

Reproductive Phenology of *Elliptio complanata* in an Upper Susquehanna River Tributary of New York

Thomas P. Franzem¹, Mark Kugler², Robin LaRochelle², Paul H. Lord¹,
Daniel Stich¹, and Andrew M. Gascho Landis^{2,*}

Abstract - *Elliptio complanata* (Eastern Elliptio) is typically an abundant species in lotic systems throughout the northeastern US; however, as with many other freshwater mussel species, some populations of Eastern Elliptio are in decline. Freshwater mussels have complex life cycles, which are important to understand for their conservation and management. The goal of this study was to determine the timing of Eastern Elliptio spawning, brooding, and glochidia release. Throughout the spawning season, we used gonad and gill extracts and drift nets to track the timing of reproduction in Otego Creek, NY, a tributary to the Susquehanna River. Females began brooding fertilized eggs by mid-May, and by early June, all females collected were brooding fertilized eggs or d-shaped glochidia. The temperature was 18 °C when all females contained glochidia. Peak glochidia-drift occurred ~1 week after we recorded the highest levels of brooding and continued at low levels for several weeks. Phenology modeling helped us to determine that accumulated thermal units was the best predictor of reproductive activity. Our work highlights the environmental cues responsible for spawning, brooding, and glochidia release in a population of Eastern Elliptio. This empirical approach to predicting reproductive activity has great potential as a tool for timing the collection of brood stock for propagation or other important conservation measures.

Introduction

Freshwater mussel species in North America have a wide range of reproductive strategies (Barnhart et al. 2008). Details of the reproductive process for many species remains unknown, and increasing our knowledge will aid in conservation. Determination of the environmental cues for spawning, brooding, and transfer of glochidia to host fish are necessary for targeted collection of brood stock for propagation efforts or for seasonal protection of specific stream reaches during vulnerable periods of the reproductive process (Price and Eads 2011). Furthermore, understanding the timing and environmental variables associated with the reproductive process could help managers promote access of a migratory host fish to mussel beds during the glochidia-release period (Fritts et al. 2012).

Elliptio complanata (Lightfoot) (Eastern Elliptio) is typically an abundant species in suitable lotic systems throughout the northeastern US; however, as with many other freshwater mussel species, some populations of Eastern Elliptio are in decline (Strayer and Malcom 2012). The proposed mechanism for Eastern Elliptio

¹Biology Department, State University of New York College at Oneonta, NY 13820. ²Department of Fisheries, Wildlife, and Environmental Science, State University of New York College of Agriculture and Technology at Cobleskill, NY 12043. *Corresponding author - gaschoam@cobleskill.edu.

recruitment failure in this context was high concentrations of un-ionized ammonia and its detrimental effect on the sensitive juvenile stage (Strayer and Malcom 2012). However, freshwater mussel population decline typically does not result because of a single variable, but rather multiple factors (Haag 2012). In this case, much is unknown about the timing of Eastern *Elliptio* reproduction, and thus it is important to consider alternate explanations for recruitment failure. Freshwater mussels have a complex life-history, and bottlenecks may occur in other parts of the reproductive cycle. Tracking gonadogenesis is important for ensuring that both sexes have gametes necessary for spawning (Saha and Layzer 2008). Confirming that females are holding glochidia in the marsupium, and understanding the process of embryo development are both required to conclude which environmental conditions are appropriate to support typical brooding patterns (Gascho Landis and Stoeckel 2016). Finally, identifying and quantifying glochidia in stream drift is useful for assessing whether female mussels are receiving the appropriate cues to initiate the process of glochidia transfer to host fish (Culp et al. 2011). Successfully completing these steps is required before the glochidia even have the chance of attaching to a suitable host fish.

The primary goal of this study was to document the reproductive phenology in Eastern *Elliptio* in the upper Susquehanna River basin, using sex ratios, the timing of spawning, brooding, and glochidia drift. Furthermore, we used our phenology results to determine which environmental factors (discharge, air temperature, solar period, and/or accumulated thermal units) were the best predictors of reproductive activity.

Field-Site Description

We conducted the Eastern *Elliptio* phenology study in Otego Creek in West Oneonta, NY (42.471182°N, 75.109362°W), starting ~30 m north of the Route 23 Bridge, and extending approximately 150 m upstream. Average stream-width was 15.4 m. The study reach was primarily pool habitat with depths varying from 0.5 m to 1.5 m; depth did not vary by more than 0.5 m in response to precipitation during the course of this study. Banks were primarily silt, and the stream bottom was a mix of cobble, gravel, and sand.

Methods

We conducted 7 surveys to document Eastern *Elliptio* reproductive phenology between 23 April and 8 July 2016. The goal for each sampling occasion was to collect a minimum of 20 mussels for gamete extracts; however, due to sampling conditions and detection, we collected <20 mussels on 2 occasions. The 20-mussel samples included mussels that were sampled repeatedly for tracking gamete development and did not represent newly captured individuals. We brought mussels to shore, where we tagged each one with 2 unique plastic Hallprint tags (Type FPN; Hallprint, South Australia, Australia), 1 on each valve. We used cyanoacrylate glue

(Seachem Flourish® glue; Seachem, Madisin, GA) to attach the tags (Hartmann et al. 2016). Tagging mussels allowed us to track embryo development within individuals and to examine for hermaphrodites; however, we did not examine all mussels on each occasion due to imperfect detection once they were returned to the stream. We pried open Eastern Elliptios with a clam knife (Dexter Russel 3" Clam Knife) to extract gametes from the gonad (Saha and Layzer 2008). We inserted an Exel Int® 3 ml disposable syringe (Exelint International, St. Petersburg, FL) with 0.5 ml sterile water into the visceral mass, toward the posterior of the mussel and removed 1 ml of visceral fluid. We stored samples on ice for transport to the lab. We inspected all mussels for swollen or enlarged gills. If gills were enlarged, we extracted gill fluid using the same technique used to extract the visceral fluid. We did not collect a gamete extract when gills were enlarged because we used enlarged gills as an indication that the mussel was female. After sampling, we returned the mussels to their original location in the stream substrate. We employed a probe at the stream edge and just off the bottom of the stream bed to measure water temperature on each visit.

On the 6 sampling occasions when we detected fertilized embryos and glochidia in female gills, we deployed a Wildco® stream drift-net (mouth opening 45 cm x 29 cm, 100-µm mesh; Wildco, Yulee, FL) immediately downstream of the mussel bed (Culp et al. 2011). We placed the net in the water for 30 min between 10 am and 3 pm and collected 2 separate samples. To limit cross contamination of the samples, we used a squirt bottle and deionized water to thoroughly rinse the net between each sampling occasion. During each sampling occasion, we measured stream flow and recorded the water height on the net to calculate the volume of water passing through the net. Samples were returned to the laboratory, where we counted glochidia in five 1-ml subsamples for each occasion. Although we did not identify glochidia to species, Eastern Elliptio is the dominant mussel species on the bed (other species were only rarely encountered) and the only short-term brooding species expected to release glochidia at this time of year.

We took visceral mass and gill samples to the laboratory for examination under a microscope. We examined the samples under 10× magnification to detect eggs and developing embryos. If neither of these were found, we placed a cover slip on the slide, and switched the magnification to 100× to examine for sperm.

Cursory analysis indicated a strong seasonal pattern in gamete expression by Eastern Elliptio in Otego Creek (Fig. 1). Therefore, we used generalized linear mixed models (GLMM) in the lme4 package (Bates et al. 2015) in R (R Core Team 2017) to quantify relationships between Eastern Elliptio reproductive phenology and several potential environmental factors of interest. We used the logit-link function to estimate the probability that individually marked mussels were reproductively active during a given sampling event based on the presence (1) or absence (0) of fertilized eggs, d-shaped glochidia (early stage glochidia as the embryo transforms into mature glochidia), or mature glochidia being brooded in female gills. All mussels collected during July no longer contained glochidia, so we assumed that this also was the case for the same mussels on 1 August to provide a post-spawning record used in phenology models. We estimated the probability of a female

mussel brooding offspring (p_j) as a linearized function of a priori combinations of accumulated thermal units (ATU), discharge (Q), mean daily air temperature (T), and photoperiod (Ph). We included a random effect of individual (j) on the intercept (β_0) to account for individual variability in timing of reproduction, and unequal numbers of observations, such that:

$$\text{logit}(p_j) = \beta_{0j} + \sum_{i=1}^k [(\beta_i)(X_i), \dots, (\beta_k)(X_k)],$$

where each β_i was interpreted as the effect of covariate X_i on p_j given that all other were held constant.

We did not collect environmental data from Otego Creek on all visits; however, we collected environmental variables for year 2016 using a variety of data sources (Table 1). We obtained daily normals for 2016 air-temperature data at station ID USC00306217 in Oneonta, NY (42.4604, -75.0643) from the National Oceanic and Atmospheric Administration National Climatic Data Center (www.ncdc.noaa.gov/cdo-web/). We used the temperature midrange for each day to represent daily air temperatures during the course of this study. We used air temperature for phenological modeling because our water temperature data did not cover the entire reproductive period, nor was it inclusive enough to allow us to calculate ATU. We calculated ATU as the sum of mean daily air temperatures greater than 0 °C (Chezik et al. 2014). We retrieved discharge data from US Geological Survey gauge station

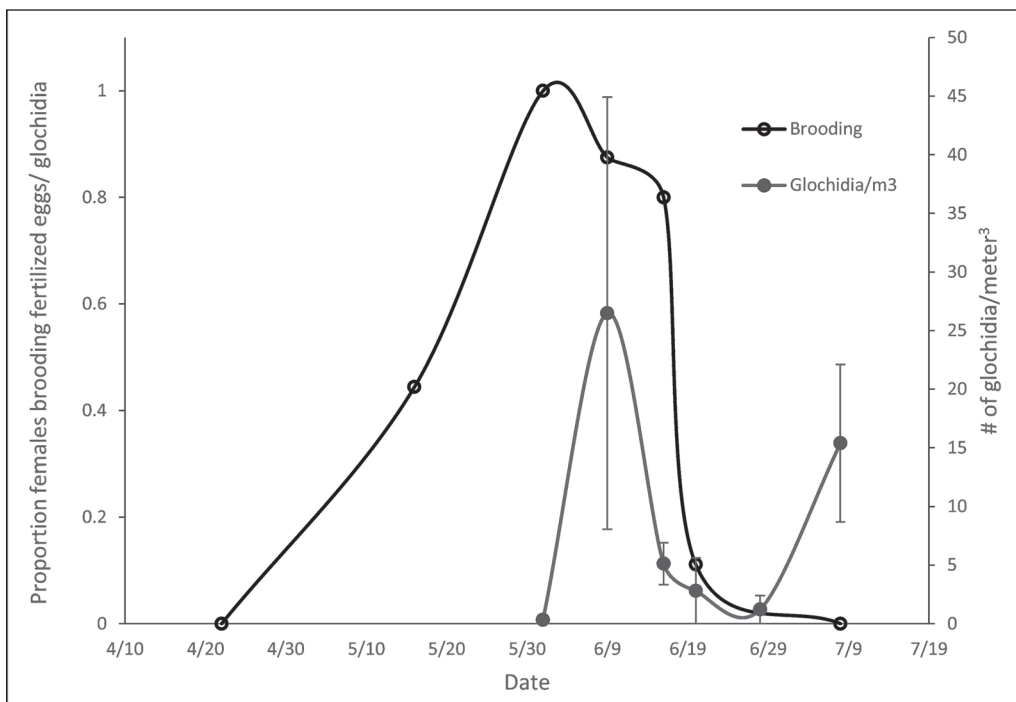


Figure 1. Proportion of Eastern *Elliptio* females brooding fertilized eggs or glochidia in their gills during the 2016 spawning season in Otego Creek (left axis). Abundance of glochidia in stream drift in Otego Creek (± 1 standard deviation, right axis).

ID 01500500 on the Susquehanna River at Unadilla, NY (42.3214, -75.3167) as a proxy for discharge near Otego Creek using the waterData package (Ryberg and Vecchia 2017) in R. Finally, we calculated photoperiod for the latitude of our sampling location using the geosphere package (Hijmans 2017) in R.

We created a set of 7 candidate models to assess the support for competing hypotheses about environmental influences on the timing of reproduction (Table 2). We created models to estimate P as a quadratic function of each of the environmental variables (ATU, Q, T, and Ph), in addition to models that included additive, quadratic effects of discharge (Q) and each of the remaining covariates (ATU, T, and Ph). We did not include combinations of ATU, T, and Ph with one another because the 3 variables were highly collinear. All explanatory variables were standardized (i.e., scaled and centered) prior to analysis to facilitate interpretation of relative effect-sizes and to reduce collinearity between explanatory variables, although we present graphical results on the real scale to ease interpretation. We used the Akaike information criterion corrected for sample size (AIC_c ; Burnham and Anderson 2002) to assess the relative support of each of our candidate models. Finally, we calculated an R^2 statistic for each model following the method of Nakagawa and Schielzeth (2013).

Table 1. Summary of discharge (Q), air temperature (T), accumulated thermal units (ATU), and photoperiod (Ph) on each survey date, along with numbers of newly marked individuals (m), recaptured individuals (r), and total number of individuals collected (n) on each day.

Date	Q (m ³ ·s ⁻¹)	T (°C)	ATU (°C)	Ph (h)	m	r	n
4/22/2016	43.32	11.95	511.20	13.69	22	0	22
5/18/2016	33.41	8.35	728.20	14.71	16	4	20
6/1/2016	16.71	16.95	964.20	15.08	10	11	21
6/9/2016	20.10	10.25	1103.35	15.22	0	9	9
6/20/2016	10.79	20.00	1267.30	15.30	6	8	14
7/8/2016	7.08	21.65	1604.15	15.14	15	14	29
8/1/2016	54.93	19.70	2084.25	14.47	0	29	29

Table 2. Model-selection statistics for candidate a priori models used to test competing hypotheses about predictors of the probability of gamete expression showing fixed effects included in each model, the number of parameters (K), the AIC_c score for each model, the difference in AIC_c between the best model and each subsequent model (ΔAIC_c), the probability that any given model was the best among those considered (w), and the R^2 for each model.

Fixed effects	K	AIC_c	ΔAIC_c	w	R^2
ATU + ATU2	4	82.80	0.00	0.72	0.96
T + T2 + Q + Q2	6	86.21	3.41	0.13	0.99
ATU + ATU2 + Q + Q2	6	86.40	3.60	0.12	0.96
P + P2 + Q + Q2	6	89.51	6.71	0.02	0.92
Q + Q2	4	91.02	8.22	0.01	0.96
P + P2	4	145.10	62.31	0.00	0.85
T + T2	4	166.86	84.07	0.00	0.16
Null model	2	177.45	94.66	0.00	0.00

Results

Throughout the phenology surveys, we captured 56 unique Eastern Elliptio; 9–29 individuals were collected in a given survey. The sex ratio was 0.85:1 (female:male) among the 56 individuals collected. We recaptured numerous individuals on multiple occasions (Table 1); thus, we had sequential gamete samples and were able to determine that 6 of the individuals were hermaphrodites, showing the presence of both sperm and eggs (or glochidia).

Spawning or brooding did not appear to have occurred by the 23 April 2016 survey. The percentage of brooding individuals increased from May through June, peaked during June, and decreased rapidly by early July (Fig. 1). Water temperature was 18.1 °C on 1 June 2016, when all females assessed were gravid. Of the 12 females assessed on that day, 10 had d-shaped glochidia and the remaining 2 had fertilized eggs. We observed no females that had more than 1 brood during our sampling period.

Drift nets deployed periodically from early June through July showed a peak in glochidia 8d after the peak in proportion of brooding females (Fig. 1). Water temperature on the day of the peak of glochidia release (15.9 °C) was 2.2 °C colder than at the time of the peak in brooding glochidia (18.1 °C), which was due to an unusual cold snap.

The quadratic effect of ATU was the best predictor of Eastern Elliptio brooding glochidia among the candidate hypotheses tested using GLMM (Table 2). According to model-selection statistics, no other hypothesis had similar support, although we noted significant, quadratic relationships between timing of female brooding and each of the other environmental predictors (discharge, air temperature, and photoperiod). Likewise, all models that included environmental predictors were better supported than the null. The second-best supported model included quadratic effects of both T and Q, indicating that brooding status may, in fact, have been cued by multiple environmental factors despite the strong relationship to ATU.

The mean probability that an individual mussel was brooding glochidia increased from the 22 April sample until the 9 June 2016 sample, after which probability of brooding declined precipitously. We noted a similar trend corresponding to seasonal increases in ATU, with a significant initial increase in P with increasing ATU, followed by rapid decrease with further increases in ATU (Table 3). Correspondingly, we predicted an increased probability of brooding until ATU of about 1000, followed by a decline in brooding thereafter (Fig. 2).

Table 3. Mean, standard error (SE), z statistic, and P -value for fixed effects (parameters) included in the best model ($p_j = \beta_0 + \beta_{ATU} \cdot ATU + \beta_{ATU^2} \cdot ATU^2$) used to describe the timing of Eastern Elliptio brooding status in Otego Creek, NY, during the 2016 spawning season.

Parameter	Mean	SE	z	P
β_0	0.9014492	0.792	1.139	0.255
β_{ATU}	-6.799847	2.621	-2.594	0.009
β_{ATU^2}	-8.79468	3.087	-2.849	0.004

Discussion

We were able to track reproductive activity, as determined by production of sperm and eggs, the development of fertilized eggs into mature brooding glochidia, and the presence of glochidia in stream drift for a population of Eastern Elliptio in Otego Creek, NY. The spawning process of these mussels appears to be highly synchronized and linked to environmental factors during spring and summer. We found significant relations between the timing of brooding glochidia and ATU, discharge, photoperiod, and air temperature experienced by individual mussels.

Water temperature can be a cue for spawning, and can alter the length of the brooding period and time of glochidia release in many species of freshwater mussels (Watters and O’Dee 2000). As a result, different populations of Eastern Elliptio may release glochidia at different times, and the timing of glochidia brooding and release may change year-to-year within a population. We observed glochidia production and release at a mean water temperature of about 18 °C during the present study. The timing of this window in relation to other seasonal cues, including those related to seasonal behavior of host species, could have important implications for managing this species in the face of changing local

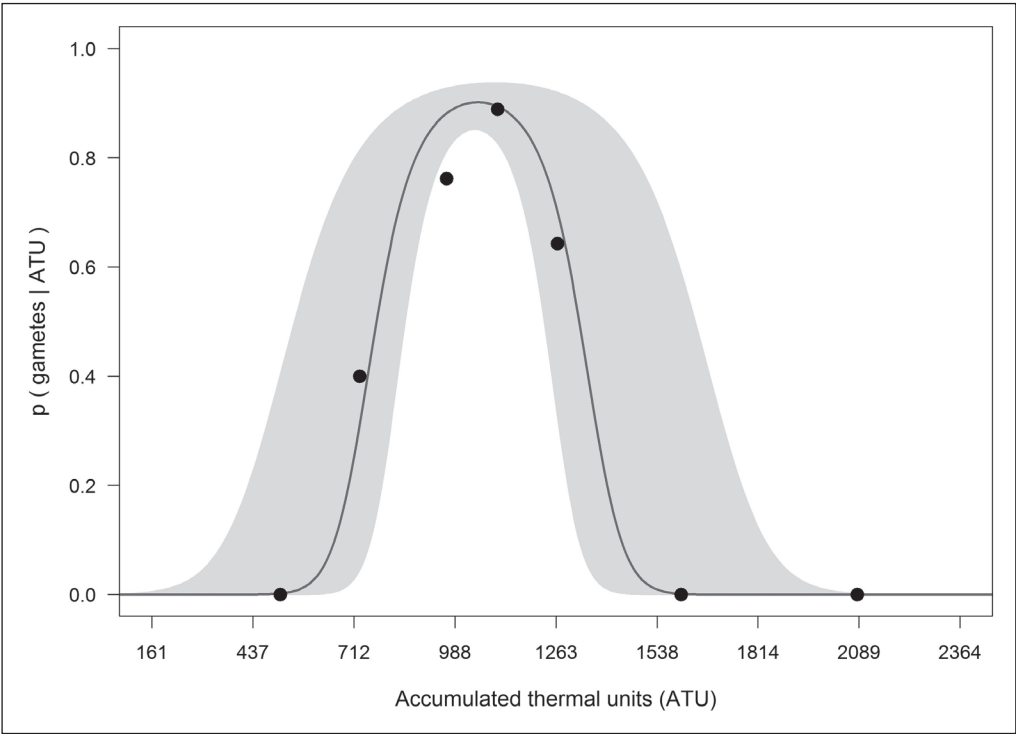


Figure 2. Quadratic relationship between accumulated thermal units (ATU) and the probability of brooding eggs or glochidia by Eastern Elliptio in Otego Creek, NY during the 2016 spawning season. The black line represents the mean model prediction, with 95% confidence intervals indicated by the gray polygon. The black circles are the observed proportion of Eastern Elliptio brooding eggs or glochidia on each sampling event.

climates. The majority of brooding occurred in a 6-week period in late spring/early summer; the dates we observed correspond very closely to the brooding period for Eastern *Elliptio* in other parts of its range (Price and Eads 2011). This relatively protracted period of brooding likely occurs at a time that allows for maximum overlap with the presence of host fish.

Despite the significant correlation of brooding status with air temperature, we found ATU was a stronger predictor of brooding glochidia than temperature, whether the latter was considered alone or in concert with other environmental predictors. Cumulative thermal experience (measured as ATU in this study) can be an important environmental predictor of life-history events such as development and reproduction in ectotherms. This finding is due to the relationship of temperature and metabolic rates when temperatures exceed minimal thresholds governing energetically taxing events such as growth, migration, and reproduction (Chezik et al. 2014, Neuheimer and Taggart 2007). While this metric has gained popularity in aquaculture during the past several decades, application to natural populations has lagged. The metric appears to hold a high degree of explanatory power, even for large-scale studies (Neuheimer and Taggart 2007), and is readily standardized to make inferences between populations (Chezik et al. 2014). In the present study, models with only ATU fixed effects explain 96% of the variability in reproductive timing of Eastern *Elliptio*, indicating that ATU has the potential to be a powerful tool for understanding population-level differences in reproductive phenology for this species. Furthermore, the approach taken here has utility for experimental design and sampling protocols because the daily-temperature data required to make predictions from model coefficients (Table 3) are widely available in real time. However, until variability in the relation between gamete expression and ATU is better understood at a landscape scale, we caution against untested application of model predictions to geographically distant regions. Accordingly, a next logical step for this work is to test these kinds of predictions on other populations.

In South Carolina, Eastern *Elliptio* is able to produce at least 2 broods per season (Price and Eads 2011). However, for our populations at a higher latitude we observed only 1 brood during our sampling period. In fact, by early July only 10% of females were observed with fertilized eggs or glochidia in their gills, the remainder had only immature eggs in their gonads. It is possible that some individuals in this Eastern *Elliptio* population can produce multiple broods, but detecting multiple glochidia-broods was beyond the goals of this study. However, during our surveys, we did not detect any incidence of multiple brooding. We also found evidence of hermaphroditism in 10% of the mussels we sampled. Simultaneous hermaphroditic populations of Eastern *Elliptio* have been observed in a Quebec Lake, with 80% of individuals containing gonad tissue from both sexes (Downing et al. 1989). It has been hypothesized that an increased prevalence of hermaphroditism is a response to low population density (Bauer 1987), especially in lakes and small streams (Haag 2012). More information about these aspects of local reproductive ecology would be helpful in conservation planning for this species. Surveys that use finer temporal resolution may be better suited for studying such patterns.

Recent work on identifying the host fish for Eastern *Elliptio* from the Susquehanna River basin determined that *Anguilla rostrata* Lesueur (American Eel) provided the highest metamorphosis success (Lellis et al. 2013). However, dams throughout the river have disrupted the migration of American Eels, such that their biomass has been drastically reduced. Work is being undertaken to reconnect American Eels with historic habitats in this system, and a fortunate byproduct of this could be exposing populations of Eastern *Elliptio* to a potentially valuable host species (SRAFRC 2014). In the future, our results could help guide efforts seeking to understand spatial and temporal overlap between Eastern *Elliptio* and this host. Our results provide insight into the basic life history of Eastern *Elliptio*, and can be used to inform future sampling of this species in the drainage.

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