitofish aggression than those from the hatchery. Nevertheless, results of Goldworthy's (2005) recent field study paralleled our laboratory studies, in terms of associations between mosquitofish presence and topminnow recruitment, growth, and size-specific survival, thereby strengthening the validity and applicability of our laboratory results. Field mesocosm studies could further enhance our understanding of interactions between these two species.

Conclusion

Our results collectively suggest that impacts of western mosquitofish on Barrens topminnows are primarily mediated through predation and injury of young life stages. Considering the fact that the relative density of these two fish in the wild is many times higher than what was used for this study (Goldworthy 2005), the predation pressure on topminnow eggs and hatchlings could be tremendous. On the other hand, the presence of mosquitofish may not affect physiological well being in adult Barrens topminnows and they may feed, grow, and reproduce normally, at least in conditions where the mosquitofish density is relatively low and food sources are not limiting.

This difference in impact of mosquitofish aggression on various topminnow size-classes has implications for its conservation and management. In particular, efforts to promote a self-sustaining topminnow population may be undermined due to recruitment failure as a result of

predation by mosquitofish. Moreover, size-biased aggression by mosquitofish suggests a minimum body size of >30 mm TL for topminnow stocking.

Our overall results are congruent with other studies highlighting threats posed by non-native *Gambusia* to native species in freshwaters around the world. Future efforts in the Barrens region could be directed towards understanding topminnow–mosquitofish interactions under a broad range of environmental conditions (e.g., Laha and Mattingly 2006), as well as developing methods to exclude mosquitofish from topminnow habitats.

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