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Walleye Larviculture in Water Reuse Aquaculture Systems

J. Alan Johnson, Kevin Kelsey, and Robert Summerfelt

Abstract Meeting of the three authors during the 2011 Midwest Fish and Wildlife Conference in Des Moines, Iowa, led to frequent communication that formed the basis for collaborating on the status of their respective production facilities. The operation techniques of the water reuse aquaculture system (RAS) facilities in Vermont and Iowa are compared and contrasted. Kelsey and Johnson present detailed descriptions of Walleye (*Stizostedion vitreum*) larviculture in innovative RASs at the fish culture facilities in Vermont and Iowa, respectively. Since 2011, intensive culture of Walleye fry/fingerlings has been conducted at the Ed Weed Fish Culture Station in Grand Isle, Vermont, with the goal of large-scale production from the facility's program inception to supplement existing extensive pond culture efforts of fingerlings that are used for sports fishing restoration. Tank volumes of 1940 L are now used in a RAS dedicated exclusively for intensive Walleye culture. Proof-of-concept techniques have been applied with successive production years to duplicate identified advances related to feed and feeding rates as well as various rearing environment conditions. After two successive years of trials in four self-cleaning tanks (2018 and 2019), as of 2020 all tanks within the system are now self-cleaning, providing optimum rearing conditions. The feeding of blended feeds through the entire culture run has also been applied since 2017. Larviculture survivals from day 1 post hatch (1 dph) through 34 dph in excess of 60% are being achieved averaging 50 mm in length, providing recruitment to the fishery that can be documented.

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The Iowa Department of Natural Resources fish hatcheries rely on surface water sources for Walleye advanced fingerling production in single-pass systems. Aquatic invasive species as well as some pathogens are present in these water sources. RAS technology with secure water sources is one solution to these challenges. A pilot-scale larviculture RAS was built at the Rathbun Fish Culture Research Facility, and fingerling production began in 2019. The larviculture RAS produced 107,800 to 139,284 fingerlings in each crop to 1.0-g size with a 77.2% survival rate over the 2019–2020 trials. Except for an outbreak of bacterial gill disease, none of the several bacterial and protozoan pathogens that frequently infect Walleye during intensive culture using traditional surface water source were observed on fish reared in RAS during the 2019–2020 trials.

Keywords Walleye · *Stizostedion vitreum* · RAS · Larviculture · Cannibalism · Vermont · Iowa

1 Introduction

Robert C. Summerfelt

1.1 Objectives

This chapter presents detailed descriptions by Kelsey (Sect. 2) and Johnson (Sect. 3) of Walleye (*Stizostedion vitreum*) larviculture in innovative reuse aquaculture systems (RASs) at the fish culture facilities in Vermont (Ed Weed Fish Culture Station (EWFCS)) and Iowa (Rathbun Fish Culture Research Facility (RFCRF)), respectively. They achieved noteworthy success for intensive larviculture of Walleye based on their many years of experience, use of science-based protocol, and application of modern RAS engineering. They address issues specific to larviculture of Walleye that are applicable to facilities using single-pass as well as water reuse systems. Their descriptions of RAS technology and system management will be immeasurably helpful for others that are seeking to transition from intensive culture using single-use flowing to reuse.

The survival and production of fingerling Walleye improved following the adoption of RAS technology at both sites. At EWFCS, larval survival in the RAS was 52–61% from hatch to 33–35 days post hatch (dph) compared with 32% average survival in ponds. In 2019, at RFCRF, a total of 107,804 fingerlings (75.2% survival rate) were produced in the RAS in production-scale tanks. At both sites, the culture

systems were designed with surface sprays to overcome the ubiquitous and serious problem of noninflation of the gas bladder (NGB) in the tank culture of many physoclistous fishes (Summerfelt 2013).

A literature review of Walleye larviculture was not a purpose of this report; therefore, citations have been intentionally limited to only serve as an aid for comprehension or to give credit to the most relevant previous work. Comprehensive literature reviews are available elsewhere (Summerfelt 2005, 2013; Summerfelt et al. 2011; Johnson and Summerfelt 2015). Likewise, technical details on water reuse technology are described elsewhere by many others (Summerfelt 1996a; Summerfelt et al. 2001; Timmons et al. 2001).

1.2 RAS Technology

The engineering technology for a reuse system includes an organized set of complementary unit processes that are needed to maintain water quality and to maximize the reuse of water from the fish culture system, with a minimum addition of freshwater to replace evaporation and water loss from clarification. Backwashing the drum filter or flushing water to prevent the accumulation of high nitrate accounts for the major water loss. The unit processes include (from Summerfelt 1996a) the following:

- Clarification for removing settleable and suspended solids
- Biofiltration for removing dissolved organics and ammonia
- Stripping carbon dioxide
- Oxygen addition to levels generally greater than saturation
- Disinfection using ozone or ultraviolet (UV) filters
- Automatic adjustment of pH

There are many options for managing each of the unit processes involved using RAS, for example, as a means for accomplishing biofiltration, the process of ammonia removal by nitrification in the filter. Alternatives include submerged biofilters, trickling biofilters, rotating biological contactors (RBC), bead filters, fluidized bed biofilters, or a moving bed bioreactor (MBBR). The latter was selected at both EWFCS (Sect. 2) and RFCRF (Sect. 3).

Major Benefits of RAS Technology

- There is reduced water consumption because a high percentage—greater than 95–99.5% in advanced systems—of water is reused after passing through the culture tanks. This allows expansion in production with the same water supply or opportunity for a facility to be sited where water supply is more limited. RAS is adaptable to small and large facilities.
- There are reduced costs for the heating and cooling of the water because only the replacement water needs treatment, whereas it is generally cost-prohibitive to control the temperature in single-pass systems.

- There are more options and reduced costs for biosecurity, such as preventing the intake of indigenous parasites such as Ich (*Ichthyophthirius multifiliis*) and microbial pathogens that cause morbidity or mortality to the cultured fish or that serve as a threat to environments that are included in the stocking plans (e.g., viral hemorrhagic septicemia virus (VHSV)). At the Rathbun Fish Hatchery, formalin treatment to control Ich is greater than 20% of the variable cost for Walleye production (Johnson and Summerfelt 2015). Another potential savings of RAS is to substantially reduce the volume of water needed, thus requiring smaller size and more effective filtration and disinfection system to treat the use of water from a surface source. The reduced water requirement can sometimes even make an economically viable use of municipal water sources for the water supply.
- Given the reduced need for large volumes of water in facilities using RAS, effective treatment of the intake water can reduce problems with aquatic invasive species (AIS). Facilities with a single-pass or partial reuse require exceptional efforts to prevent intake from surface water sources and the subsequent distribution of nonindigenous plants and animals in the water of the distribution unit. A prime example is the special effort and added expenses to prevent the distribution of Zebra Mussel (*Dreissena polymorpha*) veligers in the water used to transport fish for stocking (Edwards et al. 2002).

Disadvantages of RAS Technology

- Substantial capital investment for equipment is required for water treatment processes that allow the water to be reused in the same tank or other tanks. Although out-of-date with 1996 dollars O'Rourke (1996) has itemized investment-related costs for equipment for Walleye larviculture and fingerlings, which is not specific for larviculture in a RAS; however, exclusive of the building and land, the costs in 1996 dollars for "fixed equipment" was $33,440. A hatchery going from a flow-through system to RAS will find that some increased costs may be compensated by reduced costs for disease treatments, as well as by eliminating hatchery modifications that are often needed to prevent the intake and distribution of AIS. Also, increased production at an existing site will reduce the need for a substantial expense for expanding the current facility or for building a new one.
- RAS technology can be challenging for those with limited experience with the equipment, plumbing, and electrical service that is required. A new installation may require assistance from an engineering specialist; fortunately, however, commercial sources for the equipment are available to offer suggestions, and there are educational opportunities for hatchery personnel to gain a fundamental understanding of the technology, e.g., the short course on RAS technology taught by the staff of the Freshwater Institute, Shepherdstown, WV. That short course includes pumping and piping, fish health and biosecurity, monitoring RAS water quality, and supervisory control and data acquisition (SCADA) hardware and software. The SCADA system is used to continuously monitor and/or control water pumps, level, flow rate, and quality parameters such as dissolved oxygen,

carbon dioxide, pH, total gas pressure, oxidation-reduction potential, and temperature.

- There are many options for managing the major unit processes involved in a RAS. For example, biofiltration, the process of ammonia removal by nitrification in the filter, may be done with submerged biofilters, trickling biofilters, RBC, bead filters, fluidized bed biofilters (Timmons et al. 2001), or a MBBR. The latter was selected at both EWFCS (Sect. 6.2) and RFCRF (Sect. 6.3).
- There is an increased operating cost for the electrical power needed for pumping water, heating or cooling, UV disinfection, and providing a source of oxygen, whether produced on-site or purchased as liquid oxygen from a commercial source.
- If ever an opportunistic pathogen establishes in the RAS, it can be extremely challenging to control the pathogen. Sometimes, the best recourse has been to depopulate, disinfect, and restock, which is extremely expensive.
- Backup power, pumps, oxygen, and other critical infrastructure are often required to reduce the risk of catastrophic losses when operating RAS.

1.3 Larviculture of Walleye

The present chapter focuses on the intensive (i.e., tank) culture of Walleye from hatch to 35 dph. The first 35 dph encompasses three larval stages (prolarva, postlarva I and II), and early juvenile at about 15–18 days and a length of about 20 mm (Summerfelt 1996c). Early development of scales is visible at 24 dph but not completed until 45 days (Priegel 1964), which is an important factor affecting handling as they are more susceptible to injury before scale development.

Critical elements for Walleye larviculture were described in the Walleye Culture Manual (Summerfelt 1996c) and updated with a comprehensive literature review by Summerfelt and Johnson (2015). A detailed presentation of gas bladder inflation and noninflation of the gas bladder is described by Summerfelt (2013). An expansive body of relevant experience by hatchery personnel is difficult to access by internet search, but when available, the proceedings of the Annual Meetings of the Coolwater Fish Culture Workshop provide insight into issues and practical solutions to cultural problems reported by hatchery biologists from a cross-section of North America. Nevertheless, substantial scientific literature already exists on the culture of Walleye that spans the twentieth century and has been growing ever since.

Although the configuration of the systems at EWFCS (Sect. 2) and RFCRF (Sect. 3) differ in many details, such as the means to achieve turbid water and their feeding strategies, both systems incorporate practices essential to overcome NGB, clinging behavior, and cannibalism, which are critically important problems affecting the success of Walleye culture. The interplay of NGB and clinging behavior strongly influence the incidence of cannibalism. Summerfelt (1996c) described the methodology used to overcome these problems. Briefly, the attraction of larval Walleye to direct and reflected light has a major influence on design criteria for larviculture. A

comparison of larval Walleye behavior in laboratory aquaria from hatch to 17 dph showed that in clear water, larvae had a strong association with the sides of the aquaria, but in turbid water, larvae avoided the sides of the aquaria (Bristow and Summerfelt 1994, 1996). Importantly, larvae in turbid water had greater average swimming speeds, faster growth rates, and improved gas bladder inflation (GBI) than larvae cultured in tanks with clear water (Rieger and Summerfelt 1998). The improved performance and viability in turbid water are attributed to the changes in larval distribution as a consequence of larval reaction to diffused light in turbid water.

The other problem was NGB, dependent on the ability of the larvae to penetrate the water surface to gulp air for the first filling of their gas bladder. Walleye, other percids, and nearly all spiny-rayed fishes are physoclists, meaning that air gulped at the water surface is able to pass through the pneumatic duct for only a short interval after yolk sac absorption. Inflation cannot occur if they cannot penetrate surface tension (Rieger and Summerfelt 1998). The problem was resolved in Iowa by equipping tanks with a surface spray to homogenize the oil droplets to a size that will pass through the standpipe screen (Moore et al. 1994). The method removes oil from the surface of the culture tank during the critical period when the larvae must inflate their gas bladder. For larval Walleye, GBI takes place from the 6th to the 12th day post hatch (Marty et al. 1995). A high percentage of larval mortality occurs in this interval for fish that do not achieve GBI. Both Vermont and Iowa use surface sprays.

2 Hatchery-Scale Production of Walleye Fingerlings in a Water Reuse Aquaculture System at the Ed Weed Fish Culture Station, Grand Isle, VT

Kevin Kelsey

2.1 Walleye Culture in Vermont

Walleye are native to Lake Champlain, Vermont, and its tributaries where the species served a commercial seining fishery from the late 1800s through the early 1900s. When catches declined, culture for the fishery restoration of Walleye on Lake Champlain was undertaken beginning in 1899 when up to 100 million eggs were collected at a stripping station/hatchery established in Sandy Point on Missisquoi Bay. The resulting fry were stocked into Lake Champlain, throughout Vermont, and

Fig. 1 The Ed Weed Fish Culture Station is located on the west shore of Grand Isle in Lake Champlain (Photo courtesy of Vermont Agency of Natural Resources Engineering)

other New England states, New York, and Pennsylvania (McKenzie personal communication, Vermont Fish and Wildlife Department, retired). This site continued to provide fry for stocking into Lake Champlain until 1954. Due to the decline of Walleye harvest numbers, commercial fishing in Missisquoi Bay in Canada for Walleye ceased in 1971 (Marsden and Langdon 2012), and with recreational catch rates dropping by more than 50%, stricter regulations were being put into effect and management plans were considered and implemented.

In 1986, the Vermont Fish and Wildlife Department (VFWD) and the Lake Champlain Walleye Association (LCWA) formed an agreement to produce Walleye fingerlings using extensive rearing ponds stocked with newly hatched fry and later using intensive culture techniques to stock ponds with advanced fry. This effort was started at the Bald Hill Fish Culture Station (BHFCS) in 1992, where newly hatched fry were reared in tanks and fed *Artemia nauplii* for 5 days and then distributed to the LCWA ponds as well as ponds on the hatchery site in an effort to improve survivals. Prior to the adoption and expansion of the RAS technology, success was inconsistent with a range from no fish harvested, due to water quality or food shortage problems, to years with good harvest. The average survival in cooperative extensive ponds over the last 30 years in Vermont has been 32%.

The EWFCS came online in Grand Isle, Vermont, in 1992 (Fig. 1). The need for this facility was recognized as the fishery division's management request for cultured fish was consistently not being met and reliance on surplus from federal facilities and neighboring states was frequently unreliable. For 16 years, production

was focused on catchable Brook (*Salvelinus fontinalis*), Brown (*Salmo trutta*), Lake (*Salvelinus namaycush*), and Rainbow (*Oncorhynchus mykiss*) Trout for distribution throughout the waters of Vermont. Also, a portion of the annual production was dedicated to Lake Champlain, providing a percentage of the landlocked Atlantic Salmon (*Salmo salar*) smolts required for the lake, as well as Steelhead Trout (*Oncorhynchus mykiss*), Brown Trout, and Lake Trout.

Since 2005, when VHSV caused massive fish kills in the Great Lakes (Spickler 2007), Vermont decided to use a basin management approach to fishery efforts on Lake Champlain as it is connected to the Great Lakes drainage from the Richelieu River, a tributary of the St. Lawrence. In 2009, the production at EWFCS had become exclusive for Lake Champlain. The Walleye program was transferred from BHFCS to EWFCS in 2011; BHFCS continues to produce Walleye for Vermont waters outside the Champlain basin. The current program production objectives for EWFCS are as follows:

- 145,000 landlocked Atlantic Salmon smolts
- 58,000 Steelhead Trout smolts
- 57,000 Lake Trout yearlings
- 49,000 Brown Trout yearlings
- 165,000 advanced Walleye fry for LCWA ponds
- 200–250,000 Walleye fingerlings

The early-life stage rearing (sac fry to parr/fingerling) for all these species is done in production-scale systems using RAS technology and artificial diets exclusively. Techniques for solving problems were developed using a proof-of-concept approach wherein technology innovation in one year is validated by duplication in the following season. A detailed account of the system and techniques encompasses a culture run from the introduction of fry into tanks to fingerling harvest. The success of the system has allowed the EWFCS Vermont facility to achieve a consistent output of quality fingerlings needed to achieve program goals to enhance Sport Fish Restoration and angling opportunities for Walleye in Lake Champlain. The RAS technology allows for the control of environmental parameters critical for successful hatchery-scale production of Walleye fry, advanced fry, and fingerlings. The technique of using RAS to produce fingerlings for stocking extensive ponds managed by LCWA is carried out at EWFCS using and expanding on the same techniques that had been applied at BHFCS. The resulting fingerlings from this production are harvested in cooperation with the VFWD staff. This partnership extends beyond the ponds that the LCWA manages. Their involvement has been significant to our success. They have advocated politically, as well as financially supporting the development of intensive larviculture of Walleye in Vermont at both EWFCS and BHFCS.

2.2 System Design/Unit Processes

The system used to intensively rear Walleye fingerlings was designed to support biomass at harvest of 250–300,000, 45–50 mm Walleye fingerlings (250–300 kg) (Fig. 2). The maximum carrying capacity was estimated by an analysis of system production based on expected biomass and projected feed use to establish the requirements of the unit processes within the system (Summerfelt 1996a). Monitoring for alarm conditions such as system water levels, oxygen, temperature, and electrical function is done through the system control panel that is linked to the SCADA system that alerts day staff of alarm conditions; the three staff that live on-site 24/7 monitor overnight alarm outputs.

2.2.1 Solid Removal

General process flow begins with water gravity flowing from the tank center drain standpipe (standard tanks) or from the sidewall drain (self-cleaning tanks) to the drum filter for solid removal (Fig. 3). Tank depth is maintained by the height of the drains. The drum filter has an external bypass incorporated into the plumbing to permit water to continue flowing in the event of mechanical failure. The drum filter is sized to process 475 Lpm. Filter panels are 60 μm screen (Fig. 4).

Conducting Walleye larviculture in RAS with the addition of a turbidity agent (algae or clay) results in environmental conditions that mimic an enriched pond. This requires the "acceptance" of the presence of suspended material by both the fish and the unit processes of the system. Some solid accumulation occurs in the tanks, and

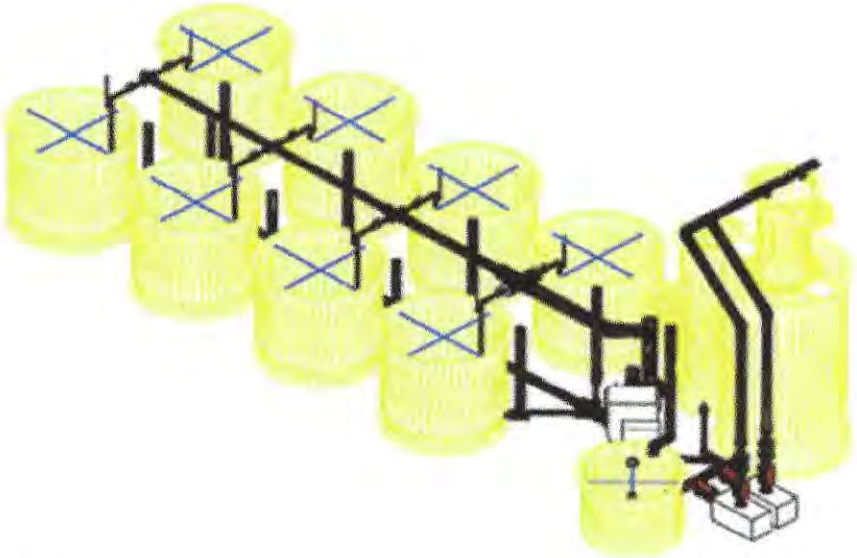

Fig. 2 A CAD drawing of a conceptual design of the intensive Walleye system at the Ed Weed Fish Culture Station (Photo courtesy of INNOVASEA)

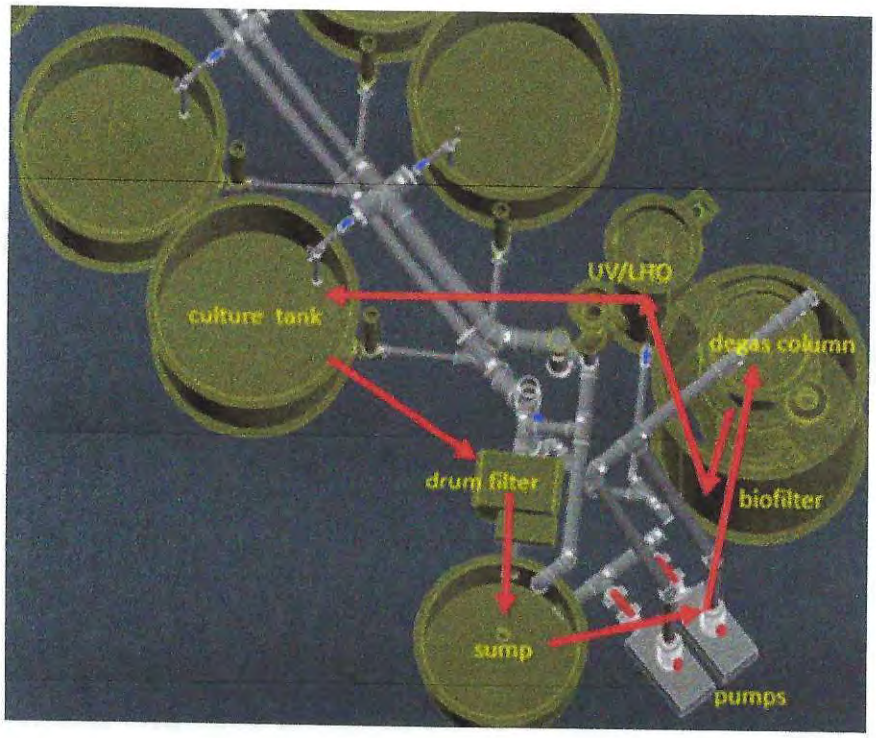

Fig. 3 Major components and process flow (red arrows) of the Walleye RAS at EWFCS. Water exits the culture tanks via surface drains to the rotary drum filter. Filtered water enters the sump where a 1-horsepower pump lifts water to the gas-stripping tower that is mounted on top of the MBBR. Water then passes by gravity through the aerated biomedia to the UV/LHO vessel and back to the culture tanks. Makeup water for the system (4–8 Lpm) is introduced to either the biofilter or sump. The overall system volume is approximately 20,000 L (CAD drawing courtesy INNOVASEA)

Fig. 4 The Hydrotech HDF-501 drum filter with a 60-μm screen manages a process flow of 475 Lpm. The flow exits the filter to the pump sump

the water has a higher quantity of both dissolved and suspended solids than typical of a single-pass system. The conditions require monitoring to maintain environmental conditions within acceptable target margins for desired growth rate and fish health. Settleable solids within the culture tanks that are greater than 100 μm settle to the tank floor and are removed manually. Finer suspended solids pass through the surface screens on the tanks and are removed by the drum filter. Algae is applied to the system (1–20 dph) to disperse the larvae and avoid clinging behavior. About 10 days after the application of algae, a biofilm of organic matter (biofloc) develops on the screens, which we try to remove as much as possible, but it dissipates completely as the system is cleared later in the culture run. Changing sizes on surface screens allows for more of this to be handled at the rotary drum as it passes through the culture tank screens. The rotary drum filter panels are 60-μm mesh. A 0.5-horsepower (hp) high-pressure pump rinses off accumulated solids from the panels to a waste trough that diverts the solids from the system.

The processed water from the drum filter discharges to the pump sump; the pump sump is the low point in the system and has an overflow standpipe to discharge exchange water that is continuously added to the system (4–8 Lpm). A 1.0-hp. pump draws water from the sump and delivers it to the top of the degassing column above the biofilter. A second pump is in standby backup with a selector switch. The backup pump will start in the event of a motor protection trip of the primary pump. A wide-angle float switch starts and stops the pump in the event of a low system water level.

2.2.2 Stripping Carbon Dioxide

Carbon dioxide was removed using a stripping column, which is designed to force large volumes of air through cascading water within an enclosed column. The stripping column is 0.9 m in diameter and 1.2 m high and is mounted on the top of the biofilter (Fig. 5). The column can process a flow of up to 475 Lpm. Water entering the top of the degassing column is uniformly distributed through a plate with antivortex crown nozzles. An inline continuous duty fan draws air upward and runs counter to the water dropping through the media. The system removes 70% of the generated carbon dioxide with each pass-through. Timmons et al. (2018) gave a recommendation for a system carbon dioxide concentration of 30–60 mg/L for warm water species and 15–20 mg/L for cool water species. We used a 15-mg/L concentration as our target limit with the recognition that levels could be as much as 30 mg/L or higher when approaching a maximum carrying capacity.

2.2.3 Biofiltration

An MBBR is used for biofiltration in the RAS at EWFCS. This type of biofilter was selected because it provides the necessary removal rate of total ammonia nitrogen (TAN) for the expected carrying capacity of the system while affording ease in maintenance and operational energy use.

Fig. 5 The gas-stripping tower is mounted on the top of the moving bed bioreactor

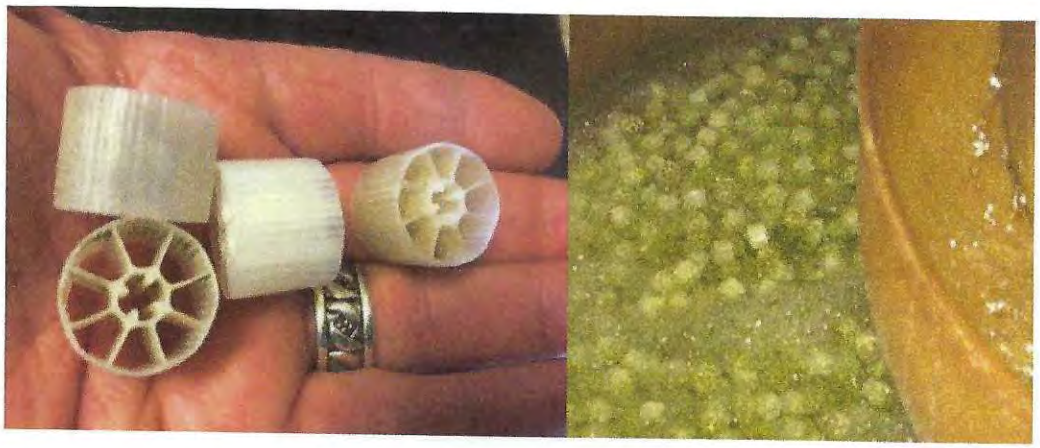

Fig. 6 MB3 media (left) was used as a surface for nitrifying bacteria in the MBBR (right)

The MBBR diameter is 1.5 m, and the height is 2.75 m. Processed water enters the top of the unit and flows downward through the media zone. A bowl below the degassing column collects and directs the water into the center of the biofilter to aid the movement of the 1.5 m^3 of MB3 polyethylene media (INNOVASEA Boston, Massachusetts) (Fig. 6). The biofilter aeration grid is powered by a 1.5-hp. regenerative blower, rolling the media up the exterior walls and down the center for

thorough mixing. Aeration provides sufficient oxygen to the microbial biofilm within the biofilter to oxidize ammonia to nitrate. A shunt valve on the supply line to the biofilter diverts air to the tanks to be used for feeders to disperse feed and box screens that have bubble curtains to discourage fry from congregating in the box area. A bottom screen retains the biofilter media. The outlet is plumbed to allow for the gravity flow of water to the vertical UV/low head oxygenator (LHO) vessel.

2.2.4 Ultraviolet Disinfection and Oxygenation

Disinfection, oxygenation, and carbon dioxide removal are achieved by three unit processes. Water from the biofilter passes to the center core of the LHO. An integral open channel vertical design UV unit in the LHO center core passes the full system flow past the UV lamp field for treatment. A minimum of 30,000 $\mu w/cm^2$ is achieved at the maximum process flow of 475 Lpm. This level of UV disinfection is effective for most pathogens of concern. Undoubtedly, some loss of effectiveness of the UV light occurs with the use of algae as a turbidity agent, which is a necessity for Walleye larviculture. However, UV dose is inversely proportional to the flow rate (Losordo and Conwell 2014); thus, in the 1–14-day interval when turbidity is being applied to the system, we assume that the system flow during that period of 208–285 Lpm is sufficient to offset the reduced efficacy caused by the turbidity. As evidenced, disease events have occurred only once in 10 years; a severe bacterial gill disease in 2013 attributed to the excessive feeding of a microdiet.

Water flow entering the LHO is dispersed by the distribution plate, thereby enhancing oxygenation by the air-water contact as it passes through the LHO. A side box standpipe prevents overflowing the LHO in the event the distribution plate is blocked and directs the water below the LHO to maintain water flow to the culture tanks. The LHO serves as a head tank for water distribution to the rearing tanks. An excess water flow overflow standpipe directs any water that is not being used in the tank gallery back to the pump sump.

2.3 Culture Tanks

The rearing units for the intensive culture of fish have been rectangular (Colesante 1996), called raceways, and circular tanks (Moore 1996; Summerfelt 1996c). Circular tanks are the predominant tank shape used for the larviculture of Walleye. The advantages of circular tanks have been described by Summerfelt (1996b), but a noteworthy feature is that they operate with a rotating flow about the center drain that concentrates solids at the bottom, and they maintain uniform water quality throughout the tank.

Two types of circular tanks are used within the system at EWFCS. Originally, eight tanks with a skirted exterior bottom and center drain sump arrangement were installed (Fig. 7a). The tank operating depth was controlled with an external

Fig. 7 Structure of tanks utilized within the system, (**a**) INNOVASEA and (**b**) Oceans Design. The dimensions of both styles of tanks were 1.5 m × 1.2 m

standpipe to facilitate draining from the sump or by the insertion of a solid wall 5-cm center standpipe to drain water from the surface. The later configuration was chosen to allow for the deposition of solids to remain in the tank bottom until they were removed with a siphon (Fig. 7a). Currently, a 1.5-m diameter tank is used with an operating depth set at 1.1 m. The tank walls have been painted black and buff-sanded to a flat finish to minimize the reflection of light that would be attractive to the photopositive fry. The floors of the tanks are light blue in color. The standpipes are retrofitted with custom surface screened drains constructed from modified plastic buckets modified to install mesh drain screens (Fig. 8b). The total surface area of drain screens provided is 0.13 m^2. The screen hole size employed is determined by the size of feed being applied and based on the developmental size of the larvae as well. Daily cleaning of the tank walls is done manually using a modified squeegee design developed at RFCRF. Floors and sumps are manually cleaned with a siphon twice daily.

In 2018 and 2019, self-cleaning tanks (Oceans Design Colorado Springs, Colorado) replaced four of the standard tanks (Fig. 7b). The self-cleaning tanks have a gear motor that moves a squeegee on the bottom and a brush on the sidewall at one rotation per hour. The squeegee moves solids to a trough in the tank bottom. The twice-daily siphoning is the same as with the standard tanks. Tank dimensions are identical to the standard tanks, and to ensure proper tank elevation, adjustable legs are used instead of tank skirts. Tank walls are a flat black finish with the tank bottom being light gray. The surface drain is an Easy Slide Larval Screen Box (Oceans Design Colorado Springs, Colorado) with a screen surface area of 0.18 m^2 mounted on the sidewall of the tank. Screen sizes are changed based on feed size and the size of the larvae (Table 1).

The operating volume of both tank styles is 1940 L. Both style tanks require attention to screen cleaning and changing screen sizes with larval development. Water exits the tanks through a surface drain—cylindrical center for standard tanks and box sidewall for self-cleaning tanks. In the last 2 years, the monitored cleaning events indicated that self-cleaning tanks take on average one-fourth of the time to

Fig. 8 Overhead photos of tanks and screens: (**a**) standard tank, (**b**) standard tank center screen, (**c**) self-cleaning tank, (**d**) screen panels of various mesh size for self-cleaning tank sidewall box

Table 1 Screen sizes used on standard and self-cleaning tanks in relation to the ranges of fry length and increased feed size

Days post hatch (dph)	Fry length range (mm)	Feed size range (μm)	Screen hole size (mm)
1–11	8–13	360–910	0.8
12–17	14–20	910–1410	1.65
18–25	21–34	910–1410	3.0
26–35	34–50	910–1800	4.0

clean than standard tanks, which is comparable to observations made by Rotman et al. (2017). Based on the increased efficiency and improved survival in self-cleaning tanks, the remaining standard tanks will be replaced with self-cleaning tanks for the 2020 culture season.

2.4 System Management

The management of systems, whether RAS, partial reuse, or flow-through, should incorporate practices and measures that address three major hurdles in Walleye larviculture: NGB, clinging behavior, and cannibalism. The presence and persistence of any of these issues alone are problematic, and in combination they will cause poor survival during and after the culture interval. Procedures utilized during culture runs at EWFCS that inhibit or reduce these specific issues are described in this subsection.

2.4.1 Temperature

Temperature is an important aspect of growth and development in larviculture. Sufficient daily temperature units (DTU) in proper proportions for critical stages of development are required to achieve optimum growth. The availability of DTU directly influences the number of dph needed to reach the target harvest size. Temperatures below 10 °C in Walleye culture can inhibit development and reduce metabolism, resulting in mortalities associated with nonfeeding behavior. During the early stages of larviculture (1–5 dph), temperatures greater than 20 °C may result in causing premature absorption of yolk sac and bacterial diseases.

Varying temperature regimes have been used in the past, with recognition that feed acceptance is greater at temperatures above 16 °C. An ideal temperature range of 16–18 °C was described by Moore (1996) to include a temperature rise at 5 dph to stimulate feeding, as described by Summerfelt (1996c). Our earlier culture runs fell within these ranges and would reach a maximum of 22 °C, similar to temperatures used by Moodie and Mathias (1996). Temperature in our RAS was controlled using a 5-cm diameter 12,000-watt in-line titanium heater that is threaded into the return plumbing line from the UV/LHO unit to the culture tanks. Temperature is monitored in the system sump, and transmission to the control panel activates and deactivates the heater based on the programmed settings. The temperature is manipulated to rise, coinciding with progressive larval stage transitions to optimize feeding and growth potential. Beginning in 2014, temperature started at 19–20 °C but was increased to 20–21 °C at 18 dph with positive results. Thereafter, a temperature of 20–22 °C was selected to reach a target size for the stocking of 1-g fingerlings, 50 mm in length in 35 days or less (Table 2).

Table 2 Temperature settings for the RAS during the 36-dph culture interval

Days post hatch (dph)	Temperature °C
1–4	19–20
5–17	20–21
18–36	21–21

2.4.2 Light

In 2011–2014, which were the first 4 years of Walleye larviculture, we used fluorescent shop lights to illuminate the tanks with a light intensity of 100–700 lux. Since 2015, light intensity has been maintained at 25 lux or less for the entire culture run. Each tank had a hooded lamp mounted 0.75 m above the water surface. The entire system was surrounded by a black curtain that eliminates external light. The subdued lighting provided in the otherwise dark environment was a strong enough attractant for the photopositive phase of the larvae but gentle enough to reduce excessive excitability observed with higher levels of light intensity. Fry began to exhibit photonegative behavior at 16–18 dph, during which point the hooded lamps were tilted at a 45°angle to reduce light intensity until the time of harvest (Fig. 9).

2.4.3 Turbidity

Turbidity is a necessity for Walleye larviculture (Bristow and Summerfelt 1994, 1996). Turbidity reduces reflected light within the culture tank and disperses the larvae, which in turn increases survival, reduces cannibalism, and enhances feed utilization and growth. The turbid environment also aids in the acceptance of artificial diets for initiating exogenous feeding, along with reducing cannibalistic tendencies as well. Except for developments reported by Johnson in this publication, an intensive culture of Walleye fry has been conducted in flow-through systems using clay to produce turbid water. However, the use of clay in a RAS was considered problematic because clay may adhere to and cover over (smother) the biofilm in the biofilter. Although clay solution has been the traditional method for producing turbidity for the larviculture of Walleye, microalgae were used to

Fig. 9 Hooded lamps are used to provide lighting to each tank (left). The hooded lamp is turned at a 45°angle as the fry transitions from photopositive to photonegative (right)

Fig. 10 Batch dosing is applied to the bowl below the system degassing column at the top of the biofilter (left). Example of turbidity created by the green water showing well-dispersed fry reacting to feed (right)

encourage the initiation of first feeding by several species of marine fish larvae (Cutts and Batty 2005). Houde (1975) described positive nutritional properties of algae by direct ingestion during the feeding of Sea Bream (*Archosargus rhomboidalis*). There is the prospect of nutritional and probiotic benefits to using algae as a turbidity agent for Walleye.

The use of algae instead of clay as the turbidity agent is a major difference between the protocol at EWFCS and that followed at RFCRF. Obtaining the concentration of algae to provide the desired turbidity is essential. Rønfeldt and Nielsen (2010) used *Chlorella* (*Chlorella pyrenoidosa*) algae paste in a turbidity experiment with Pikeperch (*Stizostedion lucioperca*). In comparison with clay, turbidity using algae was about 17 NTU (nephelometric turbidity units), while clay was close to 60 NTU. An experimental comparison of clay and algae with Walleye has not been undertaken.

In our system, an algal concentrate of *Nannochloropsis*, Nanno 3600 (Reed Mariculture Campbell, California), is employed by batch dosing directly to the system biofilter and sump (Fig. 10). Batch dosing of 800 mL of algae at 8-h intervals is applied between the biofilter and sump through 1–14 dph. Beginning at 15 dph, algae dosing is reduced by 58% with the use of a green water substitute concentrate, Sanolife GWS (Inve Aquaculture Salt Lake City, Utah), which is introduced at a batch dosing rate of 1 L every 8 h. Sanolife GWS is used exclusively for one day prior to clearing the system at 18 dph.

2.4.4 Surface Spray

The use of surface spray is required to reduce surface tension for successful GBI. Walleye larvae and that of other physoclistous fishes initially fill their gas bladder by penetrating the water surface and gulping air. NGB occurs due to the presence of a

Fig. 11 Surface spray on the radial arm of the standard tank with bucket screen removed (left), spray bar spanning a self-cleaning tank (right)

surface film that larvae cannot penetrate (Rieger and Summerfelt 1998). El Gamal (2015) noted that in the culture of White Sea Bass (*Dicentrarchus labrax*), if an oil film is allowed to accumulate within tanks, consequences such as reduced feeding and growth occur, as well as irregular swimming behavior due to negative buoyancy. Colesante et al. (1986) opined that feed is a source for surface oil, and Boggs and Summerfelt (1996, 2003) identified larval mortality as an additional source for oil accumulation. Surface skimmers are more commonly used in marine larviculture to enhance GBI by blowing low-pressure air laterally along the surface of the water to trap oil in a floating containment area for removal. High-pressure water spray that can penetrate through the surface has been the general application of choice for Walleye fry culture. Several studies have been conducted to address the hurdle of NGB. Higher percentages of GBI were documented by Barrows et al. (1993) and Clayton and Summerfelt (2010) with the use of surface spray. Johnson et al. (2008) obtained GBI rates of 93–100% at Rathbun Fish Hatchery.

In the system at EWFCS, spray bars are mounted on the tanks to span the full diameter of the tank (Fig. 11). A 3.2-cm water supply line is run directly from the biofilter driven by a 0.125-hp. submersible pump. Each arm of the spray bar is threaded to allow for slight changes in angle that can be made to control surface directional flow if desired. Five adjustable spray nozzles (Rain Drip/NDS Woodland Hills, California) are on each arm of the bar providing overlapping semi-circle coverage. The spray bars are operated for the entire culture run through harvest to keep any feed or fines from accumulating on the surface. GBI is monitored in every tank throughout the culture run and at harvest. The 5-year GBI average is 95.6%.

2.4.5 Water Quality and Quantity

Temperature, oxygen, pH, carbon dioxide, ammonia, nitrite, and alkalinity are measured. The frequency of water quality measurements within the system ranges from weekly for carbon dioxide, TAN, alkalinity, nitrite, and pH to multiple times

Table 3 EWFCS RAS water quality parameter ranges compared to recommended values for warm water species (Timmons et al. 2018)

Parameter	Recommended values	EWFCS Walleye RAS
Temperature °C	24–30	19–22
Oxygen mg/L	4–6	6–10
CO2 mg/L	30–50	15–35
TSS mg/L	20–30	10–20 (post 18 dph)
Total ammonia-N mg/L	<3	0.2–1.3
NH_3-N mg/L	<0.06	0.01–0.04
Nitrite-N mg/L	<1	0.01–0.07
Nitrate-N mg/L	'high'	Not measured
Chloride mg/L	>200	Not measured

Table 4 Tank flow rate adjustments based on developmental stages of fry. Values do not include flow from spray bar ~4 Lpm

Days post hatch (dph)	Flow Lpm
1–7	30
8–11	34
12–20	38
21–35	42

per day for in-tank oxygen levels and temperature, as well as system-wide oxygen saturation (Table 3). More frequent monitoring and measurements were required when levels of pH and in-tank oxygen began to decrease, and carbon dioxide increased with rising biomass during the final week (28–35 dph). Although suspended solids should be considered to be reduced to the lowest levels achievable in RAS, that concern had to be counterbalanced by the necessary use of turbid water culture with algae (i.e., green water) for Walleye larviculture. At EWFCS, green water was cleared at 18 dph.

Values for pH within the system range from 6.5 to 8.2 with the lower values occurring toward the end of the culture run just prior to harvest as the system reaches maximum carrying capacity. Alkalinity values range between 136 and 188 mg/L. Makeup water for the system is derived from facility processed water that is filtered to 20 μm, disinfected with UV at 100,000 mw/cm^2, and heated to 10–11 °C.

Water is continuously added to maintain adequate water quality (Table 4). Hydraulic retention time (HRT) rates between 30–60 min are considered suitable for maintaining water quality parameters with properly functioning unit processes within the system. The HRT range is under an hour at system inoculation and just over 40 min for the last 2 weeks before harvest.

Direct flows at these rates through a 5-cm pipe can exceed 30 cm/s, a velocity that would easily overwhelm fry and disrupt many processes of development due to expending extra energy to combat the current. High exchange rates cause higher current velocity; therefore, to achieve acceptable HRT and counteract current velocity, a 3.8-cm vertical inlet pipe is used with 15 holes that are 1 cm in diameter. We made 10 holes, 10 cm apart on the front side of the pipe and five holes 12 cm apart on the back side of the pipe. The holes are parallel to the tank wall, velocity is subdued

Fig. 12 A veritcal inflow pipe with 10 holes and clear tube manometer. The other side had five holes to dampen velocity within the tank

significantly with the backflow counteracting and dampening the main flow. Flow was measured by using a clear tube manometer (Fig. 12).

2.4.6 Stocking Density

In laboratory research, stocking densities of larvae were in the range of 3 to 100/L of tank volume (Summerfelt et al. 2011). Although it is important to use high density to maximize output, the higher density incurs risks of reduced survival, growth, and cannibalism. For rearing advanced fry at EWFCS to stock in LCWA cooperative ponds, a stocking density of 60 fry/L was used, with a survival rate average of 70% to 8 dph. Densities between 30 and 40 fry/L have been used for successful dry diet investigations at the RFCRF (A. Johnson, Iowa Department of Natural Resources (IDNR) personal communication). At EWFCS, from 2011 to 2014, a stocking density of 30–35 fry/L was used, but survival was poor (2–28%), perhaps due to other cultural conditions as well. Thereafter, fry stocking densities were lowered to 22–24 fry/L, which allowed for a comfortable margin for system inoculation at ~45,000 fry/tank with a potential to achieve production targets when a high level of survival to harvest was achieved.

2.4.7 Tank Hygiene

Consistent cleaning of culture tanks is critical in accomplishing positive results during larviculture. The removal of any accumulation of biofloc and fungus is necessary to maintain optimum environmental factors for both the fish and unit processes within the system. Matter accumulates from using algae for turbidity and microdiets, as well as from feces, waste feed, and mortalities. The EWFCS is staffed 16 h/day with three staff living on-site to maintain 24-h coverage. During the Walleye culture run, which occurs from mid-April to mid-June, the Walleye culture systems are staffed throughout the day, ending at midnight with an operational check of the feeders and RAS.

The screens are cleaned twice daily. Standard tank bucket screens are scrubbed in place and siphoned on the interior portion. Self-cleaning box screens have two screen panels that can be removed (Fig. 13). Parallel slots are designed so that a fresh clean screen can be inserted prior to removing the screen to be cleaned, which is brought to a sink to be hosed off and set aside for the following day. The interior of the box is cleaned with a small 1.5-cm diameter siphon.

Tank walls are cleaned continuously in self-cleaning tanks and once daily in standard tanks with a squeegee design similar to what has been used for culture tanks at RFCRF (Fig. 14). As feeding and biomass increase, inlet pipes are removed every other day for cleaning (Fig. 14). Waste feed, feces, and mortality are also removed twice a day using a 2.5-cm diameter double-valved wand (Fig. 15) designed with a clear tube section at eye level to observe waste being drawn through. The discharge of the wand is cam locked into a manifold that deposits waste and mortalities into a screen insert that holds back mortalities that are enumerated, and the waste falls through the screen into a discharge collection sump.

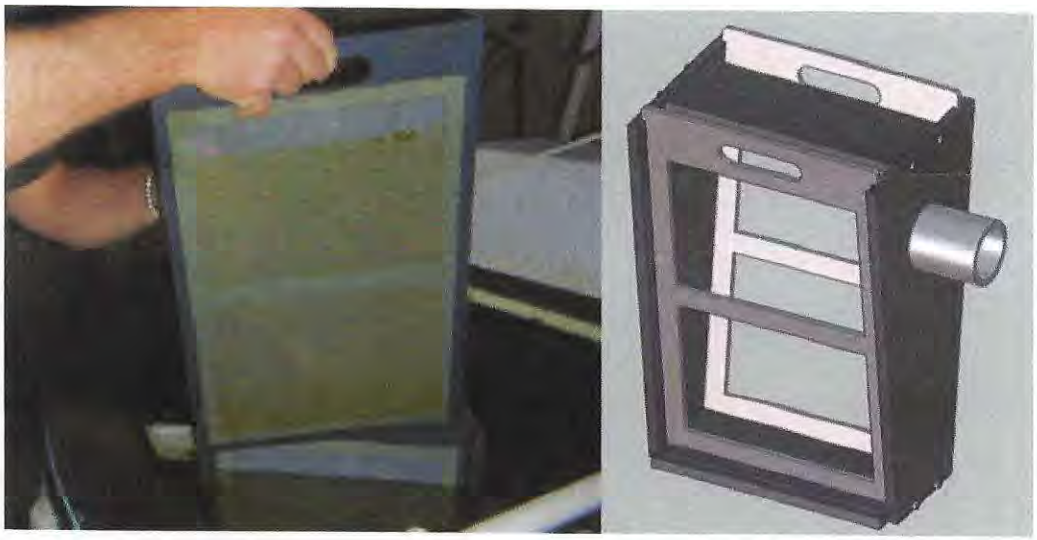

Fig. 13 The box screens were removed for cleaning in a self-cleaning tank. Note the clean screen that has been placed in the parallel slot (left). A CAD drawing of a box screen with parallel slots for screen exchanges (right) (Photo courtesy Oceans Design Inc)

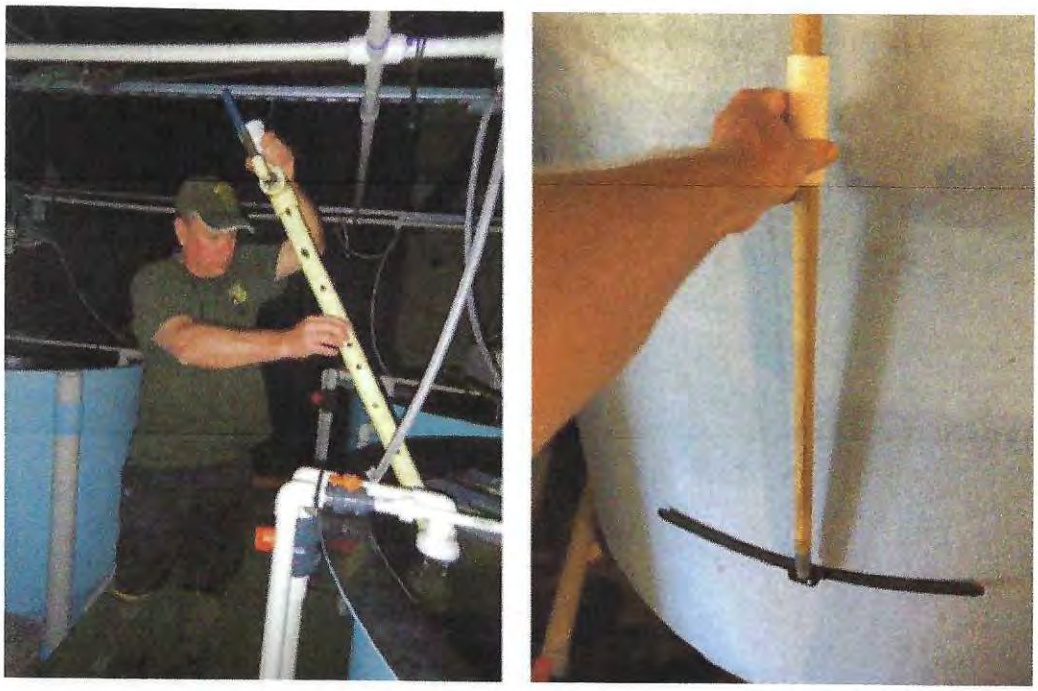

Fig. 14 The inlet pipe is removed for cleaning (left). The manual squeegee device used to clean the interior walls of standard tanks (right)

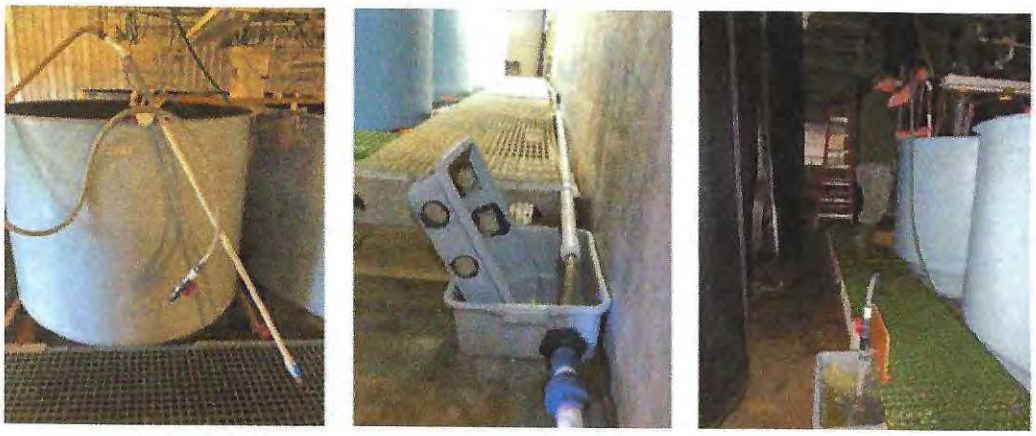

Fig. 15 A double-valved cleaning wand (left) with the vacuum head removed is used to clean tank sumps (center), and the discharge cleaning manifold and sump (right) are used for the vacuuming of standard tanks to process waste and collect mortalities

2.4.8 Feeds, Feeding Rates, and Feeders

In early research on intensive Walleye larviculture, fish were initially fed *Artemia* nauplii and zooplankton then weaned to a dry-formulated diet (Howey et al. 1980). Colesante (1996) used the protocol for large-scale production: *Artemia* were fed for one month, followed by 2 weeks of offering *Artemia* and a dry diet, after which a dry diet was fed exclusively. A modification of this technique is practiced at EWFCS.

The separate RAS that is used for producing advanced fry (8 dph) fed with *Artemia* often has surplus fry remaining after fry have been counted out for distribution to the LCWA cooperative rearing ponds. The remaining surplus of advanced fry is inventoried. To evaluate survival after stocking, fish were marked with oxytetracycline (OTC); details of this procedure are described in Sect. 2.6. Survival to fingerling harvest (33–35 dph) has ranged from 25% to 45% post OTC marking, with the bulk of mortality happening within 48 h of the mark application.

An extensive chronology of work that has been done feeding Walleye fry entirely on a dry diet appears in Summerfelt et al. (2011). Ongoing efforts in assessing diets, ratios, and rates have continued with work done by Johnson and colleagues at RFCRF. To date, comparative trials between Otohime (Japan, sourced by Reed Mariculture, Campbell, California) and Skretting Gemma (Fontaine-lès-Vervins, France) feeds indicate that Otohime performs better than Skretting Gemma. The choice to use Otohime exclusively as a diet for the EWFCS culture was based on the multiyear feeding trials at RFCRF conducted in smaller culture tanks. The rates and ratios applied from 2011 to 2014 at EWFCS were based on the findings at RFCRF (A. Johnson, IDNR personal communication). Variable results were achieved during these years because some critical feed and feeding issues needed further evaluation:

- Ratio of feed (g) to the number of fry/L—Was the feeding rate too high, thus impacting tank hygiene? Or should it be increased to enhance growth and survival?
- Diet blending—Feed trials have indicated the inconsistent performance of one diet versus another. Could there be a benefit to combining two diets that would be accepted by fry and provide desired performance?

During these earlier culture runs at EWFCS, fry were exclusively given a feed between 250 and 360 μm in size, until 7 dph, then and in combination with the larger feed until 14 dph. Though fry will consume this size feed, observations of larvae being first fed to *Artemia* in the system that is used to grow advanced fry for extensive ponds were easily consuming nauplii sizes of 350–550 μm. The smaller size dry diet created tank hygiene issues and, in the instance of the culture runs in 2013 when increases in rates were attempted, likely contributed to a bacterial gill disease outbreak, which caused significant mortality. Since 2015, the use of this feed size range was reduced and by 2018 eliminated.

When fed separately, Otohime has shown to have increased palatability in the RFCRF trials compared to Gemma; therefore, in 2015, the blending of two diets (Otohime and Skretting Gemma) was considered for first-feeding larvae. Otohime has a variable size range of crumbles and fines compared to the uniform pelleted extrusion of Gemma, and the profile of Gemma diets is more nutritionally dense compared to Otohime (Table 5). During a routine tank sampling of fry conducted through the culture runs, both diets were seen in the stomach and digestive tract by a visual examination of the transparent fry. This observation is evidence that fry will consume both diets when presented in equal proportion. Perhaps, the attractiveness of one diet may encourage the consumption of the other diet with higher nutritional

Table 5 Comparative size and nutritional values of Skretting Gemma and Otohime diets

Diet	Size range μm	% protein	% fat	% fiber	% ash
Gemma					
Wean 0.3	350–500	62	14	0.5	9
Wean Diamond 0.5	500–800	62	14	0.5	9
Diamond 0.8	800	57	14	0.2	10
Diamond 1.0	1000	57	15	0.2	10
Diamond 1.2	1200	57	15	0.2	10
Otohime					
B-2	360–650	51	11	3.0	15
C-1	580–840	51	11	3.5	15
C-2	840–1410	51	11	3.5	15
S-2	920–1800	52	14	3.5	15

Table 6 Diets, diet size, and ratios fed and the feeding rates used in 2014 and 2019 by age (dph): B-, C-, and S- are Otohime; G is Skretting Gemma; SC is Silver Cup

2014					2019								
Dph	B-1	B-2	C-1	SC 1.0	Dph	B-2	G 0.3	C-1	G 0.5	C-2	G 0.8	S-2	G 1.0
2–7	100				3	50	50						
8–9	75	25			7	35	35	15	15				
10–12	50	50			8	25	25	25	25				
13–14	25	75			10	10	10	40	40				
15–16		100			11			50	50				
17–18		75	25		13			25	25	25	25		
19–20		50	50		14			20	20	30	30		
21–22		25	75		17			15	15	35	35		
23–25			100		19			20		40	40		
26–30+			75	25	22			10		45	45		
					24					40	60		
					26					30	50	10	10
					28					15	45	20	20
					30						35	15	50
					31						20	20	60
					32–35						15	15	70

value. The blending of both diets in equal amounts through the entire culture run has been established since 2017.

Along with increases in feed size and truncating ratio transition times, feed rates were also increased well beyond rates described by Summerfelt (1996c) and diet trials conducted from 1999 to 2003 by Johnson and Rudacille (2009) (Table 6). Moving through the feed sizes and ratios faster and increasing feeding rates reduced

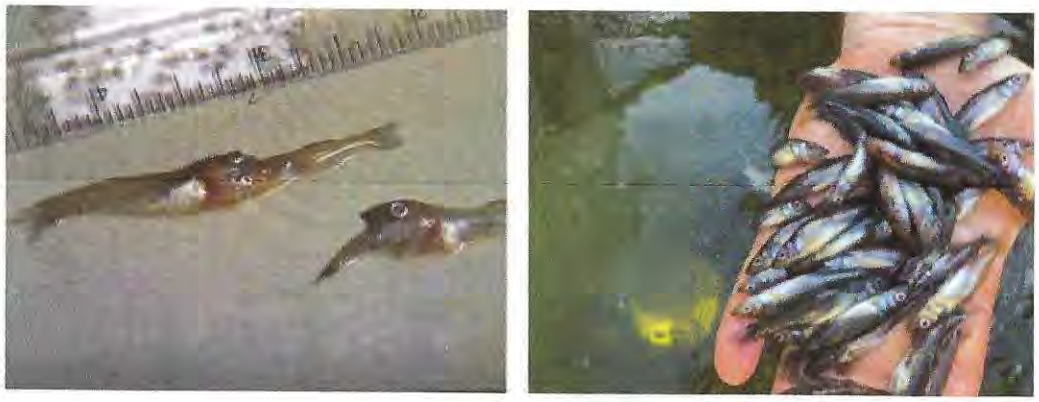

Fig. 16 (Left) Cannibalism in Walleye showing that size differential between the cannibal and prey can promote cannibalism. (Right) A random grab of fingerlings from a harvest net illustrating uniformity in fish size

the proportion of the presence of smaller size fry. These smaller fish have the potential to exacerbate cannibalistic behavior due to the substantial size differentiation (Fig. 16). Reduction of these fish from the population is attributed to the harvested population being more robust and uniform in size while still maintaining potential levels of survival to exceed 60%.

Various feeders have been described for intensive culture, such as belt feeders (McCauley 1970), vibratory (Loadman et al. 1989), and augers (Summerfelt 1996c). Moodie and Mathias (1996) had "custom built" feeders made because belt feeders lacked precision. At EWFCS from 2011 to 2013, belt feeders were mounted on tanks to deliver feed; however, small feed sizes adhered to the belt, and feed of all sizes would fall into the tank with limited dispersal. There was an obvious need for better feeders.

In recent years, feeding systems have been refined for a variety of applications for different species focusing on delivering microdiets used for dry diet exogenous weaning and feeding. In 2014, Hatchery Feeding System (HFS) feeders (Nutrakol Pty. Ltd Perth, Australia) were evaluated (Fig. 17). These feeders deliver a microdiet in doses determined by the plate size selection (single or double slot) and by the number of shots in a 24-h period that occurs as a solenoid is actuated based on selected preprogramming on the controller which can operate up to 24 feeders. The feeders are 24 volts and can be adapted to utilize interchangeable hoppers of variable capacity (250 g and 1 kg). There is also an air induction port that can be throttled to use an air current to loft and disperse feed on the tank surface.

In the event of a malfunction, backup feeders have been purchased to prevent the interruption of the 24-h feeding and so that maintenance and cleaning of the units can be accomplished. Continuous feeding is critical to avoid events of cannibalism. For instance, in 2016, at 22 dph, 50% of the feeders were inoperable for 8–12 h during the night due to an unmonitored electronic malfunction. At this stage of development, the expected daily system mortality for the eight tanks combined would be 1200–2000. The loss of these feeders resulted in a mortality of 25,000 fish 25 mm in

Fig. 17 Position of mounted HFS feeders on tanks: (left) a custom design modification of joining two 250-g hoppers together for increased capacity, (right) photo of 1-kg-size hoppers

length. Almost all mortalities had evidence of bite marks on the isthmus and caudal areas.

The rate of feed dispensed from the HFS feeders has now been elevated to closely numerically correlate to the days post hatch. For instance, in 2019, at 19–20 dph, 19 g/1000 was programmed. It should be noted that from 14 dph to harvest, hand-feeding events are also applied to the tanks 8–12 times across 16 h. The total daily amount of hand feeding applied correlates to an additional 30–35% of the calculated daily program amount of the automatic feeders. Final feed application rates toward the time of harvest exceed 45 g/1000. Feed conversion ratio (FCR) from 2017 to 2019 has averaged 0.9. Low FCR values are influenced by the large differential in water content of the feed and fish.

Data from earlier culture runs identified ranges of sizes at the time of harvest in excess of 25 mm. The propensity of Walleye fingerlings to be piscivorous at an early stage of development is key to their success in the natural setting. Creating an environment that promotes this tendency during intensive larviculture could lead to increased cannibalistic behavior that could potentially reduce harvest numbers. At EWFCS, feeding regimes have been developed to hurry the inclusion of larger size feed as that improves tank hygiene and focuses on feeding the top of the population bell curve, dropping out poor performers earlier to help reduce counterproductive cannibalistic behavior. The application of this approach may have contributed to an increase in overall growth and survival.

Our feeding rate (g feed/1000 fish) is determined by "feeding to the number." Daily mortality assessments of individual tanks are required to ensure that proper feed rates are being applied to the existing population within the tank (Fig. 18). From 1 to 7 dph, an estimate is made of 800–1000 mortalities/tank/day. By 7–9 dph, fully formed body mortalities can be identified in the waste stream and counts are begun. After rinsing the mortalities from the waste sump tray screen insert, mortalities are enumerated. Either of the following method of preference is used: shallow tray

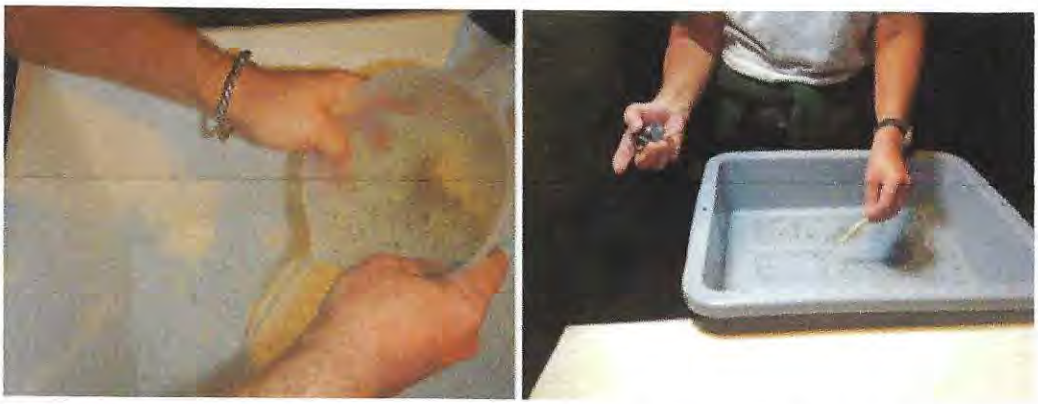

Fig. 18 Accurate accounting of tank mortalities is needed to adjust feeding rates. The beaker pour counting method is shown (left), and shallow tray is used to count mortality (right)

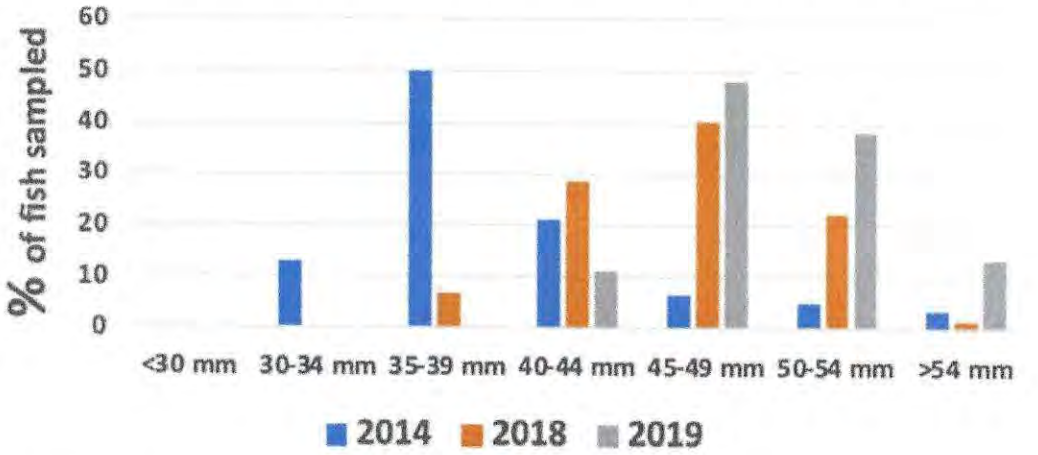

Fig. 19 Lengths of samples of 60 fish per tank measured during harvest. The stronger bimodality represented in 2014 can promote cannibalism due to the expanded ranges of size and potentially reduce survival due to the strikes and bite wounding that can be created by this size differential in the population in contrast to the greater size uniformity exhibited in 2018 and 2019

counting or slow beaker pouring (Fig. 19). With the shallow tray method, mortality from a tank is placed into a polyethene tray 40 cm × 28 cm × 12 cm with 1.2–2.5 cm of water. Mortalities are spread out on the floor of the tray, and the counting process is conducted. The beaker pour method uses a 2-L beaker filled to the halfway point with water, at which point the rinsed fry mortality from a tank is introduced. Water is added to the fill mark and then slowly poured out toward the technician to enumerate the fry. A small utensil or dissecting needle can be used to keep the fry from binding together. Additional water is added as needed to keep the fry flowing at a rate that allows each individual to be counted.

It is important to get a careful accounting of mortalities and avoid estimation so that as accurate as possible feeding rates can be applied. In dealing with cannibalism or any other unknown loss that may transpire during the first few days of the culture run, unaccounted mortality can occur. Though there can be a ranging variability

based on a number of factors, minimizing the guesswork with precise mortality counts has provided inventory accuracy from tank inoculation to harvest that averages +/− 10–12%.

2.5 *Growth and Survival*

Growth in fish length was monitored primarily using lengths at 5–7-day intervals. Vermont Fish and Wildlife Department fish health biologists sample each tank (20 fish/tank) and conduct measurements of fish, along with observations related to GBI/NGB, the prevalence of deformities, and feed utilization. The method used for samples is rapidly "trawling" two large aquarium nets toward each other. This method provides a reasonable snapshot, though it is likely that larger, faster swimming individuals avoid sampling during the last 7–10 days, indicated by length samples (60/tank) conducted at final harvest (Fig. 19). Fish weight was determined during the later stages of the culture run and at harvest (Table 7).

Survival from the initial stocking of the culture tanks to harvest is a measure of the success of larviculture. The goal at EWFCS has been to engage in intensive culture techniques using RAS to attain a consistent level of fingerling production that would result from survivals surpassing 50%. After several years of trial and error, the quality and quantity of fingerlings improved significantly beginning in 2017, which has led to consecutive years of production that has provided a substantial number of fingerlings for contribution to the Walleye program for Lake Champlain (Table 8) (Fig. 20).

2.6 *Performance Assessment of Stocked Fingerlings*

Measuring the contribution of cultured fish to the fishery is important data collected by district fisheries field staff. Marking the otoliths of cultured Walleye using OTC began in 1996 to establish the level of contribution and recruitment from the cultured fish program to the Walleye fishery. The source of fry for the RAS at EWFCS comes

Table 7 Average length and weight of 20 fish sampled from each tank during the 2019 culture run at EWFCS. At harvest (34 dph), 60 fish are measured and weighed from each tank

Days post hatch (dph)	Length (mm)	Weight (g)
6	10.52	NA
10	12.78	NA
15	18.13	NA
21	26.10	0.22
27	35.10	0.42
34	49.62	0.91

Table 8 EWFCS Walleye fingerling harvest data from 2011 to 2019

Year	EWFCS Walleye fingerling harvest trends				
	# of initial fry in system	Fingerlings harvested	% survival	Dph	Length mm
2011	344,000	20,043	5.8	41	38.1
2012	178,500	49,866	27.9	33	39.12
2013	175,190	3762	2.2[a]	35	34.29
2014	202,125	15,004	7.4	31	40.39
2015	152,500	56,344	36.9	31	37.34
2016	222,000	55,209	24.9[b]	30	37.85
2017	280,000	146,024	52.2	35	45.72
2018	320,000	194,914	60.9	33	46.1
2019	360,000	204,628	56.8	34	49.62

[a]Bacterial gill disease caused major mortality
[b]At 22 dph, 50% of tank feeders malfunctioned through the night (8–12 h), resulting in a mortality of 25,000 due to cannibalistic behavior

Fig. 20 Illustrated in this photo are uniform robust fingerlings from 2019 harvest samples

from broodstock obtained from Lake Champlain. Three tributaries in Vermont are used rotationally on an annual basis: the Poultney, Winooski, and Missisquoi rivers.

Three OTC marks are applied using a bath at a 6-h exposure (Fig. 21): a fry mark at 3 dph and an advanced fry mark at 8 dph, both of which are done at a concentration of 700 ppm, and a fingerling mark at 500 ppm is applied at the time of harvest at 34–36 dph for intensively reared fingerlings and 50–55 dph for fingerlings reared extensively in ponds. Walleye stocked at the unfed fry and advanced fry stages have shown returns that are statistically insignificant over 20 years, which is why efforts to

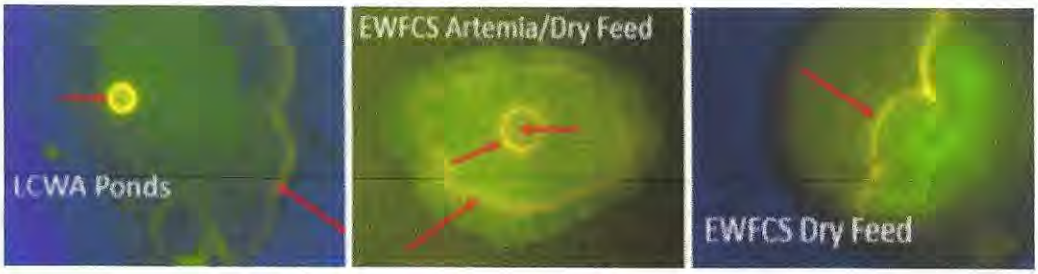

Fig. 21 Fluorescing OTC marks are observed using a Nikon E-400 microscope. LCWA extensively reared pond fingerlings have a fry and fingerling mark (left). Surplus advanced fry weaned from *Artemia* to a dry diet have a triple mark (center). Fry that are raised to fingerlings on a dry diet exclusively receive only a fingerling mark (right)

rear fingerlings have been established. A separate RAS is used for supplying the LCWA ponds with advanced fry that have been fed *Artemia nauplii* for 5 days. If there are any surplus fish in this system after the ponds have been stocked, a mark at the advanced fry stage is applied to differentiate the group having a triple mark. These fish are weaned from *Artemia nauplii* to dry feed (over a 3-day transition period) and grown to the fingerling stage.

Good (2020) describes the process of preparing and analyzing sagittal otoliths in this manner: otoliths are extracted from Walleye, wiped clean and dried, mounted concave side down to glass slides with Super Glue (Ontario, California), and allowed to harden for at least 24 h. Once the glue is cured, otoliths are gently ground on wetted 1500-grit automotive sandpaper along the sagittal plane to remove the top layers of glue and establish a level polishing surface. Specimens are sanded until the entire surface of the otolith is revealed then covered in mineral oil and viewed under a compound microscope at ×10 magnification with a reflected fiber-optic light source. Annuli are counted to estimate age.

After age estimation, otoliths are polished further using a wetted 30-μn lapping film (Precision Surfaces International, Houston, Texas), stopping occasionally throughout the process to examine the otoliths for the OTC marks until presence or absence is determined (Secor et al. 1991; Brooks et al. 1994; Fielder 2002).

Otolith inspection for OTC marks is conducted using a Nikon Eclipse E-400 epi-fluorescent microscope with fluorescent lighting and filter blocks designed to fluoresce the tetracycline marks. The Nikon E-400 microscope was outfitted with a B-3A filter cube (505-nm dichroic mirror, 420–490-nm exciter filter, and 520-nm barrier filter) and a 100-W mercury UV light source, as described by Bumguardner (1991) and Logsdon (2006). Otoliths are viewed through ×100 and ×200 magnification.

Up to 50 additional 3-year-old males are collected every year in excess of the fish that are utilized for broodstock. These males are being collected to assess otoliths for the presence of OTC marks that designate what part of the culture program contributed using the process described above. If there is no mark detected, this indicates that the fish has been naturally reproduced.

Table 9 The performance of cultured Walleye fingerlings in the Poultney River to document the contribution of intensively reared fingerlings

River	Culture method	OTC mark applied	# stocked	Return ratio (%)	*n* (OTC marked fish)	*n* unmarked fish
Poultney '20	LCWA Ponds	Fry/fingerling	78,178	18	9	0
(2017 stocking)	EWFCS Intensive	Fingerling Fry/Ad.Fr/Fing	167,745	82	42	
Poultney '17	LCWA Ponds	Fry/fingerling	69,109	66	19	10
(2014 stocking)	EWFCS Intensive	Fingerling	15,004	34	10	
Poultney '14	LCWA Ponds	Fry/fingerling	41,200	94.60	35	3
(2011 stocking)	EWFCS Intensive	Fingerling	20,100	5.40	2	

Highlights of the OTC mark analysis for 3-year-old males sampled on the Poultney River for the last three rotations are shown in Table 9. Sample years are assessing the stocking that occurred 3 years prior. The Lake Champlain Walleye program transferred to EWFCS in 2011, and efforts to work toward continued improvements were applied with each culture run. The analysis and assessment of the 2017 stocked fingerlings have just been completed. The performance of intensively reared fingerlings has developed progressively. There were three naturally reproduced fish present in sampling in 2014 and 10 present in 2017. In 2020, all fish sampled exhibited OTC marks. The reduced presence of naturally produced fish is a trend that is also being recognized on other tributaries by division biologists and could be potentially related to increased predation from invasive species such as Alewife (*Alosa pseudoharengus*) and White Perch (*Morone americana*). As investigation continues, cultured fingerlings will play an important role in the efforts to maintain a quality Walleye fishery in Lake Champlain.

3 Walleye Fingerling Production in a Reuse Aquaculture System at the Rathbun Fish Culture Research Facility, Moravia, IA

J. Alan Johnson

3.1 Walleye Culture in Iowa

Walleye are spawned and eggs incubated at three IDNR hatcheries: Fairport Fish Hatchery (FFH), Rathbun Fish Hatchery (RFH), and Spirit Lake Fish Hatchery (SLFH). In 2019, the hatcheries distributed 148 million fry (1–3 day-old prolarvae) to enhance Iowa's fishery resources. Pond fingerlings are produced at FFH and RFH while advanced fall fingerings are produced at SLFH and RFH. In 2019, the FFH located by the Mississippi River produced 472,639 pond-reared fingerlings (ca. 35–40 mm). In 2019, the RFH, located below Rathbun Lake Dam, distributed 91,240,988 fry; 748,248 pond fingerlings (ca. 40–45 mm); and 176,567 advanced fall fingerlings (mean size 225 mm). Spirit Lake Fish Hatchery, adjacent to Spirit Lake, produced 59,309,340 larvae and 21,093 tank-reared fall fingerlings (152 mm). All advanced fall fingerlings were reared from larvae stocked in a pond, harvested at about 42 mm, transferred to tanks for habituation to pelleted feed, and then grown to about 225 mm at either RFH or SLFH.

Rathbun Fish Hatchery began fish production in 1975, but intensive culture of Walleye did not begin until 1985 with the habituation of pond-reared fingerlings to pelleted feeds. Hatchery research investigations into intensive Walleye larviculture began in 1991. Larger fingerlings were desired, and the early spawning of broodstock coupled with intensive larviculture could extend the growing season to consistently produce fall fingerlings at 204 mm. The Iowa DNR's Fisheries Bureau recognized the need for a dedicated research facility and staff, providing the rationale for the development of the Rathbun Fish Culture Research Facility (RFCRF), which began operation in 1996. In 2000, six 0.04-ha research ponds and 10 0.4-ha production ponds were added to RFCRF and RFH, respectively. Production ponds are double-cropped by the production of Channel Catfish (*Ictalurus punctatus*) following Walleye harvest. All culture operations use surface water (Rathbun Lake) in single-pass culture or pond culture. Water supply to the Hatchery and Facility is from Rathbun Lake and passes through a drum screen (300 μn) before entering outdoor culture units or passing through sand filtration and UV disinfection before filling indoor tanks. In 2015, a RAS was constructed at RFCRF to evaluate pilot-scale a growout of Walleye fingerlings from 100 mm to 225 mm. The growout RAS is the largest system at RFCRF with three 10.9 m^3 tanks. Additional RASs were added for Walleye egg incubation and intensive larviculture in 2018 and 2019, respectively. The incubation RAS is a partial RAS because freshwater exchanges are needed in the absence of a biofilter and has 20 jars with 66 L of egg capacity. All RASs utilize the municipal water supply for filling, replacement, backwashing, and equipment washing; surface water is not used. Municipal water is dechlorinated by activated carbon filtration before addition to the system with one exception: drum screen backwash.

Using RAS, Walleye can be cultured from eggs to large fingerlings using municipal water that is free from AIS contamination. Other sport fish, such as Rainbow Trout and Muskellunge (*Esox masquinongy*) or yearling Walleye for future

broodstock, have been cultured in the same system when the RAS tanks were not utilized for Walleye culture. All three Iowa DNR fish hatcheries that produce Walleye utilize surface water sources (Rathbun Lake, Spirit Lake, Mississippi River) that are considered infested with Zebra Mussels and threatened by other AIS as well as surface waters that are known sources of many parasites (e.g., Ich) and pathogens. While disinfecting surface water for single-pass systems can eliminate sources of infectious pathogens or AIS, a single-pass system replaces 100% of the tank volume per hour. Comparatively, RASs rely on a small amount of makeup water volume of 5–20% of the total system volume per day. Compared to single-pass systems, the greatly reduced water requirement of a RAS substantially reduces costs and equipment to improve water quality or eliminate the ingress of pathogens or AIS into the hatchery. RAS use can overcome current limits to the production capacity of the Iowa DNR fish hatcheries that are at or near maximum capacity based on space, water quantity, and/or water quality.

The use of RAS for sport fish production is an emerging trend among government agencies. Egg incubation to food size fish production in RAS is common for many food fish species. A few studies have evaluated RAS for Walleye production (Summerfelt 1996a; Summerfelt and Penne 2007; Zarnock et al. 2010; Davidson et al. 2016), and two focused on Walleye production for stock enhancement (Aneshansley et al. 2001; Harder et al. 2014). Limitations to the successful application of RAS technology to Walleye in future hatchery renovations require applied research to establish optimal production densities, determine swimming speed tolerances, feeding regimes, and other issues. This section will describe Walleye larviculture in pilot-scale RAS at RFCRF.

3.2 System Design/Unit Process

The larviculture RAS was added to RFCRF by aquisition of a turn-key system (Fig. 22) manufactured by Oceans Design Inc. (Colorado Springs, CO). Critical system conditions such as dissolved oxygen, water levels, and electrical status are monitored by a SCADA system that alerts staff.

3.2.1 Solids Removal

Solids are removed from the tank by siphoning the waste trough once daily. Suspended solids in the tank effluent are removed by a drum screen filter (Trome, 60 microns, Londerzeel, Belgium). Culture water passing the drum filter passes on to the horizontal MBBR.

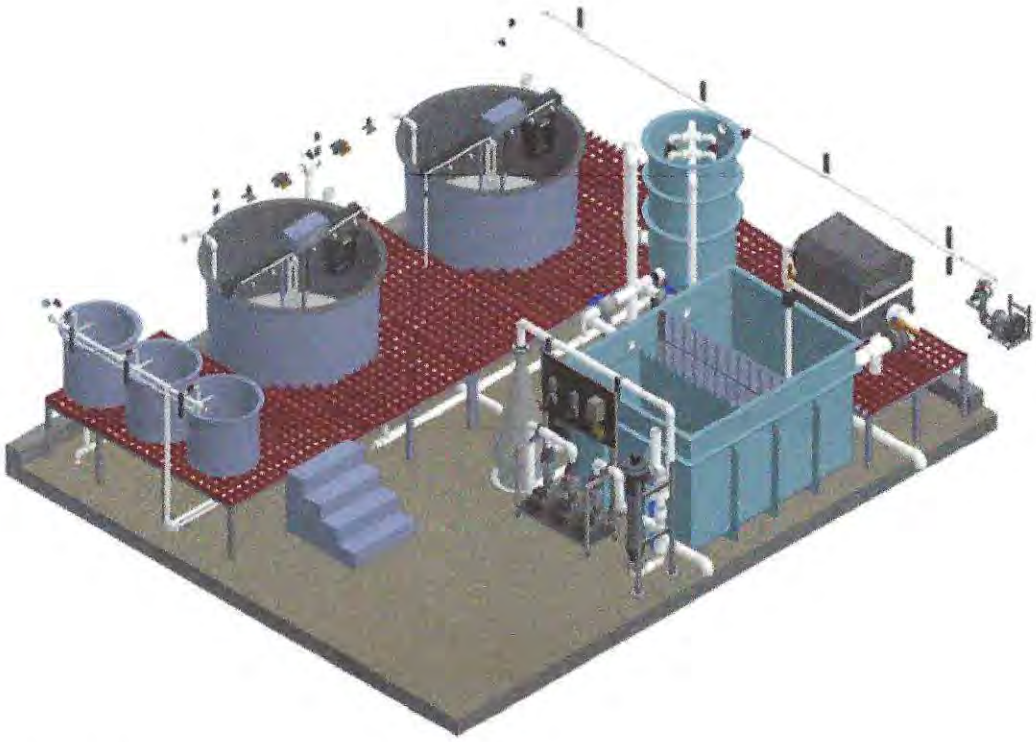

Fig. 22 Designed view of the larviculture water reuse aquaculture system at Rathbun Fish Culture Research Facility, Moravia, IA (provided by manufacturer Oceans-Design, Colorado Spring, CO). In the sequence of water flow, the components of the system are five culture tanks (three 275-L tanks and two 2400-L tanks), microscreen drum filter, biofilter and pump sump, pumps and control panel, CO_2 stripper, UV lamp array, and oxygenation cone

3.2.2 Degassing

The biofilter and pump sump are in one unit, with a media retention screen and weir wall separating the biofilter and the pump sump. A separate circulation loop pumps water from the sump through the chiller heat exchanger into the CO_2 stripper then returns water to the biofilter. A portion of the flow from the pump sump may be diverted through two inline heaters then discharged into the biofilter sump. The CO_2 stripper is filled with tube-form Nor-Pac degassing media (Oceans-Design, Colorado Springs, CO) to prevent biofouling from the combination of clay particles and biofilms that could impede water flow through the CO_2 stripper. Other types of random pack media may be more prone to plugging water flow due to biofilm and clay accumulation.

3.2.3 Biofiltration

The horizontal flow MBBR contains a 1.0-m^3 K3 media (Oceans Design, Colorado Springs, CO) that provides a total surface area of 500 m^2. Media is fluidized by air

bubble diffusers and retained by a stainless-steel screen, and water passes over a weir wall into the pump sump.

3.2.4 Ultraviolet Disinfection and Oxygenation

Reused water is pumped from the sump and through an ultraviolet disinfection unit. The unit has three lamps (390 watts total) that provide a 96-mj/cm^2 dosage (96,000 μw/cm^2), 90% UVT, and a 189-Lpm maximum system flow rate. A downflow bubble contactor oxygenates water with LOX-derived pure oxygen injection. The cone is pressurized by a modulating knife valve downstream of the cone. Pressure is generally maintained at 7–10 psi.

3.3 Culture Tanks

Production-scale larviculture tanks are 2.4 m^3 circular tanks (1.83-m diameter, 1.07-m depth) with black sidewalls and grey tank bottoms (Fig. 23; Oceans-Design; Colorado Springs, CO). Each tank has a wiper arm that pivots around the center drain driven by a gear reduction motor. The arm has a bottom squeegee wiper and a sidewall brush wiper. The wiper arm rotates at 1 rpm to sweep waste into a trough at the tank bottom extending the radius of the tank. The trough has a 1-inch drain that is only used to completely drain water from the tank during harvest. Tanks are fitted with a center drain screen and a screened box insert mounted to the tank wall and connected to a waste return line. The center screen has three parts: a 101.6-mm Sch 40 PVC pipe with cutout sections for a frame that inserts into a fitting in the bottom center of the tank, a removable plastic mesh (3.2 mm opening) to provide screen support over the frame openings, and a nylon mesh sock fitting over the plastic mesh. The nylon mesh socks used in Walleye larviculture are 0.7-, 1.0-, 1.5-, 2.0-, and 3.2-mm square openings.

3.4 System Management

3.4.1 Temperature

The water temperature for newly hatched larvae is typically maintained at 15.0–16.7 °C for 2 days post hatch. On day 5 post hatch, when larvae begin to feed exogenously, temperature is increased by 1.5–2.0 °C, which is thought to stimulate uniform feeding behavior. Temperature is gradually increased to up to 22.0 °C prior to 35 dph (Table 10).

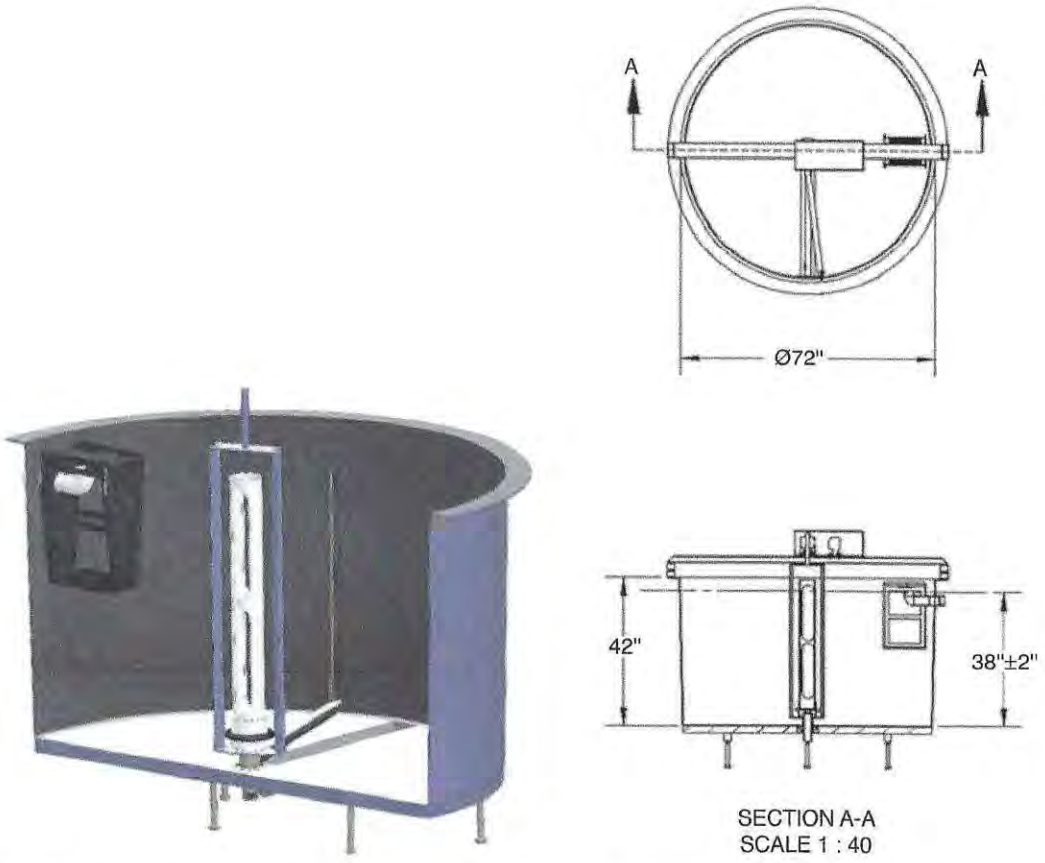

Fig. 23 Schematic cross-sectional, overhead, and side drawings of the 2.4-m^3 self-cleaning larviculture tank with dual effluents fitted with a side-wall screen box and center drain screen

3.4.2 Light

Each tank is equipped with an overhead light fixture with a full spectrum LED lamp suspended over the center of the circular tank. Light levels are measured with a lux meter and set by rheostat to 75 lux at the water surface.

3.4.3 Turbidity

Turbidity is artificially increased by the addition of a clay slurry. The clay used is the OM-4 clay (Imerys, Paris, France), a powdered clay that is mixed with water on-site (25 g/L) in a stock solution in a 113-L cone-bottom tank, aerated to keep the clay in suspension, and pumped to the culture system by a peristaltic pump. The frequency of clay addition is controlled to provide 50 NTU of turbidity during the first 21 days post hatch, and then turbidity addition is reduced over the next 3 days. The clay dissipates from the system, and turbidity levels are about 5 NTU for the remaining culture period. Turbidity levels of culture water are sampled once daily in each culture tank.

Table 10 Feeding rate (g/1000 larvae) and feed sizes fed to larval Walleye from 2 to 35 dph in relation to temperature and larval size. Feed rate expressed as % bw/d calculated as ration/(estimated weight × 1000)

	Ration	Otohime			Temperature	Length	Weight	
DPH	g 1000^{-1}	B2	C1	C2	(°C)	(mm)	(g)	%bw/d
0					16.1			
1					16.2	8.6	0.0033	
2	4.0	100			16.6	9.1	0.0044	90.7
3	4.0	100			16.9	9.6	0.0055	72.8
4	4.0	100			18.2	10.0	0.0066	60.8
5	4.0	100			19.0	10.5	0.0077	52.2
6	4.0	100			19.1	10.9	0.0088	46.0
7	4.5	100			19.2	11.4	0.0099	45.7
8	6.5	75	25		19.5	11.7	0.0105	62.1
9	8.0	75	25		19.7	12.7	0.0160	50.1
10	9.0	50	50		20.0	13.7	0.0214	42.0
11	12.0	50	50		20.3	14.7	0.0269	44.6
12	13.0	50	50		20.3	15.7	0.0324	40.1
13	14.0	25	75		20.3	16.8	0.0379	36.9
14	15.0	25	75		20.2	17.8	0.0434	34.6
15	17.0		100		20.1	18.8	0.0489	34.8
16	19.5		100		20.1	20.1	0.0643	30.3
17	20.5		100		20.4	21.4	0.0797	25.7
18	20.5		100		20.9	22.7	0.0951	21.6
19	22.0		75	25	21.2	24.0	0.1105	19.9
20	22.0		75	25	21.5	25.3	0.1259	17.5
21	23.0		75	25	21.8	26.6	0.1413	16.3
22	23.0		50	50	22.0	27.9	0.1567	14.7
23	24.0		50	50	22.0	29.4	0.1967	12.2
24	26.0		50	50	22.0	30.8	0.2366	11.0
25	28.0		50	50	22.0	32.3	0.2766	10.1
26	33.0		25	75	22.0	33.8	0.3166	10.4
27	41.0		25	75	22.0	35.2	0.3565	11.5
28	50.0			100	22.0	36.7	0.3965	12.6
29	62.0			100	22.0	38.1	0.4365	14.2
30	70.0			100	22.0	40.0	0.5214	13.4
31	80.0			100	22.0	41.9	0.6063	13.2
32	90.0			100	22.0	43.8	0.6912	13.0
33	100.0			100	22.0	45.6	0.7761	12.9
34	110.0			100	22.0	47.5	0.8611	12.8
35						49.4	0.9460	

3.4.4 Surface Spray

Surface spray nozzles provide an open area for GBI and assist in clearing feed debris trapped in the surface tension of the water. The surface spray enhances GBI (Clayton and Summerfelt 2010). Tanks have surface spray nozzles (model 73–501, Hydro-Gardens, Colorado Spring, CO) spaced evenly across the diameter of the tank by mounting to the wiper motor support frame and a second spray bar perpendicular to this spray bar.

In general, single-pass larviculture has resulted in acceptable GBI rates using one spray nozzle for a 0.76-m diameter tank. In 2019, samples of fry showed variable rates of GBI between samples obtained during culture, but the final GBI rate was 98%. In 2020, we installed a second spray bar perpendicular to the existing spray bar for a total of 12 nozzles. Rates of GBI were consistently high in 2020. The surface spray bars are supplied with pressurized system water from the oxygen cone.

3.4.5 Water Quality and Quantity

A municipal water supply is dechlorinated by passing through two activated carbon filters before entering the larviculture system as either system replacement water or drum filter backwash spray. Freshwater replacement water is 0.5–1.0 Lpm. Additional freshwater is added to the system during drum filter backwashing, but the rate of addition has not been quantified. Rathbun Lake is the water source for the local municipal system and has a low level of alkalinity (ca. 90 mg/L). Reused water inflow rates to tanks are initially set at 65% tank exchange per hour and increased to 200% tank exchange per hour at the end of the culture period. Water quality (NH_3 and pH) is measured in each tank and in the pump sump (alkalinity, NH_3, NO_3 and pH) once per week. Alkalinity is maintained above 150 mg/L with a goal of 200 mg/L by the addition of feed grade sodium bicarbonate at a rate of 25–30% of the total daily feed ration. System water is periodically tested for alkalinity and replenished to 200 mg/L.

3.4.6 Stocking Density

Eggs are incubated with water from a RAS that is supplied with dechlorinated tap water. Fry for larviculture are collected over about a 24–30-h period to minimize variation in cohort size that could lead to cannibalism. Larvae are enumerated at 2 days post hatch using a commercial fry counter (Model FCM, Jensorter; Hillsboro, OR) and stocked into tanks. The counter is calibrated according to the instruction manual, then a sample of about 500 fry is enumerated by hand and passed through the counter three times to obtain an average error estimate, and the stocking number is adjusted by the error rate to achieve the desired quantity.

Fry are concentrated in the catch tank, collected in beakers, and placed into the fry counter hopper. Fry exiting the counter are captured in a 20-L bucket with a 500-μm screen-covered window for water release. The bucket receives about 10,000 fry before larvae are transferred to the larviculture tank. In previous single-pass experiments, a stocking density of 40 fry/L was used. Currently, density has been reduced to 30 fry/L in order to produce larger (>0.6 g) size fingerlings at harvest. In the RFCRF RAS, stocking density was 30–35 fry/L in 2019 and 2020.

The offspring of Rathbun Lake broodstock, originally stocked with eggs from Spirit Lake broodstock, have been reared in single-pass larviculture with success at RFCRF. In 2019, tanks were stocked with larvae obtained from Rathbun Lake broodstock. Production, however, was limited with only two 2.4 m^3 tanks; therefore, in 2020, production was increased by rearing three back-to-back crops of fingerlings by obtaining eggs earlier and later than the normal Rathbun spawn. First crop eggs were obtained from Kerwin Reservoir, KS. The eggs were incubated and hatched at the RFCRF and cultured to 35 dph then harvested. The second crop of larvae eggs from Rathbun Lake was incubated and hatched for culture immediately following the first crop. The third crop was produced using eggs from Lake Sakakawea, ND, broodstock incubated and hatched to follow the harvest of the 35 dph Rathbun fingerlings.

3.4.7 Tank Hygiene

Solids accumulate in the waste trough in the tank bottom and are removed once per day by a siphon tube. Solids are collected in a screened basket to retain mortalities; some wastes require removal with a water spray to leave mortalities cleaned for counting. Siphoning the small trough area reduces the amount of time to clean compared to siphoning the entire tank bottom and improves the completeness of siphoning in a turbid water tank with no visibility of the tank bottom.

Center drain and sidewall box drain screens are removed and replaced with cleaned screens once daily. Typically, a few mortalities are impinged on the screen and are counted prior to cleaning. Tank water levels are elevated due to screen or effluent pipe biofilms impeding effluent flow occasionally toward the end of the culture period.

3.4.8 Feeds, Feeding Rates, and Feeders

Fish are fed Otohime B2, C1, and C2 diet sizes (Reed Mariculture, Campbell, CA), starting with B2 and progressed to C2 (Table 10). Feed size is increased by blending progressively larger feed sizes until the C2 diet is fed exclusively. Feed rates are expressed in g/1000 larvae and increased from 4 g/1000 up to 110 g/1000 fry at 35 dph.

Feed is dispensed from an ARVOTec T-drum 2000 (Huutokoski, Finland) using an industrial computer control system to continuously monitor and control the feeders. Each 2.4 m^3 tank has two feeders mounted to the wiper frame spanning the diameter of the tank. Feed is offered at 5-min intervals 24 h/day. The feed dispensing rate is measured during a set interval (100 s), and the PLC program delivers the desired ration (total g/d) at determined intervals (300 s) over a 24-h/day. Feed drops from the feeders directly into the tank without the use of a spreader.

3.5 *Growth and Survival: Culture Period*

Four culture runs, one in 2019 and three in 2020, were completed in the larviculture RAS at RFCRF with an average survival rate of 77.2%, resulting in 470,050 total fingerlings produced in the two 2400-L tanks. In 2019, a total of 107,802 fingerlings were produced in the production scale tanks with a 75.2% survival rate (Table 11). In 2020, the two production tanks were stocked and harvested successively three times, identified here by the source (number and % survival): 118,252 (75.6%) from Kerwin Lake; 139,284 (88.5%) from Rathbun Lake; and 104,712 (67.5%) from Lake Sakakawea. Over the three culture runs, GBI ranged from 96.5% to 100.0% and averaged 98.4%.

In 2019, growth rates ranged from 1.02 mm/d to 1.23 mm/d with a lower temperature of 18.7 °C resulting in slower growth rates than fish cultured at 20.3 °C average temperature in 2020. The feed conversion ratio was 1.65 in 2019. In 2020, feed conversions were 0.81–1.00 and there were observations of very little waste feed. The 2020 temperature regime increased, and the feed regime may have

Table 11 Summary of Walleye fingerling production by year and egg source in a water reuse aquaculture system in 2.4-m^3 culture tanks at Rathbun Fish Culture Research Facility, 2019 and 2020

	2019 Rathbun	2020 Kerwin	2020 Rathbun	2020 Sakakawea
Age at harvest (dph)	42	35	35	35
Final number/tank	53,901	59,126	69,642	52,356
Survival (%)	75.2	75.6	88.5	67.5
Final length (mm)	47.1	48.7	50.7	48.8
Final weight (g)	0.86	0.94	0.99	0.91
Gas bladder inflation rate (%)	98.0	99.0	100.0	96.5
Harvest density (kg/m^3)	16.0	25.4	32.3	21.1
Feed conversion ratio	1.65	0.84	0.81	1.00
Specific growth rate	15.4	16.9	16.6	16.8
Daily length gain (mm/d)	1.02	1.21	1.23	1.19
Mean temperature (°C)	18.7	20.0	20.4	20.5

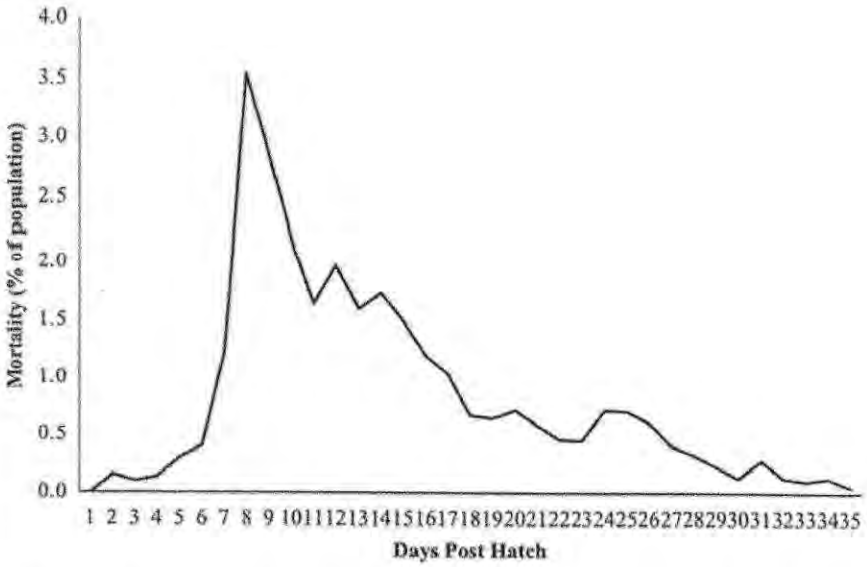

Fig. 24 Mean daily mortality rate (percent of tank population) during the 2020 three larviculture runs

been the minimum feeding rate in two runs: with 0.81 and 0.84 FCR; increased feed rates should be evaluated to determine if such a low FCR indicates underfeeding.

Mortality during the culture interval peaked at 8 dph (Fig. 24). A similar mortality peak was observed in 2019 and is commonly observed in single-pass culture experiments at RFCRF prior to RAS culture. Observations of food in the gut during the 8 dph measurement indicated high rates of feed present in the gut of sampled fish; therefore, cannibalism attempts may be the cause of mortality. Larvae were observed attacking other fish in the opercula region in an attempt to overcome the victim (Fig. 25), and this attack likely resulted in death. Trunk attacks are a greater source of mortality compared to tail attacks (Loadman et al. 1986). The main culture techniques used to minimize cannibalism are feed amount, frequency, and palatability. Some feed was observed in the waste siphoned from tanks; however, it is not known if higher feed rates may help curtail this instinctive behavior.

Feeding rates and temperature regimes have changed at RFCRF in the past 20 years based on work in single-pass larviculture experiments. In the late 1990s and early 2000s, temperature was held constant at 18.3 °C throughout a 28-day period for continuity between years of open formula diet studies. At this temperature, fingerlings reached a length of 25–28 mm. Subsequently, the focus of intensive larviculture moved toward production scale with an emphasis on growth and postlarviculture survival rates. To be competitive with Walleye pond culture growth rates at RFH, temperature regimes for larviculture were increased to obtain higher growth rates to match pond culture growth rates. Additionally, a larger fingerling size is desirable to improve survival immediately after the larviculture period when transferring to grow-out tanks. Other research at the facility demonstrated improved survival of pond-reared fingerlings of at least 0.56 g that began habituation to formulated diets in tanks (Johnson and Rudacille 2010). A predominant factor

Fig. 25 Cannibalistic behavior of 15 dph larval Walleye, shown ventral side upward, holding the victim by the throat, an attack that differs from an attempt by the predator to swallow its siblings head-on (Fig. 16)

contributing to the higher survival rates of larger fingerlings in surface water single-pass culture is likely the presence of a full complement of scales that develop at 42 mm (Priegel 1964) and that Columnaris disease (CD) is a result of mechanical damage during handling (Hussain and Summerfelt 1991).

Feeding rates utilized from 1999 to 2003 at RFCRF with BioKyowa FFK and reared at 18.3 °C began with 3-g/1000 larvae and increased to 7-g/1000 larvae by 23 mm length and fed 10% body weight per day (bw/d) at 25–40 mm length (Johnson and Rudacille 2009). In 2006 and 2007, using the Otohime diet, larvae were fed 4–7-g/1000 larvae from first feeding to 25 mm (Johnson and Rudacille 2009). Later, feeding rates were increased to match growth rates as water temperature increased. In 2012, the mean temperature in a 35-day study was 19.7 °C and resulted in 43.5-mm and 0.67-g fingerlings with a 75.8% survival rate. In that study, feed rates increased from 4-g/1000 to 6-g/1000 larvae and from 2 dph to 20 dph then changed to a 10% bw/d rate. Feed conversion ratios were not monitored in previous single-pass larviculture studies until RAS larviculture began in 2019.

Past research trials (2006–2007) at RFCRF fed Otohime B1 diet (250–360 μ; Johnson and Rudacille 2009). That size is similar to the BioKyowa FFK B400 size (250–400 μ) fed by Barrows et al. (1993). Through gradual experimentation and collaboration (Kevin Kelsey, Personal Communication, EWFCS), Otohime B1 was phased out and only Otohime B2 (360–650 μ) is offered as the first feed without observed reduction in survival rates or feed acceptance.

The temperature regime used in 2020 was modeled after a temperature regime used at the EWFCS (Lake Champlain, Vermont, Kevin Kelsey personal communication). The feeding rates used in 2020 were based on previous feed rate and growth rate observations at RFCRF and were increased substantially compared to rates used

between 1999 and 2012, particularly after seven days post hatch when the 2020 rates increased rapidly and ended with about 12.8% bw/d (110-g/1000 larvae; Table 11) rather than 10% bw/d used in previous studies. Feeding rates expressed as g/1000 larvae are used to determine the daily feed fed at RFCRF. However, these feeding rates, converted to percent body weight per day, compared with rapid larval growth rates, result in initial rates of 90.7% bw/d, decline to 45%, then rebound to 62% when the g/1000 larvae feeding rate is increased. Further research could optimize feed rates if related to survival; however, total feed costs (including shipping) were $0.016 per fish at 35 dph. Therefore, minor adjustments to the feed rate of small larvae are not likely to affect cost per fish.

These trials demonstrated that larviculture protocols and techniques previously established in single-pass culture systems could be successfully implemented on production-scale tanks in a RAS. Three geographic sources of Walleye larvae were reared in the same RAS under similar conditions with results that demonstrate that techniques for Walleye larviculture are successful in RAS regardless of larvae source.

3.6 Performance Assessment of Stocked Fingerlings

Currently, the Iowa DNR has two fishery evaluations of intensive-culture Walleye. One study is evaluating single-pass intensive-larviculture-reared fingerlings to extensive pond culture fingerlings for stocking interior rivers. Walleye fingerlings (~50 mm) cultured in a RAS may be a replacement for pond-reared fingerlings for stocking in interior rivers when pond-reared fingerlings are typically stocked. In June, the rivers are often at flood stage, and stocking at that time results in poor recruitment. Fingerlings reared on pelleted diets in tanks can be held and fed in the hatchery for a short period until river levels decline. However, natural zooplankton and benthic insect larvae in extensive pond production have a limited production capacity that requires the harvest of fingerlings when food sources in the ponds are exhausted, regardless of river level.

Poor recruitment of pond-reared Walleye fingerlings (~50 mm) stocked in constructed or natural lakes in June commonly occurs due to predation. For this reason, size has been identified as a factor in previous IDNR fishery research evaluations. Many impoundments >202.3 ha in Iowa generally receive 225-mm fall fingerlings. Walleye ≥203 mm (70 g) produced at RFH and stocked into some Iowa impoundments had mean survival rates of 45% (Mitzner 1995), and overwinter survival of these large fingerlings was twice that of fingerlings from extensive nursery lakes, which averaged 125 mm. Lower and more variable survival of 4.9–31.7% was reported for 150–178-mm average size Walleye produced at SLFH (Larscheid 1995). The IDNR Fisheries Bureau is currently comparing Walleye (225 mm) produced in RAS and fingerling fish produced in a single-pass system.

4 Discussion: Similarities, Contrasts, and Collaboration

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4.1 Similarities

The RASs at EWFCS and RFCRF employ similar but not identical unit processes for clarification, biofiltration, gas stripping, oxygenation, and UV treatment of the water. Both RASs employ the same commercial self-cleaning tanks, drum filters, and moving bed bioreactors for biofiltration. Both use surface sprays to eliminate NGB and turbid water and culture tanks with black sidewalls to prevent clinging behavior and to disperse the fish to reduce cannibalism. Otohime is fed at both facilities as the first feed using the same commercial feeders at both sites.

Both systems are obtaining better than 60% survival rate in the first 35 days with 90% or better gas bladder inflation. It is important to note that both systems have not experienced frequent outbreaks of bacterial gill disease (BGD), CD, or parasites. In single-pass culture, RFCRF Walleye fingerlings are frequently treated for the control of BGD and CD, which typically cause mortality when fish are >20 mm. During the 10 years of using a RAS at EWFCS, only one outbreak of BGD occurred—in 2013, which was due to significantly increasing the feeding rate in fish at 250–360-μm size range. The smaller dry diet had consistently created tank hygiene issues, which prompted reduced use, thereafter followed by complete elimination in 2018.

In the past, Walleye larviculture turbidity was maintained for the entire production interval, but now both EWFCS and RFCRF start with turbidity regimes then clear the system water of turbidity at 18–24 dph, respectively with no negative effects on production.

4.2 Contrasts

It is apparent that although both EWFCS and RFCRF use RASs, turbid water, and surface sprays, the details of their respective systems were developed independently, resulting in differences in the aspects of system design and in how culture techniques are carried out. The most substantial difference between facilities is how turbid water is achieved, with RFCRF using clay and EWFCS using commercial algae. Turbidity at RFCRF is developed by the addition of a slurry of inert clay, which has been practiced at the facility since its development in small-scale laboratory tanks (Bristow and Summerfelt 1994, 1996). The Blue Jay Creek Fish Hatchery (Paul

Methner, personal communications, Ontario Ministry of Natural Resources) was an early adopter of clay for use in a RAS for Walleye larviculture system, which was prior to the development of the larviculture RAS at RFCRF. Thus far, clay turbidity has proven to work with all RAS component processes at RFCRF. At EWFCS, turbidity is developed by the addition of a commercial microalgae product, which is used in the larviculture of marine species.

The biofilter at RFCRF has a horizontal tank style with an open top for accessibility to allow the clay to be siphoned from the bottom, but thus far, this has not been necessary until the system is annually disinfected. RFCRF uses K3 media, which has a similar diameter but shorter length compared to the MB3 media used at EWFCS. It is not known if using a longer cut of media with clay turbidity would clog the biofilter with biofilm and clay and prevent aeration from mixing. The system at EWFCS utilizes a vertical biofilter configuration with microalgae turbidity being directly batch dosed into the filter three times per day. Microalgae do not accumulate to create fouling in the biofilter as the cellular structure of the algae breaks down beyond 24 h.

Tank sizes are larger at RFCRF, 2.4 m^3, compared to 1.94 m^3 at EWFCS. Decisions for tank size evolved separately; the 1.83-m diameter tanks were chosen at RFCRF as the largest tank that could allow the center drain screen to be accessed by workers and because larger tanks could not fit within the space available. Center standpipe screens were desirable at RFCRF because this was similar to previous experiences on a smaller tank in single-pass culture. Additional rationale for the use of the center screen was the need for overflow points to allow surface oils to be moved out of the tank with the assistance of the surface spray bar. One side box was added to ensure that effluent capacity is adequate to prevent overflows when screen flow was reduced. The 1.52-m diameter tanks at EWFCS were chosen to match the tank diameter already in use on the system. Also, in contrast, the center screen was undesirable for EWFCS staff as cleaning had the potential to result in fish loss. Self-cleaning tanks at EWFCS were engineered to have two boxes for each tank, but only one has proven to be necessary. Tank hygiene practices vary between systems. Daily tank care at RFCRF is done with one cleaning per day. At EWFCS, two cleanings are conducted as the facility is staffed 16 h/day.

4.3 Collaboration

All three authors began communicating on Walleye larviculture techniques at the Midwest Fish and Wildlife Conference in Des Moines, Iowa, in 2011. EWFCS first reported the use of RAS for Walleye larviculture at this conference, which encouraged the collaboration between EWFCS and RFCRF. Since then, continual communication has continued before, during, and after culture runs to understand the focus and approach that each facility will conduct. The sharing of findings and results has led to advances such as the elimination of the use of B1 feed size, the application of optimum temperature regime, and determining the elimination of

turbidity to coincide with the transition of larvae from photopositive to photonegative. Communicating with each other during the culture run to discuss challenges while they are unfolding, by going back to the basics of what we are seeing and how it could be addressed with an understanding of existing biology knowledge, has been invaluable. Our partnership has resulted in advancing techniques that produce Walleye fingerlings of consistent quantity and size. This alliance has extended throughout both North America and Europe through the sharing of the knowledge and success gained with these techniques.

It is noteworthy that success has been achieved with the use of science-based protocols and modern RAS engineering for the intensive larviculture of Walleye. At the EWFCS, larval survival in the RAS was 52–61% from hatch to 33–35 dph compared with 32% average survival in cooperative extensive ponds over the last 30 years. At RFCRF, larval survival in the RAS over 2 years was 77.2% to a size of 1.0 g with gas bladder inflation of 96.5% to 100.0%.

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Robert Summerfelt thanks Kevin Kelsey and Alan Johnson for their invitation to collaborate in the preparation of this report. Personally, this has been an integrative experience of the laboratory research I have had on this topic. They (Kelsey, Sect. 2, and Johnson, Sect. 3) describe successful achievements that substantially advance prospects for hatchery-scale production methods for the intensive larviculture of Walleye, with an obvious application for on-growing Walleye and other species. Their accomplishments are a product of exceptional dedication over many years of research and development activities that have shown extraordinary resilience to accept, learn, and respond from setbacks that I know to be common with any effort to control living systems in the manner that we would like. Of course, their starting point and progress benefited from the experience of others that are cited in the text and also from unpublished studies by many hatchery persons who presented talks at the annual meetings of the Coolwater Workshop, Midcontinent Workshop, and World

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Aquaculture Society. It is as in the metaphor by Issac Newton, "If I have seen further it is by standing on the shoulders of Giants" (Chen 2013). Their success, however, has come not only from what they have learned from others but also from their own innovation in combining what was known of critical early life stages of this species and cultural systems using the latest RAS technology. Yet, all of that said, they recognize that more needs to be done.

The reference of trade names stated is to illustrate the application of these products to the culture process and does not suggest endorsement by the authors or their agency.

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