FIGURE 1. *In-line heaters with mixing cells were used to warm three of the treatment temperatures above the ambient groundwater temperature (Photo: James Barron).*

Optimization of Culture Temperatures for Larval Pacific Lamprey

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The Pacific lamprey *Entosphenus tridentatus* is one of the oldest native fish in the Pacific Northwest, USA. At an estimated 450 million years old, these lampreys have seen many other species come and go from the earth. Despite their ability to persist through numerous global mass extinction events, the Pacific lamprey is currently a species of concern in response to reductions in range and declines in abundance that have occurred within the last century due mainly to habitat destruction, dams and other barriers to passage (Close *et al*. 2002, Moser and Close 2003, Moyle *et al*. 2009).

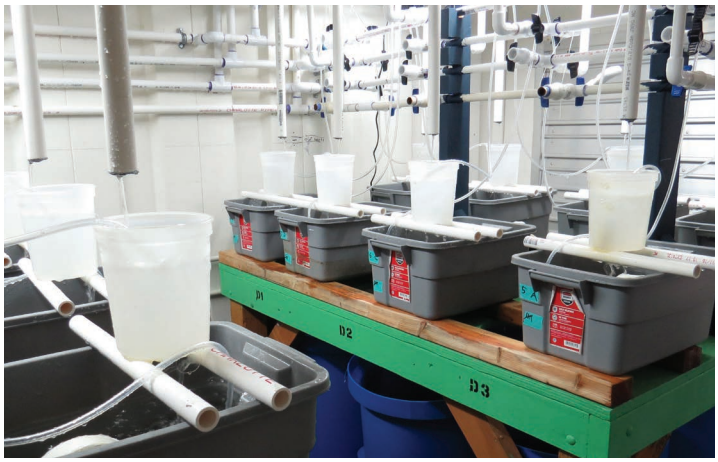

FIGURE 2. *Experimental tanks used in temperature trials, with aerated head boxes and packed columns for degassing and aeration (Photo: James Barron).*

Not only is their tenure on this planet impressive, but this species displays a staggering number of unique qualities, traits and abilities. They spend up to seven years as larvae burrowed into the beds of streams and rivers where they consume microscopic debris filtered from the water. When they eventually leave the safety of the streambed, they must not only transform their entire bodies by developing eyes, a new mouth, teeth and a silvery body, but they must also be able to persist in the saline waters of the Pacific Ocean and grow to adulthood on a diet of blood. After spending a few years attached to host fish and marine mammals in the Pacific Ocean, adults must return to freshwater where they must survive for one or two more years as they prepare to spawn. Adults can make spawning migrations as far inland as

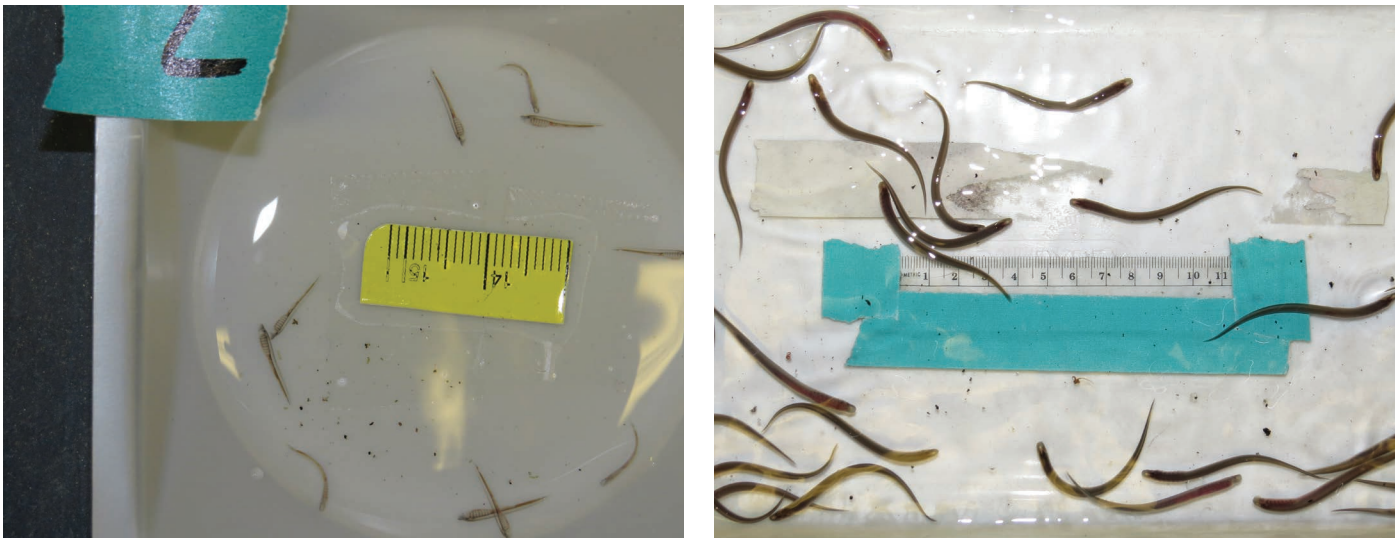

FIGURE 3. *Early larval Pacific lamprey at the beginning of a temperature trial (left). Older larvae at the end of a temperature trial (right) (Photo: James Barron).*

Idaho and can climb vertically up waterfalls they encounter along the way using their oral discs. When they arrive at their spawning grounds, they take the time to carefully build a nest by moving stones with their mouth before spawning. If a lamprey makes it this far, it will die where its life began, depositing a dose of marine nutrients into the freshwater environment.

It is no surprise given the impressive life of this iconic species that they are a critical part of the ecosystems they inhabit. They are also significant part of the culture of Native American tribes in the region, representing an important food source that is used in tribal feasts and celebrations. Warm Springs Tribal Council member Ron Suppah explains that "The Creator told the people that the eels would always return as long as the people took care of them, but if the people failed to care of them, they would disappear." Therefore, growing conservation efforts are focusing on this species and aquaculture is one aspect that may be used to supplement declining populations (CRITFC 2011).

Culture methods require further development before conservation aquaculture can be applied for this species. As with most anadromous species raised for conservation purposes, the freshwater phase is where the majority of time in captivity occurs. For Pacific lamprey most of their freshwater life occurs during the larval stage, which can take up to seven years to complete (Close *et al*. 2002). The unique biology of this species during the larval phase creates challenges that require development of unconventional culture techniques.

To enhance our ability to culture Pacific lamprey, scientists at the U.S. Fish and Wildlife Service's Abernathy Fish Technology Center (Washington State, USA) have been investigating the effects of rearing temperature on larvae at 86 and 554 days post hatch (DPH). Water temperature is a key consideration when culturing fish because it affects metabolism, development, growth, survival, and fatty acid profile of fish (Andrews and Stickney 1972, Brett 1979, Piper *et al*. 1982, Blaxter 1992, Barron *et al*. 2012). A given species can tolerate a range of temperatures, but within that range there is an optimum where metabolism functions most efficiently (Piper *et al*. 1982). The optimum rearing temperature may also shift through the life history of the fish (Piper *et al*. 1982).

Early Larval Trial (86 DPH)

Four treatment temperatures were maintained over the course of a six-week trial during the summer. Groundwater was heated to achieve treatment temperatures, with ambient groundwater being the lowest temperature tested. Inline water heaters were used to warm the single-pass water, causing several complications that required resolution before experiments could begin. First, heaters created oscillations in water temperature as they switched on and off. An inline mixing cell was installed after each heater to smooth variation in temperature before water was passed to culture tanks (Fig. 1). In addition, once water was heated, excess gasses formed that were potentially harmful to fish and could accumulate in water lines, creating a potential airlock. Bleeder valves were installed on incoming water lines to allow free gasses to purge. Packed columns followed by small aerated head boxes were added to each culture tank to strip excess gases from the incoming water and reduce the concentration of dissolved gasses from groundwater to a safe level for fish (Fig. 2).

Three replicate tanks were maintained at each treatment temperature level via incoming single-pass water. Treatments included average temperatures of 14.7, 17.3, 19.2 and 22.4 C. Rinsed and sifted sand was added to each tank as substrate for burrowing by lamprey. Fifty fish at 86 DPH and 3.7 mg average weight were stocked into each tank (Fig. 3A). Although lamprey are filter-feeders at this stage, they were fed twice per week with a slurry of yeast and larval fish diet while water flow was shut off for five hours. The substrate was flushed with water every other week during the experiment to maintain water quality within the tank.

Survival of the young larvae was high (>98 percent) at all treatment temperatures. Larvae grew quickly at all rearing temperatures tested, although final weight was reduced in fish reared at 22.4 C (Fig. 4). A lamprey-specific condition factor was calculated (Lampman *et al*. 2016) and fish had robust values regardless of rearing temperature. Total whole-body lipid was not affected by temperature and ranged from 1.9 to 2.2 percent among treatments.

Fatty acids are an important part of cell membrane structure

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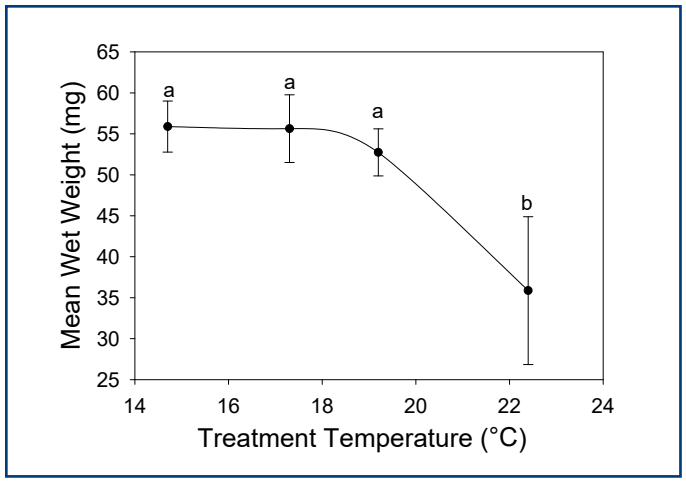

FIGURE 4. *Mean wet weight of early larval lamprey reared at different treatment temperatures for 42 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

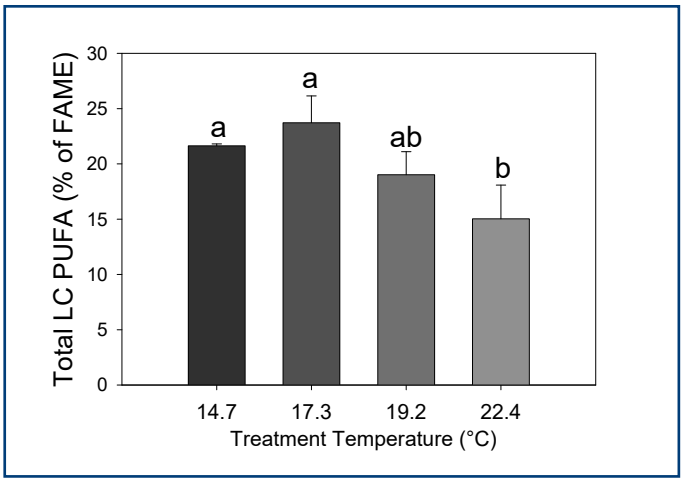

FIGURE 5. *Total long-chain polyunsaturated fatty acid (LC PUFA) content in the whole body fatty acid profile of early larval Pacific lamprey reared at different treatment temperatures for 42 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

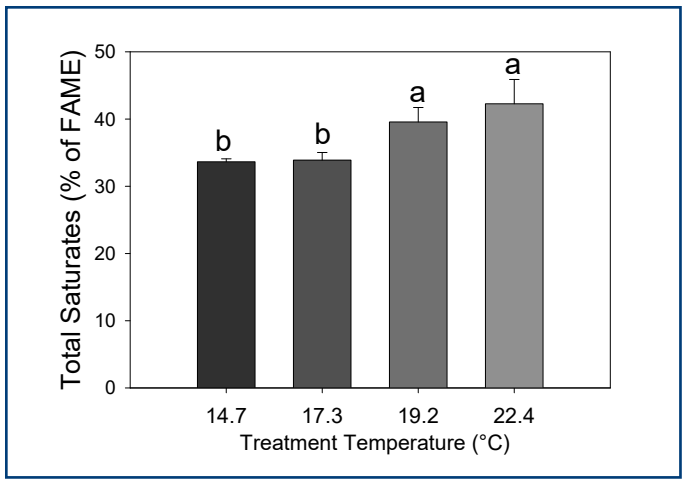

FIGURE 6. *Total saturated fatty acid content in the whole body fatty acid profile of early larval Pacific lamprey reared at different treatment temperatures for 42 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

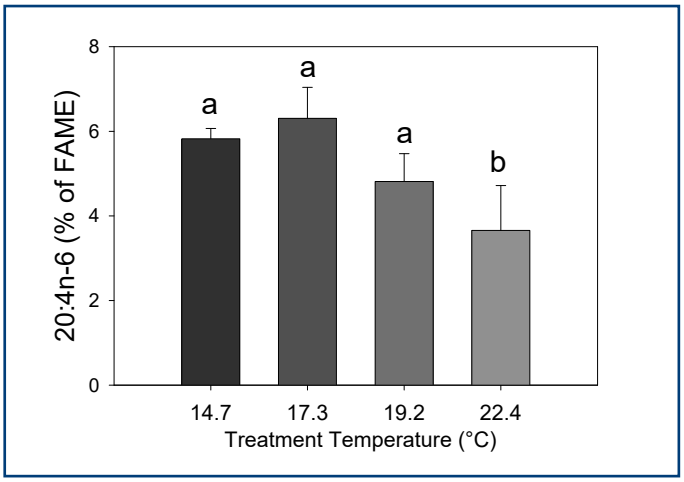

FIGURE 7. *ARA (20:4n-6) content in the whole body fatty acid profile of early larval Pacific lamprey reared at different treatment temperatures for 42 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

in fish and their specific melting points affect the fluidity of cell membranes at different temperatures. As rearing temperature increased, the fatty acid profile of lamprey larvae shifted from longer-chained, unsaturated fatty acids (Fig. 5) to saturated fatty acids (Fig. 6), the latter of which is fluid at warmer temperatures. The shifting fatty acid profiles also included a decline in key fatty acids such as arachidonic acid (ARA; Fig. 7) and eicosapentaenoic acid (EPA; Fig. 8) in larvae raised in warm water. The changes in fatty acid profile due to rearing temperature in these early larvae is similar to the response seen in other fish species (Andrews and Stickney 1972, Farkas *et al*. 1980, Neidleman 1987).

Mid-Larval Trial (554 DPH)

The second trial occurred in the winter and lasted eight weeks. Treatments included average temperatures of 12.3, 15.7, 19.4 and 22.6 C. The treatment range was broader in this trial than in the early larval trial due to the seasonally cooler ambient groundwater temperature. Every tank was stocked with 15 larvae at 554 DPH and an average weight of 156 mg (Fig. 3B). Husbandry was similar to that described for the early larval trial.

Survival of older larvae was high (>96 percent) at all rearing temperatures. Once again fish grew rapidly at all rearing temperatures, but the final length and weight of fish raised at 15.7 C were greater than fish raised at warmer temperatures (Fig. 9). Therefore, 15.7 C may be an optimal rearing temperature for older larvae. As in the early larval trial, condition factor was not affected by rearing temperature. Total whole-body lipid, although not significantly affected by temperature, was higher in the older larvae, ranging from 4.2 to 5.1 percent among treatment means.

The whole-body fatty acid profile in the older larvae was less responsive to temperature compared to the early larvae as there were no differences in total saturates, ARA or EPA due to rearing

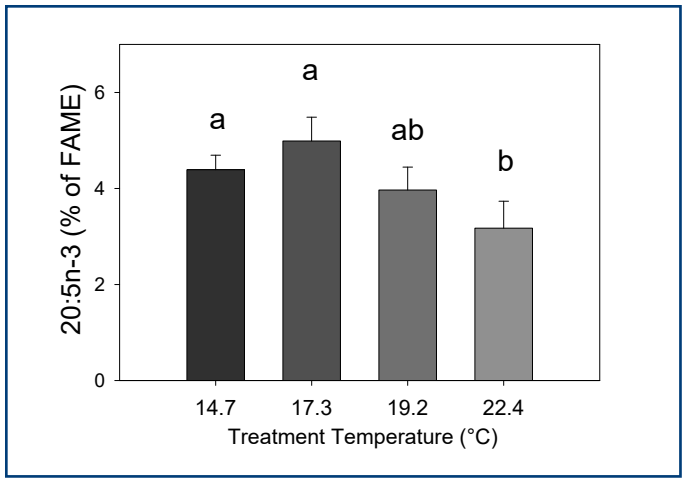

FIGURE 8. *EPA (20:5n-3) content in the whole body fatty acid profile of early larval Pacific lamprey reared at different treatment temperatures for 42 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

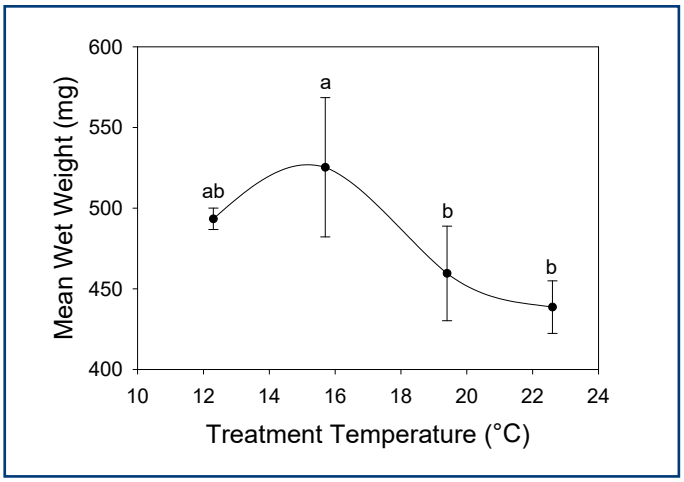

FIGURE 9. *Mean wet weight of older larval lamprey reared at different treatment temperatures for 56 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

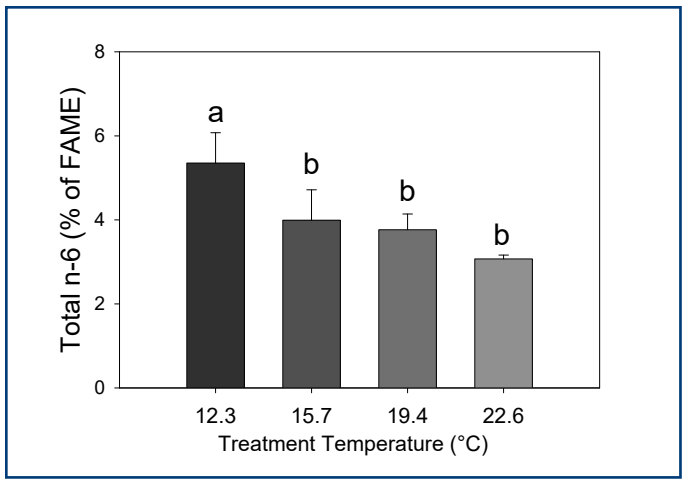

FIGURE 10. *Total n-6 fatty acid content in the whole body fatty acid profile of older larval Pacific lamprey reared at different treatment temperatures for 56 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

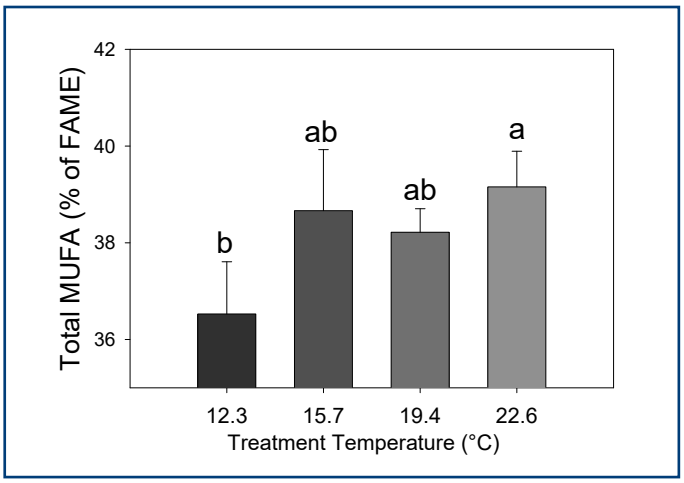

FIGURE 11. *Total mono-unsaturated fatty acid (MUFA) content in the whole body fatty acid profile of older larval Pacific lamprey reared at different treatment temperatures for 56 days. Error bars represent standard deviation (n = 3). Different letters indicate a significant difference as determined by ANOVA followed by SNK test.*

temperature, although the fatty acid profile of older larvae changed in response to rearing temperature. At lower water temperature, the amount of polyunsaturated fatty acids increased in older larvae, as exemplified by elevated total n-6 fatty acids (Fig. 10), while monounsaturated fatty acid concentrations decreased (Fig. 11). This increase in the degree of unsaturation in coldwater-reared lamprey should increase the fluidity of membranes at low temperatures. Concentrations of specific saturated fatty acids also varied as temperature changed, with lamprey in cold water increasing concentrations of 13- and 15-carbon saturates while reducing the concentrations of 12-, 14- and 16-carbon saturates. Odd-numbered saturates tend to have lower melting points than similar even-numbered saturates (Knothe and Dunn 2009) so their increased concentration in colder water may function to increase membrane fluidity.

Conclusions

This research demonstrated that larval lamprey can be cultured in water as warm as 22.6 C without increased mortality. To maintain rapid growth, rearing temperature should be around 19.2 C for early larvae and 15.7 C for older larvae. The fatty acid profile of larvae will change in response to rearing temperature and this should be taken into consideration when planning a culture program for lamprey. Fatty acids are a major source of metabolic energy for fishes, serve regulatory functions, are used to synthesize bioactive molecules and are important structural components of cell membranes. Based on changes to the fatty acid profile, younger larvae adapt more rapidly to new rearing temperatures than older larvae. In selecting a culture temperature, it may be beneficial to consider the environmental temperature after fish leave the hatchery. Ultimately this work refined our

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ability to culture larval-stage Pacific lamprey and the information gathered will be helpful in planning future culture operations.

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Notes

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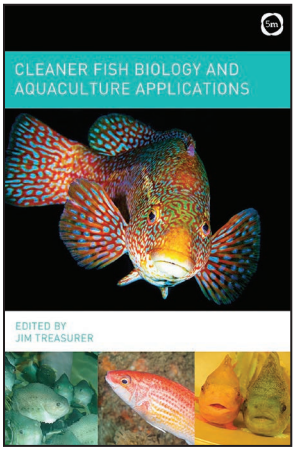

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Cleaner Fish Biology and Aquaculture Applications edited by Jim Treasurer. Cleaner fish are increasingly being deployed in aquaculture as a means of biological control of parasitic sea lice, and consequently the farming of wrasse and lumpfish, the main cleaner fish species in current use in salmon farming, is now one of the fastest expanding aquaculture sectors with over 40 hatcheries in Norway alone. Cleaner Fish Biology and Aquaculture Applications reviews and presents new knowledge on the biology of the utilized cleaner fish species, and provides protocols in cleaner fish rearing, deployment, health and welfare. The latest knowledge is presented on specialist technical areas such as cleaner fish nutrition, genetics, health, immunology and vaccinology, welfare, transport and fisheries. Specific chapters detail cleaner fish developments in the main salmon-producing countries. The book comprehensively addresses the questions of sustainability of cleaner fish use in aquaculture, bottlenecks to the optimum production of cleaner fish, and improvements and best practice in on-farm deployment methods, for optimum survival and enhanced welfare of cleaner fish.