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ARTICLE

Cisco Aquaculture Best Practices: Randomized Experiments from Eggs to Juvenile

Gregory J. Fischer^{*1} (D), Christopher Hartleb, and Kendall Holmes

Northern Aquaculture Demonstration Facility, University of Wisconsin–Stevens Point, 36445 State Highway 13, Bayfield, Wisconsin 54814, USA

Chloe Hansum² and Nathan Tintle³

Dordt University, 700 7th Street NE, Sioux Center, Iowa 51250, USA

Abstract

The Cisco *Coregonus artedi* (also known as Lake Herring), a coldwater salmonid found in the Great Lakes, is of interest to multiple agencies for restoration and conservation purposes due to its important ecological role. Further information on rearing and restocking of Cisco is needed, especially toward understanding the biological culture needs of Cisco eggs, larvae, and fingerlings. To address this gap in the literature and to provide essential fish culture information, we performed three preliminary studies in 2010 with Cisco: fertilization (wet versus dry fertilization), egg survival (pre-water-hardening versus post-water-hardening iodine treatment), and fry development (three different larval feed treatments that were commercially available at that time). The dry fertilization methodology (68%) yielded a significantly better eye-up percentage than wet fertilization (34%). Additionally, our testing revealed higher survival rates when iodophor treatment was used on fertilized eggs after water hardening (54%) in comparison with treatment before water hardening (43%). Although mean survival rates across the three diet treatments were not significantly different, larval Cisco that were fed brine shrimp *Artemia* replacement diets outperformed those fed the other diets, with the INVE Proton diet ranking highest. These early preliminary studies substantially increased the understanding of optimum culture parameters for Cisco in preparation for the widespread production of this important species, and the results provide propagation recommendations for conservation stocking programs.

The Cisco *Coregonus artedi* (also known as Lake Herring) is a coldwater salmonid found in the Great Lakes and is of interest to multiple agencies for restoration and conservation purposes. In the early 1900s, Cisco yielded large commercial harvests, but by the mid-1900s entire communities of fish were eliminated from the Great Lakes (Dryer and Beil Dryer and Beil 1964; Hansen 1990; Zimmerman and Krueger 2009). Historically, Cisco played an important role in the Great Lakes pelagic and benthic food webs (Eshenroder et al. 1995; Horns et al. 2003). The

^{*}Corresponding author: fischer@mcmjac.com

¹Present address: McMillen Jacobs Associates, 1471 Shoreline Drive, Boise, Idaho 83702, USA.

²Present address: U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office, 2800 Cottage Way, Sacramento, California 95825, USA.

³Present address: College of Nursing, University of Illinois–Chicago, 845 South Damen Avenue, Mail Code 802, Chicago, Illinois 60612, USA.

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Cisco has been identified as a species in need of conservation to promote biological diversity in the Great Lakes (Lee and Schlottmann 1988; Stewart et al. 2013; Bronte et al. 2017). Biodiversity improves the health of the entire Great Lakes ecosystem, which contributes to the social and economic benefits of the region (Great Lakes Regional Collaboration 2005). Recruitment of Cisco remains highly variable, thus restricting their full recovery (Kim and Faisal 2010). Viral hemorrhagic septicemia (VHS) infections in the Great Lakes have raised concerns over collecting and transporting Cisco eggs, and it is imperative to understand how iodophor disinfection can be utilized to prevent VHS from spreading to culture and research facilities as well as to other water bodies (Loch and Faisal 2010, 2011). Similarly, in a more recent study, Harrison (2021) highlighted multiple fish pathogens as potential contributors to the reduced health and survival of Lake Whitefish C. clupeaformis in the Great Lakes. Restoration of Cisco populations has been largely unsuccessful, providing the opportunity to raise Cisco fingerlings for future stocking efforts (Todd 1986). Because stocking success correlates strongly with fish body size at the time of release, further research on rearing practices is needed for potential restocking of Cisco into the Great Lakes (Hoff and Newman 1995; Paragamian and Bowles 1995; Szendrey and Wahl 1995). Through an evaluation of hatchery production attributes, fishery management agencies can develop propagation recommendations to further improve Cisco conservation stocking programs.

Although previous studies have been conducted to understand the stocking of early life stage Cisco, at the time of this study even less had been published about rearing Cisco to larger sizes-essential information for the success of a stocking program (Flüchter 1980; Ludsin and Devries 1997; Pangle et al. 2004). Other coldwater fish, such as Brook Trout Salvelinus fontinalis. Atlantic Salmon Salmo salar, and Rainbow Trout Oncorhynchus mykiss, have been extensively produced in aquaculture, and this information can provide the industry with a better understanding of coldwater fish culture practices (Kinnunen 2000; Fischer et al. 2009). Numerous studies on salmonids and other coldwater fish have established the peak growing conditions by identifying optimum water quality, temperature, and feed (Piper et al. 1982; Kinnunen 2000; Fischer et al. 2009; Summerfelt et al. 2013). Establishing the optimum conditions for rearing practices would assist in maximizing Cisco production and could lower overall costs, which are essential steps for achieving success in Great Lakes Cisco stocking and conservation programs.

Further study of gamete collection, egg fertilization, egg disinfection, egg survival rates, larval feeding, and postlarval development can assist in establishing the best culture practices for Cisco. Some studies conducted with Cisco that were derived from wild gametes and reared to different sizes investigated differences in incubation and rearing temperatures along with differences in food sources in an effort to optimize the survival and growth of wild Cisco (Hale 1970; McCormick et al. 1971; Colby and Brooke 1973; Brooke and Colby 1980). These past studies identified gaps in coregonine culture practices that needed to be addressed in order to move forward with the culture of this species for stocking and conservation work at the time of this preliminary project. More research has been done recently with Cisco and other coregonids, but at the time of this research project in 2010, our research was considered groundbreaking and helped to provide baseline information on intensive culture of Cisco for hatchery propagation.

To address the gaps that had been identified in the literature at the time of this study, three related experiments were conducted with Cisco in 2010, each examining a different area or life stage of culture practices (i.e., egg fertilization, egg survival, and fry development). The three experiments employed random sampling that compared two or more treatments to determine best practices and the impact of different culture protocols. The first experiment attempted to better understand the impact of fertilization technique on egg survival by using both wet and dry fertilization methods (Piper et al. 1982; Malison and Held 1996; Johnston 2008). The second experiment examined egg survival using pre-water-hardening and postwater-hardening iodophor treatment to address fish health concerns. The third experiment evaluated Cisco fry development by using larval diets that were available at the time of the study. These experiments were meant to provide baseline data for Cisco culture, as interest in this species was increasing for potential restoration or rehabilitation in the Great Lakes region.

METHODS

Experiment 1: egg survival by fertilization technique.— Eggs were collected from Cisco adults captured in commercial gill nets near the Apostle Islands area of Lake Superior in early December 2010. On the boat, eggs from four females were immediately stripped and equally divided into two stainless-steel bowls. Pooled milt from eight males was equally introduced into one bowl by utilizing a wet method of fertilization and into the other bowl by utilizing a dry method (Piper et al. 1982; Malison and Held 1996; Johnston 2008). For the dry fertilization method, ova and milt are collected in separate containers and no water is allowed in contact with them until ready for fertilization. Water is added to activate both eggs and sperm; once in contact with water or ovarian fluid, sperm become motile and actively seek out ova and will enter through a small opening in the egg called the micropyle for approximately 10–60 s before they run out of energy

(Johnston 2008). With the wet method, ova and milt are collected together into a "wet pan" with water present, thus activating both eggs and sperm immediately (Johnston 2008). This process was repeated a total of five times, for a total of five replicates of each method. In total, 20 females and 40 males were utilized for this experiment. Following the removal of eggs and milt, the 60 adults were sacrificed and sent to the U.S. Fish and Wildlife Service's La Crosse Fish Health Center (Onalaska, Wisconsin) for bacteriology and virology testing in accordance with American Fisheries Society Bluebook standards (AFS–FHS 2014). Results for *Aeromonas salmonicida, Yersinia ruckeri, Renibacterium salmoninarum*, infectious pancreatic necrosis virus, and VHS virus (VHSV) tests were negative.

After fertilization, eggs were disinfected with iodophor (100 mg/L for 15 min; Syndel, Ferndale, Washington) and were transported to the Red Cliff Tribal Fish Hatchery (Red Cliff, Wisconsin). Eggs were placed in McDonald bell hatching jars (6 L) for incubation. The egg volume of each of the 10 jars was measured and recorded; the volume of eggs ranged between 460 and 500 mL. Eggs were incubated at 7.7°C with flow-through, degassed and aerated groundwater. The flow rate was initially 2.5 L/min and was slowly increased to 4.6 L/min as the eggs developed. Water conditions were monitored daily for dissolved oxygen (average = 10.5 mg/L), total dissolved gases, and temperature with a portable Model TBO-DL6F meter (Common Sensing, Clark Fork, Idaho). A Pinpoint pH meter (American Marine, Ridgefield, Connecticut) was used for measuring pH daily (pH range = 7.2-8.2). Eggs were regularly treated with formalin (Paracide F; Argent Chemical Laboratories, Redmond, Washington) at a concentration of 1,670 mg/L maintained for 15 min (Piper et al. 1982) with a drip feed to limit fungus growth. Dead eggs were siphoned off daily. At 33 d postfertilization (DPF), eyed eggs were measured by using volumetric displacement. Percent eve-up was calculated as the 33-DPF egg volume (mL) divided by the 0-DPF egg volume.

Experiment 2: egg survival by iodine treatment.— Additional eggs for experiment 2 were collected in the same manner and at the same time as the eggs for experiment 1. All eggs were fertilized using the dry method (Piper et al. 1982; Malison and Held 1996). Fertilized eggs were subsequently divided equally into three groups with six replicates per group and were exposed to a one-time treatment with one of three disinfection methods based on information from various hatchery managers and resources: 100-mg/L iodophor for 55 min before water hardening (PRE), 100-mg/L iodophor for 10 min after water hardening (POST), or no treatment (CONTROL). Eggs were transported to the University of Wisconsin–Stevens Point (UWSP) Northern Aquaculture Demonstration Facility (NADF; Red Cliff, Wisconsin). The egg volume of each replicate was adjusted as described earlier (experiment 1), and eggs were placed in McDonald jars (6 L) for incubation.

The 18 jars were connected in-line to provide global control over water quality. Water quality measurements were taken daily throughout the incubation period. Temperature ranged from 7.5°C to 8.0°C, pH varied from 7.29 to 8.16, and levels of dissolved oxygen remained adequately high (O₂ saturation > 81%) throughout the study. Dead eggs were siphoned off daily.

Formalin treatments were administered to all jars during incubation. At 26 DPF, the number of eyed eggs was estimated by using volumetric displacement. Percent eyeup was calculated as the 26-DPF egg volume (mL) divided by the 0-DPF egg volume.

Experiment 3: fry development by feed.— The feeding experiment was conducted in three single-pass, 1,000-L tanks with 0.285-m³ inserts, each having six individual 0.036-m³ compartments. Tanks were supplied separately with heated and aerated groundwater via a 25.4-mm water delivery line. Flow to each compartment was maintained at 2.0 L/min. Water quality was measured daily; average water temperature was 10.5° C, dissolved oxygen concentration was maintained at greater than 9.0 mg/L, total dissolved gas pressure was maintained at less than 101%, and average pH was 7.8. Based on previous studies available at the time of this work (Pangle et al. 2003), all measured water quality variables were within optimal ranges for Cisco production.

Each compartment within a tank was stocked by handcounting 200 fry, resulting in a total of 1,200 fry/tank. The fry density in each tank was 5.5 fry/L. Fry were 7–9 mm long at the time of initial stocking. Based on the small number of fry that were stocked and the volume of rearing space that was available, tank densities were considered irrelevant for this study. Compartments within each tank were considered pseudoreplicates (6 replicates/tank). Dividers in the tanks separated each group of fish from the feed treatments. Each of the three tanks was assigned one of the three diets described in Table 1. Diets were all sized, if needed, by utilizing a tabletop coffee bean grinder and wire screening to accommodate the gape size of the fry and were considered small enough for fry to eat. Fry were fed to satiation four times daily by hand (>25% of calculated total body weight) for 48 d. At the completion of the experiment, 20 fish from each compartment were randomly dip sampled and measured for length and weight.

Statistical analyses.—One-way ANOVA and twosample *t*-tests were used when variability within the groups being compared was similar, whereas the Kruskal– Wallis test and Dunn's test were used when substantial differences in within-group variability were observed. The significance level was set to 0.05 for all tests. When analyzing data from experiment 3 for differences in fish length

TABLE 1. Nutritional components (protein, fat, and moisture) and granule size of the three commercially manufactured diets that were fed to Cisco in experiment 3.

Variable	Artemac ^a	Proton ^b	Silver Cup Moist ^c
Protein (%)	57	54	52
Fat (%)	19	15	14
Moisture (%)	7	7	10
Granule size (mM)	100-500	200-400	200

^aAquafauna Bio-Marine, Inc. (Hawthorne, California).

^bINVE Aquaculture, Inc. (Ogden, Utah).

^cNelson and Sons, Inc. (Murray, Utah). The Silver Cup feed was prepared with a portable coffee bean grinder and then collected with a 200–300-µM sieve.

among diet groups, a linear mixed-effects model was used to predict fish length by diet, with a random effect for compartment.

RESULTS

Experiment 1: Egg Survival by Fertilization Technique

The mean 33-d eye-up percentage for eggs in dry fertilization jars was 67.6% (SD = 10.4%; minimum = 59%, maximum = 85.0%; n = 5) compared to a mean of 34.2% for eggs fertilized with the wet fertilization technique (SD = 10.0%; minimum = 18.9%, maximum = 44%; n = 5). Importantly, every jar of eggs subjected to the dry fertilization technique had a higher percent eye-up than all of the jars containing wet-fertilized eggs (Figure 1). The difference in eye-up egg percentage was on average 33.4 percentage points better for the dry-fertilized eggs (95% CI = 18.6–48.3%; P = 0.0008).



FIGURE 1. Percentage survival (eye-up) of Cisco eggs based upon dry or wet fertilization techniques. Error bars show standard error.

Experiment 2: Egg Survival by Iodine Treatment

The average percent egg survival was 56.6% in the CONTROL group, 53.7% in the POST group, and only 42.9% in the PRE group (Figure 2). However, variation in the egg survival percentage differed greatly between the groups (CONTROL: SD = 0.097; POST: SD = 0.082; PRE: SD = 0.016). To account for the large differences in within-treatment group variability, a Kruskal–Wallis test was used to examine for overall group differences. The Kruskal–Wallis test yielded a *P*-value of 0.011. To evaluate the potential for pairwise group differences, post hoc Dunn's tests were used. These tests identified significant differences between the PRE group and both the CON-TROL group (P = 0.002) and the POST group (P = 0.016) but not between the POST and CONTROL groups (P = 0.22).

Experiment 3: Fry Development by Feed

Mean survival across the three diet groups after 48 d was not significantly different (Artemac: 53.3%; Proton: 56.3%; Silver Cup [SC] Moist: 51%; Kruskal–Wallis rank-sum test: P = 0.23), but there was a large difference in the variability across diets (Figure 3A), with SDs of 1.04 for Artemac, 4.2 for Proton, and 6.8 for SC Moist.

In contrast, there were differences in average weight (ANOVA: P < 0.0001; Figure 3B), with significant differences between all three diet groups (P < 0.05). Proton-fed fish had the largest average weight (0.029 g; SD = 0.0023 g), followed by Artemac-fed fish (0.023 g; SD = 0.0044)



FIGURE 2. Percentage survival of Cisco eggs depending on the three treatments (control, post-water-hardening iodophor treatment [post-WHIT], and pre-water-hardening iodophor treatment [preWHIT]). Post hoc Dunns tests were used to compare all pairwise groups. These tests identified significant differences between the PRE group (Y) and both the CONTROL group (P = 0.002) and the POST group (P = 0.016), but not between the POST and CONTROL groups (P = 0.22) (Z). Error bars show standard error.



FIGURE 3. (A) Percentage survival, (B) average weight (g), and (C) length (mm) at 48 d posthatch for Cisco that were fed three diets (Artemac, Proton, and Silver Cup [SC] Moist). In (A), mean survival across the three diets after 48 d was not significantly different (Artemac: 53%; Proton: 56.3%; SCMoist: 51%; Kruskal–Wallis rank sum test (P = 0.23), with the similarity of the groups denoted by the letter Z. In (B), all three treatment groups were significantly different than each other in pairwise tests using linear mixed effects models, denoted by having different than each other in pairwise tests using linear mixed effects models, denoted by having different than each other in pairwise tests using linear mixed effects models, denoted by having different letters for each treatment letters for each treatment.

and SC Moist-fed fish (0.011 g; SD = 0.0022). Similarly, significant differences in length were observed in relation to diet (overall mixed-effects model: P < 0.0001; Figure 3C), and mean length was significantly different between all pairs of diets (P < 0.0001). In particular, Proton-fed fish had the greatest mean length (18.78 mm; SD = 1.99), followed by Artemac-fed fish (17.06 mm; SD = 1.79) and SC Moist-fed fish (13.69 mm; SD = 1.19).

Although treatment was confounded by system, efforts to maintain similar characteristics in each compartment were effective. In particular, no statistically significant differences were found in mean water quality characteristics across the three tank systems during the study (ANOVA: P = 0.17, 0.61, 0.26, 0.44, and 0.99 for temperature, dissolved oxygen, oxygen saturation, total dissolved gases, and pH, respectively).

DISCUSSION

Egg Survival by Fertilization Technique

Maximizing the survival of eggs when collecting from a wild broodstock for conservation purposes is a concern of fish managers and researchers alike for various reasons. Often, ripe fish are only available for a short period of time or it may be extremely difficult to collect large numbers of mature broodstock that are capable of supplying the optimum number of eggs for the purpose. Methodology that allows for the maximum survival of eggs collected from deepwater, lake-spawning fish such as Cisco is valuable given the short time window and difficulty in collecting broodstock in the Great Lakes during the early winter period from October to December.

The dry fertilization method generated significantly better eye-up percentages (68%) than the wet fertilization method (34%). Dry fertilization offers more exact environmental control and timing over how the eggs are fertilized with milt from specific males as well as better control with the amount of water that is used to activate the milt (Piper et al. 1982; Malison and Held 1996; Johnston 2008). Dry fertilization methodology has historically provided better egg fertilization and eye-up percentages for Walleye Sander vitreus. Arctic Char Salvelinus alpinus. Atlantic Salmon. and Brook Trout at UWSP NADF (Fischer et al. 2009; UWSP NADF, unpublished data). Dry fertilization is highlighted as having more successful fertilization rates for a variety of species and is often recommended (Piper et al. 1982; Malison and Held 1996; Johnston 2008: 31-33; Ontario Ministry of Natural Resources 2009).

Egg Survival by Iodine Treatment

Effective management of biosecurity in a hatchery setting is always challenging, especially when dealing with wild broodstock and the introduction of eggs into a

production facility (Piper et al. 1982; Bebak 1996; Santigosa et al. 2020). Managers are often interested in specific checkpoints to control the introduction of various viruses (e.g., VHSV and others; Kim and Faisal 2010; Harrison 2021) and pathogens, thereby protecting the existing stocks in facilities. The use of iodophors with unfertilized and fertilized eggs has been widely accepted for many species of fish (Piper et al. 1982) but had not been explored with Cisco at the time this study was conducted. With increased interest and concern over multiple fish pathogens and potential effects on various Great Lakes fish populations, it is increasingly important to understand the effects of various chemical treatments on fish eggs and the different stages at which treatment may occur (Loch and Faisal 2010; Kim and Faisal 2011; Faisal et al. 2012; Groocock et al. 2012; Harrison 2021). Recent studies have indicated that iodophor treatments may reduce the hatch rate and survival rate for Walleye and Northern Pike Esox lucius (Tuttle-Lau et al. 2010). Groocock et al. (2012) found that although VHSV-exposed Walleye eggs were treated with various levels of iodophor (50 and 100 mg/L) during incubation through hatch, VHSV persisted longer on Walleye eggs than previously known. These results reinforce the need for careful disinfection of eggs and potentially indicate the need for applying various treatment regimens in disinfection protocols. The results of our testing revealed that utilizing iodophor (100 mg/L for 15 min) on fertilized eggs after water hardening (i.e., POST) resulted in higher survival (54%) than treatment with an iodophor concentration of 100 mg/L for 55 min before water hardening (PRE; 43%). There was no significant difference in survival between the CONTROL group (56.6%; with no iodine treatment) and the POST group (54%; Figure 2). Survival of the CONTROL was lower than anticipated based on our experience and may be attributable to difficulty during the egg collection and fertilization processes for this study on the boat in inclement weather. We opted for higher levels of iodophor for eggs in the treatments due to various discussions with several hatchery managers. The label for Ovadine specifies an application of 50 mg/L for 30 min or 100 mg/L for 10 min, but the hatchery managers indicated that they routinely used 100 mg/L for 15 min and higher for treatment of eggs due to concerns about VHS at the time of this study. We acknowledge that prefertilization treatment and postfertilization treatment of eggs accomplish different objectives and have different outcomes related to vertically transmitted diseases, such as VHSV and others. More research and discussion on prefertilization and postfertilization iodophor egg treatments with Cisco are needed to fully understand the efficiency of this treatment protocol, the survival of treated eggs, and the ensuing larval development. Recently, Harrison (2021) conducted research related to this topic with

Lake Whitefish, which may prove valuable to others in this arena to review.

Fry Development by Feed

Mean Cisco survival rates across the three diets evaluated were not significantly different after 48 d. Fish that were fed the INVE Proton diet had the highest survival, growth, and condition factor throughout the entire project. The Proton diet was designed as a brine shrimp Artemia replacement diet. Although little information is available on rearing larval Cisco on formulated feeds, it appears that advances in commercial feeds through higher quality, a greater variety of protein sources, and delivery form resulted in improved growth and production for larval Cisco. Earlier literature focusing on other species, such as Walleye, Muskellunge E. masquinongy, and Northern Pike (Colesante et al. 1986; Szendrey and Wahl 1995; Larscheid et al. 1999), suggested that fish receiving formulated feed exhibited lower survival and growth than fish that were fed natural diets. In those earlier studies, formulated diets were not able to fulfill the nutritional needs of the larval fish species being reared. More recent studies (Johnston 2008) have indicated otherwise with the new, improved larval diets that are available for fish culture use. Our results show that commercially available formulated diets can provide adequate nutrition for the rearing of larval Cisco. At the time of this study, very few commercial larval diets were available or known for Cisco. Newer commercial diets are now available that have been successfully used for many fish species in combination with intensive feeding strategies and systems (Bruner and DeBruyne 2021).

Fish density was not identified as a limiting factor in this study. Larval fish of other species are reared with higher fish densities utilizing similar systems. Walleye are commonly reared at densities of 20–40 fish/L with the use of commercial diets and intensive tank systems (Bruner and DeBruyne 2021).

Although dividers separated each group of fry from the other groups and feed treatments, with each compartment having an independent water supply and effluent drain, the replicates were considered pseudoreplicates because the fish were present in the same rearing tank. We feel that this study still provides valuable Cisco culture information and insight into various feeds and strategies that were available at the time of this study in 2010. We also believe that the use of new formulated feeds and strategies for commercial-based hatchery production of Cisco results in less labor, higher reliability, and better biosecurity.

Conclusions

Application of the dry methodology is recommended, when possible, in acquiring and fertilizing Cisco eggs for production and conservation purpose to maximize

broodstock utilization and egg collection efforts. From this research, we recommend treating Cisco eggs after fertilization and water hardening to maximize egg survival when using iodophor treatments to alleviate fish health concerns regarding Cisco eggs collected from a wild broodstock, but we understand that some agencies have separate protocols that may not support this practice. More research should be conducted to examine the actual success of iodophor treatments in destroying specific viruses on coregonine species. Based on our study, we recommend using a modern Artemia replacement diet for early life stage rearing of Cisco under the culturing conditions applied in this study. We note that more research and project work are necessary to understand the optimal culture parameters and strategies for optimizing Cisco production in a hatchery setting.

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ORCID

Gregory J. Fischer D https://orcid.org/0000-0001-7222-3594

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