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Survival and spawning success of American shad (*Alosa sapidissima*) in varying temperatures and levels of glochidia infection

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Abstract Temperature fluctuations and climate change impacts may substantially affect spawning success of fish, especially migratory species with a limited spawning window. Factors affecting American shad (*Alosa sapidissima*) spawning success and survival were investigated at different temperatures and periods (peak- and late-spawning periods) during the Connecticut River, USA, spawning migration in 2017. Wild caught American shad were exposed to constant temperatures regimes of 15, 18, 21, 24 and 27 °C for 2 weeks. During the peak-spawning period, an increase in temperature (15–24 °C) was shown to increase spawning success factors, including spawning probability, number of eggs, and fertilization success, but decreased egg size. Temperatures between 18 and 27 °C did not affect these factors during the late-spawning period. Glochidia infection by the alewife floater (*Anodonta implicata*) was much higher in the late-spawning period and significantly decreased the survival of American shad. Further research should investigate the parasite-host relationship between the alewife floater and American shad to determine annual variability of glochidia infections and how they affect American shad from physiological and passage perspectives. Higher temperatures were shown to increase spawning success of American shad during the peak-spawning period, but temperature had no effect during the late-spawning period. However, any effect during the late-spawning period may have been masked by a high level of glochidia infection.

Keywords Fish egg · Fertilization success · *Anodonta implicata* · Fish passage · Parasite-host relationship

Introduction

American shad (*Alosa sapidissima*) is a widespread and abundant anadromous fish native to the western North Atlantic Ocean that makes spawning migrations in rivers from Florida, USA, to the St. Lawrence River, Canada (Limburg et al. 2003). They display a clinal variation in life history and reproductive traits that includes percent of repeat spawners (iteroparous vs. semelparous), size, maximum age, and annual fecundity (Glebe and Leggett 1981a; Limburg et al.

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2003; Olney and McBride 2003). American shad spawn across a wide temperature range (Walburg and Nichols 1967; Limburg et al. 2003), and temperature is considered the primary factor for the timing of spawning as temperatures gradually warm in northern locations (Leggett and Whitney 1972).

How temperature influences American shad spawning timing, energetics, and iteroparity has been well studied (Leggett and Whitney 1972; Glebe and Leggett 1981b; Leonard et al. 1999). However, how temperature affects other important factors affecting spawning success such as spawning probability, number of spawns, and fertilization success is unknown. Additionally, how environmental effects, such as temperature, affect survival of juvenile American shad has been often studied (Crecco and Savoy 1985; Leach and Houde 1999; Bayse et al. 2020), but environmental effects are less clear for post-spawn adult survival.

For iteroparous American shad populations, several factors affect emigrating adult survival including temperature, discharge, predators, migration distance, and passage efficiency (Raabe and Hightower 2014). How these factors interact is likely to have large consequences on American shad survival. American shad either do not feed or do not effectively feed during migration (Walter and Olney 2003), which gradually reduces energy resources. Over long migrations at high temperatures, energy resources are further reduced (Castro-Santos and Letcher 2010). Thus, the combination of a long migration and relatively high temperature would more rapidly use resources, a condition that likely reduces the survival of an emigrating American shad (Raabe and Hightower 2014). Other factors can further complicate these relationships, including increased predation at low-discharge conditions (Raabe and Hightower 2014) and increased exposure to high temperatures during passage delay (Castro-Santos and Letcher 2010; Bayse et al. 2019). Another potential factor that may influence American shad survival is parasitism.

Glochidia are a parasitic, larval stage of freshwater mussels from the superfamily Unionacea (Kat 1984). The alewife floater (*Anodonta implicate)* disperses its glochidia via a mucous net that entraps glochidia into fish gills, allowing it to distribute juveniles upriver as its host migrates to spawning habitat (Kat 1984). Glochidia from the alewife floater were reported to expand in range in the Connecticut River from the Hartford, CT, USA, area prior to 1970 to below Bellows Falls, Vermont, USA, in 1984 (approximately 200 river km; Smith 1985). This range expansion was considered to follow the passage improvements of clupeids that serve as host for the parasitic larva (Smith 1985). In 2016, glochidia of the alewife floater were reported as being prevalent on American shad gills in the Connecticut River during their spawning migration and may have been responsible for high mortality at hatcheries (S. Hanlon, 2020, U.S. Fish and Wildlife Service, personal communication; Connecticut River Atlantic Salmon Commission (CRASC) 2016).

The objective of this study was to determine how temperature affects the spawning success and survival of American shad in the Connecticut River during the spawning migration at two periods of the migration. Temperature is a driving factor for determining many facets of the American shad spawning migration, but it is unclear how it directly affects egg production and fertilization success. American shad experience a wide range of temperatures during the freshwater portion of their migration, and Connecticut River American shad spawn during a short period of time (6–8 weeks) that coincides with a large temperature increase, typically increasing from 10 to 22 °C (Leonard and McCormick 1999).

The variability in temperature of the Connecticut River during the American shad migration makes this a relevant population to investigate how temperature affects spawning. Here, we isolated and investigated several factors in a laboratory setting that could be affected by American shad spawning at different temperatures including egg production and size, fertilization success, and survival. Additionally, how temperature affects the survival of spawning adults is also unclear and likely interconnected with other factors, such as sex, lipid content, and glochidia infection, which ultimately would affect spawning success. Here, we considered these factors and investigated how temperature, sex, lipid content, and glochidia infection affect the survival of spawning American shad. The results from this study add to the understanding of temperature's role in the American shad spawning migration, such as migration timing and post-spawn survival, and are of particular interest in light of increasing temperatures from climate change and anthropogenic thermal inputs.

Material and methods

Fish collection

Migrating, adult American shad were collected from a fish lift on the Connecticut River at Holyoke Dam, Holyoke, MA, USA (139 river km). Two fish collections were made to be representative of the peak-spawning period (trial 1; 19 May to 2 June 2017) and late-spawning period (trial 2; 14 June to 28 June 2017). For each trial, approximately 100 fish were collected and transported to S.O. Conte Anadromous Fish Research Laboratory (U.S. Geological Survey; USGS) in Turners Falls, MA, USA, via a fish transport truck (1000 L) specifically designed to carry adult American shad and supplied with recirculating, oxygenated Connecticut River water. Twenty fish, 10 of each sex, were indiscriminately placed in each of four experimental tanks, for a total of 80 fish. Sex was determined by external characteristics (e.g., external visual of gonad, body size) which are not 100% accurate, so the final sex ratio of each group varied slightly.

Experimental tanks

Fish were held in four identical 4.6-m outdoor tanks with flow-through Connecticut River water at 30 $L{\cdot}min^{-1}$. Fish were not fed, which is typical of American shad's behavior during freshwater migration (Walter and Olney 2003; Bayse et al. 2018). Water temperature was regulated by heat pumps (5 HP Titan Air Cooled Heat Pump, Aqua Logic, San Diego, CA, USA) connected to each tank. Supplemental aeration was provided, maintaining oxygen levels near saturation (90–100%). A 10.2-cm drain in the middle of the tank with a 5.1-cm metal grating kept fish from leaving but allowed water and eggs to exit. Eggs were collected from a gravity-fed collection tank (1.5 m) that had a mosquito netting collection frame approximately 15 cm below the water surface that was connected to the 4.6-m tank via the aforementioned drain.

Peak-spawning temperature treatments were matched to historical records for peak periods of the Connecticut River American shad migration (Leggett and Whitney 1972), where the reported mean, peak-spawning temperature was 19.5 °C—the mean of our four temperature treatments (15, 18, 21, and 24 °C). The upper and lower range of our treatment temperatures for peak-spawning also matched those of historical records (Leggett and Whitney 1972). Our goal was to encompass the range of possible spring increases in temperature, such as a rapid and sustained increase (e.g., the 15 to 24 °C treatment), versus the scenario of cooler temperatures that would be associated with a rain event and/or reduced ambient temperatures (e.g., final temperatures of 15 and 18 °C). Fish collection for trial 1 was timed with the ambient river temperature reaching the lower range of peak-spawning temperature, 15 °C, measured by a HOBO Water Level Data Logger (Onset, Bourne, MA) at Holyoke Dam and coincided with an accelerated increase in river temperature from ~ 10 to 15 °C (Fig. 1). Fish were held overnight at 15 °C and then temperature was increased 2 °C per day until desired temperature was reached and then that temperature was maintained, final temperatures of (15, 18, 21, and 24 °C). The late-spawning period (trial 2) represented a similar temperature scenario (18, 21, 24, and 27 °C), but, 2 weeks later, is associated with later spawning conditions for temperature (approaching 30 °C) and time (July; Walburg and Nichols 1967). Ambient river temperature was 21 °C at the beginning of trial 2, and similarly to trial 1 began after an accelerated increase in river temperature from ~ 14 to 21 °C (Fig. 1). Temperatures were increased/decreased 2 °C per day to meet target temperatures.

Egg sampling protocol

American shad typically spawn from dusk and through the night (Greene et al. 2019). Thus, each morning, spawned eggs were collected from each tank. The entire mass of eggs from each tank, with excess water drained, was weighed and two approximately 2-g subsample replicates were randomly taken to determine total eggs, fertilization success, and egg size from each tank. The total number of eggs was averaged between subsampled replicates and total egg number was extrapolated by weight for each tank per day. The number of fertilized and unfertilized (translucent versus opaque; Ross et al. 1993) eggs was determined for each subsample and averaged between replicates. Fertilization success was determined as the percent of fertilized eggs d^{-1}. Egg diameter d^{-1} was

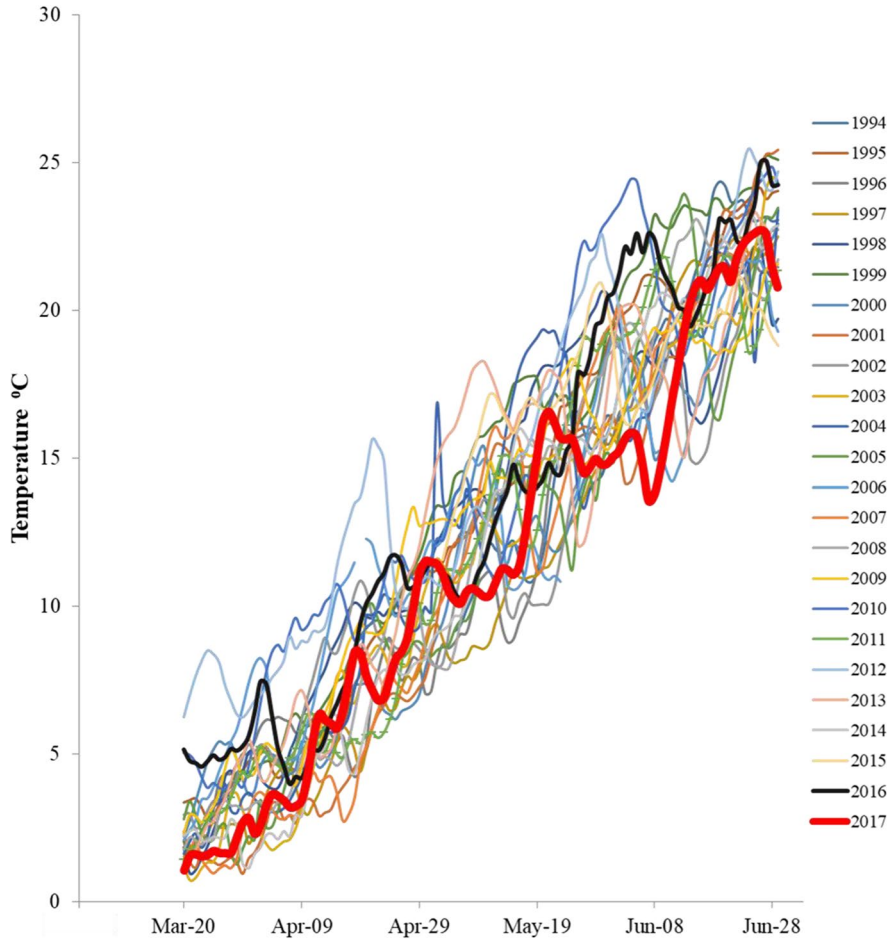

Fig. 1 Historical temperatures during the American shad (*Alosa sapidissima*) migration collected at Holyoke Dam, Massachusetts, USA, from 1994 to 2017. A different color is used for each year, and the bold red line is the year of the current study, 2017. A break in a line represents missing data

considered the average of two replicates of 30 fertilized eggs.

Fish sampling protocol

Dead fish were removed from tanks twice daily, in the morning and at midday; otherwise, fish were not disturbed to facilitate spawning. After removal, fish mass was taken to the nearest g, measured for fork length (FL), and sex was determined. Lipid measurements were taken via a fat meter (Distell Model 692 Fish Fat Meter, Distell, Inc., West Lothian, Scotland, UK) following methods for American shad described in Bayse et al. (2018, 2019).

Glochidia infection was documented with an ordinal approach to determine the level of infection of glochidia on fish gills, where "0" was no glochidia cysts, "1" was a small amount, "2" a medium amount, and "3" where the majority of the gills were covered in glochidia cysts. At the end of each trial (14 days), fish were sacrificed and the above methods were followed.

Statistical analysis

The number of eggs per temperature treatment was analyzed with generalized linear models (GLM) in R statistical software (R Development Core Team 2009). Egg data were continuous and skewed to the right, enabling modeling with a gamma distribution. However, given the large number of zeros (non-spawning events) and the inability to use a gamma distribution when data contain zeros, a hurdle model approach was used. A hurdle model is a two-part model which considers the dependent variable when zero and when not. Thus, when no spawning occurred (i.e., zero eggs), a binary logit model was used to determine the likelihood of a spawning event taking

place. When a spawning event occurred, the number of eggs was modeled with a gamma distribution using a log link. Independent variables included temperature and day, and their interaction term. Additionally, female fecundity is correlated with size, so an offset was used in the model to incorporate different female weights. The best model was selected based on the minimum Akaike information criterion value with a correction for small sample sizes (AICc). AICc was determined using the AICctab function in the bbmle package (Bolker 2017). A $\Delta AICc_i < 2$ provides substantial support that the *i*-th model(s) are the best fit model(s) (Burnham and Anderson 2004). When one model had a $\Delta AICc < 2$, it was considered the best model and sufficiently different from the other models tested. When multiple models had a $\Delta AICc < 2$, AICc weights were investigated to determine the best model (Wagenmakers and Farrel 2004). Differences in the number of eggs between temperature treatments were compared using a post hoc general linear hypotheses test using the glht function in the multcomp package (Hothorn et al. 2016).

Fertilization success was modeled with a beta regression using the function betareg in the package betareg (Zeileis et al. 2016). Independent variables included temperature and day, and their interaction term. The best model was selected via the AICc methods described above, and a post hoc analysis between temperature treatments was performed via a least square means test, using the function lsmeans in the package lsmeans (Lenth 2017).

Egg diameter was compared with a multiple regression, where independent variables included temperature and day, and their interaction term. The best model was selected via the AICc methods described above, and a post hoc Tukey HSD test was performed between temperature treatments following an analysis of variance (ANOVA).

American shad survival probability was investigated with survival analysis. Survival probability was modeled using the coxph function from the package survival (Therneau and Lumley 2014). Independent variables considered included temperature, sex, lipid content, and glochidia infection. Fish that survived the entire 14-day trial were considered censored observations. The best model was chosen using the AICtab function, and the model selection criteria explained above. Further model interpretation was conducted via plotting model results under varying scenarios with the ggsurvplot function in the package survminer (Kassambara et al. 2017; Bayse et al. 2019).

Results

Fish collection

A total of 81 fish were included in trial 1, 38 were female with a mean FL of 45.0 cm and standard deviation (SD) of ± 23.4 and 43 were male with a mean FL of 41.3 and SD of ± 18.5. There were at least 9 females in each tank and up to 12 males in a tank (Table 1-Supplemental). For trial 2, 80 fish were included, 40 of which were female that had a mean FL of 45.4 cm and a SD of ± 27.0 and 40 males with a mean FL of 41.1 cm and a SD of ± 18.2. Each tank had 20 fish with a 1:1 sex ratio (Table 2-Supplemental). Once tanks reached target temperatures in trial 1, the mean (SD) temperature for the four treatments was 15.3 (0.3), 17.8 (0.1), 21.3 (0.2), and 24.2 (0.2) °C, respectively, and for trial 2, 18.4 (0.3), 21.4 (0.4), 24.4 (0.2), and 26.6 (1.4) °C, once target temperatures were reached.

Number of eggs

There was a maximum of 56 possible spawning events (4 tanks over 14 d) for each trial. Trial 1 had 32 spawns and the likelihood of a spawning event without consideration for temperature was 57.1% (44.1–69.6%, 95% confidence intervals (CIs)) and trial 2 had 16 spawning events, and the likelihood of a spawning event when not considering temperature was 28.6% (17.9–41.2% CIs). When considering temperature, trial 1 had a similar spawning event probability for 18 °C and up (> 76%), but 15 °C had a lower probability, 28.6%, which was significantly different from 24 °C (Fig. 2). Trial 2 also had the lowest spawning event probability for the lowest temperature (18 °C; 14.3%) and similar probabilities for the three highest temperatures (> 62.1%); however, no statistical differences were determined ($p > 0.05$; Fig. 2).

In trial 1, the number of eggs increased with temperature (Fig. 3), and the best model included the temperature variable (Table 1). The 15 °C treatment had 4 spawns and the number of mean ± SD eggs was 12,525 ± 8,378, which was significantly lower

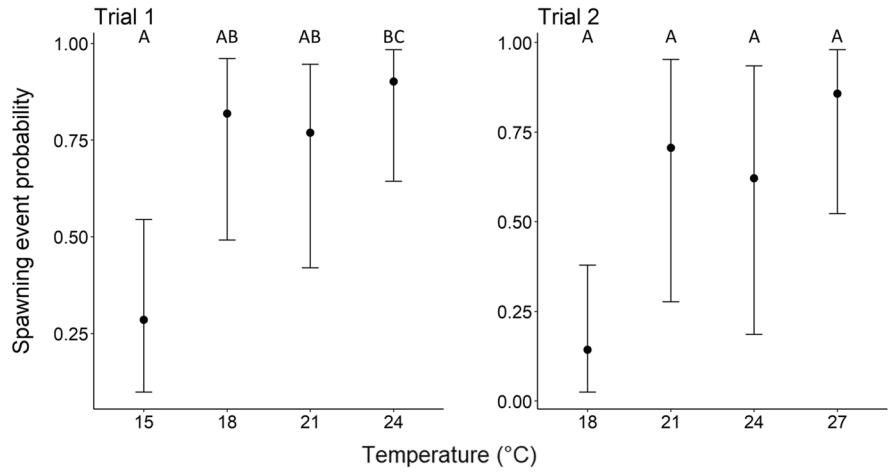

Fig. 2 Spawning event probability of American shad (*Alosa sapidissima*) in the Connecticut River, USA, at different temperatures for trials 1 and 2. Circles are predicted means and the error bars represent 95% confidence intervals. Different letters report differences between temperatures from a general linear hypothesis post hoc test

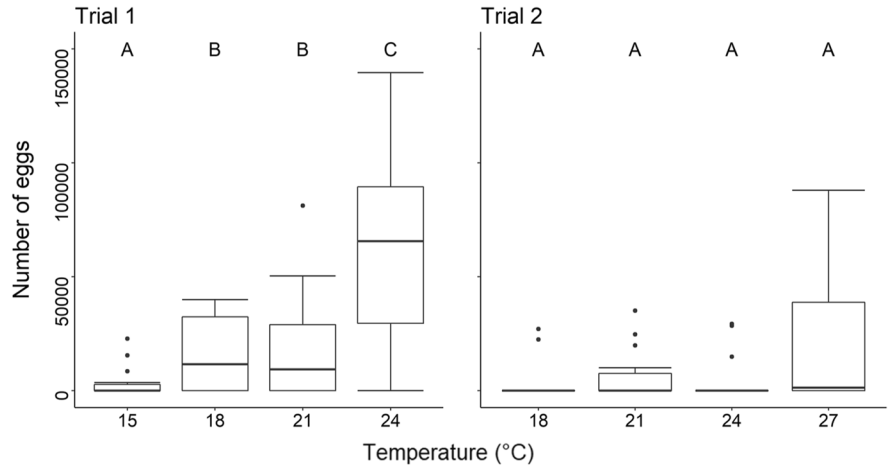

Fig. 3 Box plots of the raw number of eggs spawned from American shad (*Alosa sapidissima*) in the Connecticut River, USA, calculated at different temperatures for trials 1 and 2. The line in the middle of the box is the 50th percentile (median), the bottom of the box is the 25th percentile, top of the box 75th percentile, the bottom "whisker" is the 25th percentile − 1.5 * interquartile range, and the top "whisker" is the 75th percentile + 1.5 * interquartile range. Circles are values beyond the range of the whiskers. Different letters report differences between temperatures from a general linear hypothesis post hoc test

than all other treatments ($p < 0.05$; Fig. 3). The 18 and 21 °C treatments were similar. The 18 °C treatment had 9 spawns, versus 8 for the 21 °C treatment. The mean number of eggs for the 18 °C treatment was 25,426 ± 13,126 and the 21 °C treatment 34,311 ± 24,370, which was not significantly different (Fig. 3). The highest temperature tested in trial 1, 24 °C, had the most mean eggs (80,340 ± 35,787) over 11 spawning events.

For trial 2, the 3 lowest temperature treatments (18, 21, 24 °C) produced similar results, 2, 4, and 3 spawning events and mean number of eggs 24,733 ± 3,276, 22,401 ± 10,404, and 24,184 ± 8,049, respectively. The highest temperature, similar to trial 1, produced the most spawns (7) and highest number of mean eggs (37,624 ± 28,552), but this higher amount was not significant ($p > 0.05$; Fig. 3). The best model contained day-only, as the number of eggs produced per spawn increased throughout the trial (Table 2).

Table 1 Independent variables included in models, Akaike information criterion (AIC) or AICc, delta-AIC (dAIC) or dAICc, and AIC or AICc weight for each model from trial 1 analyses

Egg number			
Independent variables	AICc	dAICc	Weight
Temperature	732.6	0.0	0.816
Temperature + Day	735.6	3.0	0.181
Temperature*Day	743.6	11.0	0.003
Null model	755.0	22.4	< 0.001
Day	757.5	24.9	< 0.001
Fertilization success			
Independent variables	AICc	dAICc	Weight
Temperature + Day	− 42.6	0.0	0.735
Temperature	− 39.0	3.6	0.121
Null model	− 37.8	4.7	0.069
Day	− 37.2	5.3	0.052
Temperature*Day	− 35.6	6.9	0.023
Egg diameter			
Independent variables	AICc	dAICc	Weight
Null model	236.1	0.0	0.447
Day	237.2	1.2	0.250
Temperature	237.3	1.3	0.239
Temperature + Day	240.0	3.9	0.064
Temperature*Day	250.5	14.4	< 0.001
Survival probability			
Glochidia			
Independent variables	AIC	dAIC	Weight
Null model	17.2	0.0	0.219
Lipid content	18.1	0.9	0.140
Glochidia	18.8	1.5	0.101
Sex	19.2	2.0	0.081
Temperature	19.2	2.0	0.081
Lipid content + Glochidia	19.7	2.5	0.063
Sex + Adjust	19.9	2.6	0.059
Temperature + Lipid content	20.1	2.8	0.053
Sex + Glochidia	20.8	3.5	0.038
Temperature + Glochidia	20.8	3.5	0.038
Temperature + Sex	21.2	4.0	0.030
Sex + Lipid content	21.4	4.2	0.027
Temperature + Lipid content + Glochidia	21.7	4.4	0.024
Temperature + Sex + Lipid content	21.8	4.6	0.022
Temperature + Sex + Glochidia	22.7	5.5	0.014
Temperature + Sex + Lipid content + Glochidia	23.4	6.1	0.010
No glochidia			
Independent variables	AIC	dAIC	Weight
Null model	69.6	0.0	0.422
Sex	70.7	1.1	0.243
Lipid content	71.6	2.0	0.156
Sex + Lipid content	72.7	3.1	0.091
Temperature	74.3	4.7	0.041
Temperature + Sex	75.4	5.8	0.023

Table 1 (continued)

Temperature + Lipid content	76.3	6.7	0.015
Temperature + Lipid content + Sex	77.4	7.8	0.009

Fertilization success

In trial 1, fertilization success increased with temperature and over the 14-day trial; thus, the best model included both temperature and day (Table 1). Collinearity was not an issue, and the variance inflation factor (VIF) was ~ 1, which is a low number and not near approaching values to be concerned for collinearity (> 5; James et al. 2013). Mean fertilization success was the lowest for the 15 °C treatment (mean 9.0% per day, range 0.8–25.5%), and similar for the 18 (mean 12%, range 1.6–53.4%) and 21 °C (mean 11.9%, range 0.1–66.2%) treatments. The 24 °C treatment had the highest fertilization success, mean 35.9% and a range of 2.5–82.2% (Fig. 4). Post hoc analysis showed no differences between 15 and 21 °C treatments, and that the 24 °C treatment had significantly higher fertilization success than the other groups ($p < 0.05$; Fig. 4).

Trial 2, generally, had reduced fertilization success as temperatures increased. Mean fertilization success per day for the 18 °C treatment was 20.5% (range 1.0–40.0%), 37.6% (range 7.5–92.8%) for 21 °C treatment, 11.1% (range 2.0–27.0%) at 24 °C, and 9.7% (range 2.0–36.7%) at 27 °C. The best model was the null model, showing that neither temperature nor day significantly affected fertilization success; this was further confirmed with post hoc analysis ($p > 0.05$ for all groups; Table 2; Fig. 4).

Egg diameter

In trial 1, egg diameter had a mean of 3.1 mm ± SD 0.3. The null model was the best model (Table 1). Temperature-only and day-only models were within 2 AICc, but according to AICc weights, the null model had 78.8% and 87.0% more AICc weight than the other two models, respectively (Table 1). Egg diameter for the two lowest temperature treatments was significantly larger than the highest two treatment temperatures ($p > 0.05$; Fig. 5), which may have been affected by larger females being present in the lower temperature tanks (Table 1-Supplemental). For trial 2, egg diameter had a mean of 3.0 mm ± SD 0.3. The best model was the null model, and the post hoc test revealed no differences between temperatures ($p > 0.05$; Table 2; Fig. 5).

Glochidia and survival

In trial 1, eight fish (10%) died before the end of the 14-day experiment. Of all the fish in trial 1, one fish was observed to have no glochidia, and most fish had infection levels of 1 (60, 74%; Fig. 6), followed by level 2 (11, 14%), and 3 (3, 4%; Figs. 6, 7). Glochidia infection was undetermined for six fish. Separate model routines were investigated. One model routine included fish that had information for every variable of interest ($n = 75$), excluding fish that did not have a glochidia infection determination. The second included every fish ($n = 81$), but did not consider glochidia's effect on survival. For the first model routine, the null model had the lowest AIC (Table 1). Four other models were within 2 AIC, but the null model had at least a 56.4% higher AIC weight than the other models (Table 1). For the second model routine, the null model also had the lowest AIC; however, models that contained sex-only and lipid content-only were within 2 AIC (Table 1). According to AIC weight, the null model had 73.4% and 170.4% higher AIC weight, thus providing the best fit (Table 1). Given that the null model was the best fit for both model routines, it can be considered that none of the measured variables had any significant effect on survival. The null model including all fish is illustrated in Fig. 8.

Trial 2 had more fish die during the 14-day trial ($n = 61$) and higher levels of glochidia infection (Fig. 7). No fish had zero glochidia, 20 had a level 1 infection (25%), 27 at level 2 (34%), and 24 at level 3 (30%; Fig. 7). Glochidia infection was not determined for nine fish. Separate model routines were investigated as described previously. The first model routine included fish that had information for every variable ($n = 71$), excluding fish with no glochidia infection determination. The model with the lowest AIC included only the variable glochidia infection (Table 2). Two other models, glochidia infection + lipid content and glochidia infection + sex, had

Table 2 Independent variables included in models, Akaike information criterion (AIC) or AICc, delta-AIC (dAIC) or dAICc, and AIC or AICc weight for each model from trial 2 analyses

Egg number			
Independent variables	AICc	dAICc	Weight
Day	361.9	0.0	0.841
Temperature + Day	365.4	3.6	0.141
Temperature*Day	369.6	7.7	0.018
Null model	378.8	16.9	< 0.001
Temperature	380.9	19.0	< 0.001
Fertilization success			
Independent variables	AICc	dAICc	Weight
Null model	− 13.6	0.0	0.812
Day	− 10.5	3.1	0.171
Temperature	− 5.3	8.3	0.013
Temperature + Day	− 3	10.6	0.004
Temperature*Day	10.6	24.2	< 0.001
Egg diameter			
Independent variables	AICc	dAICc	Weight
Null model	10.2	0.0	0.880
Day	14.1	3.9	0.120
Temperature	26.2	16.0	< 0.001
Temperature + Day	37.2	27.0	< 0.001
Temperature*Day	88.2	78.0	< 0.001
Survival probability			
Glochidia			
Independent variables	AIC	dAIC	Weight
Glochidia	370.8	0.0	0.390
Lipid content + Glochidia	371.5	0.7	0.277
Sex + Glochidia	372.5	1.6	0.173
Sex + Lipid content + Glochidia	373.4	2.6	0.109
Temperature + Lipid content + Glochidia	376.4	5.5	0.025
Temperature + Sex + Glochidia	377.0	6.2	0.018
Temperature + Sex + Lipid content + Glochidia	378.3	7.5	0.010
Lipid content	389.7	18.9	< 0.001
Sex	390.2	19.3	< 0.001
Sex + Lipid content	390.2	19.4	< 0.001
Null model	390.7	19.9	< 0.001
Temperature + Lipid content	393.0	22.2	< 0.001
Temperature + Sex + Lipid content	393.6	22.8	< 0.001
Temperature + Sex	394.0	23.1	< 0.001
Temperature	467.7	96.9	< 0.001
Temperature + Glochidia	472.5	101.6	< 0.001
No glochidia			
Independent variables	AIC	dAIC	Weight
Lipid content	467.3	0	0.261
Sex + Lipid content	467.4	0.1	0.25
Sex	467.7	0.4	0.209
Null model	468.7	1.4	0.132
Temperature + Lipid content	470.6	3.3	0.05
Temperature + Sex + Lipid content	470.7	3.4	0.047

Table 2 (continued)

Temperature + Sex	471.6	4.3	0.031
Temperature	472.5	5.2	0.02

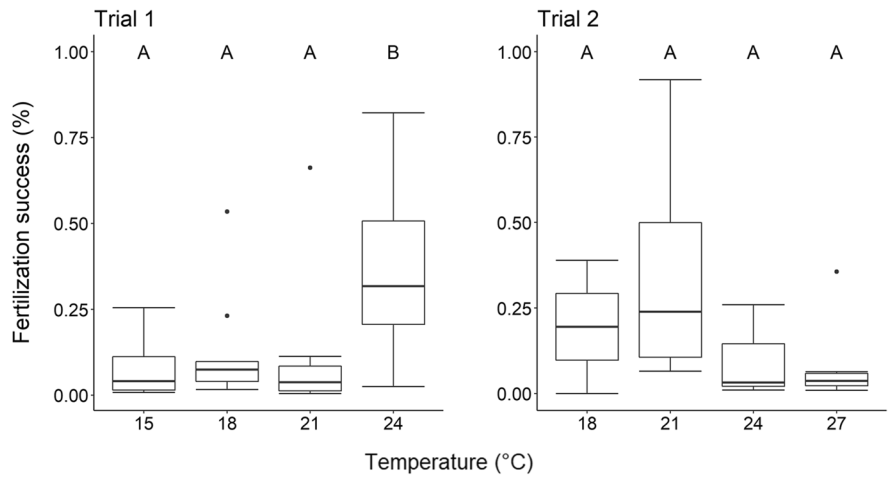

Fig. 4 Box plots of the raw percentage of fertilization success of American shad (*Alosa sapidissima*) eggs in the Connecticut River, USA, at different temperatures for trials 1 and 2. The line in the middle of the box is the 50th percentile (median), the bottom of the box is the 25th percentile, top of the box 75th percentile, the bottom "whisker" is the 25th percentile − 1.5 * interquartile range, and the top "whisker" is the 75th percentile + 1.5 * interquartile range. Circles are values beyond the range of the whiskers. Different letters report differences between temperatures from a least square means post hoc test

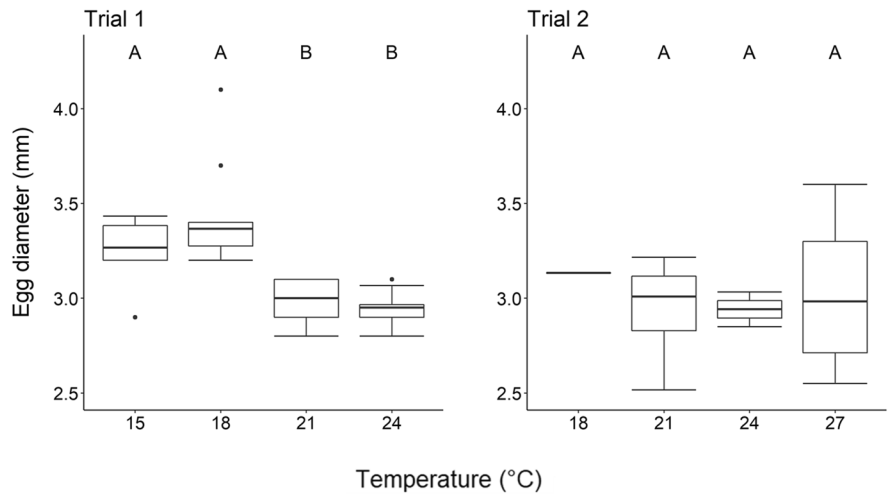

Fig. 5 Box plots of the raw egg diameters from American shad (*Alosa sapidissima*) in the Connecticut River, USA, at different temperatures for trials 1 and 2. The line in the middle of the box is the 50th percentile (median), the bottom of the box is the 25th percentile, top of the box 75th percentile, the bottom "whisker" is the 25th percentile − 1.5 * interquartile range, and the top "whisker" is the 75th percentile + 1.5 * interquartile range. Circles are values beyond the range of the whiskers. Different letters report differences between temperatures from a Tukey HSD post hoc test

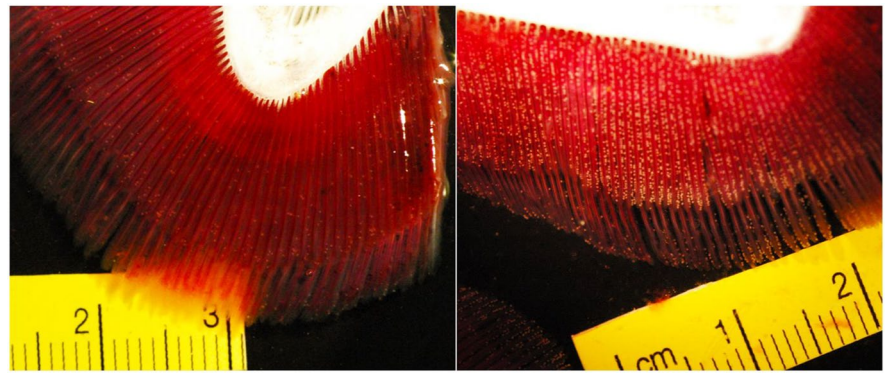

Fig. 6 Photos of American shad (*Alosa sapidissima*) gills infected with glochidia cysts (small, white circles). The left photo is a level 1 infection, and the right photo is a level 3 infection

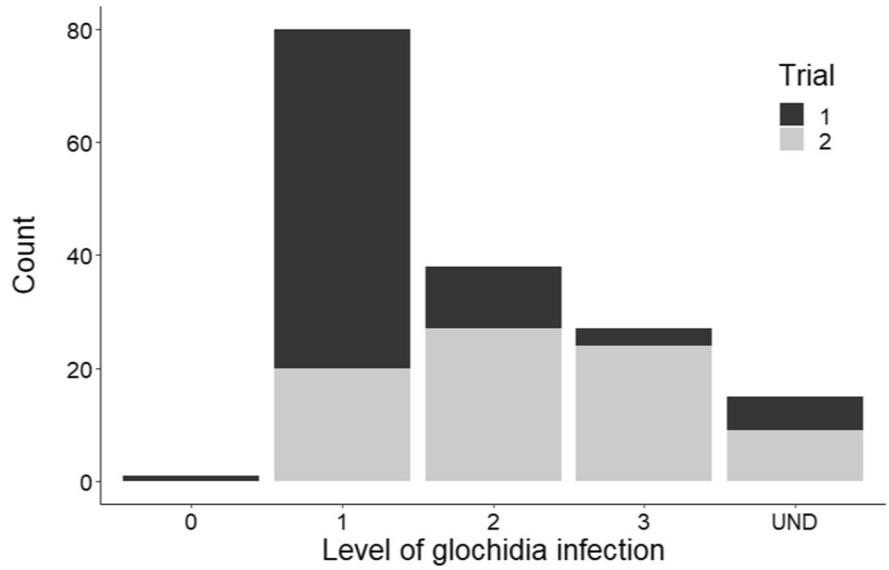

Fig. 7 Levels of glochidia infection found on American shad (*Alosa sapidissima*) gills in the Connecticut River, USA, for trials 1 and 2

AIC values within 2 AIC; however, AIC weights of the glochidia infection-only model were 40.8% and 124.6% higher, respectively (Table 2). Thus, the glochidia infection-only model was the best fit. As glochidia infection level increased, survival significantly decreased (Fig. 8). For example, a 50% survival probability was observed at day 5 for fish with a glochidia infection level of 3 and day 9 for fish with a level 2 infection, and a 50% survival probability was not reached for a level 1 infection (Fig. 8). The second model routine, which included all fish ($n = 80$) and did not consider glochidia, had four models with AIC values within 2: the null model, model containing lipid content-only, sex-only, and lipid content + sex. AIC weights did not indicate a model as having an obviously better fit than any other model within 2 AIC (larger percentage of AIC weight; Table 2). Thus, each model was investigated with plots. These plots showed little effect from the variables contained in the best models (i.e., overlapping confidence intervals), and since the null model was a competitive model, it can be inferred that the variables considered had little effect on survival. Temperature was not included in any of the best models and showed little effect when plotted.

Discussion

How temperature affects spawning American shad is often interrelated with several factors, which can vary during a single migration, or over several migrations, as environmental factors vary. Earlier research with American shad from the Connecticut River found

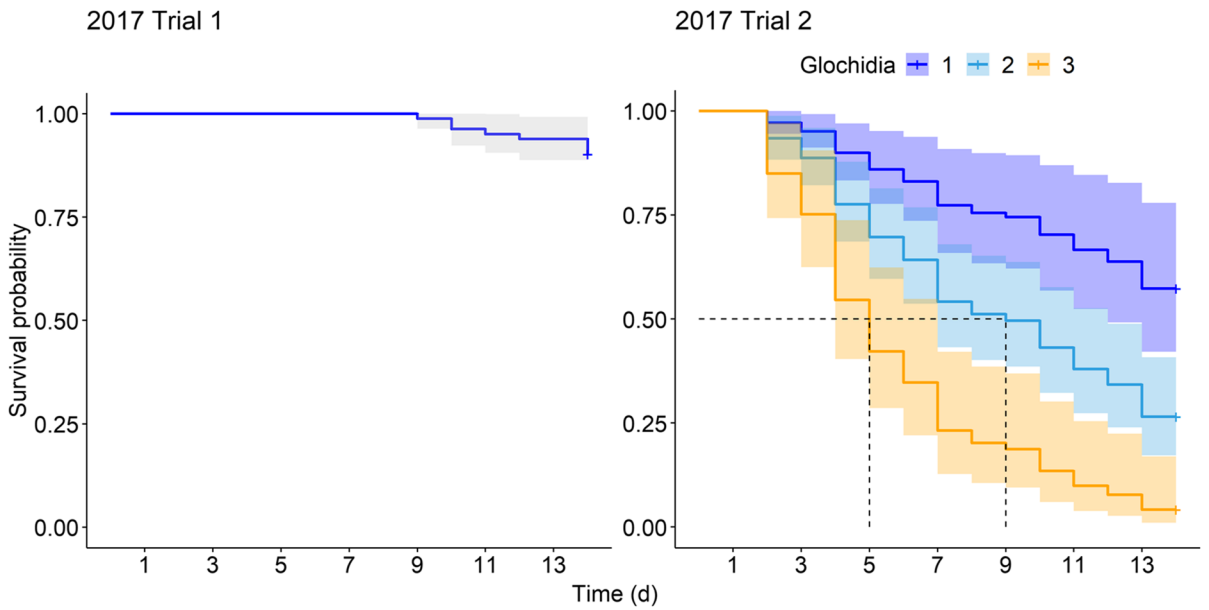

Fig. 8 Survival probability for American shad (*Alosa sapidissima*) in trial 1 represented by the null model (left). The blue line is the modeled proportion and the gray shading is the 95% confidence intervals. Censored observations are denoted by crosses. Survival probability for American shad in trial 2 represented by a model only considering glochidia infection (right). Each line represents the modeled proportions for each observed level of glochidia infection and the shaded area around the line are the 95% confidence intervals. Dashed lines indicate a 50% survival probability. Censored observations are denoted by crosses

little difference between egg production at ambient temperatures from peak- to late-spawning periods, but survival was significantly reduced for American shad in the late-spawning period (Bayse et al. unpublished results). Building off these results, the present study aimed to understand what temperature's role was (if any) in spawning production and success, and if the increased temperature was the driving factor that reduced American shad survival in the late-spawning period, and what other factors may have influence.

How temperature affected American shad spawning differed between the two spawning periods. During the peak-spawning period (trial 1; 15 to 24 °C), higher temperatures were shown to have a higher number of eggs spawned, higher fertilization success, lower egg size, and no effect on survival. Conversely, during the late-spawning period (trial 2; 18 to 27 °C), temperature was shown to not have an effect on any of the tested factors. Interestingly, the lowest temperatures tested during each spawning period produced the lowest spawning event probability and the lowest number of eggs. Similarly, Leim (1924) observed that spawning stopped when water temperature dropped suddenly from 16 to 10 °C. These results indicate that when temperatures drop (trial 2) or remain low (trial 1), spawning is reduced.

Increased temperatures showed positive effects during trial 1 for spawning probability, number of eggs, and fertilization success, which suggests warmer temperatures during peak-spawning is beneficial to American shad from a population sustainability perspective. However, similar to what was reported in Bayse et al. (2019), there is ambivalent evidence for warmer water. Bayse et al. (2019) showed that in warmer temperatures, American shad were more motivated to pass barriers (i.e., dams), but passage capacity was shown to be reduced for large females. Thus, American shad having a higher motivation to pass barriers is an improvement from the population sustainability perspective, but reducing the passage of highly fecund females would be considered a detriment. Similarly, our reported increases in spawning probability, number of eggs, and fertilization success versus reduced egg size have corresponding concerns. An increase in spawning probability will inherently increase the number of spawned

eggs, which would have direct, positive effects on the American shad population, as would increased fertilization success. However, warmer temperatures also showed a decrease in egg size. There is variation among factors that affect egg size, typically egg size increases with mother age/length and warmer temperatures, but this is not always the case (Johnston and Leggett 2002). Egg size could potentially have a direct effect on larval feeding success and survival, as it does for other species (Moodie et al. 1989), which at warmer temperatures ultimately would be a negative for the American shad population.

Glochidia infection likely had a stronger effect on the spawning success of American shad than temperature during the late-spawning period, perhaps even masking any observable temperature effects due to its large influence on the survival of American shad. In trial 1, 74% of fish had a level 1 infection and only eight fish died (9.9%). Conversely, in trial 2, infection levels 2 and 3 were much higher (64% of total) and a significantly higher number of fish died ($n=61$), where higher levels of infection led to significantly lower levels of survival. Notably, ambient river temperatures at the start of trial 2 were similar to observed temperatures of alewife floater glochidia release (20 °C; Davenport and Warmuth 1965). Temperature has been considered the main driver of ending the American shad spawning migration in the Connecticut River (~26 °C; Walburg and Nichols 1967), effectively ending upstream migration and fish actively spawning; however, glochidia infection may also play a role at the end of the spawning period by increasing mortality in the Connecticut River.

How glochidia affect their hosts can range from negligible (Nezlin et al. 1994) to negative effects (e.g., reduced respiration; Karna and Millemann 1978), and can include changes in behavior (e.g., reduced migration distance; Horký et al. 2014), altered thermoregulation (Horký et al. 2019), or asphyxiation (Karna and Millemann 1978). Levels of infection are typically low (average of 8%; Trdan 1981), but can be as high as 100% with an individual being infected with over 4,000 glochidia (Dartnall and Walkey 1979). High infection rates likely result from the combined effects of high-level host specificity with large densities of mussels (parasite) and fish (host) (Trdan 1981). There may be direct interference of high infection rates of glochidia on the respiratory function of the gill simply by their physical blocking of water movement through respiratory lamellae. Since the capacity to deliver oxygen has been linked to thermal tolerance (Pörtner and Knust 2007), there may be important interactions between glochidia infection and thermal tolerance and performance. However, we did not observe any interaction between survival in different temperature regimes and the level of glochidia infection in the present study.

Little can be found in the scientific literature on how the alewife floater's glochidia affect its host, with most studies focusing on its range and association with clupeid species (Davenport and Warmuth 1965; Smith 1985). In an ecological connectivity sense, the presence of the alewife floater has been considered a positive observation (Smith 1985). Historically, the alewife floater has had its range reduced by dams that have restricted its dispersal mechanism, which comes via migrating clupeids (Kat 1984). The reduced passage capacity of the hosts directly led to reductions in the alewife floater habitat. Thus, the presence of the alewife floater has been deemed a sign of successful fish passage restoration (Hall et al. 2012; Smith 1985), which should be considered in the context that many of the endemic freshwater mussel populations are endangered or declining (Williams et al. 1993). The alewife floater has a very synchronized relationship with clupeid migrations, releasing glochidia when waters warm; glochidia were observed to be released in a laboratory at 20 °C (Davenport and Warmuth 1965). The high levels of glochidia infection on American shad reported here and in CRASC (2016) may be common in the Connecticut River and gone unnoticed or could be a result of a combination of a relatively large American shad migration in 2017, high density of alewife floaters, and a well-timed release of glochidia. Other factors, such as passage delay, may have artificially increased fish density and led to high levels of infection.

Some limitations should be considered from our study design. We aimed to minimize handling at collection to reduce any spawning or survival bias, but this reduced our knowledge of individual fish information (e.g., change in lipid content) over the course of the experiment until they died or were sampled. Additionally, we have no information on the history of each individual in terms of what took place once it entered the river. This restricts our results from individual fish data and history. For example, we do not know when or if each fish spawned

during the experiment, if or how much it had previously spawned, nor its history upon entering the river (e.g., number of days migrating up river, any passage delay). There could be potentially large differences in the number of days each fish spent in the river, and the accrual of thermal days (the aggregate sum of mean daily temperature [°C] for each day upstream; Raabe and Hightower 2014; also called degree-days), which could have affected individual survival and spawning success results. Potentially, thermal history could be inferred from lipid content, as lower lipid levels would be an indicator of a longer time spent in the river (Leonard and McCormick 1999; Bayse et al. 2018); however, lipid content was not an important indicator for survival in our analysis.

In summary, temperature was shown to influence spawning success for American shad during the peak-spawning period, but not during the late-spawning period. In the peak-spawning period, warmer temperatures resulted in increased spawning success, but smaller eggs, which may have mixed impacts on larval and juvenile survival. Temperature effects may have been masked in the late-spawning period due to a high level of glochidia infection, which significantly decreased survival of American shad. Future work could focus on how temperature affects the late-spawning period, especially in the context of high and low glochidia infections. If glochidia infections are low during the late-spawning period, then temperature may have a larger effect on the factors tested here, either positively or negatively. Additionally, more work is needed to understand the parasite-host relationship between American shad and the alewife floater. High infection rates, as observed in this study, could negatively affect American shad populations by reducing survival, migration distance, and spawning success.

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Author contribution SMB: conceptualization, methodology, validation, investigation, data curation, formal analysis, visualization, writing (original draft), writing (review and editing). AMR: conceptualization, methodology, validation, investigation, data curation, visualization, writing (review and editing). SDM: funding acquisition, conceptualization, methodology, validation, investigation, data curation, visualization, writing (review and editing).

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Data availability Data available upon request.

Code availability Code available upon request.

Declarations

Ethics approval All experiments were carried out under US Geological Survey Institutional Animal Care and Use Committee Guidelines under protocol no. C09076.

Consent to participate Not applicable.

Consent for publication Not applicable.

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