Optimizing Triploid Walleye Production in Colorado

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Abstract

Walleye (*Sander vitreus*) provide important recreational and commercial fisheries throughout the United States and Canada. In Colorado, more walleye are stocked every year than any other species of fish. Use of sterile triploid fish in Colorado is an important management strategy for protecting native endangered fish species from naturally reproducing populations of introduced predators like walleye. Triploid fish are created by exposing eggs to hydrostatic pressure shock, temperature shock, or chemical treatments after fertilization to prevent the extrusion of the second polar body. This results in sterile triploids because chromosomes are unable to synapse correctly during division. Hydrostatic pressure has been one of the more effective methods for inducing triploidy in walleye, and the technique is currently evolving. A 2.7-L capacity electric pressure chamber manufactured by TRC Hydraulics was used to produce triploid walleyes using hydrostatic pressure with eggs collected from Pueblo Reservoir, Colorado. The TRC hydraulic press has many advantages over manually-operated pressure chambers used to produce triploid fish including its portability, large capacity, safety, and increases in hatching survival and induction rates. Here, the methods used to create triploid walleye using the TRC hydraulic press are described. In addition, times to initiation (TIs; time between fertilization to when eggs were subjected to pressure) were varied between 4 minutes and 8.5 minutes to determine the optimal TI for maximizing induction and hatching success rates. Our results suggest that triploidy in walleye is maximized with a TI of approximately 7.5 minutes, whereas hatching success is maximized with a TI of just over 8 minutes. Because the goal of the triploid walleye production program in Colorado is focused on maximizing induction rates, we recommend inducing triploidy at approximately 7.5 minutes to meet this objective.

Introduction

Throughout the United States and Canada, walleye provide important recreational and commercial fisheries (Becker 1983). In Colorado, more walleye (*Sander vitreus*) are typically stocked every year than any other species of fish. For example, in 2013 over 40 million walleye were stocked in Colorado in contrast with about 11 million rainbow trout (*Oncorhynchus mykiss* and their crosses) and 9 million kokanee salmon (*Oncorhynchus nerka*). Walleye are one of the most sought after species of fish in the state due to their availability, palatability, and potential to reach trophy size. Although this popular sportfish species is targeted by anglers, walleye are not native to Colorado. Walleye natural reproduction now occurs in some systems in Colorado. However, in other systems where walleye are desirable, natural reproduction is limited or absent. In these cases, walleye populations are maintained and enhanced through stocking.

Despite their popularity, walleye represent a novel predator in Colorado and they can have impacts on native fish populations (USFWS 2009). More specifically, walleye escapement from reservoirs has occurred in the past, and movement of naturally reproducing predators from reservoirs into rivers has been problematic for some native endangered and threatened species. In these instances and in others where control of walleye populations (maintaining appropriate densities) is desirable, there has been growing interest to create and make use of sterile triploid walleye to curb natural reproduction and help regulate walleye populations.

Creating sterile fish for management purposes has been practiced for decades, and more recently Colorado Parks and Wildlife (CPW) has begun to use sterile triploid walleye to address targeted management issues within the state. Triploid fish are created by exposing eggs to treatments (e.g., hydrostatic pressure shock, temperature shock, chemical) after fertilization to prevent the extrusion of the second polar body. This causes sterility because chromosomes are unable to synapse correctly during division (Thorgaard 1983; Strickberger 1985). The result is an extra set of chromosomes, or triploidy instead of diploidy.

Hydrostatic pressure shock has been used to induce triploidy in walleye. This technique is currently evolving, and walleye are a relatively recent addition (Malison et al. 2001) to the list of species for which this approach has been formally described. The success of hydrostatic pressure to produce viable triploid fish is dependent on the timing and magnitude of pressurization, which is species-specific, so control of these factors is crucial (Abiado et al. 2007). The objective of this study was to determine the most effective time between fertilization and when eggs were pressurized to 9,500 PSI to optimize induction rates, increase survival rates, and produce viable triploid walleye.

Source of Gametes – Pueblo Reservoir

Pueblo Reservoir (Pueblo, Colorado) is a 1,880 hectare reservoir managed primarily for sport fishing and recreational opportunities in southern Colorado. Sportfish in the reservoir include walleye, wiper (*Morone chrysops* x *Morone saxatillis*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), spotted bass (*Micropterus punctulatus*), channel catfish (*Ictalurus punctatus*), flathead catfish (*Pylodictis olivaris*), blue catfish (*Ictalurus furcatus*), black crappie (*Promoxis nigormaculatus)*, white crappie (*Promoxis annularis*), bluegill (*Lepomis macrochirus*), yellow perch (*Perca flavescens*), and rainbow trout. A number of other species can be found in the reservoir, including green sunfish (*Lepomis cyanellus*), common carp (*Cyprinus carpio*), gizzard shad (*Dorosoma cepedianum*), and white sucker (*Catostomus commersonii*). Walleye spawn in the reservoir from mid-March to mid-April, and spawning operations conducted by CPW in the reservoir support over 50% of the state's walleye production on an annual basis.

Eggs and spermatozoa used to produce triploid walleye for the years 2010 to 2014 were collected from spawning walleyes caught in gill nets set parallel to the shoreline once reservoir temperatures reached 6.1°C, generally corresponding to the last week of March. Walleye captured in gill nets were transported by boat in aerated hauling tanks to a centrally-located boathouse for spawning. Each day of the spawning operation, male walleye were sorted into a separate holding tank where they were held until stripped (number of days held varied), whereas female walleye were sorted into ripe and green groups. Ripe fish were spawned on the day captured. Green females were held for up to three days to allow them to become ripe, and spawning status was checked on a daily basis. Therefore, gametes collected on any given day, including the days in which triploid walleye were produced, were from a combination of males and ripe females captured from the lake that day, males held over from previous capture days, and females that had ripened in the boathouse holding tanks.

Spawning Operations

Females were spawned into a plastic spawning container, with two to four females spawned per batch. Males (two to four) were used to fertilize the eggs according to the dry method (Piper et al. 1982). Eggs and sperm were mixed for 90 seconds in reservoir water filtered through a 25 micron sock filter.

A stock solution of 800 mg/L tannic acid (Sigma Chemical Co., St. Louis, Missouri) was added to filtered reservoir water at an equal volumetric rate to attain a 400 mg/L tannic acid wash concentration. Fertilized eggs were washed for 90 seconds to reduce egg adhesiveness, and goose (*Branta* sp.) feathers were used to stir the eggs to prevent clumping. Tannic acid was decanted from the spawning container, and filtered reservoir water was used to rinse the eggs one to two times to remove acid and other spawning debris. Note that the use of Fuller's Earth for preventing adhesion is not recommended as it has been attributed to lower hatch rates in triploid walleye, and requires more time to decant during the rinsing process, potentially delaying target times for egg pressurization. Diploid eggs were poured into a fine-mesh, screen-bottomed basket and placed in a bath of filtered reservoir water for water hardening (1 hour). Eggs used to create triploids were retained in the spawning container while the pressure chamber was prepared for use.

Pressure Shock Treatment Methods

A 2.7-L capacity electric pressure chamber manufactured by TRC Hydraulics (New Brunswick, Canada) was used to pressure shock the walleye eggs. To prepare the chamber for use, filtered reservoir water was run through the chamber for several minutes to acclimate chamber temperature to that of the reservoir as the chamber generally cooled to the ambient air temperature overnight. The top and bottom valves of the chamber were closed prior to egg transfer to ensure that water and eggs would not be lost. A 3 mm mesh egg basket (standard from TRC Hydraulics) was placed in the chamber to hold the walleye eggs. Note that a basket with 3 mm mesh does allow the passage of some eggs from the basket during treatment. This can be prevented by using a basket with finer mesh (e.g., 1.5 mm; can be custom ordered from TRC Hydraulics). The chamber was filled half full with filtered reservoir water so that eggs were not exposed to air or damaged by impacting the bottom of the egg basket at any point while being transferred.

Walleye eggs were transferred to the chamber using a 2.8 L wide-spout plastic pitcher with equal parts filtered reservoir water and eggs. Eggs were poured directly into the seated egg basket within the chamber and residual eggs were rinsed from the pitcher. Care was taken to prevent eggs from being caught on the upper lip of the chamber. Once eggs had settled within the egg basket, filtered reservoir water was used to fill the chamber so that there was no air inside the chamber once the plug was inserted (air would compress during pressurization, potentially preventing the chamber from reaching full pressure). To complete chamber preparation, the plug was inserted, the top valve of the chamber was opened to allow the plug to seat, and the plug was rotated clockwise to seat it within the locking arms, preventing accidental release while under pressure. Finally, the top valve was closed to create the vacuum needed for pressurization.

Hydrostatic pressures exceeding 8,000 PSI have produced higher rates of triploid induction in walleye and other fish species (Malison et al. 2001; Kozfkay et al. 2005; Abiado et al. 2007); CPW used a pressure of 9,500 PSI to induce triploidy in walleye. Pressurization of the chamber to 9,500 PSI required 45 to 50 seconds, depending on temperature. As such, the chamber operator timed the initiation of pressurization to correspond with the goal time of initiation (TI; time between fertilization and when eggs were under 9,500 PSI) for a given trial or year. Eggs remained under 9,500 PSI for ten minutes. Following pressure shock treatment, the hydraulic valve was opened to depressurize the chamber, and the top valve was opened to release the vacuum so that the plug could be removed after pressure was released. The egg basket containing the eggs was removed from the chamber, and the lower plug was opened to drain the chamber through a fine mesh filter that caught any remaining eggs that had made it through the egg basket during treatment. Eggs were transferred to a fine-mesh, screen-bottomed basket and placed in a bath of filtered reservoir water to complete the water hardening procedure (1 hour). Following water hardening, diploid and triploid eggs were transferred to a five gallon bucket for transport back to the main hatchery building for incubation, hatching, and rearing.

Methods of preparation and timing differed slightly from year to year. In 2010, TI occurred at approximately four minutes after fertilization. In 2011, TI was increased with each batch of eggs to

determine the TI needed to optimize both induction and hatch rates. Batches were run at a TI of 5.5, 6.5, 7.5 and 8.5 minutes with a water temperature of 6.1°C. Batches with shorter TIs (i.e., 5.5 and 6.5 minutes) were generally only rinsed once following tannic acid treatment, whereas eggs were rinsed twice with the longer TIs. Aside from these exceptions, methodology remained the same among TIs. In 2012, 2013, and 2014, a TI of 7.5 minutes at a water temperature of 6.1°C was used to pressure shock walleye egg batches.

Egg Incubation and Rearing

Egg incubation occurred at the CPW Pueblo Hatchery (Pueblo, Colorado). The hatchery is supplied by well water, with temperatures ranging between 8.9 and 14.4°C. Upon arriving at the hatchery, egg size was assessed using a Von Bayer trough (Piper et al. 1982). The number of eggs per liter was calculated to determine the initial number of eggs per hatching jar where they were held for the duration of the incubation period (approximately 300 degree days; Piper et al. 1982). Eggs were treated with hydrogen peroxide at a concentration of 500 ppm on a daily basis to prevent fungal infections. Dead eggs were allowed to float out of the jars during the incubation period, or were siphoned off when they floated to the top.

Once eggs were eyed, the remaining eggs in a jar were stirred using increasing and decreasing flows until they were uniformly mixed. A 6.3 mm glass tube was inserted into the center of the rolling mass of eggs and used to pull out between 600 and 800 eggs to determine the ratio of live to dead eggs, providing the hatching success percentage for each trial. Egg volume from each jar was recalculated to account for the dead eggs removed during incubation, and the hatching success rate was applied to this final volume. Following this procedure, jars were transferred to hatching tanks to complete incubation and hatch.

Ploidy Analysis

One-day-old walleye fry were shipped live from the CPW Pueblo Hatchery to Virginia Commonwealth University where the ploidy analysis was performed using batches of approximately 100 fry. Each trial or year was analyzed separately. Ploidy was determined using the flow cytometry method in which the florescence of a dye used to stain the samples is quantified into channels indicating diploidy or triploidy (Thorgaard et al. 1982; Ewing et al. 1991; Harrel et al. 1998). Ploidy determination took only one day to complete, and the results were used to guide fry stocking upon receipt. Groups in which induction was high were stocked in locations where triploidy was imperative to management, whereas those groups in which induction was low were used in locations where triploidy was not required to meet management objectives.

Statistical Analyses

In 2010, CPW used the TRC hydraulic press to increase hydrostatic pressure to 9,500 PSI approximately 4 minutes after fertilization (n = 4 batches). In 2011, the same process was used, but eggs were pressurized at 5.5, 6.5, 7.5 and 8.5 minutes (n = 4 batches). In 2012, 2013 and 2014, eggs were pressurized 7.5 minutes after fertilization in all cases (single batches each year). Based on data collected from these trials, statistical analyses were conducted to: 1) determine the optimal TI to maximize triploidy induction, 2) determine the optimal TI to maximize egg hatching success, 3) determine if there was a difference in triploidy induction rates between walleye eggs with a TI of 4 minutes (2010) versus those with a TI of 7.5 minutes (2011 – 2014), and 4) determine if there was a difference in egg hatching success between walleye eggs with a TI of 4 minutes (2010) versus those with a TI of 7.5 minutes (2011 – 2014). Mean inductances and hatching success (by batch of pressurized eggs) were compared to avoid pseudoreplication associated with sample duplicates.

The TIs needed to optimize triploidy inductance (analysis 1) and egg hatching success (analysis 2) were determined by solving the first derivative of second order polynomial equations (quadratics) of the best fit lines for egg hatching success and triploidy inductance (respectively) as functions of TI (5.5, 6.5, 7.5

and 8.5 minutes). This was done with data collected in 2011. A comparison of triploidy induction rate (analysis 3) between walleye eggs with a TI of 4 minutes (2010; n = 4) versus those with a TI of 7.5 minutes (2011 – 2014; n = 1 for each year) was conducted using a two-sample *t* test assuming unequal variance. The same was done to compare the egg hatching success of these two groups (analysis 4).

Results

Based on the data collected in 2011 using the TRC hydraulic press, the optimal TI for walleye eggs to maximize triploidy induction (analysis 1) was approximately 7 minutes and 33 seconds. Further, the optimal TI for walleye eggs to maximize egg hatching success (analysis 2) was approximately 8 minutes and 10 seconds (Figure 1).

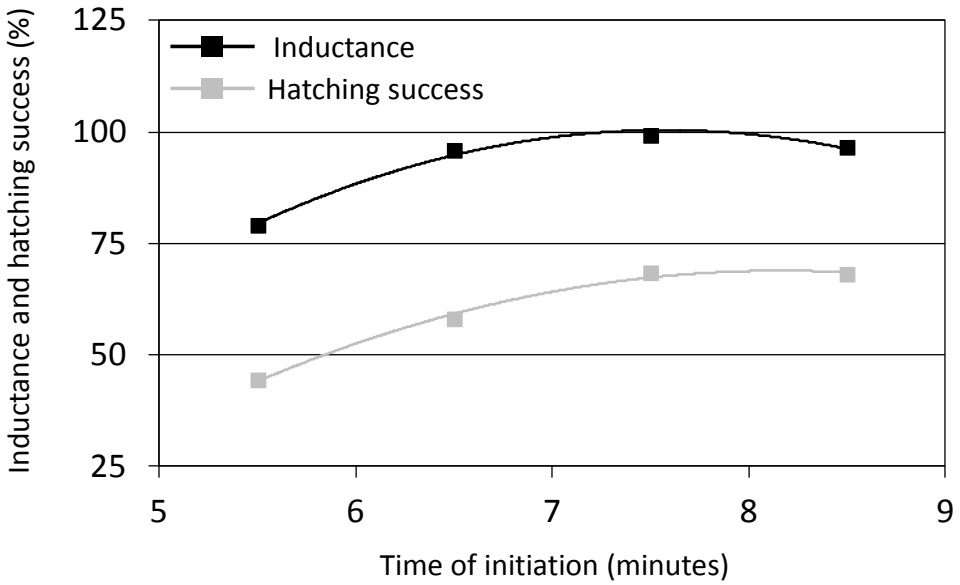

Figure 1. Percent walleye triploidy inductance and egg hatching success as functions of time of initiation (TI). Inductance is represented in black and egg hatching success is represented in gray. Solid lines are best fit quadratic equations.

The comparison of triploidy induction rates between walleye eggs with a TI of 4 minutes (2010) versus those with a TI of 7.5 minutes (2011 – 2014) indicated that triploidy induction rates were approximately 12% lower for eggs pressurized 4 minutes versus 7.5 minutes after fertilization (85 and 97%, respectively; one –tail *t* test, *t* statistic = 3.19, n = 4, 4, p = 0.02; analysis 3). The comparison of hatching success between walleye eggs with a TI of 4 minutes (2010) versus those with a TI of 7.5 minutes (2011 – 2014) indicated that hatching success rates were 30-35% lower for eggs pressurized 4 minutes versus 7.5 minutes after fertilization (23 and 56%, respectively; one –tail *t* test, *t* statistic = 4.82, n = 4, 4, p < 0.01; analysis 4).

Discussion

The TRC hydraulic press used in this study has many advantages over manually-operated pressure chambers used to produce triploid fish in previous studies including its portability, large capacity, and increases in hatching survival and induction rates (Abiado et al. 2007). Abiado et al. (2007) showed that triploid saugeye (*Sander vitreus* x *S. canadensis*) hatching success increased from 59.3 to 81.6%, and induction rates increased from between 80 and 96.7% to 100% when using the 2.7-L TRC hydraulic press compared to a 1-L manually-operated pressure chamber. Due to the advantages of the TRC hydraulic press, it has also been used to successfully induce triploidy in Atlantic salmon (*Salmo salar*; O'Flynn et al. 1997), lake trout (*Salvelinus namaycush*; Kozfkay et al. 2005), and now, walleye.

The success of hydrostatic pressure to produce viable triploid fish is dependent on the timing and magnitude of pressurization, which is species-specific, so control of these factors is crucial (Abiado et al. 2007). For example, pressures ranging from 9,000 to 9,500 PSI have been used to successfully induce triploidy in lake trout (Kozfkay et al. 2005), whereas pressures as low 8,000 PSI have been used to induce triploidy in walleye (Malison et al. 2001). Similarly, duration at which eggs are under pressure have varied greatly among species, from five minutes in lake trout (Kozfkay et al. 2005), to up to 30 minutes in walleye (Malison et al. 2001). As such, protocols used to develop triploid fish are constantly evolving.

As the production of triploid walleye is relatively new compared to other species (Malison et al. 2001), techniques for maximizing induction and hatching success rates continue to advance. Unfortunately, induction of triploidy through hydrostatic pressure tends to lower hatching success regardless of time to initiation (Garcia-Abiado et al. 2001). In Colorado, diploid hatching success rates are higher than maximized hatching success rates observed in triploid walleye (85 versus 56%, respectively). However, if maximization of hatching success is the goal of a particular triploid walleye production program, our results suggest that hatching success is maximized at a TI of 8 minutes and 10 seconds using 9,500 PSI. In addition, hatching success appears to increase with an increase in TI, with hatching success rates increasing by 12% with an increase in TI from 4 to 7.5 minutes. Similar increases have been observed by other states using similar protocols. For example, the Kansas Department of Wildlife, Parks and Tourism (KDWPT) achieved an increase in hatching success from 29 to 46% when increasing time of initiation from 4 to 7.5 minutes at 9,500 PSI (pers. comm.). Survival at a time of initiation of four minutes has ranged from 7.6 to 18.2% for triploid saugeye (Abiado et al. 2007) to 63.3 to 73.3% for triploid walleye (Malison et al. 2001). Therefore, it is likely that hatching success of triploid walleye is dependent upon a number of different factors including, but not limited to, time to initiation, duration of pressurization, temperature, egg quality, and hatchery rearing conditions.

One consistency among percid triploid induction methodology up to this point has been the initiation of pressurization at 4 minutes post-fertilization (Malison et al. 2001; Abiado et al. 2007). Success regarding triploid induction rates has varied within the percids at this TI. For example, Abiado et al. (2007) achieved 100% triploid induction rates in saugeye treated in the TRC hydraulic press at 9,000 PSI for durations of 5, 12, and 16 minutes, whereas Malison et al. (2001), using 8,000 PSI, achieved induction rates in walleye of 72.2% and 100% at durations of 15 and 30 minutes, respectively. Our results suggest, however, that induction rates were 30-35% lower at a TI of 4 versus 7.5 minutes when using higher pressures and lower durations. In fact, induction rates are maximized at a TI of 7 minutes and 33 seconds when using 9,500 PSI for a ten minute duration. Other states have noted a change in successful induction rates when increasing from a TI of 4 to 7.5 minutes. For example, KDWPT obtained induction rates of only 93% at a TI of 4 minutes, but increased induction rates to over 99% when increasing the TI to 7.5 minutes at 9,500 PSI (pers. comm.). Likewise, the Montana Fish, Wildlife and Parks Fort Peck Hatchery found that induction rates of 98.5% were achieved when using a TI of 8 minutes at 9,500 PSI (pers. comm.).

Triploid fish are used in a variety of management situations, and have been found to be useful for the control of overpopulation (due to sterility), for increasing growth in juveniles, and for extending survival and improving growth in mature fish (Tiwary et al. 2004). Although maximizing both triploidy induction rates and hatching success is desirable, the management goals for many agencies require that triploid induction rates be as high as possible to guarantee that sterile fish are being stocked. For example, triploid walleye are used in reservoirs on the western slope of Colorado where escapement and reproduction in rivers could present a risk to endangered native species (USFWS 2009). Under controlled culture situations, triploid fish tend to exhibit similarities in growth relative to diploids (Myers and Hershberger 1991; Galbreath et al. 1994). However, triploid saugeye have exhibited differences in foraging behavior, capturing smaller prey with lower reward, and exhibiting lower capture efficiencies and greater foraging times, all of which can affect growth, and ultimately survival, if similar behaviors are seen in other percid species (Czesny et al. 2002). Despite this, triploid walleye appear to survive and grow well in Narraguinnep Reservoir in the San Juan drainage of southern Colorado, where biologists found up to three year classes of triploid walleye, with the largest being 593

mm total length, in 2013, four years after triploid walleye stocking had commenced in the reservoir (pers. comm.). Therefore, it appears that triploid walleye are surviving after being stocked, providing important recreational fishing opportunities while meeting the management objectives of protecting threatened and endangered native fish species in the event of escapement.

Our results suggest that triploidy in walleye is maximized with a time of initiation of approximately 7 minutes and 33 seconds, whereas hatching success is maximized with a time of initiation of 8 minutes and 10 seconds. We acknowledge that there is error in the interpolation of these values and they are specific to the circumstances described here. However, these values could be used as targets or starting points, and refinement and development of situation-specific ranges for these values are encouraged. It is important to note that techniques were selected to optimize triploidy induction due to its relative importance for the management objectives of the State of Colorado, compared to hatching success. Therefore, we recommend inducing triploidy at approximately 7 minutes and 30 seconds to meet this objective.

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