Maine Alexa (Orcid ID: 0000-0002-9382-2948)

Probiotics improve survival and growth of larval Pacific Lamprey *Entosphenus tridentatus* in laboratory culture

Alexa N. Maine[1,2], Mary L. Moser[3], Aaron D. Jackson[4], and Frank Wilhelm[5]

[1]Confederated Tribes of the Umatilla Indian Reservation, Department of Natural Resources, Pacific Lamprey Research and Restoration Project, 500 Tausick Way, Walla Walla, WA 99362

[2]University of Idaho, College of Natural Resources, Environmental Science Graduate Program, 875 Perimeter Drive MS 1138, Moscow, ID 83843

[3]Fish Ecology Division, Northwest Fisheries Science Center, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, WA 98112

[4]Confederated Tribes of the Umatilla Indian Reservation, Department of Natural Resources, Pacific Lamprey Research and Restoration Project, 46411 Timine Way, Pendleton, OR 97801

[5]University of Idaho, College of Natural Resources, Department of Fish and Wildlife Sciences, 875 Perimeter Drive MS 1138, Moscow, ID 83843

Corresponding author: alexamaine@ctuir.org

[A]Abstract

Pacific Lamprey *Entosphenus tridentatus* is a First Food for members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and other Columbia Plateau tribes in the Pacific Northwest. Declines in Pacific Lamprey abundance have prompted restoration efforts, including development of artificial propagation. Laboratory rearing of larvae has focused on maximizing survival and growth to conserve resources and increase production. To test the hypothesis that bacterial supplements increased survival and growth of first-feeding larval Pacific Lamprey, we conducted two controlled experiments. First, a probiotic supplement (EPI-CIN G2, Epicore Bionetworks, Eastampton, New Jersey) was added to a standard food ration (yeast and Otohime mix) at two levels (2 and 5 mg/L) in a replicated, randomized design. Growth at 10 weeks was measured and larvae fed probiotics, at both levels, grew significantly faster (2 mg/L: 11.0 µm/day; 5 mg/L: 13.3 µm/day) than controls that were fed the standard ration alone (6.6 µm/day). Larvae that received the probiotic supplement also had higher survival (2 mg/L: 36%; 5 mg/L: 44%) than those fed the standard ration (24%). Next, a different cohort of larval lamprey was fed the same two levels of probiotic (at the same rate as in the first experiment), but in larger rearing tanks and for 28 weeks. In this experiment, overall growth rates were lower than in the first experiment (2 mg/L: 4.6 µm/day; 5 mg/L: 5.7 µm/day; control: 3.4 µm/day); but, both growth and survival (2 mg/L: 71.4%; 5 mg/L: 78.6%; control: 55.7%) were highest in the treatments with probiotic. Moreover, in both experiments we observed the highest growth in the probiotic treatments that also had high larval density. This suggests that probiotics may help overcome density-dependent growth, a common problem in lamprey culture. Successful artificial propagation and culture of Pacific Lamprey is vital to the long-term restoration goals of this imperiled First Food.

[A]Introduction

In the Pacific Northwest, Pacific Lamprey *Entosphenus tridentatus* is considered a First Food, foods of traditional and cultural significance, by members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and other Columbia Plateau tribes (Quaempts et al. 2018). Unfortunately, Pacific Lamprey populations have declined dramatically due to impoundments, intentional poisonings, irrigation diversions, host availability, and habitat alterations (Close et al. 2002; Murauskas et al. 2013; Clemens et al. 2017; Lampman et al. 2021).Therefore, harvest opportunities have diminished or ceased (Close et al. 2002) over the last 50 years. As part of a multipronged approach to Pacific Lamprey restoration, artificial propagation has been developed to provide larval lamprey for research and to supplement populations (CRITFC, 2018).

For other lamprey species, propagation has been used to supply organisms for research of evolutionary development (Kuratani et al. 2002; York et al. 2019), to develop control methods (invasive Sea Lamprey *Petromyzon marinus* in the Laurentian Great Lakes; Ciereszko et al. 2005; Wagner et al. 2006; Li et al. 2007; Johnson et al. 2009), and to produce Arctic Lamprey *Lethenteron camtschaticum* (Hokkaido Fish Hatchery, 2008), European River Lamprey *Lampetra fluviatilis* (Kujawa et al. 2017), and Korean Lamprey *Eudontomyzon morii* (Feng et al. 2018; Almeida et al. 2021) for population supplementation. Low survival and growth are factors that limit production-level laboratory propagation of all lamprey species (Lampman et al. 2016; Lampman et al. 2019; Moser et al. 2019). This motivates our research because Pacific Lamprey propagation is also limited by these issues as it relies on techniques used in the culture of other lamprey species.

As Pacific Lamprey burrow into freshwater substrates as part of an extended filter-feeding larval stage (several years), mortalities can go undetected and causes of such events are difficult to pinpoint (Lampman et al. 2016; Lampman et al. 2021). Low survival and growth of larval Pacific Lamprey in laboratory culture has led to the investigation of alternative methods to improve rearing success (Lampman et al. 2016; Barron et al. 2020). In a pilot experiment, a microbial supplement (conditioned water from older larval lamprey cultures) was added to the normal ration and larvae showed slight improvements in growth.

The addition of coconut filter mats (a potential substrate for microbes) improved survival over multiple cohorts (Lampman et al. 2016; Maine et al. 2017). Moreover, larval Pacific Lamprey that received effluent from a salmonid hatchery grew faster and larger than did those that were raised without such a source of microbes and nutrients (Barron et al. 2020). These observations piqued our interest to determine if a commercially available probiotic supplement could increase survival and growth of larval Pacific Lamprey in culture.

Probiotics are live or dead microorganisms (commonly bacteria) that contribute to intestinal or environmental (in the case of aquatic environments) microbial balance (Nayak, 2010; Hai, 2015). In aquaculture, they are used to improve survival, growth, immune response, or disease resistance, and are applied with food or directly into the water (Zhou et al. 2009; Nayak, 2010). Commercially available probiotics for aquaculture are formulated to perform certain functions in the aquatic environment depending on the individual species or mixture of species present in the product. We chose a commercially sourced probiotic product containing *Bacillus*, *Lactobacillus*, and *Acetobacter* species (EPI-CIN G2, Epicore Bionetworks, Eastampton, New Jersey), bacterial genera known to confer benefits in aquaculture (Table 1). While this probiotic was readily available in a shelf-stable container, any non-pathogenic bacteria could potentially be used as a probiotic.

Probiotics impart both direct and indirect (synergistic) benefits to cultured organisms. The microbes we used for probiotic supplementation potentially confer benefits via the following main mechanisms: 1) improving feed conversion efficiency and gut function, 2) acting as a direct food source, 3) imparting pathogen resistance, 4) increasing the production of enzymes, antibiotics, and acids, 5) enhancing immune responses, and 6) competing with pathogens (Nayak, 2010; De et al. 2014). Numerous studies have shown improved growth, survival, and/or increased immune response of adult and larval fishes that are reared with commercially available (e.g. commercially mass- or batch-cultured strains) or cultured (e.g. bacteria cultured from adult intestines to be fed to larvae of the same species) probiotics (Table 2). Probiotics have also been effective in increasing the survival and growth of other aquatic organisms, such as sea cucumber, marine mussels, seahorses, and shrimp (Table 2).

The possibility of enhanced survival and growth from probiotic supplementation is of particular interest in Pacific Lamprey culture given a standard feed for rearing larval Pacific

Lamprey has been developed (Barron et al. 2016), but in high-density cultures, larval lamprey exhibit density-dependent growth (Mallatt, 1983; Rodriguez-Munoz et al. 2003; Lampman et al. 2016). Although the standard ration can be increased to improve production capacity, (Lampman et al. 2016) this requires careful monitoring to avoid fouling, especially in static or recirculating systems. The use of probiotics to increase survival and/or growth has a lower risk of water-quality degradation compared to increasing the food ration (i.e. probiotics can provide supplemental nutrition with additional water quality or competitive exclusion benefits). Probiotic supplements could also provide a more consistent food source through the development and maintenance of a diverse and healthy microbial community compared with only providing a food ration.

Beneficial microbes play an important role in critical aspects of aquaculture such as the absorption of CO_2, oxygen production, the decomposition of organic matter in sediments, and the reduction of nitrogenous wastes (reviewed in Zhou et al. 2009). While they are especially important to maintain high water quality and the cycling of nitrogen, microbes also convey antifungal protection and pathogen control for some fish species (Lowery et al. 2015). Boeker and Geist (2016) found that larval lamprey, through their burrowing activities, play a significant role in structuring the microbial community in river substrate.

Because larval lamprey live in the substrate, and interact with the environment at the interface between the substrate and the water column, they likely rely on local benthic microbes to provide food and ecological services. This may be especially important when larvae are unable to filter feed from the water column due to high water velocity or turbidity during high water events or during periods of low stream productivity (Yap and Bowen, 2003; Moser et al. 2019). Based on these observations, we hypothesized that the addition of a commercially available probiotic to feed in Pacific Lamprey cultures would increase both the survival and growth of first-feeding larvae.

[A]Methods

We used Pacific Lamprey larvae propagated by the CTUIR at the Walla Walla Community College Water and Environmental Center (WEC) in our experiments. Adult lamprey were collected at lower mainstem Columbia River dams (e.g. John Day Dam, Rkm 347) and held over winter. In two separate spawnings in 2018 and 2019, ripe adults were hand-stripped to collect gametes, and the eggs were fertilized at the WEC following the methods of Lampman et al. (2016). The embryos were incubated in static 10-L tanks of well water with aeration in a 13.0 ± 1.5°C water bath, a temperature chosen to reflect natural stream temperatures at that time.

[C]Probiotic supplement experiment in 2018.—In 2018, 200 larvae aged 29 days post-fertilization (average length 8.55 mm, SD= 0.51 mm; yolk sac had been mostly absorbed and larvae were starting exogenous feeding) were randomly collected from a holding chamber and placed into 20, new (never used) 1-L glass beakers (*n* = 10 larvae/beaker) with source water (conditioned well water). Prior to use, the beakers were rinsed with source water, and randomly assigned to one of three treatments: control (no probiotic; *n* = 10 replicate beakers), T1 (2 mg/L probiotic; *n* = 5 replicate beakers), or T2 (5 mg/L probiotic; *n* = 5 replicate beakers). The probiotic treatments used EPI-CIN G2 powdered commercial aquaculture probiotic (Epicore Bionetworks, Eastampton, New Jersey), applied during once weekly feedings. Each beaker (105 mm in diameter) was aerated and contained 1.5 cm (in depth) sieved and autoclaved sand (grain size 149–595 μm) for a sediment volume of 1.27×10^{-4} m^3. A 5 × 5-cm piece of filter mat (latex-coated coconut fiber spawning mat with polyester backing; Spawntex mat, Pentair AES, Apopko, Florida) was placed on top of the sand to provide cover. At 24-hour after transfer, the beakers were checked for survival and any mortalities (visible on the sediment surface) were replaced with live larvae so that the densities in all of the beakers were equal (10 larvae/L, 1,154.9 larvae/m^2, and 78,740.2 larvae/m^3) at the start of the experiment.

The larvae were fed a weekly ration of 80% yeast (Red Star Baking Yeast, Lesaffre Yeast Corp. Milwaukee, Wisconsin) and 20% larval fish food (Otohime A1, Marubeni Nisshin Feed Co. Ltd. Tokyo, Japan) at a concentration of 250 mg/L (Barron et al. 2016). The food was prepared separately for an entire treatment group each week (control: 2,500 mg food; low probiotic [T1]: 1,250 mg food, 10 mg probiotic; and high probiotic [T2]: 1,250 mg food, 25 mg probiotic) by emulsifying in source water using a blender. Control beakers (n = 10) each received 250 mg of food; T1 (n = 5) each received 252 mg of food–probiotic mixture and T2 (n = 5) each received 255 mg of food–probiotic mixture. Feedings were preceded by a 200 mL water change in each beaker using source water. An additional 200 mL water change was also completed each week that was not associated with feeding.

All of the beakers were held in a randomized order in a 13.5 ± 1.0°C water bath for ten weeks. At the end of the experiment, larvae (aged 98 days post-fertilization) were removed from each beaker by stirring the sediment with a blunt probe and using a dip net to capture them. The larvae were counted to assess survival and final density for each treatment. Surviving larvae were photographed with a calibrated scale (Figure 1) and individual body length (to the nearest 0.01 mm) was measured for up to 20 randomly-sampled larvae from each treatment using ImageJ (NIH, version 1.52a; Schneider et al. 2012). Individual larvae from each treatment were not measured due to the stress of anesthetizing and handling individual larvae as well as the time needed to complete the task.

[C]Probiotic supplement experiment in 2019.—In 2019, 210 larvae aged 31 days post-fertilization with an average length of 9.31 mm (SD = 0.22 mm) were collected from a holding chamber and randomly placed with source water into three static, aerated 10-L polycarbonate Cambro CamWear pans (53 × 32.5 cm, water depth 9–10 cm; Cambro Manufacturing, Huntington Beach, California). Each pan contained sieved and washed sediment (grain size 149–595 µm) to a depth of 7.5 cm (sediment volume: 1.29×10^{-2} m^3) and a pan-sized filter mat (Spawntex mat). After a 24-hour acclimation period, any mortalities were replaced with live larvae so that the densities were equal in all of the tanks (7 larvae/L; 406.4 larvae/m^2; 5,418.5 larvae/m^3).

The larvae in each pan received a standard food ration of 250 mg/L (yeast:Otohime, 80:20; as in 2018) once weekly. The food was blended with source water and added to the pans after a 2-L (20% of the total pan volume) water change with source water. The probiotic-supplemented treatments received powdered commercial aquaculture probiotic (Epicore Bionetworks EPI-CIN G2), applied weekly with food at the same levels (per volume) as were used in the 2018 experiment. Larvae in the control pan received 2,500 mg of food; those in the T1 pan received 2,500 mg food and 20 mg probiotic; and those in the T2 pan received 2,500 mg food and 50 mg probiotic. An additional 2-L water change also was conducted weekly, not associated with feeding. The pans were held in a water bath to maintain the temperature at 14.0 ± 1.0°C for the duration of the experiment.

The survival and body length of larvae were assessed at 77, 178, 200 (only T2), and 226 (only T1 and control) days post-fertilization during the 28-week experiment by removing all of the surviving larvae from each pan (replacing them back into their respective pans after assessments were completed). The larvae were netted from the water column in each pan after stirring the sediment with a blunt probe. On each occasion, the number of surviving larvae in each treatment was recorded and 20 randomly subsampled individuals were photographed for measurement using the same methods as in 2018.

[C]Statistical analysis.—Logistic regression was used to analyze survival between the treatments (Warton and Hui 2011) and a one-way analysis of variance (ANOVA) was used to compare body lengths between the treatments at the end of the experiment. This was followed by a Tukey's HSD post hoc test to identify which treatments differed. All of the analyses were completed in R (version 3.5.1, R Core Team, 2020) using the STATS package (version 3.5.1, R Core Team, 2020).

[C]Instantaneous growth rate.—To account for measurements made on different dates, we computed instantaneous growth rate (G) based on Wootton (1990), Hopkins (1992), and Crane et al. (2019):

$$G = [\ln(L_2) - \ln(L_1)] / (t_2 - t_1) \times 1{,}000,$$

where G is the instantaneous growth rate (μm/day), $\ln(L_2)$ is the natural logarithm of the average length (mm) of larvae in a given tank at an intermediate or ending period (t_2), and $\ln(L_1)$ is the natural logarithm of the average length of larvae in that tank at the start of the experiment (t_1).

[C]Water quality.—Water temperature, pH, and dissolved oxygen were monitored and recorded weekly in each system by using a Vernier handheld computer and sensors (Vernier, Beaverton, Oregon) while semi-quantitative colorimetric Hach test strips were used to measure nitrate/nitrite and ammonia (Hach Company, Loveland, Colorado).

[A]Results

[B]Probiotic supplement experiment in 2018

Survival was 36% (416 larvae/m^2; 28,347 larvae/m^3; means rounded to nearest whole numbers) and 44% (508 larvae/m^2; 34,646 larvae/m^3) in the 2 mg/L (T1) and 5 mg/L (T2) probiotic treatments, respectively, which was significantly higher (logistic regression, $p = 0.014$, $n = 200$) than the 24% (277 larvae/m^2; 18,898 larvae/m^3) in the control group (Figure 2).

Larvae that were supplemented with either dose of probiotic grew significantly larger than those in the control group (control: 13.6 ± 1.4 mm; T1: 18.5 ± 3.0 mm; and T2: 21.5 ± 2.6 mm, mean final length ± SD; $F_{2, 61} = 65.34$, $p = < 0.001$). The Tukey HSD test indicated that the larvae in the T2 (5 mg/L) treatment grew significantly larger than control larvae ($p < 0.001$), and they were also significantly larger ($p < 0.001$) than T1 larvae (Figure 3). Larvae from both treatments receiving probiotic doses had faster growth rates than controls (control: 6.6 µm/day; T1: 11.0 µm/day; and T2: 13.3 µm/day). The larvae in the probiotic-supplemented treatments did not show density-dependent growth, growing larger than the control group despite the higher densities relative to the control group as the experiment progressed (Figure 4).

[B]Probiotic supplement experiment in 2019

Survival differed between larvae in the control (55.7%; 226 larvae/m^2; 3,018 larvae/m^3; means rounded to nearest whole numbers) and the probiotic treatments (T1: 71.4%; 290 larvae/m^2; 3,869 larvae/m^3; and T2: 78.6%; 319 larvae/m^2; 4,259 larvae/m^3), at 178 days post-fertilization. A lapse in aeration in the T2 probiotic treatment resulted in mortality of the entire tank at 200 days post-fertilization when dissolved oxygen dropped to 0.9 mg/L (Figure 5). To account for this mortality event, we estimated T2 growth from 200 to 226 days using the instantaneous growth rate, to allow for final length comparisons among treatments (Figure 6). The final lengths of the larvae in both probiotic treatments (T1: 23.2 ± 2.9 mm; or T2: 26.4 ± 5.3 mm) were higher than controls (18.4 ± 3.4 mm). The larvae in treatments receiving probiotic also grew at a faster rate than controls (T1: 4.6 µm/day, and T2: 5.7 µm/day; control: 3.4 µm/day, Figure 7). Changes in instantaneous growth rates at different points in the experiment may be a natural product of larval growth, but insufficient data exists on growth rates for larvae of this age to make comparisons or conclusions. As observed in 2018, larvae in probiotic-supplemented treatments grew larger than did those in the control group, despite having higher ending densities (Figure 8). No statistical test was completed for these particular experiments given the experimental design of housing each treatment in a single large tank and larvae were not measured or tracked individually. Though this experiment was not statistically analyzed and was not replicated, it provides additional support for the probiotic supplementation in a variety of rearing environments.

[B]Water quality

There were no observed differences among the three treatments for any of the water quality parameters for either the 2018 or the 2019 experiments (Table 3).

[A]Discussion

Pacific Lamprey populations have been negatively affected by anthropogenic changes, and tribal restoration efforts rely on small numbers of broodstock for propagation annually. Maximizing the survival and growth of cultured larvae will further reduce the number of broodstock needed. Previous rearing efforts of Pacific Lamprey have had mixed success and low survival rates (CTUIR, unpublished data). This may have been linked to the use of UV sterilization and/or chemical disinfection of the culture water, as these practices have been shown to promote low bacterial diversity, pathogen control, and stability in other aquaculture settings (de Carvalho, 2017; Brugman et al. 2018). The experiments reported here suggest that use of a probiotic could improve survival and growth such that large production scales of larval Pacific Lamprey are possible with relatively low levels of wild broodstock collection.

The higher survival and growth of Pacific Lamprey in the probiotic treatments compared to the controls, suggests that the probiotics provided a benefit in the culture of larvae. Similar results from the two different larval cohorts (2018, 2019) and rearing environments (1L, 10L chambers) further strengthens this conclusion. Our results also suggest that there is a positive relationship with probiotic dose; the 5-mg/L dose produced better survival and faster growth compared to the 2-mg/L dose. It may be that the higher probiotic dose provided more micro-organismal food to the larvae or conferred a higher level of synergistic (indirect, see Introduction) benefits than did the lower dose. Gut microbes can produce amino acids and enzymes to aid in feed conversion (Burr et al. 2005; Nayak, 2010; De et al. 2014; Table 1). It is possible that this mechanism resulted in the increased survival and growth we observed, but further investigation is needed to determine optimal probiotic dose, whether this effect is observed in different rearing conditions (i.e., production scale rearing), and the pathways that are involved for Pacific Lamprey.

Larval growth rates in these two experiments were higher than in previous years also using recirculating systems (Maine et al. 2019) and were similar to those reported for flow-through operations (Barron et al. 2016). Larval Pacific Lamprey reared in the same recirculating system in 2016 and 2017 had an average instantaneous growth rate of 2.1 µm/day (Maine et al. 2019; Moser et al. 2019). The addition of filter mats in 2018, led to an increase in the average instantaneous growth rate to 5.6 µm/day (Maine et al. 2019) which may have been due to the filter mat providing an increased surface area on which microbial growth developed. Growth rates between 3.2 and 10.4 µm/day were found in a flow-through system with larvae of a similar age and over a comparable growth period (Barron et al. 2016). Barron et al. (2020) reported a growth rate of 5.7 µm/day for yearlings that were fed 500 mg/L over 63 days (twice our standard ration) and growth rates as high as 6.5 µm/day for larvae that were reared in effluent water with no supplemental feed. They observed growth rates as high as 8.4 µm/day for larvae that were reared in effluent plus supplemental feed. Similarly, Maine et al. (2019) found growth rates of 4.2 µm/day for subyearling larvae that were reared in a recirculating system and 5.4 µm/day for those reared in polyculture with teleosts. These results suggest that probiotics or other microbial supplementation may be a cost-effective method to improve the survival and growth of lamprey larvae in dense laboratory cultures.

We observed improved survival of Pacific Lamprey larvae aged 32–98 d (2018) and 29–226 d (2019) when supplemented with probiotics. The survival rates in larval lamprey are not well studied, especially those for sub-yearlings. Survival rates of Pacific Lamprey larvae from 30–90 days post-fertilization varied from 0–50% in a variety of rearing conditions at different facilities (reviewed in Lampman et al. 2016). Survival typically declines after the first-feeding stage (approximately 45 days post-fertilization) from over 90% survival before first feeding to an average of 35% thereafter. Hence, the time of first feeding has been identified as a significant bottleneck to lamprey rearing in the hatchery environment (Lampman et al. 2016). The higher survival rate observed in this experiment for cultures supplemented with probiotics as compared to controls suggests that the addition of probiotics may be a method to overcome this survival bottleneck.

Other lamprey species also exhibit low survival at first feeding (Moser et al. 2019), suggesting that this feature may be inherent to cultured lamprey. For example, Hansen et al. (1974) found that larval survival in Sea Lamprey was between 11.6% and 36.5% in the first year of life. Rodriguez-Munoz et al. (2001) indicated that the maximum survival rate of larval Sea Lamprey was 43% during the 3 months after first feeding. Higher survival (55–100%) has been observed in Pacific Lamprey larvae from first feeding to 1 year of age in a recirculating system that contained Speckled Dace *Rhinichthys osculus* (Maine et al. 2019). The higher survival rate we observed suggests that the larvae benefited from probiotic supplementation. It is possible that microbes introduced in feed for other fish, are important in overcoming early larval mortality, especially when larvae are switching to exogenous feeding.

Probiotic supplementation significantly increased growth of larvae compared to controls when reared at high densities. The densities used in our experiments (407–1,155 larvae/m^2) were higher than densities typically observed in the wild (< 1–32 larvae/m^2) but lower than those recommended for supplementation production (4,042–6,811 larvae/m^2; Moser and Close, 2003; CRITFC, 2018). Larval lamprey exhibit density-dependent growth (Mallatt 1983; Murdoch et al. 1991; Rodriguez-Munoz et al. 2003), which has hampered the production-level numbers of larvae needed for restoration. The use of probiotics to reduce or overcome density-dependent growth (density is inversely proportional to growth) in the culture larval lamprey could significantly increase production and decrease the facility space needed to grow them. Additionally, the use of condition factor as a metric to determine growth improvements could be considered, though it was not used in this study due to the small size of fish and need for finer scale equipment than was on hand. Future research should further explore the use of probiotics in overcoming density–dependent growth at the densities recommended for production-level rearing of larvae.

Water quality did not differ between the probiotic treatments and controls in either year of study, suggesting that increases in survival and growth were not related to water quality. Water quality is often linked to the development of disease outbreaks in aquaculture (Padmavathi et al. 2012), thus improving water quality in the culture environment is a delicate balance between controlling harmful, and promoting beneficial, microorganisms (Sayes et al. 2018). Probiotics have been used to improve water quality through mechanisms such as increased nutrient cycling, inhibition of potential pathogens, and decreased build-up of nitrogenous waste compounds (Kim et al. 2005; Lalloo et al. 2007; Padmavathi et al. 2012). The frequent water changes as part of our study protocol likely contributed to a stable and suitable water quality, and we conclude that mechanisms other than water quality improvement were at play in the observed increases in survival and growth of larval lamprey.

It is likely that the larval lamprey obtained some nutritional benefit from the supplemented microbes and the fortified microbial community in this experiment. They could have obtained other benefits from the probiotics, such as increased feed digestibility, production of enzymes, or a positive immune response, similar to those reported with the use of probiotics in other fishes (Robertson et al. 2000; Bagheri et al. 2008; Cerezuela et al. 2013; Munir et al. 2016). Larval lamprey are suspension feeders, using primarily bacteria, detritus, and diatoms as food (Moore and Beamish 1973; Moore and Potter 1976; Yap and Bowen 2003). Larval lamprey feed primarily at night from within or just outside their burrows, and they possibly take in nutrients from sediment pore water at other times (CTUIR, unpublished data; Applegate 1950; Moser et al. 2019). It is unknown how much of their total intake is from subsurface versus surface feeding, which should be explored in future research. Understanding their feeding behavior could help determine the optimal method of probiotic application: via food, water, or mixed into the sediment.

Probiotics could help provide larval lamprey with the type and size of food that are optimal for growth in the laboratory. Larvae have been shown to survive in cultures with only bacteria or organic detrital material as a food source, though this has not been explored rigorously (Moore and Potter 1976; Sutton and Bowen 1994; Nelson and Nelle 2007; reviewed in Lampman et al. 2021). Moser et al. (2019) reported that small (<50–100 µm) food-particle size is important for growth of first-feeding larvae. Probiotic supplements and microorganisms fit this size requirement and could help offer and maintain a diversity of small particles for larvae during this sensitive period of development.

Other mechanisms by which probiotic supplementation conferred benefits to the larvae in this experiment are unknown, but they could include competitive exclusion of pathogens, increased immune response, or directed development of gut or mucosal surface fauna. Certain microbes can competitively exclude harmful bacteria (Yong 2016), and probiotics could serve this purpose for larval lamprey in the laboratory. Of the three genera in the EPI-CIN G2 probiotic we used, the *Bacillus* and *Lactobacillus* species are known to provide benefits to aquaculture organisms via competitive exclusion, including faster growth and higher nutrient uptake rates than are observed in the presence of pathogenic bacteria (e.g. Lalloo et al. 2010) as well as the production of antibacterial compounds (e.g. Lash et al. 2005; Table 1).

Improved immune responses are known to occur as a result of probiotic use in aquaculture (e.g. activation of immune defenses and protective effects against pathogens from probiotics containing *Bacillus* or *Lactobacillus*, as reviewed in Balcazar et al. 2006). Similar to jawed fishes, jawless fishes like lamprey are thought to require activation of the innate immune system to initiate adaptive immune responses (Kasamatsu 2012). Giri et al. (2012) found that probiotics improved innate immunity in teleost fishes, and this may be another benefit of probiotic use. Outside of the laboratory, larval lamprey appear to be relatively resistant to disease or infection, as compared with the juvenile and adult life stages (Jackson et al. 2019). However, fungal, parasitic, and pathogenic infections have been reported in dense larval cultures in the laboratory (Lampman et al. 2019; Lampman et al. 2021). It is possible that the burrowing behavior of larvae could increase their exposure to pathogens or parasites in the wild, potentially inducing immune responses that lower disease risk at that life stage.

The internal and external mucosal surfaces of fishes are known to host a diverse microbiota, which play important roles in disease control (Lowery et al. 2015). In larval fishes, microbiota in culture water are important, as they help to establish an internal microbial community during early development (Egerton et al. 2018; Jiang et al. 2019). Larval lamprey are thought to obtain their gut microbiota entirely from their environment (Rogers et al. 1980). The use of probiotics in lamprey culture might direct the development of the external mucosal surface or gut microbiomes in newly hatched and first-feeding larvae. Moser et al. (2020) conducted a microbial inoculant experiment, which used different water sources to incubate Pacific Lamprey embryos. They reported no differences in survival or growth between treatments using microbe-rich water and those using conditioned or unconditioned well water. The mechanism for the colonization of the lamprey gut by microbes is not well understood, and, while the results from Moser et al. (2020) documented no apparent benefits from this practice, there were also no obvious disadvantages of early microbial inoculation. In other cases, the absence of disinfection during larval rearing resulted in increased risk of fungal infections (Lampman et al. 2016; Jackson et al. 2019). Future studies should assess the ontogeny of the external mucosal and gut microbiomes to identify the time at which exposure to microbes is most important and to determine the role of disinfection in lamprey incubation and early larval rearing.

Findings from this study could have direct benefit for lamprey culture and management by increasing early survival and growth, which should improve overall survival in a culture setting and/or in the wild. Identification of lamprey-specific microorganisms could be used to develop probiotic agents to direct gut microbiome development in early larval lamprey, prepare larvae for out-planting through inoculation with wild-type microorganisms, or confer immune benefits prior to release or for research. Further research is needed to investigate differences in gut and mucosal surface microbiomes of wild and laboratory-reared larval lamprey. Identifying and culturing specific bacteria that are isolated from wild larval lamprey could allow for the identification of microbes most important to lamprey and, ultimately, lead to the preparation of lamprey-specific probiotic supplements. This would be especially prudent for production operations that are struggling with low survival rates during the first-feeding bottleneck. Particularly of interest in the context of holistic restoration of declining lamprey species, identifying lamprey-specific microbes could elucidate the degree to which larval lamprey link benthic and water-column organisms through trophic connections, broadening our collective understanding of their ecological role in freshwater systems. Biotic connections are important in the laboratory for improving conservation aquaculture techniques and for successful habitat and biological community restoration in the field. This study demonstrates that use of microbial community supplementation can enhance conservation aquaculture for Pacific Lamprey.

[A]Acknowledgments

We thank Tristan Shonat, Makana Stone, Tessa Irvine, Frankie Gerraty, Evan Marquardt, Natalie Gregorius, Jerrid Weaskus, Kanim Moses-Conner and Raul Montoya for their help in the laboratory and the field. We thank Dave Stockdale and Walla Walla Community College's Water and Environmental Center staff for facility and operational support. Gene Shippentower, Gary James, Debbie Docherty, Julie Burke, and Celeste Reves provided administrative support and guidance. While major support for this project was provided by the Bonneville Power Administration, this project benefited from U.S. Bureau of Reclamation, Chelan County Public Utilities District, and University of Idaho support.

[A]References

Almeida, P. R., H. Arakawa, K. Aronsuu, C. Baker, S.-R. Blair, L. Beaulaton, A. F. Belo, J. Kitson, A. Kucheryavyy, B. Kynard, M. C. Lucas, M. Moser, B. Potaka, A. Romakkaniemi, R. Staponkus, S. Tamarapa, S. Yanai, G. Yang, T. Zang, and P. Zhuang. Lamprey fisheries: History, trends, and management. *Journal of Great Lakes Research*, 2021, vol. 47, supplement 1, pp. S159-S185.

Applegate, V. C. Natural history of the Sea Lamprey (*Petromyzon marinus*) in Michigan. U.S. Fish and Wildlife Service, Special Scientific Report: *Fisheries*, 1950, vol. 55, pp. 1-237.

Bagheri, T. S., A. Hedayati, V. Yavari, M. Alizade, and A. Farzanfar, Growth, survival and gut microbial load of rainbow trout (*Oncorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. *Turkish Journal of Fisheries and Aquatic Sciences,* 2008, vol. 8, pp. 43-48.

Balcázar, J. L., I. De Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell, and J. L. Muzquiz, The role of probiotics in aquaculture. *Veterinary Microbiology*, 2006, vol. 114, no. 3-4, pp. 173-186.

Barron, J. M., R. G. Twibell, H. A. Hill, K. C. Hanson and A. L. Gannam, Development of diets for the intensive culture of Pacific Lamprey. *Aquaculture Research*, 2016, vol. 47, pp. 3899-3906.

Barron, J. M., K. C. Hanson, R. R. Headley, K. A. Hawke, R. G. Twibell, and A. L. Gannam, Evaluation of effluent waste water from salmonid culture as a potential food and water supply for culturing larval Pacific Lamprey *Entosphenus tridentatus*. *Aquaculture*, 2020, vol. 517, pp. 734-791.

Boeker, C. and J. Geist, Lampreys as ecosystem engineers: Burrows of *Eudontomyzon* sp. and their impact on physical, chemical, and microbial properties in freshwater substrates. *Hydrobiologia*, 2016, vol. 777, no. 1, pp. 171-181.

Brugman, S., W. Ikeda-Ohtsubo, S. Braber, G. Folkerts, C. M. Pieterse, and P. A. Bakker, A comparative review on microbiota manipulation: Lessons from fish, plants, livestock, and human research. *Frontiers in Nutrition*, 2018, vol. 5, pp. 80.

Burr, G. D., Gatlin III, and S. Ricke, Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. *Journal of the World Aquaculture Society*, 2005, vol. 36, no. 4, pp. 425-436.

Cerezuela, R. M., Fumanal, S. T. Tapia-Paniagua, J. Meseguer, M. Á. Moriñigo, and M. Á. Esteban, Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus aurata* L.) specimens. *Fish and Shellfish Immunology*, 2013, vol. 34, no. 5, pp. 1063-1070.

Chang, C. I., and W. Y. Liu, An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68 and *Bacillus toyoi*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. *Journal of Fish Diseases*, 2002, vol. 25, no. 5, pp. 311-315.

Clemens, B.J., Beamish, R.J. Coates, K.C. Docker, M.F. Dunham, J.B. Gray, A.E. Hess, J.E. Jolley, J.C. Lampman, R.T. McIlraith, B.J. and Moser, M.L. 2017. Conservation challenges and research needs for Pacific Lamprey in the Columbia River basin. *Fisheries*, vol. 42, no. 5, pp. 268-280.

Close, D. A., M. S. Fitzpatrick, and H. W. Li, The ecological and cultural importance of a species at risk of extinction, Pacific Lamprey. *Fisheries*, 2002, vol. 27, no. 7, pp. 19-25.

Craft, J., A. J. Stanford, and M. Pusch, Microbial respiration within a floodplain aquifer of a large-scale gravel-bed river. *Freshwater Biology*, 2002, vol. 47, pp. 251- 261.

Crane, D. P., D. H. Ogle, and D. E. Shoup, Use and misuse of a common growth metric: Guidance for appropriately calculating and reporting specific growth rate. *Reviews in Aquaculture*, 2019, pp. 1-6.

CRITFC (Columbia River Inter-Tribal Fish Commission), Master Plan: Pacific Lamprey artificial propagation, translocation, restoration, and research. Conceptual phase to address Step 1—Master Plan review elements. Prepared by: CRITFC, YN, CTUIR, NPT. March 23, 2018.

Dagá, P., G. Feijoo, M. T. Moreira, D. Costas, A. G. Villanueva, and J. M. Lema, Bioencapsulated probiotics increased survival, growth and improved gut flora of turbot (*Psetta maxima*) larvae. *Aquaculture International*, 2013, vol. 21, no. 2, pp. 337-345.

De, D., T. K. Ghoshal, R. Ananda Raja, and S. Kumar, Growth performance, nutrient digestibility and digestive enzyme activity in Asian seabass *Lates calcarifer* juveniles fed diets supplemented with cellulolytic and amylolytic gut bacteria isolated from brackish water fish. *Aquaculture Research*, 2015, vol. 46, no. 7, pp. 1688-1698.

De Carvalho, C., Biofilms: Microbial strategies for surviving UV exposure. *In* Ultraviolet light in human health diseases and environment. Advances in Experimental Medicine and Biology, 2017, vol. 996, pp. 233-239.

Douillet, P. A., and C. J. Langdon, Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). *Aquaculture*, 1994, vol. 119, no. 1, pp. 25-40.

Egerton, S., S. Culloty, J. Whooley, C. Stanton, and R. P. Ross, The gut microbiota of marine fish. *Frontiers in Microbiology*, 2018, vol. 9, pp. 873.

Falcinelli, S., S. Picchietti, A. Rodiles, L. Cossignani, D. L. Merrifield, A. R. Taddei, F. Maradonna, I. Olivotto, G. Gioacchini, and O. Carnevali, *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Scientific Reports, 2015, vol. 5, pp. 9336.

Feng, B., T. Zhang, F. Wu, S. Chen, and A. Xu, Artificial propagation and embryonic development of Yalu River lamprey, *Lampetra morii*. *Acta Biochimica et Biophysica Sinica*, 2018, vol. 50, no. 8, pp. 828-830.

Giri, S. S., S. S. Sen, and V. Sukumaran, Effects of dietary supplementation of potential probiotic *Pseudomonas aeruginosa* VSG-2 on the innate immunity and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish and Shellfish Immunology*, 2012, vol. 32, no. 6, pp. 1135-1140.

Ghosh, K., S. K. Sen, and A. K. Ray, Characterization of Bacilli isolated from the gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. *Journal of Applied Aquaculture*, 2002, vol. 12, no. 3, pp. 33-42.

Gram, L., J. Melchiorsen, B. Spanggaard, I. Huber, and T. F. Nielsen, Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. *Applied Environmental Microbiology*, 1999, vol. 65, no. 3, pp. 969-973.

Hai, N. V. Probiotic in aquaculture: Current status and outlook. *Journal of Applied Microbiology*, 2015, vol. 119, pp. 917-935.

Hanson L., H. E. L. King Jr, J. H. Howell, and A. J. Smith, A culture method for Sea Lamprey larvae. *Progressive Fish-Culturist*, 1974, vol. 36, pp. 122–128.

Hokkaido Fish Hatchery, Manual for artificial hatching of Arctic Lamprey. Inland Water Resources Department, Hokkaido Fish Hatchery, Hokkaido, Japan [in Japanese]. 2008.

Hopkins, K. D. Reporting fish growth: A review of the basics. *Journal of the World Aquaculture Society*, 1992, vol. 23, no. 3, pp. 173-179.

Jackson, A. D., M. L. Moser, S. T. Onjukka, S. LaPatra, K. M. Lujan, C. Samson, M. G. White, M. Blair, L. Rhodes, R. Lampman, A. N. Maine, and J. C. Jolley, Occurrence of pathogens in Pacific Lamprey (*Entosphenus tridentatus*). *Reviews in Fish Biology and Fisheries*, 2019, vol. 29, pp. 653-668.

Jiang, Y. Y., Wang, Z. Zhang, M. Liao, B. Li, X. Rong, and G. Chen, Responses of microbial community structure in turbot (*Scophthalmus maximus*) larval intestine to the regulation of probiotic introduced through live feed. *PLoS ONE*, 2019, vol. 14, no. 5, pp. 1-17.

Johnson, N. S., S.–S. Yun, H. T. Thompson, C. O. Brant, and W. Li, A synthesized pheromone induces upstream movement in female Sea Lamprey and summons them into traps. *Proceedings of the National Academy of Sciences USA*, 2009, vol. 106, pp. 1021-1026.

Kasamatsu, J. Evolution of innate and adaptive immune systems in jawless vertebrates. *Microbiology and Immunology*, 2013, vol. 57, no. 1, pp. 1-12.

Kesarcodi-Watson, A. Probiotic bacteria for hatchery production of Greenshell mussels, *Perna canaliculus*. *Doctoral dissertation*, University of Technology, Sydney, 2009.

Kim J. K., K. J. Park, K. S. Cho, S. Nam, T. Park, and R. Bajpai R, Aerobic nitrification–Denitrification by heterotrophic bacillus strains. *Bioresource Technology*, 2005, vol. 96, pp. 1897-1906.

Klakegg, Ø. S., Myhren, R. A. Juell, M. Aase, K. Salonius, and H. Sørum, Improved health and better survival of farmed lumpfish (*Cyclopterus lumpus*) after a probiotic bath with two probiotic strains of Aliivibrio. *Aquaculture*, 2020, vol. 518, pp. 734810.

Kujawa R., D. Fopp-Bayat, B. I. Cejko, D. Kucharczyk, K. Glińska-Lewczuk, K. Obolewski, and M. Biegaj, Rearing river lamprey *Lampetra fluviatilis* (L.) larvae under controlled conditions as a tool for restitution of endangered populations. *Aquaculture International*, 2017, vol. 26, pp. 27–36.

Kuratani, S., S. Kuraku, and Y. Murakami, Lamprey as an evo-devo model: Lessons from comparative embryology and molecular phylogenetics. *Genesis*, 2002, vol. 34, no. 3, pp. 175-183.

Lalloo, R., G. Moonsamy, S. Ramchuran, J. Görgens, and N. Gardiner, Competitive exclusion as a mode of action of a novel Bacillus cereus aquaculture biological agent. *Letters in Applied Microbiology*, 2010, vol. 50, no. 6, pp. 563-570.

Lalloo R., S. Ramchuran, D. Ramduth, J. Gorgens, and N. Gardiner, Isolation and selection of *Bacillus* spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. *Journal of Applied Microbiology*, 2007, vol. 103, pp. 1471-1479.

Lampman, R. T., A. N. Maine, M. L. Moser, H. Arakawa, and F. B. Neave, Lamprey aquaculture successes and failures: A path to production for control and conservation. *Journal of Great Lakes Research*, 2021, vol. 47, no. 1, pp. 201-215.

Lampman, R., M. L. Moser, A. D. Jackson, R. K. Rose, A. L. Gannam, and J. M. Barron. 2016. Developing techniques for artificial propagation and early rearing of Pacific Lamprey (*Entosphenus tridentatus*) for species recovery and restoration, in A. Orlov and R. J. Beamish, editors. *Jawless Fishes of the World,* American Fisheries Society, Bethesda, Maryland, pp. 160-194.

Lash, B. W., T. H. Mysliwiec, and H. Gourama, Detection and partial characterization of a broad-range bacteriocin produced by *Lactobacillus plantarum* (ATCC 8014). *Food Microbiology*, 2005, vol. 22, pp. 199-204.

Li, W., M. Twohey, M. Jones, and M. Wagner, Research to guide use of pheromones to control Sea Lamprey. *Journal of Great Lakes Research*, 2007, vol. 33, pp. 70-86.

Lin, T., X. Liu, D. Xiao, D. Zhang, Y. Cai, and X. Zhu, *Lactobacillus* spp. as probiotics for prevention and treatment of enteritis in the lined seahorse (*Hippocampus erectus*) juveniles. *Aquaculture*, 2019, vol. 503, pp. 16-25.

Lowrey, L., D. C. Woodhams, L. Tacchi, and I. Salinas, Topographical mapping of the rainbow trout (*Oncorhynchus mykiss*) microbiome reveals a diverse bacterial community with antifungal properties in the skin. *Applied Environmental Microbiology*, 2015, vol. 81, no. 19, pp. 6915-6925.

Luis-Villasenor, I. E., M. E. Macias-Rodriguez, B. Gomez-Gil, F. Ascencio-Valle, and A. I. Campa-Cordova, Beneficial effects of four *Bacillus* strains of the larval cultivation of *Litopenaeus vannamei*. *Aquaculture*, 2011, vol. 321, pp. 136-144.

Ma, Y. X. L., Y. Li, M. Li, W. Chen, P. Y. Bao, Z. C. Yu, and Y. Q. Chang, Effects of dietary probiotic yeast on growth parameters in juvenile sea cucumber, *Apostichopus japonicus*. *Aquaculture*, 2019, vol. 499, pp. 203-211.

Maine, A. N., A. D. Jackson, and M. L. Moser, Development of artificial propagation methods for Pacific Lamprey (*Entosphenus tridentatus*). Annual Research Report for BOR/CTUIR Cooperative Agreement R17AC00167. Submitted to U.S. Bureau of Reclamation, Boise, Idaho, 2017.

Maine, A. N., A. D. Jackson, and M. L. Moser, Development of artificial propagation methods for Pacific Lamprey (*Entosphenus tridentatus*). 2018 Annual Research Report submitted to U.S. Bureau of Reclamation, Boise, Idaho, 2019.

Mallatt, J. Laboratory growth of larval lampreys (*Lampetra* (*Entosphenus*) *tridentata* Richardson) at different food concentrations and animal densities. *Journal of Fish Biology*, 1983, vol. 22, no. 3, pp. 293-301.

Moore, J. W., and F. W. H. Beamish, Food of larval Sea Lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). *Journal of the Fisheries Board of Canada*, 1973, vol. 30, no. 1, pp. 7-15.

Moore, J. W., and I. C. Potter, Aspects of feeding and lipid deposition and utilization in the lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). *The Journal of Animal Ecology*, 1976, vol. 45, no. 3, pp. 699-712.

Moser, M. L. and D. Close. Assessing Pacific Lamprey status in the Columbia River Basin. *Northwest Science*, 2003, vol. 77, no. 2, pp. 116-125.

Moser, M. L., J. B. Hume, K. K. Aronsuu, R. T. Lampman, and A. D. Jackson. 2019. Lamprey reproduction and early life history: Insights from artificial propagation, in M. F. Docker, editor. *Lampreys: Biology, conservation and control*, Vol. 2. Springer, Dordrecht, Netherlands, pp. 187-245.

Moser, M. L., A. N. Maine, and A. D. Jackson, Development of artificial propagation methods for production of juvenile Pacific Lamprey (*Entosphenus tridentatus*) for the use in research associated with Section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan. 2019 Annual Report from NOAA Fisheries to the Public Utility District No. 1 of Chelan County, Wenatchee, Washington, 2020.

Munir, M. B., R. Hashim, Y. H. Chai, T. L. Marsh, and S. A. M. Nor, Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings. Aquaculture, 2016, vol. 460, pp. 59-68.

Murauskas, J. G., A. M. Orlov, and K. A. Siwicke, Relationships between the abundance of Pacific Lamprey in the Columbia River and their common hosts in the marine environment. *Transactions of the American Fisheries Society*, 2013, vol. 142, no. 1, pp. 143-155.

Murdoch, S. P., F. W. Beamish, and M. F. Docker, Laboratory study of growth and interspecific competition in larval lampreys. *Transactions of the American Fisheries Society*, 1991, vol. 120, no. 5, pp. 653-656.

Nayak, S. K., Probiotics and immunity: A fish perspective. *Fish and Shellfish Immunology*, 2010, vol. 29, no. 1, pp. 2-14.

Nelson, M. C., and R. D. Nelle, Juvenile Pacific Lamprey use of a pollution abatement pond on the Entiat National Fish Hatchery. Final Report, U.S. Fish and Wildlife Service, Leavenworth, Washington, 2007.

Opiyo, M., A. J. Jumbe, C. C. Ngugi, and H. Charo-Karisa, Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Scientific African*, 2019, vol. 4, pp. e00103.

Padmavathi P., K. Sunitha, and K. Veeraiah, Efficacy of probiotics in improving water quality and bacterial flora in fish ponds. *African Journal of Microbiology Research*, 2012, vol. 6, pp. 7471-7478.

Paerl, H., J. Dyble, P. Moisander, R. Noble, M. Piehler, J. Pinckney, T. Stepp, L. Twomey, and L. Valdes, Microbial indicators of aquatic ecosystem change: Current applications to eutrophication studies. *FEMS Microbiology Ecology*, 2003, vol. 46, pp. 233-246.

Quaempts, E. J., K. L. Jones, S. J. O'Daniel, T. J. Beechie, and G. C. Poole, Aligning environmental management with ecosystem resilience: A First Foods example from the Confederated Tribes of the Umatilla Indian Reservation, Oregon, USA. *Ecology and Society,* 2018, vol. 23, no. 2, pp. 29.

Queiroz, J. F. and C. E. Boyd, Effects of a bacterial inoculum in channel catfish ponds. *Journal of the World Aquaculture Society*, 1998, vol. 29, no. 1, pp. 67-73.

R Core Team, R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*, Vienna, Austria. <https://www.R-project.org>, 2020.

Ringø, E. and O. Vadstein, Colonization of *Vibrio pelagius* and *Aeromonas caviae* in early developing turbot (*Scophthalmus maximus* L.) larvae. *Journal of Applied Microbiology*, 1998, vol. 84, no. 2, pp. 227-233.

Robertson, P. A., W. C. O'Dowd, C. Burrells, P. Williams, and B. Austin, Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Aquaculture*, 2000, vol. 185, no. 3-4, pp. 235-243.

Rodriguez-Muñoz, R., A. G. Nicieza, and F. Braña, Density-dependent growth of Sea Lamprey larvae: Evidence for chemical interference. *Functional Ecology*, 2003, vol. 17, pp. 403-408.

Rogers, P. A., A. R. Glenn, and I. C. Potter, The bacterial flora of the gut contents and environment of larval lampreys. *Acta Zoologica*, 1980, vol. 61, no. 1, pp. 23-27.

Sayes, C., Y. Leyton, and C. Riquelme. 2018. Probiotic bacteria as a healthy alternative for fish aquaculture, in S. Savic, editor. *Antibiotic Use in Animals*. Intech Open, London, pp. 115-132.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 2012, vol. 9, no. 7, pp. 671-675.

Silva, E. F., M. A. Soares, N. F. Calazans, J. L. Vogeley, B. C. do Valle, R. Soares, and S. Peixoto, Effect of probiotic (*Bacillus* spp.) addition during larvae and postlarvae culture of the white shrimp *Litopenaeus vannamei*. *Aquaculture Research*, 2012, vol. 44, no. 1, pp. 13-21.

Stanford, J. and J. Ward, An ecosystem perspective of alluvial rivers: Connectivity and the hyporheic corridor. *Journal of the North American Benthological Society*, 1993, vol. 12, pp. 48-60.

Sutton, T. M. and S. H. Bowen, Significance of organic detritus in the diet of larval lampreys in the Great Lakes basin. *Canadian Journal of Fisheries and Aquatic Sciences*, 1994, vol. 51, no. 11, pp. 2380-2387.

Wagner, C. M., M. L. Jones, M. B. Twohey, and P. W. Sorensen, A field test verifies that pheromones can be useful for Sea Lamprey (*Petromyzon marinus*) control in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 2006, vol. 63, pp. 475-479.

Wang, Y. Use of probiotics *Bacillus coagulans*, *Rhodopseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings. *Aquaculture Nutrition*, 2011, vol. 17, no. 2, pp. e372-e378.

Warton, D. I. and F. K. Hui, The arcsine is asinine: The analysis of proportions in ecology. *Ecology*, 2011, vol. 92, no. 1, pp. 3-10.

Wootton, R. J. Dynamics of population abundance and production, in *Ecology of Teleost Fishes*. Springer, Dordrecht, 1990, pp. 238-281.

Yap, M. R. and S. H. Bowen, Feeding by northern Brook Lamprey (*Ichthyomyzon fossor*) on sestonic biofilm fragments: Habitat selection results in ingestion of a higher quality diet. *Journal of Great Lakes Research*, 2003, vol. 29, pp. 15-25.

Yong, E, *I contain multitudes: The microbes within us and a grander view of life*. Ecco, HarperCollins Publishers, New York, New York, 2016.

York, J. R., E. M. J. Lee, and D. W. McCauley, The lamprey as a model vertebrate in evolutionary developmental biology, in M. Docker, editor. *Lampreys: Biology, Conservation and Control.* Springer, Dordrecht, 2019, pp. 481-526.

Zhao, W., Y. Liu, M. Latta, W. Ma, Z. Wu, and P. Chen, Probiotics database: A potential source of fermented foods. *International Journal of Food Properties*, 2019, vol. 22, no. 1, pp. 198-217.

Zhou, Q., K. Li, X. Jun, and L. Bo, Role and functions of beneficial microorganisms in sustainable aquaculture. *Bioresource Technology*, 2009, vol. 100, no. 16, pp. 3780-3786.

[A]Tables

Table 1. Mechanisms observed in use of probiotics containing genera of bacteria present in the probiotic (EPI-CIN G2) in cultured aquatic organisms.

Genus	**Mechanisms**	**References**
Bacillus	Increased intestinal enzyme activity, competitive exclusion via rapid growth, inhibition of growth of pathogenic bacteria, decreased nitrogenous waste	Wang 2011; Luis-Villasenor et al. 2011; Lalloo et al. 2007; Lalloo et al. 2009
Lactobacillus	Increased intestinal enzyme activity, increased growth performance due to decreased cholesterol and increased fatty acid levels, inhibition of growth of pathogenic bacteria via bacteriocin protein secretion	Wang 2011; Falcinelli et al. 2015; Lash et al. 2005
Acetobacter	Synthesis/fixation of nitrogen, production of acetic acid	Zhou et al. 2009; Zhao et al. 2019

Table 2. Studies investigating probiotic use in cultured aquatic organisms.

Species	**Life stage**	**Route of exposure**	**Metrics improved by probiotic application**	**Reference**
Lumpfish *Cyclopterus lumpus*	Larvae	Water	Survival, growth, disease resistance	Klakegg et al. 2020
Rohu *Labeo rohita*	Fingerlings	Food	Growth, feed conversion	Ghosh et al. 2004
Rohu	Juveniles	Food	Growth, feed utilization, immune function	Giri et al. 2013
Turbot *Scophthalmus maximus*	Larvae	Water	Survival	Ringo and Vadstein 1998
Turbot	Larvae	Food	Survival, growth	Daga et al. 2013
Rainbow Trout *Oncorhynchus mykiss*	Adult	Water	Disease resistance	Gram et al. 1999
Rainbow Trout	Fry	Food	Survival, growth	Bagheri et al. 2008
Channel Catfish *Ictalurus punctatus*	Adult	Water	Survival, growth	Queiroz and Boyd 1998
Atlantic Salmon *Salmo salar* Rainbow Trout	Fingerlings	Food	Disease resistance	Robertson et al. 2000
European Eel *Anguilla anguilla*	Adult	Food	Disease resistance	Chang and Lui 2002
Sea cucumber *Apostichopus japonicus*	Juvenile	Food	Growth, enzyme activity	Ma et al. 2019
Pacific oyster *Crassostrea gigas*	Larvae	Food	Growth	Douillet and Langdon 1994
Greenshell mussel *Perna canaliculus*	Larvae	Water	Survival, disease resistance	Kesarcodi-Watson 2009
Lined seahorse *Hippocampus erectus*	Juvenile	Food	Survival, growth	Lin et al. 2019
White shrimp *Penaeus vannamei*	Larvae	Food and water	Survival, growth	Silva et al. 2011

Table 3. Mean (range) of measured water quality parameters during larval Pacific Lamprey survival and growth experiments using two different concentrations of a probiotic (EPI-CIN G2) in 2018 and 2019.

	Temperature (°C)		**Dissolved Oxygen (mg/L)**		**pH**		**Ammonia (mg/L NH_3-N)**		**Nitrite (mg/L NO_2-N)**		**Nitrate (mg/L NO_3-N)**	
	2018	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
Control (no probiotic)	13.98 (13.5-14.6)	13.59 (13.1-13.9)	8.70 (8.1-9.0)	8.78 (7.9-9.2)	7.6 (7.3-7.8)	7.6 (7.3-7.9)	0.24 (0.1-0.4)	0.25 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.0)
T1 (2 mg/L probiotic)	13.92 (13.7-14.4)	13.61 (13.1-14.2)	8.78 (8.2-9.1)	8.79 (7.9-9.1)	7.6 (7.2-7.9)	7.6 (7.2-8.0)	0.25 (0.1-0.4)	0.22 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.5)
T2 (5 mg/L probiotic)	13.96 (13.4-14.6)	13.6 (13.3-14.6)	8.83 (8.1-9.1)	8.80 (7.9-9.1)	7.6 (7.4-7.8)	7.6 (7.3-7.9)	0.26 (0.1-0.4)	0.24 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.0)

[A]Figure captions

Figure 1. Digital photograph of larval lamprey used to obtain lengths.

Figure 2. Density of beakers (larvae/m^2) at the end of the 2018 probiotic experiment (experiment lasted 69 days). Densities (rounded to nearest whole number) at the start of the experiment were 1,155 larvae/m^2 for each beaker. The 25th and 75th percentiles are defined by the vertical extent of the box, while the thickest line (inside the box for C, top of the box for T1 and T2) represents the mean value. The whiskers mark the maximum and minimum values. Values outside the whiskers are considered outliers. The treatments are as follows: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement.

Figure 3. Box and whisker plots of mean larval length (mm) as a function of treatment for the 2018 experiment (69 days in length). The 25th and 75th percentiles are defined by the vertical extent of the box, while the line inside each box represents the mean value. The whiskers mark the maximum and minimum values. The treatments are as follows: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement. Different letters above each treatment note significant differences as a result of the Tukey's HSD post hoc test.

Figure 4. Final larval lamprey lengths (in mm) as a function of final larval density (larvae/m^2) at the end of the 2018 experiment (69 days in length). The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered to separate overlapping values.

Figure 5. Density of tanks (larvae/m^2) during the 2019 probiotic experiment. Densities (rounded to nearest whole number) at the start of the experiment were 407 larvae/m^2 (774 larvae/m^3) for each tank. Larvae were assessed at 77, 178, 200, and 226 days post-fertilization during the 2019 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The T2 treatment larvae all died on day 200 post-fertilization and growth was extrapolated for that group.

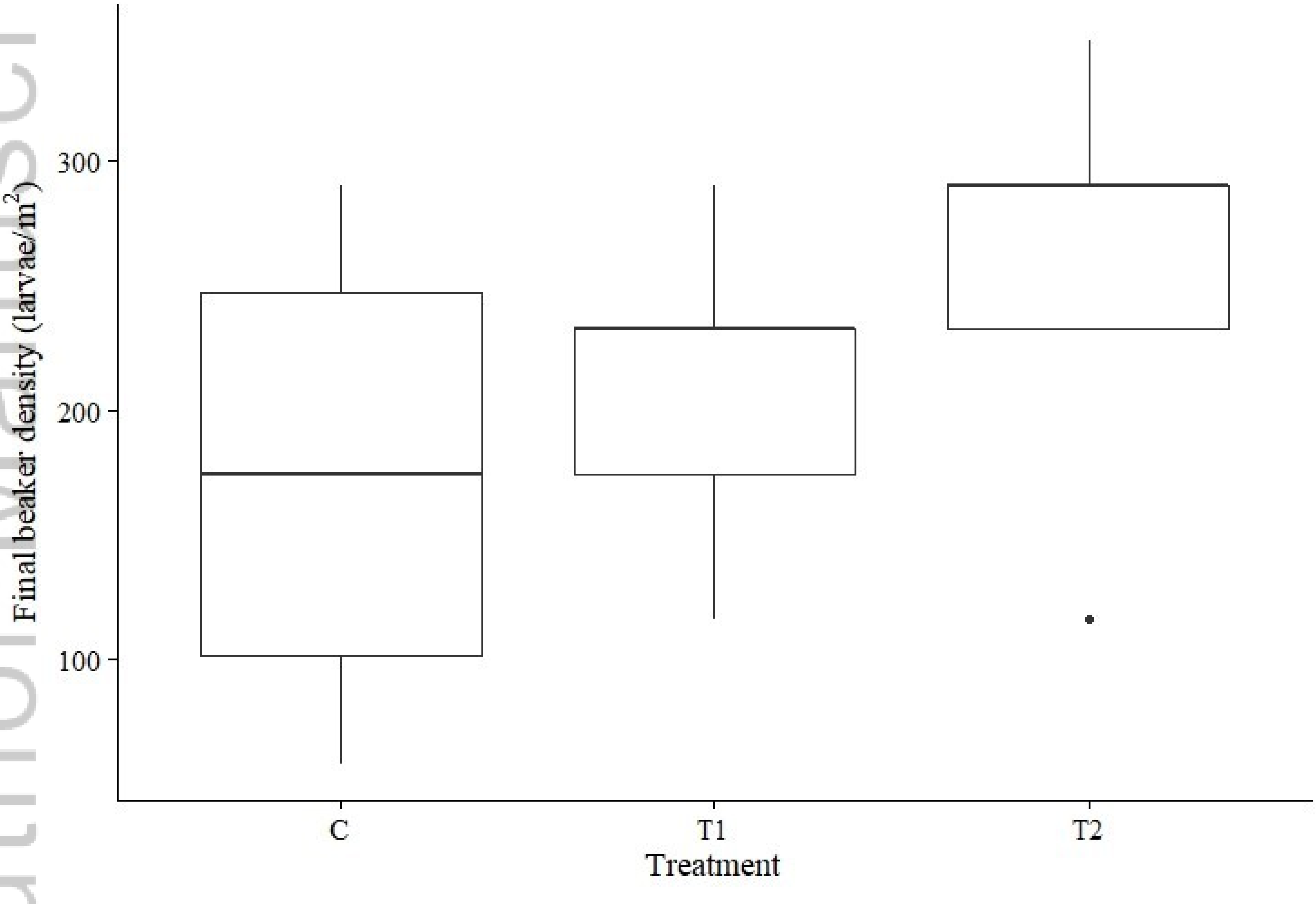
Figure 6. Box and whisker plots of mean larval length (mm) as a function of treatment for the 2019 experiment: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement. Higher probiotic dose (T2) lengths were extrapolated from 200 to 226 days using their instantaneous growth rate to estimate final length. The 25th and 75th percentiles are defined

by the vertical extent of the box, while the line inside each box represents the mean value. The whiskers mark the maximum and minimum values. Values that are outside the whiskers are considered outliers.

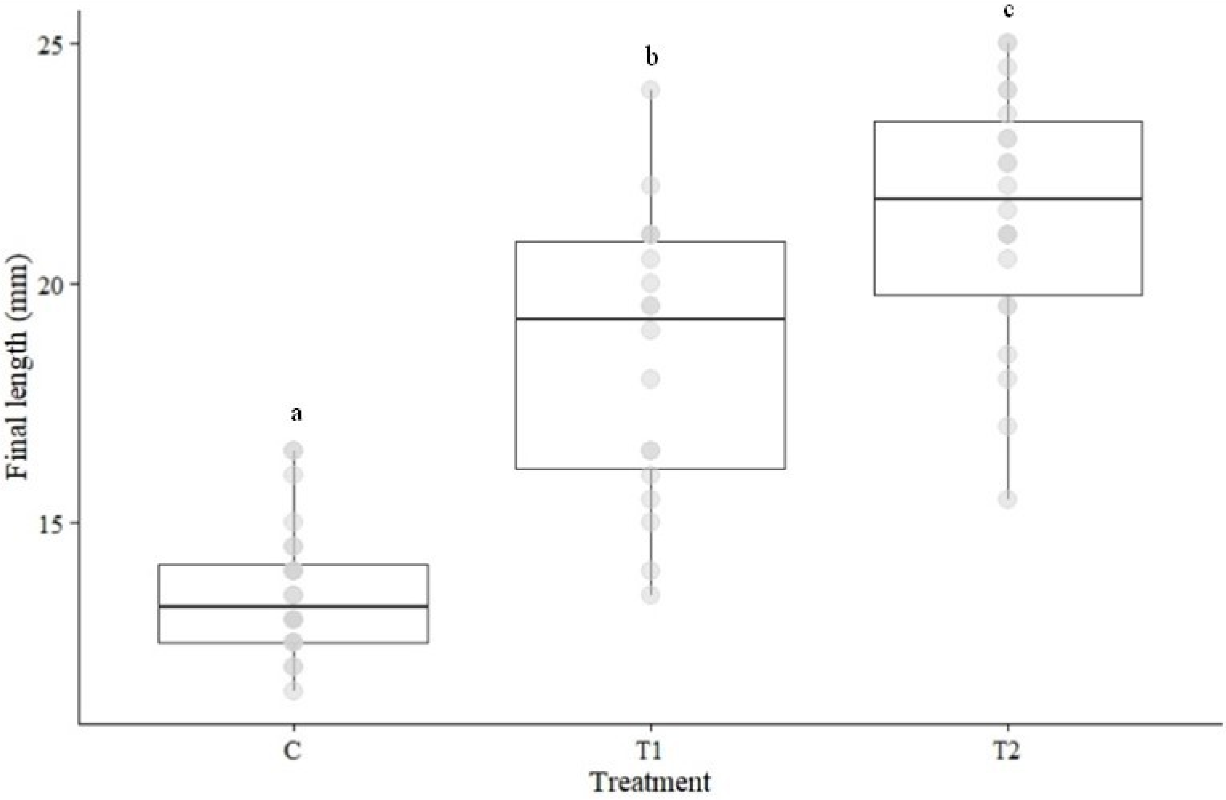
Figure 7. Instantaneous growth rate (µm/day; mean) of larvae as a function of days post-fertilization. Larvae were assessed at 77, 178, 200, and 226 days post-fertilization during the 2019 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered at each assessment period to separate overlapping values.

Figure 8. Final lengths (in mm) of larval lamprey as a function of final tank density in 2019. The treatments are: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered to separate overlapping values.

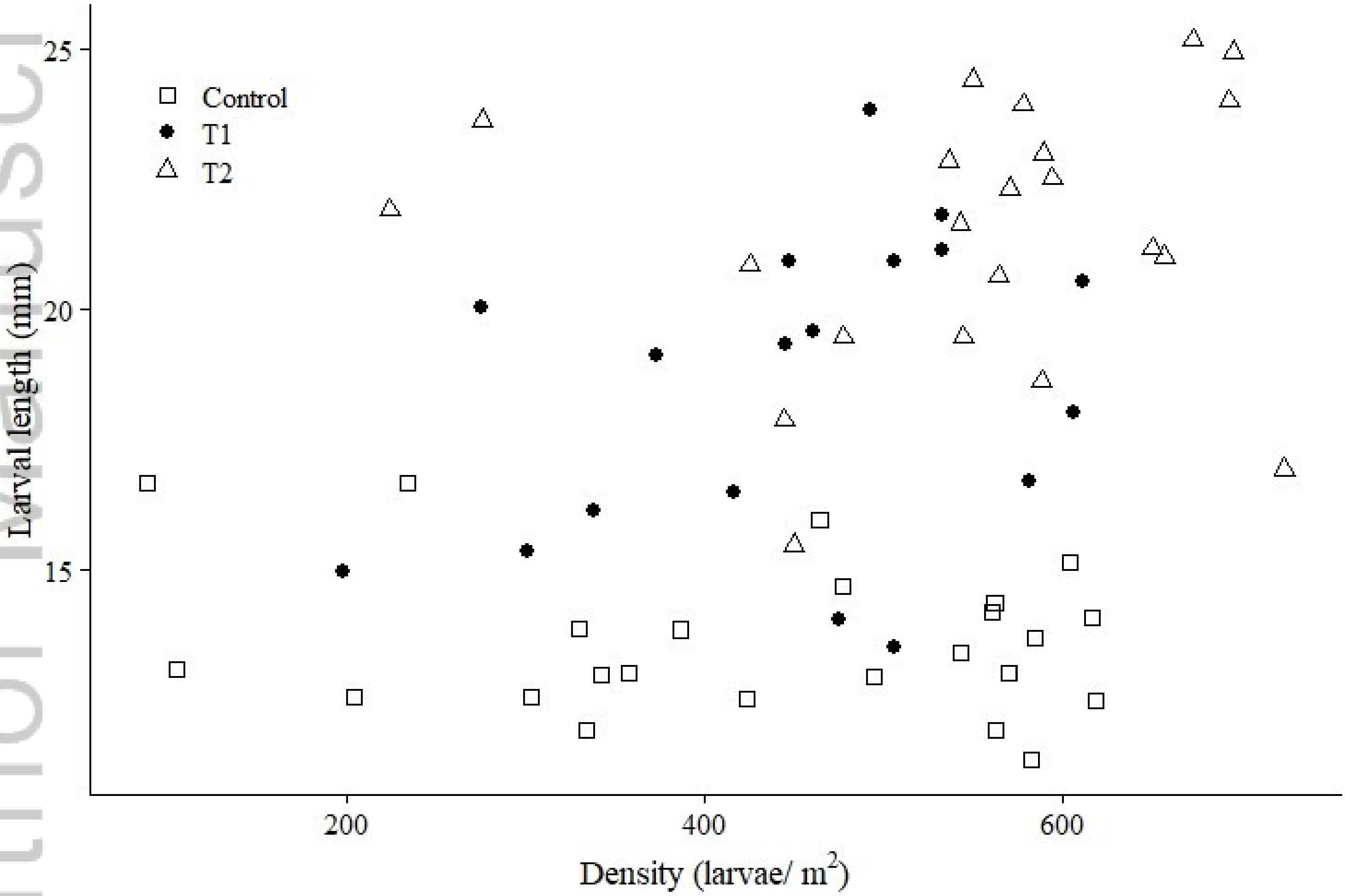
Maine et al_Fig 1.jpg



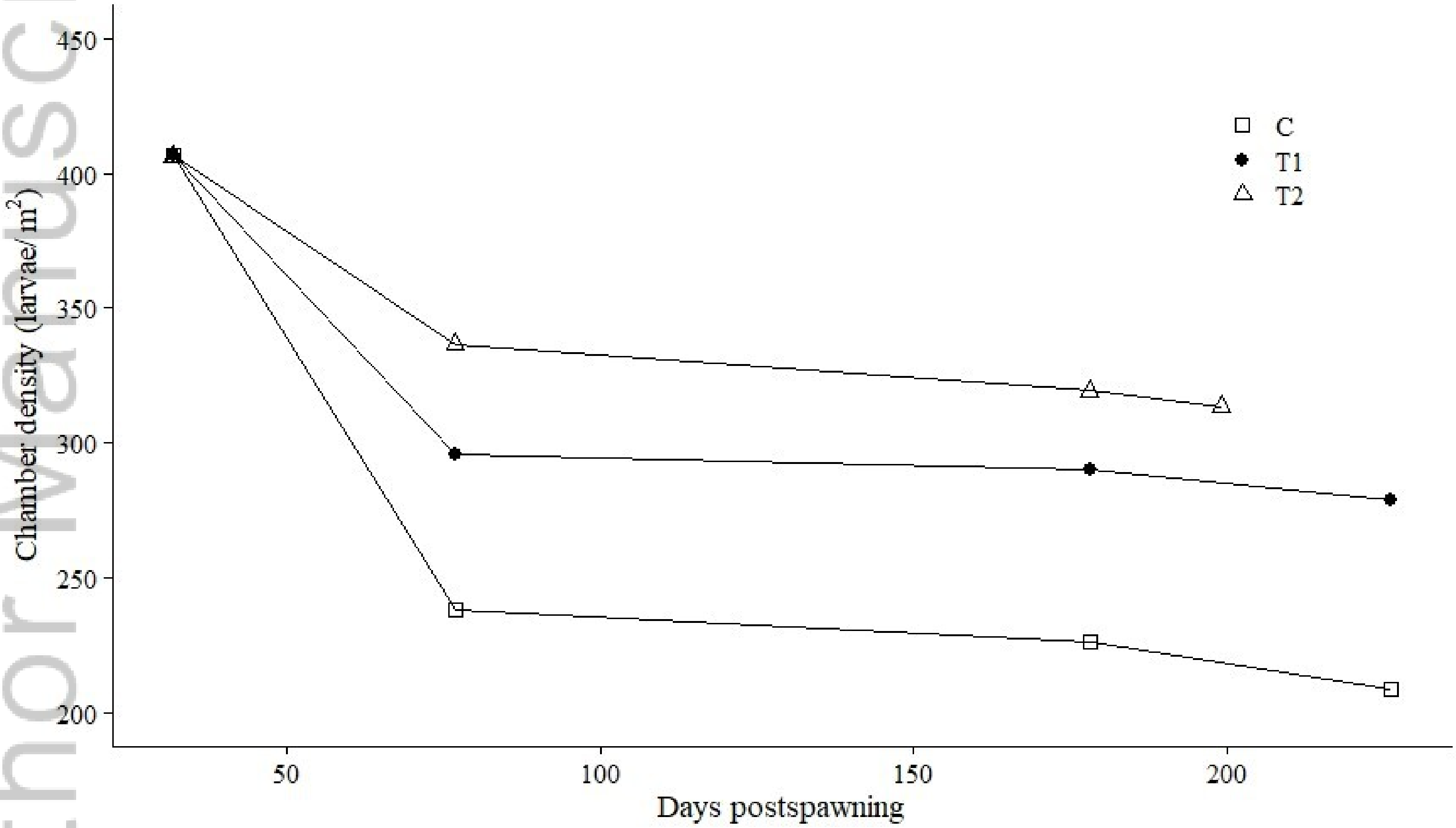
Maine et al_Fig 2.jpg



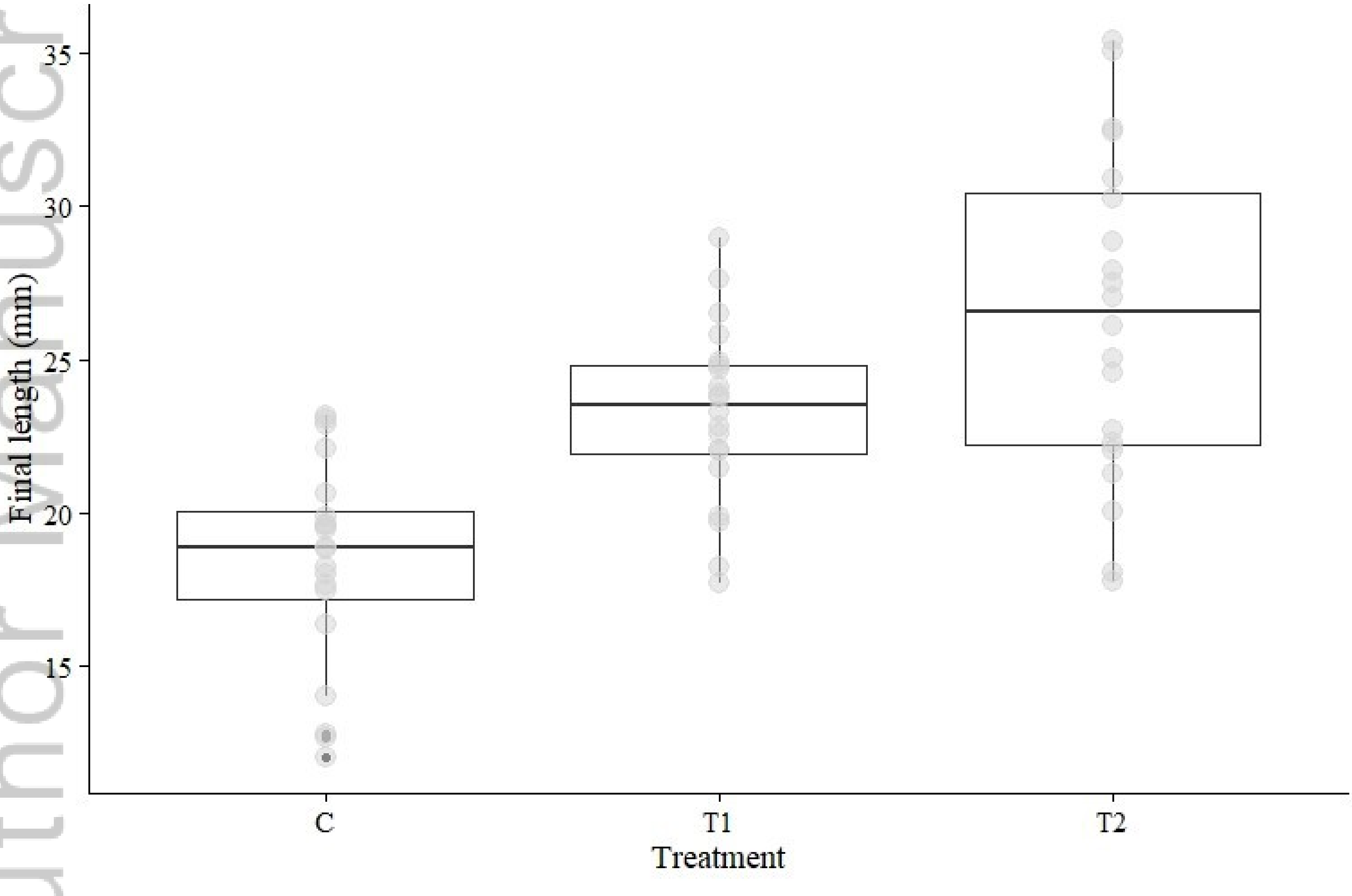
Maine et al_Fig 3.jpg



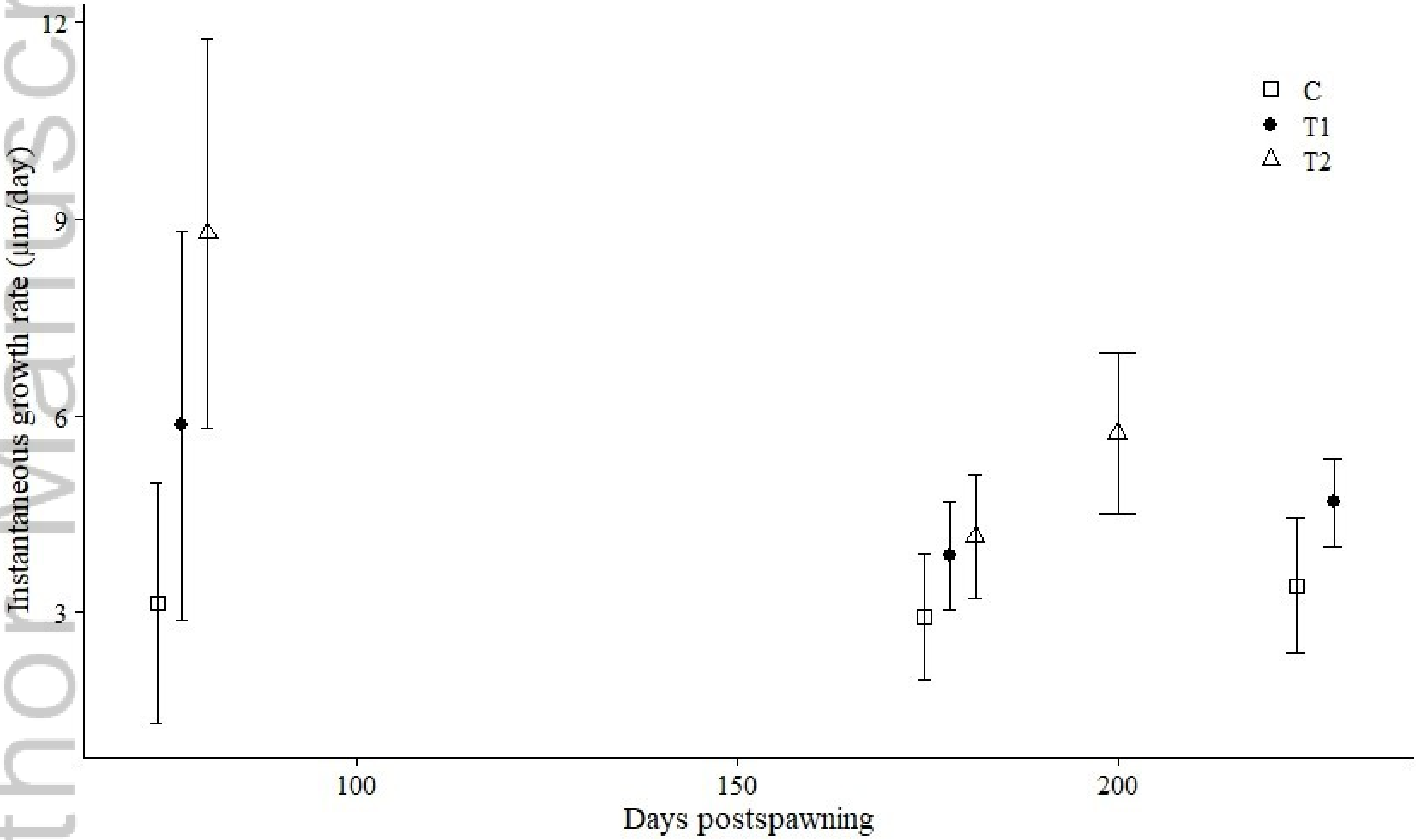
Maine et al_Fig 4.jpg



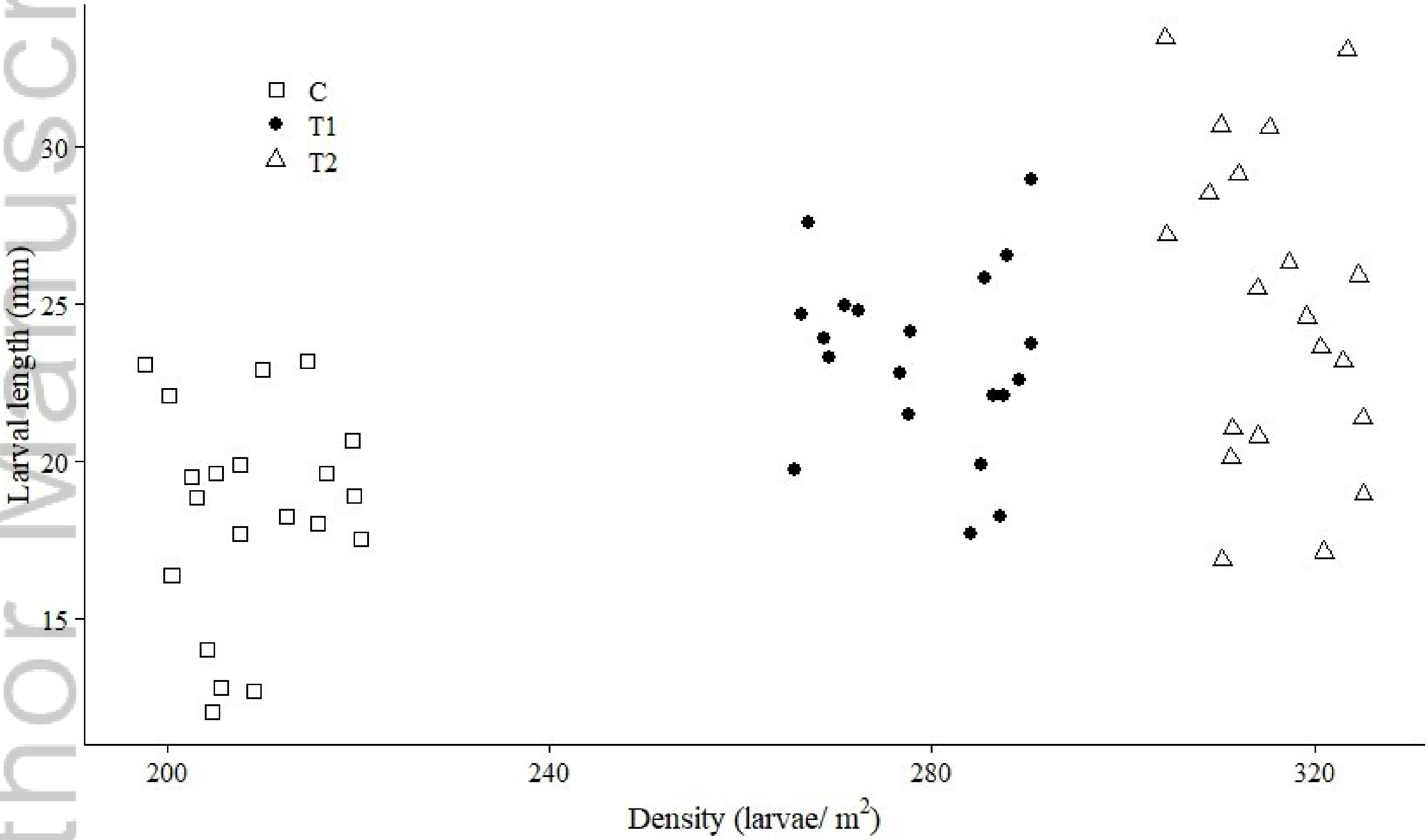
Maine et al_Fig 5.jpg



Maine et al_Fig 6.jpg



Maine et al_Fig 7.jpg



Maine et al_Fig 8.jpg