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Sex-Specific Hatch and Growth Patterns in Young-of-the-Year Atlantic Silversides (Menidia menidia, Atherinopsidae) From Mumford Cove, Connecticut

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ABSTRACT

The Atlantic silverside (*Menidia menidia*) is an important semelparous forage fish along the North American Atlantic coast and a well-known model for experimental studies. However, ecological and evolutionary theories, such as the adaptive significance of temperature-dependent sex determination (TSD), have yet to be tested using *M. menidia* juveniles from their natural habitat. Additionally, how the characteristics of survivors at the end of their growing season are shaped by growth and mortality is insufficiently understood. This study used otolith microstructure analysis to determine sex-specific size-at-age, growth, and hatch date distributions in young-of-the-year juveniles sampled monthly between October-December 2015 in Mumford Cove in northeastern Long Island Sound. I hypothesized that sexual size dimorphism was due to differences in age, with female survivors hatching earlier during the spawning season than males, because TSD in this species produces increasingly male-biased sex ratios with increasing temperatures. While females were nearly 2 cm larger in total length, I found this sexual dimorphism was not due to differences in age but differences in growth rates, as female survivors had similar hatch dates but grew on average 37% faster than males. Furthermore, realized sex ratios of fall survivors were considerably less male-biased than expected, based on monitored temperature conditions and TSD values from published common garden experiments. Selective survival of faster growing females over males may account for these more balanced sex ratios. However, growth back-calculations also revealed the existence of both slow- and fast-growing female phenotypes in the survivor population and a selective disappearance of fast-growers from the study area between October and December. The existence of different growth strategies and the presence of slow-growers into December suggest multiple modes of selection and trade-offs between slow growth and size at the time of migration.

INTRODUCTION

Individuals within a population experience variable biotic and abiotic conditions that shape the characteristics of the survivors. In the majority of fish species, small changes in the interaction of these factors influence early life survival and can lead to large fluctuations in recruitment and subsequent year-class strength (Hjort, 1914; Anderson, 1988). Mortality in the early life stages of fish can exceed 99% (Peterson and Wroblewski, 1984; Bailey and Houde, 1989; Cowan et al., 1996), placing strong selective pressure on successful traits enhancing the probability of survival. Over a century of research on variable fish early life mortality has resulted in a suite of recruitment hypotheses on variability to better predict what drives fluctuations of marine fish populations.

High mortality rates in fish early life stages may appear random, but they most often remove individuals selectively from the population. Selective mortality can be biased against smaller or larger body size or growth rate, which are products of intrinsic (genetic) and external factors (e.g., temperature, food availability) (Miller et al., 1988; Cushing, 1990; Hare and Cowen, 1997). A critical stage occurs when fish larvae need to transition from endogenous yolk reserves to exogenous feeding (Hjort, 1914), and mismatches of this 'critical period' with pulses of prey abundance can lead to starvation and subsequent recruitment failure (Cushing, 1975; Cushing, 1990). Yet, predation is the largest source of mortality, not just for early life stages, but often for a fish's entire lifespan (Peterson and Wroblewski, 1984; Bailey and Houde, 1989). Predator physiology and behavior are important in determining which individuals are removed from a population, especially when individual variation in traits is high (Bailey and Houde, 1989; Paradis et al., 1996). Additionally, differences in larval behavior can impart survival advantages (Fuiman and Cowan, 2003).

There are multiple related hypotheses regarding how larval growth influences the probability of survival, collectively referred to as growth-mortality hypotheses (Anderson, 1988). The 'stage duration' hypothesis states that faster growing larval cohorts outgrow the window of highest vulnerability faster and therefore experience lower cumulative mortality rates (Cushing, 1975; Houde, 1987; Leggett and Deblois, 1994). The 'bigger is better' hypothesis states that faster growing larvae attain larger sizes-at-age than their slower growing conspecifics and therefore experience reduced starvation and predation risk (Ware, 1975; Anderson, 1988; Miller et al., 1988). By growing at a faster rate, individuals are larger at metamorphosis, can more effectively evade predators, and outgrow 'windows of vulnerability' set by gape limitations of predators (Bailey and Houde, 1989; Cowan et al., 1996; Paradis et al., 1996). Further, predation mortality has been shown to be inversely related to growth rate independent of size or stage, as stated by the 'growth-selective predation' hypothesis (Takasuka et al., 2003).

Individual variation in size and growth can arise due to many factors, both intrinsic and extrinsic, including genetic capacity for growth, age, temperature, prey abundance, and density-dependent effects. While fast growth is often advantageous, it can also be metabolically costly. This leads to tradeoffs, as allocation of energy to maximizing growth constrains the metabolic scope of an individual, resulting in reduced swimming performance and predator escape responses (Arendt, 1997; Arnott et al., 2006). Additionally, temperature has a direct, positive relationship with metabolic rate (Houde, 1989; Gillooly et al., 2001). An increase in prey consumption is therefore needed to sustain elevated growth rates caused by increases in temperature (Houde, 1989). Temperature also has a negative relationship with development time (O'Connor et al., 2007) and has been shown to be positively correlated with mortality (Houde, 1989; Pepin, 1991).

Temperature can also interact with other stressors, such as pH or dissolved oxygen, which may lead to increased metabolic costs (Gobler and Baumann, 2016; Murray and Baumann, 2018).

While most work has focused on larval fish mortality, it is imperative to understand selective survival during the juvenile stage as well. In some species, year class strength is determined in the early juvenile, rather than the larval stage (Leggett and Deblois, 1994; Bailey et al., 1996; Campana, 1996; Cowan et al., 2000; Baumann et al., 2006). Juveniles have completed metamorphosis, so vulnerability to predation is not associated with changes in morphology, pigmentation and behavior that occur as larvae (Sogard, 1997). The magnitude of size differences is often larger in juveniles, as differences in growth rate have accumulated over time. This pronounced individual variation in traits provides an opportunity for selection to occur. Additionally, juveniles can be quantitatively sampled using a beach seine, whereas sampling smaller fish often requires vessel-deployed equipment.

Otolith microstructure

If growth contributes to predation vulnerability, individual growth histories must be estimated to determine which characteristics are related to survival. While growth can be measured directly in the laboratory as weight or length gains per unit time, this is more difficult in field samples, as age cannot be controlled. This is an important limitation, as instantaneous growth rates are positively correlated with age (Pepin et al., 2001). Otolith microstructure analysis is a powerful tool to estimate individual age and daily somatic growth (Pannella, 1971; Brothers et al., 1976; Campana and Neilson, 1985). Otoliths are paired structures located within the inner ears of all teleost fishes, composed of crystalline calcium carbonate and a matrix of the protein otolin (Murayama et al., 2002). Each of the three otoliths pairs (lapillae, sagittae, asteriscii) are embedded

in sacs of endolymph fluid and surrounded by sensory cells, which aid in maintaining equilibrium and provide auditory functions (Popper and Lu, 2000). As part of a fish's statoacoustic sensory system, otolith size generally increases proportionally to fish size (Brothers et al., 1976).

The utility of using otoliths to estimate individual growth histories lies in the manner in which they grow. Differential deposition of calcium carbonate and protein creates zones that appear either translucent or dark, corresponding to growth and non-growth over a 24-hour period, respectively (Campana and Neilson, 1985). As such, one increment constitutes both dark and light zones and represent one day of growth. This daily periodicity in otolith microstructures has been verified for most fish taxa (Brothers et al., 1976; Campana and Neilson, 1985). By enumerating daily increments, individual age can be estimated, provided age of first increment formation is known (most commonly at hatch). Since otolith and fish size are correlated, measuring the width of each increment provides a proxy for daily somatic growth rate. The biological intercept method can be used to back-calculate length-at-age for each day of an individual's growth history (Campana and Neilson, 1985; Campana, 1990).

Individual growth histories become most insightful when correlated to environmental histories, e.g., temperature (Campana and Neilson, 1985). The influence of other abiotic or biotic factors affecting growth are also potentially detectable through changes in relative increment widths. Additionally, selective mortality of growth rates, such as predation on slower-growing individuals, could be examined by comparing the widths of the same-age increments of individuals sampled successively from a population (Hare and Cowen, 1997; Baumann et al., 2003). A shift in the distribution of growth rates would then be interpreted as selective removal of individuals with fast or slow growth.

Study species

The Atlantic silverside, *Menidia menidia* (Atherinidae), is a small forage fish commonly found in salt marshes and coastal waters along the east coast of North America, ranging from northern Florida (~30 °N) to the Gulf of St. Lawrence (~46 °N) (Jessop, 1983; Conover and Present, 1990). As an abundant inshore forage fish, this species is ecologically important as prey for larger, commercially and recreationally targeted species (Bayliff Jr, 1950; Conover and Ross, 1982). *M. menidia* is a semelparous batch spawner with semilunar spawning periodicity (Conover and Ross, 1982; Conover, 1985). The spawning season varies with latitude, but in Long Island Sound (~41°N) spawning typically begins in early May and continues until early July (Conover and Present, 1990). Repetitive batch spawning requires vast energy resources, after which almost all adults succumb to predation, given that two year old adults are very rare (Austin et al., 1975; Conover and Ross, 1982). Eggs are laid on submerged aquatic vegetation, and typically hatch within 6-12 days depending on temperature (Bengtson et al., 1987; Tewksbury and Conover, 1987). Larvae and early juveniles remain in nearshore waters throughout the summer and fall, until water temperatures fall below 12°C, when growth ceases and migration to deeper, typically offshore waters occurs (Conover and Murawski, 1982; Conover and Ross, 1982). Severe, size-selective mortality occurs during this overwintering phase, often exceeding 90% (Conover and Ross, 1982) and favoring larger individuals with greater accumulated energy reserves (Schultz et al., 1998).

M. menidia has become an important model organism for experimental research on a variety of eco-evolutionary questions, including fisheries-induced evolution (Conover and Munch, 2002), toxicology (Roark et al., 2005), local adaptation (Conover and Heins, 1987a; Conover and Present, 1990; Schultz et al., 1998), and impacts of climate change (DePasquale et al., 2015;

Murray et al., 2017). Growing seasons in northern populations are typically 6 months or less, while southern populations experience less severe seasonality in temperatures and do not undergo a winter migration and avoid winter mortality (Conover and Present, 1990; Schultz et al., 1998). In response, growth capacity exhibits countergradient variation across latitudes, where northern populations have genetically faster temperature-specific growth rates than southern conspecifics (Conover and Present, 1990). Other traits under local adaptation in *M. menidia* include vertebral number (Billerbeck et al., 1997), accumulation of lipids (Schultz and Conover, 1997), and swimming performance (Billerbeck et al., 2001). These local adaptations occur despite high gene flow that occurs when populations mix in winter habitats (Conover, 1998; Clarke et al., 2010).

Additionally, the Atlantic silverside was the first documented fish species to exhibit temperature-dependent sex determination (TSD) (Conover and Kynard, 1981), the degree of which decreases with latitude (Conover and Heins, 1987a; Duffy et al., 2015) and depends on parentage (Conover and Heins, 1987b). Experiments showed that the development of females is favored at lower temperatures, while temperatures of 17°C and above produce increasingly male-biased sex ratios. The temperature-sensitive period occurs late in the larval stage, when total length is between 8-21 mm (Conover and Fleisher, 1986). The current paradigm for the adaptive significance of TSD in *M. menidia* is that it extends the growing season for females, as female determination is favored at colder temperatures seen earlier in the year (Conover, 1984). A longer growing season would allow females to attain larger sizes prior to overwintering, increasing the likelihood of winter survival (Conover and Ross, 1982; Schultz and Conover, 1997). Further, this imparts a fitness advantage, as body size is hyperallometrically related to fecundity in female fishes (Wootton, 2012; Barneche et al., 2018).

Yet, TSD patterns in *M. menidia* have been determined by laboratory studies and have yet to be observed in wild-caught juveniles. Initial experiments showed that the level of TSD under genetic control varied with growing season length (Conover and Heins, 1987a), which required rearing individuals from different populations under common garden conditions, i.e., artificial laboratory settings. However, wild silversides experience stochasticity in their environment, and variability in extrinsic factors can impact survival and thus influence sex ratios. To assess the paradigm of TSD in wild silversides, both age and sex of individuals must be known. While larvae can be aged using otoliths, sex cannot be readily determined until the juvenile stage when gonads are sufficiently developed. Further, age and growth patterns of older *M. menidia* juveniles, as inferred from otolith microstructure analysis, have not been studied. Barkman (1978) confirmed growth increments in the *M. menidia* otoliths were deposited daily, and Barkman and Bengtson (1987) used microstructure analysis to assess daily growth, yet otolith analyses have not been extended to silversides older than 79 days post-hatch (dph). Thus, it has yet to be conclusively demonstrated that the sexual size dimorphism at the end of the growing season is due to differences in hatch dates, as predicted by TSD (Conover and Kynard, 1981; Conover, 1984). Additionally, it is unknown how mortality shapes the characteristics and sex ratios of juvenile survivors, but selective mortality in *M. menidia* larvae is known to occur with respect to size and growth (Juanes and Conover, 1994; Lankford et al., 2001).

Objectives

Hence, my study was motivated by earlier work on the Atlantic silverside, aiming to employ the power of otolith microstructure analysis to test the paradigm of adaptive significance of TSD in wild *M. menidia*. I hypothesized that females would be larger in total length than males,

and otolith-derived back-calculated hatch dates would show this dimorphism was due to differences in age, with females older than males. A second objective of these otolith analyses was to infer the growth characteristics of the juvenile silverside population that survived until the end of the growing season. I hypothesized that individuals with slower growth rates would be selectively removed from the population over time, as predicted by the growth-mortality hypotheses. Thirdly, estimates of length-at-age coupled with continuous temperature monitoring in the study area enabled estimation of temperature during the sex-determining period of each individual, when back-calculated length was between 8-21 mm. Since the *M. menidia* spawning season extends into early July, when temperatures are biased toward determination of males, I expected to find strongly male-biased sex ratios at the end of the season, following the laboratory predictions of Conover and Heins (1987a).

Study area

My study area was Mumford Cove, a small embayment in northeast Long Island Sound in Groton, Connecticut USA (41.32°N, 72.02°W, Figure 1). The 0.5 km^2 embayment has an average depth of 1 m with a shoreline comprised mostly of salt marsh and a 500 m sandy beach along the eastern shore (Vaudrey et al., 2010). Mumford Cove has semi-diurnal lunar tides with a mean tidal range of approximately 0.8 meters (NOAA tide gauge in New London, CT, station ID: 8461490), which flow into the cove via a ~615 m inlet flanked by sand bars that opens to Fisher's Island Sound. This tidal flushing creates a dynamic diel cycle of variable pH, dissolved oxygen, and temperature conditions. The subtidal habitat within Mumford Cove is currently dominated by eelgrass (*Zostera marina*), while shorelines excluding the beach are composed of marsh grass

(*Spartina* spp.). This vegetation provides spawning and nursery habitat for *M. menidia* and many other local fish species.

MATERIALS AND METHODS

Environmental monitoring and specimen collection

Temperature conditions (°C) within Mumford Cove were monitored continuously with a Eureka-Manta Sub2 probe in 30-minute intervals. The probe was deployed in the deepest region along the western side of the cove (Figure 1) on a subsurface buoy approximately 0.5 m above sandy bottom (average depth of 1.89 meters). Deployment alternated between 3 and 6 weeks, after which the probe was exchanged with a newly calibrated probe and renewed battery power. When removed from the water, the probe was cleaned of biofouling, data were downloaded, and the probe was recalibrated to check for drift. Prior to deployment, each probe was calibrated for salinity (FisherScientific conductivity standard 50,000 µS/cm).

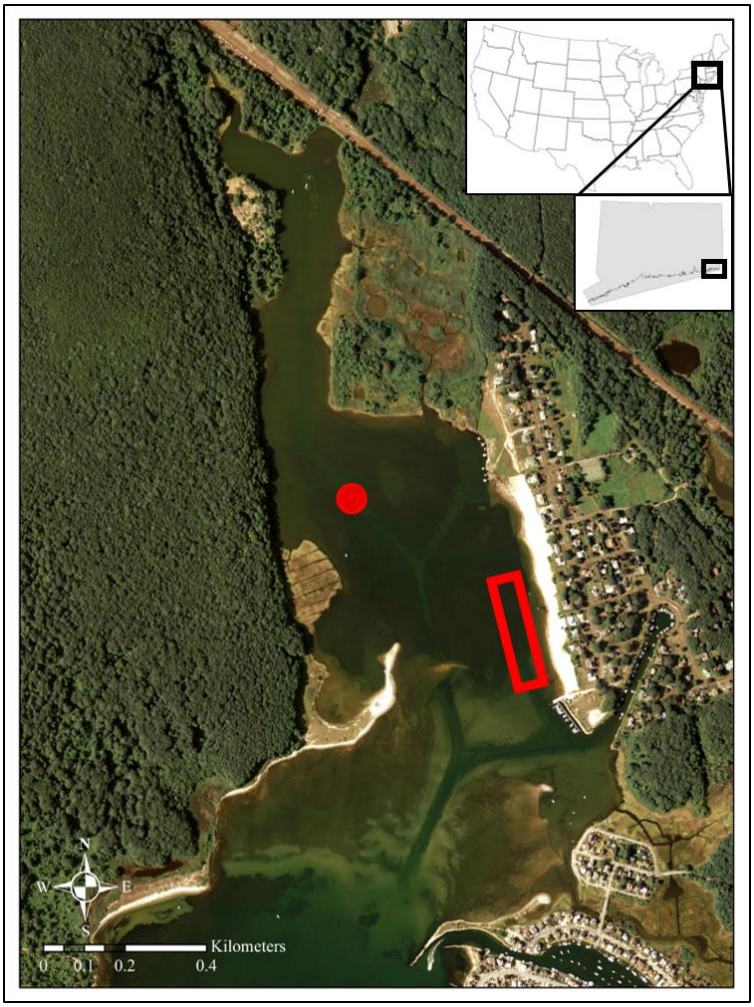

Figure 1. Satellite photo of the study area, Mumford Cove, Connecticut. The red dot represents the location of the probe which collected abiotic data. The red rectangle represents where specimens were collected with a beach

Four gaps were present in the data series due to battery and probe failures, including one 34-day gap (June 18-July 22). Temperature during this 34-day gap was estimated via linear regression between the Mumford Cove dataset and a 42-year time series from Millstone Power Station, in Waterford, CT, approximately 13 km west of Mumford Cove. The power plant monitors the water temperature on their intake pipes, which feed into Niantic Bay, an embayment slightly larger than Mumford Cove (4.2 km^2). Linear regression was performed with data from 2015-2016, with Millstone daily mean temperature as the independent variable (n=730) and Mumford Cove daily mean temperature as the dependent variable (n=537; Figure 2). A strong correlation was found between the two datasets (r^2=0.92, F=6024.9, p<0.001, MSE=3.56), allowing for temperature in Mumford Cove to be estimated as:

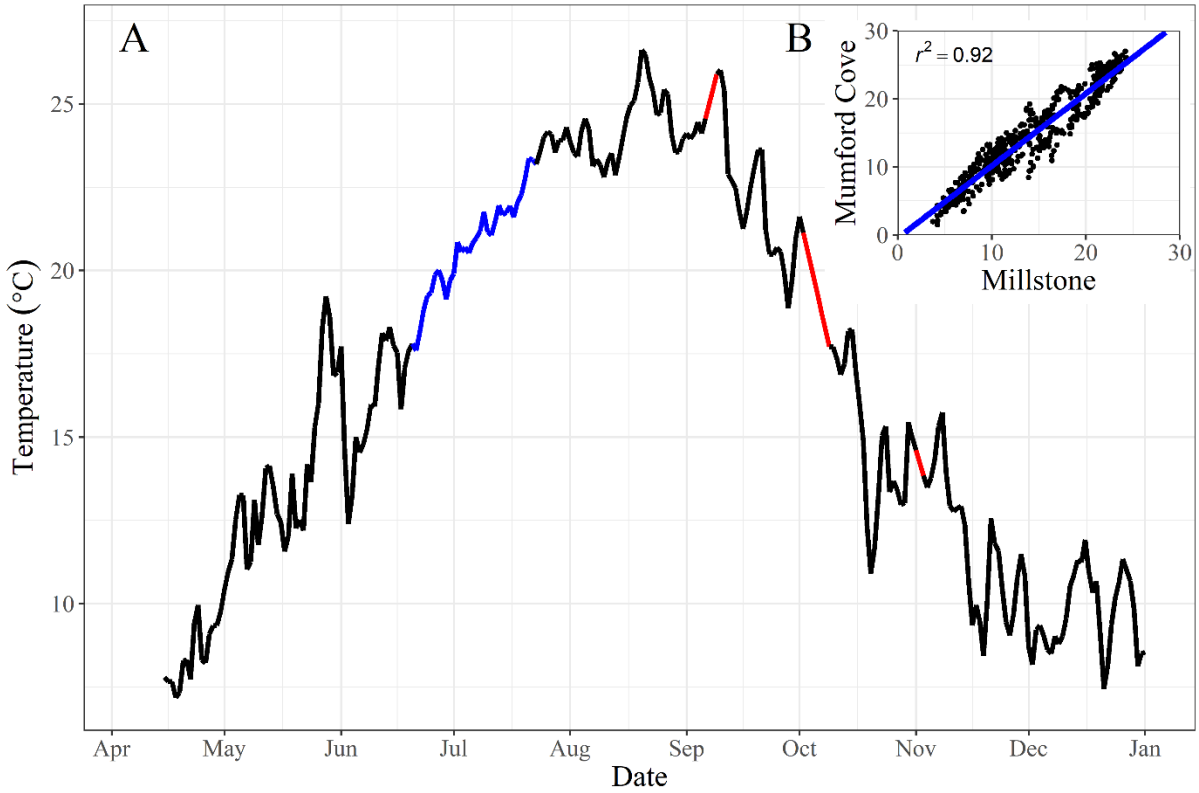

Figure 2. (A) Mean daily water temperature (°C) within Mumford Cove, CT from 2015, beginning in April. Data were continuously collected via a probe deployed ~1m from the bottom, with short data gaps estimated by linear interpolation (red lines). (B) A 34-day gap (blue line) in June-July was estimated via linear regression with a strongly correlated nearby temperature record from Niantic Bay, CT.

$$T_{Mumford} = 1.07(T_{Millstone}) - 0.54$$

Temperatures were reconstructed in this manner for the 34-day gap, while the three other data gaps were shorter in duration (<8 days) and were estimated via linear interpolation.

Menidia menidia were sampled from Mumford Cove three times (October 23, November 20, and December 18 of 2015) using a 30×2 m beach seine with 3 mm mesh. Fall collection dates ensured capture of juvenile young-of-the-year, while minimizing capture of adults spawners. On each collection date, two hauls were conducted along different sections of the beach on the eastern shore of Mumford Cove. Timing of the beach seine surveys was selected to coincide with a flood tide. All silversides were euthanized, enumerated, measured for total length (TL) to the lower 0.5 cm using a measuring board and subsequently preserved in a -20°C freezer.

Otolith processing and microstructure analysis

From each collection date, 100 individuals were selected for otolith microstructure analysis, for a total of 300 juveniles. A stratified random sampling design was used to obtain individuals across the entire size distribution of each collection. Each individual was assigned a unique identification number and measured for TL to the nearest 0.1 mm, wet weight (wW, nearest 0.01 g), sexed via visual inspection of gonads, and both sagittal otoliths were extracted. Sagittae were mounted on microscope slides using CrystalBond ™ 509 thermoplastic cement. The left or right sagitta was randomly chosen for analysis, unless a difference in clarity was observed, or if one sagitta became cracked while polishing. Each otolith was polished using 9 µm followed by 3 µm lapping film (3M®) in a circular motion until the entire reading axis was clearly visible under 400X magnification using a Nikon Eclipse E400 compound microscope. Following polishing, a

drop of immersion oil was placed on each otolith and let absorb for 3-24 hours to enhance the clarity of growth increments.

Otoliths were measured and read laterally across the sagittal plane of the otolith (Figure 3). Ageing started at the nucleus and extended to either the dorsal or ventral edge, depending on which region was most visible. This was not the longest axis on the otolith, as the convex shape of the otolith made the reading plane to the posterior edge too difficult to polish. While this reading axis differed from convention (Barkman and Bengtson, 1987; Campana, 1992), it was consistently the clearest axis that conformed to the expected linear otolith-fish size relationship ($Length_{fish}=185.7(Length_{otolith})-17.8$; $r^2=0.76$; $p<0.001$). Every increment, from the nucleus of the otolith to the periphery along the chosen axis, was measured and enumerated by marking its outer edge using Image Pro® Premier (V9.1) software and Lumenera® Infinity2-2 digital camera.

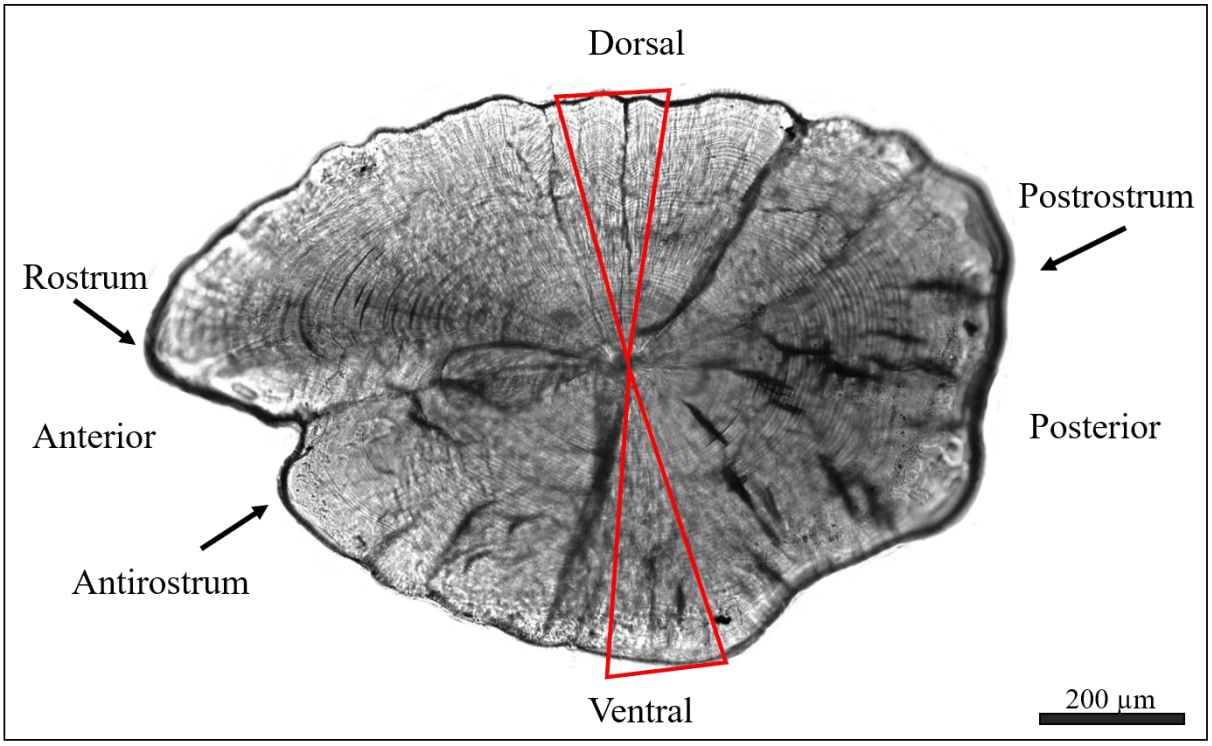

Figure 3. *M. menidia.* Polished, sagittal otolith of a juvenile (aged= 109 d) shown under 400X magnification. Major morphometric features are labelled. The counting axis for microstructure analyses started at the nucleus and extended to either the dorsal or ventral edges, outlined in red.

Multiples images were taken with different focal planes and merged to maximize clarity of different regions of the otolith. Because otoliths were too large to fit one image under 400X magnification, multiple images were taken by moving the stage without adjusting the focus to ensure precision of measurements. In addition to increment width and number, other measurements included cumulative sagitta width (µm), and diameter of the hatch-check (µm). The last growth increment was assumed to be incomplete and was excluded from growth analyses. Age post-hatch was determined for each individual as the number of all growth increments, assuming daily increment formation (Barkman, 1978; Barkman and Bengtson, 1987). Hatch date was calculated by subtracting age from the date of collection. The formation date of each increment was calculated as:

$$date\ of\ increment\ formation\ =\ sample\ date - age + \ increment\ number$$

thus the first increment represents the day after hatching.

Of 300 otoliths, six were found unreadable, making the total sample size for otolith analyses 294 (101 from Oct 23, 97 from Nov 20, 96 from Dec 18). Due to the stratified random sampling design, less frequent size classes were overrepresented in the otolith subsamples compared to the size distribution in the seines for each collection (Figure 4). The least frequent size classes in October (11.5-11.9 cm, n=4; 12.5-12.9 cm, n=1), November (4.0-4.4 cm, n=3; 9.5-9.9 cm, n=5) and December (4.5-4.9 cm, n=1) were not represented in the subsamples for otolith analysis.

To correct for over- and underrepresentation of certain size classes in the otolith subsamples, a sex-specific scale factor was applied to each size class. For each collection, I determined the sex ratio of each size class and used it to scale overall sex ratios of each collection.

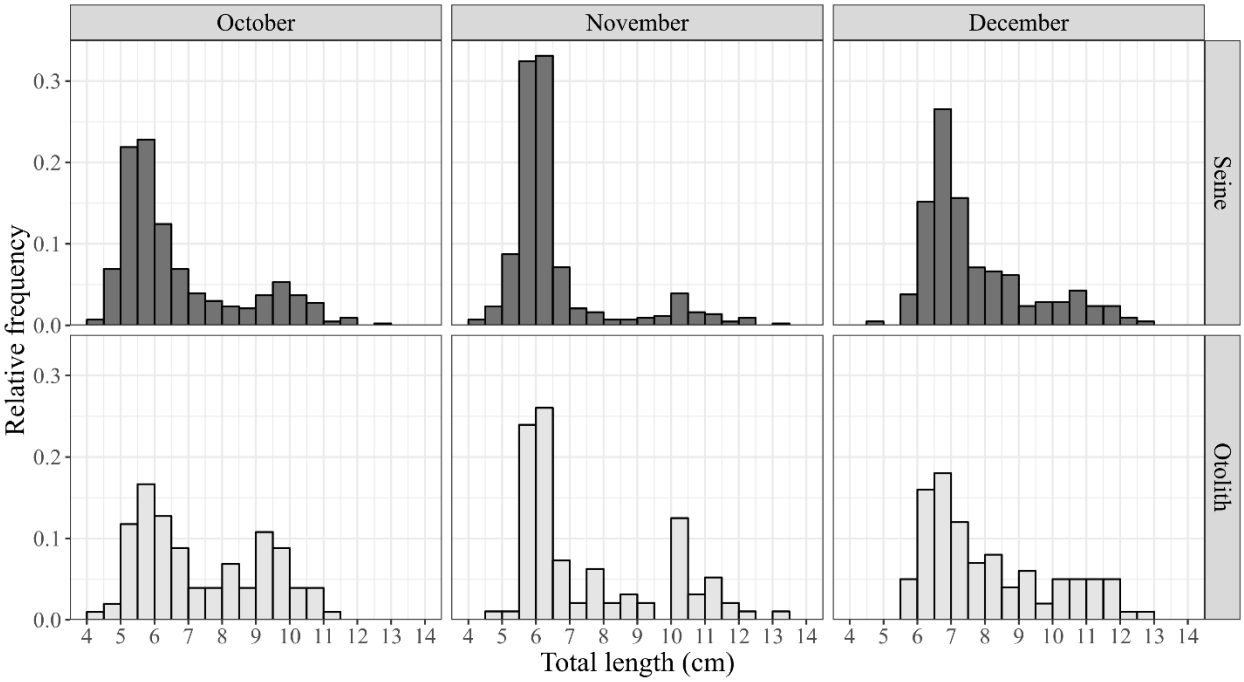

Figure 4. *M. menidia.* TL frequency distributions of juveniles captured by beach seine during October, November and December 2015 (upper panels, dark gray) and those subsampled for the otolith microstructure analysis (lower panels, light gray).

Sex-specific frequencies of each size class (*i*) were then scaled by the relative frequencies of that size class in the otolith sample and in the seines for each sex and each collection date using the ratio:

$$(relative\ frequency\ of\ size\ class\ i)_{seine}\ /\ (relative\ frequency\ of\ size\ class\ i)_{otolith}$$

Similarly, hatch distributions were scaled to the size frequency of the population sampled during each collection date.

Otolith readings were quality-controlled by estimating ageing precision and reader bias via within and across reader calibrations, using a ~10% subsample of analyzed otoliths (30 out of 294, 10 from each collection). An experienced reader independently read the same images for each otolith within the calibration set, and this was repeated with the original reader. Precision was estimated by calculating the average percent error (APE) with the second reader's estimates and original reader's first and second counts (Chang, 1982; Campana and Jones, 1992). In all but 2

instances, the APE was less than 10%, and the overall APE was 6%. However, age estimates from the second reader did not randomly deviate from those of the first reader, but were higher in all but one instance (mean difference = 14.1 days). The within-reader APE was 3.6%.

Both the mean and variance of increment width are known to be correlated with age (Barkman and Bengtson, 1987; Pepin et al., 2001), hence the widths of increments formed at the same date by individuals of different age were not immediately comparable. I used two methods to overcome this problem. First, individuals were grouped into 2-week hatch intervals, given the semilunar spawning periodicity in this species (Conover, 1985) (Table 1). Second, increment widths were standardized to zero mean and unit deviation:

$$z_{ij} = \frac{x_{ij} - x_j}{s_j}$$

where x_{ij} is the increment width (µm) of individual i at age j, while x_j and s_j are the mean and standard deviation at age j of all individuals within the otolith sample, respectively. This calculation was done for males and females separately to evaluate sex-specific growth patterns. Standardizing increment width to zero mean and unit deviation statistically removes the influence of age on increment width and thus allows deviations in age-detrended increment width to be observed among different cohorts. Standardized increment widths were used to examine potential patterns of selective mortality, i.e., by comparing the distributions of standardized increment widths between subsequent collections. Since the distribution of standardized increment widths violated parametric assumptions, shape and changes in the distributions were qualitatively analyzed by plotting the sex-specific 10^{th}, 50^{th} and 90^{th} percentiles for each day of increment formation. Comparing percentiles between successive collections (Oct-Dec) allows potential changes to be interpreted as the selective disappearance of either fast- or slow-growing individuals from the population. Thus, if individuals with slow growth rates selectively disappeared from the

Hatch Interval	Date	Julian Day	Total n		Relative Frequency	Relative Frequency (scaled)
			Male	Female		
1	6/7 – 6/20	158 -171	7		2.4%	2.6%
			1	6		
2	6/21 – 7/4	172 – 185	17		5.8%	5.0%
			9	8		
3	7/5 – 7/18	186 – 199	59		20.1%	20.3%
			34	25		
4	7/19 – 8/1	200 – 213	70		23.8%	22.6%
			33	37		
5	8/2 – 8/15	214 – 227	50		17.0%	17.9%
			31	19		
6	8/16 – 8/29	228 – 241	46		15.6%	15.4%
			26	20		
7	8/30 – 9/12	242 – 255	39		13.3%	14.3%
			24	15		
8	9/13 – 9/26	256 - 269	6		2.0%	1.7%
			3	3		
Total			**294**			
			161	**133**		

Table 1. Summary of sex-specific sample sizes within each back-calculated hatch interval from otolith subsamples. The relative frequency has been scaled to be representative of the size distribution captured in the seines, based on the ratio of relative frequencies of 0.5cm size classes between the seine and individuals within the otolith subsample. Dates are from the year 2015.

population between October and December, the overall distribution of standardized increment widths would shift towards wider increments in successive collections.

Length-at-age (L_a) was back-calculated using the biological intercept method (Campana, 1990):

$$L_a = L_c + (O_a - O_c) * (L_c - L_h) * (O_c - O_h)^{-1}$$

where L_a is length at age *a* (mm), L_c is total length at catch (mm), O_a is otolith radius at age *a* (µm), O_c is otolith radius at catch (µm), L_h is length at hatch (mm), and O_h is otolith radius at hatch (µm). I used an L_h value of 5 mm based on previous studies (Barkman and Bengtson, 1987; Bengtson et al., 1987; Murray et al., 2014). Using daily L_a back-calculations, instantaneous daily growth rate

was back-calculated for each individual using the following equation, where j represents age in days post hatch:

$$(La_j - La_{j-1})$$

Statistical analyses

Data were tested for normality and homogeneity of variance to determine whether parametric or nonparametric tests were appropriate. When analyzing traits across different collections or hatch intervals, analyses of variance (ANOVA) or a Kruskal-Wallis tests were used. Within each collection and/or hatch interval, sexes were assumed to be independent and pooled variance two sample t-tests or Mann-Whitney tests were used to test for significant differences between males and females. Nonparametric tests were used when comparing TL distributions, while parametric tests were performed on hatch and age distributions. For comparisons of larval growth rates, the first 30 increments (30 dph) were averaged. A repeated mixed-effects ANOVA was conducted to determine interactions between back-calculated daily growth rates, age, sex, and collection date. All statistical analyses were performed using SPSS Statistics (IBM, V20) and the statistical software R (cran.r-project.org, V3.5) with RStudio (V1.1.453) including tidyverse, cowplot, ggpubr and lubridate packages.

RESULTS

Seasonal temperature conditions in Mumford Cove

During the study period in 2015, temperature showed large seasonal, diel, and tidal variability (Figure 2). Maximum temperature of 28.4°C occurred on August 19 at 19:30, while the coldest temperature of 7.6°C occurred on December 6 at 04:30. Daily temperature fluctuations

were most severe on June 15, when the temperature fluctuated 6.5°C (14.9-21.5°C). Salinity ranged from 27.2-32.5 psu.

August was the warmest month of 2015 with a mean monthly water temperature of 24.3°C. Excluding January-March, when silversides overwinter in deeper waters, Mumford Cove was coldest in December, with a mean temperature of 9.8°C. The largest monthly increase in water temperature occurred between June and July, when mean temperature increased from 17.3 to 22.3°C (Figure 2). Conversely, the largest monthly temperature decrease occurred between September and October, with mean temperatures decreasing from 22.6 to 16.1°C. The first mean daily temperature to surpass 17°C occurred on May 31, while temperatures were consistently > 17°C after June 10. Mean daily temperature reached 19°C on June 23, and remained at least 19°C until July 7, when temperatures surpassed the 21°C TSD threshold.

On October 23, the day of the October collection, mean water temperature was 15.3°C. On this day, the lunar cycle was a waxing moon, 4 days prior to a full moon. On November 20, mean water temperature was 12.6°C. The lunar cycle for the November collection was also waxing, 5 days prior to a full moon. On December 18, the day of the final collection, mean water temperature was 10.7°C. The moon was at its first quarter, one week between the new and full moons.

Size distributions and sex ratios

A total of 434 *M. menidia* with a mean TL (±SD) of 6.4 ±1.8 cm were captured during the October sampling (Table 2). The majority of fish (45%) were 5-6 cm in TL. A smaller, second mode in TL was observed, with 23 individuals within the 9.5-9.9 cm size class, representing 5% of the sample. This multimodality gave the distribution a positive skew (Table 2). The male to female ratio was 60:40%.

In November, a total of 435 silversides were collected with a mean TL of 6.3 ±1.6 cm (Table 2). The most numerous size classes were 5.5-6.4 cm, comprising 66% of the overall catch. While much smaller in abundance, there was a second mode of larger silversides 10.0-10.4 cm in TL (3.9% of total catch), making the size distribution strongly skewed to the right (Table 2). The male to female ratio was 77:23%.

In December, juvenile silverside abundance decreased to 211 individuals with a mean TL of 7.5 ±1.6 cm (Table 2). Similar to the other collections, the December TL distribution was also skewed to the right. Silversides of 6.0-7.4 cm TL comprised 57% of the total catch, with a mode in the 6.5-6.9 cm size class (27%). The male to female ratio was 63:37%.

Total Length (cm)	**Oct**		**Nov**		**Dec**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
n	434		435		211	
	259	175	336	99	132	79
Mean	6.423		6.276		7.469	
	5.768	7.391	5.772	7.985	6.848	8.506
Median	5.50		6.0		7.0	
	5.500	7.000	5.500	6.500	6.500	8.00
SD	1.804		1.557		1.624	
	1.0595	2.204	0.7752	2.206	1.0244	1.8972
Range	8.5		9.0		8.0	
	5.5	8.0	7.0	7.5	7.0	6.5
Skewness	1.168		2.116		1.178	
	1.620	0.267	2.645	0.437	1.537	0.314
Kurtosis	0.255		3.942		0.567	
	2.841	-1.375	13.005	-1.332	3.759	-1.194

Table 2. Descriptive statistics for total length (cm) of male and female *M. menidia* collected via beach seine on October 23, November 20, and December 18 of 2015.

Sexual size dimorphism

Regardless of collection, females were consistently less abundant and substantially larger than males (Table 2). Size distributions of all three collections suggested at least two cohorts, with modes below and above 8 cm. In October, 94% of males but only 56% of females were smaller than 8 cm, whereas 6% of males and 44% of females were larger than 8 cm (Figure 5A). Mean female TL was 7.4 cm, while males measured on average 5.8 cm (Figure 5B; Mann-Whitney, standardized U=7.08, p<0.001). The female size distribution was clearly bimodal (modes at 5 and 9.5 cm) and had the widest range (Table 2), whereas the male distribution was unimodal and highly skewed to the right (Figure 5A). Mean TL in November was 5.8 and 8.0 cm for males and females, respectively (Figure 5B; Mann-Whitney, standardized U=10.63, p<0.001). Approximately 56% of females were <8 cm, whereas 97% of males were <8 cm. Silversides >8 cm were almost exclusively female. The female TL distribution in November had modes at 6 and 10 cm (Figure 5A), while the male distribution was heavily skewed to the right, with data heavily concentrated in the right tail (kurtosis=13.005; Table 2). Females collected in December had a mean TL of 8.5

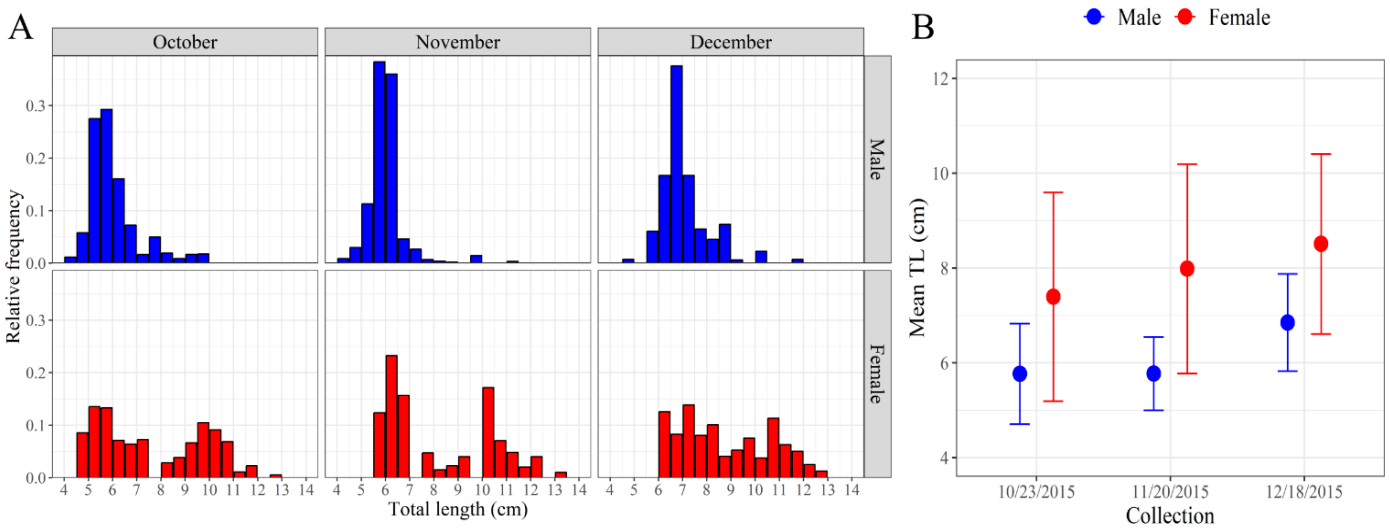

Figure 5. *M. menidia*. (A) Sex-specific TL distributions of juveniles collected during three successive collections in Mumford Cove, CT. (B) Mean TL ± SD.

cm, while males were on average 6.8 cm (Figure 5B; Mann-Whitney, standardized U=6.41, p<0.001). 84% of males and 43% of females were <8 cm (Figure 5A). Conversely, females were twice as abundant as males within larger size classes in December. Across all collections, sex ratios of silversides >8 cm were greater than 70% female (Figure 6A).

Mean TL increased across collections, with the largest increase occurring in males between November and December (Figure 5B). With males and females combined, the distribution of TL in the December collection was significantly larger than both the November and December collections (Kruskal-Wallis with Dunn-Bonferroni, df=2, p<0.001), while the distributions were

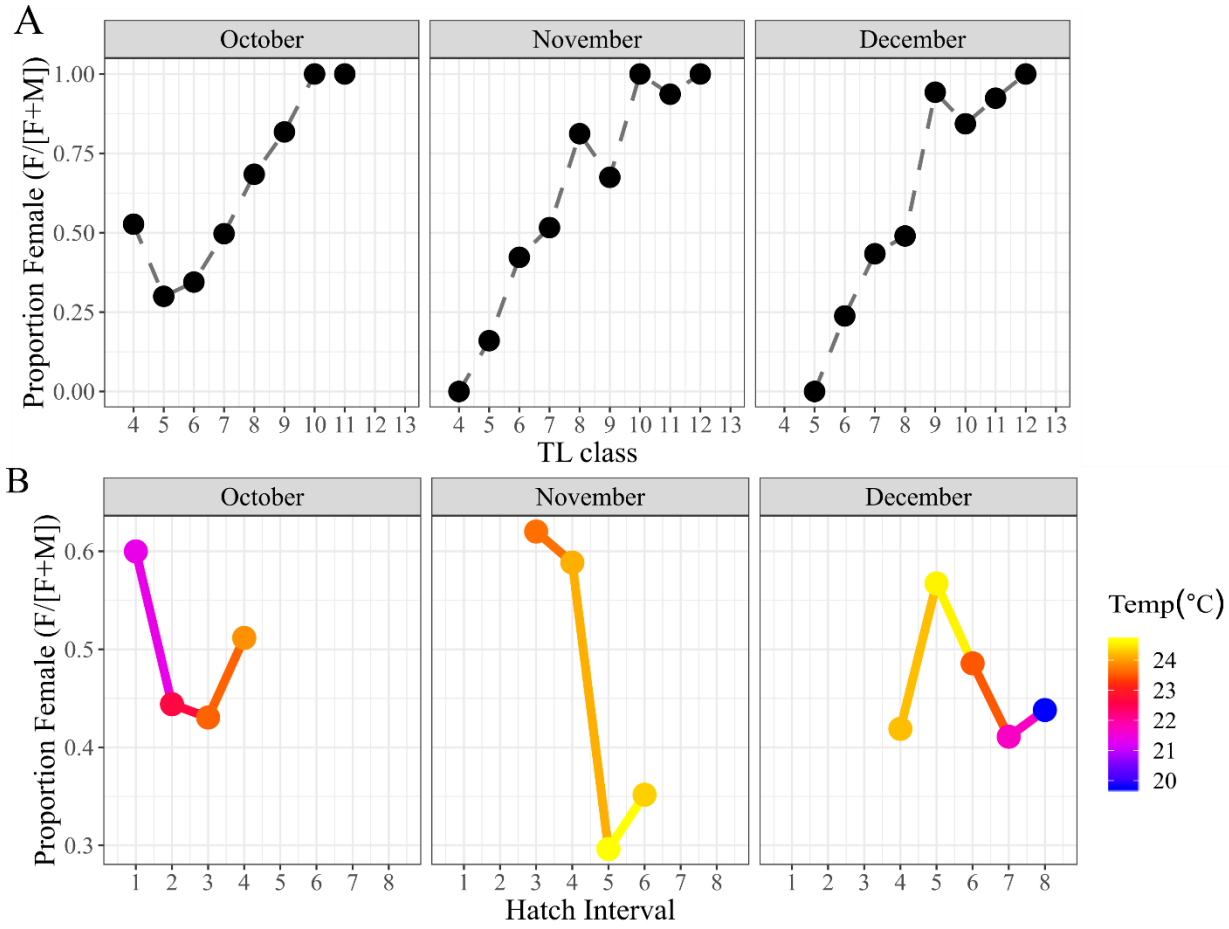

Figure 6. *M. menidia.* (A) Length-specific proportions of female juveniles collected during October, November and December 2015. (B) Female proportions within each biweekly hatch interval. Colors represent the mean temperatures (°C) within the sex-determining period of each hatch interval.

not different between October and November (p=1.00). The same pattern occurred for the male TL distributions across collections. For females, TL in December was the largest, but the difference was significant only when compared to female TL in October (Figure 5B; Kruskal-Wallis with Dunn-Bonferroni, df=2, p<0.001). Females from November were larger than those collected in October (p=0.031).

Hatch dates and age

Combining males and females, estimated mean hatch date (± SD) for all samples was August 3 ±22 days, with a mean age of 109.0 ±12.4 days post-hatch (dph). The range of hatch dates was 101 days, with a hatching period lasting from 6/11 until 9/20. Based on this range, individuals were placed into 8 biweekly hatch intervals (Table 1). Mean hatch dates increased successively for each collection. With males and females combined, mean ± SD hatch dates for each collection were 7/11 ±11.2 days, 8/3 ±10.3 days and 8/26 ±13.3 days for October, November, and December, respectively. When collections were combined, mean hatch dates were not significantly different between males and females (t-test, df=289, t=0.96, p=0.34). Mean female hatch date was August 1 ±21.8 days, while mean male hatch date was August 4 ±22.1 days. Likewise, females were on average 109.9 ±12.6 days old, while mean male age was 108.2 ±12.2 dph (t-test, t=-1.21, df=289, p=0.23). Overall, males ranged in age from 85-154 dph, while female age ranged from 86-150 dph. The earliest hatch dates were 6/11 for females, and 6/20 for males, while the latest hatch dates were 9/17 and 9/20 for females and males, respectively.

The mean hatch date of silversides sampled in October was July 11 for both males (SD=10.1) and females (SD=12.5) (Table 3; t-test, t=0.12, df=98, p=0.91). The oldest and youngest females hatched on 6/11 (age=134 dph) and 7/29 (86 dph), while males hatched from

6/20 (125 dph) to 7/30 (85 dph). However, 79% of males and 76% of females from this collection hatched between July 5-August 1 (Figure 7). Both the male and female hatch distributions were slightly skewed to the left (Table 3). Mean male age within this collection was 103.5 ±10.1 dph, while mean female age was 103.8 ±12.5 dph.

For the November collection, mean hatch date was August 4 ±10.4 days for males, and August 1 ±10.1 days for females (Table 3). This 3-day difference in mean hatching was not significant (t-test, t=1.63, df=94, p=0.11). The oldest female hatched on 7/12 (131 dph), while the youngest female hatched on 8/22 (90 dph). The oldest and youngest males hatched on 7/7 (136 dph) and 8/25 (87 dph), respectively. Peak hatching for silversides collected in November occurred

Julian day	**Oct**		**Nov**		**Dec**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
n	53	47	55	42	53	43
Mean	192.47	192.21	216.20	212.76	238.82	236.71
Median	192.00	196.32	215.00	211.60	242.00	237.40
SD	10.132	12.460	10.374	10.077	13.943	12.441
Range	40.00	48.00	49.00	41.00	65.00	58.00
Skewness	-.381	-.737	-.513	.028	-1.064	-.888
Kurtosis	-.155	-.158	-.510	-.640	1.200	1.185

Table 3. Descriptive statistics for back-calculated hatch dates of male and female *M. menidia* collected on October 23, November 20, and December 18 of 2015. Data are Julian days of hatching.

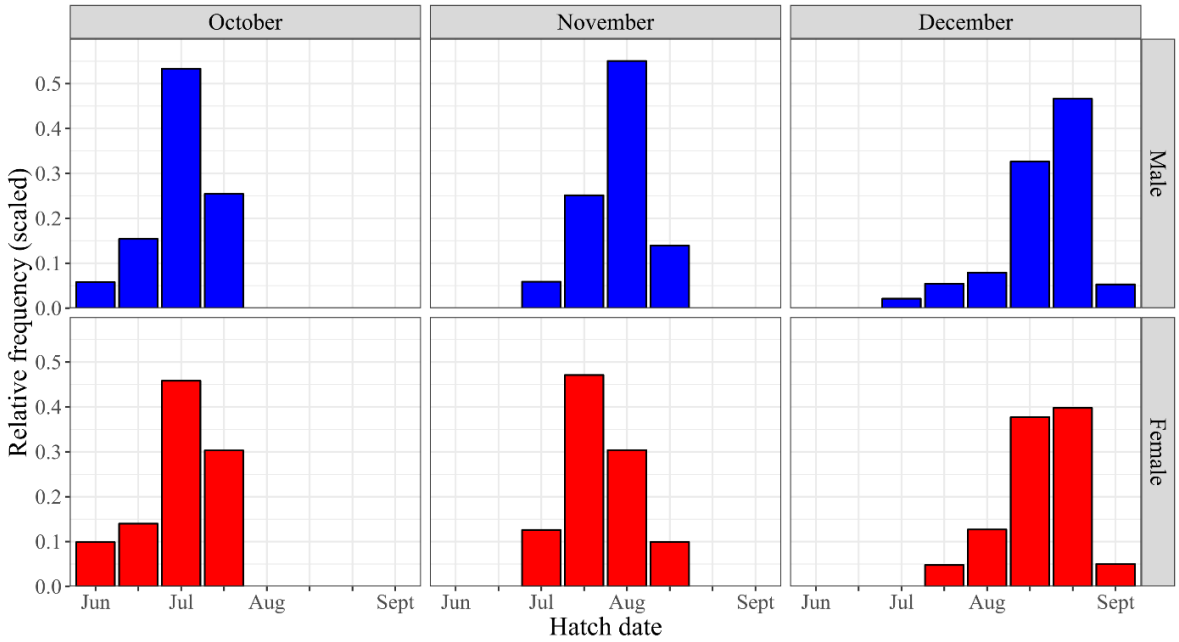

Figure 7. *M. menidia.* Sex-specific back-calculated hatch distributions of male (blue) and female (red) juveniles collected during October, November, and December 2015. Data are relative frequencies of scaled sample sizes hatched within each hatch interval.

between July 19- August 15, when 80% of males and 77% of females hatched (Figure 7). Within this collection, the male distribution was more variable, with a larger range and standard deviation, and a slight left skew (Table 3). Mean ages within the November collection were 107.8 ±10.4 dph and 111.2 ±10.1 dph for males and females, respectively.

Individuals sampled in December had mean hatch dates of August 27 ±13.9 days (males) and August 25 ±12.4 days (females) (Table 3; t-test, t=0.77, df=94, p=0.44). Male hatch dates ranged from 7/17 (154 dph) to 9/20 (89 dph). Females in this collection hatched from 7/21 (150 dph) to 9/17 (92 dph). The December collection was the most variable, with standard deviations of approximately two weeks, and a hatching range of approximately two months (Table 3). Both male and female hatch distributions were skewed to the left more strongly than other collections (Figure 7). Peak hatching occurred between August 16 and September 12, with 79% of males and 77% of females hatching in this period. Mean male age within this collection was 113.2 ±13.9 dph, while mean female age was 115.3 ±12.4 dph.

Hatch distributions of females from each collection were significantly different from each other (Figure 7; 1-way ANOVA with Tukey HSD, $F=160.20$, $df=2$, $p<0.001$). Females from the October collection hatched significantly earlier than those of November and December collections, while the November females hatched significantly earlier than December females ($p<0.001$). The same pattern was observed for males, where each successive collection had later hatch dates, with significant pairwise comparisons (Figure 7; 1-way ANOVA with Tukey HSD, $F=210.8$, $df=2$, $p<0.001$). Additionally, age was significantly different across collections for both males and females (1-way ANOVA with Tukey HSD, $df=2$, male: $F=9.2$, $p<0.001$; female: $F=11.1$, $p<0.001$). Males from October were significantly younger than those collected in December ($p<0.001$), yet there was no difference in age with those collected in November ($p=0.14$). However, November males were significantly younger than December-collected males ($p=0.046$). In October, females were significantly younger than those collected in November and December ($p=0.01$, $p<0.001$), yet there was no significant age difference between November- and December-collected females ($p=0.26$).

There was a negative trend in the proportion of females with increasing hatch intervals, where individuals hatched later in the season were less likely to be female (Figure 6B). For the October and November collections, the proportion female was negatively related to the mean temperature during the sex-determining period, when back-calculated length was 8-21 mm. However, silversides collected in December had the opposite trend, where individuals hatched later experienced colder temperatures and were less likely to be female (Figure 6B). Within the December collection, females were more frequent (57%) only during the 5^{th} hatch interval (8/2-8/15), when temperatures during the thermosensitive period averaged 24.7°C. Within the October and November collections, the sex ratio of earlier-hatching silversides was the highest, 60% or

greater, when temperatures were 21.3-23.6°C. Regardless of collection, all fish experienced temperatures greater than 19°C during their thermosensitive period.

Back-calculated growth rates

Throughout the entire growth trajectory, females exhibited higher mean growth rates than males, regardless of collection. Mean female back-calculated instantaneous growth rate (±SD) of all collections was 0.66 ±0.27 mm day^{-1}, while mean male growth was 0.48 ±0.20 mm day^{-1}, 27% slower than female growth rate. The mean maximum instantaneous growth rate was 1.36 mm day^{-1} for females, substantially greater than that of males (1.03 mm day^{-1}). Females grew significantly faster than males across increments, but no significant interaction of sex, increment number, and collection date was found (Repeated mixed-effects ANOVA, increment×sex: $F_{98,125}=3.27$, $p<0.001$; increment×sex×collection: $F_{196,252}=1.06$, $p=0.337$). With all collections combined, females grew faster than males immediately after hatching, and growth remained significantly faster until 126 dph (Mann-Whitney, standardized $U<-2.12$, $p<0.034$).

Within the October collection, larval growth rates during the first 30 dph, were similar between males and females (Figure 8A). At approximately 15 dph, back-calculated growth rates diverged, with females growing at a substantially higher rate than males for the remainder of the trajectory. For the November and December collections, females grew faster from 1 dph and throughout the remainder of the growing season, until approximately 126 dph (Figure 8A). Across collections, the shapes of the growth trajectories were similar between males and females, but female growth accelerated faster with age. Mean back-calculated growth peaked at >1.0 mm day^{-1} for females between 45-48 dph within each collection. Maximum mean daily growth for males was 0.79-0.87 mm day^{-1}, with the peak occurring slightly earlier in the trajectory at 38-42 dph

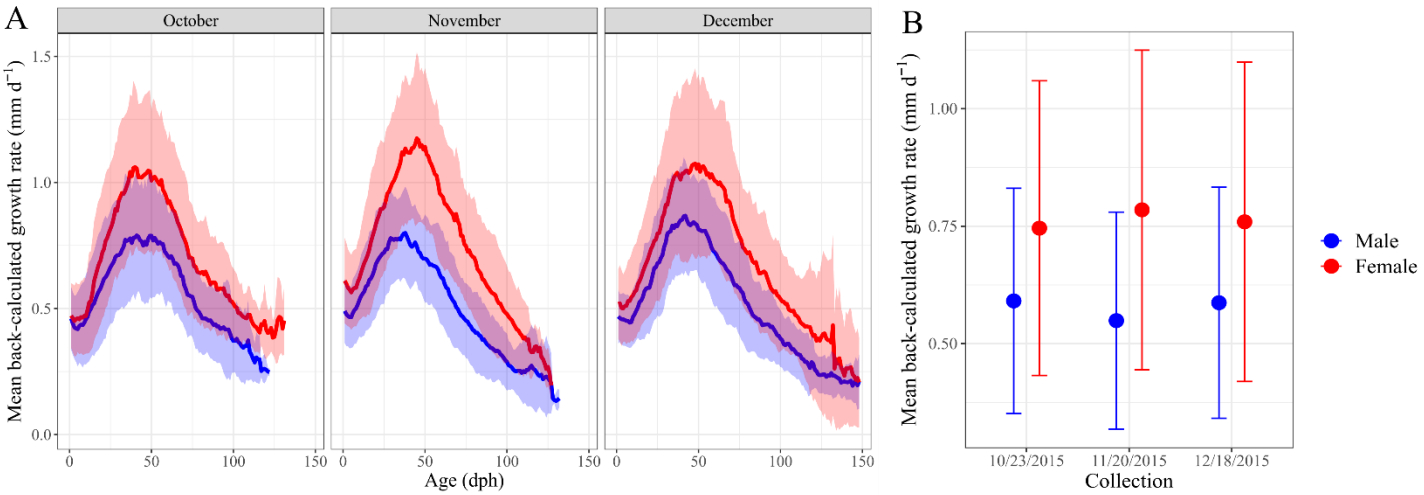

Figure 8. *M. menidia.* Sex-specific back-calculated growth rates of juveniles collected in October, November, and December 2015. Solid lines and shading represent daily means ± SD for each day post hatch (dph, omitted where n<3). (B) Mean ± SD of back-calculated growth throughout the entire trajectory in (A).

(Figure 8A). Averaged across the entire growing season, females grew at least 0.12 mm day^{-1} faster than males within each collection (Figure 8B; Mann-Whitney, standardized U> 24.91, p<0.001). Yet, throughout all collections, female back-calculated growth rates were more variable than males.

Sex-specific differences in growth remained when samples were analyzed across separate hatch intervals and collection dates (Figure 9A). When dividing into hatch intervals, growth trajectories can be analyzed in relation to the Julian day of increment formation, since each hatch interval contains fish of approximately the same age. Hatch intervals 1 from October, and 3 from December contained a single male, and thus sex-specific comparisons could not be made for these intervals. Males hatched within the fourth hatch interval and captured in December grew faster than females for approximately 34 days, from Julian days 239-272, the only period in which this occurred (Figure 9A, Mann-Whitney, standardized U=0.15, p=0.88). For all other hatch intervals

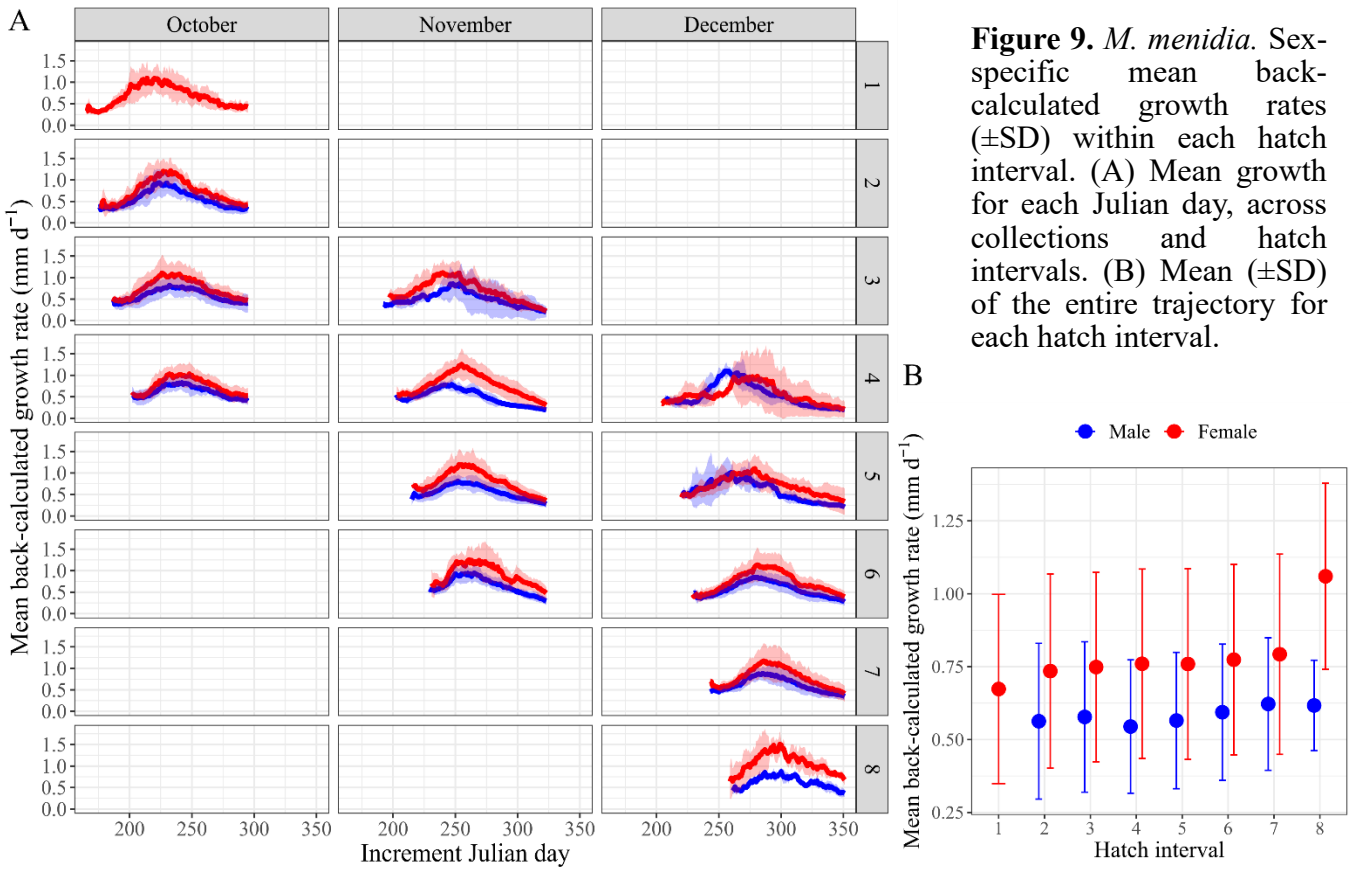

Figure 9. *M. menidia.* Sex-specific mean back-calculated growth rates (±SD) within each hatch interval. (A) Mean growth for each Julian day, across collections and hatch intervals. (B) Mean (±SD) of the entire trajectory for each hatch interval.

and across all collections, mean female back-calculated growth was significantly faster than that of males, averaged over all increment Julian days (Mann-Whitney, standardized U>8.14, p<0.001). Across collections, mean growth for each hatch interval was 0.67-1.06 mm day^{-1} for females, and 0.54-0.62 mm day^{-1} for males (Figure 9B). When larval growth (1-30 dph) was analyzed across Julian days and divided into collections and hatch intervals, sex-specific differences in mean back-calculated growth rates were reduced (Figure 10A). For each collection, males and females hatching at approximately the same time exhibited nearly identical growth patterns for the first ~20 dph, after which females began to grow at a faster rate. Yet within each hatch interval, larvae that became females grew significantly faster on average than larvae that became males (Figure 10B; Mann-Whitney, standardized U>3.66 p<0.001).

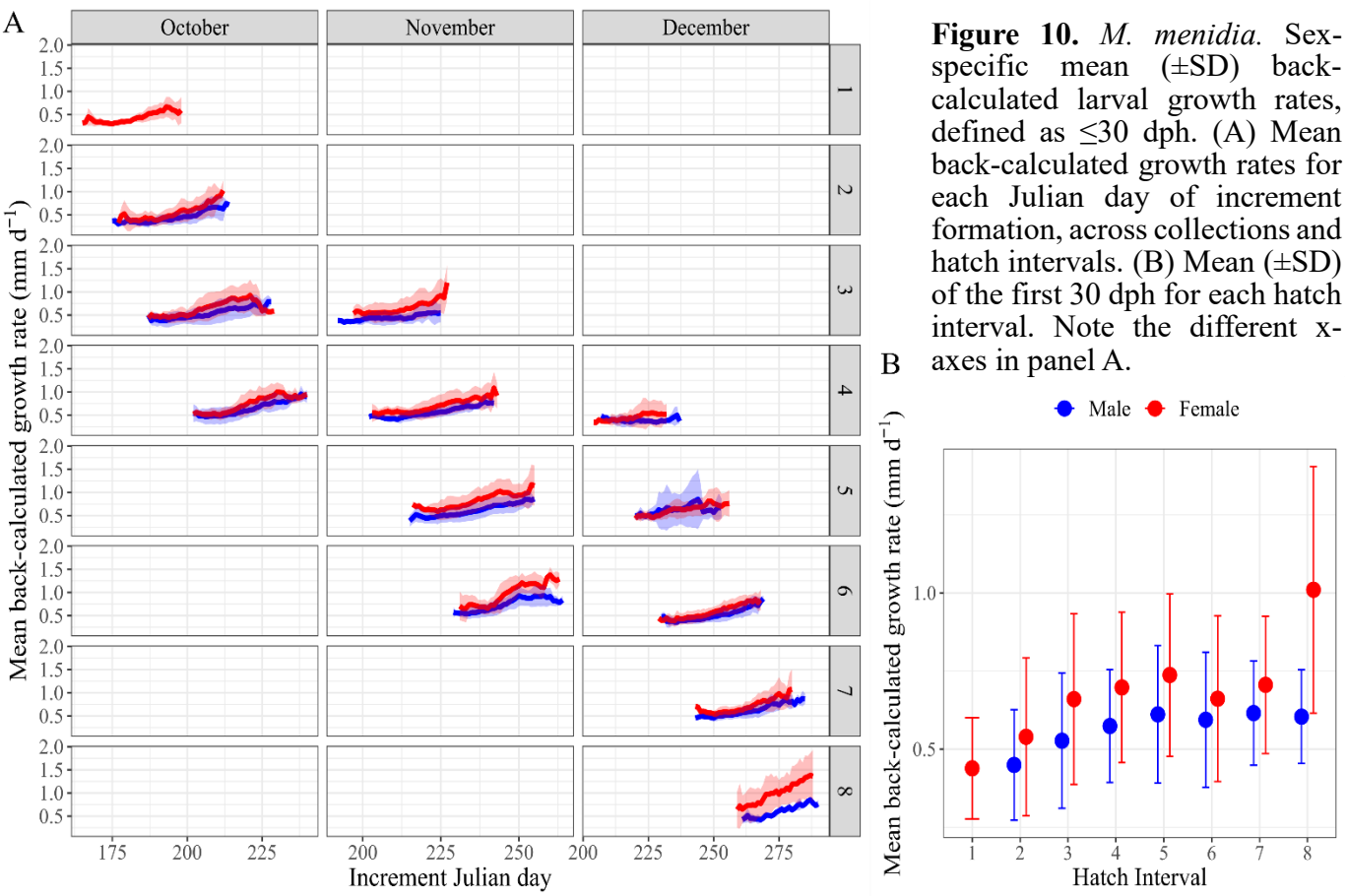

Figure 10. *M. menidia.* Sex-specific mean (±SD) back-calculated larval growth rates, defined as ≤30 dph. (A) Mean back-calculated growth rates for each Julian day of increment formation, across collections and hatch intervals. (B) Mean (±SD) of the first 30 dph for each hatch interval. Note the different x-axes in panel A.

These growth differences accumulated over time, leading to considerable sex-specific differences in back-calculated length at age (L_a). For each collection, male and female back-calculated L_a diverged beginning at approximately 38 dph (Figure 11). This divergence was most pronounced within the November collection, where mean female L_a was at least 2cm larger than that of males beginning at 75 dph. Even when increment widths were standardized to remove autocorrelation with TL, females grew faster than males throughout most of the trajectory. Mean back-calculated larval growth was positively correlated to temperature for males and females collected in October and November (Figure 12). This relationship was weak for November-collected larvae, as these individuals experienced a narrow temperature range during the first 30 dph. Back-calculated growth rates of larvae from the December collection exhibited a negative relationship to temperature, growing fastest at temperatures 21-22°C. These colder temperatures

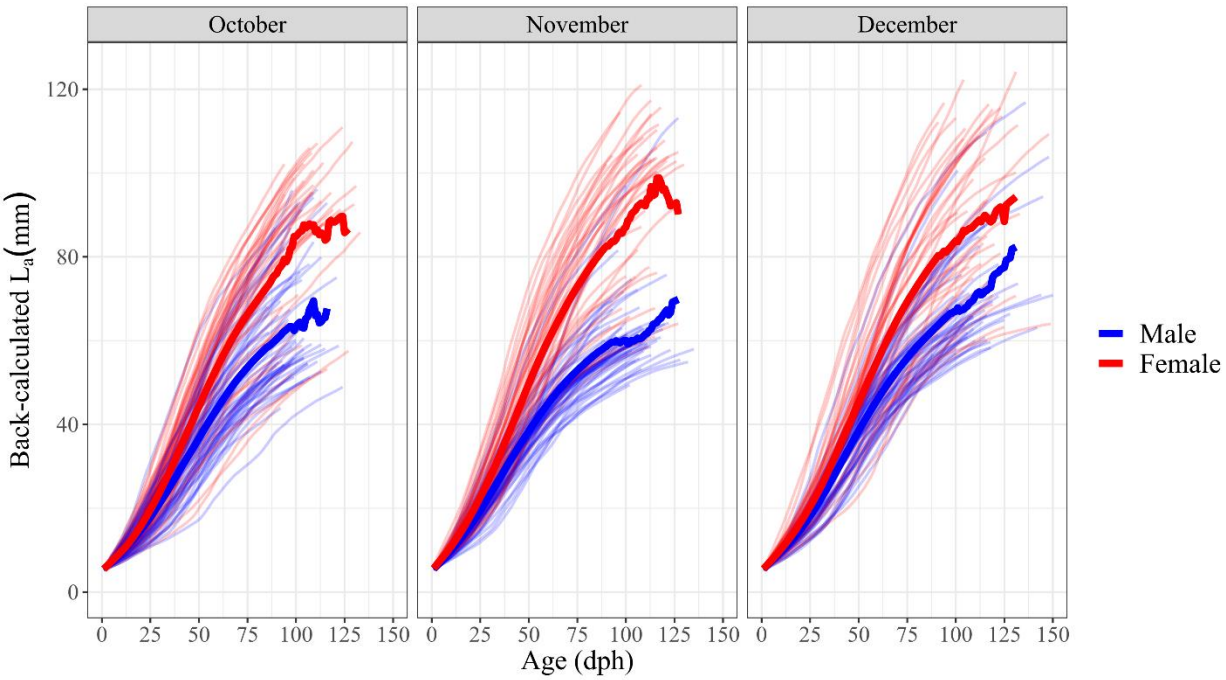

Figure 11. *M. menidia.* Back-calculated length-at-age (L_a) of male and female juveniles from each collection. Thin lines represent individual L_a trajectories, thick lines represent mean L_a for each increment number (= dph).

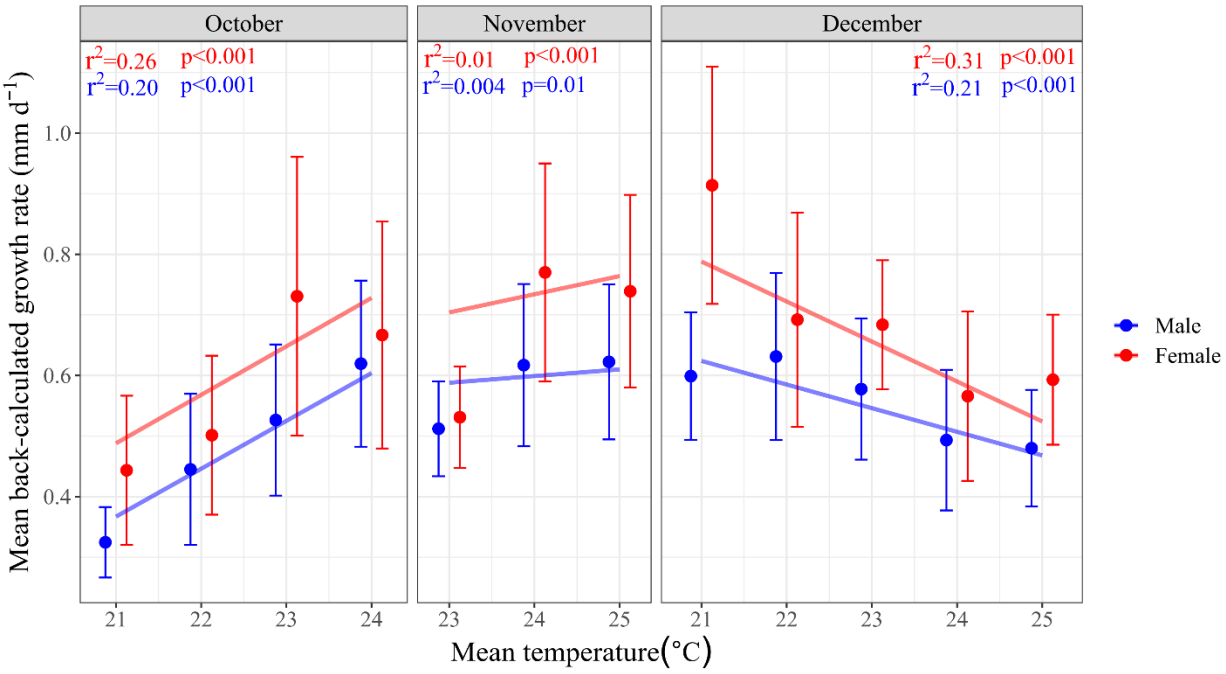

Figure 12. *M. menidia.* Relationship between temperature and sex-specific back-calculated larval growth rates (30 dph) for each collection. Individual temperature histories were binned into 1°C intervals and plotted against mean larval growth rates of individuals within each temperature bin. Lines are sex-specific linear regressions of individual temperature and growth histories, with statistics from each regression in an upper corner.

were associated with reduced growth in larvae collected in October and November. Regardless of collection, female larvae grew faster than male larvae at all temperatures (Figure 12).

Female variability

The female size distribution failed normality tests in each collection, as each TL distribution had two modes (Figure 5; Shapiro-Wilk, df≥43, p=0.01). Due to this bimodality, females were divided into two separate size bins: smaller females whose total length was less than 8 cm (abbreviated <8 cm), and larger females with TL equal to or greater than 8 cm (8+ cm). Within both the October and November collections 56% of females were <8 cm. In the December collection, the majority of females were 8+ cm in TL (57%).

Within the October collection, large and small females had significantly different hatch distributions, with large females hatching earlier in the season and thus significantly older (Figure

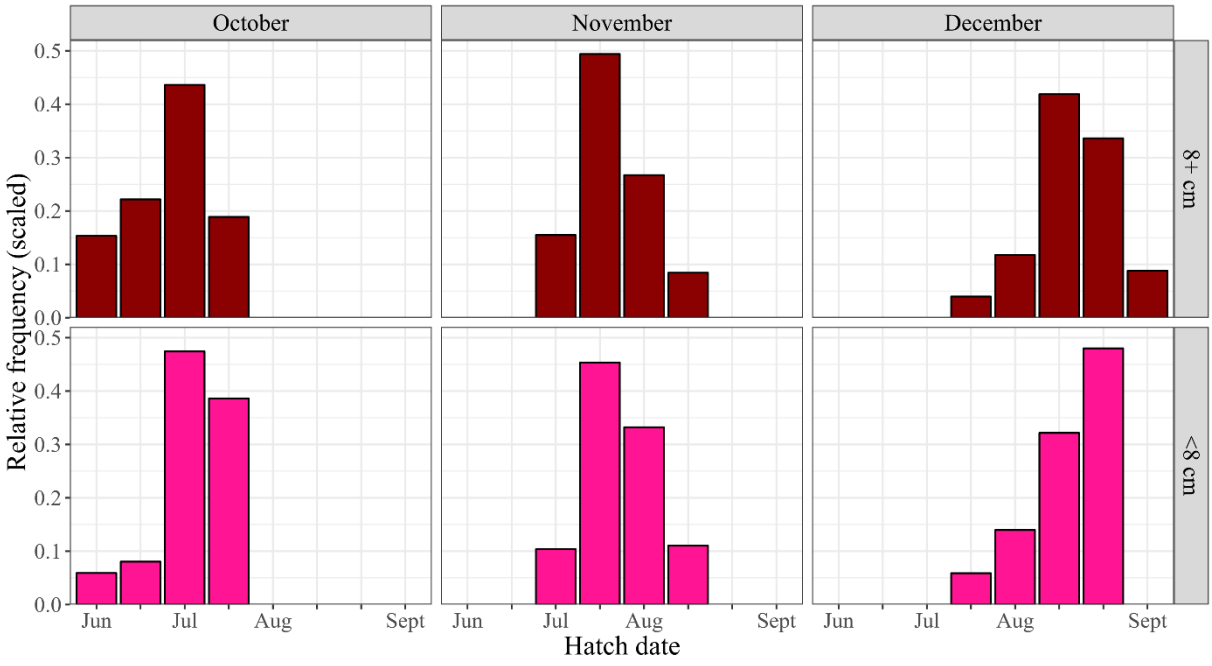

Figure 13. *M. menidia.* Distribution of back-calculated hatch dates for female *M. menidia* 8+ cm (dark red) and <8 cm TL (pink) from each collection. Data are relative frequencies of scaled sample sizes within each hatch interval.

13; t-test, t=3.17, df=45, p=0.003). Mean hatch dates were 7/16 (99.3 dph) ±10.9 days and 7/5 (110.0 dph) ±12.0 days for <8 and 8+ cm females, respectively, with a difference of 11 days. The majority of small females hatched in mid-July, with 86% hatching in hatch intervals 3 and 4, while 66% of large females from this collection hatched in late June-early July (hatch intervals 2 and 3). The <8 cm hatch distribution was slightly skewed to the left, but was less variable than the 8+ cm hatch distribution (Figure 13).

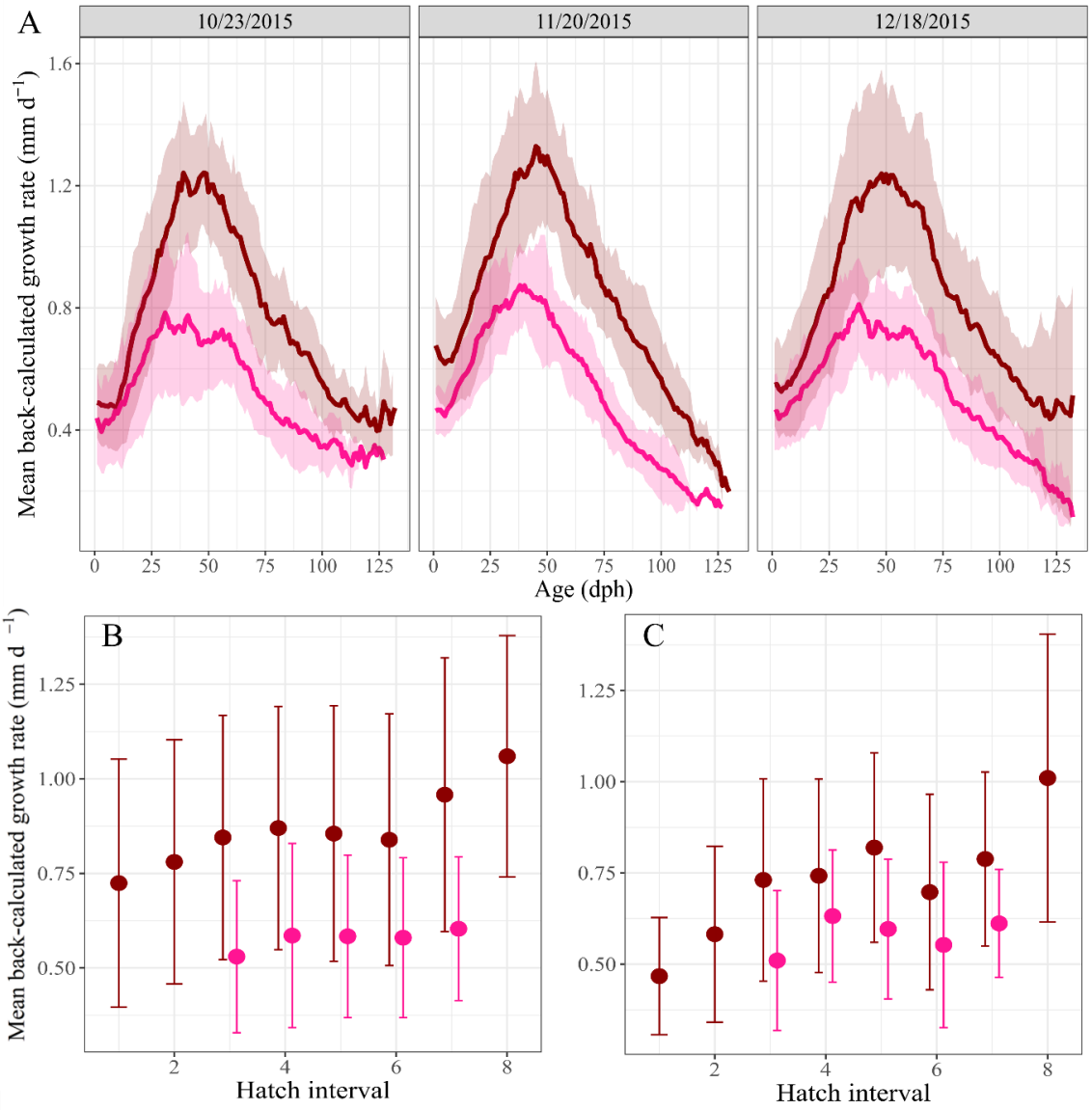

Figure 14. *M. menidia*. (A) Mean (±SD, shading) of back-calculated growth trajectories for females <8 and 8+ cm in TL from each collection date. (B) Mean growth (±SD) of females hatched in each hatch interval. (C) Mean larval growth (≤30 dph) across hatch intervals.

For the November and December collections, however, differences in size between small and large females were not due to differences in age (Figure 13). Differences in hatch distributions and age between female size bins in both later collections were not significant (t-test, $t \geq 1.464$, $df \geq 40$, $p \geq 0.151$). Mean hatch dates for November females were 8/3 (109.3 dph) ±9.7 days for <8 cm and 7/29 (113.8 dph) ±10.2 days for large females. The 8+ cm females had a longer hatching range of 41 days, where small females had a range of 32 days. Within the November collection, 76% of females, both large and small, hatched in late July - early August, in hatch intervals 4 and 5 (Figure 13). Within the December collection, small females had a mean hatch date of 8/24 (115.7 dph) ±12.7 days, while large females had a mean hatch date of 8/25 (115.0 dph) ±12.5 days (t-test, $t=-0.174$, $df=41$, $p=0.863$). Despite the long left tail, >75% of females in both size bins hatched in August, within hatch intervals 6 and 7 (Figure 13). The December collection was the most variable, with hatch ranges of 48 and 57 days for <8 and 8+cm females, respectively, and standard deviations in excess of 12 days.

Within each size bin, females from each collection had significantly different hatch distributions (1-way ANOVA, <8 $F=69.7$, 8+ $F=103.4$, $df=2$, $p<0.001$). For both small and large females, October females hatched earliest and December females latest, with November females in between, with the differences all being significant (Tukey's HSD, $df=2$, $p<0.001$). However, comparing age between females from different collections was significant for <8 cm females only (1-way ANOVA, $F=12.6$, $df=2$, $p<0.001$). Within the small female size bin, the October collection is significantly younger than the other collections (Tukey's HSD, $df=2$, $p=0.006$). Small females collected in November were 7 days younger on average than small females from December, yet this difference was not significant (Tukey's HSD, $df=2$, $p=0.161$). For large females, age did not vary significantly with collections (1-way ANOVA, $F=1.05$, $df=2$, $p=0.36$).

Despite having similar ages, small and large females attained differences in TL due to differences in their growth rates. Within each collection, mean growth rate was significantly slower for females within the <8 cm TL size bin (Figure 14A; Mann-Whitney, standardized U<-26.7, p<0.001). All females from the October collection grew at a similar rate for the first ~10 dph, but subsequently growth rates started to diverge (Figure 14A). Within the November and December collections, mean back-calculated growth for 8+ cm females was faster for the entire trajectory, with the largest difference in growth compared to small females occurring at approximately 50 dph. Even within hatch intervals, mean growth rate was significantly higher for 8+ cm females, regardless of when they hatched (Figure 14B; Mann-Whitney, standardized U<-10.72, p<0.001). Larval growth (<30 dph) analyses indicate that within each hatch interval, differences in growth strategies were apparent even as hatchlings (Figure 14C, Mann-Whitney, standardized U<-6.07, p<0.001).

Selective mortality

Selective mortality was assessed by evaluating patterns in 10^{th}, 50^{th} and 90^{th} percentiles of standardized increment widths. Silversides collected in October hatched earliest, and overlapped with individuals collected in November during hatch intervals 3 and 4 (Figure 15). Silversides from all collections hatched within hatch interval 4, making July 19-August 1 the only hatching period where individuals experienced the same environmental conditions from hatch until capture. Only individuals from the November and December collections hatched in intervals 5 and 6. Silversides captured in October and November grew at similar rates throughout the growing season, regardless of when they hatched (Figure 15). Among silversides hatched within interval 4, individuals captured in December initially grew considerably slower, but by Julian day 259 (9/16)

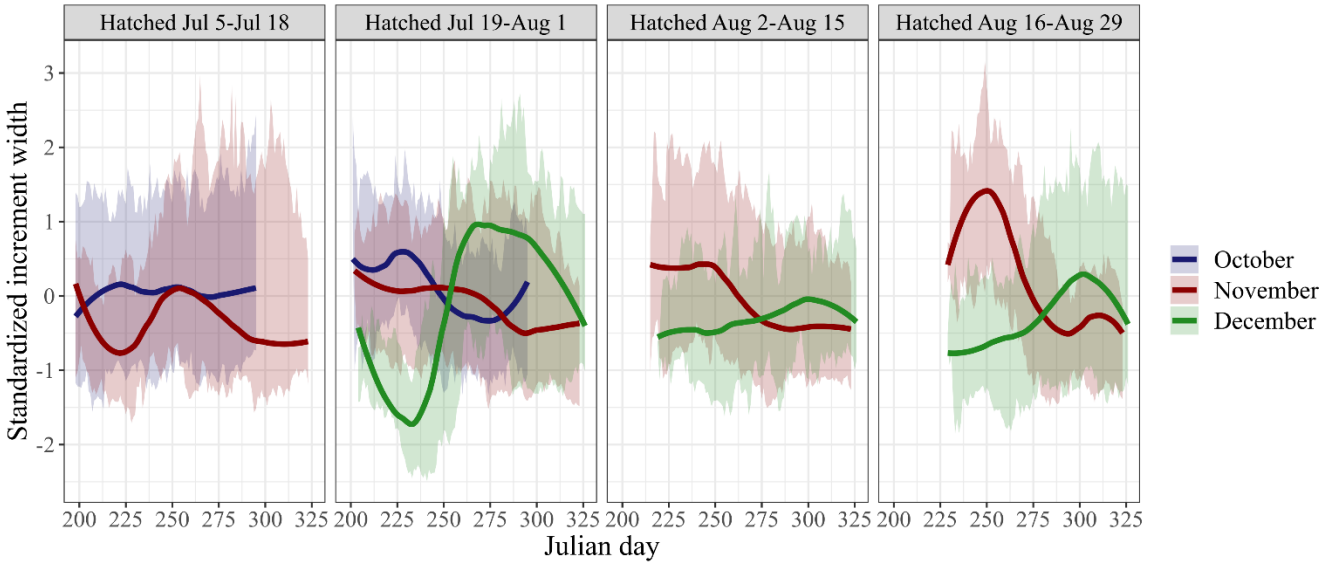

Figure 15. *M. menidia.* Standardized increment widths by collection (colors) for individuals hatched between July 5-Aug 29, 2015 (intervals 3-6). Increment widths were standardized separately for males and females. Lines and shading correspond to Loess-smoothed (span=0.5) medians and 10th and 90th percentiles, respectively.

began to grow faster than individuals from the other collections. This trend was also observed within hatch intervals 5 and 6, with silversides that were captured in December growing slowest at the beginning of the growing season, indicating that between November-December, initially faster growing individuals selectively disappeared from the surviving population.

DISCUSSION

This study utilized otolith microstructure analysis to infer hatch distributions and sex-specific growth patterns in juvenile Atlantic silversides (*Menidia menidia*) surviving to late fall and early winter in nearshore waters of eastern Long Island Sound. I hypothesized that females were larger than males principally because they hatched earlier during the season, given that this semelparous species exhibits temperature-dependent sex determination (TSD) (Conover and Kynard, 1981; Conover, 1984). TSD produces increasingly male-biased sex ratios with increasing

temperature (Conover and Kynard, 1981) and is assumed to be adaptive because it affords earlier born females a longer growing season, a larger body size, and thus enhanced fitness via higher fecundity after maturation (Barneche et al., 2018). My findings, however, showed that the observed sexual dimorphism in length was not due to differences in age but differences in growth rates, as female survivors had similar hatch dates but grew on average 37% faster than males. Furthermore, realized sex ratios of fall survivors were considerably less male-biased than expected from monitored temperature conditions and TSD values from published common garden experiments. Selective survival of faster growing females over males may account for these balanced sex ratios. However, growth back-calculations also revealed both slow- and fast-growing female phenotypes persisting in the surviving population and a selective disappearance of fast-growers from the study area between October and December.

Growth strategies

For each of the three collections in 2015, females were larger but not older than their male conspecifics. Therefore, the observed size dimorphism was achieved through sex-specific differences in growth rates (Figure 8). Sexual size dimorphism is common in fishes (Parker, 1992) and known for *M. menidia* (Kendall, 1901; Bayliff Jr, 1950; Conover and Ross, 1982), but it has generally been attributed to TSD in this species (Conover, 1984). Regardless of collection or hatch interval, females grew significantly faster than males (Figure 9). Only during a single instance (fish collected in December and hatched between July 19 and August 1), female growth rates were not significantly different. Longitudinal back-calculations of length-at-age revealed that these differences in growth rates began during the early larval stages, continued throughout ontogeny and resulted in substantial length differences (Figure 11). Provided that all analyzed silversides

hatched and then remained within Mumford Cove until capture, they all would have experienced similar temperature and feeding conditions. This suggests that the observed growth differences have a genetic basis in *M. menidia*.

Local adaptation of growth capacity has been well studied in *M. menidia* and is known to exhibit countergradient variation along the latitudinal gradient of its geographical range (Conover and Present, 1990). Importantly, this indicates that all but the most northern populations exhibit submaximal growth rates. If winter mortality and fecundity were the sole drivers of selection, it would be expected that energy allocation strategies maximizing growth would be favored. While large females exhibited fast growth, it appears that males and smaller females have submaximal growth, a strategy that must have advantages to compensate for potential reductions in winter survival due to smaller sizes. The differences in growth rates between males and females suggest that fast- and slow-growers are utilizing different strategies for growth, and that growth-mortality tradeoffs may be occurring. The bimodality within females is further evidence, as small females may experience selection similar to that of males. Submaximal growth may allow for less risky foraging behavior (Chiba et al., 2007) and greater flexibility in energy allocation towards predator avoidance and escape (Billerbeck et al., 2001; Lankford et al., 2001; Munch and Conover, 2003).

Predation is the primary source of mortality during the early life stages of fish, generally exceeding 99% (Peterson and Wroblewski, 1984; Bailey and Houde, 1989; Cowan et al., 1996). Faster growing and larger individuals may be better at escaping predation and exceed gape limitations (Cowan et al., 1996; Paradis et al., 1996) and therefore have a higher probability of survival (bigger-is-better hypothesis). Faster growth also reduces the duration of the most vulnerable life history stages and increase the likelihood of survival (stage-duration hypothesis; Leggett and Deblois, 1994; Houde, 1997). A majority of studies have supported the bigger-is-

better and stage-duration hypotheses, resulting in mortality inversely related to size and growth (Ware, 1975; Peterson and Wroblewski, 1984; Miller et al., 1988; Baumann et al., 2003; Baumann et al., 2007).

In the Atlantic silverside, size-selective winter mortality of the young-of-the-year comprises a third important process, because larger individuals are more likely to survive over winter than smaller conspecifics (Schultz et al., 1998; Munch et al., 2003). Winter temperatures inhibit growth (Conover and Ross, 1982; Conover and Present, 1990), while a scarcity of food cause overwintering silversides to rely on internal energy reserves or risk starvation. In response, juvenile *M. menidia* have evolved to rapidly accumulate lipids in somatic tissue prior to migrating. Individuals with smaller body sizes have fewer stored lipids, which leads to an energy deficit and winter mortality (Schultz and Conover, 1997). Since females are larger in size, this size-dependent winter survival must be biased toward females. Large size confers an additional evolutionary advantage, as fecundity increases hyperallometrically with female body size (Wootton, 2012; Barneche et al., 2018). Since egg production demands a larger allocation of energy than sperm, this evolutionary concept applies primarily to females.

Given the clear fitness advantage of faster growth and larger body size at the end of the growing season (Schultz et al., 1998), it is intriguing that my study also found evidence for persisting slower-growing males and females. This suggests the existence of trade-offs and/or alternative modes of selection on the fitness landscape of juvenile silversides. Despite the general veracity of growth-mortality hypotheses (Anderson, 1988), for some fish species including *M. menidia*, there is evidence that smaller individuals could be more effective at evading predators. As typical forage fish, silversides school and feed in the open water that lacks refuges from predators. Silversides do not have predator deterrents, such as sharp spines or defensive chemicals,

so escaping predation is solely achieved via burst swimming and fleeing. Thus, any reductions in burst swimming speed will diminish the ability to escape predation. Billerbeck et al. (2001) found that *M. menidia* from fast-growing northern populations exhibited slower burst and prolonged swimming speeds compared to slow-growing southern populations. Reduced swimming performance increased predation risk for fast-growing juveniles in both short-term (Lankford et al., 2001) and long-term (30 days) experiments (Munch and Conover, 2003). Size selective predation biased toward larger individuals has also been documented in other fish species, such as the congener *M. beryllina* (Litvak and Leggett, 1992; Gleason and Bengtson, 1996). Consuming a large meal, as is required for large fish to maintain fast growth rates, is metabolically costly, diverting energy away from predator evasion (Arnott et al., 2006). Further, this tradeoff between intrinsic growth rate and swimming speed was found using *M. menidia* reared for multiple generations in a common garden environment, implying a genetic basis (Billerbeck et al., 2001; Lankford et al., 2001). This suggests that slow growth seen by males and small females may be adaptive, despite likely reductions in overwinter survival.

Winter mortality is likely the tradeoff for any survival advantage gained earlier in the season due to enhanced predation evasion in slow-growing silversides. Some small individuals must survive the overwintering phase, as these genotypes would otherwise be removed from the population. Most previous *M. menidia* experiments tested traits along a latitudinal gradient and sourced silversides from the two ends of their range: Nova Scotia and South Carolina. While closer to Nova Scotia, Connecticut may be an intermediate between the two extremes of adaptive pressures due to intermediate seasonality at this latitude. Additionally, fast growing northern populations mix with slow-growing southern populations during the offshore overwintering phase,

allowing gene flow to occur (Conover, 1998; Clarke et al., 2010). Thus, populations of silversides within Long Island may contain a mix of slow- and fast-growing genotypes.

I found that *M. menidia* juveniles that survived in Mumford Cove until December exhibited slower growth earlier in the season compared to those collected in October and November (Figure 15). Faster growers selectively disappeared from the population between October and December. I argue that this is unlikely to be a result of predation mortality. Fast-growing silversides were present in the October and November seines, indicating these individuals successfully evaded predation during the summer months, when predators are most abundant. Rather, I hypothesize that faster growing silversides selectively disappeared from the study area because they began migrating earlier into deeper, warmer waters. Outmigration of *M. menidia* occurs in late fall, when water temperatures fall below 8-12°C (Conover and Murawski, 1982; Conover and Present, 1990). Monitoring in Mumford Cove showed that mean daily temperatures were consistently below 10°C after November 24. Faster growing silversides likely were of sufficient body size to migrate, and did so between the November and December collections of this study. Size distributions of a biweekly beach seine survey in Mumford Cove indicate *M. menidia* above 12.5 cm in TL were present until December 4 (Baumann, unpublished data), whereas these size classes were not captured in the December collection. This indicates that larger individuals with faster growth rates began their migration before the time of the last collection.

The persistence of slow-growing individuals in Mumford Cove in December may indicate a release from predation mortality due to slow growth, as these slow growers survived until the end of the growing season. Smaller fish may remain in coastal waters beyond the temperature threshold for migration in an attempt to engage in compensatory growth prior to winter migration (Schultz et al., 2002). Compensatory growth occurs after a period of reduced growth, after which

growth is maximized to compensate for earlier losses (Ali et al., 2003). The negative relationship between temperature and back-calculated larval growth rates for silversides that survived until December may indicate compensatory growth was occurring (Figure 12). This relationship to temperature was surprising, as it was opposite in sign from individuals collected in earlier seines and from previous experiments (Conover and Present, 1990). December-collected silversides were the youngest (Figure 7) and hatched during the period of peak water temperatures. As the temperature declined into the fall, these larvae were apparently able to grow at faster rates compared to larvae from other collections at the same temperature (Figure 12). If compensatory growth was occurring, it suggests later-hatching larvae were food-deprived and/or temperature-stressed (Ali et al., 2003). For larvae that hatched latest in the season, selection may act on maximizing growth, such that they may still reach a sufficient size for overwintering. This could explain frequent observations of silversides in coastal waters in winter months, where small size and lack of energy reserves likely precluded migration (Baumann, unpublished data; Schultz and Conover, 1997).

While two growth and energy allocation strategies may exist in the Mumford Cove population of *M. menidia*, this study was limited to juveniles that survived until fall and early winter. Predator abundance is high in coastal waters during the silverside growing season. Predators such as the striped bass (*Morone saxatilis*) and Atlantic needlefish (*Strongylura marina*) are often captured in summer beach seines in Mumford Cove (Baumann, personal observation), while other predators, such as bluefish (*Pomatomus saltatrix*) are abundant and known to feed on juvenile silversides (Friedland et al., 1988; Juanes et al., 1994). Predation on fish larvae follows a dome-shaped curve with prey/predator size ratios, with maximum predation vulnerability occurring when prey are 10% of the predator's length (Paradis et al., 1996). While this will shift

over time and vary depending on predator species, predation likely removed an unknown portion of genotypes from the population prior to the beginning of this study. Yet, if predators are selective, individuals both above and below the threshold body size may survive. Conflicting selective pressures on growth would presumably act as stabilizing selection, favoring intermediate growth genotypes. While there was substantial variation of growth rates in this study, most individuals could either be placed in the fast or slow growth category, regardless of sex. Conversely, disruptive selection could be occurring, and individuals with intermediate growth rates are not surviving. Further, the shape and magnitude of selection can change throughout ontogeny, which may act to maintain genetic variation and promote growth-mortality tradeoffs (Gagliano et al., 2007).

Sex ratios

Estimated male:female ratios were 60:40%, 77:23%, and 63:37% during the October, November and December collections, respectively, which is less male-biased than expected based on laboratory-derived TSD values for this species. Experiments have found that the proportion of *M. menidia* females from this latitude decreases from approximately 50% at 15°C to 5% at 28°C (Conover and Heins, 1987a). On average, the onset of the thermosensitive period for TSD (8-21 mm, Conover and Fleisher, 1986) was reached within 10 dph for each hatch interval and lasted an average of 20.5 days. While 8-21 mm in TL is a wide range, the limits of the temperature sensitive period are likely more narrow for each individual (Conover and Fleisher, 1986). Temperature monitoring in Mumford Cove suggested that the oldest individuals (1st and 2nd hatch intervals) experienced mean temperatures of 21.3°C and 22.6°C, respectively during their thermosensitive period. The majority of juvenile survivors, however, hatched after July 5 and thus experienced mean temperatures of 23.6-24.7°C during their sex-determining period. Individuals hatched after

9/13 experienced declining temperatures throughout their sex-determining period, with a mean of 19.7°C. In the laboratory, these temperatures produce only 12-24% females (Conover and Heins (1987a)), which contrasts with the less male-biased sex ratios among fall survivors, particularly in October and December (Figure 6). In November, observed sex ratios were closer to expectations, possibly due to larger females leaving the study area to begin winter migration. The more balanced sex ratios suggest that mortality was sex-specific, where survival was biased toward females. A similar trend was observed in 2017, with male:female ratios in Mumford Cove of 45:55% in October and 66:34% in November, where larger females were observed in October but not November (Dupuis, unpublished data).

The degree of TSD varies in response to decreasing growing season lengths with latitude, from genetic sex determination in the north to mostly temperature-sensitive in southern populations (Conover and Heins, 1987a; Lagomarsino and Conover, 1993). However, temperature sensitivity is nonlinear and can vary on spatial scales as small as 58.2 km, especially near latitudinal breakpoints in the climatic cline in level of TSD (e.g. ~40°N) (Hice et al., 2012; Duffy et al., 2015). Even in southern populations, sex determination is partly genetic in 20-30% of individuals (Duffy et al., 2015). This suggests that silversides from our study area (~41°N, middle of the geographical distribution) have intermediate levels of TSD, due to moderate interannual variability in temperatures and an intermediate length of their growing season. Previous work has found 40% of individuals at this latitude have reduced temperature sensitivity (Conover and Heins, 1987a; Hice et al., 2012; Duffy et al., 2015), which could explain the more balanced sex ratios of fall survivors. Alternatively, the higher-than-expected proportions of females could be due to selective survival, given that females grew faster and attained larger sizes than males, which is consistent with the bigger-is-better hypothesis (Miller et al., 1988; Leggett and Deblois, 1994).

A third potential explanation for the more balanced sex ratios could be immigration of females from other populations. *M. menidia* have been found as far as 170 km offshore in the winter, suggesting they have the potential to swim long distances (Conover and Murawski, 1982). While young-of-the-year silversides likely remain in the most productive nearshore waters throughout the growing season, larger juveniles are certainly capable of swimming long distances. Since this study was conducted in the fall, some individuals from other locations could have immigrated into Mumford Cove en route to their overwintering habitats. Migrant silversides might have arrived from further west in Long Island Sound, or potentially been entrained in currents from fall storms from further east.

Otolith microstructure analysis

Overall, otolith microstructure analysis was an effective and useful tool for back-calculating hatch days and daily growth rates in *M. menidia*. Microstructure analysis is routinely used in juveniles of many teleosts (Campana and Neilson, 1985; Jones, 1992; Sogard, 1997 and references therein), but for silversides it had so far been restricted to young larvae and juveniles up to 79 days post hatch (Barkman, 1978). The reason for this potentially lays in the unusual morphometry of sagittal otoliths in older *M. menidia* juveniles and adults, making otherwise time-tried procedures unsuccessful in this case. For example, the unusual convex curvature of the otoliths precluded polishing them from both sides (Campana and Neilson, 1985; Secor et al., 1992), because doing so would have made either the increments near the core or increments near the edge indiscernible. Furthermore, otolith microstructure analysis generally aims to discern increments along the otolith's longest axis, whereas in this species only a shorter lateral axis was consistently clear enough to read (Figure 3). Finally, decreasing fall temperatures likely suppressed

growth and led to very narrow and difficult to discern increments at the otolith edge, increasing the likelihood of age underestimation (Campana et al., 1987). As such, it is likely that the age estimates decreased in accuracy from October to December, with estimates from silversides collected in December most likely to be underestimated.

Given these challenges, the average percent error (APE) of 6% between independent otolith readers was deemed acceptable, while the within-reader provided an APE <4%. However, the age estimates of the experienced second reader (Baumann) were consistently higher than those of the original reader, averaging +14.1 days and thus indicating a potential age underestimation of approximately 2 weeks. Differences in back-calculated age between collections may also point to age underestimation. Given that the collections were approximately 30 days apart, average age of silverside survivors was expected to increase by ~30 days from one collection to the next, assuming a common hatching period. However, mean back-calculated ages differed among collections by almost two weeks, with each successive collection having significantly later back-calculated hatch dates (Figure 7).

During every collection, survivors were younger than expected, given that the spawning season of *M. menidia* in Long Island Sound (and similar latitudes) occurs between May and early July (Bayliff Jr, 1950; Bengtson, 1984; Conover and Present, 1990). A biweekly beach seine survey in Mumford Cove since 2015 has observed silversides in spawning condition between late April and early July (Baumann, personal communication). While some of the survivors collected in October were estimated to have hatched in June, silversides collected in November and December all hatched after July 1. The right tail of the December hatch distribution extended into September and thus well beyond the known spawning period for *M. menidia*. Even if corrected by a potential age underestimation of two weeks, my results suggest that silversides continue to

reproduce well beyond July, potentially in areas other than Mumford Cove. Conversely, there was a lack of surviving juveniles from May, even though spawning-ripe silversides have been collected from Mumford Cove during this period (Murray et al., 2017; Murray and Baumann, 2018; Snyder et al., 2018). These results suggest that earlier hatched silversides either migrated out of Mumford Cove prior to October or were selectively removed from the population throughout the growing season.

Summary and future directions

My study was focused on silverside juveniles surviving to fall and early winter months in nearshore waters of Long Island Sound, but extending the sampling period and evaluating growth patterns of *M. menidia* from earlier in the season would be valuable, even though silverside < 30 mm TL are not quantitatively sampled in beach seines and are challenging to sex. As an alternative, a light trap has recently been successfully deployed in Mumford Cove (Stark, unpublished data), which could be used to test whether *M. menidia* spawning indeed extends into August, as was suggested by this study.

Annual forage fish such as *M. menidia* likely exhibit large interannual fluctuations in population size (Conover and Ross, 1982), which is why the observed pattern should be tested again during subsequent years. Additionally, similar methods could be used to evaluate growth histories of silversides along the latitudinal gradient of their geographic range, which would determine if the observed sex-specific growth patterns vary with seasonality and the level of TSD. A central assumption of my study was that the silverside population consisted solely of Mumford Cove residents, but migrants from other areas with potentially other growth characteristics might have been present as well. Stable isotope analysis from muscle tissue or otolith microchemistry

can be used to assess fish movement patterns, yet these methods are only viable if individuals encounter different water chemistries, often along a salinity gradient, or isotopically distinct food webs (Hobson, 1999; Elsdon et al., 2008). Mumford Cove waters are almost full-strength marine (29-32 psu), which limits the utility of otolith chemistry analysis, and food sources across the region are likely too isotopically similar. Further, physical tags, such as external markings or internal fluorescent marks on otoliths, are generally of little utility in forage fish with very high natural mortality rates. DNA microsatellite analysis could provide insights, but it requires migrants to immigrate from genetically distinct populations (Hansen et al., 2001). However, recent advances in whole genome sequencing offer promising new insights into the genomic basis of gene flow and local adaptation in this species.

Overall, the intriguing findings of my study demonstrate the power of field-testing ecological theories developed from laboratory experiments. Decades of laboratory and field studies have elucidated evolutionary trade-offs and patterns of local adaptation in *M. menidia* (Conover and Present, 1990; Schultz et al., 1998; Lankford et al., 2001). However, this study is the first to apply otolith microstructure analysis to juvenile *M. menidia* surviving until late fall and early winter in their natural environment. Hence, this study builds on the collective knowledge by demonstrating that patterns in wild silversides are more complex than previously thought. The mechanism responsible for sexual size dimorphism in the young-of-the-year was sex-specific growth differences in rates, not age as previously assumed. Additionally, the existence of different growth strategies and the presence of slow-growers into December suggests multiple modes of selection and trade-offs between slow growth and size at the time of migration. This warrants further investigation, as the presumed adaptive significance of temperature-dependent sex-determination may not apply to all individuals, and sex-specific differences in growth need to be

considered. The successful use of otolith microstructure analysis to evaluate sex-specific characteristics of surviving young-of-the-year shows that this method can be used to further elucidate these evolutionary questions in *M. menidia*.

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