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EXPERIMENTAL INCUBATION OF FISH EGGS IN A MOIST-AIR ENVIRONMENT

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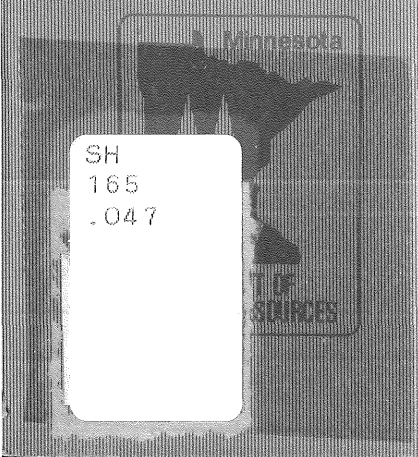

Division of Fish and Wildlife

Experimental Incubation of Fish Eggs in a Moist-Air Environment [1/]

by

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ABSTRACT

Incubation of fish eggs in moist air was investigated as a water-saving alternative to high-volume water circulation used in some fish hatcheries. Eggs of walleye, tullibee and white sucker developed normal appearing embryos with high survival (>80%) to initial hatching when moist air of suitable temperature was circulated through the egg mass and eggs were washed at 6 or 8 hour intervals to remove accumulated wastes. Moist air appears to be a suitable environment for incubating fish eggs but difficulties in the transition of fry from air to a water medium during hatching may preclude its use on a production basis for fishes having protracted hatching periods.

INTRODUCTION

Minnesota's walleye management program includes incubation of 200 to 300 million eggs annually for stocking fry into rearing ponds and public waters. Eggs are incubated in hatching jars on vertical racks (batteries) stationarily housed in special buildings. Operation of a jar-hatchery without water recirculation uses approximately 600 volumes of water daily per unit volume of eggs. More than one million gallons of water is circulated through 500 quarts of eggs during 15 days of incubation. The high water volume used in contemporary hatcheries restricts hatching locations

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[1/] Completion Report, Study 120, D-J Project F-26-R Minnesota

to sites having an adequate supply of surface or ground water and requires high-capacity pumps, heaters, and filters to circulate and condition water for egg incubation. Use of moist air as an alternative incubating medium, appeared to offer a means for substantial reduction in water used which might lead to development of a more efficient and versatile incubation system. Experimental incubation of fish eggs in air was undertaken with the objective of detecting and solving problems inherent to egg incubation in a moist-air environment as a preliminary step in determining the feasibility of an air incubation system. Modifications in experimental methods occurred throughout the investigation as dictated by findings of previous experiments. Therefore, experimental methods are described, in part, with the findings of this report for clearer presentation to the reader.

METHODS

Incubation experiments began in spring 1978 at the Detroit Lakes fisheries station and continued through spring 1980. Walleye was the target species for air incubation studies, but tullibee and white sucker eggs were also experimentally incubated to extend investigational periods and to project findings toward development of a system capable of incubating eggs of a variety of fishes.

Eggs were fertilized by conventional hatchery methods, mucked with bentonite to avoid clumping, washed, and allowed to water harden before being placed into incubation containers. Egg containers were screen-bottom open trays, screened containers suspended vertically and screen-bottom acrylic plastic tubes (Fig. 1). Air temperatures during incubation were regulated by holding egg containers in an upright refrigerator-type

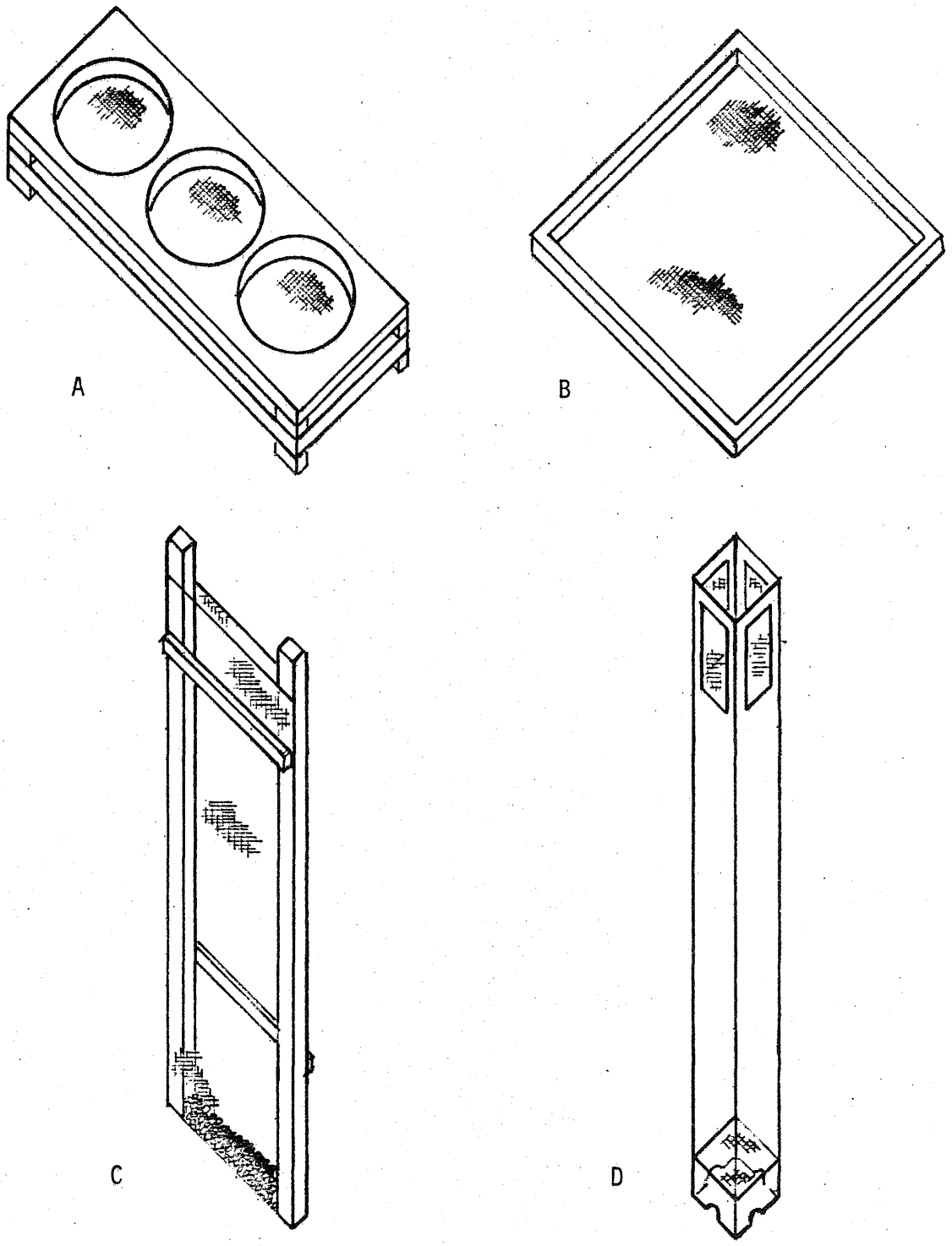

Fig. 1. Egg containers used in experimental incubation of fish eggs. (A), (B) screen-bottom trays, (C) vertical screened container, (D) incubation tube.

incubator (Precision Scientific Incubator, Model 805). [2/] Walleye and sucker eggs were incubated within their natural temperature range for incubation. Tullibee eggs were held at higher than natural temperatures to reduce the normal 160-day incubation period.

Low volume circulation of moist air was provided with diaphragm-type aquarium pumps which pumped air from outside the incubator and bubbled it through water contained within the incubator.

Waste products of metabolism and decomposition were removed at various intervals by washing egg masses with water in wash cycles of five minute durations.

Fungus growth on eggs was controlled with 17-minute treatments in solutions of 1 part formalin to 600 parts water at 48-hour intervals. Samples of 150 to 300 eggs from experimental containers and from eggs incubated in hatching jars on the production battery were examined under low-power magnification during early cleavage stages and the percentages of fertilized eggs recorded. Embryo survival in experimental containers and hatching jars was compared in samples taken periodically throughout incubation. However, since dead eggs in hatchery jars on the production battery lost weight and stratified above the developing eggs it became increasingly difficult to obtain representative samples for comparative survival estimates. Success of experimental incubation was therefore evaluated directly from the estimated percentages of eggs in experimental containers which began cleavage that survived to the time that hatching

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[2/] Mention of brand name does not imply endorsement by the Minnesota Department of Natural Re sources.

commenced. Experimental environments which were unfavorable for incubation resulted in high mortalities, the causes for which were usually apparent. Conditions for incubation were considered to be favorable if more than 80 percent of fertilized eggs developed to the hatching stage.

Walleye fry from eggs incubated in an air environment to the hatching stage were reared during summer months in natural ponds. Fingerling walleyes sampled in summer collections and trapped in fall harvests were examined for physical abnormalities which might have been attributable to incubation in air.

FINDINGS

Air Incubation Prior to Hatching

Walleye eggs incubated on screen-bottom trays stacked horizontally in the incubator (Fig. 1,A) developed normally in moist air (10-13°C) for five days. Retarded embryo development was detected in eggs on the sixth day, followed by rapid mortality in subsequent days. Middle and lower layers of eggs showed highest mortality which was attributed to air blockage from interstitial water retained after each 8-hour washing cycle. Because of the high mortality this experiment was terminated.

In a second trial, walleye eggs were incubated on three 33X33 cm screen bottom trays (Fig. 1,B) at 11-14°C. Trays containing 500, 1000 and 1500 cc of eggs were washed with water at 8-hour intervals and interstitial water was drawn from egg masses by mopping the outer screen surfaces of tray bottoms with a cellulose sponge. With interstitial water cleared from eggs after each wash cycle, eggs in the 1500 and 1000 cc developed to initial hatching ($S > 0.8$) on the ninth day of incubation. Survival was lower ($S \leq 0.6$) in the tray containing 500 cc of eggs because the smaller

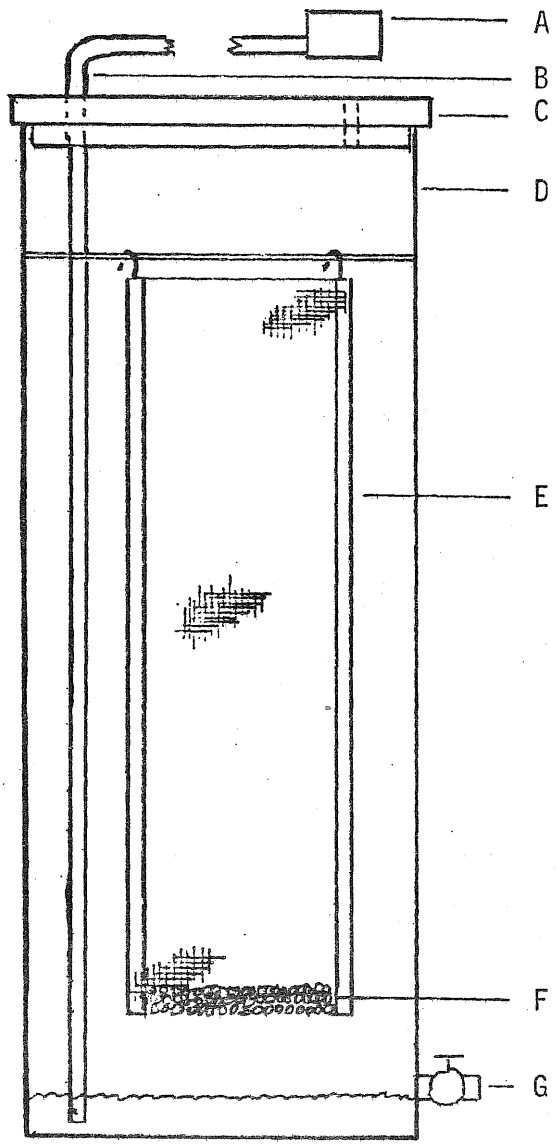

Fig. 2 System for experimental incubation of fish eggs in water-saturated air. (A) air pump, (B) air line, (C) removable top, (D) polyethylene tank, (E) egg container, (F) gravel, (G) water drain outlet.

mass of eggs was not sufficient to prevent some egg esiccation in air which was less than saturated with water. Eggs in the 1500 and 1000 cc trays also lost turgidity between washing cycles but osmotically replenished interior water during 5-minute washing periods.

Failure to maintain a water-saturated air environment in this experiment resulted from condensation and freezing of moisture on the incubator's cooling coils. This was corrected in subsequent experiments by enclosing egg containers within a polyethelene tank within the incubator (Fig. 2). Eggs retained turgidity between washings when air was bubbled through water in the bottom of the tank and allowed to escape through a small hole in top.

The necessity for removing interstitial water was experimentally demonstrated, but mechanical removal (sponging) presented design problems for any practical air incubation system involving large numbers of eggs. An alternative method for clearing eggs of interstitial water was tested in screened containers which supported vertical egg columns 1.3 X 9 X 36 cm (Fig. 1,C). Gravity drainage cleared interstitial water from all but the lower 2.5 cm of the egg column. Drainage of the entire egg mass was accomplished when lower eggs in the column were replaced with 2.5 cm of aquarium gravel. Vertical screened containers holding 230, 300 and 400 cc of walleye eggs were suspended within the polyethylene tank and incubated at 11°C. The tank was filled with water and drained at 6-hour intervals to flush waste products from the eggs. Walleye eggs developed to initial hatching ($S > 0.8$) on the 14th day of incubation.

White sucker eggs were also successfully incubated in this sytem to initial hatching ($S > 0.8$) in 9 days at 13°C.

To test the duration of embryo development in air without removal of accumulated wastes, tullibee and walleye eggs were air incubated in

separate trials without a periodic washing cycle. Tullibee eggs in water-saturated air at 9°C. retained their turgidity and appeared to develop normally with high survival to the stage of beginning eye pigmentation on the 23rd day of incubation but suffered high embryo mortality on the 24th day. Eggs in lots which were washed daily developed with high survival for 26 days, but daily washings were of insufficient frequency to maintain high embryo survival throughout the entire incubation period.

Walleye eggs incubated in water-saturated air at 11°C developed to shortly before detectable eye pigmentation in seven days without washing metabolic wastes from the egg mass, but suffered high embryo mortality on the eighth day of incubation.

Hatching of Fish Eggs in Air

When air-incubated walleye eggs commenced hatching, most fry were dead when washed from egg masses at 8 or 6-hour intervals. By increasing the washing frequency to 3-hour intervals, most fry washed from egg masses were alive and displayed normal swim-up patterns of early behavior. However, as the rate of hatching increased the structure of the egg mass in air began to collapse closing interstitial spaces and resulted in unacceptably high mortality of both hatched and unhatched embryos. To avoid high mortality in air incubation containers eggs were returned to circulated water in hatchery jars for hatching or the eyed-eggs were stocked directly into ponds. Fingerling walleyes collected from ponds in summer sampling and in fall harvest appeared normal in physical development.

White sucker eggs incubated in air in vertical screen containers began to hatch on the 9th day and completed hatching in less than 6 hours. Fry which escaped through the screen mesh survived in water retained in the lower level of the tank. Most fry which were unable to escape from the screen con-

tainer were dead when examined six hours after hatching.

Egg Incubation in Aerated Water

Experiments showed that fish eggs can be successfully incubated in air up to hatching but require a suitable water environment for successful hatching. Transition from air to a water environment presents problems in design and operation of a practical large scale incubation system. Rather than continue experiments with air incubation systems, an experimental incubator was constructed in which eggs were incubated in water with air bubbled through the egg masses to provide oxygen and to circulate eggs and water.

Egg incubation tubes of 1/8-inch acrylic plastic were 81 cm in length including a 13 cm top section of Nitex HC 3-600 screen (Fig. 1,D). Tubes of two designs, square or round in cross section, were tested. Round tubes were 5.7 cm inside diameter and square tubes were of similar inside dimension. Tube bottoms of Nitex screen material retained eggs in the tubes and allowed air and water to circulate through the egg masses. Twelve incubation tubes were placed side to side in three rows of four tubes each within a thermally insulated water bath tank (Fig. 3). The base of each tube rested on the upper surface of an air manifold chamber with air-jet holes positioned to direct air into the tube base below the screen bottom. Two aquarium pumps (Whisper 800 Air Pump)[2/] circulated air through the system. Semicircular openings in the tube base allowed tubes to straddle 3/8-inch water lines which were connected through a control valve to the hatchery water system. Water-jet holes in the water lines directed water flow into the base of each tube. Accordingly, each incubation tube in the water bath was provided means for air and/or water flow through the incubating egg mass. Water containing metabolic wastes was discharged through a water surface outlet in the insulated tank. Initial incubation trials with

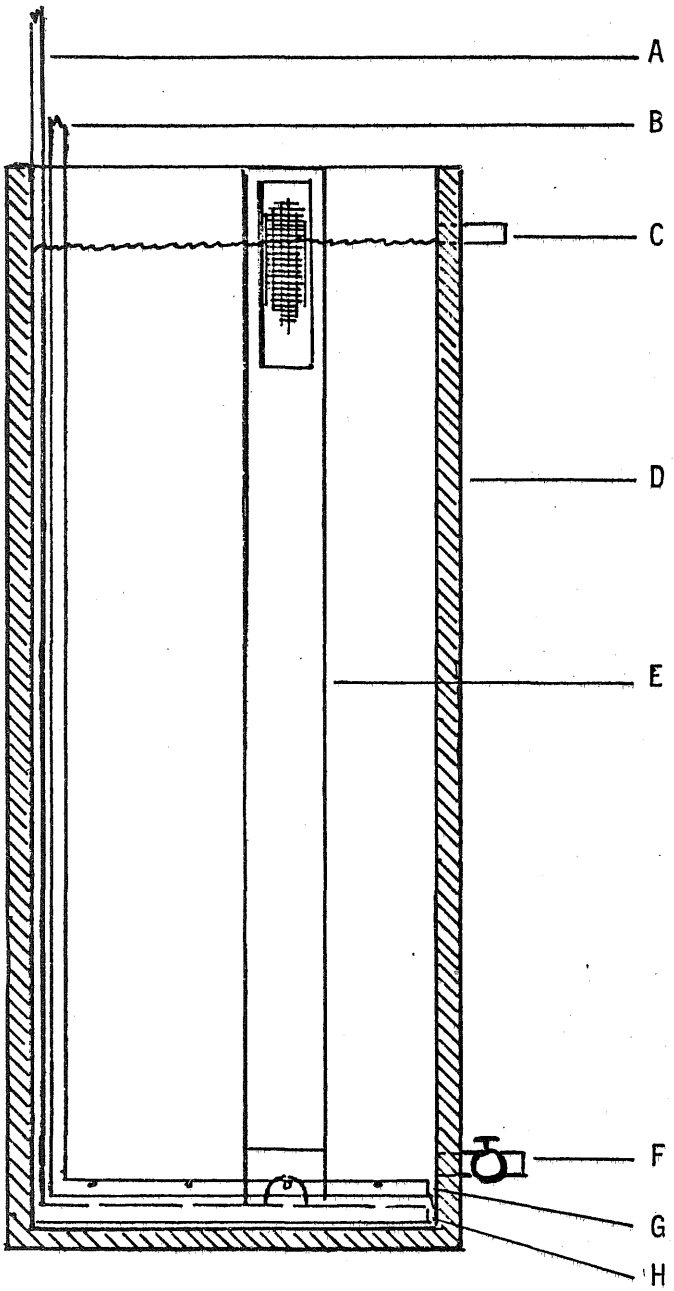

Fig. 3. Experimental incubator with air and water circulation systems. (A) air line, (B) water line, (C) water surface outlet, (D) insulated tank, (E) incubation tube, (F) water drain outlet, (G) water manifold, (H) air manifold.

tullibee eggs resulted in high mortalities from too vigorous air circulation. Eggs were killed from physical shock in the sensitive developmental stages before blastopore closure. When air-flow was controlled to a gentle bubbling action, tullibee eggs were successfully incubated to initial hatching (S> 0.8) in 63 days at 2.8 - 6.7°C. Eggs were successfully incubated in both round and square tubes but fry hatched in tubes did not survive.

Walleye eggs (12.75 quarts) incubated in this system developed to initial hatching (S> 0.8) in 15 days at 10-11°C. Water exchange at a flow of 11 liters per hour prevented a toxic accumulation of wastes. Dissolved oxygen was maintained near saturation through 13 days of incubation and then dropped to 6-7 ppm O_2 prior to hatching.

Walleye fry hatched in aerated incubation tubes died soon after hatching. Increasing the water exchange, from 11 liters per hour to 176 liters per hour, failed to prevent fry mortality when air was bubbled through the tubes. Fry survived when air circulation was turned off and oxygenated water was circulated through each tube at a flow rate of approximately 250 ml per minute.

High fry mortality in aerated incubation tubes appears to have resulted in part from physical damage sustained by fry while trapped within the egg mass, but underlying causes were not fully investigated.

DISCUSSION

Moist air appears to be a suitable medium for incubating fish eggs (walleye, tullibee and white sucker) throughout incubation prior to hatching. Normal embryo development and high survival can be attained in systems which provide: 1) means for removing interstitial water to allow sufficient air circulation through the egg mass, 2) suitable incubation temperature,

3) removal of toxic wastes in periodic flushings with water, and 4) control of fungus growth with periodic fungicide treatments.

Eggs retain turgidity in water-saturated air but lose interior water in unsaturated air. However, eggs can be successfully incubated in air somewhat less than saturated with water if washed at sufficiently frequent intervals and allowed adequate time in each washing cycle to regain their turgidity.

Eggs in early stages of development appear to develop normally in moist air for days without washing accumulated wastes from the egg mass, but when embryo development approaches the eyed stage, wastes must be removed at washing intervals not exceeding eight hours to maintain high survival. Egg masses which contain a high proportion of dead eggs may require more frequent washing to avoid toxic conditions from products of decomposition.

Design and construction of water efficient air incubation systems for fish eggs appears to be feasible and would be of practical use in fish culture if management goals can be achieved by stocking eyed-eggs or if the transition from air to water in the hatchery can be facilitated by inducing eggs to hatch in a short time period. Eggs of the white sucker which completed hatching in less than 6 hours are more adaptable to culture in an air incubation system than walleye eggs which have a more protracted hatching period. Hatching of walleye eggs extends for 2 or 3 days before the remaining unhatched eggs can be induced to hatch. It appears that a suitable air incubation system for walleye eggs requires a separate system for water circulation during hatching. Rather than pursue development of a dual system, future efforts to design less costly and more efficient incu-

bating equipment will be redirected toward the design and testing of a compact and portable water-recirculating incubator. Water volume requirements for operation of such a system are expected to be approximately the same as the quantity of wash-water required for a system incubating fish eggs in air.

Although the air incubation system presented unresolved problems in effecting the transition of fry from an air to a water medium on a production basis, awareness among fish-culturists that fish eggs can be successfully incubated in air throughout the entire incubation period prior to hatching may suggest innovative uses in fish research or management.