ZOOPLANKTON DIETS FOR CAPTIVE BROOK STICKLEBACK LARVAE: A COMPARISON OF *ARTEMIA* AND *ROTIFERA*

By

Bill Pevey

Abstract

Sticklebacks are a group of small, spined fish that are frequently cultured in laboratory settings for their use in genetic and other biological research. More research is needed regarding the husbandry of captive brook sticklebacks (*Culaea inconstans*), a species that is far newer to lab rearing than the more common threespine stickleback (*Gasterosteus aculeatus*). In this study, which was conducted as the focus of a graduate internship in aquatic husbandry, larval brook sticklebacks were fed one of three diet treatments: live brine shrimp nauplii, live L-type rotifers (*Brachionus plicatilis*), or a commercial diet paste made of dead rotifers. The majority of the brine-shrimp-fed larvae survived, whereas both groups of rotifer-fed larvae died before the end of the 32-day period. The low survival rate of the rotifer-fed fish could be attributed to insufficient nutrient content in the rotifers or inability of the larvae to find them. This result indicates that it is inadvisable to raise brook stickleback larvae on a rotifer-based diet, as some other species of cultured fish have been.

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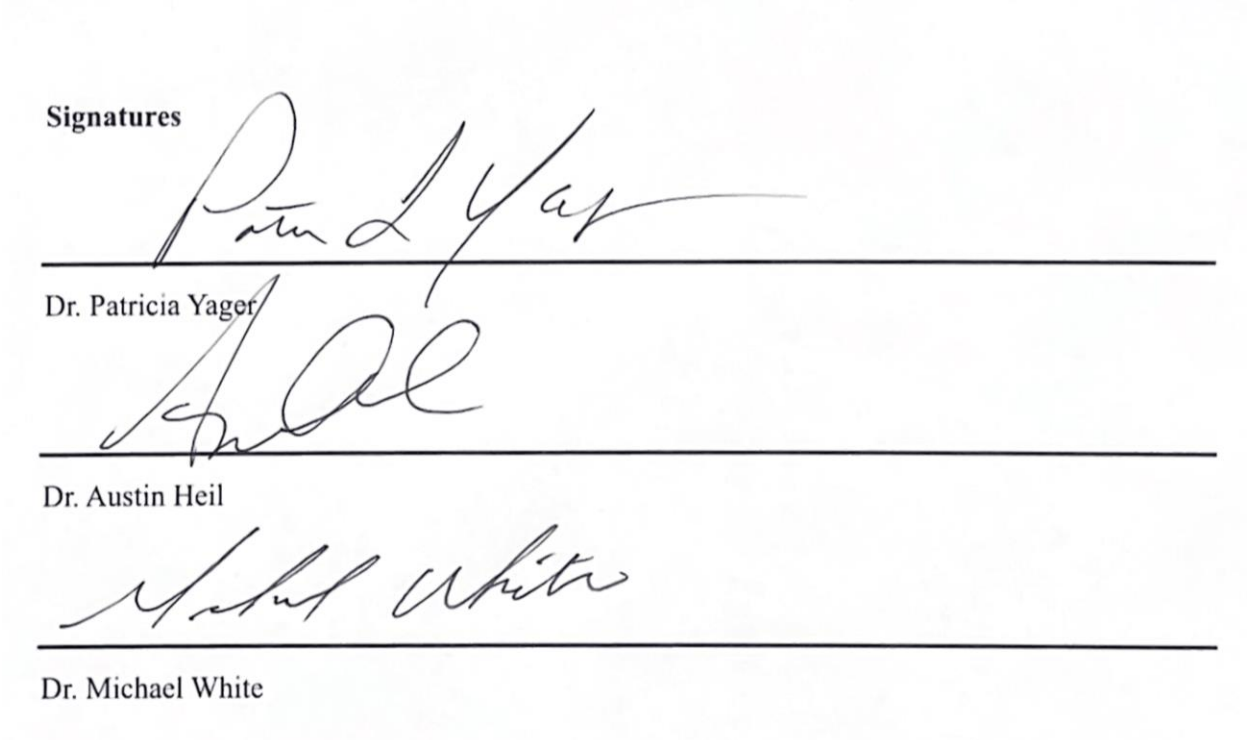
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Internship & Experience

The internship and research project for my master's program were carried out from May 2025 to the present. I began by consulting with Shannon Fox and Dr. White to find questions regarding stickleback husbandry that were yet to be addressed and could benefit from dedicated study. Given the lack of research into the husbandry of brook sticklebacks (*Culaea inconstans*), I decided to focus my research on improving their survivorship in lab settings, both to benefit the success of the lab and the wellbeing of the fish. The lab (and many others like it) had always raised stickleback larvae on a diet of live brine shrimp (*Artemia franciscana*) nauplii, but the question was raised whether live rotifers would lead to greater larval survival rates.

I began my project by collecting literature on rotifers and methods of culturing them. Throughout June of 2025, I started a culture of L-type rotifers (*Brachionus plicatilis*) and worked to make them healthy and reproductive. The culture crashed once as I was learning the correct amounts of food to give them, but I grew from that experience and eventually established a healthy culture (Figure 2). I also maintained brine shrimp hatchers (Figure 3) previously used by the lab, which hatch brine shrimp eggs so the 24-hour nauplii can be fed to fish. We also purchased Roti-Feast®, a commercial rotifer diet, to test if it would be possible to raise larvae on non-live food. After counting the number of rotifers or brine shrimp per milliliter in each diet, I created larvae diet plans in volume of food per fish per day, so it could be applied to tanks with any number of fish. These practices allowed me to build aquaculture skills in growing live food, as well as experience in diet formation. Both of these skills will be beneficial in my planned career path in aquarium science and animal care.

Eggs and sperm from wild-caught brook sticklebacks arrived in early June, and we crossed them in the lab to produce embryos. After they hatched, I cared for the larval fish in

mason jars and later small zebrafish tanks (see Methods section). Performing the daily maintenance and husbandry of these small systems allowed me to improve my skills in fish care, especially because this project allowed me to take responsibility over creating operating procedures. I also gained more experience with research and data analysis, as well as adjusting real-world lab procedures based on the results of research.

This work adds to the academic community's knowledge about the husbandry of captive larval sticklebacks, especially brook sticklebacks, which are understudied. These results can inform the care of brook sticklebacks in Dr. White's lab, as well as contributing to more efficient research using sticklebacks in genetics, ecology, marine science, and other fields. Researchers have an ethical obligation to constantly strive to improve the care of their model animals, and I am grateful to contribute to the study of captive fish care.



Figure 1: Experimental setup for larvae study

Introduction

Brook sticklebacks (*Culaea inconstans*) are a species of small, estuarine and freshwater fish native to cold waters in the eastern portion of North America and its coastline (Cryster, 2020). Given concerns about their invasive presence in western North America (Wieker et al., 2016) and increasing interest in using them for genetic study on morphology and development (Mee et al., 2025), it is essential to better understand their needs when raised in laboratory settings. While threespine sticklebacks (*Gasterosteus aculeatus*) have long been the most popular stickleback species used for research due to their genetic traits and ease of culturing (Blaker et al., 2022), increasing interest in the study of brook sticklebacks means that research into their care is needed.

When rearing stickleback larvae, diet is often composed of live brine shrimp (*Artemia franciscana*) nauplii (Blaker et al., 2022), as the omnivorous fish respond well to the movement of live food of that size. However, this is just the standard feed for threespine sticklebacks. Brook stickleback larvae are smaller and more demersal than threespines (Winn, 1960), so it is worth consideration whether a different food could be optimal for this species. Rotifers are a smaller type of brackish-water zooplankton that are commonly used to feed small larval fish of similar size to brook sticklebacks (Allen et al., 2016). This study measured the survival rate of nine populations of lab-hatched brook stickleback larvae during their first 32 days post-hatch and examined if brine shrimp are truly the optimal feed for captive brook sticklebacks, or whether the smaller rotifers are the better option. It was hypothesized that the live rotifer diet would lead to higher survival rates. In addition, it compared larval survivorship consuming a non-live rotifer feed to those eating live prey, as the use of commercial feeds could be a cost-effective and time-

saving way for laboratories to raise brook sticklebacks.

Methods

Small cultures of *Artemia franciscana* (brine shrimp nauplii) and *Brachionus plicatilis* (L-type rotifers) were grown and maintained for use in feeding (Figures 2 and 3). Brine shrimp were hatched and harvested daily to ensure maximum nutritional value of the nauplii, and nauplii were stored in 15-mL tubes and refrigerated to increase their longevity (Léger et al., 1983). Rotifers were cultured in a 15L bucket and fed RGComplete™ (a commercial algae feed for enrichment of rotifers). Daily maintenance was performed on the rotifer culture to remove waste products, as well as partial harvests every other day, which is essential for the growth of rotifer colonies (Lubzens, 1987).



Figure 2: 15L culture of *Brachionus plicatilis.* Rotifers were cultured in 16ppt water cycled by a hang-on-back filter and aerated with an airline.



Figure 3: Small disc hatcher for brine shrimp eggs. After hatching, nauplii are attracted to light in the center of the disc, where they can be harvested.

Brook stickleback eggs and sperm were harvested from wild-caught fish from Pine Lake in Turnbull Wildlife Refuge, Cheney, Washington on 6/12/2025. The gametes were preserved

and shipped to Georgia, where they were crossed in petri dishes in the lab. The resulting embryos were maintained in petri dishes with daily water changes and removal of unviable eggs. When the eggs hatched on 6/25, the newborn larvae were moved into nine aerated 1.5-L mason jars with 25-39 fish per jar (Figure 4). Water was kept at 17-18°C, and the jars received partial water changes twice per week. After six days post-hatch, larvae were transferred to nine 1.4-L recirculating zebrafish breeding tanks (Aquaneering^{TM} product number ZT140) (Figure 5), where they lived for the remainder of the study.



Figure 4: Jar set up for initial larval rearing.



Figure 5: Tanks used for days 7-32 of the trial.

Tanks were randomly assigned to one of three diet treatments, with three tanks assigned to each diet. Tanks (jars) began with 25-39 larvae per tank, with each tank containing larvae from a single clutch of eggs, and thus, the same genetics as their tankmates. Larvae were fed either *Artemia franciscana* (brine shrimp) nauplii, *Brachionus plicatilis* (L-type rotifers), or Roti-Feast® (a commercial feed made of dead L-type rotifers). Brine shrimp were kept at a density of approximately 1000/mL, live rotifers at a density of approximately 300/mL, and dead rotifers (from Roti-Feast®) at a density of approximately 300/mL. Because of the higher concentration, larvae fed brine shrimp were fed a smaller volume than the ones fed rotifers. When they began exogenous feeding (~4 days post-hatch), the larvae were fed three times per day, each time given

0.25 mL brine shrimp, 0.5 mL live rotifers, or 0.5 mL Roti-Feast®, depending on their assigned diet. Rations were re-evaluated weekly, and if all the food in a tank was consumed within 30 minutes, that tank's ration was increased by 0.25 mL. The tanks were checked daily for mortalities, and any dead larvae were removed and noted, along with their tank and diet.

Data Analysis

Larval survival over time was analyzed using MATLAB R2025a to produce Kaplan-Meier (KM) survival curves, a Weibull survival model, and cumulative mortality curves in order to visualize larvae survival and mortality rates. In addition, the median survival rate for each diet was plotted, along with 95% confidence intervals for each. A pairwise log-rank test was performed to assess the significance of differences between treatments.

Results

The brine shrimp diet generated the highest larval survival rates (Figure 6). All of the larvae fed Roti-Feast® died by day 10 post-hatch in a one-day mortality event. The larvae fed live rotifer lived slightly longer in general and declined less sharply, but they, too, had all died by day 18 post-hatch. Over 50% of the fish fed brine shrimp survived to the end of the 32 days, with a median survival time of 25 days. Survivorship differed significantly between the diets (Table 1; $p < 0.01$ for each pairwise comparison).

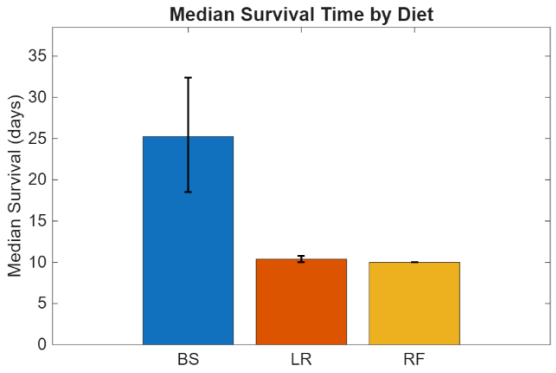


Figure 6: Median survival time for the brine shrimp (BS; blue), live rotifer (LR; orange), and Roti-Feast (RF; yellow) diets with a 95% confidence interval.

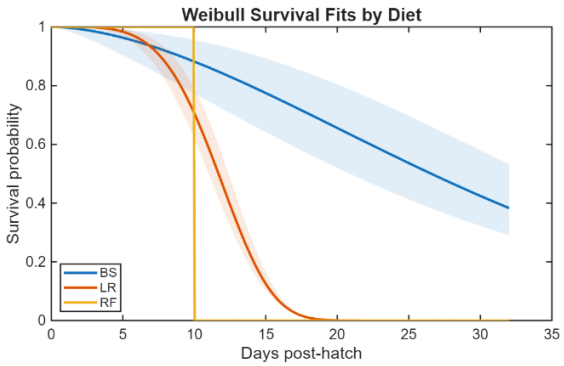


Figure 7: Weibull survival curves for the brine shrimp (BS; blue), live rotifer (LR; orange), and Roti-Feast (RF; yellow) diets.

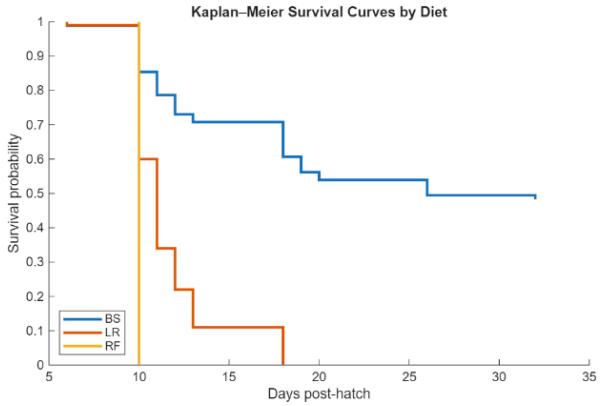


Figure 8: Kaplan-Meier survival curves for the brine shrimp (BS; blue), live rotifer (LR; orange), and Roti-Feast (RF; yellow) diets.

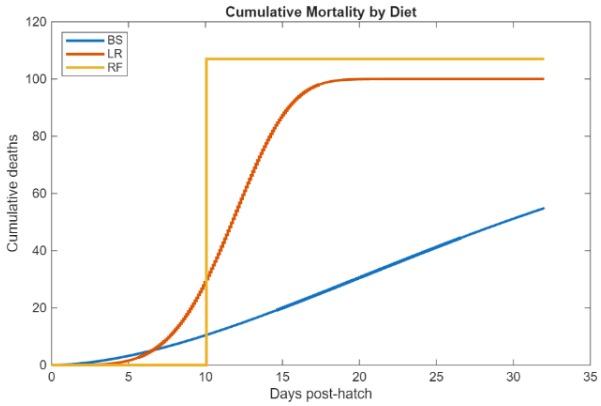


Figure 9: Cumulative mortality for the brine shrimp (BS; blue), live rotifer (LR; orange), and Roti-Feast (RF; yellow) diets.

Log-Rank Test	**p-value**
BS vs LR	3.11e-15
BS vs RF	1.82e-11
LR vs RF	1.01e-5

Table 1: Results of the pairwise log-rank test between the brine shrimp (BS), live rotifer (LR), and Roti-Feast (RF) diets.

Discussion

The survival rates of the larvae consuming brine shrimp were similar to those of other captive larval fish fed brine shrimp in their first month of life (Lipscomb et al., 2020; Ehrlich et al., 1989; Policar et al., 2007). Larval survivorship of the brine shrimp diet group was also similar to stickleback survival rates in both captive (Breckels & Neff, 2013) and wild (Macdonald et al., 1995; Walton et al., 2007) populations.

It was unexpected that the larvae fed the live rotifer diet would have such low survivorship. It was hypothesized that smaller prey item (rotifers being smaller than brine shrimp nauplii) would be easier for the brook stickleback larvae, which are smaller than threespine stickleback larvae, to eat.

The failure of the live rotifer larvae could have been due to multiple factors. Though live rotifers are widely considered to be a beneficial food source for young larval fish (Lubzens et al., 1989), they must be enriched with the proper macro and micronutrients in order to provide fish with a complete diet (Lubzens, 1987). Though the rotifers in this study's culture were alive and had a high reproductive rate, it is possible that they did not consume sufficient algae (in the form of RGComplete™) to provide all the nutrients that the larvae needed. This possibility would explain why the live rotifer fish generally lived several days after starting exogenous feeding (indicating that they were likely at least consuming some rotifers), but a lack of nutrition could have led to their eventual decline. A rotifer culture fed live algae may have provided better nutrient content (Lubzens, 1987), but the difficulty of maintaining an algal culture in a small lab setting may have outweighed any benefits.

The rotifers' smaller size may have given them a slower sink rate when compared to the brine shrimp. Combined with their more limited swimming ability, slower sink rate could have led to them being swept out of the tanks before larvae resting on the bottom could access them. The flow in the tanks was kept as low as possible (inflow at a few milliliters of water per second), but because the brook stickleback larvae are more demersal (Winn, 1960), they may have been unable to access rotifers in the water column before they were circulated out of the tanks. In the future, research into novel live foods like blackworms or benthic microworm species which may be more readily consumed by demersal larvae (Bouguenec, 1992) is recommended.

While it was expected that the commercial food would lead to lower survival rates in the larvae, the sharp decline of all the fish fed Roti-Feast® was noteworthy. The deaths of all the larvae in that treatment group at relatively the same time indicates that the larvae likely never ate the Roti-Feast®, as compared to the limited consumption of live rotifers. This result could be due to larval fish being less likely to recognize non-moving particles as food (Cahu & Zambonino Infante, 2001) or due to newly-hatched larvae lacking the proper digestive enzymes to break down tougher, nutrient-rich commercial food (People Le Ruyet et al., 1993). Thus, if the larvae in this study did successfully ingest the Roti-Feast®, their underdeveloped digestive systems may have been unable to digest and use any of it, leading to starvation.

Due to these findings, it is not recommended to use rotifers (live or dead) as the main food source for newborn brook stickleback larvae in small-scale lab settings. It is possible that a different method for culturing rotifers would result in them being more nutritious for the larvae, but that may be more labor-intensive than is feasible for small laboratories. Inert commercial foods are also not likely to be successful without developments in feeding and culture methods.

In the future, other new live foods may lead to higher survival rates, but at present time, brine shrimp nauplii appear to be a sufficient food source for captive brook stickleback larvae.

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