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ECOLOGY OF *MENIDIA MENIDIA* LARVAE IN TWO TEMPERATE ESTUARINE LITTORAL HABITATS

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ECOLOGY OF MENIDIA MENIDIA LARVAE IN TWO TEMPERATE

ESTUARINE LITTORAL HABITATS

BY

MIRANDA LOPEZ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

MASTER OF SCIENCE THESIS

OF

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UNIVERSITY OF RHODE ISLAND 2013

ABSTRACT

The Atlantic silverside (Menidia menidia) is one of the numerically most abundant fish species in estuaries along the East Coast of North America, but its ecology during the first two weeks post-hatch has not been described. Therefore ecological investigations into appropriate sampling methods, preferred habitats, feeding ecology and growth of these larvae will contribute valuable information to our knowledge base for this species. The upper reaches of two Rhode Island, USA, estuaries, with differing levels of anthropogenic inputs, were the study sites for this project. Previous studies have shown that during early spring *M. menidia* adults ripen for spawning by feeding exclusively on zooplankton. The zooplankton community in Upper Point Judith Pond (UPJP) is dominated by polychaete larvae, indicating a eutrophic environment, whereas the Upper Pettaquamscutt River (UPR) is dominated by crustaceans, indicating a relatively pristine environment. To assess and describe the habitat ecology of *M. menidia* larvae during their first two weeks of life in the littoral zone, four goals were set: (1) determine depth distribution of *M. menidia* larvae from both estuaries: (2) assess abundance and distribution of *M. menidia* larvae between estuaries; (3) compare feeding habits of the larvae in the two estuaries through gut content analysis; (4) compare growth of larvae in the two estuaries via age-length relationships based on otolith analysis. Of the four sampling devices used to collect larvae, the circular quadrat, which sampled the land-water interface, the aquarium net, which sampled water from 0.3 - 0.4 m depth, and the small plankton net, which sampled water from 0.4 - 0.5 m depth collected many larvae. A large plankton net, which sampled water > 1 m depth, did not. This indicates that M.

menidia larvae can be found from the shoreline interface to 0.5 m depth. Analysis of collection data indicated a zero-inflated Poisson distribution, suggesting a patchy distribution of larvae in the field. Gut content of larvae between estuaries differed markedly, with 76.2% of the larval diet at UPR consisting of copepod eggs and 72.5% of that at UPJP consisting of copepod nauplii. The slopes of the age-length regressions of the larvae between estuaries were not significantly different, indicating that growth rates did not differ. These results provide new information on the feeding habits, growth, and distribution of *M. menidia* during its first two weeks of life in the field.

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PREFACE

This thesis is being submitted in manuscript format. There is one chapter for this thesis and two appendices. The title of the manuscript is, "Ecology of *Menidia menidia* larvae in two temperate estuarine littoral habitats". This manuscript will be submitted to *Estuaries & Coasts*, with co-authors Gavino Puggioni and David Bengtson.

Appendix I describes a laboratory experiment designed to determine if size classes of *Menidia menidia* larvae have a depth preference when residing in the littoral zone. Appendix II lists the catch data for *Menidia menidia* larvae collected from the Upper Pettaquamscutt River and Upper Point Judith Pond.

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MANUSCRIPT - 1

Ecology of Menidia menidia larvae in two temperate estuarine littoral habitats

Prepared for submission to Estuaries & Coasts

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INTRODUCTION

Estuaries are physically complex habitats due to influences from the ocean and freshwater drainage. Simultaneously, they serve as nursery habitats for a critical phase of the life cycle of numerous species of fish. To provide a favorable habitat for larval and juvenile development, many adult fish spawn near coastal habitats (i.e. tidal inlets, bays, passes, and estuaries) (Patillo et al. 1997). Other adult fish species migrate from the open ocean to estuaries to spawn. Some species of adult fish spawn in structured habitat so that the risk of predation is reduced and food availability for larvae is high (Beck et al. 2001; Boesch and Turner 1984; Heck and Thoman 1981; Rooker and Holt 1997; Weinstein 1979). Such habitats include shallow, near-shore environments that may or may not have submerged aquatic vegetation.

The Atlantic silverside (*Menidia menidia*) is an estuarine species that occurs from Nova Scotia to Florida (Middaugh 1981) and is one of the numerically most abundant fish species in those estuaries. Although *M. menidia* has no commercial fishery value, it serves as a forage species for commercially important fish such as bluefish, striped bass, and Atlantic mackerel (U.S. Fish and Wildlife 1983). From March to December, *M. menidia* occupy estuaries while during the winter months they migrate to open water. The life cycle and spawning behavior of *M. menidia* have been described in detail (Conover and Kynard 1984; Koltes 1984; Middaugh et al. 1981; Moore 1980). For example, Middaugh (1981), showed that *M. menidia* spawn during on a lunar cycle. In addition, Bengtson et al. (1987) investigated the relationship between maternal length and egg diameter. Research investigating the habitat ecology of *M. menidia* has been restricted to the juvenile and adult life stages (Barkman et al.

1981; Bengtson 1984). Adults spawn on grasses in the intertidal zone (Middaugh1981). In Rhode Island, USA estuaries, spawning occurs between May and early July(Huber and Bengtson 1999).

In the upper reaches of two local estuaries, the Pettaquamscutt River (UPR) and Point Judith Pond (UPJP), the zooplankton communities are quite different in early spring, when adult *M. menidia* return from the winter in an emaciated condition and feed on zooplankton to ripen for spawning (Bengtson 1982; 1984). The zooplankton community in UPR is dominated by crustaceans at this time, indicating a fairly pristine environment, and UPJP is dominated by polychaete larvae, indicating a eutrophied environment (Bengtson 1982). Given the propensity of marine larval fish to feed on copepods, an a priori assumption might be that *M. menidia* in UPR feed on higher quality prey than do those in UPJP. Volson (2012) has examined effects of nutritional quality of zooplankton prey from these two estuaries on adult *M. menidia* and their eggs, along with the hatching length of their larvae after incubation in the laboratory. Surprisingly, length-at-hatch was greater for fish from UPJP than it was for fish from UPR in each of two years. It remains unclear whether greater length-athatch translates into different growth rates of the larvae during the first two weeks of life in the field.

Although we know a great deal about *M. menidia* juveniles, adults and developing embryos in the field, we know nothing about the habitat ecology of larvae during their first 2-3 weeks of life in the estuary (e.g. depth distribution in the estuaries). Further, the feeding ecology of larval *M. menidia*, in the field, is undocumented (U.S. Fish and Wildlife 1983). Therefore, one focus of this research

was to determine food consumption by *M. menidia* larvae during their first two weeks post-hatch in the littoral zone. Since this is one of the numerically most abundant fish species in estuaries along the East Coast of the U.S., investigations of larval ecology will contribute valuable information to our knowledge base for this species.

The goals of this study were to: (1) determine the depth distribution of *M. menidia* larvae; (2) compare abundance and distribution of *M. menidia* larvae between estuaries; (3) compare feeding habits of the larvae in the two estuaries through gut content analysis; (4) compare growth of larvae in the two estuaries via age-length relationships based on otolith analysis. Distribution and abundances of *M. menidia* larvae were determined by collecting the species in the littoral zone from both estuarine environments using four sampling devices at different depths. Gut content analysis and the age-length relationship using otoliths of the *M. menidia* larvae were determined from field samples. The null hypotheses tested were: all of the devices can be used to collect *M. menidia* larvae from the littoral zone in both estuaries; there is no difference in densities of *M. menidia* larvae between estuaries; and there is no difference in the diet of *M. menidia* larvae between estuaries; and there is no difference in growth (age-length relationship) of *M. menidia* larvae between estuaries.

MATERIALS AND METHODS

I. Field Sampling

1. Site Description

Field collections took place in the upper portions of two estuaries: Point Judith Pond (UPJP) and the Pettaquamscutt River (UPR). The estuaries are approximately 5 km apart, located in Washington County, Rhode Island, USA and have different physical characteristics (Table 1). Point Judith Pond is a shallow coastal lagoon, one of seven along the southern coast of Rhode Island, connected to Block Island Sound by a breachway (Lee 1980). The Pettaquamscutt River is a flooded river valley that is approximately 227 hectares (Gaines 1975).

2. Abundance and Distribution Sampling

To determine distribution patterns and densities, i.e., abundance per cubic meter, of *M. menidia* larvae in the field, a variety of sampling methods were investigated. The sampling methods included four sampling devices: (1) a cylindrical quadrat with a diameter of 0.5 m to sample the shoreline interface between land and water, (2) an aquarium net, 19.05 cm X 26.03 cm with 500- μ m mesh, to collect samples in water that was about 0.35 m deep, (3) a plankton net with a diameter of 0.2 m, length of 0.6 m, and 200- μ m mesh to collect samples in water from 0.4 - 0.5 m deep, and (4) a second plankton net with a diameter of 0.5 m, length of 1.8 m, and 100- μ m mesh to collect samples in water that 1 m deep. Due to the different dimensions of the devices, each was only used at the depths mentioned above.

At both estuaries, collections occurred seven days after the May 20, 2012 new moon and continued for two weeks and again for a second two-week interval seven days after the June 4, 2012 full moon. Sampling began at 6:30 AM and continued throughout the day until all 64 tows and plots were complete or until weather conditions prohibited further sampling. Collections starting at 6:30 AM were important for determining foraging habits as this is after *M. menidia* larvae have begun to feed for the day. Each device was used at four locations within UPJP and UPR.

Collections on and after June 14th in UPJP were sampled from one location because this estuary had the same benthic structure and was treated as one location. The quadrat was haphazardly tossed four times at the shoreline interface. Both plankton nets were pulled along 10 m transects in replicates of four. Finally, the aquarium net was pushed along a 10 m transect in replicates of four. However, on and after June 14 the aquarium net was pushed along a 1 m transect in replicates of four. Typically, the devices were used one at a time in each location; not alternated. The quadrat sampled 0.01 m³ of water at the shoreline interface. For field collections made before June 14th, the volume of water filtered by the aquarium net was 0.49 m³. For field collections made on and after June 14th, the aquarium net filtered 0.049 m³ of water. The small plankton net filtered 0.32 m³ of water. Finally, the large plankton net filtered 1.98 m³ of water.

Larvae that were collected for laboratory analysis were euthanized using MS-222 mixed in seawater (90 g/mL), then preserved in either 95% ethanol (for otolith analysis) or 10% straight formalin mixed in seawater (for gut content analysis). Each larva collected in the field was measured to the nearest hundredth of a millimeter for total length (TL) using a dial caliper.

III. Laboratory Work

1. Gut Content Analysis

Foraging habits of *M. menidia* larvae were determined by gut content analysis of preserved *M. menidia* larvae collected from the field. In the laboratory, the gut was gently pulled apart and examined in a 50-mm Sedgwick-Rafter counting cell under a compound microscope. Each prey item was tallied and identified to the lowest

possible taxon. From UPJP, a total of 58 guts were examined. From UPR, a total of 51 guts were examined. All larvae dissected, for both estuaries, were between 4.18 mm and 9.36 mm (TL). The number method was used to show food type as a percentage of the total gut contents of each larva (Zacharia and Abdurahiman 2004). Each taxon was represented as a percent of the total gut contents for all the larvae dissected for each estuary.

2. Otolith Analysis

In the lab, one of the sagittal otoliths was extracted from each larva, placed on a microscope slide with one drop of immersion oil (Grade A from Cargille Laboratories), photographed using a light microscope camera (at 100X or 400X magnification), and the rings counted using methods of Barkman (1978). Otoliths were first examined using light microscopy (400 X magnification) to count daily growth rings to determine age (days). Measurements of diameters of the sagittae were taken as a proxy for growth. At a later time, a second reading was completed by the same observer from the photographs taken of the sagittal otoliths. Six pre-hatch rings were subtracted from the total number of daily rings on each sagittal otolith (Barkman, 1978). The sagittae did not require additional processing because the core was visible. The relation of the number of daily rings (age) to larval length (TL) was determined for each estuary. The slopes of these linear relationships provided an estimate of growth (mm/day) of larvae in each of the estuaries. All measurements were in micrometers (μ m) using the computer software program ImageJ[®] (Abràmoff et al. 2004). Seventeen larvae were examined from UPJP and 19 larvae from UPR.

IV. Statistical Analysis

The relationship between age and length was determined for each estuary and the slopes of these regressions were analyzed with an analysis of covariance (ANCOVA). A chi-square analysis was applied to the gut content data to determine any significant difference in feeding habits of *M. menidia* larvae between estuaries.

Distribution and abundance data collected from the field were analyzed using a Zero-inflated Poisson model (White and Bennetts 1996). This type of generalized linear model assumes that the outcomes that have a zero value are due to two processes, collecting larvae vs. not collecting larvae. Not collecting larvae results in an outcome of zero. If larvae were collected, then the outcome becomes count data.

$$Logit(Y_p = extra \ 0) = \hat{\beta}_{01} + \hat{\beta}_{quadrat}H_p + \hat{\beta}_{aquarium \ net}I_p + \hat{\beta}_{small \ plankton \ net}J_p + \hat{\beta}_{large \ plankton}$$
$$_{net}K_p$$

$$\begin{split} Log(E(Y_p)) &= Log\;(\mu_p) = \widehat{\beta}_0 + \widehat{\beta}_{site}A_p + \widehat{\beta}_{date}B_p + \widehat{\beta}_{depth}C_p + \widehat{\beta}_{quadrat}D_p + \widehat{\beta}_{aquarium\;net}E_p + \\ &\widehat{\beta}_{small\;plankton\;net}F_p + \widehat{\beta}_{large\;plankton\;net}G_p \end{split}$$

Where Y_p is equal to the number of larvae collected, $\hat{\beta}_{01}$ and $\hat{\beta}_0$ are the intercepts, and $\hat{\beta}_{quadrat, aquarium net, small plankton net, large plankton net, site, date, depth are the coefficients for each predictor. The predictors included in the part of the model that analyzes when a larva was not collected are <math>H_p$ which represents the quadrat, I_p which represents the aquarium net, J_p which represents the small plankton net, and K_p which represents the large plankton net. The intercept was set at zero for this part of the analysis. The predictors included in the part of the model that analyzes when larvae were collected are A_p which represents the sites (UPR and UPJP), B_p which represents the duration of sampling (date), C_p which represents the depth of the water, D_p which represents the

quadrat, E_p which represents the aquarium net, F_p which represents the small plankton net, and G_p which represents the large plankton net. For this part of the analysis, the intercept could not be set at zero.

RESULTS

I. Field Abundance and Distribution

Average density and the number of larvae collected by each device are shown in Table 2. Only one *M. menidia* larva was collected with the large plankton net. The quadrat, aquarium net, and small plankton net all collected more larvae compared to the large plankton net. This indicates that *M. menidia* larvae are generally not in waters greater than one meter depth, but can be found in waters less than 0.5 m depth in the littoral zone. Further, the quadrat, the aquarium net, and small plankton net can all be used to collect *M. menidia* larvae from the littoral zone of estuaries.

Distribution and abundance of *M. menidia* larvae from the field are represented as the frequency of occurrence of the number of larvae collected (Figure 2). The distribution of the data follows a Poisson curve with a high frequency of zeros (Figure 2). This suggests that *M. menidia* larvae have a patchy distribution in the littoral zone. It also shows how often and how many *M. menidia* larvae were collected per tow (Figure 2). For field collections made before June 14th, up to 69 larvae were collected per tow (Figure 2A). For field collections made on and after June 14th, up to fifteen larvae were collected per tow (Figure 2B).

The Zero-inflated Poisson analysis shows which predictors in this study influenced the number of larvae collected, as well as which predictors influenced the zeros in the data (Tables 3, 4). Before June 14th, the presence of larvae in the littoral

zone in both estuaries correlated with date, depth, and all sampling devices (Table 3). By the middle of June, fewer larvae were collected from the littoral zone in UPR and UPJP compared to the beginning of this sampling season ($\hat{\beta}_{date} = -0.10, \chi^2 = 46.01, p$ < 0.0001, Table 3). The number of larvae collected increased with depth in the littoral zone ($\hat{\beta}_{depth} = 1.34, \chi^2 = 35.16, p < 0.0001, Table 3$). The quadrat, aquarium net, and small plankton net collected larvae in statistically significant amounts ($\hat{\beta}_{quadrat} =$ 291.32, $\chi^2 = 131.62, p < 0.0001, \hat{\beta}_{aquarium net} = 6.73, \chi^2 = 243.79, p < 0.0001, \hat{\beta}_{small}$ plankton net = 7.63, $\chi^2 = 58.92, p < 0.0001, Table 3$), as indicated by the positive coefficients. However, the large plankton net did not catch *M. menidia* larvae in statistically significant amounts ($\hat{\beta}_{large plankton net} = -2.22, \chi^2 = 17.71, p < 0.0001, Table$ $3), as indicated by the negative coefficients. The quadrat (<math>\hat{\beta}_{quadrat} = 2024.99, \chi^2 =$ 1959.45, p < 0.0001, Table 3) and the aquarium net ($\hat{\beta}_{aquarium net} = 36.29, \chi^2 = 2748.10, p < 0.0001, Table 3)$ influenced the presence of zeros in the data.

For field collections made on and after June 14th, depth, the quadrat, and the aquarium net influenced the number of larvae collected from the littoral zone in UPR and UPJP (Table 4). The number of larvae collected increased with depth in the littoral zone ($\hat{\beta}_{depth} = 5.28$, $\chi^2 = 12.96$, p < 0.05, Table 4). The quadrat and aquarium net did not consistently collect larvae in statistically significant amounts ($\hat{\beta}_{quadrat} = -247.90$, $\chi^2 = 4.03$, p < 0.05, $\hat{\beta}_{aquarium net} = -59.65$, $\chi^2 = 4.68$, p < 0.05, Table 4), as indicated by the negative coefficients. This suggests that larvae were most likely in waters between 0.4 and 0.5 meters depth in the littoral zone. No predictors significantly influenced the presence of zeros for this data.

One of the goals of this project was to determine if the density of *M. menidia* larvae differed between the estuaries. The results of the Zero-inflated Poisson analysis for site indicated that UPR had a higher density of *M. menidia* larvae compared to UPJP, for the entire sampling period.

II. Gut Content Analysis

Feeding habits of *M. menidia* larvae between estuaries were significantly different ($\chi^2 = 622.7$, p < 0.0001). In UPJP, copepod nauplii made up 72.5% of total gut content (Figure 3). In UPR, *M.menidia* larvae consumed mostly copepod eggs, which made up 76.2% of the total gut content (Figure 3).

III. Otolith Analysis

Results from the ANCOVA show a significant relationship between length of larvae and age for fish from both estuaries (p < 0.0001, Figure 4). Based on the age-length regressions, larvae grow 0.65 mm/day in UPR and 0.66 mm/day in UPJP (Figure 4). The results from the ANCOVA show no significant difference (p = 0.8147) in the age-length relationship of larvae between estuaries (Figure 4).

DISCUSSION

This is the first report of the field ecology of *M. menidia* larvae, even though laboratory studies on this larval species have been conducted for decades (e.g., Austin et al. 1975; Middaugh and Lempesis 1976; Morgan and Prince 1977; Deacutis 1978; Bengtson 1985; Lankford et al. 2001). Field collections from the littoral zone of UPR and UPJP during the summer of 2012 showed that this larval fish can be collected at

the shoreline interface to waters 0.5 m deep, can be collected with a variety of sampling devices, and displays a patchy distribution. The two estuaries sampled differed with regard to abundances of *M. menidia* larvae and the prey consumed by those larvae, but the larvae grew at the same rates regardless of those differences.

The quadrat, aquarium net, and small plankton net collected more larvae compared to the large plankton net with the aquarium net collecting the most M. menidia larvae both in absolute and per-volume terms. Hildebrand (1922) and Middaugh et al. (1981) documented *M. menidia* adults spawning in shallow grassy areas. This study suggests that *M. menidia* larvae stay in the littoral zone after hatch. Lindsay et al. (1978) sampled ichthyoplankton in the Indian River, Delaware, USA and noted that the low abundance of atherinid larvae was not representative of their high abundance as juveniles and adults. Of the mid-channel water column that they sampled from 0-45 cm, atherinid larvae occurred mostly within the top 5 cm (their study included *M. menidia*). They concluded, based on data from Breder and Rasquin (1950) on the confamilial species *Atherina stipes*, that atherinid larvae are positively phototaxic, which explains their presence very close to the surface. Their finding that larvae can occasionally be collected from mid-channel (as well as our finding that one larva was collected at a depth > 0.5 m) indicates that some larvae stray from the apparently preferred shallower water.

Occupation of very shallow waters by *M. menidia* larvae likely provides them with protection from predators. *Pomatomus saltatrix, Morone saxatilis, Morone americana*, and *Caranx hippos* have all been collected from the Pettaquamscutt River estuary and used in predation experiments on larval and juvenile *Menidia beryllina*

(inland silverside) (Gleason and Bengtson 1996). In Point Judith Pond, predators include *Morone saxatilis* and *Fundulus majalis* (Pfeiffer-Herbert 2007).

Menidia menidia larvae collected from the field followed a zero-inflated Poisson model, suggesting that these larvae are not distributed evenly in the littoral zone, but have a patchy distribution. Many fishes display a patchy distribution because of ocean processes (Pepin et al. 2003) and social behavior (Maynou et al. 2006), such as spawning, predator pressures, and feeding. Understanding why marine larvae have a patchy distribution has been a focus of many studies. Hewitt (1981) proposed that larvae display a patchy distribution because it benefits schooling, a behavior displayed in the juveniles and adult life stages. Shaw (1960, 1961) showed that *M. menidia* begin to school around 11 - 12 mm SL; later research by Shaw and Sachs (1967) showed that optomotor responses, proposed to be involved with schooling, are present in newly hatched *M. menidia*. Since schooling is widely thought to reduce predation pressure, the development of such behavior likely allows *M. menidia* larvae to enter water that they occupy as newly hatched larvae. Future research should include collecting spatial data to determine where these patches of larvae are in the littoral zone and the size of each group.

Results from the gut content data show that *M. menidia* larvae in UPR consume mostly copepod eggs, whereas those in UPJP consume mostly copepod nauplii. Volson (2012) found a high abundance of calanoid copepods in UPR and a varying zooplankton community in UPJP, where from early spring (April to early May) to late spring (June), the dominant zooplankton present switches from polychaete larvae to copepods. Most of the sampling for this study took place in late

spring, when the polychaete larvae had already settled and were not available to, or not preferred by, the larvae. The exact species of copepod that the eggs came from, for this study, was not determined.

These findings agree with previous research on *M. menidia*. In the Pataguanset Estuary in Connecticut, Cadigan and Fell (1985) found that one of the most commonly occurring food items in the guts of adult *M. menidia* were copepods. Further, research by Gilmurray and Daborn (1981) showed that small-sized *M. menidia* consumed smaller zooplankton species, such as copepods. In UPR and UPJP it may be that size class of *M. menidia* is also an important factor in prey selection. Fernandez-Diaz et al. (1994) showed that mouth size of larval *Sparus aurata* correlates to the size of prey it consumes. For this study, the prey items found in the gut contents of larval *M. menidia* may be due to the available zooplankton in the estuary and mouth size.

It is necessary to stress the importance of future monitoring of the zooplankton communities in both of these estuaries, because of impacts due to anthropogenic influences. Increased nutrient levels in the water can change the community composition of estuaries (Pinckney et al. 1998), including UPJP and UPR. UPJP has a high abundance of polychaete larvae (Bengtson 1982), and is more eutrophic than UPR (Table 1). However, as stated previously there are shifts in the dominant species of zooplankton in this estuary, which can lead to different predators entering the estuary (Purcell 2012). For example, during the sampling year of 2005, an unusually high abundance of Lion's Mane jellyfish were present in UPJP (Volson *pers. comm.*). Few to no *M. menidia* adults and juveniles were collected in seine hauls that spring. It was determined that the jellyfish were consuming the zooplankton community in

UPJP. The mechanisms that regulate this phenomenon are not well understood, but eutrophication has been suggested as one factor (Purcell 2012).

It has long been known that otoliths can be used as a proxy for fish growth. The significant age-length relationship for *M. menidia* larvae in the current study has been previously shown in work by Barkman (1978).

Between estuaries, there was no significant difference in the age-length relationship of *M. menidia* larvae. According to the regression coefficients in the agelength equations, the larvae grow at 0.65 - 0.66 mm/d. Barkman et al. (1981) found that over a length range of about 12 – 90 mm, *M. menidia* grow at 0.84 mm/d, based on an otolith age-length relationship, whereas Mulkana (1964) estimated a growth rate of 7-11 mm/month (0.23 - 0.37 mm/d) based on length-frequency analyses of a cohort. Volson (2012) found that larval length at hatch was significantly greater for *M. menidia* larvae in UPJP than UPR. The results from the present study indicate that a greater length at hatch does not translate into faster growth for larval *M. menidia*. Temperature influences the growth of fish. In particular, for *M. menidia*, temperature is one factor that determines sex and size during the larval stage (Conover and Kynard 1981). Research by Conover and Kynard (1981) showed that warmer temperatures, $17^{\circ} - 25^{\circ}$ C, produce more male fish compared to cooler temperatures, $11^{\circ} - 19^{\circ}$ C, which produced more females. In addition, the male fish produced in those warmer temperatures tended to be smaller in size compared to female *M. menidia* (Conover and Kynard 1981). Water temperatures in UPJP are cooler than the water temperatures in UPR, even during the summer months (Volson 2012) when sampling occurred for this study (Table 1). Despite a larger length-at-hatch for *M. menidia* in

UPJP, the cooler water temperature in this estuary may have resulted in a slower growth rate for the larvae. As a result, larval growth was not greater in UPJP compared to UPR.

Nutritional quality of the prey items might also influence the growth of *M*. *menidia* larvae. Previous research has shown that zooplankton from UPJP are more lipid-rich compared to zooplankton from UPR (Volson 2012). On the other hand, copepod eggs, with more yolk, might be expected to have a higher lipid content than do copepod nauplii. In any case, this difference in gut content between the estuaries did not influence larval growth during the time samples were collected for this study. Furthermore, the number of copepod nauplii and copepod eggs consumed by *M*. *menidia* larvae on an hourly or daily timescale in both estuaries is not known. As a result, any further remarks concerning the effect of the nutritional quality of prey on *M. menidia* larvae cannot be made.

The "growth-mortality" hypothesis states that larger fish have higher survivability than smaller fish (Anderson 1988). However, Gleason and Bengtson (1996) found that for inland silversides (*Menidia beryllina*) the smaller individuals have higher survival rates than larger individuals. If *M. menidia* larvae in UPJP are not growing significantly faster, once in their larval life stage, compared to larvae from UPR, then we cannot expect larvae in UPJP to have a higher survival rate. Despite having no difference in growth, and thus survival of *M. menidia* larvae, between the estuaries, density of larvae did differ. UPR had higher densities of *M. menidia* larvae compared to UPJP.

Summary

The larval life stage is an important life stage to the recruitment of adult populations. Studies on this fish are not important simply because of its abundance, but its role as a forage fish for fisheries species and how these fish influence the energetics of estuaries. The goal of this study was to describe the larval ecology of *M. menidia*. Larvae from both estuaries displayed a patchy distribution and were collected with the quadrat, aquarium net, and small plankton net. Diet differed between estuaries with UPR larvae eating mostly copepod eggs and UPJP larvae consuming mostly copepod nauplii. The growth of larvae collected in UPR was not significantly greater than that of larvae collected from UPJP, indicating that a larger length at hatch, based on the previous study of Volson (2012) does not translate into faster growth in the larval life stage. We hope that the initial information presented here will stimulate estuarine researchers to further examine the larval ecology of this important component of estuarine ecosystems.

| Parameters | Time | Depth (m) | Upper Pettaquamscutt River | Depth (m) | Upper Point Judith Pond |
|----------------------|----------------------|-----------|----------------------------|-----------|--------------------------------|
| Temperature | May 2012 | 0.1 | 19.5 | 0.5 | 19.8 |
| (° Celsius) | Jun 2012 Jul 2012 | | 24 27 | | 21 25 3 |
| | 0012012 | Average: | 24 | | 22 |
| Salinity | May 2012 | 0.1 | 16.5 | 0.5 | - |
| (ppt) | Jun 2012 Jul 2012 | | 15 16 | | 27.5 30.5 |
| | | Average: | 16 | • | 29 |
| Fecal Coliform | May 2012 | | < 10 | | 478 |
| (per 100 mL) | Jun 2012 Jul 2012 | | < 10 < 10 | | 189 84 |
| Enterococci | May 2012 | | < 10 | | 124 |
| (per 100 mL) | Jun 2012 Jul 2012 | | < 10 124 | | 20 30 |
| Dissolved Phosphorus | May 2012 | 0.5 | 5 | 0.5 | 7 |
| (µg/L) | Jun 2012 Jul 2012 | | < 3 4 | | 8 29 |
| Ammonium-Nitrogen | May 2012 | 0.5 | 45 | 0.5 | 60 |
| (µg/L) | Jun 2012 Jul 2012 | | 40 25 | | 45 75 |
| Total Phosphorus | May 2012 | 0.5 | 16 | 0.5 | 42 |
| (µg/L) | Jun 2012 Jul 2012 | | 23 35 | | 72 107 |

Table 1. Physical parameters of the Upper Pettaquamscutt River and Upper Point Judith Pond from May to July of 2012 from URI Watershed Watch.

Table 2. Description of catch data for each sampling device used in the Upper Pettaquamscutt River (UPR) and Upper Point Judith Pond (UPJP) before June 14, 2012 (Table A) as well as on and after June 14, 2012 (Table B). Also included in each table are descriptions of the volume of water filtered by each sampling device.

| A. | | | | | | | |
|--------------------|--------------------|-----------------|------------------|--|-------------------|--|--|
| | Volume water | Total Number of | Larvae Collected | Average Density of Larvae (# fish / m^3) ± S.E. | | | |
| | sampled per tow | UPR | UPJP | UPR | UPJP | | |
| Quadrat | 0.01 m^3 | 33 | 2 | 0.23 ± 1.43 | 0.01 ± 0.10 | | |
| Aquarium Net | 0.49 m^3 | 152 | 311 | 2.55 ± 6 | 10.11 ± 25.30 | | |
| Small Plankton Net | 0.32 m^3 | 35 | 3 | 0.05 ± 0.21 | 0.11 ± 0.76 | | |
| Large Plankton Net | 1.98 m^3 | 0 | 1 | 0 | 0.01 ± 0.07 | | |

| B. | | | | | | | |
|--------------------|---------------------|-----------------|------------------|--|-----------------|--|--|
| | Volume of water | Total Number of | Larvae Collected | Average Density of Larvae (# fish $/ m^3$) \pm S.E. | | | |
| | sampled per tow | UPR | UPJP | UPR | UPJP | | |
| Quadrat | 0.01 m^3 | 8 | 1 | 0.05 ± 0.33 | 0.02 ± 0.11 | | |
| Aquarium Net | 0.049 m^3 | 25 | 24 | 6 ± 33.97 | 20.16 ± 47.57 | | |
| Small Plankton Net | 0.32 m^3 | 2 | 0 | 0.07 ± 0.47 | 0 | | |
| Large Plankton Net | 1.98 m^3 | 0 | 0 | 0 | 0 | | |

Table 3. The parameter estimates for the Zero-inflated Poisson model from field samples collected before June 14, 2012. The top table includes parameters from the ZIP model that analyzes all data values greater than zero, i.e. when a larva was collected. Site refers to the Upper Pettaquamscutt River and Upper Point Judith Pond. Date represents the duration of sampling, May 30, 2012 until June 13, 2012. The Wald Chi-Square statistic tests if the probability of collecting a larva is significantly influenced by site, depth, date, the quadrat, the aquarium net, the small plankton net, and the large plankton net. The bottom table includes the parameters influencing the probability of not collecting a larva is significantly affected by each of the sampling devices.

| | Analysis Of Maximum Likelihood Parameter Estimates | | | | | | | | | | | | |
|--------------------|--|----------|----------------|-----------------|--------------|-----------------|-------------|--|--|--|--|--|--|
| Parameter | DF | Estimate | Standard Error | Wald 95% Confid | dence Limits | Wald Chi-Square | Pr > Chi Sq | | | | | | |
| Intercept | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | | | | | | | | |
| Site | 1 | -0.7790 | 0.1007 | -0.9763 | -0.5816 | 59.85 | <.0001 | | | | | | |
| Date | 1 | -0.1039 | 0.0153 | -0.1339 | -0.0739 | 46.01 | <.0001 | | | | | | |
| Depth (m) | 1 | 1.3441 | 0.2267 | 0.8998 | 1.7884 | 35.16 | <.0001 | | | | | | |
| Quadrat | 1 | 291.3240 | 25.3932 | 241.5542 | 341.0939 | 131.62 | <.0001 | | | | | | |
| Aquarium Net | 1 | 6.7282 | 0.4309 | 5.8836 | 7.5728 | 243.79 | <.0001 | | | | | | |
| Small Plankton Net | 1 | 7.6333 | 0.9945 | 5.6842 | 9.5824 | 58.92 | <.0001 | | | | | | |
| Large Plankton Net | 1 | -2.2153 | 0.5264 | -3.2471 | -1.1835 | 17.71 | <.0001 | | | | | | |

| | Analysis Of Maximum Likelihood Zero Inflation Parameter Estimates | | | | | | | | | | | | | |
|--------------------|---|----------|----------------|---------------|---|---------|--------|--|--|--|--|--|--|--|
| Parameter | DF | Estimate | Standard Error | Wald 95% Cont | Wald 95% Confidence Limits Wald Chi-Squ | | | | | | | | | |
| Intercept | 1 | -17.2746 | 0.3014 | -17.8653 | -16.6839 | 3285.32 | <.0001 | | | | | | | |
| Quadrat | 1 | 2024.991 | 45.7462 | 1935.330 | 2114.652 | 1959.45 | <.0001 | | | | | | | |
| Aquarium Net | 1 | 36.2855 | 0.6922 | 34.9288 | 37.6421 | 2748.10 | <.0001 | | | | | | | |
| Small Plankton Net | 0 | 62.2735 | 0.0000 | 62.2735 | 62.2735 | | • | | | | | | | |
| Large Plankton Net | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | | | | | | | | | |

Table 4. The parameter estimates for the Zero-inflated Poisson model from field samples collected on and after June 14, 2012. The top table includes parameters from the ZIP model that analyzes all data values greater than zero, i.e. when a larva was collected. Site refers to the Upper Pettaquamscutt River and Upper Point Judith Pond. Date represents the duration of sampling, June 14, 2012 until June 25, 2012. The Wald Chi-Square statistic tests if the probability of collecting a larva is significantly influenced by site, depth, date, the quadrat, the aquarium net, the small plankton net, and the large plankton net. The bottom table includes the parameters influencing the probability of not collecting a larva is significantly affected by each of the sampling devices.

| | Analysis Of Maximum Likelihood Parameter Estimates | | | | | | | | | | | | |
|--------------------|--|----------|----------------|--------------|-----------------|-----------------|------------|--|--|--|--|--|--|
| Parameter | DF | Estimate | Standard Error | Wald 95% Cor | nfidence Limits | Wald Chi-Square | Pr > ChiSq | | | | | | |
| Intercept | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | | • | | | | | | |
| Site | 1 | 1.4755 | 0.5281 | 0.4404 | 2.5105 | 7.81 | 0.0052 | | | | | | |
| Date | 1 | -0.0371 | 0.0646 | -0.1636 | 0.0895 | 0.33 | 0.5658 | | | | | | |
| Depth (m) | 1 | 5.2823 | 1.4675 | 2.4060 | 8.1585 | 12.96 | 0.0003 | | | | | | |
| Quadrat | 1 | -247.903 | 123.5552 | -490.067 | -5.7396 | 4.03 | 0.0448 | | | | | | |
| Aquarium Net | 1 | -59.6478 | 27.5598 | -13.664 | -5.6315 | 4.68 | 0.0304 | | | | | | |
| Small Plankton Net | 1 | -16.8952 | 9.2335 | -34.9926 | 1.2021 | 3.35 | 0.0673 | | | | | | |
| Large Plankton Net | 0 | 1.7209 | 0.0000 | 1.7209 | 1.7209 | | | | | | | | |

| | Analysis Of Maximum Likelihood Zero Inflation Parameter Estimates | | | | | | | | | | | | | |
|--------------------|---|----------|----------------|-------------|-----------------|-----------------|------------|--|--|--|--|--|--|--|
| Parameter | DF | Estimate | Standard Error | Wald 95% Co | nfidence Limits | Wald Chi-Square | Pr > ChiSq | | | | | | | |
| Intercept | 1 | 8.6700 | 8.4818 | -7.9541 | 25.2940 | 1.04 | 0.3067 | | | | | | | |
| Quadrat | 1 | -571.863 | 849.9277 | -2237.69 | 1093.965 | 0.45 | 0.5011 | | | | | | | |
| Aquarium Net | 0 | 146.905 | 173.1981 | -486.367 | 192.5570 | 0.72 | 0.3963 | | | | | | | |
| Small Plankton Net | | -17.5081 | 27.4089 | -71.286 | 36.2123 | 0.41 | 0.5230 | | | | | | | |
| Large Plankton Net | 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | | | | | | | | | |



Figure 1: Map and aerial photographs of the upper portions of the Pettaquamscutt River estuary (left image) and Point Judith Pond (right image). Arrows point to the approximate sampling locations in each estuary.

Figure 2: The frequency of occurrence of the total number of *Menidia menidia* larvae collected from the Upper Pettaquamscutt River (UPR) and Upper Point Judith Pond (UPJP). Graph A represents field collections made before June 14, 2012; while Graph B represents field collections made on and after June 14, 2012. For both graphs, red triangles represent the probability estimates following the Zero-inflated Poisson model. The blue dots, in each graph, represent the observed relative frequencies of the total number of larvae collected from the field. Both graphs also show how many *Menidia menidia* larvae were collected from the field in one tow.





Figure 3: Gut contents of *Menidia menidia* larvae for UPR (n = 51) and UPJP (n = 58). Each taxon is represented as a percent of the total gut contents for all larvae collected in each estuary. Results from the chi-square analysis show a significant difference in feeding habits of *Menidia menidia* larvae (p < 0.0001).







Figure 4: Growth of *Menidia menidia* larvae collected from UPJP (open circles) and UPR (diamonds). Linear regressions represent the age-length relationship of *Menidia menidia* larvae from UPJP (dashed line), y = 0.66x + 2.98, and UPR (solid line), y = 0.65x + 3.06. In the linear equations Y = total length in millimeters and X = age in days. Results from the ANCOVA analysis show no significant differences in the slopes of the age-length relationship of larvae between estuaries (p = 0.8147). However, age is a significant indicator of the size of larvae (p < 0.0001).

APPENDIX I. DEPTH PREFERENCE EXPERIMENT

A mock littoral zone was created in the laboratory to determine if different size classes of *M. menidia* larva prefer a certain depth when residing in the littoral zone. Larval *M. menidia* used for the experiment were spawned from four gravid male and four gravid female adults; collected in the field. In the lab, the adults were stripspawned, their fertilized eggs incubated, and the larvae reared according to methods described by Barkman and Beck (1976). During incubation and for the duration of the experiment, room temperature was kept at a constant 22°C with an 18 h light: 6 h dark cycle. After seven days of incubation, the newly hatched larvae were added to one of the three aquaria. To the first aquarium, 54 larvae were added. To the second aquarium, 34 larvae were added. And to the third aquarium, 40 larvae were added.

Each aquarium was 113.56 liters (76.2 cm X 30.4 cm X 31.7 cm) and lined with sand that was sloped 12°. A piece of acrylic glass, 0.64 cm thick, was cut to the dimensions of the aquarium and sealed over the sand (Figure 5). The main purpose of the sand was to act as a support for the acrylic glass. The purpose of creating a slope was to mock the natural slope of the littoral zone found along the shores of Point Judith Pond and the Pettaquamscutt River. Each aquarium was divided into three sections according to depth. The shallow area ranged from 1.27 to 3.80 cm deep, the middle area ranged from 3.80 cm to 7.62 cm deep, and the deep area ranged from 7.62 cm to 10.16 cm deep. To ensure that the larvae would not be disturbed by the entrance of the observer, a black tarp was hung in front of the aquaria. During the experiment, larvae were fed *Artemia* nauplii every other day *ad libitum*. The experiment ran for

two weeks. Three observations per aquarium were made daily to determine the number of larvae in each depth zone.

The exact number of larvae in each depth was unknown through most of the experiment, therefore; the number of larvae in each depth zone could only be estimated. Initial populations in each aquarium were determined by tallying the number of dead larvae removed from each tank each day.

Although the larvae were too delicate for determination of total length at the beginning of the experiment, length-at-hatch of larvae from these estuaries is about 4.5 mm (Volson 2012).



Figure 5: Representation of aquarium used for depth preference experiment.

Analysis & Findings

A replicated test of goodness of fit (heterogeneity test) was constructed to determine any significant differences in depth preferences by larvae. Results showed no significant differences in depth preference by the *M. menidia* larvae ($G_T = 11.8$, p > 0.05).

Contrary to observations in other laboratory settings (Bengtson, *pers. comm.*), the larvae did not seem to prefer specific depths over time. It was postulated that the

larvae would prefer the shallow zone or be found very close to the water's edge, on the day of hatch. As time progressed, and the larvae grew, we expected them to move into the deeper zones of the aquaria. However, there did not appear to be a pattern of preference for the duration of the experiment.

APPENDIX II MENIDIA MENIDIA LARVAE CATCH DATA

Menidia menidia larvae were collected from the upper portions of two Rhode Island estuaries, the Pettaquamscutt River (UPR) and Point Judith Pond (UPJP). A variety of sampling methods were investigated to determine optimal sampling methods for this larval fish. The four sampling devices included: (1) a cylindrical quadrat with a diameter of 0.5 m to sample the shoreline interface between land and water, (2) an aquarium net, 19.05 cm X 26.03 cm with 500 μ m mesh, to collect samples in water that was about 0.35 m deep, (3) a plankton net with a diameter of 0.2 m, length of 0.6 m, and 200 μ m mesh to collect samples in water from 0.4 - 0.5 m deep, and (4) a second plankton net with a diameter of 0.5 m, length of 1.8 m, and 100 μ m mesh to collect samples in water that was slightly greater than 1 m deep. Due to the different dimensions of the devices, each could only be used between the depths described above.

Field sampling was complete when the entire 64 tows and plots were finished; unless weather conditions prohibited further sampling. Each device was used at four locations within UPJP and UPR. However, collections on and after June 14th in UPJP were sampled from one location because this estuary had the same benthic structure and therefore was treated as one location. The field data was divided by date because a different sampling method was used. Therefore, field collections made before June 14, 2012 (Table 1) as well as on and after June 14, 2012 (Table 2) are provided.

| Quadrat | | | Upper I | Pettaquams | cutt River | | | Upper Point Judith Pond | | | | |
|----------------------------|---------|--------|---------|------------|------------|---------|---------|-------------------------|--------|--------|--------|---------|
| Number of Replicates | 5/30/12 | 6/1/12 | 6/4/12 | 6/6/12 | 6/8/12 | 6/10/12 | 6/13/12 | 6/3/12 | 6/5/12 | 6/7/12 | 6/9/12 | 6/12/12 |
| 1 | 0 | 0 | - | - | - | 0 | - | 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 6 | 0 | 2 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 13 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 1 | 0 | 0 |
| 14 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 19 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 |

Table 1. Number of *M. menidia* larvae collected with the quadrat, aquarium net, small plankton net, and large plankton net from the Upper Pettaquamscutt River and Upper Point Judith Pond before June 14, 2012. A dash indicates that the device was not used.

| Aquarium Net | | Upper Pettaquamscutt River | | | | | | | | Upper Point Judith Pond | | | |
|----------------------------|---------|----------------------------|--------|--------|--------|---------|---------|--------|--------|-------------------------|--------|---------|--|
| Number of Replicates | 5/30/12 | 6/1/12 | 6/4/12 | 6/6/12 | 6/8/12 | 6/10/12 | 6/13/12 | 6/3/12 | 6/5/12 | 6/7/12 | 6/9/12 | 6/12/12 | |
| 1 | 0 | 0 | - | - | - | 0 | - | - | 12 | - | 16 | 1 | |
| 2 | 3 | 0 | - | - | - | 0 | - | - | 13 | - | 0 | 4 | |
| 3 | 0 | 4 | - | - | - | 0 | - | - | 36 | - | 9 | 0 | |
| 4 | 11 | 2 | - | - | - | 0 | - | - | 5 | - | 0 | 1 | |
| 5 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | 69 | - | 0 | 0 | |
| 6 | 1 | 0 | 0 | - | 5 | 4 | 0 | - | 18 | - | 0 | 1 | |
| 7 | 1 | 0 | 0 | - | 0 | 0 | 1 | - | 28 | - | 0 | 0 | |
| 8 | 0 | 0 | 2 | - | 1 | 3 | 0 | - | 5 | - | 0 | 0 | |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 1 | - | 1 | 0 | |
| 10 | 0 | 0 | 3 | 0 | 18 | 0 | 1 | - | 0 | - | 0 | 3 | |
| 11 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | |
| 12 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | |
| 13 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | - | 0 | - | 0 | 2 | |
| 14 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | - | 0 | - | 0 | 2 | |
| 15 | 3 | 0 | 8 | 0 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | |
| 16 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | - | 0 | - | 1 | 2 | |
| 17 | 2 | 0 | 13 | 14 | 0 | 0 | 2 | - | 0 | 2 | 3 | 0 | |
| 18 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | - | 0 | 0 | 7 | 0 | |
| 19 | 0 | 0 | 6 | 11 | 1 | 3 | 0 | - | 0 | - | 3 | 6 | |
| 20 | 7 | 0 | 2 | 0 | 0 | 3 | 0 | - | 0 | - | 56 | 4 | |

Table 1 cont'd.

| Small Plankton Net | | | Upper F | ettaquams | Upper Point Judith Pond | | | | | | | |
|----------------------------|---------|--------|---------|-----------|-------------------------|---------|---------|--------|--------|--------|--------|---------|
| Number of Replicates | 5/30/12 | 6/1/12 | 6/4/12 | 6/6/12 | 6/8/12 | 6/10/12 | 6/13/12 | 6/3/12 | 6/5/12 | 6/7/12 | 6/9/12 | 6/12/12 |
| 1 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | - | 0 | 0 |
| 2 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | - | 0 | 0 |
| 3 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | - | 0 | 0 |
| 4 | 0 | 0 | - | - | - | 0 | - | 0 | 0 | - | 0 | 0 |
| 5 | 0 | 0 | 1 | - | 0 | 0 | 11 | 0 | 0 | - | 2 | 0 |
| 6 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 7 | 0 | 0 | 0 | - | 0 | 5 | 0 | 0 | 0 | - | 1 | 0 |
| 8 | 0 | 0 | 0 | - | 0 | 1 | 0 | 0 | 0 | - | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 11 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 12 | 0 | 3 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | - | 0 | - |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 15 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 16 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | - | 0 | 0 |

Table 1 cont'd.

| Large Plankton Net | | | Upper F | Pettaquams | scutt River | Upper Point Judith Pond | | | | | | |
|----------------------------|---------|--------|---------|------------|-------------|-------------------------|---------|--------|--------|--------|--------|---------|
| Number of Replicates | 5/30/12 | 6/1/12 | 6/4/12 | 6/6/12 | 6/8/12 | 6/10/12 | 6/13/12 | 6/3/12 | 6/5/12 | 6/7/12 | 6/9/12 | 6/12/12 |
| 1 | 0 | 0 | - | - | - | 0 | - | 1 | - | 0 | - | 0 |
| 2 | 0 | 0 | - | - | - | 0 | - | 0 | - | 0 | - | 0 |
| 3 | 0 | 0 | - | - | - | 0 | - | 0 | - | 0 | - | 0 |
| 4 | 0 | 0 | - | - | - | 0 | - | 0 | - | 0 | - | 0 |
| 5 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | 0 | - | - |
| 6 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | 0 | - | - |
| 7 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | 0 | - | - |
| 8 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | 0 | - | - |
| 9 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | - | 0 | - | - |
| 10 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | - | 0 | - | - |
| 11 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | - | 0 | - | - |
| 12 | 0 | 0 | 0 | - | 0 | - | 0 | 0 | - | 0 | - | - |
| 13 | 0 | - | 0 | - | 0 | - | - | 0 | - | 0 | - | - |
| 14 | 0 | - | 0 | - | 0 | - | - | 0 | - | 0 | - | - |
| 15 | 0 | - | 0 | - | 0 | - | - | 0 | - | 0 | - | - |
| 16 | 0 | - | 0 | - | 0 | - | - | 0 | - | 0 | - | - |
| 17 | 0 | - | 0 | - | - | - | - | 0 | - | 0 | 0 | - |
| 18 | 0 | - | 0 | - | - | - | - | 0 | - | 0 | 0 | - |
| 19 | 0 | - | 0 | - | - | - | - | 0 | - | 0 | 0 | - |
| 20 | 0 | - | 0 | - | - | - | - | 0 | - | 0 | 0 | - |

Table 1 cont'd.

| Quadrat | | Upj | per Pettaqu | amscutt R | iver | Upper Point Judith Pond | | | | | | |
|----------------------------|---------|---------|-------------|-----------|---------|-------------------------|---------|---------|---------|---------|---------|---------|
| Number of Replicates | 6/15/12 | 6/17/12 | 6/19/12 | 6/21/12 | 6/23/12 | 6/25/12 | 6/14/12 | 6/16/12 | 6/18/12 | 6/20/12 | 6/22/12 | 6/24/12 |
| 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 6 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 7 | 0 | 0 | 0 | 3 | 0 | - | - | - | - | - | - | - |
| 8 | 1 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 9 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 10 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 11 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 12 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 16 | 0 | 4 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |

Table 2. Number of *M. menidia* larvae collected with the quadrat, aquarium net, small plankton net, and large plankton net from the Upper Pettaquamscutt River and Upper Point Judith Pond on June 14, 2012 and after. A dash indicates that the device was not used.

| Aquarium Net | | Uŗ | oper Pettaqu | amscutt R | iver | Upper Point Judith Pond | | | | | | |
|----------------------------|---------|---------|--------------|-----------|---------|-------------------------|---------|---------|---------|---------|---------|---------|
| Number of Replicates | 6/15/12 | 6/17/12 | 6/19/12 | 6/21/12 | 6/23/12 | 6/25/12 | 6/14/12 | 6/16/12 | 6/18/12 | 6/20/12 | 6/22/12 | 6/24/12 |
| 1 | 0 | 1 | 0 | 0 | 0 | - | 1 | 0 | 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | - | 1 | 0 | 0 | 3 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 11 | 0 | 1 |
| 4 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 1 | 1 | 4 |
| 5 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 6 | 0 | 0 | 0 | 1 | 0 | - | - | - | - | - | - | - |
| 7 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 8 | 3 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 9 | 0 | 0 | 0 | 0 | 1 | - | - | - | - | - | - | - |
| 10 | 0 | 0 | 1 | 0 | 0 | - | - | - | - | - | - | - |
| 11 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 12 | 0 | 1 | 0 | 0 | 2 | - | - | - | - | - | - | - |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 16 | 0 | 0 | 15 | 0 | 0 | 0 | - | - | - | - | - | - |

Table 2 cont'd.

| Small Plankton Net | | Upp | er Pettaqu | amscutt R | iver | Upper Point Judith Pond | | | | | | |
|--------------------------|---------|---------|------------|-----------|---------|-------------------------|---------|---------|---------|---------|---------|---------|
| Number | 6/15/12 | 6/17/12 | 6/10/12 | 6/21/12 | 6/22/12 | 6/25/12 | 6/14/12 | 6/16/12 | 6/18/12 | 6/20/12 | 6/22/12 | 6/24/12 |
| Replicates | 0/13/12 | 0/17/12 | 0/19/12 | 0/21/12 | 0/23/12 | 0/23/12 | 0/14/12 | 0/10/12 | 0/10/12 | 0/20/12 | 0/22/12 | 0/24/12 |
| 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 1 | 1 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 6 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 7 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 8 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 9 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 10 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 11 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 12 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |

Table 2 cont'd.

| Large Plankton Net | | Upp | er Pettaqu | amscutt R | iver | Upper Point Judith Pond | | | | | - | |
|----------------------------|---------|---------|------------|-----------|---------|-------------------------|---------|---------|---------|---------|---------|---------|
| Number of Replicates | 6/15/12 | 6/17/12 | 6/19/12 | 6/21/12 | 6/23/12 | 6/25/12 | 6/14/12 | 6/16/12 | 6/18/12 | 6/20/12 | 6/22/12 | 6/24/12 |
| 1 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | - | - | 0 | 0 | - | - | - | - | - | - | - |
| 6 | 0 | - | - | 0 | - | - | - | - | - | - | - | - |
| 7 | 0 | - | - | 0 | - | - | - | - | - | - | - | - |
| 8 | 0 | - | - | 0 | - | - | - | - | - | - | - | - |
| 9 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 10 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 11 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 12 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 13 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 14 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 15 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |
| 16 | 0 | - | 0 | 0 | 0 | - | - | - | - | - | - | - |

| Tabl | le 2 | con | ıt'd. |
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| | | | |

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