PRELIMINARY DATA ON USE OF THE INLAND SILVERSIDE, MENIDIA BERYLLINA, TO CONTROL MOSQUITO LARVAE^{1, 2}

D. P. MIDDAUGH, 3 P. G. HESTER, 4 M. V. MEISCH 5 AND P. M. STARK 5

ABSTRACT. A study of procedures for spawning and culture of the inland silverside, Menidia beryllina, was conducted. The efficacy of young Menidia, 20 to 22 and 31 to 33 days old, to control mosquito larvae was determined in the laboratory with first and second larval instars of the saltmarsh mosquito Aedes taeniorhynchus. Feeding trials were run at salinities of 1, 5, 15 and 25%. Field trials were also conducted to determine if Menidia would effectively control Culex quinquefasciatus in brackish water impoundments.

INTRODUCTION

Many species of fishes have been utilized in efforts to control mosquito larvae in aquatic environments. Since the early 1900's, use of the mosquitofish, Gambusia affinis (Baird and Girard), has been promoted as a measure for control of several different species of mosquitoes which breed in a variety of aquatic habitats (Hildebrand 1919, Howard 1925, Krumholz 1948, Hoy et al. 1971). However, the introduction of Gambusia, as well as other species of fishes, has met with varying degrees of success because of habitat incompatibility, the distribution of mosquito larvae within habitats, and the feeding habits of the introduced fishes (Hoy et al. 1972, Bence 1982, Haas and Pal 1984).

In the United States, ephemeral brackish water habitats can produce large numbers of pestiferous and disease vector mosquitoes (Harrington and Harrington 1961). Rapid fluctuations in water temperature, salinity and dissolved oxygen limit the number of species of fishes that are able to live and thrive in these habitats.

The inland silverside, *Menidia beryllina* (Cope), is a ubiquitous resident of estuaries from Massachusetts to Texas. It is euryhaline, living in freshwater and at salinities greater

than 35 % (Hubbs et al. 1971, Echelle and Mosier 1982). It is also eurythermal, reproduction occurs at temperatures from approximately 15 to 30°C (Hubbs et al. 1971, Hubbs 1982). In addition to estuaries, the inland silverside is found in numerous freshwater lakes and reservoirs (Cook and Moore 1970, Moyle et al. 1974, Johnson 1975, Chernoff et al. 1981). Our study was designed to determine the potential of this fish for control of mosquito larvae in laboratory feeding trials conducted at several different salinities and in brackish water impoundments under seminatural conditions in the field.

MATERIALS AND METHODS

Brood Stocks. Adult inland silversides, M. beryllina, were collected on April 16 and 17, 1984 approximately 2 km south of the Interstate 10 bridge at Bay Point in Blackwater Bay (Escambia Co., Florida). The salinity, measured with a refractometer, ranged from 1 to 3 % o/oo and temperature was 18.5 to 18.7 °C. Collections were made with a 10 m \times 1 m \times 5 mm mesh bag seine. Menidia beryllina were carefully transferred (by hand) from the bag portion of the seine (which was not beached) to buckets of water and then to a 500 liter aerated holding tank. On each collection day, approximately 150 fish were transported to the laboratory.

Fifty individuals (sex ratio 1:1) were placed in each of two 1.3 m diameter brood tanks in a recirculating spawning system. During spring and early summer the sex of individuals is easily determined by exerting gentle pressure on the abdomen. This pressure results in extrusion of a few eggs or sperm. A detailed description of the spawning system and operational parameters for Menidia was provided by Middaugh and Hemmer (1984). In brief, 5 % oo salinity water was pumped from a reservoir-filter system through each brood tank, producing a mean surface current velocity of 8 cm/sec. A gravity flow drain returned water to the reservoir via a filter unit containing layers (surface to bottom) of aquarium filter fiber, activated charcoal, crushed oyster shell and coarse pea gravel. Cool

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³ U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, FL 32561.

⁴ Department of Health and Rehabilitative Services, West Florida Arthropod Research Laboratory, P. O. Box 15277, Panama City, FL 32406.

⁵ Department of Entomology, University of Arkansas, Fayetteville, AR 72160. Published with the approval of the Director, Arkansas Agricultural Experiment Station.

white fluorescent lamps, used for all laboratory tests, provided 175–200 lux light intensity. The photoperiod was 13L:11D. Brood tanks were shielded from outside disturbances with light-tight curtains.

Adults in each brood tank were fed Tetramin® SM80 flake food four times daily. Each tank received 8 g of food at 0800, 4 g at 1100, 4 g at 1400 and 8 g at 1600 hr. On weekends, a single 8 to 12 g daily feeding was provided, generally in mid-morning. Brood tanks were syphoned clean of excess food, and the aquarium filter fiber and activated charcoal changed weekly. Water temperature ranged from 23 to 25°C. Approximately 5% of the water in the system was replaced with salinity adjusted water (5 % of oo) each week.

A spawning substrate made of polyester aquarium filter fiber, 15 cm long × 10 cm wide × 10 cm thick was suspended just below the surface of the water and in contact with the side of each brood tank. Substrates were removed from the brood tanks and checked for eggs between 0800 and 1200 hr daily. A new spawning substrate was then suspended in each tank for subsequent removal the following day. Daily egg production in each brood tank was determined for 13 days by removing spawning substrates and counting the total number of eggs present.

CULTURE OF YOUNG Menidia. Developing eggs were removed from the surface of spawning substrates. No effort was made to tease individual eggs from the substrate, rather, concentrations of eggs and accompanying substrate were removed from the main body of the spawning substrate with forceps. To determine the percentage hatch, a subsample consisting of 6 replicates of 30 newly fertilized (<8 hr old) eggs was maintained in covered 100 ml glass beakers with 40 ml of 5 % oo salinity water at 25±1°C. The remaining eggs and attached polyester substrate were placed in an 8 liter wide-mouth glass jar containing 6 liters of salt water at 5 %oo salinity and 25±1°C under a 13L:11D photoperiod, light intensity 2400 lux. An airstone at the bottom of the vessel provided gentle aeration. Air was trapped in the polyester spawning substrate which then floated at the water's surface.

Eggs incubated in the 8 liter wide-mouth glass jar hatched 6 to 8 days after fertilization. Larval *Menidia* were fed the mixohaline rotifer, *Brachionus plicatilis* (0. F. Mullër), *ad libitum* for 4 days. Rotifers were cultured using the procedures of Theilacker and McMaster (1971).

On the fourth day after hatching, 500 young fish were counted and poured into an 80 liter glass aquarium containing salt water at 5 % and 25 ± 1 %. They were fed newly hatched <12

hr old Artemia nauplii at the rate of 5000 nauplii/liter of salt water, twice daily. A Dyna Flo® filter system was run for 2 hrs, twice weekly, to help maintain water quality. Plankton netting (mesh size 400 µm) was attached to the filter intake siphons to keep young fish from passing into the filter. This netting had to be cleaned frequently to ensure proper water flow. Dead Artemia nauplii were syphoned from the tank as necessary. A group of 500 young Menidia was easily maintained in the 80 liter aquarium up to an age of 30 days.

SALINITY TOLERANCE, GROWTH AND FEEDING TRIALS. Laboratory experiments were conducted to determine the ability of young *Menidia* to tolerate rapid changes in ambient salinity. We also monitored growth and the feeding efficacy of young fish presented first and second instars of larval *Aedes taeniorhynchus* (Wiedemann).

In salinity tolerance tests, 50 8-day-old fish were transferred from the 80 liter holding aquarium, 5 % ooo salinity, and water temperature 25±1°C, to 8 liter wide-mouth jars containing 7 liters of water at the following salinities: 1, 5, 15, 25 and 35 % o. Transfers were made using a 3 mm I.D. × 40 cm glass tube equipped with a rubber squeeze bulb. When young Menidia were transferred with a dip net, there was high mortality. Transfers to respective salinities were instantaneous with no acclimation period. Water temperature was maintained at 26±1°C. Fish in each container were fed live Artemia nauplii (washed with fresh water) at the rate of 5000 nauplii/liter of water each morning (0800-0900 hr) and afternoon (1500-1600 hr).

Survival was monitored daily. A subsample of 10 fish was taken from the 80 liter aquarium at the time of transfer to different salinities and sacrificed to establish an initial size. Additional subsamples of 5 fish were taken from each salinity on the first day of each three day feeding trial with mosquito larvae (i.e., when fish were 20 and 31 days old). Fish were preserved in 4% neutral buffered formalin and subsequently weighed and measured.

Feeding trials were initiated with fish reared in the different salinities when individuals were 20 and 31 days old. Two fish from each salinity regime were placed in a glass battery jar containing 4 liters of water adjusted for salinity and temperature to match holding aquaria. The photoperiod was 13L:11D, light intensity was 2400 lux.

Trials with young Menidia and Ae. taeniorhynchus were of 3 days duration. We increased numbers of first and/or second instar mosquito larvae on successive days; counting and removing any residual larvae before add-

ing the next higher number. Consumption rates of mosquito larvae by *Menidia* at each salinity were computed as the average number consumed/fish/day. After each 3-day feeding trial, fish were returned to their respective 8 liter wide-mouth jars. All fish cultured at the respective salinities of 1, 5, 15 and 25 % owere sacrificed when 34 days old and preserved in 4% neutral buffered formalin for final growth measurements.

Larval Ae. taeniorhynchus used in feeding trials were hatched from eggs hydrated in 1 to 2 liters of 5 % oos salinity water at 27°C. Hatched mosquito larvae were fed a 3:2 mixture of desiccated liver and yeast.

Brackish water impoundments. Field tests were conducted at the West Florida Arthropod Research Laboratory (WFARL), Panama City, Florida to determine the potential of *Menidia* as a biological control agent against larval populations of *Culex quinquefasciatus* Say under seminatural conditions. Tests were designed to evaluate the ability of the fish to feed in vegetated and open water areas as well as to determine the number of fish required to obtain acceptable control.

Tests were conducted in concrete plots 3 m² and 0.6 m deep. The bottom of each plot was covered with soil to a depth of 0.15 m and flooded with water to a depth of 0.30 m above the soil. Each plot was equipped with automatic float valves and overflow stand pipes for control of the water level. Bay water from the adjacent Robinson Bayou was pumped into the plots and the salinity adjusted to 5 % os even days prior to the addition of mosquito egg rafts. All plots were protected with removable wooden frames covered with Fiberglas screen to reduce the entry of predacious aquatic insects.

The field test, initiated on August 1, 1984 utilized young Menidia. Fish in each test were measured for total length (TL) and converted to standard length (SL) by dividing the TL by 1.2 to provide continuity in the data (Hubbs and Dean 1979). A cohort of 5 fish, selected to include minimum and maximum sizes, contained individuals ranging from 23 to 28 mm SL and 140 to 210 mg wet weight. Two test plots were stocked with 15 fish each and a third plot served as a control. One plot contained heavy emergent vegetation, Panicum repens, and one no vegetation to compare larval control under the two extreme conditions. The daily addition of 50 mosquito egg rafts (producing approximately 5,000 Cx. quinquefasciatus larvae) began 11 days prior to stocking with fish and continued until August 31. Fifty ml of a 3:2 mixture of desiccated liver and yeast were added to each plot on July 24 and 27, and August 15 to ensure that adequate food was available to sus-

tain the larval mosquito population. The 5 week test, was terminated on September 7, 1984.

The efficacy of *Menidia* in controlling mosquito larvae was evaluated by taking dipper samples (450 ml) at 10 predetermined stations in each plot. The larval population in both the control and *Menidia* plots were sampled daily Monday through Friday of each week during the test.

The evaluation method was not used to extrapolate to the actual population of mosquito larvae but as an indication of larval density. Factors possibly affecting the sampling technique were overcast, time of day and wind velocity. Criteria for acceptable mosquito control were based on less than 10 larvae dipped per plot that consisted primarily of the first and second instars. Water temperature was monitored in one plot with a constant temperature recording device. Other water quality variables monitored included salinity, pH and dissolved oxygen (DO).

Statistical analyses (SAS Institute Inc. 1982) were conducted where appropriate. These analyses are identified and reported with the results.

RESULTS

LABORATORY SPAWNING AND CULTURE. Brood stock *Menidia beryllina* spawned each day during a 6-week observation period in this study. The daily yield of eggs during 13 days when counts were made ranged from 659 to 4,649, $\bar{x} = 2.316$.

The percentage hatch in six groups of eggs incubated at 5 % on and 25 ± 1 °C ranged from 77 to 97%, $\bar{x}=89$ %. During days 4 to 8 of life and prior to instantaneous transfer to lower or higher salinities, *Menidia* survival in the 80 liter holding aquarium was 85%.

SALINITY TOLERANCE AND GROWTH. Survival patterns for young *Menidia* (8 through 34 days old) at salinities from 1 to 35 % oo are shown in Fig. 1, along with the time of scheduled feeding trials with *Ae. taeniorhynchus*. Fish were able to tolerate instantaneous changes in salinity when transferred from 5 % ot 1, 5, 15 or 25 % oo. However, none lived for more than 3 days at 35 % os salinity.

Growth patterns of young *Menidia* followed the general trend of survival at respective salinities. Young fish transferred from 5 % of the same salinity at 8 days of age and monitored through 34 days old showed the greatest increase in SL and weight (Table 1). The SL and wet weight of 34 day-old *Menidia* cultured at 4 salinities were significantly different (ANOVA, P < 0.001). Tukey's post-hoc analyses ($\alpha = 0.05$) revealed that SL and wet weight of

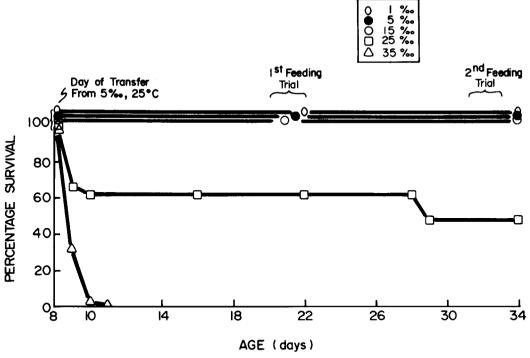


Fig. 1. Survival of 8-day old *Menidia beryllina* instantaneously transferred from 5 % o/oo at 25±1% to different salinities. Intervals when feeding trials were conducted with *Aedes taeniorhynchus* are also shown.

Menidia cultured at 5 % osalinity was significantly greater than in fish reared at 1, 15 and 25 % o. While there was no difference in the SL of 34 day-old fish maintained at 1, 15 and 25 % osalinity, the wet weight of individuals cultured at 1 and 15 % owas significantly greater than for fish at 25 % oo.

LABORATORY FEEDING TRIALS. Menidia readily preyed upon Ae. taeniorhynchus larvae at

salinities ranging from 1 to 25 % o/oo. The first feeding trial was initiated with 20 day-old fish (Fig. 2A), the second with 31 day-old individuals (Fig. 2B). Each trial lasted 3 days. Feeding was lowest at each salinity on day 1 but almost all Ae. taeniorhynchus larvae were consumed on days 2 and 3, indicating that Menidia were not satiated. Moreover, a one-way analysis of variance (ANOVA) of arc-sine transformed con-

Table 1. Mean standard length (SL) in millimeters and wet weight (WT) in milligrams of inland silversides, Menidia beryllina, used in feeding trials with saltmarsh mosquitoes Aedes taeniorhynchus. A subsample of 10 fish was weighed and measured on day 8. Subsequent samples of 5 fish were taken on days 20 and 31. The final samples taken on day 34 were comprised of 10 fish from each salinity.

	Salinity ⁰ /oo											
		1			5			15			25	
Age and event	N	SL	WT	N	SL	WT	\overline{N}	SL	WT	\overline{N}	SL	WT
8 days old—transferred from 5°/00, 25°C to												
respective salinities	10	7.3	4	_	7.3	4	_	7.3	4		7.3	4
20 days old—1st feeding trial	5	12.4	14	5	13.6	22	5	12.8	17	5	12.6	16
31 days old—2nd feeding trial	5	17.3	49	5	16.6	44	5	16.4	42	5	15.7	36
34 days old—study terminated	10	17.9	58	10	19.2	72	10	17.9	57	10	16.9	39
$\bar{\mathbf{x}}$ gain in size												
8 days old—34 days old		10.6	54		11.9	68		10.6	53		9.6	35
x % increase		145	1350		163	1700		145	1325		132	875

sumption data demonstrated that there was no significant difference in the number of mosquito larvae consumed at each salinity in the respective feeding trials with 20-22 day-old fish (P = 0.57) and 31-33 day-old individuals (P = 0.11).

Brackish water impoundments. Test plots contained Cx. quinquefasciatus in all developmental stages with pretreatment counts exceeding 100 larvae dipped/plot (Fig. 3). Following addition of Menidia on August 1, the larval population of mosquitoes decreased by the 6th day in the open water plot and by the 8th day in the vegetated plot. Dipper counts indicate that acceptable control was achieved in both treated plots 12 days after fish were introduced. During the 5 week study, the test plots yielded significantly different numbers of mosquito larvae (ANOVA, P < 0.001). Tukey's post-hoc test ($\alpha = 0.05$) revealed significantly greater numbers of mosquito larvae in the control plot, with no difference in the number of larvae found in the open and vegetated plots.

Recently hatched *Menidia* were observed in both plots at the termination of the test. Although it was difficult to determine when reproduction first occurred, it seems unlikely that

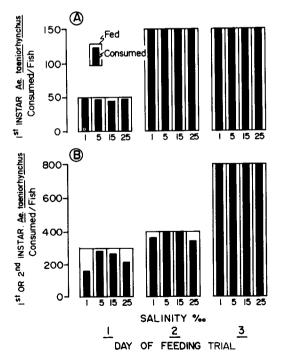


Fig. 2 (A) Results of the three day feeding trial conducted at salinities of 1, 5, 15 and 25 % owith Aedes taeniorhynchus and 20–22 day-old Menidia, (B) results of the three day feeding trial with 31–33 day-old fish.

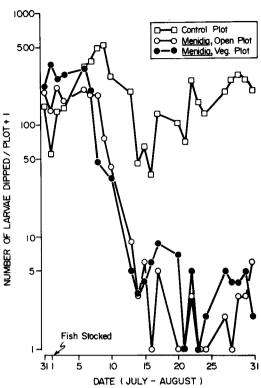


Fig. 3. Effectiveness of *Menidia beryllina* for control of *Culex quinquefasciatus* larvae in open and vegetated brackish water plots.

it took place prior to the 12th day posttreatment. Survival and growth data for *Menidia* recovered from the brackish water test-plots are summarized in Table 2.

Water quality data recorded during the test showed an average temperature of 29°C with lowest temperatures occurring at the beginning and end of the test period. Salinity in all plots was 5 % oo. The pH at the beginning of the test was 7.2, 7.4 and 9.4, respectively, for the control, open and vegetated plots. Minimal DO values of 1.7 and 2.6 mg/liter, measured in the vegetated plot, indicate that *Menidia* are able to tolerate low oxygen conditions.

DISCUSSION

Our study and earlier research (Middaugh et al. 1985) has demonstrated that long term maintenance and repeated spawning of brood stock *Menidia beryllina* in the laboratory is feasible. Moreover, young fish from a single parental stock can be cultured under environmental conditions suitable for subsequent stocking in habitats with a wide range of salinities. Our laboratory data on the frequency of reproduc-

Table 2. Summary data for adult Menidia beryllina stocked in open water and vegetated plots at the West Florida Arthropod Research Laboratory, Panama City, Florida.

Event and	Plot					
variable	Open water	Vegetated				
No. stocked						
Aug. 1, 1984*	15	15				
No. recovered on						
Sept. 7, 1984	12**	13***				
% recovered	80	87				
Size-stocked on						
Aug. 1, 1984						
\overline{x} Std. length (mm)	25.2	25.2				
\overline{x} Wet weight (mg)	160.0	160.0				
Size-recovered on						
Sept. 7, 1984						
\overline{x} Std. length (mm)	42.3	39.2				
x Wet weight (mg)	830.0	573.0				
% Increase in size		0,000				
\overline{x} Std. length (mm)	68	56				
\overline{x} Wet weight (mg)	319	256				

* A random sample (N = 5) from pond-reared fish was taken for initial growth measurements.

** All fish recovered ($\tilde{N} = 12$) were used for final growth determination.

*** Fish sample limited for growth determination (N=3) due to fire ant mutilation prior to recovery from vegetated area.

tion in Menidia beryllina agree with observations from nature. Hubbs (1976) reported that nearly all females in Lake Texoma produce egg clutches daily during June and July.

The daily number of eggs produced in brood tanks ($\bar{x}=2,316$) is below the expected production for 25 feral females. This number of females would be expected to produce approximately 500 eggs/female/day or 12,500 eggs/brood tank/day if the observations of Hubbs et al. (1971) on spawning frequency and fecundity are correct. We suspect that limited food supplies and the carrying capacity of our brood system may have inhibited egg production.

The appetite of the relatively small Menidia for mosquito larvae can be attributed to their feeding behavior in nature. In Clear Lake, California, Menidia feed primarily during daylight hours and apparently have a stomach retention time of about 1 hour (Elston and Bachen 1976). Therefore, 12 to 14 daily feeding sessions would be expected (Hubbs 1982). Menidia is carnivorous, consuming cladocerans, dipterans and copepods (Saunders 1959, Elston

and Bachen 1976, Lucas 1982). Considering that females in Lake Texoma reproduce daily. with individuals generating approximately 90 clutches of eggs between April 1 and July 1, it is not surprising that they have a voracious appetite. This reproductive effort constitutes about a 7.5% daily loss of each female's biomass which must be rapidly replenished in order to maintain day-to-day egg production (Hubbs 1982). The euryhaline and eurythermal nature of Menidia beryllina (Hubbs et al. 1971, Hubbs 1982, Middaugh et al. 1985) indicate that it should live and thrive in a diverse set of habitats. Indeed, it is found in freshwater lakes and reservoirs in Florida, Mississippi, Louisiana, Texas, Arkansas, Oklahoma, Tennessee and California (Mense 1967, Johnson 1975, Chernoff et al. 1981. Echelle and Mosier 1982). It is also resident in estuaries from Massachusetts to Texas (Robbins 19697, Johnson 1975).

In Texas, it has been collected from numerous coastal sites at salinities of 0 to 35 % (Gunter 1945, Echelle and Mosier 1982). Simmons (1957) found *Menidia beryllina* in the Upper Laguna Madre, Texas at a salinity of 75 % and reported it as abundant at 45 % (1906).

Laboratory salinity tolerance tests and field experiments conducted with *Menidia* in small brackish water plots indicate that this fish could be a useful biological control agent of mosquitoes that breed in coastal areas where salinities range for 0 to 25 % oo. Control was obtained in experimental plots with an initial stocking rate of 15 fish per plot even though approximately 5,000 *Cx. quinquefasciatus* eggs (larvae) were added daily. The rapid growth rate of *Menidia* in these plots may be attributed to the large number of mosquito larvae available as food.

Growth of *Menidia* to sexual maturity in brackish water plots was unexpected. It is possible that rapid attainment of sexual maturity and the very high reproductive potential of males and females could yield a successful strategy for control of mosquitoes in brackish water habitats. The introduction only a limited number of sexually mature adults and subsequent rapid production of large numbers of carnivorous offspring should help to ensure adequate control of mosquito larvae. Hubbs (1982) calculated that each female in Lake Texoma, Oklahoma produced approximately 835 eggs daily during the 3- to 4-month breeding season. Thus, the reproductive potential of

⁶ Saunders, R. P. 1959. A study of the food of the Mississippi silverside, *Menidia audens* (Hay), in Lake Texoma. M.S. Thesis, University of Oklahoma. 43 pp.

⁷ Robbins, T. W. 1969. A systematic study of the silversides *Membras* Bonaparte and *Menidia* (Linnaeus) (Atherinidae, Teleostei). Ph.D. Dissertation. Cornell University, Ithaca, New York. 282 pp.

Menidia far exceeds that of Gambusia. Assuming production of 104 Gambusia fry (Hoy and Reed 1970) on each of 6 occasions at 24 day intervals (Krumholz 1948), a single female would have a maximum reproductive potential of approximately 624 young during a 144 day period. In contrast, Menidia beryllina, conservatively producing 500 eggs daily for 90 days would possess the potential for production of approximately 45,000 young during a mosquito breeding season.

Besides showing promise as a control measure for mosquito larvae, growth rates seen in brackish water habitats and a very high reproductive capacity suggest that *Menidia beryllina* has potential as a forage fish for larger carnivores.

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