

Chapter 2

Reproductive Biology and Spawning

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An understanding of the basic mechanisms that control reproduction is an important prerequisite for the efficient commercial culture of any species. The case studies that accompany this chapter deal primarily with techniques to artificially propagate walleye. In order to furnish the reader with the underlying rationale for the development of these specific techniques, we have provided a brief description of the reproductive biology of walleye in the first half of this chapter. The second half summarizes walleye propagation techniques and highlights some of the different spawning, incubation, and hatching protocols used by walleye culturists.

Walleye reproduction

Sexual maturity in walleye is dependent on both the size and age of the fish and, therefore, dependent to some extent on ambient water temperatures and food availability (Ney 1978). In the wild, males generally mature at 2–4 years and at a size greater than 11 in (280 mm) in total length (TL). Females mature at 3–6 years and at a size greater than 14 in (360 mm) in TL (Scott and Crossman 1973). With proper control of water temperature and photoperiod, captive broodstock can be successfully spawned (Nagel 1985; unpublished data). Intensive culture can provide improved food availability and environmental conditions that are more conducive to rapid growth than those generally found in the wild, thereby resulting in early maturation (our unpublished observations).

Walleye spawn annually in the spring, when water temperatures reach 38–44°F (3.6–6.7°C), which is shortly after ice breakup in most locations (Becker 1983). The spawning season may begin as early as

February in the southern extremes of the walleye's range, and end as late as July in the northern extremes (Hokanson 1977). Peak spawning activity occurs at water temperatures of 42–50°F (5.6–10°C; Becker 1983), and generally lasts for 1–2 weeks at any one location. The level and duration of peak spawning activity can be affected by rapid changes in the weather.

Courtship behavior has been described by Ellis and Giles (1965). In brief, it is characterized by approaches and contacts, with no continued relationship between any particular pair of fish. The spawning act culminates in a rush to the surface by a female and two flanking males, with other males in close pursuit (Priegel 1970), and a turning or pushing of the female onto its side which indicates spawning has occurred.

Eggs are broadcast onto suitable substrates, and walleye do not build nests or provide any parental protection (Becker 1983). Typical spawning sites include gravel and rubble shoals (Johnson 1961), gravel bottoms of inlet streams and rocky wave-washed shallows (Becker 1983), or flooded wetland vegetation (Priegel 1970). Females can completely spawn in one night (Ellis and Giles 1965). O'Donnell (1938) observed that 200–300 eggs were released during each spawning act, with the acts repeated at 5-min intervals. The number of eggs produced and released is related to body size; it averages 27,000/lb (60,000/kg; Stickney 1986), and ranges from 12,500–55,000/lb (28,000–120,000/kg; Smith 1941; Wolfert 1969).

Like most temperate freshwater fishes, the reproductive processes of walleye are controlled by environmental cues, and the annual reproductive cycle is geared so that

progeny are produced in the spring when conditions are most favorable for their survival (Redding and Pattiio 1993). Seasonal variations in photoperiod and water temperature elicit gonadal and hormonal changes that result in spawning. The annual cycle for walleye can be characterized by changes in gonadosomatic indices (GSIs; the ratio of gonad weight to total body weight), gonadal histology, and serum levels of steroidogenic hormones, particularly testosterone (T) and 11-ketotestosterone (11-KT) in males and estradiol-17 β (E₂) in females. The following description of the annual cycle of walleye (Malison et al. 1994b) pertains to fish captured from central Minnesota, and the timing of specific stages of gonadal development may vary by one month or more in walleye from other latitudes.

Just after spawning, males exhibit low GSIs and the testes are comprised primarily of nongerminal tissue. A few scattered lobules of spermatogonia are present at the periphery of the testes. Serum levels of T and 11-KT are low at this time. By late summer, there is little increase in GSIs or androgen levels, but in the majority of males an increased number of spermatogonia and a few spermatocytes can be observed. By early fall, increased spermatogenic activity causes GSIs to increase. In mid-fall, approximately one-half of the germ cells appear to be mature spermatozoa and GSIs continue to increase. Additionally, increases in serum levels of T can be measured. By midwinter, almost all of the germ cells present are spermatozoa, and some milt can be expressed. By early spring, there are few additional changes in the physical appearance of the testes, but continued increases in the serum levels of T as well as elevations in serum 11-KT can be measured. Finally, at spawning, sharp decreases in the GSIs and both androgen levels occur, as spermatozoa are expelled and the gonads return to mostly non-germinal tissue.

Ovarian development in walleye is group synchronous, (Malison et al. 1994b), meaning that two populations of oocytes at different developmental stages can be distinguished at some point during the annual reproductive cycle (Nagahama 1983). One of these populations consists of a heterogeneous group of small oocytes, and the other consists of a group of larger oocytes developing synchronously for the next spawning season.

Just after spawning, the GSIs of female walleye are low, and ovaries are comprised of oogonia and nonvitellogenic oocytes in equal numbers. A few

unspawned vitellogenic oocytes are still present, and serum E₂ levels are low. By late spring, ovaries are already filled with a large number of nonvitellogenic oocytes, indicating that female walleye have a relatively short post-spawning quiescent period. By midsummer, yolky vesicles can be observed in most of the oocytes. During autumn, with the onset of exogenous vitellogenesis and a corresponding increase in mean oocyte diameters, GSIs rise rapidly and serum E₂ levels increase sharply to their highest levels. Increases in oocyte diameters and GSIs continue until late January, when the oocytes appear to be near maturity (Malison et al. 1994b). Serum E₂ levels steadily decline throughout winter and spring.

Shortly before spawning, the oocytes undergo final maturation and ovulation. There is some evidence that 17 α , 20 β -dihydroxyprogesterone (17-20P) is the steroid responsible for the final maturation process in walleye (Pankhurst et al. 1986; Barry et al. 1995) which occurs just prior to spawning. The stages of final oocyte maturation are characterized by changes in the germinal vesicle (GV), which breaks down during maturation, and the coalescence of the oil droplet (Figure 1, opposite page). Maturing oocytes can be classified into five distinct stages: (1) those having a centrally located GV with many small oil droplets present; (2) those showing migration of the GV, in which the GV is located between the center and the periphery, and numerous oil droplets are still present; (3) those having a GV located at the periphery, and fewer, larger oil droplets are present; (4) those in which germinal vesicle breakdown (GVBD) has occurred, and the oil droplet has coalesced; and (5) oocytes that have ovulated and have been expelled from the follicle.

Spawning occurs shortly after ovulation, and the release of the eggs results in rapid decreases in GSIs. Eggs must be fertilized within 2 min after contact with water. Sperm are active and viable for less than 1 min after contacting water. The eggs have an adhesive coating that can act to attach them to the substrate over which they have been broadcast. Over the next several hours, the eggs swell to about twice their original size, a process known as “water hardening”, and lose their adhesive quality (Becker 1983). The development of walleye eggs (Figure 2, page 138) is greatly dependent on temperature. Niemuth et al. (1959) observed that walleye eggs hatch in 26 d at a water temperature of

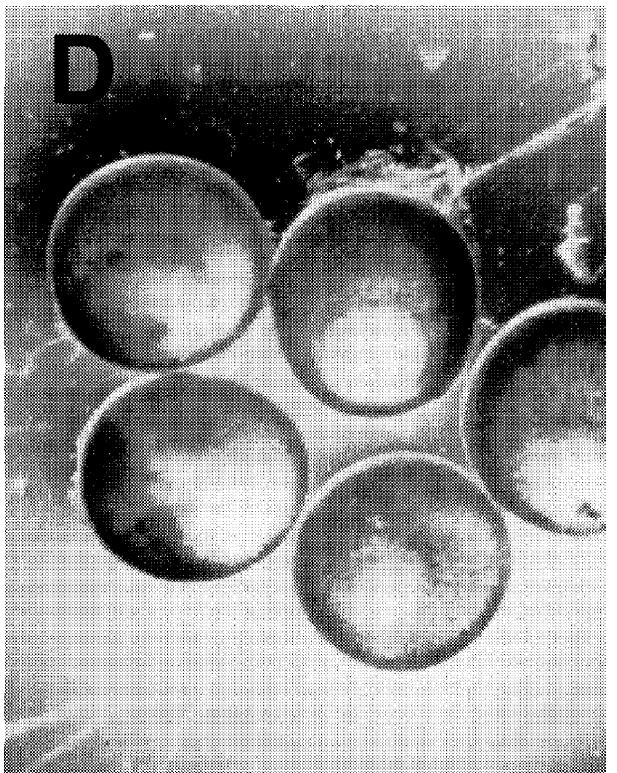
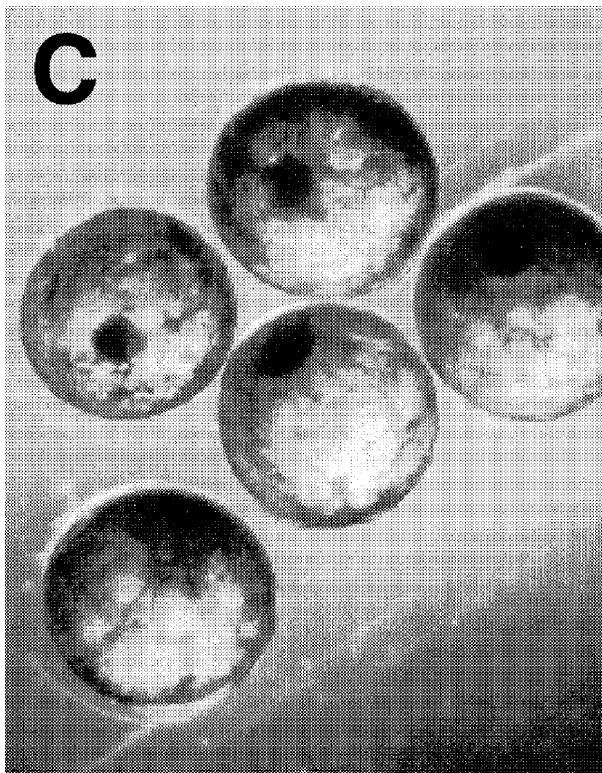
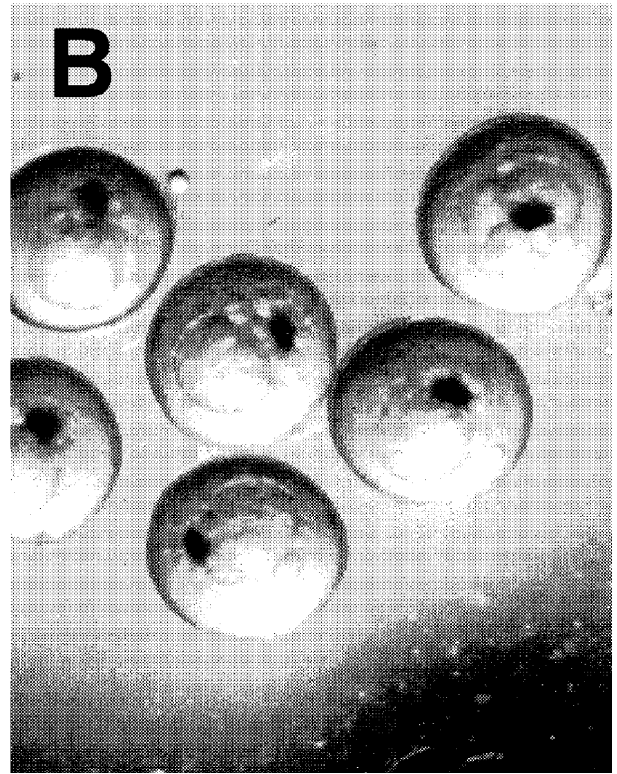
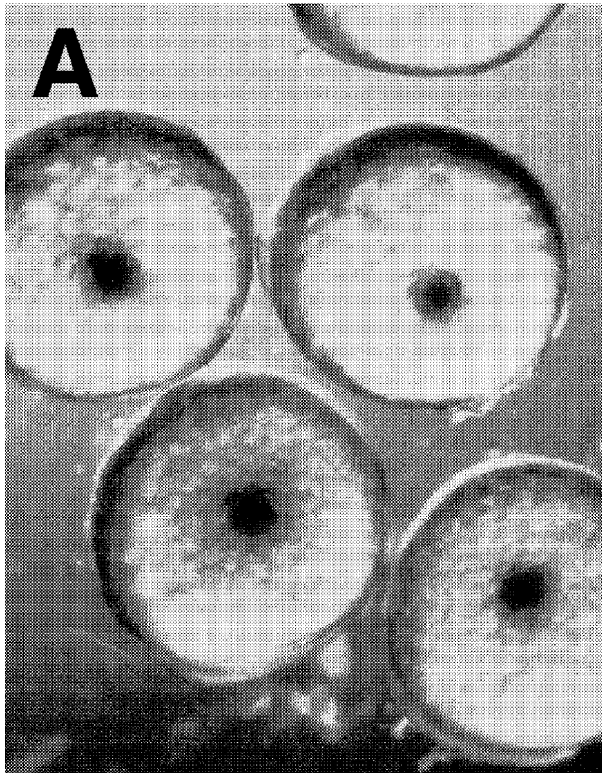


Figure 1. Migration and breakdown of the germinal vesicle during the final maturation of walleye oocytes. A) centrally located germinal vesicle (GV), B) migrating GV, C) peripheral GV, D) germinal vesicle breakdown (GVBD).

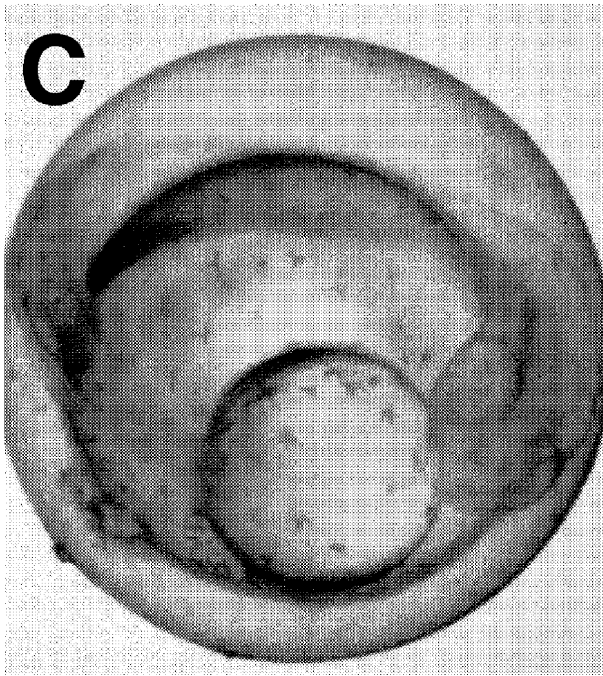
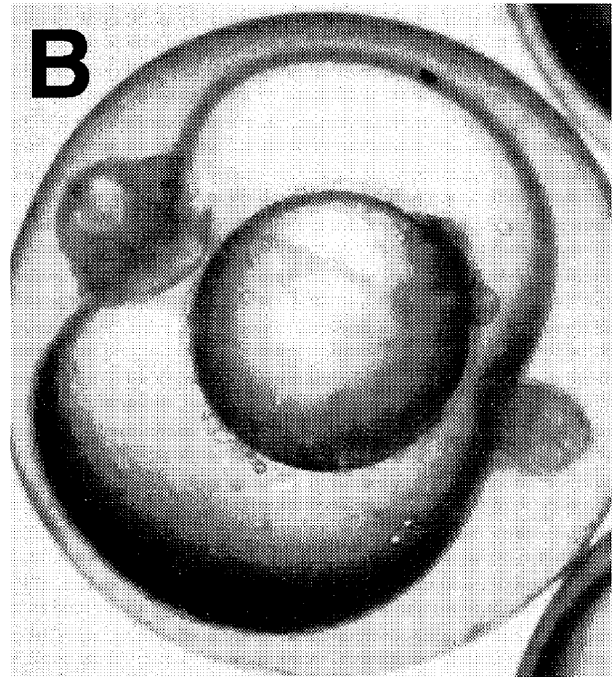
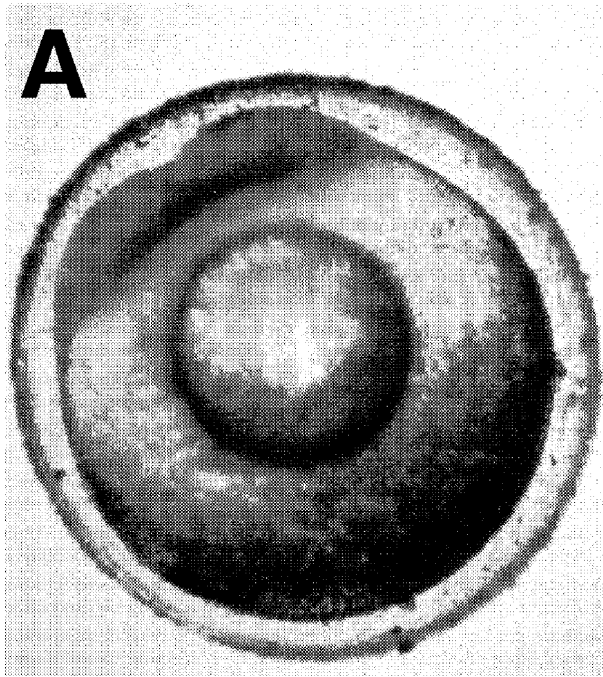
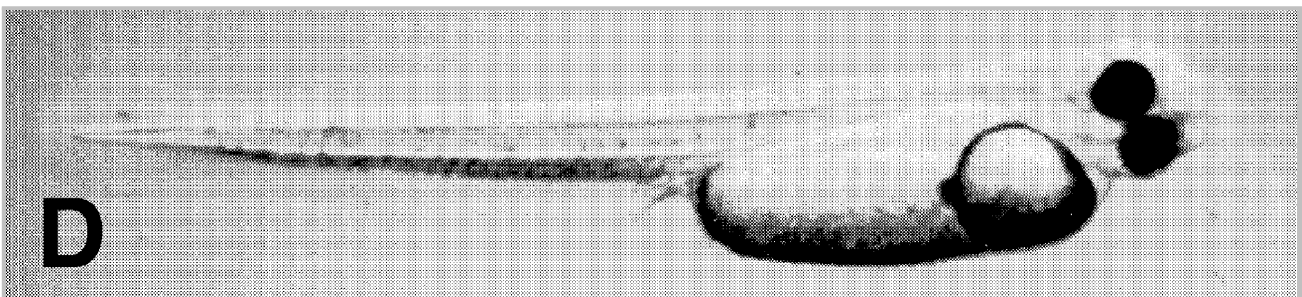


Figure 2. Development of walleye embryos incubated on a slowly rising temperature regimen (50–60°F) that leads to hatch in 11–13 d. A) Approximately three and one-half hours after fertilization the first cleavage furrow forms, and one cell becomes two. The cell cap is developing in the upper left corner of the egg. Most of the remainder of the egg consists of yolk and a centrally-located oil droplet. B) Four d after fertilization, the developing embryo is wrapped around the yolk and oil droplet. The head and eyes are in the upper left corner of the photograph. C) A walleye egg seven d after fertilization, the head of the developing fish is in the right-hand side of the photograph. D) A newly-hatched walleye fry.



40°F (4.4°C), in 21 d at 50–55°F (10–12.8°C), and in 7 d at 57°F (13.9°C).

At hatch, walleye fry average 0.3 in (7.6 mm) in TL and can range in size from 0.25–0.37 in (6–9.5 mm) in TL (Priegel 1970). Generally, the yolk sac is absorbed by the time the fish reach 0.37 in (9.5 mm) (Houde 1969). According to Forney (1966), fry are photopositive and pelagic, and undergo the transition from endogenous to exogenous feeding 1–6 d after hatching. Others, however, have observed that walleye fry do not have a functional mouth until they are at least 3 d old (Robert Summerfelt, Iowa State University, personal communication).

Walleye propagation

Propagation of walleye begins with obtaining broodstock. Although a few hatcheries have captive broodstock, most operations depend on the capture of wild walleye broodstock. The case studies that accompany this chapter describe various broodstock collection methods, such as electroshock, gill nets, and a variety of trap nets. Generally, the methods are tailored to the spawning site and experience of the collection crew. In all cases, however, an emphasis should be placed on minimizing the stress on the fish while maximizing the quality and quantity of gametes collected.

The first fish to be caught during the spawning run are generally males, and early in the season males produce copious amounts of milt. Late in the season, males can become difficult to locate, and can contain much smaller volumes of milt. This problem can be resolved by using one of several sperm extenders and storage techniques (see some of the accompanying case studies). To insure high fertilization rates, the quality of semen should be determined prior to its use. Contaminants such as feces, mucous, and particularly water can significantly affect both the duration that milt can be successfully stored and its fertilization capacity. Sperm viability or motility are usually characterized by activating a sample with water and estimating the percentage of motile spermatozoa and the duration of their activity. Techniques to measure sperm motility are described in some of the case studies accompanying this chapter.

Most walleye culturists prefer to work with ripe and running females; i.e., females whose eggs have under-

gone ovulation. Although careful handling is required to reduce egg loss, these fish are immediately available for spawning, and because they can be quickly returned to the wild they are subjected to minimum handling stress.

Several walleye culturists have described procedures for using “green” or unripe females. Tested protocols range from simply allowing green females to ovulate in captivity, to injecting them with various gonadotropic hormones. The identification of the stage of final oocyte maturation (described above) can be beneficial when working with green females, and by doing so one can decide to keep a green female for ripening in captivity or to release it for possible recapture. Oocytes can be easily collected from green females with a cannula; the oocytes are then placed into a clearing solution (ethanol:formalin:glacial acetic acid; 6:3:1, v/v) and after 3–5 min observed under a microscope or hand lens. Fish having eggs in the early stages of final maturation (prior to stage 3) often will not ripen in captivity without the use of hormones. At our laboratory, we do not keep females unless the eggs have at least reached stage 4. It is important to note, however, that our captured broodstock are held in indoor tanks. Other holding facilities, such as net pens in a natural waterway, may allow less mature fish to ripen. Another important factor when holding wild walleye is the degree of stress to which the fish are subjected. Repetitive handling and prolonged captivity can result in high levels of mortality either during captivity or after release.

Several hormone treatments can induce ovulation in green female walleyes. Hormone preparations like human chorionic gonadotropin (HCG), des-Gly¹⁰-[D-Ala⁶]-LHRH-ethylamide (LHRHa), carp pituitary extract, and several maturational steroids have been successfully used to induce final oocyte maturation in many fish species including walleye (Donaldson and Hunter 1983; Goetz 1983; Barry et al. 1995). Although such hormone treatments can be effective and are generally not harmful to the fish, current FDA regulations severely restrict the use of hormone injections in walleye. Another potential problem with using hormones is that poorly timed or improperly dosed treatments can be ineffective or induce ovulation prior to final oocyte maturation, the latter resulting in non-fertile eggs. Several successful methods of using hormones to induce spawning in walleye are discussed in the accompanying case studies.

Recent studies conducted by investigators from the University of Wisconsin-Madison and the University of Nebraska-Lincoln (Malison et al. 1994a) have shown that environmental manipulations and hormone injections can be successfully used to advance spawning in walleye. In one study, over 100 adult walleye were captured in the autumn and stocked into ponds at the Gavins Point National Fish Hatchery, Yankton, South Dakota. In late January, February, and March (as much as 2 months before normal spawning) groups of these fish were transferred to indoor tanks at the Calamus State Fish Hatchery, Burwell, Nebraska. Water temperature was raised from 2-10°C over a 1 week interval; photoperiod was held at 12h light: 12h dark; fish were subjected to one of four injection regimens: saline control (d 0 and 2), HCG (150 IU/kg on d 0 and 500 IU/kg on d 2), LHRHa (35 IU/kg on d 0 and 100 IU/kg on d 2), or HCG at 150 IU/kg on d 0 and 17-20P at 2 mg/kg on d 2. Over 60% of the females treated with HCG or LHRHa spawned 6-8 d after injection. Eggs from these fish were fertilized, incubated, and hatched; the survival rates of these fry were comparable to those produced during the normal spawning season (Robert Summerfelt, Iowa State University, personal communication). As a result of these studies, the production of walleye fry can now be extended for over 3 months of the year. The availability of walleye fry during this interval should increase the efficiency of existing intensive fry culture systems (e.g., by allowing double- or triple-cropping), aid public and private hatcheries in their efforts to produce larger walleyes for stocking, and facilitate research on the intensive culture of walleye fry.

Methods for stripping and fertilizing walleye eggs generally follow one of two protocols. One technique is the dry method, in which eggs and sperm are mixed together before being activated by the addition of water. The second technique is the wet method, in which eggs or sperm are stripped into water, and the other gametes are quickly added. Each technique has its advocates. Proponents of the dry method cite better quality control and improved hatch rates, whereas those who espouse the wet method assert that they have a more efficient production-line operation. Regardless of the method used, care and attention to detail can make the difference between a 20% and 80% hatch rate. Because of such differences, egg stripping and fertilization techniques are critical steps in the propagation of walleye.

As previously mentioned, walleye eggs become highly adhesive shortly after they are exposed to water. During artificial propagation, eggs will stick to one another if left untreated. Such clumped eggs will not roll properly in incubation jars, and even small clumps can interfere with oxygen distribution, resulting in smothered eggs. Large numbers of dead eggs provide an excellent substrate for fungus, which can ultimately lull all the eggs in a jar.

The case studies that accompany this chapter describe a variety of methods to alleviate clumping during spawning. Suspensions of compounds such as bentonite (clay), Fuller's earth, starch, or pond muck can be applied to the eggs; the fine particles coat the eggs and reduce egg-to-egg adhesion. Other methods include the use of a protease solution, which is effective but quite expensive when used on a large scale (Krise et al., 1986; Krise 1988), or a tannic acid solution (Colesante and Youmans 1983). The oldest known method is simply to continually stir the eggs for an extended period of time (up to 4 h), which remains an effective but extremely manpower intensive technique.

Following treatment to prevent clumping, the eggs are allowed to water harden for 2-3 h. During this process, eggs are normally held undisturbed in buckets, coolers, or fine-meshed nets, and during this time the eggs swell to about twice their original size. After water hardening, the eggs are loaded into incubators. The most common incubators used for walleye eggs are jars supplied with an upwelling flow of water. The continuous water flow rolls the eggs, it provides oxygen, and removes metabolites. As mentioned previously, the time from fertilization to hatch is highly dependent on temperature and can range from 7-26 d or more. During incubation, eggs are normally treated with formalin (we use a 1:1000 static bath for 15 min once daily) to inhibit fungal growth. Hydrogen peroxide is currently being evaluated as a replacement for formalin in this regard.

Eggs are usually counted by volumetric displacement after water hardening, with sub-samples of eggs from different batches measured by hand or with calibrated egg troughs that provide an estimate of the number of eggs/quart (L). Walleye eggs generally average 80,000-150,000 eggs/qt (85,000-160,000 eggs/L), and egg size varies both within and among walleye populations. Moodie et al. (1989) suggested that larvae from small eggs within a population had relatively high

mortality rates, but that among populations small egg size was not a disadvantage.

One of the most critical times for walleye culture is during hatch. A jar of walleye eggs can take from several hours up to several days to completely hatch. The process can be accelerated to some extent by increasing the water temperature by several degrees once the eggs begin to hatch. In most facilities fry are allowed to swim out of the hatching jars and into a centralized collection tank. Care must be taken to prevent screens from plugging, and the deterioration of water quality that can result from the accumulation of egg shells, oil droplets, and dead eggs and fry. Solutions to this problem include redundant screens, self-cleaning designs (e.g., bubble curtains adjacent to screens), and a good measure of attentiveness.

Acknowledgments

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Walleye Spawning Operations at Bonny Reservoir, Colorado

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Introduction

Bonny Reservoir is a 1,924-acre (778.6-ha) plains reservoir constructed and operated by the United States Bureau of Reclamation. The Colorado Division of Wildlife and Colorado Division of Parks and Outdoor Recreation own the conservation pool and manage fish, wildlife, and recreation. It is located on the South Fork of the Republican River in Yuma County, 27 mi (43.44 km) northeast of Burlington, Colorado.

Walleye spawning at Bonny historically starts on or about March 20. Thirty to 45 million walleye eggs are collected annually from wild captured broodfish. Average hatching rate is 65–75 %.

Methods

A two-slip boat house in a protected cove adjacent to the dam is modified for walleye spawning. A floor is constructed over one boat slip that contains four, 4 x 4 x 4 ft x 1 in (1.22 x 1.22 x 1.22 m x 25.4 mm) mesh holding nets, a 100-gal (378.5-L) holding tank for receiving broodfish, a spawning table, and an oblong shallow (50 gal, 189 L) tank for floating egg boxes during the egg hardening process. A submersible electric pump provides water for all phases of the spawning process. Overhead PVC pipes and valves deliver water where needed.

Standard neutral buoyancy 2.5-in (63.5-mm) bar mesh gill nets, 6 x 200 ft (1.83 x 60 m) are used to capture brood size walleye. Nets are deployed daily at sunset parallel to the dam riprap and in sand/rock rubble shoreline areas. Net placement is in 3–6 ft (0.91–1.83 m) deep water.

Gill nets are checked at 0700 h by a boat crew of 3–5 people. Two crew members pull the nets while 1–2 personnel remove broodfish. The sex of a netted fish is

noted before removing, because removal techniques are different due to average size and body shape differences between sexes. Males are usually removed from the nets head first, while females are backed out of the net to minimize egg loss. Standard net picks are used and additional care is given when in the opercular area to prevent gill damage. Gravid females that are expelling eggs while in the net are held tail up or on their back, and the vent is blocked with a finger to prevent further egg loss while handling. Brood fish are placed in a 100-gal (378.5-L) tank containing lake water and supplied with 1–2 L oxygen/min dispensed with airstones. Fresh water is supplied as necessary with a 12-v pump. A burlap tank cover is used to reduce potential stress from bright sunlight.

The standard project boat is 18 ft (5.4 m) long, powered by a 150 hp outboard engine. The size and power of this equipment is deemed necessary due to payload, distance from the boathouse (1–1.5 mi, 1.61–2.4 km), and potential foul weather conditions.

Broodfish are returned to the boathouse when the carrying capacity of the tank is reached. The actual number of broodfish in any haul varies due to fish size and sex ratio, but generally 25–50 fish can be held safely in the tank. Fish are transferred to a holding tank inside the boathouse. The tank is supplied with recirculating lake water and provided with 1–2 L oxygen/min. An overhead counter-weighted submersible pump is lowered to empty and refill the boat tank for additional trips to the nets. Broodfish are sorted by sex and ripeness. Males are placed in a 4 x 4 x 4 ft (1.22 x 1.22 x 1.22 m) holding net (1-in ,25.4-mm bar mesh) suspended through the floor of the boat house. “Green” females are held in a similar holding net. They are checked each morning and ripe fish are stripped. Fish that are still green are marked with a hole punch in one

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lobe of the caudal fin and held back another day. Females that have not ripened after two such marks are released. Males as needed are placed in a tub with oxygen and recirculating water on the spawning table.

The dry method of spawning is used in Colorado. A female and the spawning pan is wiped dry and eggs are extruded from the fish into the pan by firm pressure to the abdomen. We have found that wearing rubber surgical and/or wool gloves and using a closed finger rocking motion from the tips of the fingers to the back of the hand is an effective method for stripping eggs. This technique is thought to be less harmful to the fish. Less scale loss and mucus production is observed compared with other techniques that have been used. However, personnel with small hands may have difficulty using this technique. Large females (8-15 lb, 3.6-6.8 kg) are always handled by the head and tail rather than by the tail only. Handling a large fish by the tail only can result in skeletal damage.

Fresh semen from two males is used to fertilize the eggs. Extended semen is used when males are in short supply. Fertilized eggs are covered with water and stirred for 90 s with a feather. Water is then decanted from the eggs. This step is done as quickly as possible, and can require additional repetitions if blood or fecal matter is present. Clumping will occur if too much time is spent cleaning the eggs.

After fertilization and cleaning, a mixture of Fullers Earth and water (3-4 cups Fullers earth to 1 gal (3.785 L) of water) is added to the eggs and stirred for an additional 90 s to remove the sticky matrix that causes eggs to clump during incubation. The mud solution is decanted and the eggs are washed with clean water to remove residual mud, blood, and excreta.

Eggs are then poured into a Saran cloth (32 x 32 mesh/in) covered egg box. Each box is capable of holding 2-3 million eggs. The boxes float in a shallow oblong tank that is supplied with oxygen and lake water. Minimum water hardening time is 1 h. After the eggs are water-hardened, they are dipped from the egg boxes and placed into an appropriate sized cooler. Coolers should not be filled more than two-thirds full. Additional egg swelling can exceed the cooler capacity and oxygen depletion can result in substantial losses. Fresh water is added to completely fill the containers and lids are secured. The coolers are then transported to the Wray, CO hatchery for tempering, enumeration, and jar incubation in well water,

Bonny Reservoir has consistently produced 30-45 million fertilized eggs annually. The average hatching rate is 65-75 %.

Walleye Spawning in Michigan

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Michigan Department of Natural Resources has been utilizing walleye from the Muskegon River as a source of walleye eggs for many years. Fish migrate upstream and are captured by DC electrofishing below a barrier dam (Figure 1). Spawning techniques have evolved to the point where we can normally rely on >80% development to the eyed egg stage (eye-up in 1986 was only about 27%). Between 80 and 100 million eggs are taken annually.

Broodfish are selected and captured by district field crews when the Muskegon River water reaches 36°F (2.2°C). Fish are then sorted by sex visually and taken to a spring-fed pond which is 50°F (10°C). Most females are “green” upon arrival, and are all placed in 54 ft³ (1.53 m³) holding nets. Average weight of the females is about 7 lbs (3.2 kg).

Fish are collected daily from the river and transported to the holding nets until 500–600 fish have been captured. Females in each net are examined daily to check for ripeness and ripe females are spawned daily until egg-take goals are met. Experience has shown that all fish will be ripe within 2–3 d after capture. The various apparatus are illustrated in Figure 2.

Ripe fish are anesthetized prior to spawning. Spawning is done on a small dock at the pond. No protection from wind, sun, rain or snow is provided for the eggs during stripping. Anesthetized fish are rinsed in fresh water and wiped with a towel before spawning. One female is stripped into a stainless steel 5 qt (4.75 L) pan (Figure 3, next page). The number of eggs per female ranges 160,000 to 200,000. If a female produces > 200,000 eggs, the eggs are usually split between two pans for fertilization.



Figure 1. Electrofishing as a source for collection of broodfish on the Muskegon River, Michigan.

Prior to 1990, two males would be stripped into a pan of eggs and a small amount of water (about a cup) was added. The eggs were stirred for 5–10 seconds and then allowed to set approximately 2 min before being poured into a 5-gal (18.9 L) pail with Fuller’s earth and water.



Figure 2. Equipment for collecting walleye for eggs.



Figure 3. Stripping eggs from wild walleye in Michigan.

If eggs were allowed to set for more than 2 min, they would begin to stick together. The Fuller's earth solution was prepared by adding one cup (8 fluid ounce capacity) of Fuller's earth to about 3.5 gal (13.25 L) water.

Beginning in 1990, a new fertilization technique was used. Semen from two males is rapidly stripped into a 1.6 qt (1.5 L) plastic jar full of water. This mixture is vigorously shaken, then poured into a pan of freshly

stripped eggs. The mixture of eggs and solution of semen are stirred with a feather, then allowed to set for 30 sec. If eggs begin to stick before 30 sec, they must be poured into the Fuller's earth mixture immediately or they will form clumps. It is believed that fertilization is almost instantaneous. The Fuller's earth mixture is stirred with a feather as the eggs are poured into it. This procedure is repeated until 8 or 10 females have been stripped and fertilized, and the entire mass of eggs put into a 5-gal (18.9 L) pail.

After the last pan of eggs has been added to the Fuller's earth mixture, the pail of eggs is allowed to set for a maximum of 5 min. At this time, the eggs are poured into a floating 30 ft³ (0.850 m³) holding net (with 0.0625 in [1.59 mm] mesh) to allow the eggs to water harden. Eggs from up to 100 females are put into each net. The net also serves to wash excess Fuller's earth out of the eggs. The eggs are packaged for shipment 1 hr after the final lot of eggs have water hardened. Fish that have been stripped are held in 54 ft³ (1.53 m³) net pens in the spring-fed pond for 24 h and are then returned to the Muskegon River.

Water-hardened eggs are dipped from the net using one of the spawning pans. Eggs are put into 12 x 10 x 9.5 in (305 x 254 x 241.3 mm) Styrofoam boxes. A box is initially filled about half full with water. Eggs are gently poured into the box until the water overflows. At this point, water is poured off the eggs and this process continues until the eggs in the box are about 1 in (25.4 mm) from the top. The eggs are just covered with water for shipment. Each box contains about 16 qts (15.1 L) of eggs. The trip to the hatchery is approximately 100 miles (160.9 km) and takes about two h.

Upon arrival at the hatchery, the eggs are measured into plastic hatching jars. Five qts (4.73 L) of eggs are added to a jar, and the volume of eggs in this jar is used as a guide for filling the remaining jars. The eggs are siphoned into the jars. The jars are then put on the hatching battery to incubate the eggs. Each jar is supplied with 1.06qt/min (1 L/min) of 50°F (10°C) spring water. Eggs will not be rolled at this low flow.

Eggs are treated daily with formalin at concentrations of 1:600 for 15 min. On day ten, treatments are discontinued, and water flow is increased until the eggs roll gently. This allows dead eggs to migrate to the top of the egg mass and allows them to be siphoned. It may take 3–4 d before most dead eggs will roll to the top. During this period, eggs should be monitored for fungus (*saprolegnia*) infestation. If fungus does occur, formalin treatment should be repeated.

In 1986, the average eye-up was 27%. In 1990, 1991, and 1992, the mean had risen to 63%, 78%, and 83% respectively.

Eggs will be very near hatch on the 15 d of incubation. At this time eggs are transferred to a heated water 62°F

(16°C) battery. After about 3 d at this temperature, eggs will hatch. Hatching fry are directed into rectangular 9.7 x 13 ft (2.91 x 3.9 m) long troughs where the egg shells settle. If tannic acid is used to eliminate egg adhesiveness, egg shells will not break down at hatch and settle in the troughs. The overflow from this trough leads into a 8-ft (100ft³, 2.8 m³) circular tank. The egg shell settling trough is covered with a black cover and a light bulb is placed at the outlet. Fry are attracted to the light and are quickly pulled into the overflow. The carrying capacity of the circular holding tank is about three million fry. Fry are held in these circular tanks for 2–3 d and are then shipped to rearing ponds or stocked.

The same Styrofoam containers that were used to transport eggs are used to ship fry. Approximately 2 gal (8 L) of water and 50,000 fry are put into a plastic bag in the Styrofoam container. Fry are measured using volumetric displacement in a 500 ml graduated cylinder. The number of fry/ml is determined using a 3–4 ml sample of fry in a 10 ml graduated cylinder and averaging 3–5 samples from each tank. After adding the fry, oxygen is added and the bag is sealed.

Collection and Spawning of Walleye Broodfish and Incubation of Fertilized Eggs

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Introduction

Walleye is one of the most sought after coolwater sport fish in Pennsylvania. To supply the fish needed for population enhancement, the Pennsylvania Fish and Boat Commission is committed to a large-scale walleye spawning activity that supports a diverse walleye culture effort. Fertilized walleye eggs are also traded for species of fish from other states and the U.S. Fish and Wildlife Service that the Pennsylvania Fish and Boat Commission needs but cannot produce for its fisheries management goals.

Methods

Most of the walleye eggs come from a wild population in the 2,500-acre (1,012-ha) Pymatuning Sanctuary Lake, which is closed to the public, located near the town of Linesville, Pennsylvania (Figure 1). The Linesville Fish Culture Station is situated on the shores of Pymatuning Sanctuary and has conducted walleye spawning operations every spring since the early 1950s.

Collection of adult walleye begins in March or early April as soon as the ice melts. The water temperature at that time is 36–38°F (2–3°C). Eight to 10 trap nets are set throughout the sanctuary by a two-man crew working from a pontoon boat. The Pennsylvania trap net (Figure 2) is constructed with knotless nylon sewn on 4-ft (1.2-m) square aluminum frames. The net size is 1-in (25.4-mm) (bar measure) mesh in the crib and 1.5-in (38.1-mm) mesh in the 125-ft (38.1-m) lead. The frames in the crib ensure that the net will not collapse when it is set in shallow water, and also facilitates removal of fish by preventing the net from collapsing when it is lifted onto the deck of the pontoon boat (Figure 3, next page). Net locations are on

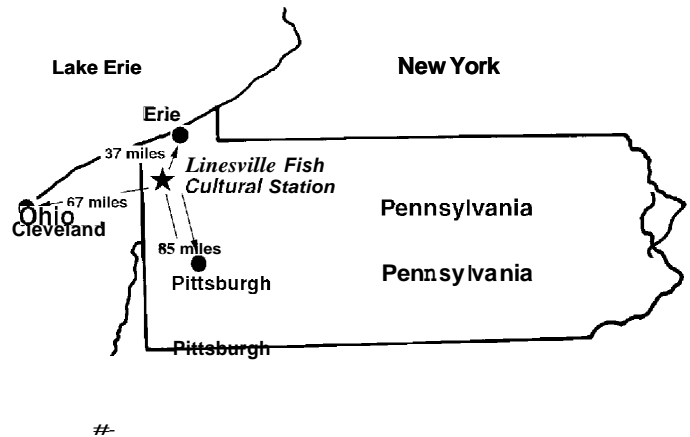


Figure 1. Location of the Linesville Fish Culture Station.

gravel and sand bars, preferably in water 5–7 ft (1.5–2.1 m) deep. The net opening faces shoreward, with the lead extending to the shore.

Initially, net catches contain a high percentage of males and few ripe females. The proportion of females in the catch increases as water temperature rises to 45°F (7°C) and peak catch usually lasts 7–10 d. Males become less abundant toward the end of this period. Nets are checked daily, and adult walleye are trans-

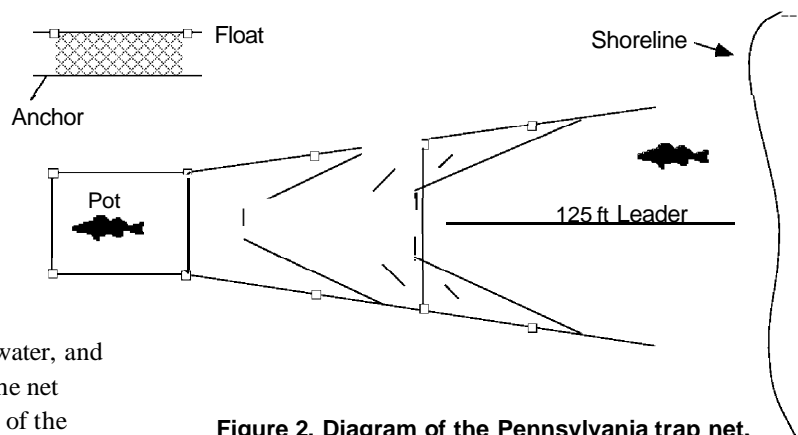


Figure 2. Diagram of the Pennsylvania trap net.



Figure 3. Checking a trap net.

ported back to the hatchery in onboard tanks. Fresh water is provided to maintain oxygen levels by means of a pump and manifold system. At Linesville Fish Culture Station, an overhead hoist system lifts the tanks off the boat (Figure 4), and positions them over the holding tanks, and the walleye are released into the 3.5-m³ (126-ft³) tanks by mechanically lifting an end and pouring out the fish and water. Fish are sorted into the category of males, ripe females (flowing eggs), and green females (no flowing eggs), then placed in separate tanks.

Green females are checked daily, and usually ripen within 3 d. Warmer well water (51°F, 11°C) is added to speed up the ripening process. The ripe females are spawned as soon as sorting is completed. It has been learned from experience that it is best not to delay spawning of ripe females as they will lose a large number of eggs in the holding tanks, especially if it is overnight.

A large female may produce as many as 300,000 eggs. The number varies with the size of the fish; 25,000–40,000 eggs for each pound (55,000–88,000/kg) of fish. On the average, each female spawned from the

Pymatuning Sanctuary yields about 0.5 quart (0.47-L) or approximately 70,000 eggs. Egg size is related to the size of the female (Table 1). The average walleye egg from the Pymatuning Sanctuary is 0.078-in (1.98-mm) in diameter or 140,000 eggs/qt (132,440 eggs/L).

Males and ripe females are placed in separate tubs containing 132ppm Finquel® for several min until completely anesthetized and then rinsed in clean water so that no anesthetic comes in contact with eggs or semen. Eggs are expressed into a wet pan by a gentle stroking motion from behind the gills to the vent while slightly arching the back upwards (Figure 5). The eggs from 2 females are stripped into the same pan. Simultaneously, the semen from two



Figure 4. Tanks with adult walleye being lifted off the boat.

Table 1. Walleye egg size as related to size of female from Pymatuning Sanctuary.

Female length		Female weight after spawning		Egg diameter		Eggs	
(in)	(mm)	(lb)	(kg)	(in)	(mm)	(per qt)	(per l)
15.0	381	1.0	0.5	0.072	1.83	181,300	171,510
18.5	470	2.2	1.0	0.075	1.91	160,400	151,738
20.0	508	2.7	1.2	0.082	2.08	122,730	116,103
23.0	584	4.5	2.0	0.084	2.13	114,172	108,007
28.0	711	7.0	3.2	0.090	2.29	92,826	87,813

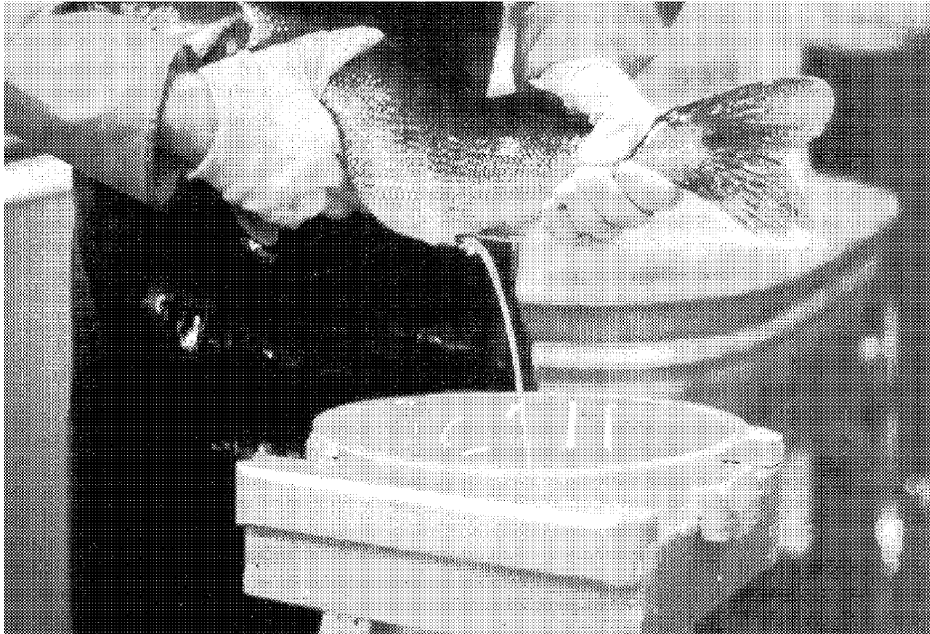


Figure 5. Expressing eggs into plastic basin.

males is added. Semen from 2 males is used as a precaution against the possibility of a male having non-motile sperm. Each male has a sufficient quantity of milt to be used more than once if needed. However, ample males are usually available and reuse of males is seldom necessary except at the end of the run. Walleye have rough ctenoid scales, and if many fish are stripped, these scales can cause abrasions on the hands. Cotton gloves can be worn to prevent this from occurring.

After mixing eggs and semen, a small amount of water is poured into the pan to activate the sperm. The sperm and eggs in the pan are then gently mixed by a circular motion of the hands for 2 min. The fertilized eggs are then poured into a 5-gal (19-L) plastic bucket, 1/3 filled with well water. No more than 8-qt (7.6-L) of eggs are placed in a bucket. The eggs and water in the bucket are gently stirred with a turkey feather for 15–20 min to keep the eggs from clumping. During this time period, the water is changed

frequently to remove excess sperm, egg shells, ovarian material, and other foreign matter.

After this period of constant stirring and water exchange, the eggs are poured into a 25 gal (95-L) oak keg which contains a 68-oz (2-L) scoop of Fuller's earth in 2-gal (7.6-L) of water. No more than 24 qt (23-L) of eggs are placed in a keg. The Fuller's earth slurry prevents the eggs from clumping while they water harden. Eggs are stirred every 5–10 min in order to keep the Fuller's earth in suspension. After 45 min, the keg is tilted slightly and well water is

added at the bottom of the keg via a hose. All the Fuller's earth must be flushed away prior to the next step. After an hour, the eggs have doubled in size and water hardened sufficiently to be placed in incubator jars. Before being placed in the jars, the eggs are strained through a 1/4-in (3.2-mm) screen to remove any clumped eggs or any other material that might interfere with water movement in the jars. Walleye eggs may be

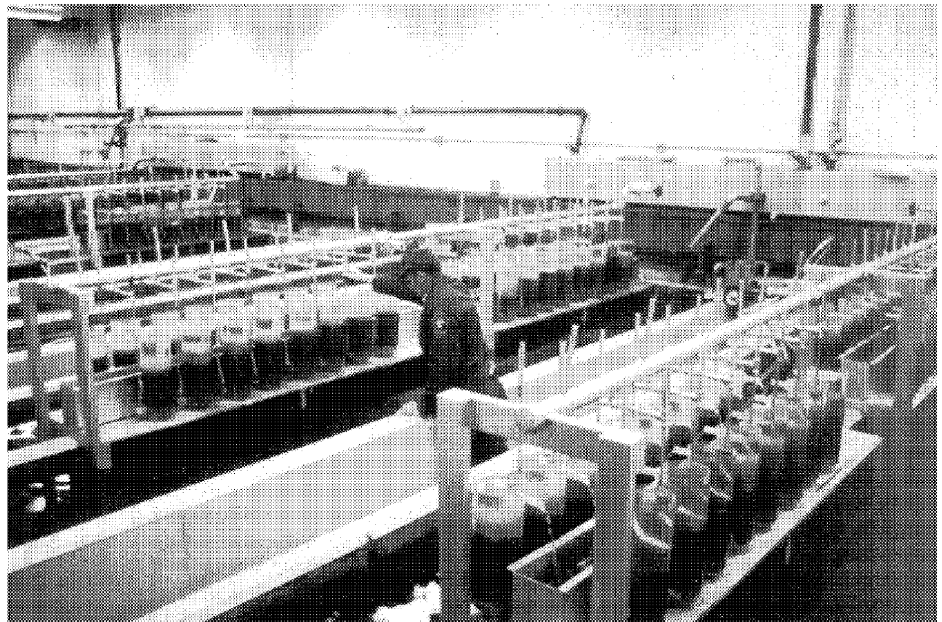


Figure 6. Incubator batteries at the Linesville Fish Culture Station.

Chapter 2 — Reproductive Biology and Spawning

shipped in water, 8 qt (7.6-L) eggs per an equal volume of water in sealed plastic bags with pure oxygen added. Eggs may also be shipped moist in Heath style boxes, 4-qt (3.8-L) of eggs/tray.

Four-qt (3.8-L) of eggs are placed in each May-Sloan jar in each of four 36-jar incubating batteries (Figure 6). Approximately 0.75-gpm (2.8-Lpm) of water enters each May-Sloan jar at the bottom through a hose and spigot. This flow is regulated to ensure a continuous gentle movement of the eggs. Fine screens are placed at the top of each jar to prevent eggs from washing away with the outflow. Annually, 1,400–2,400 females are spawned, and these fish produce 700–1,200-qts (662–1135-L) of eggs (98–168 million eggs).

The water supply for egg incubation is from wells located on the grounds at the Linesville Fish Culture Station. Water from the Pymatuning Sanctuary Lake, a shallow, extremely fertile lake with a very high plankton production has been used, but problems were encountered with temperature fluctuations and siltation.

After a week of incubation, dead eggs become evident. Dead eggs are more buoyant than live eggs, they rise to the top of the incubator jars, and are siphoned off. Eggs are treated daily with a 1:600 formalin solution (1,667 ppm) for 17-min to prevent fungal infestations.

The incubation period varies with water temperature. The incubation period is 21 d with eye-up occurring in 12–15 d, in well water of 51°F (11°C) at the Linesville Fish Culture Station. There have been successful hatches in 12 d when water temperature was 65°F (18°C). Our experience indicates that a higher hatching percent can be achieved, with fewer abnormalities, if water temperature is 50–52°F (10–11°C). If the incubation period is extended by lowering the water temperature, the percent of hatch will decrease.

Once hatching begins, it usually takes 3–7 d for all the eggs in a lot to hatch. Most of the eggs hatch during the middle of that time period. The retaining screens are removed from the incubator jars as soon as the eggs begin hatching and the fry are allowed to flow out into the battery discharge trough and then into holding tanks. Eggs can be force-hatched by interrupting the water flow into the incubating jars for about 5–15 min and by then restoring flow. Care must be taken when force-hatching eggs, because if too many hatch at the same time an excessive accumulation of egg shells can clog retaining screens in the fry holding tanks. Air from a compressor is bubbled at the retaining screen to constantly remove organic material. Just prior to hatch, egg volume is recorded, and this is used to determine percent hatch, which ranges from 70–95%.

Incubation of Walleye Eggs at Garrison Dam National Fish Hatchery

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Introduction

Garrison Dam National Fish Hatchery is located in central North Dakota and has been operated by the U.S. Fish and Wildlife Service since 1964. Currently the hatchery has three buildings used for the production of cold water species and 64, 1.5 acre (0.6 ha) earthen ponds used for the production of warm and cool water species. The hatchery incubates about 48 million walleye eggs a year that are collected from wild stocks, primarily from Lake Sakakawea and Devils Lake, North Dakota. Survival to the eye stage averages 47%, but varies, depending upon the source of the eggs.

Because not all ponds can be stocked at once due to the number of ponds that can be filled or drained at a time, it is necessary to stagger the hatching of the eggs to coincide with pond filling. Controlling the incubating water temperature allows the hatchery to prolong the incubation of some walleye eggs, allowing a crop of northern pike fingerlings to be raised in a particular pond in May, followed by walleye fingerlings in June. Prolonging incubation is also used to time hatching until the weather and water temperatures are favorable and zooplankton production is sufficient to support the walleye fry.

Methods

Before spawning starts, an incubation plan is developed to project how many green eggs are needed, based on the requests made by fishery biologists. This information, along with the number of ponds that will be used and the date that they will be ready for stocking, determines how the eggs will be divided among the incubators.

Spawning begins in early April. Fertilized eggs are transported from the spawning areas to the hatchery in coolers. The eggs are tempered if there is more

than 8°F (13.3°C) difference between the water temperature in the incubator and the water temperature used to transport the eggs. Each incubation jar has been calibrated and marked in 0.5 qt (0.47 L) increments to allow for easy inventory. Four qt (3.8 L) of eggs are ladled from the cooler into each jar, and the water flow is adjusted in each jar until the eggs roll uniformly.

Because the hatchery's water supply comes from Lake Sakakawea, fungus infestations would be a problem unless kept under control during incubation by treating the eggs with formalin (1,667 ppm for 15 min every other day). Because the lake temperature is approximately 35°F (1.7°C) when spawning starts, some of the water is heated by electric boilers to provide warmer water for incubation. Each incubator is supplied with heated and unheated water and the flow of each is controlled to produce the desired water temperature.

The eggs are hatched in nine incubation units. Each unit contains 28–40 incubation jars (Figure 1). Heated water is degassed by flow-through a packed column and is

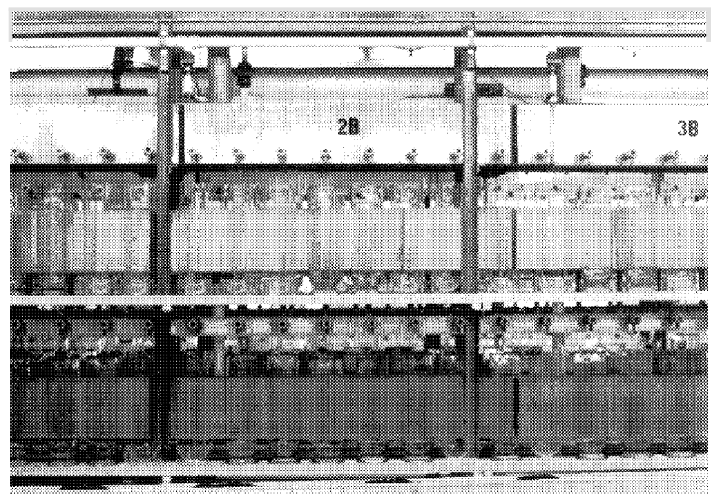


Figure 1. Side view of incubator 2B.

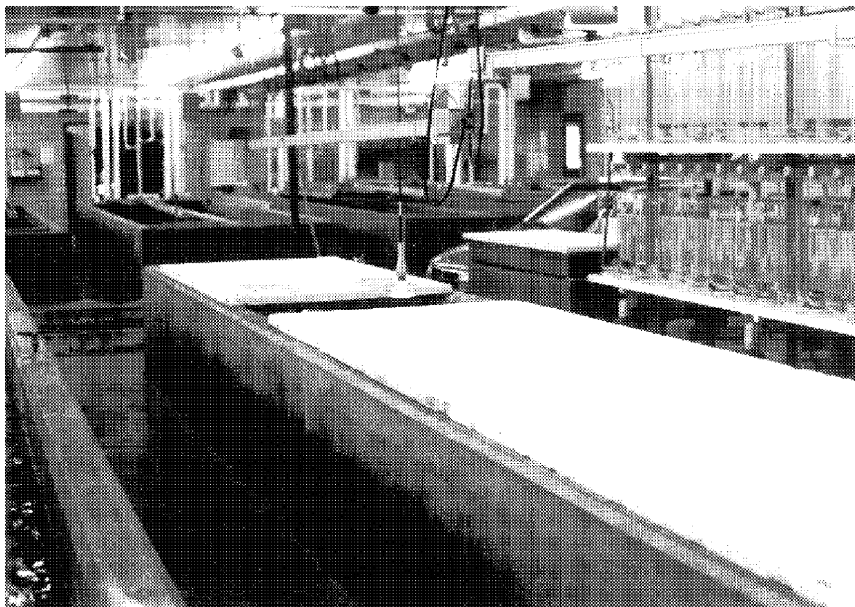


Figure 2. View of the tank used to catch the fry and the flood light used to congregate the fry.

mixed with unheated water before it enters the upper trough if a cooler temperature is desired. By mixing the water in this way, the water temperature of each incubator can be different and adjusted to within 0.5°F (0.28°C) of a desired temperature. Water temperatures are recorded daily for each lot of eggs and adjusted if necessary. As the hatching date approaches, the eyed eggs are inventoried using the increments on the jars and monitored so that the hatching date can be recorded. Past records have been used to generate a computer program that determines what temperature the eggs should be incubated to obtain hatching at a predicted date.

When an extended incubation period is desired, the eggs are incubated at a minimum of 46°F (7.8°C) for the first 5 d to allow for initial embryo development. The temperature can be lowered after this 5 d period until the eggs are close to hatching. A day before hatching, the temperature is increased to a minimum temperature of 56°F (13.3°C) so the fry will be active enough to escape their egg shells. This procedure has allowed the incubation period to be extended to 42 d. There has not been a noticeable reduction in the percent eye up in eggs that were incubated using this procedure,

but an increase in fry mortality has been observed. It is not yet known if this is due to the low incubation temperatures or because the water temperatures were not increased before the fry started to hatch. A comparison of the survival rate of these fry in ponds has not been made with fish whose incubation period has not been prolonged.

When hatching starts, the water flow from the incubator is redirected from the drain line into a "catch tank". The water flow through the incubator directs the fry out of the jars, through the troughs, and into the tank.

The catch tank has a wire screen that is covered with polypropylene filter cloth (1,024 openings/in², 159

openings/cm²) to prevent the fry from escaping. To help keep the screen from becoming clogged with egg shells and other debris, a 0.25 in (0.6 cm) perforated rubber hose is attached to the bottom, upstream side of the screen and connected to an air supply. The agitation from the air bubbles keeps the screen relatively free of debris. Debris is also siphoned from the bottom of the tank periodically using a 0.75 in (1.9 cm) hose when the tank has relatively few fry.

Fry are concentrated into one area of the tank for harvest. The tank is covered except for a small area which is illuminated by a 150-watt flood light hung about 3 in (7.6 cm) above the water. (Figure 2). Fry are attracted to the light and congregate in the immediate area, which keeps them away from the screen and makes their removal easier.

Fry are removed from the catch tank when they are 1–4 d old. Fry are captured using a dip net made of dacron cloth. Fry are enumerated using the volumetric displacement method and placed into buckets. Oxygen is supplied to the buckets by small air stones that are hooked up to an oxygen bottle. Fry are then quickly transported to the ponds and stocked.

Stripping, Fertilizing, and Incubating Walleye Eggs with Big Redd Incubators

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Introduction

The St. Croix Chippewa Indians of Wisconsin raise and stock fingerling walleyes to maintain populations in lakes harvested by Tribal members. Staff of the Tribe's Natural Resources Department have collected, spawned, and incubated walleye eggs for five years. Wild broodstock are captured with fyke nets during the walleye spawning season, the eggs and sperm are stripped, and the *dry* method used for fertilization. Fertilized eggs are incubated in Big Redd Incubators (Big Redd Incubators, Inc., Frazee, MN). After hatching, the fry are stocked in a natural pond. Harvest begins 4- to 5-wk later when the fingerlings are about 2 in (5 cm) long.

Spawning wild broodfish

Spawn collection is often coordinated with the Wisconsin Department of Natural Resources (WDNR) survey of adult walleye populations. The WDNR sets fyke nets in lakes in mid-April after the ice has thawed. The net crew notifies us when the peak of spawning approaches. We collect the ripe fish after the WDNR crew has taken their survey data. Because our 3-person crew collects the spawn and monitors the incubators, we try to strip all the spawn we need in 1 d.

Broodstock are separated by sex, then placed in separate 72-gal (272 L) steel basins in a jon boat. The water in the basins is not aerated, but is changed frequently. Spawning equipment in the boat includes a wooden spawning bench 15 x 36 x 16 in (38 x 91 x 41 cm) (width x

length x height) with a circular hole cut in one end of the top to secure the 5-qt (5 L) plastic pan used for fertilization. The diameter of the hole allows the lip of the pan to rest on the bench surface. Two round pans are used to hold the spawn, one steel 1.5-gal (5.7 L) bucket to clean the fertilized eggs, and one 18-gal (68 L) square steel basin for claying the eggs.

Before collecting the gametes, the small bucket and one pan are half filled with lake water. The second pan is placed dry in the hole cut for it in the spawning bench. Milt from one male is stripped into the dry pan in the spawning bench. For stripping both males and females, the fish's head is held between the spawn collector's upper arm and side so that the arm from the elbow down remains free (Figure 1). With the other hand, the spawn collector grasps the fish just above the tail. The fish is held belly-down with its back arched over the



Figure 1. Stripping eggs from a female walleye.

basin. Milt or eggs are expressed by pressing the fish's abdomen firmly with the free hand beginning forward of the vent and working back toward it. Stripping is stopped if the milt (or eggs when stripping females) does not flow freely or if blood is seen. Care is also taken to prevent fish slime, water, or other matter from entering the pan. The male is released into the lake after stripping once, however, large males may be retained and stripped twice if few males are collected. A female is then stripped into the pan containing milt. If less than 2–3 cups (473–710 mL) of eggs are collected, a second female is stripped. After collecting the eggs, milt from another male is stripped over the eggs. Fertilization must be accomplished within about 2 min of stripping.

The milt and eggs are mixed by placing the fingers of one hand firmly against the bottom of the pan and stirring rapidly without lifting or touching the sides of the pan. After mixing to a homogenous color, water from the second pan is added to the spawn and this mixture of water, eggs, and milt is poured from pan to pan 3–4 times. To minimize egg clumping or sticking to the pans, the pans are shaken while the fertilized eggs are poured. At this point fertilization is complete.

The eggs are washed free of mucus and semen by pouring them into the small bucket (1.5 gal, 15.7L) previously half filled with water. The eggs are swirled to prevent sticking by twisting the bucket. While swirling, half the water is decanted and replaced with fresh lake water. Rinsing is continued until the water in the bucket is clear.

The rinsed eggs are poured into a clay suspension and mixed thoroughly. The clay suspension is prepared in the square 18-gal (68 L) basin half filled with lake water. A handful of wet bentonite is added and stirred into suspension until the water feels slippery. Too much clay is better than not enough. The clay sticks to the eggs and prevents the eggs from clumping.

We repeat the procedure until we have stripped all available fish or have fertilized 10–20 qt or L of eggs from 15–30 female walleye.

The clay/egg basin is placed in shallow water in shade and the eggs are hardened in the clay suspension. The suspension is mixed periodically. The eggs take about 2 h to harden at 48–50°F (8.9–10°C). Eggs are tested for hardness by squeezing them between the thumb and

forefinger. If they do not break, they are hard. When the eggs have hardened, they are poured into a 12 x 4 in (30.5 x 10.2 cm) deep window screen box and all the clay is rinsed off with lake water. Clean eggs are placed into an 18-gal (68-L) plastic ice-chest in a layer no more than 4 in (10 cm) deep with most of the balance of the ice-chest filled with lake water. It usually takes about 8 hr between the first fertilization and arrival at the incubators.

Incubation

We used two Big Redd Incubators (Figure 2) to hatch our walleye eggs. Each incubator can hold 11 qts (9.9 L) of walleye eggs. Because the incubators are portable, they can be set up where the best water quality is found and dismantled for cleaning and storage after the eggs have hatched. Since 1990, a temporary hatchery has been set up in the facilities of the Tribal Construction Company. The water source is a well. In

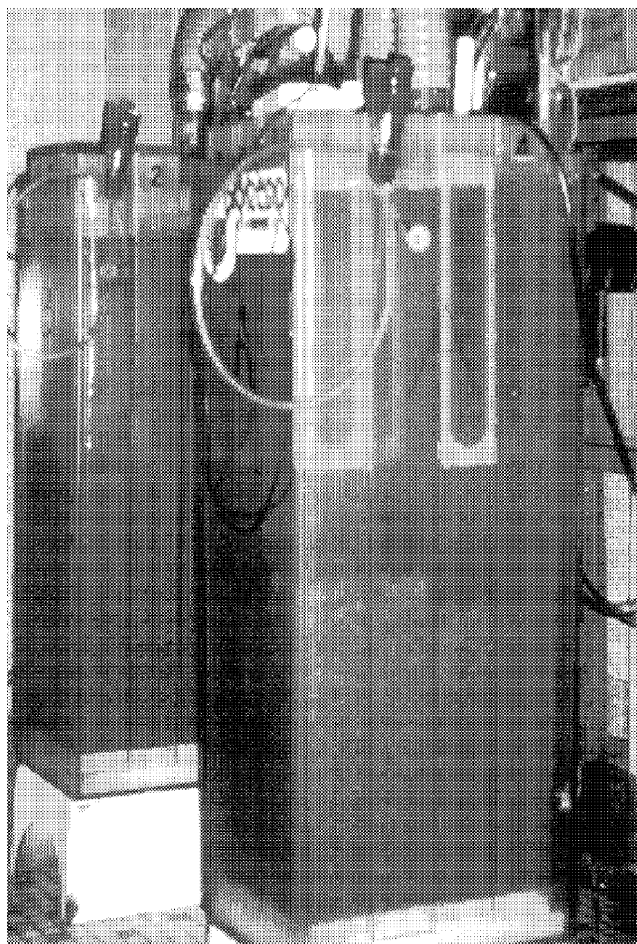


Figure 2. Big Redd Incubators loaded with walleye eggs.

1990, water samples were taken before the water entered the incubator. In 1994, water was sampled directly from the incubators prior to egg introduction. The different sampling locations account for the differences in water quality (Table 1).

Table 1. Well water quality of well used for incubating walleye eggs in Big Redd Incubators, 1990 and 1994.

Year	Temperature (°C)	pH	Dissolved oxygen (ppm)	Carbon dioxide (ppm)	Iron (ppm)
1990	7.8	8.0	7.0	8.8	
1994	9.8	7.0	6.2	8.8	0.2

The incubators are connected separately to a water line with clear ³/₁₆ in ID (4.8 mm) plastic tubing, and after passage through the incubators, water is discharged to a floor drain. Compressed air is generated with an AC, 115-V, 60-Hz air pump that supplies each incubator independently. A DC air compressor and a 12-V marine deep-cycle battery is available in case of electrical failure.

The basic incubator structure is a 9 x 12 x 30 in (23 cm x 30 cm x 76 cm) clear plastic holding tank. The tank holds 11 clear plastic removable tubes mounted on a base plate through which water circulates (Figure 3). The tubes are 2.5 x 2.5 x 30 in (6.4 x 6.4 x 76.2 cm). Each of the 11 tubes can hold a quart or liter of eggs, but we do not use the two interior tubes because the eggs cannot be observed from the side of the tube. The tank's standpipe, with three water level settings, piezometer, and airlift assembly fits into the twelfth space. The airlift assembly circulates and oxygenates the water with perforated plastic tubing connected to the air line at the bottom of the tank.

Before eggs are placed in the incubators, they are gently rolled through a screen box with ⁵/₃₂ in (4.0 mm) mesh to break up any remaining clumps. At the same time, the eggs are gradually brought to the same temperature as the incubator water by the addition of warm or cool water. The WDNR V-trough method and walleye egg count chart, modeled after the Von Bayer method but specific for walleye, is used to determine the number of

eggs/qt (Table 2 and Figure 4). We measure about 0.95-qt (900-ml) of eggs into each tube.

Once the eggs are in the incubators, we monitor air flow, water exchange rate, piezometer head level, dissolved oxygen, water temperature, and pH every 4 h, until the fry are removed. Carbon dioxide is measured once a day. We do not monitor ammonia because traces of formalin used to treat the eggs for fungus interfere with the performance of the ammonia test kit. Differences between Incubator A and B (Table 3) occur because each incubator operates as a separate unit, with individual water, air intake lines, and controls.

Air flow is determined by measuring the head of water in the piezometer above the tank's water level. Air flow varies with the stage of incubation. Eggs are loaded with a piezometer reading of 0.47 in (12 mm), incu-

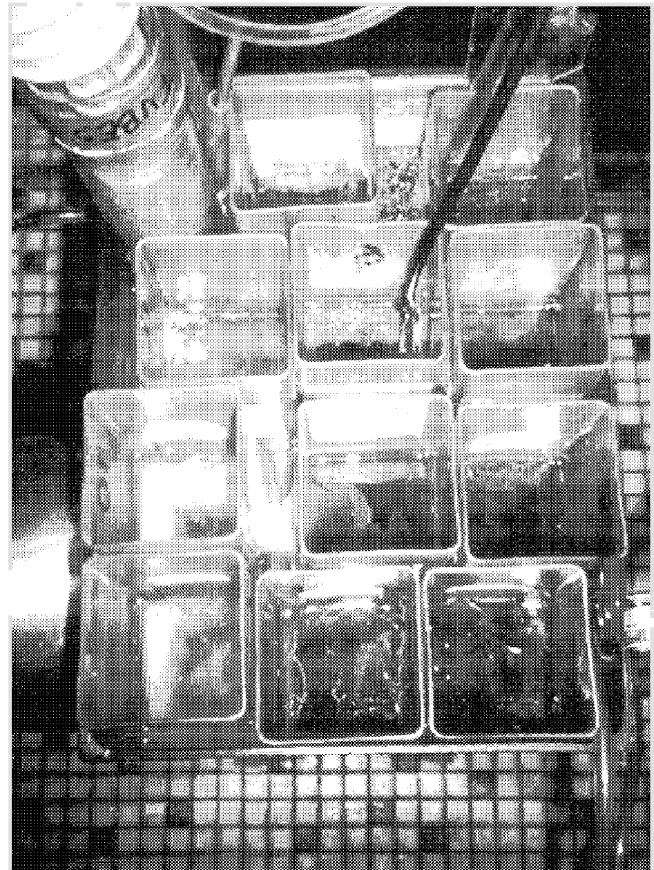


Figure 3. Top view of a Big Redd Incubator showing 11 incubating compartments.

Table 2. Walleye egg count chart, Wisconsin Department of Natural Resources, used by St. Croix Tribal Natural Resource Department.^a

No. in trough	No. per quart	No. per liter	No. in trough	No. per quart	No. per liter
38	16,960	17,928	58	61,925	65,460
39	18,240	19,281	59	65,680	69,429
40	19,680	20,803	60	67,670	71,533
41	21,744	22,985	61	71,899	75,993
42	23,140	24,461	62	74,146	78,378
43	25,197	26,635	63	78,927	83,432
44	26,901	28,436	64	84,130	88,932
45	28,764	30,406	65	86,904	91,865
46	30,801	32,559	66	92,826	98,125
47	32,268	34,110	67	95,990	101,469
48	34,647	36,625	68	99,297	104,965
49	37,265	39,392	69	102,762	108,628
50	39,161	41,396	70	110,190	116,480
51	41,186	43,537	72	118,346	125,101
52	44,494	47,034	74	127,333	134,601
53	46,899	49,576	76	137,251	45,086
54	49,480	52,304	78	148,220	56,681
55	52,254	55,237	80	160,400	69,556
56	55,239	58,392	82	173,950	83,879
57	58,456	61,793	84	189,070	99,862

^a The number of hardened eggs in a single row in a 6-in trough.

bated prior to hatching at 0.98 in (25 mm), and hatched out at 1.69 in (43 mm). Fry are held in the tank with a piezometer reading of 1.69 in (43 mm). During the fungicide treatments, the air flow is adjusted so that the piezometer reads 0.98 in (25 mm). Air flow is controlled by a valve in the air supply tube. Each tank also has an airstone, controlled by an auxiliary air supply valve, to increase dissolved oxygen if necessary.

The water exchange rate is measured at the tank’s outlet and is controlled with two valves per incubator in the water supply tube. We use the manufacturer’s water flow recommendation of 500 mL/min as a minimum and manipulate the water flow primarily to control temperature. In the small room where the incubators are held, altering room temperature also controls tank temperature. The incubators are equipped with aquarium heaters but they are fragile, and have broken

inside the tank. They also crowd the tank. Temperature is more effectively controlled by manipulating water flow and room temperature.

Dissolved oxygen is measured with a two-probe electric meter. Temperature and pH are measured with a battery-operated meter in each tank. Both dissolved oxygen and pH meters operate continuously. Carbon dioxide is measured by titration.

The hardest water quality parameters to maintain within desirable limits have been dissolved oxygen and temperature. Dissolved oxygen drops significantly during the hatch and when fry are in the incubators. When DO drops to 5 ppm, we use the air compressor to increase oxygen levels. Pure oxygen could be used for aeration, but this has not been necessary. Water flow

and room temperature are adjusted as needed, usually several times a day, to maintain the desired water temperature. We have never had problems maintaining other water quality parameters.

Two to three d after loading the eggs into the incubators, both tanks are treated for 15 min with 4,500 ppm formalin to control fungus. We have used concentrations of 2,200–3,300 ppm in previous years, but they were ineffective. After the 15 min treatment, the water is drained from the tanks to the lowest standpipe level by removing the upper section of the standpipe. The tank is flushed for 10 min, with both main and auxiliary water valves fully open. After flushing, the standpipe is replaced and the tank is refilled. The fungicide treatment is repeated every 48 h until the fry are removed from the incubators. Most unfertilized eggs that move

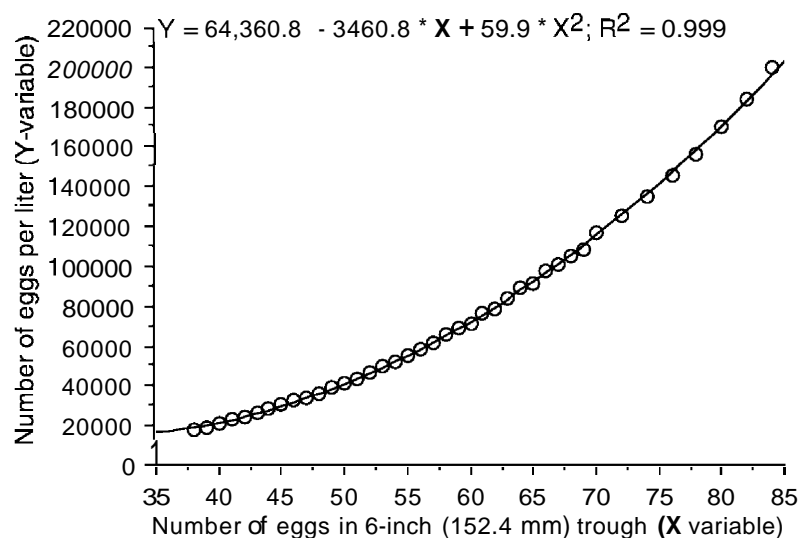


Figure 4. Curvilinear relationship between Von Bayer though counts of walleyes and number of eggs/L. A 2nd degree polynomial gives a good fit to the relationship.

to the top of the egg mass can be siphoned off. The volume of dead eggs is measured.

During 1990–1994, the average operating water temperature was 51.3°F (10.7°C). At that temperature, the eggs eye-up in 9 d (173.7 TU, where TU= temperature - 32°F x days) and begin to hatch in 13–14 d (251–277 TU). We induce complete hatch 3 d after hatching begins by pouring 129–145°F (54–63°C) tap water into the airlift assembly at about 1 qt/min (0.9 Wmin) until the tank water temperature has increased by 37°F (3°C). The increased temperature is maintained for 20 min by the addition of more hot water. After 20 min, the water temperature is allowed to return to the normal operating range of 50–53°F (10–11.7°C). The rapid hatching that follows generates foam where aeration agitates the water. The incubators are equipped with flexible tubing that fits over the airlift assembly to withdraw foam. Hatching is complete by day 18. We keep the fry in the

incubators four more days. The number of fry is determined by subtracting the volume of siphoned dead eggs from the initial volume of eggs placed into each tube.

The average hatch rate is 78%, and has ranged from 73–87%. The fertilization rate cannot be deduced from the hatch rate because eggs lost to fungus are included in the volume of dead eggs. However, in 1994, when there was almost no fungal growth during incubation, the mean hatch rate was 83%. In 1992, when all fertilized eggs died, probably due to temperature shock, the WDNR gave us eyed eggs to hatch. The average hatch rate, starting with eyed eggs, was 96%. Extrapolating from this observation suggests that loss to fungus infection ranges from 4–5%.

We do not transport fry in the incubators although Big Redd literature says it's possible. We tried it once, using a DC air pump, but were unable to maintain air or water flow through the tanks. We transport eggs in boxed, 10-gal (38 L) plastic bags half filled with water that is supersaturated with oxygen.

Cleaning the Big Redd Incubators is time consuming because they have to be completely dismantled. All incubator parts and other equipment that comes into contact with eggs or fry must be sterilized with an overnight soak in a 20-ppm solution of 70% active chlorine. Everything is soaked a second night in household rust remover (sodium hydrosulfite and bisulfite). The chlorine is removed in a third overnight soak of sodium thiosulfate solution four times more concentrated than the chlorine bath. Finally, every piece is washed with dish soap and water and thor-

Table 3. Average water quality parameters maintained in Big Redd Incubators during spring walleye incubation, 1994.

Incubator	DO (ppm)	Temperature (°C)	pH	C02 (ppm)	Flow rate (mL/min)
A	6.6	10.8	7.1	6.6	816
B	6.1	10.7	7.0	6.8	812

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oroughly rinsed. The incubators are stored at room temperature in their original boxes.

Big Redd Incubator operation is most labor intensive during and after the hatch because the egg shells must be removed manually, and fry that are removed with them must be sorted and returned to the tank. If the eggshells are not removed, they will impede circulation and eventually cause the incubators to overflow.

Siphoning dead eggs is also time consuming. The incubators must be monitored regularly to adjust water and air flow and to measure water quality. Although they require considerable attention, they can be set up anywhere where there is suitable water and a drain. The incubators and associated equipment require very little space. Big Redd Incubators have been indispensable in establishing St. Croix's walleye culture program.

Stripping Walleye Eggs Collected from Speared Fish and Incubation of Eggs using Big Redd Incubators

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Introduction

Tribally regulated spearfishing usually occurs in Northern Wisconsin on treaty ceded lands during mid-April through early May when walleyes are in spawning condition. Fish are speared at night when they have congregated in the shallows to spawn, with lights shined into the water to reflect the eyes of the walleye. Tribal spearfishing harvest accounts for about 4% of walleyes taken from all lakes in the treaty ceded territory.

Tribal spearfishing is controversial. The perspective of many Wisconsin anglers is that fish are for recreation and should be taken only by hook and line angling methods, and that it is ethically wrong to harvest spawning fish. Tribal harvesters, however, view fish as a food source and spearing as a means to maintain a traditional way of life. In the late 1980s, treaty protesters tried to disrupt the tribal spearfishing season. Incidents of harassment included rock throwing, racial insults, gun shots, and the use of cement walleye decoys to damage spears.

In 1989, a cooperative effort was established between the Cable, Wisconsin area Fish for the Future organization and the Bad River and Red Cliff Tribes to ease tensions between Indian and non-Indian user groups. Walleye gametes from tribally speared fish were collected from inland lakes and fertilized eggs were transported to tribal hatcheries for incubation. Once incubated, fry were transported back to the Cable area to culture ponds tended by the conservation club and grown to fingerling size. To maintain genetic integrity, gametes were collected from lakes with poor natural reproduction and the resultant fish stocked directly back into those lakes or into lakes in the same watershed. A similar effort with the Eau Claire Lakes Conservation Club began in 1992.

Methods and results

There was initial concern that water would enter the body cavity through the spear holes and it would harden the eggs before they could be mixed with milt. Therefore, initially in these cooperative efforts, gametes were collected on the lake, as soon as a female was speared. It was soon discovered, however, that there was ample time to spawn fish at the boat landing. Most veteran tribal spearfishermen had become proficient in spearing and speared most of their fish in the head region. Also, by stripping fish at the landing, every ripe fish could be spawned.

Walleye eggs are adhesive and must be mixed with an inert substance such as clay or starch to prevent them from sticking together. Bentonite clay was obtained from a local well driller and mixed with water to the consistency of a thin milkshake. A blender was found to provide a better product than either stirring the mixture by hand or by shaking it up. The clay mixture was stored in gallon (3.8 L) water/milk jugs until used. At the boat landing, biological data were collected from the speared fish by the Great Lakes Indian Fish and Wildlife Commission. After the data were obtained from a ripe female, it was spawned using the *dry* method, and the milt of three or more ripe males, was used to fertilize her eggs. Availability of ripe females is the limiting factor, as males make up approximately 85% of the speared fish because males tend to congregate in larger numbers and remain on the spawning grounds longer. When the weather was bitterly cold or heavy snow was falling, eggs were collected using the wet method to help protect them from the elements. An enamel or stainless steel pan was filled with two to three inches (5.1–7.6 cm) of lake water and the eggs and milt were expressed into the pan.

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The spawn was stirred after the milt was added to the eggs, then the clay-water mixture was immediately added to wash the eggs from the pan into a plastic 5 gal (18.9 L) bucket filled with approximately 6 in (15.2 cm) of lake water. Fresh water was added to the bucket and was also used to rinse the pan clean. The fertilized eggs were gently stirred in the bucket with a rubber gloved hand for a few minutes. Enough water was added to the bucket to allow for water absorption by the eggs. The fertilized eggs were stirred occasionally throughout the night/morning, as more eggs were added. Fresh lake water was added to the bucket with each returning fisherman to allow for absorption. The fertilized eggs were transported in the 5-gal (18.9-L) buckets to the Red Cliff Tribal Fish Hatchery where the eggs were rinsed clean and bits of fish viscera, from spear perforations, and other debris were removed with tweezers.

The hatchery used for the incubation of the walleye eggs was a temporary cold-water facility with a 6 in (15.2 cm) well that produced 100 gal/min (378.5 L/min). Water temperature was a constant 47–48°F (8.3–8.9°C). Financial restraints precluded heating the water for use with standard hatching jars, therefore, Big Redd Incubators (Figure 1) were used. This incubator uses recirculating water, therefore, it does not require much water and it was practical to heat the water to desirable walleye incubation temperatures.

Incubators were set up and operated at least one week before the expected start of the tribal spearing season to establish the water temperature at the water replacement flow rates. These rates were set by flow meters attached to the system. Upon arrival, 1 quart (0.95 L) of eggs was measured into each of the 11 tubes in each incubator. The incubation tubes in the Big Redd incubators are individually numbered to identify the source of the eggs. The incubator operates with an airlift mechanism. A small air compressor is provided by the manufacturer. A head of approximately 1 in (2.54 cm) was maintained during incubation. About 1 pint (473 mL) of water/minute is recirculated through each incubation tube. Water in this system is only used to limit the buildup of metabolites and aids in temperature control.

A 250-watt submersible aquarium heater was used in each incubator to maintain and control water temperature. Replacement water flow was regulated to obtain a water temperature of 53–55°F (11.7–12.8°C) for incubation.

Daily operating procedures involved checking and cleaning the screen in the incubator, determining water temperature and dissolved oxygen levels, treating the eggs with formalin if required, reporting quantities (volume) of dead eggs removed, and recording the replacement water flow.

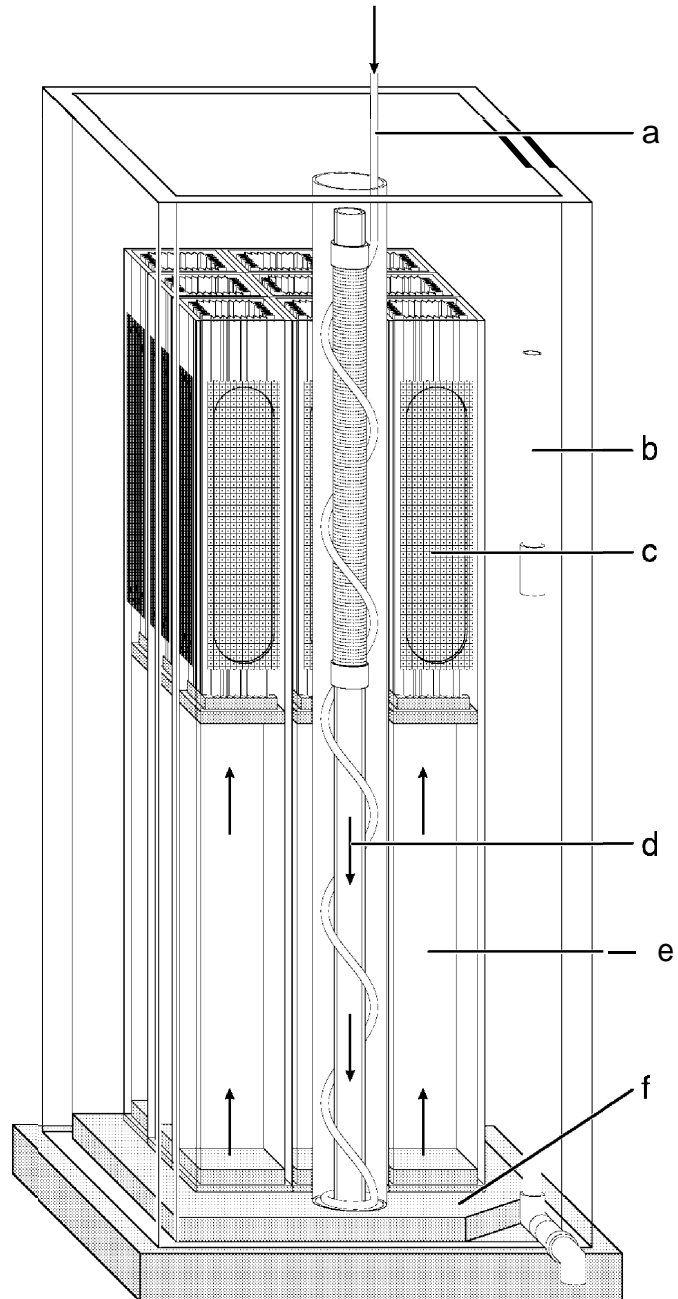


Figure 1. Schematic illustration of Big Redd incubator; a. air line, b. adjustable standpipe, c. screened water discharge windows, d. water return tube, e. incubation tubes, and f. manifold for water distribution to individual incubation tubes.

Using these methods, we averaged **83%** fertilization rates; the rates ranged from 40 to 90%. Variation in fertilization was dependent upon the conditions under which the gametes were collected. Highest mortality occurred during the beginning of the tribal spearfishing season when fish were not fully ripe. High mortalities also occurred if gametes were collected on extremely cold nights.

To control *Saprolegnia*, formalin fungicide treatments were started 2–3 d after the introduction of eggs and were repeated every 48 h. Dead eggs that accumulated at the tops of the incubation tubes were removed by siphon to help prevent fungal infestations.

Egg shell collectors were inserted into the incubation tubes before hatching commenced. This kept egg shells and dead eggs from plugging the outlet screens. Flexible pipe was also placed on top of the airlift assembly at the beginning of hatching to remove the foam that is a by-product of the hatching process.

Eggs start to hatch in about two weeks. The auxiliary air stone was also turned up to produce more oxygen during hatching. Three days after initial hatching eggs were induced to hatch rapidly by increasing the water temperature by 4° or 5°F (2°–3°C) in an **18 min** period. Attempts were made to stock fry about 3 d after hatching in order to prevent cannibalism, Ten percent of the fry were immediately returned to the lakes from which they came strictly for public relations. The

remaining fry were placed into fingerling production ponds.

Several problems were encountered with the incubation system. Airlocks often developed underneath the incubation tubes which resulted in poor movement of eggs. Plunging the tubes up and down usually sufficed to remove trapped air. Some tubes would not sit firmly on the manifold nipple. When this occurred, a heavy object, such as a brick, was placed on top of the incubator to help hold the incubation tubes down. In the event of a power outage, the backup system provided by the manufacturer would start a backup air compressor, but no fresh water would enter the incubator. If the hatchery does not have a backup power supply, to keep water flowing, the eggs could be heated to lethal temperature. Eggs may also clump at the bottom of the incubation tube and decrease circulation throughout the tube. A stiff plastic tube or wire with a loop at the end was used to break up clumps as they occurred. Hatching rates ranged 0–87%; failures were largely the result of power outages at the hatchery facility.

Acknowledgments

The line drawing (Figure 1) of the Big Redd Inc. incubator was redrawn by Rex Heer, Iowa State University Media Graphics, from a figure supplied by Donald Olson, Bid Redd Incubators, Inc. Bayfield, Wisconsin. I am thankful to them for their permission to use this illustration.

Stripping, Fertilizing, and Incubating Walleye Eggs at a Minnesota Hatchery

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Introduction

Walleye have been cultured in Minnesota since the late 1800s. In 1993, about 510 million walleye eggs were collected from ten sites and incubated in 11 hatcheries. Of the 355 million fry that were hatched, 280 million were stocked in 226 lakes and streams, and 75 million were stocked in 240 fingerling production ponds. The Pike River egg take site and hatchery in northeastern Minnesota is one of the largest in Minnesota. This case study describes current techniques used to capture broodfish, fertilize eggs, and incubate walleye eggs at the Pike River facility

Pike River site

The Pike River is located in northeastern Minnesota. It is 41 mi (66 km) long, has a drainage area of 192 mi² (497 km²), and is a tributary to Lake Vermilion, a 40,557 acre (16,413 ha) lake with low acid neutralizing capacity (total alkalinity 20–35 mg/L). The river has one small impoundment and is stained with tannin, so that it warms quickly in the spring. In normal years, the river becomes ice free about a week before Lake Vermilion. A site near the mouth of the Pike River has been used as a walleye egg take site from about 1890 to 1946 and from 1971 to the present time. The river at this site is about 100 ft (32 m) wide with a maximum depth of 10 ft (3 m). The current at this site varies widely with lake stage, spring snowmelt, and rain. A hatchery near the egg take site has four stacked hatching batteries with a total capacity of 1,000 qt (946 L) of eggs, or about 122 million eggs.

Collection of broodstock

Broodfish capture begins as soon as enough ice has left the river channel to allow the trap and associated structures to be put in the river. At this time the river temperature is about 40°F (4°C) but the lake is still ice covered. The average egg take starting date from 1985

through 1994 was 17 April, and the average ending date was 28 April. During the spawning season, virtually the entire spawning run of walleye is funneled into a pound net trap by lead nets spanning the mouth of the river. An average of 3,870 females and 16,926 males are captured annually. Several investigations suggest this run comprises about 5% of the Lake Vermilion adult walleye population. About 67% of the captured females are stripped; they yield on average about 1,040 qt (984 L) of eggs. Each stripped female yielded on average 53,984 eggs; the eggs average 122,442/qt (129,383/L).

Trapping

Six square platforms holding the trap, a work deck, and four fish-holding cribs, each 18 x 18 ft (5.5 x 5.5 m) exterior dimension, are bolted together, floated to one side out of the main body of current, and tied in place by cables extending to opposite shores (Figure 1). The downstream platform near the thalweg holds the fish trap. The fish-holding cribs are made of 1.5 in (3.8 cm)



Figure 1. The Pike River site used to collect walleye broodstock. Nearly 21,000 walleye are collected annually by Minnesota Department of Natural Resources personnel at this site using a large trap net. The facility includes a work deck and four fish-holding cribs (18 x 18 ft). Workers strip and fertilize eggs on the work deck.

bar mesh nylon, 6 ft (1.8 m) deep; one crib is used to hold males and three are used to hold females.

Long nylon lead nets, 12 ft (3.7 m) deep with 1.5 in (3.8 cm) bar mesh, are sewn onto each side of the trap net throat; the other ends are attached downstream to steel pins on opposite shores. Chain, weighing 15 lb/ft (22.4 kg/m), is sewn onto the bottom line of these lead nets with twine, 20 ft (6.1 m) at a time, and lowered into the water from a large pontoon boat with a power winch. Float logs are tied to the top line of each lead net to suspend the nets from the bottom of the river channel to the water surface. A nylon mesh funnel extends from the lead nets to the interior of the trap, allowing fish to enter.

Fish handling

Unripe females are held in cribs for 3 d; holding walleye longer than this results in excessive fin wear and reddened skin. Each morning the crib holding the third-day females is emptied; ripe females are placed in 18 gal (68 L) tubs and held until stripped, and unripe females are released upstream of the trap. Similarly, the second-day and first-day females are sorted, with unripe females placed in the just-emptied crib, using fish slides to facilitate the move. Males from the previous day's trap catch (in the male holding crib) are used to provide milt during this operation. Lastly, the trap is emptied and sorted. Only 19% of females in the trap are ripe, while an additional 48% ripen within the 3-d holding period. Males are held for 1 d, so 2 day's catch of males is available during each day's egg stripping, ensuring an adequate supply of milt.

The number of walleye entering the trap follows a bell curve over the 10-d run, with numbers doubling each day early in the run and halving each day at the end. Early in the run, males outnumber females by as much as six to one, but late in the run, males outnumber females by only two to one. Sizes of both males and females are larger early in the run.

In most years, females first ripen when midday water temperatures warm to about 42°F (6°C). The peak of the egg take occurs when water temperatures are 46–50°F (8–10°C); the egg take is essentially over when water temperatures reach 52–54°F (11–12°C). Eggs taken during the middle of the spawning run typically have survival rates about 5% higher than those obtained at the beginning or end of the run (79% vs 74%).

Hatchability declines for eggs fertilized at temperatures warmer than about 54°F (12.2°C).

There are obvious opportunities for selection to occur during this egg take operation: river-run spawners and open water spawners; large and small fish; early spawners and late spawners. At present, selective pressures are not considered during our hatchery operations except for restricting the stocking of fry to waters within the Hudson Bay watershed or to waters with no natural reproduction.

Stripping and fertilizing

Eggs are fertilized using the wet method. About 0.5 qt (0.5 L) of river water (pH about 7.0) is added to a flat-bottomed, 14 in (35 cm) diameter, 4 in (10 cm) deep polyethylene pan. The water is stirred by rotating the pan. White pans are used to reduce absorption of heat from the sun. After an initial squirt of milt from a male into the pan, eggs from 3–6 females are sequentially stripped into the pan along with milt from 6–12 males which are simultaneously stripped into the pan, yielding a slurry of eggs, sperm, and water 1.0–1.5 in (2.5–3.5 cm) deep. Milt is added every 15 s for 1 min after the last female is stripped to ensure active sperm are present while the micropyles of the eggs are open.

Stirring continues for another minute prior to the addition of filtered pond-bottom muck which is added to remove adhesiveness of the eggs and prevent clumping in the incubation jars. About 0.5 qt (0.5 L) of muck slurry is added to the pan of fertilized eggs and the mixture is swirled for approximately 2 min. The eggs can then be rinsed immediately or can be left immobile for a few minutes before rinsing. Various types of bentonite-water suspensions (9% bentonite by weight) have also been used as declumping agents.

Dry fertilization is not used because water drips into pans from fish, hands, or rain. Moreover, our attempts at using a dry or nearly dry method did not improve survival to the eyed-egg stage, 80.0% for dry method compared with 84.8% for the wet method. The dry method may be useful when there is a shortage of males and sperm has to be conserved, when extra time is available to ensure dry stripping operations, and when slightly lower egg survival rates are acceptable.

Water hardening

After treatment with muck, eggs are poured into a cradle that floats in the river, and they are rinsed to remove muck. The cradle is 6 x 16 x 30 in (15 x 41 x 76 cm) (depth x width x length), with the bottom rounded towards the ends. The entire bottom is covered with brass screen which has 58% open area and 24 openings/in (9.4 openings/cm).

Eggs are worked to one end of the cradle while rinsing, then they are placed in 18 gal (68 L) tubs to water-harden for approximately 4 h. The tubs are filled two-thirds full of river water and eggs are added until the tub is one-third full of eggs. The eggs will swell until the tub is one-half full of eggs. The water is changed every hour during this 4 h hardening period.

Eggs are considered to be fragile during the water hardening process, consequently, they are treated gently during this period. Several times, however, we have shipped freshly fertilized eggs to hatcheries several hours away by placing them loose in water into coolers or plastic cans. These eggs developed normally and they had survival rates similar to eggs taken the same day and hardened in the usual manner.

Incubation

After water hardening, the tubs are transported a short distance to the hatchery and eggs are placed in 5 qt (4.7 L) plastic hatching jars. Each day's egg take is incubated separately so survival can be tracked. The jars are then placed on hatching batteries. Water flow is adjusted in each jar so that the egg mass is buoyed a few inches by upwelling and the eggs are gently rolling. Each jar requires 0.8–1.0 gal/min (3–3.8 L/min) of flow.

Egg size

Egg sizes are measured 12–18 h after fertilization, using a 6 in (15.2 cm) Von Bayer trough. Past measurements have shown no consistent change in egg sizes at 4, 8, 24, 48, 96, and 168 h post-fertilization. Samples of eggs are collected with a 0.5 in (1.3 cm) tube. The tube is pushed vertically to the bottom of the jar, the top of the tube is covered with a thumb, and the tube withdrawn. Three counts are made for each egg lot and the average count is interpolated on a Von Bayer chart to determine the number of eggs per quart. Egg sizes are

larger at the beginning of the spawning run (110,190/qt) than at the end of the run (127,333/L), probably due to the larger size of females at the beginning of the run.

Water supply

The water source for the hatchery is the Pike River, which warms faster than area lakes, often reaching 65°F (18°C) during the later stages of egg incubation. Goals during incubation are to delay the hatch, to allow a gradual warming of incubation water to match the temperature of lakes to be stocked when the eggs hatch, and to minimize temperature swings during incubation. The intake water has sufficient oxygen and contains low levels of minerals. Water is pumped into a 10,000 gal (37,850 L) holding tank on a knoll above the hatchery from which it flows by gravity to the hatchery. An adjustable chiller with no recirculation allows hatchery water to be cooled as much as 10°F (5.6°C) below ambient.

Temperature units for development and hatching

Hatching battery temperatures are taken at 4 h intervals. Daily Temperature Units (DTU's), expressed as the average daily temperature (°F) minus 32°F, are calculated for each egg lot. The number of DTU's at eye-up, appearance of first fry, and completion of hatch are recorded for each egg lot. Seven years of data shows the average time to hatch is 27 d with a range of 22–31 d. Incubation begins at about 46°F (8°C) and temperatures are gradually increased until hatch occurs at about 57°F (14°C). Average DTU accumulations are: 206 to eye-up, 307 to first appearance of fry, and 478 to completion of the hatch. More DTU's are needed when incubation temperatures are cool than when they are warm. The linear formula for this relationship is: days to complete hatch = $23.2 + 0.104 \times \text{DTU's}$ ($R^2 = 0.64$).

Removal of dead eggs

Dead eggs often develop saprolegnia fungus, usually after about 5 d. Dead and fungused eggs are lighter than live eggs and they will gradually segregate to the top of the egg mass in the hatching jars. Dead and fungused eggs are siphoned into a screen box, with 0.125 in (3.2 mm) apertures, that is floating in a tub of water.

Over several siphonings, about 10% of the live eggs will be drawn out of the jars along with the dead eggs. These eggs are saved with the following procedure: When several quarts of fungused and live eggs have been drawn into the screen box, the box is suspended

above the water in the tub, and a stream of water from the siphon tube is used to break apart the fungus clumps and force all eggs through the screen into the tub. Water is then poured out of the tub through overflow screens, the eggs are returned to the hatching jars and placed on the hatching battery, and water is started through them. After several minutes the live eggs will remain on the bottom and the dead eggs will again separate. Dead eggs and fungus “fluff” are siphoned off and discarded.

One day after embryonic eyes are visible (eye-up) virtually all dead eggs have been removed. Eggs are then poured into a tub, the jars are brushed clean, and eggs are placed into hatching jars at the rate of 2 qt/jar (1.9 L/jar) instead of the initial rate of 2.5 qt/jar (2.4 L/jar). This lower incubation density is needed because the eggs tend to buoy higher in the jar after eye-up. The survival rate for each lot is calculated by dividing the remaining eyed-egg volume by the initial egg volume. Virtually all eyed-eggs hatch.

If the hatchery water warms quickly during incubation, gas bubbles may come out of solution, attach to eggs, and float them out of the hatching jars. To overcome this problem we turn down the flow rates to the jars, reduce the rate at which the water is allowed to warm, or install agitators or packed-column diffusers.

Fry holding tanks

After hatching, fry and egg shells pass to the lowest tank on the hatching battery and through a pipe to the fry holding tanks. The fry tanks have a total capacity of 900 gal (3,400 L), but they are inadequate to hold all

the fry (24 million) from a battery even if water is chilled and the flow increased. To handle all fry that hatch, fry are occasionally removed for stocking during the hatching period. Brass screens, with 0.027 in (0.68 mm) circular apertures), are placed in front of the outlets at each end of the fry tanks and bubblers made from 0.5-in (1.3 cm) CPVC plastic plumbing tubes with 0.09 in (2.4 mm) perforations are placed in front of the screens to prevent fry and egg shells from clogging the screens.

Once hatched, fry are removed from the fry tank and stocked into lakes or fingerling production ponds within 48 h to prevent holding mortality. Fry are dipped with a net made from fine nylon mesh with 150 μ m apertures and 51% open area. The net is 2 x 3 ft (0.6 x 0.9 m) and is attached to two 1.0 in (2.5 cm) wooden dowel rods. When dipped, fry are gently rolled in the net for several seconds to let excess water drain. When the stream of water from the net becomes discontinuous and starts to form drops, fry are poured into a tub of water and weighed with a digital scale.

Fry enumeration

We have attempted many methods of measuring fry including volumetric displacement and weighing. Most such samples yielded about 90,000 fry/lb (198,000/kg, or 5 mg each), but weights were highly variable. We collect the samples during the midway point of each pour from the net because the early portion of each pour contains more water than later portions. At present, we use a standard rate of 100,000 fry/lb (220,000 fry/kg, or 4.5 mg each).

Comparative Storage Methods and Fertility Studies of Walleye Semen

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Introduction

The timing and duration of the natural breeding season of walleye is limited by environmental and ecological conditions. Often, males ripen before females, which requires capture and holding of male walleye in the hatchery until females ripen, however, this may affect reproductive physiology of the male and sperm viability. To circumvent this problem, it is possible to refrigerate or cryopreserve semen for later use. The objective of this report is to describe methods for the storage of semen and its use in fertilization of walleye eggs. Effective use of these techniques can improve the reliability of fertilization and facilitate walleye breeding programs.

Methods

Samples of seminal fluid were obtained from male walleyes at the Rathbun Fish Hatchery and Spirit Lake Fish Hatchery during April and May, 1991–1994. Males were anesthetized with tricaine methanesulfonate (Finquel™) and semen was collected by using semen aspiration apparatus (Moore 1996). Semen samples were collected in calibrated plastic test tubes, the volume of semen measured, then the semen transferred to 40 ml, tissue culture flasks (Figure 1), that have a 25 cm³, growth area for transportation from the hatchery to Iowa State University. The flasks are durable, reusable,

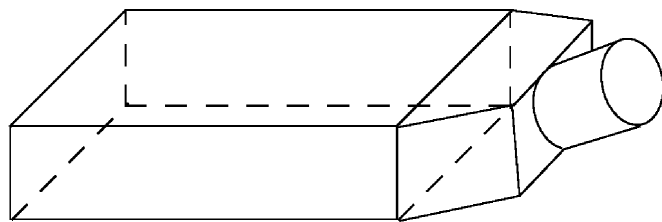


Figure 1. Standard tissue culture flasks used for storing and transporting walleye semen.

and excellent for transporting and storing samples. Best results were obtained when semen and all containers were kept on wet ice (ice allowed to partially melt, 0°C).

Analysis of untreated semen involved the measurement of volume, pH, sperm concentration, and a dye exclusion test. Sperm concentrations were determined by mixing 25 µL of semen with 25 ml of a saline solution (usually extender 7), and by using a hemacytometer to obtain counts. The average count was multiplied by 50,000 to obtain the concentration of spermatozoa (spermatozoa/ml). The dye exclusion test was used to determine the concentration of living (viable) spermatozoa. An eosin solution (0.113 g of Eosin B/100 ml of extender) was mixed (1:5) with semen and sperm counts were made of the semen-eosin solution in a hemacytometer. Because only dead spermatozoa absorbed the dye, the percentage of viable spermatozoa could be determined.

Sperm motility is used as an indicator of sperm viability of fresh and stored semen samples. Motility was determined by using the drop method. A drop of semen about 1.0 mm in diameter was placed on a coverslip and a large drop (about 0.05 ml) of dechlorinated water was placed over the smaller drop of semen resulting in a rapid dilution of the semen. With high-quality semen, an explosive motility which persisted for about 30 s, occurred following addition of water. However the percentage of samples having sperm motility percentage decreased with age of the samples.

To evaluate stored samples, a sperm motility index (*smi*) was used to determine the percent of sperm that were motile: 6 = explosive and 100% sperm motility; 5 = 75-100%; 4 = about 50% of the sperm were motile; 3 = 5 to 25%; 2 = <5%; 1 = <1%; 0 = no motile sperm. In all cases, motility was recognized as forward motion of

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spermatozoa, not vibratory motion. Sperm motility was determined as soon as possible, usually within 5–15 sec, after addition of water to the drop of semen.

Initially, semen samples were stored undiluted (raw), at a depth no greater than 0.3 mm (usually 3-5 ml of seminal mixture) in the tissue culture flasks. These flasks were placed flat in a refrigerated incubator (1°C), and the lids were loosened to provide air exchange. At intervals over 28 d, small amounts of semen were removed from the flasks to determine sperm motility.

Results and discussion

The volume of undiluted raw semen collected from each male averaged 3.6 ml, with a maximum of 7.0 ml (Table 1). The sperm concentration averaged 38.6×10^9 spermatozoa/ml. The pH of the

semen was 8.5. The dye exclusion test averaged 94.1%, indicating that almost all spermatozoa were viable. The combination of high sperm count and high viability indicated that the fresh semen was of high quality.

Undiluted semen did not store well, as indicated by a rapid drop in the percentage of sperm motility after 3 d (Table 2).

Table 1. Analysis of undiluted walleye semen.

	Average	Range	Samples
volume (ml) per male	3.6	2.0-7.0	49
sperm concentration (x10 ⁹)	38.6	24.0-59.4	31
dye exclusion (%) ^a	94.1	86.2-99.6	12

^a a measure of sperm viability

Table 2. Analysis of semen quality after storage at 1°C for 3–28 d: % survival (%S) and the average sperm motility index (smi).

	n	Od		3 d		6-7 d		14 d		19-21 d		28 d	
		%S	smi	%S	smi	%S	smi	%S	smi	%S	smi	%S	smi
Loosened lids													
undiluted	10	100	5.2	90	5.0	10	2.0	0					
extended	6	100	5.0	100	5.0	100	5.0	83	5.0	83	4.5	17	1.0
Tightened lids													
extended	6	100	5.0	100	5.0	100	5.0	100	5.0	83	2.4	0	
Extended^b													
1:1	10	100	6.0	100	5.0	100	5.0	100	4.4	0			
1:2	44	100	5.5	98	4.7	91	4.8	70	4.9	45	3.5	2	1.0
Extenders^b													
Ext7	21	100	5.6	95	4.5	81	4.8	52	4.4	10	2.0	0	
Rathbun's Ext	20	100	5.6	100	5.0	100	5.0	81	4.8	33	4.3	0	
Ext13	13	100	5.6	100	4.9	100	4.8	92	4.9	62	3.1	7	1.0

^a Average sperm motility index (smi) in dechlorinated tap water: 6 = explosive, 100% motility; 5 = 75-100% motility; 4 = ~50% motility; 3 = 5-25% motility; 2 = <5% motility; 1 = e1% motility; 0 = no motility.

^b Samples stored in flasks with loosened (48) and tightened (6) lids.

Three extenders were evaluated: extender 7, Rathbun's extender, and extender 13 (Table 3). Extender 7 and the Rathbun formula are modifications of an extender described by Erdahl and Graham (1980). Extender 13 is a simple saline solution (0.88% NaCl) with 1% penicillin-streptomycin. Samples were extended and tested in a 1:1 and 1:2 ratio (semen:extender) and with tightened and loosened lids (Table 3). Although both ratios of semen:extender maintained high-motility samples for 14 d, we selected the 1:2 ratio for further studies. No differences were determined in sperm motility of semen samples stored in flasks with either tightened or loosened lids. However, experience of the senior author with storage of white bass and striped bass semen indicated that storage in containers with loosened lids was detrimental.

All three extenders maintained semen in good condition for 14 d, but a higher percent of samples (81% and 92%) had a higher *smi* (4.8 and 4.9, respectively) with the Rathbun walleye extender and extender 13 compared with extender 7 (52% and 4.4 *smi*). In general, we found that extending seminal fluid which dilutes the sample and reduces the concentration of spermatozoa is

more important in maintaining the viability of stored semen than chemical composition of the extender. Semen diluted with extender 13, a saline solution, had higher survival and *smi* than undiluted semen (Table 3). Although antibiotics were used in all extenders, they may not be necessary because the semen contains natural antibiotics.

Samples of extended semen that were stored 2–14 d were used to fertilize freshly collected walleye eggs (Tables 4, 5). Eggs were stripped into a dry pan. About 4 ml sample of seminal fluid from one flask was used to fertilize about 60 ml of eggs. Three d post-fertilization, aliquots of 25 fertilized eggs were counted. All samples were maintained until after hatching. These experiments demonstrated that the viability of walleye semen that had been diluted with extender and maintained at 1°C for two weeks fertilized 75–99% of walleye eggs (Tables 4, 5).

We evaluated two media for cryopreservation of spermatozoa. Cryogenic medium A consisted of 10 g glucose/L of Rathbun's extender, 7.5 mg/ml PRO-FAM, 4 mg/ml BSA, and 7% dimethyl sulfoxide (DMSO).

Cryogenic medium B consisted of 7.5 g glucose and 2.5 g sucrose/L of Rathbun's extender, 4 mg/ml BSA, egg yolk (1.5 ml yolk/100 ml extender), and 7% DMSO. Fresh semen was mixed 1:2 with cryogenic medium in a 5 ml centrifuge tube (1°C). The mixture was drawn into freezing straws (0.25 ml), the ends sealed and straws placed on a flat piece of dry ice for 30 min. Straws with frozen semen were stored in the vapor area of a liquid nitrogen chamber. After about one year of storage, the semen in the straws was thawed by holding under running tap water. The ends were cut off and content empty onto 2 oz (56.7 g) of freshly collected walleye eggs. Development was followed until hatching and the fertilization rate was measured 3 d post-hatch (Table 6). The fertility rate

Table 3. Composition of extenders. Extenders were mixed, pH adjusted, then used or frozen.

Extenders	Ext 7	Rathbun's	Ext 13
CaCl ₂ ·2H ₂ O	0.103 g	0.117 g	
MgCl ₂ ·6H ₂ O	0.220 g	0.134 g	
NaHCO ₃	0.235 g		
Na ₂ HPO ₄		0.236 g	
KCl	2.558 g	1.872 g	
NaCl	5.780 g	6.578 g	8.760 g
glucose		10.000 g	
pyruvate	6.000 g		
citric acid	0.100 g	0.100 g	
bicine ^a		20 ml	
HEPES	2.380 g		
ddH ₂ O	1000 ml	1000 ml	1000 ml
KOH(1.27g/100ml)	10 ml	10 ml	
pen-strep ^b	10 ml		10 ml
pH	7.6	9.0	7.6

^a5.3g/100ml

^bpenicillin-streptomycin: Sigma, P0906: 5000 units of penicillin, 5 mg streptomycin per ml of 0.9% NaCl.

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using semen that had been stored one year was 31.3%. We think that with some improvement of techniques, this percentage could be significantly higher.

University of Minnesota, Minnesota Sea Grant Program 102, St. Paul.

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Table 4. Fertility of walleye semen mixed 1:2 in extender 7 or extender 13 and stored 2–13 d. The control semen was fresh and undiluted semen. The percentage of fertilized eggs for both extender and control was determined 3 d postfertilization.

No. days stored	Number of Trials	Extender 7	Extender 13	Control	Extender/Control ^a
2	1	99.2		97.6	100.2
4	1	84.0		88.0	95.5
4	2	88.0		88.0	100.0
5	1		97.6	97.6	100.0
6	1	59.2		93.6	63.2
6	2	68.0		93.6	72.6
7	1		77.6	88.0	88.2
8	1	90.4		95.2	95.0
8	2	77.4		95.2	81.3
9	1		71.2	93.6	76.1
10	1	85.6		96.0	89.2
10	2	78.4		96.0	81.7
11	1		76.8	95.2	80.7
13	1		97.6	96.0	101.7

^aThe percentage of fertilized eggs from semen stored in extender was divided by percentage of fertilized eggs from control semen

Table 5. Fertility of semen stored in 1:2 Rathbun's extender compared with control, undiluted semen. The percentage of fertilized eggs for both extender and control was determined 3 d postfertilization. (modified Moore 1987).

Days stored	Number of Trials	Rathbun's Extender	Control	Extended Control ^a
10	2	93.6	97.2	96.3
11	2	92.0	92.8	99.0
12	2	89.2	90.8	98.2
13	2	90.8	94.8	95.8
14	2	89.2	90.4	98.7

^aThe percentage of fertilized eggs from semen stored in extender was divided by percentage of fertilized eggs from control semen

Table 6. Fertility of walleye eggs fertilized with cryopreserved semen and fresh (control) semen.

Cryogenic media	Number of females	Number of eggs	Percent hatch of cryo-preserved semen	Percent hatch of control semen	Percent hatch from cryopreserved semen relative to control ^a
A	1	95	24.0	59.2	40.5
B	1	108	13.6	59.2	22.3
total	2	203	18.5	59.2	31.3

^aThe percentage of fertilized eggs from cryopreserved semen was divided by percentage of fertilized eggs from control semen

Use of Semen Extenders for Walleye

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Introduction

The Fisheries Bureau of the Iowa Department of Natural Resources (DNR) has two hatcheries dedicated to the production of walleye. The hatcheries at Spirit Lake and Rathbun hatch about 115 million fry and raise 310,000, 5–7-in (12.7–17.8-cm), fingerlings annually. In addition, 500,000, 2 in (5.0 cm) walleye are raised at satellite facilities.

Each spring, DNR hatchery personnel collect large numbers of wild, spawning size walleye. Because males often become ripe several days before females, males may be held in the hatcheries for two to three weeks. This period of captivity may lead to thick, viscous semen, reduced fertility, and mortality. Since 1987, the stripping of male walleye prior to female readiness and the preservation and cold storage of semen has enhanced hatchery efficiency, reduced male handling loss, and increased egg fertility. Preserved (extended) semen has been used successfully at both the Spirit Lake and Rathbun hatcheries. This paper will discuss the methods used at Rathbun and introduce the basics of walleye semen preservation.

General semen collection and storage methods

Walleye broodstock are collected by gill net, transported to the Rathbun Hatchery, sorted by sex, and held in raceways. Prior to stripping, males are anesthetized in water with 200–300 ppm carbon dioxide, rinsed in fresh water, wiped dry, and held ventral side up. Semen is forced from the fish with gentle external pressure along the gonad area and semen is collected using a water-powered sink aspirator (Figures 1 and 2). Only enough suction is used to gently move the semen through the collection tubes because too strong a suction will kill

the sperm. Semen are activated in water, and they have a short period of motility after activation (<1 min), therefore, during the aspiration process, semen is kept free from contamination by water or urine.

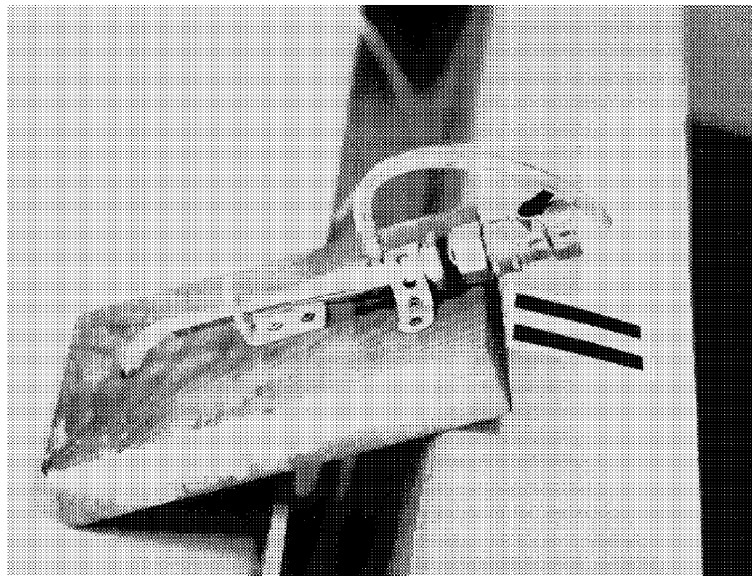


Figure 1. Water powered sink aspirator used to collect walleye

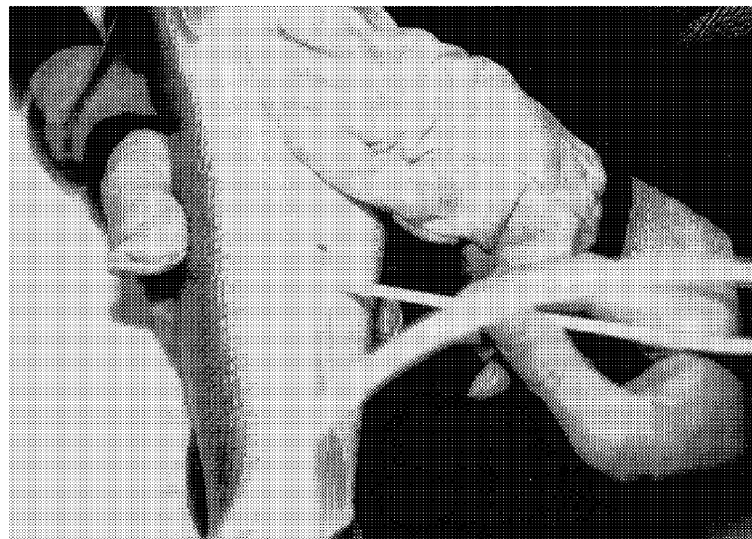


Figure 2. Stripping semen from a walleye with gentle external pressure.

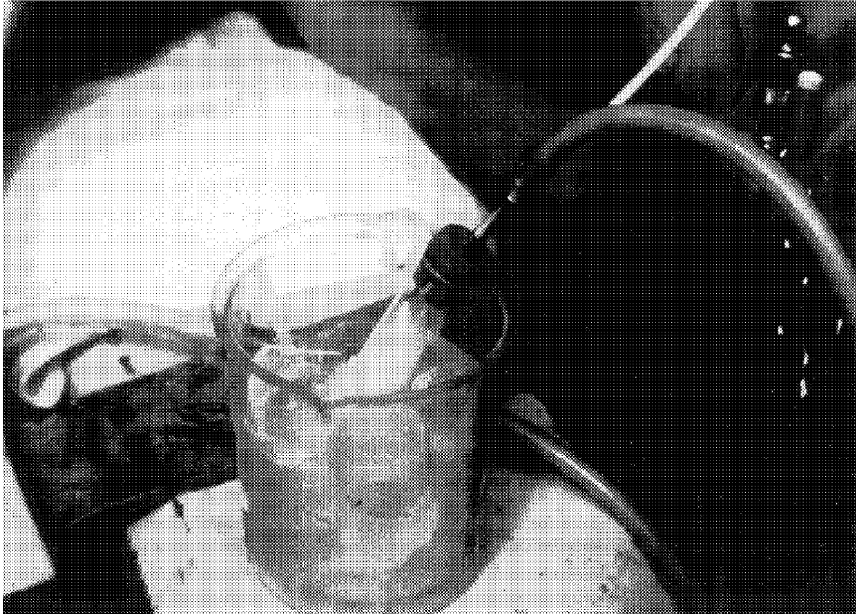


Figure 3. Collecting semen which flows from the aspirator directly into a test tube that is cradled in an ice water bath.

Semen is aspirated into 10-ml vials that are held in an ice-water bath (Figure 3) at a temperature of 34–39°F (1.0–4.0°C). Immediate cooling of the semen prevents premature sperm activation and prolongs cell life. For refrigerated storage, 3.0 ml of semen is placed in a 25-cm² tissue culture flask, followed by 6.0 ml of extender (Figure 4) (Table 1, opposite page). The mixture of semen and extender is gently swirled. To prevent sperm activation, the extender is always added to the semen. Total semen-extender volume is 9.0 ml. To allow for gas exchange, the maximum storage depth per flask is 2.0–3.0mm, and flask lids are screwed on but left loosened. Flasks are stored in a refrigerator at 33–36°F (0.6–2.2°C). To prevent the semen and extender from separating, flasks are gently swirled on a daily basis. Semen may be stored in this manner for up to 14 d with little loss of fertility; however, beyond 14 d, results may be variable.

During the stripping of female walleye, semen flasks are removed from the refrigerator and placed in

an ice-water bath. One 9-ml flask of extended semen is used for each group of eggs to be fertilized; about one quart (0.9 L) of eggs is maximum volume. The extended semen is poured onto the dry eggs, mixed gently with a feather or finger, and lake water added to activate sperm cells. Use of extended semen during spawning has increased egg fertility by 5%.

Walleye semen may also be frozen for long-term storage and used to preserve genetic lines. To freeze walleye semen, the extender formula given in Table 1 is modified by reducing the amount of glucose to 15.0g, and adding 5.0 g. mannitol, 4 mg/ml bovine serum albumin, 7.5 mg/ml protein, and 7% dimethyl sulfoxide to the mixture.

Semen is collected in the same manner as in the refrigerated semen process, and mixed with the modified extender at a 1:2 ratio of semen to extender. The semen-extender mixture is aspirated into 0.25-ml

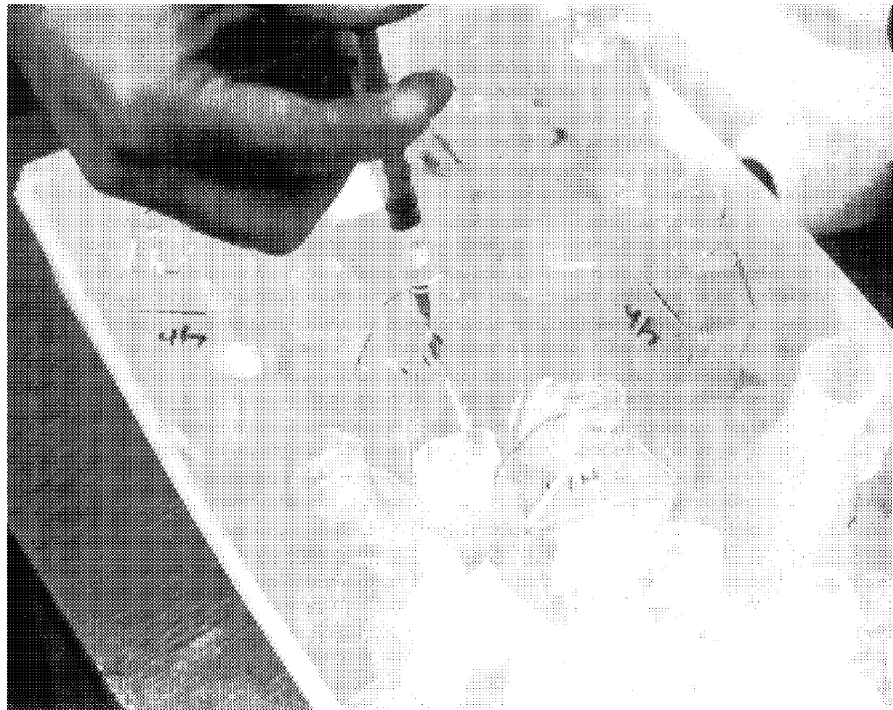


Figure 4. Semen is measured into tissue culture flasks and extender added.

plastic straws, sealed with a cryo-sealer, placed on dry ice for 30 min, and stored in liquid nitrogen.

To use the cryopreserved semen, the straws are placed in 70°F (21.1°C) extender, then mixed with eggs immediately upon thawing. Approximately 5 straws are used per 2 fl oz (59.1 ml) egg volume. Fertility rates ranging **48–83%** have been achieved using cryopreserved sperm.

Table 1. Composition of the extender added to walleye semen used at Rathbun and Spirit Lake Fish Hatcheries.

Constituent	Amount ^a
Distilled water	1,920 ml
Calcium chloride dihydrate (CaCl ₂ •2H ₂ O)	0.234 g
Magnesium chloride (MgCl ₂ •6H ₂ O)	0.267 g
Sodium phosphate dibasic (Na ₂ HPO ₄)	0.472 g
Potassium chloride (KC1)	3.744 g
Sodium chloride (NaC1)	13.155 g
Glucose	20.000 g
Citric acid monohydrate HOCCOOH (CH ₂ COOH) ₂ •H ₂ O	0.200 g
Sodium hydroxide (NaOH) solution ^b	40 ml
Bicine solution ^c	40 ml

^a Total volume equals 2000 ml.

^b NaOH solutions is 1.27 g NaOH/100 ml water.

^c Bicine (N,N-bis[2-hydroxyethyl]glycine) solution is 5.3 g bicine/100 ml water.

Field Utilization of Extended Semen

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Introduction

In 1949, the Colorado Division of Wildlife (CDOW) introduced walleye into artificial lakes of Colorado on the eastern side of the Rocky Mountains. They are now a popular sport fish with Colorado anglers. Because natural recruitment has been inadequate to sustain a sport fishery, the CDOW developed a large-scale program of stocking artificially propagated walleyes into Colorado's eastern man-made impoundments.

Traditionally, Bonny Reservoir and, more recently, Pueblo and Horsetooth Reservoirs have been the major sites for walleye spawning operations by the CDOW. Broodstock collection on these reservoirs has provided between 50 and 200 million eggs annually for use by the CDOW. In the mid-1980s, however, adequate fry production was impaired because insufficient numbers of ripe males were captured. The shortage of male walleye seems to be the consequence of selective angling mortality for males which are more vulnerable to anglers' catch than females.

To increase the availability of male walleye, CDOW personnel established a walleye population in Marston Reservoir, which is controlled by the Denver Water Department and is not open to public angling. This walleye population provides a greater proportion of male fish. Milt from Marston Reservoir walleye is collected, preserved with semen-extender, then shipped to spawning operations that require additional semen.

Field operations

Spawn collection site

The initial step of the spawning operation is to set up a site on the shoreline for spawn-taking. The ideal site has a permanent building located adjacent to the shoreline that can be used annually to obtain milt, eggs, or both. A source of dechlorinated running water would also be ideal, but it is not a necessity. Because a permanent shoreline site is not always available, a wall tent is helpful. It is extremely important, however, to conduct walleye stripping operations in some form of shelter, because precipitation and sunlight can compro-

mise the results. The spawn-taking area will also require at least two sturdy tables, one to keep the spawning equipment out of the mud, and the other to strip males. It is important to have a table that is a comfortable height (adjustable height preferred), tubs that can hold at least 15 gal (56.8 L) of water, paper towels to wipe off fish and equipment, rubber gloves, a supply of 10mL syringes, an oxygen bottle and hose hook-up, microscope with slides to check sperm motility, and a portable electrical generator.

Broodstock collection methods

Traditionally, CDOW biologists captured walleye with gill and trap nets, however, we have found that night electrofishing is more efficient. Personnel first spotlight groups of spawning walleyes along rip-rap and shallow rocky shorelines, then electrofish these areas. Electrofishing spawning aggregation can provide up to 50 male walleye per hour of electrofishing. Although there was initially some concern that electrofishing would reduce the viability of the gametes, a difference in the hatching percentage of eggs fertilized with semen of walleye collected with electrofishing and passive gear has not taken place.

Semen collection

Semen is collected and extended at the spawn taking area. At Marston Reservoir, which is closed to public fishing, male walleye are anesthetized with 100 mg/L of Finquel, but on public waters, a different anesthetic is needed because Finquel requires a withdrawal period of 21-days before treated fish can be released. When using Finquel to anesthetize fish, we wait until fish are motionless, then they are removed from the anesthetic solution, briefly dipped in fresh water, then wiped with paper towels. It is essential that no moisture be present on the fish or any of the supplies utilized in this operation to avoid activating the sperm before it is mixed with the eggs. Milt is extracted from the walleye into a small plastic cup that rests in a larger cup containing an ice bath, which keeps the milt temperature between 33 and 35°F (0–1°C). The composition of the extender that we use is the same as that described

by Moore (1987, 1996). It is maintained at a temperature close to that of the milt.

It is important to prevent contaminating milt in the collecting cup. The longer it remains in the open the more chance it has of becoming contaminated. Large quantities of milt in the collecting cup are at greater risk of contamination. A guideline for determining the amount of milt collected is that male walleye on average produce 3 mL, 1.25 mL, and 0.5 mL on their first, second, and third daily strippings, respectively. These numbers can be used to judge the amount of semen that has been collected within the cup until personnel are able to use visual estimates.

To prepare a 1:2 semen:extender ratio, we place 8 mL of milt in a plastic sandwich container (Figure 1) and add 16 mL of extender with a syringe. We collect sufficient milt to fill at least two of the plastic sandwich containers, then we use a dry syringe to remove the amount needed to make the desired semen:extender ratio. We store extended semen in 24 mL quantities because this volume produces a shallow film in the container and allows for good oxygen transfer. After air is blown into the container, the container is quickly sealed, labeled with the date and semen:extender ratio then placed in a cooler filled with cubed ice for storage. The stored semen is effective for fertilization for 10 d if it is oxygenated daily with pure oxygen, sheltered from all moisture, and stored at temperatures 33–46°F (0.6–7.7°C). Even when stored at 46°F, which may seem high, extended semen has produced satisfactory results.

Sperm motility of all extended semen should be checked with a microscope (400X) before use. This can be done by placing a thin film of extended milt on a slide and observing sperm motility (movement) when water is added. Batches of extended semen that are considered to be good will have nearly 100% motile sperm cells, and motility will last at least 30 sec.

Four mL of extended milt are used to fertilize 1 L of eggs by the dry method (Figure 2). Eggs fertilized with extended semen are treated similarly to those

fertilized by fresh semen, as far as water hardening, storage, and hatching are concerned.

Problems

Sperm activation by accidental contamination with water is of major concern. Water contamination occurs from wet gloves that were utilized to dip fish in fresh water after Finquel bath, filling the larger ice bath cup too full and pushing water over the edge of the cup holding the milt when extracting the milt for preservation, and placing tubs used for anesthetizing and recovery where water from splashing fish may contaminate the milt. Finally, after extended semen has been successfully preserved in plastic sandwich containers the container must be securely fastened to prevent contamination from melting ice.

Another concern is fecal material contaminating extended semen. It is common for fecal material to be expelled when stripping semen, especially during the second and third strippings when additional pressure is needed to expel semen. Fecal material reduces the quality of extended semen and should be removed immediately with a dry spoon. Blood in semen is also a common sighting, however, blood is not considered detrimental to semen quality and need not be removed.

We think that extended semen must be oxygenated daily (Figure 1) to ensure that sperm cells will have

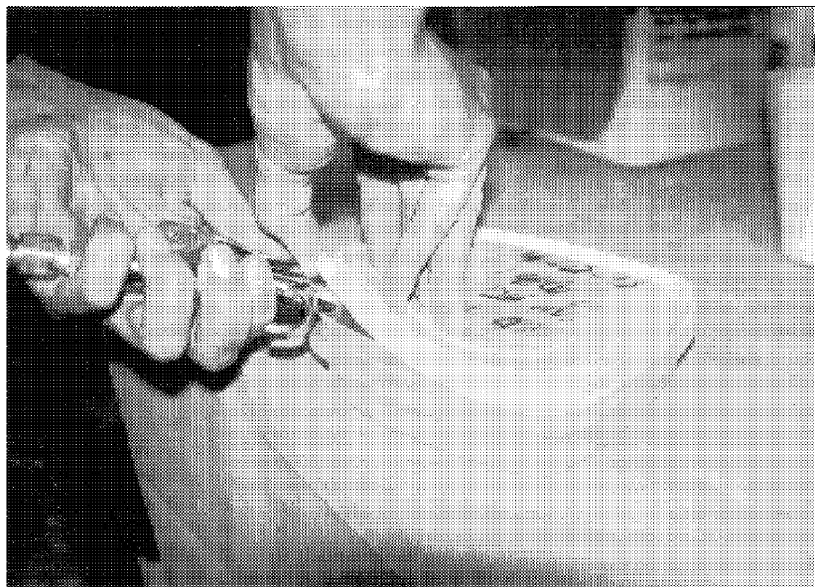


Figure 1. Addition of pure oxygen to a plastic sandwich container containing extended semen (1 part semen: 2 parts extender).

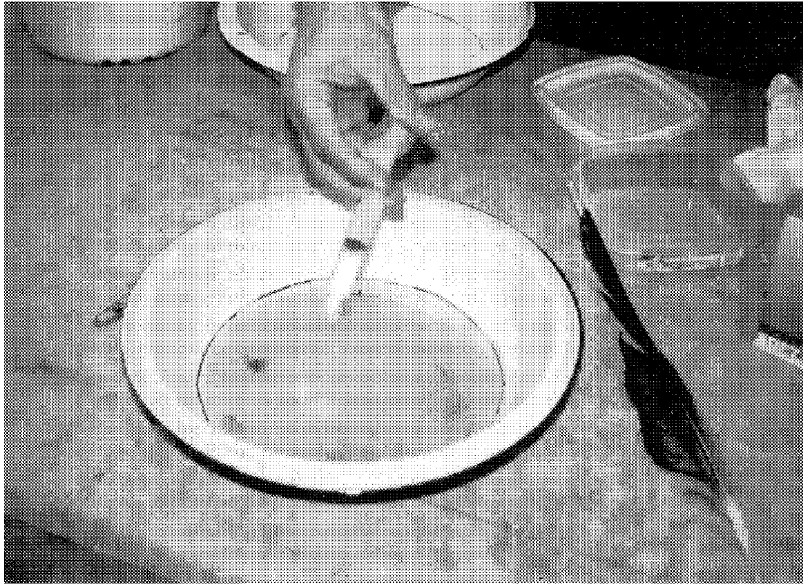


Figure 2. A syringe with 4 ml of extended semen is used to fertilize 1 L of eggs by the dry method.

adequate oxygen to sustain life, even in their cold, almost dormant state. It is the opinion of some personnel that the initial oxygenation should be sufficient to maintain quality sperm cells, and they think that subsequent oxygenation is not only unnecessary, but that the handling of the extended milt may be stressful and increase the chances of contamination by water. Daily oxygenation may not be necessary, but until further research is conducted, it is suggested that containers be reoxygenated daily.

A condensate will often be present on the lid of the plastic sandwich containers holding the semen, but this condensate has not stimulated sperm motility when it mixes with the extended semen. However, it is probably advisable to be careful to avoid dripping the condensation into the semen container.

Accidental freezing of stored semen has been observed in field operations. This may reduce semen viability. Preserved semen should be kept between 33 and 46°F (1–8°C). We found that crystallization starts in the 1:2 semen:extender ratio at 26.4°F (-3.1°C) and solidification can be accomplished at 23°F (-5.0°C). Semen that has undergone partial freezing or solidification of the extended semen should be used only as a last resort. Extended semen that has been partially frozen or solidified results in less than optimum motility when viewed under the microscope. A propane heater placed within the structure where extended semen is kept may prevent freezing from occurring on colder nights.

Extended semen must be checked for motility before it is utilized. Many field personnel forget the importance of insuring the quality of extended milt they are using. All containers must be checked to avoid contamination of extended semen.

Handling procedures

Undoubtedly, capture and handling of walleye utilized in spawning operations will be stressful to the fish. Typically, males are stripped for three consecutive days and then released. To evaluate post-spawning mortality of walleye, we held a group in a culture pond for 45 d after spawning. We observed 22% mortality in this group. Since that observation, we have taken steps to decrease mortality from spawn-taking operations. If ample males are collected, it is suggested that they be released after only two strippings. The third stripping provides minimal amounts of milt which is often more dilute and contaminated with fecal material. If practical, males should be stripped on alternate days. This practice allows the males to rejuvenate milt, and they will provide more milt on alternate days than when they are stripped in two consecutive days.

Spawning operations that collect adequate numbers of males can use the first stripping for fresh milt fertilization and milt from the second stripping for preparation of extended semen. Even with adequate numbers of males, extended semen is a valuable back-up supply. When insufficient numbers of males are available to use only fresh semen, then extended semen at 1:2, 1:4, or 1:6 ratios should be used.

Advantages and disadvantages of extended semen

The use of extended semen requires additional labor, it is time consuming, and stored semen can become contaminated with water or feces. However, experiments conducted at Marston Reservoir during the 1993 and 1994 spawning seasons proved the value of extended semen. Over two years: 63.7% of eggs (9.2 million) fertilized with fresh semen hatched; 59.8% of eggs (2.2 million) fertilized with 1:2 semen:extender

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hatched; 60.7% of eggs (2.4 million) fertilized with 1:4 semen:extender hatched; and 66.9% of eggs (2.8 million) fertilized with 1:6 semen:extender hatched. These results show that extended semen is just as effective as fresh semen.

In Colorado, we do not plan to replace fresh semen in spawning operations entirely with extended semen. We consider use of extended semen an alternative that field biologists can use effectively in special circumstances. When these opportunities arise, however, extended semen becomes especially valuable. Although a higher hatching percentage was obtained with fresh semen in the first two-thirds of the spawning operation, in the last one-third of the season, gamete quality decreases and thus lower hatching percentages occur. However, hatching success of eggs fertilized with extended semen has been less variable and extended semen results in higher hatching percentages in the last one-third of the spawning season. Thus, the practical advantage of extended semen is the capability to preserve gametes collected earlier in the spawning season for use when fresh semen quality decreases or is less available.

Extended semen can be transported from one spawning site to another. It is far easier to transport extended semen than live fish. Transported males often become stressed and fail to produce sufficient amounts of milt. Extended semen is especially advantageous for making

hybrid crosses. Hybrid walleye are prepared using sauger semen and walleye eggs. Extended semen eases the transportation process of male gametes and allows for crosses of geographically isolated populations. Also, extended semen can be used for mating different genetic stocks.

Colorado Division of Wildlife has utilized extended walleye semen since 1988 and found it to be comparable to the results obtained by fresh semen. This experience has allowed field personnel to obtain confidence in the extended semen product. Extended semen is a reliable source of milt as long as the appropriate steps are taken during its preparation, storage, and use.

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Hormone Induced Spawning of Walleye

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Introduction

It is often difficult to capture walleye in “running-ripe” condition because that stage of spawning is of short duration. Walleye culturists in Illinois often collect hard (green) broodfish and attempt to bring them into spawning condition in the hatchery. This case study describes how hormone injections can be used to accelerate the ripening process. Several different types of hormone preparations have been used to successfully advance oocyte maturation and/or induce ovulation in walleye. The hormones include human chorionic gonadotropin (HCG), carp pituitary extract, luteinizing hormone (LH), luteinizing hormone-releasing hormone (LH-RH), and pimozide. The use of such hormones had variable success, with low hatchability of eggs often occurring. Use of hormones in fisheries is not approved by the U.S. Food and Drug Administration (FDA). The work reported here was conducted under an investigational new animal drug (INAD) permit issued by FDA. FDA is currently monitoring a pharmaceutical company funded study on the safety of HCG and a study on the efficacy of HCG.

During the maturation phase of the ova, a germinal vesicle migrates from the center to the periphery of the eggs and breaks down prior to ovulation (GVBD). For walleye, this process may begin during February and require up to 2.5 months to complete. When females are injected with appropriate maturing hormones, such as HCG, the germinal vesicle migration and subsequent GVBD may take 96 h or less.

Objectives

The objective of this study was to determine the dose of HCG that needs to be interperitoneally injected into female walleye that have eggs at various stages of maturation to maximize both the number of females that ovulate and the hatching success. We also wanted to minimize the length of time wild, walleye broodfish

were held in the hatchery. Hatch rates of the HCG injected females were compared with females that were “running ripe” at the capture site and those that ovulated in the hauling tank on their way to the hatchery. In addition, time to ovulation and percentage of injected females that ovulated were compared with non-injected, hard females that were allowed to ripen in hatchery raceways.

Materials and methods

The study was conducted at Jake Wolf Memorial Fish Hatchery, Manito, Illinois. Walleye broodfish were collected from Heideke Lake, Lake Shelbyville, and Kaskaslua River above Lake Shelbyville during April of 1987, 1988, and 1989. Fish were collected when water temperatures ranged from 42–52°F (5.6–11.1°C). Female walleye were initially classified into three groups:

- Group 1. Fish that were “running-ripe” at the capture site;
- Group 2. Fish that ovulated in route to the hatchery and were stripped immediately upon reaching the hatchery;
- Group 3. Fish that had firm ovaries (hard condition).

Hard females were catheterized with a 0.12-in (3-mm) O.D. glass tube to collect a sample of ova for determination of developmental stage. Subsequently, the females were injected with various doses of HCG. The egg samples were placed in a modification of Stockard’s preservation solution until they could be examined for stages of development. Egg stages were classified by degree of oil globule coalescence, egg membrane opacity, and position of the germinal vesicle (GV). Eggs from injected females (group 3) were thus classified into seven stages of maturity:

Stage 3.0. Many small oil droplets; membrane opaque and granular; GV centered.

Stage 3.5. Some oil droplet coalescence; membrane opaque and granular; GV off center.

Stage 3.75. Oil droplets fewer and larger; some membrane clearing; GV migrating and approaching periphery.

Stage 4.0. Single, large oil globule with few, smaller droplets; oocyte membrane translucent; most eggs with GV peripheral.

Stage 4.5. Single, large oil globule; few smaller droplets on periphery of large globule; oocyte membrane slightly granular; GV break down (GVBD) beginning.

Stage 4.75. Oil globule similar to stage 4.5; egg membrane not completely transparent; GVBD complete.

Stage 5.0. Oil globule usually completely coalesced; oocyte membrane transparent; GVBD and ovulation complete.

Walleye eggs staged at 3.0–3.75 were immature, and eggs staged at 4.0–5.0 were nearly mature.

Group-3 (hard) females were intra-peritoneally injected with HCG at doses of either 0, 50, 150, 250, or 500 IU/lb (0, 110, 330, 550, or 1100 IU/kg) of female weight during 1987–89 (Table 1). At the time of injection, each fish was marked with an individually numbered tag for later identification. After hormone injections, the fish were placed in concrete raceways with water temperature of about 54°F (12.2°C) and checked twice daily for ovulation. In 1987, walleye received only one injection of HCG of 150 IU/lb (330 IU/kg). In 1988 and 1989, fish which had not ovulated 48 h after the first injection were injected a second time. In 1988, initial injections were at either 50 or 500 IU/lb (110 or 1100 IU/kg) and second injections, if required, were at 500 IU/lb (1100 IU/kg). In 1989, females were given initial doses of HCG at either 50, 250 or 500 IU/lb (110, 550, or 1100 IU/kg), and second doses were at the level of the first. Some group-3 (hard) females were allowed to ripen without injection in the raceways.

Ovulation was determined to have occurred when eggs would freely flow from the vents with gentle, posterior stroking of the fish's abdomen. Standard "dry method" was used to fertilize the eggs. Refrigerated sperm, which had been mixed with extender solution at a ratio of 1 ml sperm to 2 ml extender (Moore 1987), was used during the experiments. Each batch of extended semen was examined for motility prior to its use. In a previous experiment, we ascertained that using 0–9 d old refrigerated, extended semen solution resulted in mean hatch rates that were similar to rates when fresh sperm

was used. We also compared the amount of semen solution used per 100 ml of eggs with the hatch rate. There were no differences in mean hatch rates between 0.25, 0.5, 1.0, 1.5 or 2.0 ml of solution/100 ml of eggs. For this experiment, we used 0.5 ml of 1:2 sperm solution/100 ml of eggs.

Following fertilization, eggs were mixed in a bentonite solution to remove adhesiveness, water hardened for about 1 h, and incubated in 16.8-oz (0.5 L) glass cylinders that simulated the McDonald hatching jars that are commonly used by hatchery personnel. In a previous study, we determined that egg viabilities could be microscopically quantified accurately at either 6 h, 3 d, or at 8 d after fertilization. In this study, egg viability was quantified 3–4 d after fertilization.

Results

During the three-year study, HCG dose, egg maturation stage at time of initial injection, and handling of broodfish were monitored to examine their effects on egg maturation and viability. There were no significant differences among mean egg viabilities, time-to-ovulation, or percent ovulation among injection dose treatment groups, whether the walleye required only the initial injection or an additional injection after 48 h (Table 1). Therefore, data from various injection dose trials were combined in the following analysis.

Walleye "running ripe" (group 1), and spawned at collection sites or immediately after arriving at the hatchery (group 2), had higher mean egg viabilities than hard females (group 3) injected with HCG or held in the hatchery raceways without injection (Table 1). Egg viability of "running-ripe" females that were stripped at walleye collection sites was 76%, and 66% for the females that were transported to the hatchery prior to stripping. Mean egg viabilities of injected and non-injected females (group 3) were lower than "running ripe" fish, but egg viability of females injected with HCG was 37% compared with 45% of hard fish held without injection at the hatchery.

Although egg viability was not affected by the HCG dose levels of 50–500 IU/lb (110–1100 IU/kg), degree of egg ripeness at the time of injection did have an effect on egg viability. Eggs of non-ripe walleye given HCG injections to promote ovulation were staged as immature and mature. When the walleye were initially

Table 1. Comparison of ovulation percentage, days to ovulation, and egg viabilities of walleye females injected with various doses of human chorionic gonadotropin or uninjected. Numbers in parentheses represent number of females in treatment.

Initial HCG injection (IU/lb)	Second HCG injection (IU/lb)	Ovulation (%)		Days to ovulation		Mean hatch (%)	
		Ima	Nmb	Ima	Nmb	Ima	Nmb
Injected							
50 (35)	50 (22)	91 (23)	100 (12)	4.6 (21)	2.5 (12)	27.0 (18)	39.5 (10)
50 (14)	500 (5)	100 (7)	100 (7)	4.3 (7)	1.4 (7)	34.4 (7)	45.4 (7)
150 (23)		100 (17)	100 (6)	4.2 (17)	1.5 (6)	29.6 (17)	59.3 (6)
250 (30)	250 (21)	76 (21)	100 (9)	4.0 (16)	2.6 (9)	31.6 (15)	38.3 (7)
500 (55)	500 (35)	100 (36)	90 (19)	3.8 (36)	3.1 (17)	35.8 (34)	50.4 (17)
	Mean	93 (104)	96 (53)	4.0 (97)	2.4 (51)	31.4 (91)	48.0 (47)
Non-Injected							
	Running ripe at site		NA		NA	76.0 (5)	
	Running ripe at hatchery		NA		NA	65.6 (28)	
	Hard		23 (44)		4.6 (41)	45.0 (16)	

a Walleye with eggs at immature stages (3.0–3.75) at the time of initial HCG injections.

b Walleye with eggs at nearly mature stages (4.0–5.0) at the time of initial HCG injections.

injected, eggs staged as nearly mature had a mean hatch rate of 48.0%, while eggs staged as immature had a mean hatch rate of 31.4%.

Injections of HCG (irrespective of dosage) significantly increased the percentage of fish which ovulated and, depending on egg stages, reduced the period of time until ovulation. During the three year period, a total of 157 female walleye were given HCG injections of 50, 150, 250, or 500 IU/lb (110, 330, 550, or 1100 IU/kg).

Including fish which were given a second injection, 94% of the females injected with HCG ovulated within 5 d (Table 1). In contrast, only 23% of 44 females taken to the hatchery and held without injections ovulated within 7 d. Walleye with immature eggs required a mean of 4.0 d to ovulate after injection, and fish with nearly mature eggs at initial injection required only a mean of 2.4 d to ovulate.

Conclusion

- Use of HCG is effective for inducing ovulation of walleye captured with unripe eggs. HCG injections effectively shortened the time to ovulation for walleye that were not “running-ripe” upon arrival at the hatchery and induced a higher percentage of walleye to egg ovulation than fish held without injections.
- Egg hatch percentages were similar for injected and non-injected walleye that required a holding period prior to egg ovulation, however holding stress on female fish may adversely affect viability of their eggs.

Acknowledgments

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