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Separating the effects of intra- and interspecific age-structured interactions in an experimental fish assemblage

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Abstract We documented patterns of age-structured biotic interactions in four mesocosm experiments with an assemblage of three species of co-occurring fishes from the Florida Everglades, the eastern mosquitofish (*Gambusia holbrooki*), sailfin molly (*Poecilia latipinna*), and bluefin killifish (*Lucania goodei*). These species were chosen based on their high abundance and overlapping diets. Juvenile mosquitofish and sailfin mollies, at a range of densities matching field estimates, were maintained in the presence of adult mosquitofish, sailfin mollies, and bluefin killifish to test for effects of competition and predation on juvenile survival and growth. The mesocosms held 1,200 l of water and all conditions were set to simulate those in Shark River Slough, Everglades National Park (ENP), USA. We placed floating mats of periphyton and bladderwort in each tank in standard volumes that matched field values to provide cover and to introduce invertebrate prey. Of 15 possible intra- and interspecific age-structured interactions, we found 7 to be present at the densities of these fish found in Shark River Slough marshes. Predation by adult mosquitofish on juvenile fish, including conspecifics, was the strongest effect observed. We also observed growth limitation in mosquitofish and sailfin molly juveniles from intra- and interspecific competition. When maintained at high densities, juvenile mosquitofish changed their diets to include more cladocerans and fewer chironomid larvae relative to low densities. We estimated size-specific gape

limitation by adult mosquitofish when consuming juvenile mosquitofish and sailfin mollies. At high field densities, intraspecific competition might prolong the time period when juveniles are vulnerable to predation by adult mosquitofish. These results suggest that path analysis, or other techniques used to document food-web interactions, must include age-specific roles of these fishes.

Keywords Food web · Age-structure · Competition · Predation · Cannibalism

Introduction

A large number of biotic interactions are possible among the species in even a small assemblage (Wootton 1994). Age-structured interactions within and among species greatly expand the list of possible interactions, even when those known to be unlikely are excluded. For example, the difference in size between juveniles and adults may result in a predator-prey relationship between age classes of species that compete for food as similar-sized adults (Wilbur 1988). Cannibalism is probably under-appreciated as a factor in population and community dynamics (Polis 1981). Few studies attempt to consider both intra- and interspecific age-structured biotic interactions (Winemiller and Polis 1996), though overlooking the complete array of interactions may limit the ability to predict how changing environmental conditions will affect the community (Wootton 1994).

In size or age-structured populations, the influence of intra- or interspecific competition on juvenile growth rates can have important effects on population dynamics and community structure by shaping age-specific mortality patterns (Reznick and Endler 1982; Persson 1988; Polis et al. 1989; Abrams and Rowe 1996; Reznick et al. 1996). The relative strength of intraspecific and interspecific interactions plays a central role in population and community regulation (Connell 1983; Sih et al. 1985; Polis et al. 1989). Intra- or interspecific competition and predation may occur simultaneously in size-structured

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assemblages and may yield complex or unexpected community dynamics. Predation on potential competitors (intraguild predation) can have effects beyond the outcomes predicted by direct competition or predation alone and can result in unstable dynamics or alternative stable states (Holt and Polis 1997). For example, Strauss (1991) found that damage caused to a plant by one species of herbivorous beetle facilitated the oviposition of another competing species, thereby increasing its competitor's population density. Thus, the interactive effects of competition and predation have the potential to produce a rich array of outcomes that can not be predicted by examining either process alone (Bender et al. 1984; Polis et al. 1989).

We examined the relative importance of intra- and interspecific competition and predation in an experimental assemblage of small fishes that inhabit freshwater marshes of the Florida Everglades. We chose to study three abundant and co-occurring species that share overlapping diets to separate the relative strengths of their interactions. These species included two poeciliids, the eastern mosquitofish (MF), *Gambusia holbrooki*, and the sailfin molly (SM), *Poecilia latipinna*, and one fundulid, the bluefin killifish (BFK), *Lucania goodei*. The MF consumes both larval and neonate fish and invertebrates, as well as plant and detrital material (Loftus and Kushlan 1987; Belk and Lydeard 1994; Winkleman 1996). BFK feed primarily on small invertebrates, and SM are herbivores that feed primarily on algae (Loftus and Kushlan 1987). Thus, BFK and SM potentially compete for a portion of the MF diet, and juveniles of all three species are potential prey for adult MF. We identified 15 potential interactions among these three species based on their feeding ecology (Fig. 1A), and developed four experiments to test the relative strength of each of these interactions. Previous studies (Belk and Lydeard 1994; Schaeffer et al. 1994) have addressed the importance of predation and interspecific competition among MF and another co-occurring poeciliid fish, *Heterandria formosa*. This study differs from the previous work in three ways. First, it addresses the importance of interspecific competition and predation in relation to population age-structure. Second, the effects of competition and predation are measured directly from growth and survival data rather than inferred from population size structure. Finally, this study examines the importance of intraspecific competition.

There are contrasting views about the ability to scale up mesocosm studies of experimental assemblages to field conditions, and about their overall usefulness in understanding field ecology (e.g., Carpenter 1996; Wilbur 1997). As an alternative, statistical techniques have been developed that permit estimating biotic interaction webs from field experiments and sampling data (e.g., Wootton 1994; Ives et al. 1999), but these inevitably suffer from the problems of multicollinearity (Petraitis et al. 1996). We advocate a mixture of field and mesocosm experiments and the analysis of field time-series as the most powerful approach to understanding the complexity of

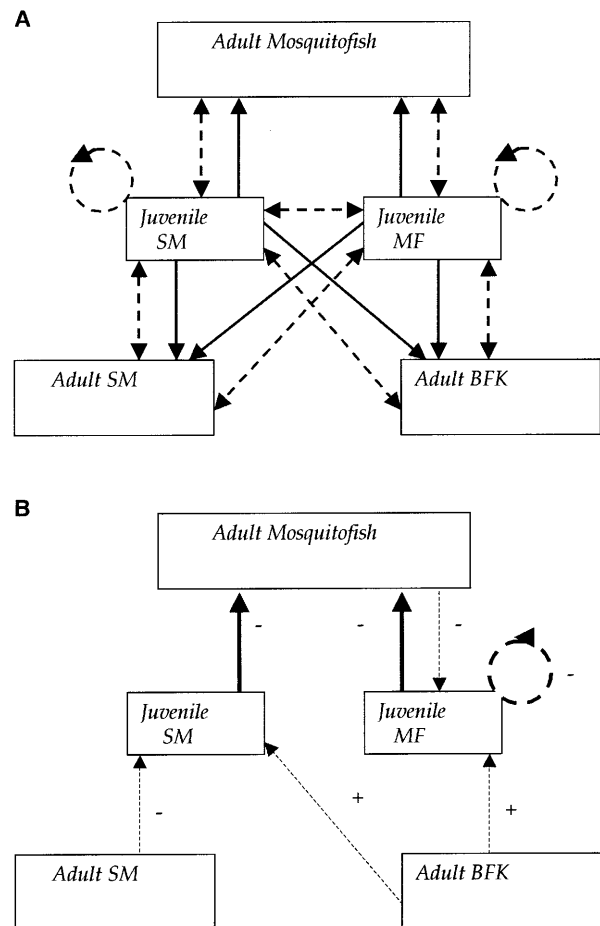


Fig. 1 **A** Conceptual model of interactions among fish species examined in this study. This model does not address all possible, or even all likely, interactions. For example, we were unable to obtain enough juvenile bluefin killifish (BFK) to include them in the study. One-way *solid arrows* indicate predation of adult fish on juveniles, and two-way *dotted arrows* indicate competition for food resources. **B** Conceptual model indicating interactions among fish species found to be significant in this study. The strength of interactions, based on the effect sizes measured, is indicated by thickness of arrows. Strong interactions are depicted with *bold arrows*, weak interactions are depicted with *thin arrows*. The +/- indicates a positive or negative interaction

species interactions and, ideally, to develop a predictive understanding of community regulation. The mesocosm studies reported here are instructive about the dynamics of age-structured biotic interactions. Our use of realistic densities of animals and experimental conditions are intended to complement a collection of studies seeking to describe natural food webs in the Florida Everglades (Trexler et al. 2000).

Materials and methods

Natural history

We selected our study species and experimental designs based on the natural history of fish in the Florida Everglades, USA. Everglades marshes are spatially extensive, shallow (usually <1 m

deep) wetlands that derive their water from seasonal rainfall and overland sheet flow. Approximately 85% of the annual rainfall occurs during the wet season, May through October, though there is marked interannual variation (Fennema et al. 1994). In many years, large portions of the marsh dry from November to May, leaving only the lowest elevations inundated, and confining aquatic animals to refugia in alligator ponds or solution holes. The seasonal hydrology of the Everglades shapes its aquatic communities along patterns of hydroperiod (Loftus and Eklund 1994), defined as the number of days per year that a given area is inundated.

The fish community in Everglades marshes is numerically dominated by small fish species [<8 cm standard length (SL) at maturity], primarily cyprinodontoids. Large piscivorous fishes (>8 cm SL at maturity), such as sunfishes (*Lepomis* spp.), largemouth bass (*Micropterus salmoides*), gar (*Lepisosteus platyrhincus*), and yellow bullhead (*Ameiurus natalis*), are sparsely distributed. Their influence on community structure is poorly known and may be limited to relatively long-hydroperiod areas (Kushlan 1976; Loftus and Eklund 1994; Jordan and McCreary 1996; Trexler et al., 2000). Our study focused on three of the most abundant and widespread fishes in Everglades marshes (Loftus and Kushlan 1987). Between 1992 and 1995, MF comprised 30% of the fishes (mean density = $4.9\text{--}7.5\text{ m}^{-2}$, $n=3$ sites) collected at typical long-hydroperiod sites in the Shark River Slough (SRS), the main drainage through Everglades National Park (ENP). BFK comprised 21% (mean density = $3.4\text{--}6.6\text{ m}^{-2}$, $n=3$ sites), and SM comprised 4% (mean density = $0.6\text{--}2.2\text{ m}^{-2}$, $n=3$ sites) of the fishes at those sites. The density estimates were taken from 5 sampling months throughout the year (February, April, July, October, and December). The average density of fishes was 18.5 m^{-2} (SE = 0.3 , range $0\text{--}99\text{ m}^{-2}$, $n=1,292$ samples collected by 1 m^2 throw trap; Trexler et al. 1996). The relative mix of these species varied seasonally and spatially depending on hydroperiod and nutrient status of local sites (Loftus and Eklund 1994; Trexler et al., 2000). Thus, the interactions we examined experimentally are likely to represent conditions experienced by these species in the marsh.

Mesocosm experiments: overview

We conducted four experiments in mesocosm tanks housed at the Daniel Beard Natural Resources Center, ENP. We used 36 cement tanks, each with a capacity of 1,200 l and a surface area of 1.06 m^2 . The tanks were filled with well water to a depth of 45 cm to match the typical wet-season water depths of many marshes in ENP. Each tank was fitted with a screen cover to exclude predators such as dragonfly naiads. We then manipulated the abundance and composition of adult and juvenile fishes in the tanks (Table 1). The mixtures of fishes in the experimental tanks were representative of the diversity in composition and density indicated by field data. Because of their high relative abundance, we focused on juvenile MF as competitors while adult MF acted as predators, competitors, or both. The first experiment ran for 18 days and the final three experiments each ran for 14 days.

All experimental conditions were chosen to match field conditions in SRS as closely as possible. Dense floating mats of periphyton and bladderwort spp. are characteristic of many Everglades marshes (McCormick et al. 1998) and provide cover for small fishes and invertebrates. A floating algal mat covering 12% of the surface area was added to each tank to provide cover and a food base. We examined the tanks for 7 days prior to starting each experiment to ensure that fish were not accidentally introduced with the vegetation. Field data from 2 years in SRS show that the water-surface coverage by floating algal mats ranged from 1.5% to more than 85%, with an average of 37%. The volume of floating mat ranged from 264.0 to $4,800.0\text{ ml/m}^2$, with an average volume of $2,585.0\text{ ml/m}^2$ and biomass of 36.7 g/m^2 (Geddes, unpublished data). The 12% cover in our tanks yielded an average volume of $1,750.0\text{ ml/m}^2$, and a biomass of 22.8 g/m^2 . The percent cover and biomass of floating algal mat in our experimental tanks was lower than average, but within the range of that found in the field.

Table 1 Number of replicates used in each mesocosm experiment. All control tanks contained 0 adult fish, and the density of adults and juveniles in each treatment is indicated in parentheses. BFK Bluefin killifish, MF mosquitofish, SM sailfin molly

Experiment 1. All tanks contained 5 juvenile SM

Juvenile	Adult treatments			
	Control	MF (5)	SM (5)	BFK (5)
Low (2)	3	3	3	3
Med (5)	3	3	3	3
High (12)	3	3	3	3

Experiment 2. All tanks contained 5 juvenile SM, juvenile size >experiment 1

Juvenile	Adult treatments			
	Control	MF (5)	SM (5)	BFK (5)
Low (3)	4	4	4	4
High (12)	4	4	4	4

Experiment 3. All tanks contained 5 juvenile SM.

Juvenile	Adult treatments			
	Control	MF (5)	MF (5) + SM (5)	MF (5) + BFK (5)
Low (2)	3	3	3	3
High (12)	3	3	3	3

Experiment 4.

Adult treatments		
Juvenile treatment	Control	MF (5)
Low MF (3)/Low SM (3)	6	4
Low MF (3)/High SM (12)	4	6
High MF (12)/Low SM (3)	6	4

We examined the gut contents of all fish in the tanks to determine the degree of dietary similarity and the availability of invertebrate prey during the experiments. The gut contents were analyzed by removing the stomachs of the specimens preserved in 10% formalin, followed by 70% ethanol, and examining the contents under a dissecting microscope. We identified the invertebrates to the family level, estimated their dry mass, and preserved the stomach contents in 70% ethyl alcohol.

Mesocosm experiment 1

Experiment 1, conducted in August 1997, tested the relative importance of both age-structured predation and competition, but was designed to be most sensitive to the effects of predation. Juvenile SM at one density were reared with three densities of juvenile MF, and in four combinations of adult fish: no adults present (Control), adult MF present, adult SM present, or adult BFK present (Table 1). To measure the effects of competition and age-structured predation, we recorded survivorship and growth of juvenile fish across all treatments and densities.

We varied the density of juvenile MF over three levels. The low-density treatment received 2 juvenile MF, the medium level

(5) was representative of the mean number of juvenile MF found in the field, and the high level (12) was the maximum density found at a nutrient-enriched site in SRS. The density of juvenile SM was 5 per tank in all treatments, high for this species but within the range commonly seen. SM were kept at higher than average densities to increase the likelihood of observing an effect of this herbivore, should one exist. The density treatments were crossed with all four adult treatments.

At the beginning of the experiment, we measured the standard length of all fish to the nearest 0.5 mm, and individuals were chosen to be as similar in size as possible. The mean size of adult MF was 24.6 mm (SE=0.76), the mean size of juvenile MF was 8.6 mm (SE=0.08), and the mean size of juvenile SM was 9.1 mm (SE=0.08). At the end of the experiment all tanks were drained and the fish collected. The fish were preserved in 10% formalin and later transferred to 70% ethanol. The number of fish in each tank was counted and each fish was measured. In treatments containing adult MF and SM, we used only females because female MF are usually more aggressive than males, and sex ratios of poeciliids are often female-biased (Meffe 1985; Snelson 1989; personal observations). We used only females that had recently given birth to avoid reproduction, which would have increased the number of juveniles in the tank.

Mesocosm experiment 2

We conducted a second mesocosm experiment in July 1998 to relax the effects of predation and more fully address the effects of competition. This experiment was conducted in the same fashion as the previous experiment, with two exceptions (Table 1). First, based on results from experiment one, we eliminated the medium density of juvenile MF and increased the low density to 3 individuals. Second, size of the MF was manipulated (average adult size =17.8 mm, SE=0.18; average juvenile size =9.7 mm, SE=0.05) so that the juveniles were slightly larger than the adults could consume, based on average gape size (see below). This increased the probability that juveniles would survive until the end of the experiment, allowing comparisons of juvenile growth across all treatments.

Mesocosm experiment 3

In August 1998 we conducted a third tank experiment to test for the effects of adult interactions on age-structured predation. This experiment differed from the previous two in that various combinations of adult species were placed in tanks with juveniles (Table 1). The objective was to test for the effects of predation under conditions that are closer to those found in natural habitats (i.e., several species of adults interacting). We placed one or two male MF and SM per tank, whereas adult poeciliids in previous experiments were all female. The mean size of adult MF in this experiment was 28.5 mm (SE=0.52) and the mean size of juvenile MF was 9.2 mm (SE=0.06). Otherwise, this experiment was conducted similarly to the previous two.

Mesocosm experiment 4

The final tank experiment (conducted in July 1999) was designed to test for competition between juvenile SM and MF by decreasing the predation efficiency of adult MF. In the previous three experiments, cover for the juvenile fish consisted exclusively of the floating algal mat and associated dead macrophytes. In this experiment, artificial vegetation was added to the tanks to increase the amount of cover available to juvenile fish, and thus decrease the effectiveness of adult MF as predators. The artificial vegetation was constructed of strips of black polyethylene plastic tied onto a steel mesh frame. One mesh frame containing 120 plastic strips was placed on the bottom of each tank, resulting in 112 strips/m². The artificial vegetation strips floated in the water column and

reached the surface, thus simulating the abundant submerged vertical stems found in SRS (average stem density of spikerush, *Eleocharis* spp., =100.1 m⁻², SE=3.4, *n* =749, range 0–549 m⁻²). As in the previous experiments, a floating algal mat was added to the tanks covering approximately 12% of the surface area.

In this experiment, juvenile MF and SM were exposed to one of three density combinations: low MF/low SM, low MF/ high SM, and high MF/low SM (Table 1). The densities of fish in this experiment were the same as in previous experiments with the low density consisting of three individuals and high density consisting of 12 individuals. All juvenile density combinations were crossed with treatments containing 0 MF or 5 MF adults. Because we were unable to obtain large numbers of juvenile fish for this experiment, replication of treatment combinations was unequal. In addition, the starting date of our 9 replicates was staggered by 4 days because of problems collecting enough fish. The mean size of adult MF in this experiment was 24.0 mm (SE=0.51) and the mean size of juvenile fish was 8.4 mm (SE=0.07).

Gape limitation

We conducted a gape-limitation study during the summer of 1998 to determine the maximum-sized juvenile poeciliid fish that could be consumed by an adult MF. This experiment was important in determining the size of juvenile MF in experiment 2 that would not be consumed by adult MF placed in the tanks. Also, it permitted us to determine the amount of time (based on growth rates) that juvenile fish are vulnerable to predation by adult MF. We measured 9 male and 18 female MF to the nearest 0.5 mm standard length and placed them individually into 19-l aquaria. The adult fish were fed flake food and allowed to acclimatize to aquarium conditions for 4 days. After the acclimatization period, a single juvenile MF or SM was measured to the nearest 0.5 mm and placed in the tank with the adult. Each feeding trial was allowed to run for 5 min. If the adult fish consumed the juvenile within that time period, the trial was repeated 2 days later using a juvenile between 0.5 and 1.0 mm longer. These trials were repeated until the juvenile fish placed in the tank reached a size that could no longer be consumed by the adult. Thus, each observation was the maximum-sized juvenile consumed by a particular adult MF.

Statistical analyses

Survivorship of juvenile MF and SM was analyzed using a loglinear model for the analysis of categorical data. The survivorship data were binomially distributed and did not meet the assumptions of ANOVA for continuous variables. The loglinear models were decomposed to find which factors yielded the most parsimonious explanation of the data (Fienberg 1980). This analysis yields estimates of standardized deviates from the expectations when all treatments are equal, as a measure of the magnitude of treatment effects (Fienberg 1980).

Growth of juvenile MF and SM was analyzed in a two-factor analysis of variance and a Tukey multiple-comparison post-hoc test. Growth rate was estimated from the difference between the average size of fish placed in a tank at the beginning of the experiment and the average size of fish removed at the end of the experiment. Type III sums of squares were used to account for unequal replicates in experiment four. A Box-Cox test was performed to determine which transformations, if any, were needed to fulfill assumptions of normality and homoscedasticity for subsequent analyses (Sokal and Rolf 1981). We report the coefficients of determination (CD) for significant terms in the analyses, calculated by dividing the treatment sums of squares by the total sums of squares for the model. This indicates the minimum percentage of variation attributable to each factor in the model, and provides a measure of effect size (Richardson 1996).

Gut contents were analyzed to determine the relative consumption of each prey category identified. The percent dry masses of the gut contents of all fish in each tank were pooled and analyzed

using analysis of variance to determine if diets changed with our treatments. The angular transformation was used to fulfill the assumptions of ANOVA (Zar 1999) and the CD was reported for significant terms.

Results

Mesocosm experiment 1

The presence of adult MF had a strong negative effect on survivorship of both MF and SM juveniles (Fig. 2). Log-linear analysis revealed that treatment was a significant predictor of juvenile mortality for both species (MF: $\chi^2_1=15.43$, $P<0.001$; SM: $\chi^2_1=13.61$, $P<0.001$). Examination of the standardized deviates indicated that the adult MF treatment produced the greatest deviation from expected values of survival for both MF juveniles (mean deviate across juvenile densities: Control =1.02, MF =-3.40, SM =1.15, BFK =0.28) and SM juveniles (mean deviate: Control =0.82, MF =-3.29, SM =0.54, BFK =1.00). This indicated that adult MF were responsible for the high juvenile mortality in this experiment. Juvenile MF in high-density MF tanks had higher rates of mortality than juveniles in tanks with low densities (deviate in MF treatment: low density =-1.99, medium density =-3.43, High density =-4.77); every juvenile SM was consumed in the MF treatment tanks, no matter the density. The survivorship of juvenile MF in high-density tanks was 11.7% lower than in low-density tanks. However, the low and high density treatments remained distinct during the course of the experiment.

Intraspecific competition by juvenile MF limited their growth rates in our tanks. Analysis of growth indicated that an increasing density of juvenile MF had a significant negative effect on their growth (Fig. 3A; $F_{2,18}=13.32$, $P<0.001$, $CD=0.48$), but had little effect on juvenile SM growth ($F_{2,18}=1.91$, $P=0.177$). The presence of adult SM or BFK had no significant effect on growth of juvenile MF (Fig. 3B; $F_{2,18}=3.0$, $P=0.07$) but did have a significant effect on the growth of juvenile SM ($F_{2,18}=6.77$, $P=0.006$, $CD=0.35$). The growth of juvenile SM during the experiment did not differ significantly between the control (no adult) treatment and the adult SM treatment, but was approximately 52% faster in the treatment containing only BFK adults compared to the adult-SM and control treatments.

Stomachs of both adult and juvenile MF were found to contain invertebrates and algal material. Nearly 80% of juvenile MF ate invertebrates, with adult midges and cladocerans constituting the majority of the invertebrate diet, 40% and 11% by dry mass, respectively. No juvenile MF had empty stomachs. There were no significant differences in the percent composition of midges in the diet of juvenile MF across treatments ($F_{2,24}=0.705$, $P=0.504$). Juveniles did significantly increase consumption of cladocerans at higher densities ($F_{2,24}=5.310$, $P=0.012$, $CD=0.30$). Seventy-seven percent of adult MF had invertebrates in their stomachs, with adult midges and gastropods comprising the majority of their diet. No

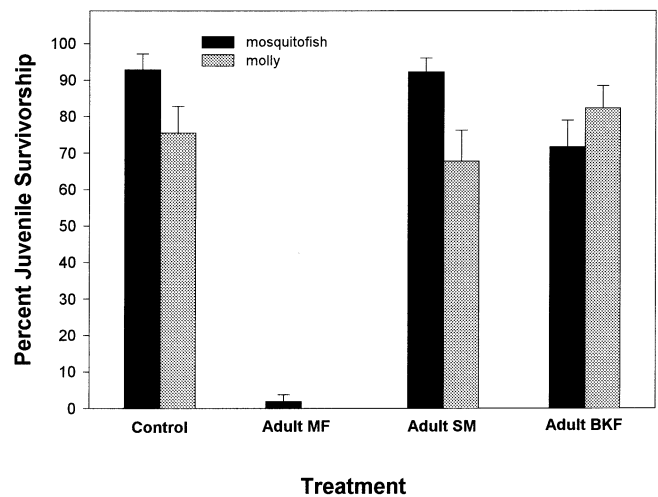


Fig. 2 Survivorship of juvenile mosquitofish (MF) and sailfin molly (SM) across treatments in experiment 1. Error bars indicate 1 SE

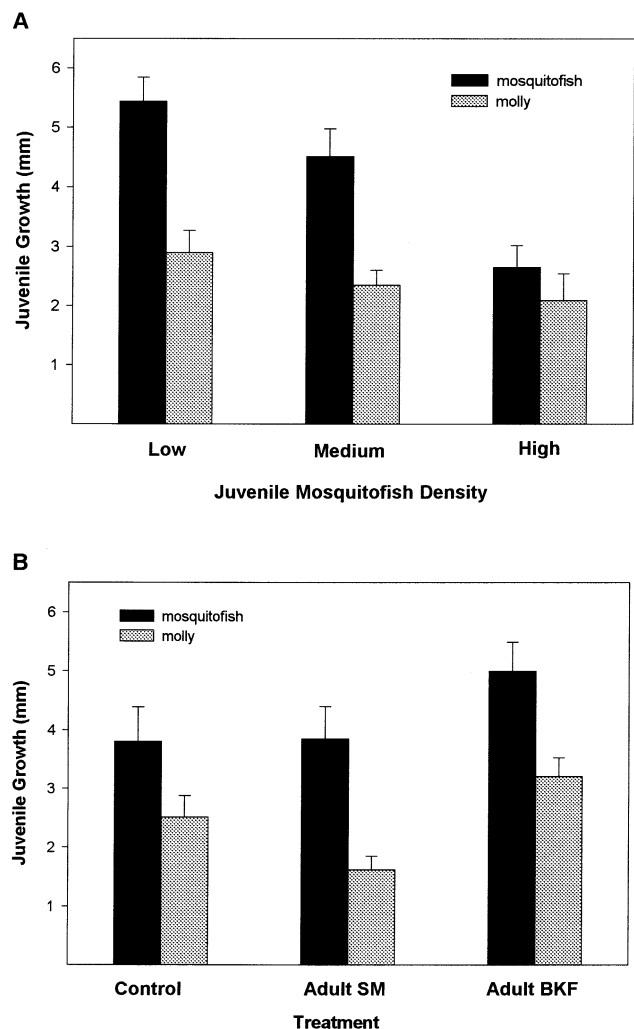


Fig. 3A, B Growth of juvenile MF and SM in tank experiment 1. A Estimates of growth of juvenile fish across the three densities of juvenile MF. B Estimates of growth of juvenile fish across three adult treatments. Error bars indicate 1 SE

adult MF had empty stomachs. Both juvenile and adult MF also fed on beetle larvae, eggs, mites, and algae, but in relatively low proportions. There were no significant differences in the diets of adult MF at any density of juvenile MF ($F_{1,6}=0.917$, $P=0.449$).

While BFK stomachs contained some algal material, they primarily contained a variety of small invertebrates. Relatively large invertebrate types, such as midges, were virtually absent from their diets. Cladocerans were the dominant food item, approximately 43% of prey by dry mass, with mites making up an additional 13% of the diet. Beetle larvae, eggs, gastropods, and algal material made up the remaining 44% of the stomach contents. Only one adult BFK was found to have an empty stomach. All adult and juvenile SM had full guts containing only algal material.

Mesocosm experiment 2

We used smaller adult MF (17.8 mm mean standard length, $SE=0.18$) in this experiment than in experiment 1 to minimize the predatory effect of adult MF on survivorship of juveniles. Loglinear analysis indicated that juvenile density did contribute significantly to mortality of juvenile MF ($\chi^2_{1}=168.20$, $P<0.001$). However, the actual differences in survivorship were small. The mean survivorship for juvenile MF in low-density tanks was 95.9% ($SE=2.0$), while the survivorship in the high-density tanks was 91.7% ($SE=2.8$). As in experiment one, the densities remained distinct.

As in the first experiment, the density treatment had a strongly negative effect on the growth of juvenile MF (Fig. 4A). Juvenile MF exhibited significantly less growth in the high-density treatments compared to the low-density treatments. Density explained 44% of the variation in growth (Table 2). Over the course of the experiment, the mean growth of juvenile MF was 5 mm (0.35 mm/day) in the low-density tanks, and 2.5 mm (0.179 mm/day) in the high-density tanks. The presence of adults did not significantly affect juvenile MF growth, though there was a trend of relatively slow growth by juvenile MF in tanks containing adult conspecifics (Fig. 4B). Contrary to juvenile MF, the presence of adult conspecifics significantly decreased juvenile SM growth (Table 2, Treatment effect). Juvenile SM exhibited the slowest growth in tanks containing adult SM, and the fastest growth in tanks with BFK (Fig. 4B). The density of juvenile MF did not significantly affect juvenile SM growth (Table 2).

The stomach contents of MF at the end of this experiment included a variety of invertebrates, suggesting that invertebrates were available to them over the entire duration of the experiment. Both adults and juveniles fed on algal and invertebrate material, though invertebrates were the dominant item. Sixty-seven percent of juvenile MF ate invertebrates, and the remaining fish had only algal material (31.2%) or empty guts (1.8%). Adult midges and cladocerans constituted the majority of the inverte-

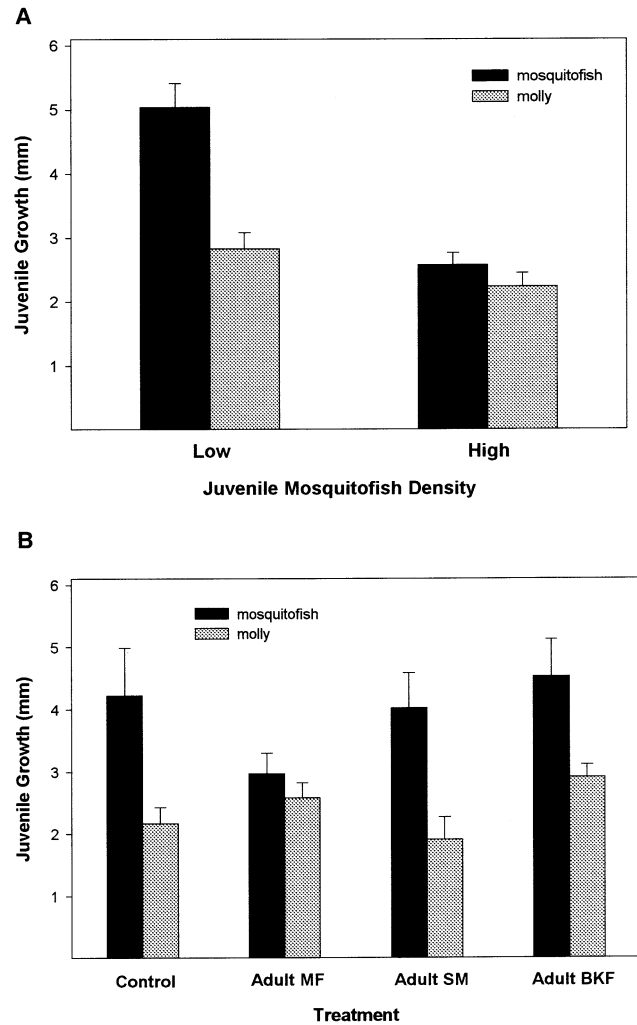


Fig. 4A, B Growth of juvenile MF and SM in tank experiment 2. **A** Estimates of growth of juvenile fish across the two densities of juvenile MF. **B** Estimates of growth of juvenile fish across all four adult treatments. Error bars indicate 1 SE

Table 2 Results of analysis of variance for growth of juvenile MF and SM in experiment 2

MF source	df	SS	MS	F	P	CD ^a
Treatment	3	10.859	3.620	2.496	0.084	
Density	1	39.316	39.316	27.116	0.0001	0.44
Den. × Treat.	3	2.907	0.969	0.668	0.580	
Error	24	34.798	1.450			
SM source						
Treatment	3	0.571	0.190	3.297	0.038	0.29
Density	1	0.087	0.087	1.506	0.232	
Den. × Treat.	3	0.065	0.022	0.372	0.774	
Error	24	1.386	0.058			

^a Coefficient of determination (see Materials and methods)

brate diet. Although the percent dry mass of midges in the diet of juvenile MF declined 62% from the high-density tanks to the low-density tanks, the difference was not significant ($F_{3,27}=1.303$, $P=0.29$). However, cladocerans in juvenile stomachs increased significantly from

17.7% in the low-density tanks to 50.2% in the high-density tanks ($F_{1,27}=8.51$, $P=0.007$, $CD=0.22$).

Eighty-seven percent of adult MF contained some invertebrate material in their stomachs, and the remaining 13% of adult fish fed only on algal material. Midge accounted for 44% of the invertebrate diet of adult MF from tanks containing low densities of juvenile MF, and 8.9% of the adult diet in tanks containing high densities of juvenile MF. Although there was a large decline in midge consumption by adult MF between low and high-density tanks, this difference was not significant ($F_{1,6}=2.11$, $P=0.20$). Similarly, the change in consumption of other invertebrates (primarily gastropods) did not differ significantly between low and high-density tanks ($F_{1,6}=2.59$, $P=0.16$). No adult fish had an empty stomach at the end of the experiment. All SM had full stomachs containing only algae.

Mesocosm experiment 3

Adult MF, either alone or in combination with other species, decreased survivorship of juvenile MF and SM by 60% or more relative to the control tanks ($\chi_1^2=8.35$, $P<0.0001$). Survival of juveniles was very high in the control tanks (MF 80%, SM 100%). As in the previous experiments, loglinear analysis indicated that density was a significant factor in predicting mortality in juvenile MF ($\chi_1^2=81.27$, $P<0.0001$). The mean survivorship for juvenile MF in low-density tanks was 27% ($SE=9.0$), while the survivorship in the high-density tanks was 24% ($SE=0.1$). Since the overall survivorship of juvenile MF was low in this experiment, we do not report growth or diet analyses of the small number of survivors. Also, the results were in agreement with experiments 1 and 2.

Mesocosm experiment 4

As in previous experiments, the survivorship of juvenile fishes was lower in tanks containing adult MF than in predation-control tanks lacking adult MF ($\chi_1^2=79.59$, $P<0.0001$). In the presence of adult MF, there was no significant difference in the mean survivorship of either species of juvenile fish (MF survivorship =45.3% and SM survivorship =34.5%; $F_{1,25}=1.42$, $P=0.244$). For juvenile MF, there was a significant treatment by density interaction effect on survivorship ($\chi_1^2=6.45$, $P=0.039$).

Also consistent with all previous experiments, there was a significant negative effect of density on the growth of juvenile MF (Table 3). Juvenile MF grew 4.55 mm ($SE=0.41$) in low-density tanks, but only 3.18 mm ($SE=0.47$) in high-density tanks. The growth rate of juvenile MF was relatively low in the presence of high densities of juvenile SM, but the effect was not significant (Table 3). SM did not display intraspecific competition; the density of juvenile SM had no significant effect on their growth (Table 3). The growth of juvenile SM was 3.59 mm ($SE=0.41$) in tanks with low densities of

Table 3 Analysis of variance table for growth of juvenile MF and SM in experiment 4

MF source	df	SS	MS	F	P	CD ^a
Treatment	1	0.088	0.088	0.559	0.463	
Density	2	1.215	0.608	3.863	0.038	0.28
Den. × Treat.	2	0.766	0.383	2.436	0.113	
Error	20					
SM source						
Treatment	1	0.0001	0.0001	0.002	0.963	
Density	2	0.628	0.314	2.321	0.127	
Den. × Treat.	2	0.136	0.068	0.501	0.614	
Error	18	2.435	0.135			

^a Coefficient of determination (see Materials and methods)

SM and 3.68 mm ($SE=0.44$) in tanks with high densities of SM.

Invertebrates and algal material were present in the guts of both juvenile and adult MF, with invertebrates predominating. Midges and cladocerans respectively made up 35.7% and 39.9% of the invertebrate dry mass in juvenile MF stomachs. There was no significant difference in the percent composition of invertebrates in juvenile MF guts among any treatments, and only one juvenile MF was found to have an empty stomach. Midges constituted 52.7% of the invertebrate dry mass in adult MF stomachs. All juvenile SM had full guts containing 100% algae.

Gape limitation

The gape limitation study revealed a linear relationship between MF body size and the size of the juvenile that could be consumed. The largest juvenile MF consumed was 14.5 mm by a 43.0 mm male MF (Fig. 5A). MF generally do not grow larger than about 26.0 mm standard length in SRS marshes, and this fish was taken from brood stock maintained in artificial pools. The smallest adults used in this portion of the study were 19.0 mm standard length, and the maximum size juvenile MF consumed by either male or female MF of this size was 7.5 mm. There was no difference in the ability of adult male or female MF to consume juveniles at this size class. In larger adults (>25 mm), females consumed larger juvenile fish than males, but this difference was not significant (size by sex interaction $F_{1,21}=0.069$, $P=0.79$). Field data from SRS indicated that approximately 27% of the MF population consists of adults greater than 20 mm SL, with an average size of 22.1 mm (Trexler, unpublished data). The results of the gape-limitation study indicate that juvenile fish are vulnerable to this average-sized adult until they reach a standard length between 8.5 and 9.0 mm. We found no significant difference in the ability of MF to consume either juvenile SM or MF (Fig. 5B; $F_{1,15}=3.55$, $P=0.078$). However, adult MF of a given size consistently consumed juvenile MF that were slightly longer than juvenile SM; the lack of

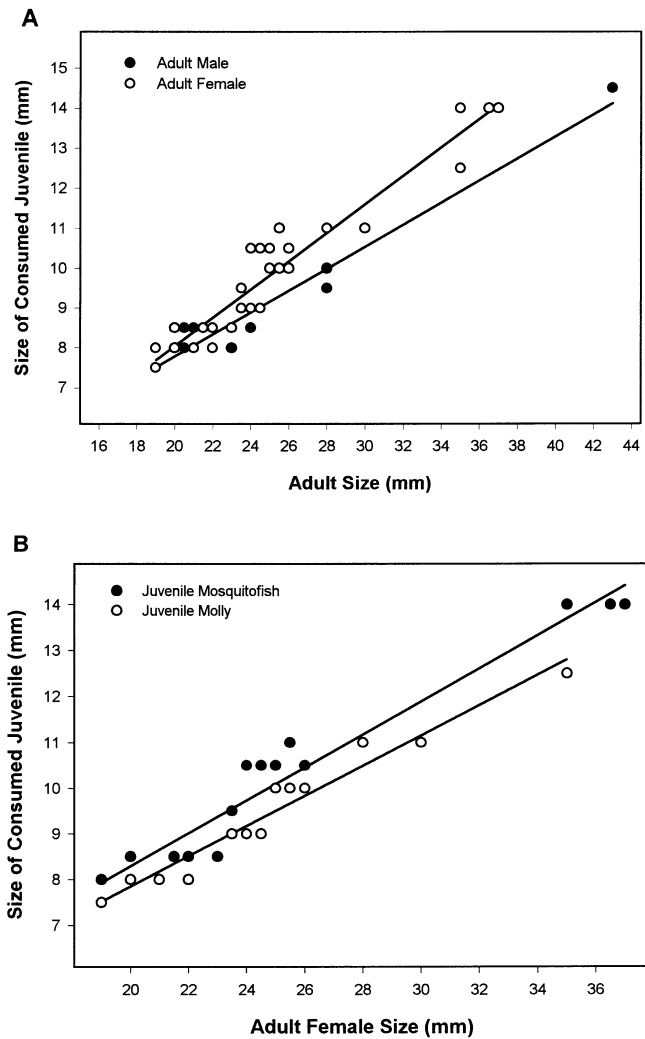


Fig. 5A, B Gape limitation results illustrating the relationship of the maximum size juvenile fish an adult MF of a given size can consume. **A** Maximum-sized juvenile fish that could be consumed by adult male and female MF. At larger sizes, females could consume larger juvenile fish than males of an equivalent size, although this difference was not significant. **B** Maximum-sized juvenile MF and SM that could be consumed by adult female MF. Adult females could consume juvenile MF that were slightly longer than juvenile SM but this difference was not significantly different

significance may have been a problem of low sample size.

Discussion

Of the 15 possible interactions examined in this study, only 7 were found to be present (Fig. 1B). Of these 7, only 3 could be described as strong interactions under our study conditions, based on the magnitude of effects documented. The strong interactions were predation by adult MF on both juvenile MF and SM, and intraspecific competition among juvenile MF. Weak interactions that we detected included intraspecific competition be-

tween adult and juvenile MF, and between adult and juvenile SM. Finally, adult BFK actually enhanced growth of both juvenile SM and MF, possibly as a result of nutrient dynamics in our tanks from increased fish biomass (Geddes 1999).

Some expected effects not observed surprised us by their absence, while others were not expected and their absence is trivial. For example, we expected neither predation among the juveniles, nor by SM adults which are known to be herbivores. More interesting were the absence of interspecific competitive effects by either adult or juvenile SM, and the presence of only weak intraspecific competition in this species between adults and juveniles. SM are generally much less abundant in the Everglades than MF or BFK. However, at nutrient-enriched locations they may reach densities up to 16.6m^{-2} , over 390% higher than the average density in SRS, and well above our experimental densities. Intraspecific competition for food may be present for SM under such circumstances. Adult BFK had minimal effects on any other species in our study, presumably because their diet of small crustaceans had minimal overlap with the other two species. Adult MF displayed no interspecific competitive effects in our tanks. We did not test for intraspecific competition in adult MF, SM, or BFK in these studies, but there is evidence for density-dependent limitation of adult MF growth across the range of densities employed in these experiments (Geddes 1999). Additional evidence of competition comes from our diet analyses. Juvenile MF included more cladocerans in their diets in the high density tanks than in the low density ones in experiments 1 and 2, and adult MF diets indicated a similar change in experiment 2 that was not significant.

Several studies have noted that MF will consume their own offspring (Nesbit and Meffe 1993), fueling speculation of interesting feedbacks in their own population dynamics. We noted near-zero survivorship of MF juveniles in experiments 1 and 3, and 45.3% in experiment 4 with MF adults present. Not surprisingly, increasing cover in experiment 4 increased the survivorship of juvenile MF. Perhaps more interesting was that juvenile SM had a 34.5% survivorship, indicating little, if any, avoidance of conspecifics by adult MF. The overall survivorship of juvenile fish in 0 MF adult treatments, including treatments containing SM and BFK adults, was greater than 90% in all of our experiments. Based on gut content analyses from wild-caught fish, Nesbit and Meffe (1993) suggested that cannibalism was uncommon in a population of *G. holbrooki* from South Carolina, and they proposed that previously reported cannibalism rates may be an artifact of confined laboratory conditions. However, gut-content data may under-represent cannibalism because small fish are digested more rapidly than other MF food items (Winkleman and Aho 1993). Our tanks had a capacity of 1,200 l, contained cover, had densities of fish comparable to those found in the field, and had alternative invertebrate prey available. Therefore, confinement effects and hunger probably had little

influence on the predation rates we observed. Experiment 4 demonstrated that greater habitat structural complexity in the field, compared to the experimental tanks, could reduce the overall predation rates below what we recorded.

Because MF are often the most abundant fish in Everglades marshes, their predation on juvenile fish arguably represents one of the strongest predatory interactions in this community. MF have demonstrated their potential to be important predators in other ecosystems by changing community composition after their introduction (Hurlbert and Mulla 1981; Meffe et al. 1983). Experimental studies have also indicated that MF can directly decrease their prey's abundance, or even drive them locally extinct (Hurlbert et al. 1972; Bence and Murdoch 1986; Belk and Lydeard 1994; Schaefer et al. 1994; Harris 1995; Goodsell and Kats 1999). MF appear to be opportunistic predators of fish juveniles or larvae in the Everglades. They congregate around the nests of substrate-spawning fish and consume eggs and larvae (X. Pagan and W. Loftus, unpublished data), though randomly collected individuals rarely have fish larvae in their guts (A. Jelenszky and J. Trexler, unpublished data). Because MF often comprise over 30% of the fish in Everglades marshes, even a relatively low predation rate on juvenile fish could have important effects on the fish community (Meffe 1985).

The results from our experiments suggest that competition for food resources may have important implications for MF populations, but less so for SM or BFK at the densities we studied. The growth rates of juvenile MF in the low-density tanks was 0.35 mm/day, while the growth rate of juvenile MF in high-density tanks was almost 50% lower (0.18 mm/day). Juvenile fish become too large for the average size adult MF to consume at approximately 8.5–9.0 mm standard length. The growth rate in the high-density treatment required a period of 8–11 days for juvenile MF to reach 9 mm, in contrast to the 5–7 day period in the low-density tanks. Haake and Dean (1983) estimated growth rates of MF in SRS from otoliths, and found that they grew an average of 0.20 mm/day during the first 14 days of life. Juvenile MF reared in enclosures in the field exhibited growth rates between 0.12 and 0.24 mm/day, with an average of 0.14 mm/day (Loftus, unpublished data). These data from fish in the field demonstrate that the growth of MF in our high-density treatment was closest to growth rates under natural conditions. This suggests that juvenile MF in the low-density tanks underwent competitive release and were able to increase their rate of growth, compared to field conditions. The importance of food limitation for juvenile fishes probably lies in the ability of juveniles to grow out of the size at which they are most vulnerable to predation. Thus competition and predation appear to have strongly interactive effects for MF.

Juvenile MF and SM exhibited the greatest growth rates in tanks containing adult BFK (experiments 1 and 2), indicating that adult BFK actually had a positive effect on juvenile fish growth. The reason for this en-

hanced growth is unclear, especially for juvenile MF that have similar dietary preferences to adult BFK. One explanation may be diet separation. The gut contents analysis showed that the highest percentage of midges were found in the diet of juvenile MF in tanks containing adult BFK, where adult BFK stomachs contained large numbers of small aquatic invertebrates such as cladocerans and mites. Adult BFK preying heavily on smaller aquatic invertebrates such as cladocerans may force juvenile MF to consume larger prey items such as midges. The growth of juvenile SM may have been enhanced by increased nutrient cycling by adult BFK (Vanni 1997), leading to increased algal food resources for the juvenile SM. Tanks containing adult SM also may have had increased nutrient recycling (compared to tanks with no adult fish) but adult SM were also grazing on algae, lowering food availability for juveniles.

Though we only examined 3 fish species from a community of over 30 (Loftus and Kushlan 1987), our efforts revealed a diversity of age-structured interactions. Further untangling of the web of interactions in this community would require consideration of a discouraging array of potential regulatory paths. Multivariate statistical techniques summarizing community changes over environmental perturbations may be the only practical way to further characterize the complexity of field communities. This study suggests that fish species should not be considered as single variables in those analyses. Age-structured interactions should be included to capture their role in community dynamics (Polis et al. 1989; Wilbur 1997), possibly by categorizing species into at least two size classes based on predation vulnerability. Among other benefits, this would permit inclusion of feedback relationships in MF, including cannibalism. Our mesocosm studies suggest that intraspecific competitive relationships may be important in the Everglades, at least for some species. Resource levels may be relatively low in this oligotrophic ecosystem (Turner et al. 1999), possibly yielding food limitation for the fishes at lower densities than in other ecosystems with the same species.

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