Inability of Hormone Treatment in Wild-Caught Female Walleyes to Increase Egg Ripening

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Abstract – Wild-caught *Sander vitreus* Mitchill (Walleyes) collected during natural spawning are often used to procure gametes. However, some wild-caught females are not ripe (green) and typically are held in hopes that they will ripen. In hatchery settings, the injection of the hormone human chorionic gonadotropin (hCG) has resulted in a portion of green females ripening within a few days. An increase in ripe Walleyes would increase the number of gametes collected and potentially reduce the resources needed during spawning operations. Our objectives were to determine if the percentage of green female Walleyes that ripened while held in in-lake net pens was higher for fish injected with hCG (injected) than those not injected (control) and to assess the probability of ripening with increases in water temperature and Julian date for at-large, injected, and control fish at two South Dakota lakes (Middle Lynn Lake and Piyas Lake). We injected hCG (500 IU/kg) into freshly caught green females (n = 197 at Middle Lynn Lake, n = 40 at Piyas Lake) and held each day's fish in a separate in-lake net pen; controls (n = 40 at Middle Lynn Lake, n = 20 at Piyas Lake) were placed in separate pens, and fish in all pens were checked daily for ripening. The percentage of injected females ripening (58.9%) was less at Middle Lynn Lake than the percentage of control females ripening (77.5%), and no difference was identified at Piyas Lake where 53.7% of injected fish ripened and 60.0% of controls ripened. At each lake, the probability of female Walleye being ripe increased with water temperature and Julian day for daily caught at-large, injected, and control fish. While previous studies in controlled environments have demonstrated that hCG injections increased the proportion of green females ripening, our results indicate that using hCG in Walleye field spawning operations did not increase the number of eggs collected and would not have reduced resources needed during spawning.

Introduction

Sander vitreus Mitchill (Walleye) is an important sport fish species across its range. Because of its importance, agencies spend considerable resources on Walleye management. A substantial component of Walleye management for many agencies is the stocking of fry and hatchery-reared juveniles. Fisheries management agencies annually spend substantial resources (economic and staff days) to collect and fertilize eggs to meet yearly Walleye stocking requests. In 2006, an estimated 869 million Walleyes were stocked in North America by 5 Canadian and 36 American jurisdictions, and increased numbers were expected to be stocked in future years (Kerr 2008).

To meet their stocking needs, agencies frequently collect Walleye broodstock from wild populations for artificial spawning. Female Walleyes follow group-synchronous ovarian development (Barton and Barry 2011), allowing for the collection of a high number of gametes over a relatively short period of time. Walleyes spawn shortly after ice-out at northern latitudes with peak spawning activity occurring when the photoperiod is appropriate and water temperatures are between 5.6 °C and 10.0 °C (Summerfelt et al. 2011). Natural spawning generally occurs over suitable substrate in shallow water near shore, making Walleyes

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vulnerable to capture with a variety of fish collection gears (Piper et al.1982, Summerfelt et al. 2011). Ideally, high numbers of ripe females can be collected in a short time to lessen the resources needed to achieve egg collection goals. The duration of spawning can be variable depending on spring weather conditions, which can alter the timing and extent of natural spawning. Cold springs may delay Walleye spawning while warm springs may advance it (Summerfelt et al. 2011). An extended spawning season may occur when warm weather initiates spawning activity followed by a cold front that stops the spawning until waters warm (Summerfelt et al. 2011). Rapid weather changes can also affect the level and duration of peak spawning activity (Malison and Held 1996).

During artificial spawning operations, the ovulation status of female Walleyes is determined when they are collected. Ripe females, those that have undergone ovulation, typically are artificially spawned the same day as capture to prevent egg loss. Females that are not ripe are considered green and may be held for several days in a hatchery setting or within a lake in hopes that they will ripen (Harvey and Hood 1996, Thompson 1996). Held green Walleyes are generally checked daily, and females that ripen are artificially spawned, while those that do not ripen within a set number of days are released back into the lake. Thompson (1996) reported that 48% of held green females collected from the Pike River, Minnesota, ripened within 3 days at the Pike River Walleye spawning facility.

A substantial number of the female Walleyes collected during artificial spawning operations may be green and held to allow them to potentially ripen. Increasing the percentage of green females that ripen within the allotted time could shorten the time needed to collect eggs and possibly the number of lakes needed for procuring eggs. For example, in 2016, 50 of the 94 green female Walleyes held in net pens failed to ripen at Middle Lynn Lake, South Dakota (M. Ermer, South Dakota Department of Game, Fish and Parks, Webster, SD, unpubl. data). If these fish had ripened, an additional 6.2 million eggs (based on a mean of 123,173 eggs per female at Middle Lynn Lake in 2016) could have potentially been collected from Middle Lynn Lake in 2016.

Agencies have used different methods to try to ripen green female Walleyes for spawning. In Pennsylvania, green female Walleye broodstock collected from Pymatuning Sanctuary Lake (water temperature 7 ºC) were placed in warmer well water (11 ºC) at the Linesville Fish Culture Station to speed the ripening process (Harvey and Hood 1996). Several hormone treatments, including human chorionic gonadotropin (hCG), have been used to induce ovulation in female Walleyes (Malison and Held 1996). Injections of hCG significantly increased the percent of wild-caught green Walleye ripening over non-injected fish held at Jake Wolf Memorial Fish Hatchery, Manito, Illinois, and it was believed that use of hCG could reduce the time period to ovulation (Heidinger et al. 1996). Additionally, the authors found the percent egg hatch to be similar between injected and non-injected green Walleyes but lower than for wild-caught ripe fish.

Placing green female Walleyes in water warmer than the lake water from which they are collected currently is not an option in South Dakota and likely not for many other agencies because of distances between spawning and hatchery locations. Previous results with intramuscular injections of hCG, albeit in hatchery settings with warmer water, suggest that the percentage of lake-held green females ripening could increase with the use of hCG. An increase in the number of ripe Walleyes could result in fewer days to collect gametes and(or) lakes necessary for artificial spawning operations, which would result in a fiscal savings for agencies. We are unaware of any field studies examining the use of hCG treatments in wild-caught Walleyes held in lake water. Our objectives were to determine if the percentage of green female Walleyes that ripened while held in in-lake net pens was higher for fish injected with hCG than those not injected (control), and to determine the probability of a female being ripe across temperatures and Julian dates for at-large, injected, and control fish at two South Dakota lakes.

Materials and Methods

Collection of broodstock Walleyes was completed at two natural lakes in northeast South Dakota during 27 April-5 May 2022, with the exception of 30 April. The two lakes were Middle Lynn Lake (Day County, 420 hectares) and Piyas Lake (Marshall County, 792 hectares). Two sizes of modified-fyke nets were used to collect broodstock at Middle Lynn Lake. The smaller of the two net types had two 0.9 x 1.5-m frames, three 0.9-m diameter hoops, a single throat, a 0.9 x 15.2-m lead, and 19-mm knotted mesh. The large net had two 1.5 x 1.8-m frames, four 1.5-m diameter hoops, a double throat, a 1.5 x 30.5-m lead, and 19-mm knotless mesh. Only the smaller modified-fyke net was used at Piyas Lake.

Modified-fyke nets were checked daily and the surface water temperature was recorded each morning from the middle of each lake using a depth finder (Lowrance HDS-7). Collected Walleyes were dip netted from the cod end of the modified-fyke nets, placed in stock tanks with oxygenated lake water, and transported to a central spawning location on each lake. Ripeness of female Walleyes was determined by applying pressure to their abdomen (Summerfelt et al. 2011). Eggs were stripped from ripe females on the day they were collected. Green females were weighed (g) to determine the amount of hCG to inject. Intramuscular injections of Chorulon® (freeze-dried preparation of hCG reconstituted in a sterile diluent) were administered at 500 IU/kg fish weight (Grizzle and Xu 1996, Heidinger 1996) using a 20-gauge needle attached to a 3-ml syringe. The injections were made ventral to the dorsal fin, and multiple sites were used when the dosage exceeded 1 ml to minimize Chorulon discharging from the injection site. Control females were randomly selected from available green females on 3 days (27 April and 2, 3 May) at Middle Lynn Lake and 2 days (28 April and 1 May) at Piyas Lake. The control fish group on each of these days consisted of 10-15 green females, which did not receive an injection and were held in an in-lake net pen. We did not complete "sham" injections on the controls because we were interested if hCG-injected fish ripened at a higher rate than the standard protocol of holding green females in in-lake net pens.

Injected fish were held in different net pens each day, and separate net pens were used for control fish. Holding pens were made of a knotless (6-mm bar measure) nylon mesh bag (1.5-m deep) hung from a 1.3 x 2.0-m metal conduit frame held above the water level with a series of floats attached to the conduit frame. Holding pens were anchored next to the spawning site and were protected from prevailing winds. Ripeness of all held fish was checked daily and those not ripening were held up to 4 days. Weights of ripe fish (injected and control) were recorded, and their eggs stripped before being released back into each respective lake. All fish that failed to ripen during the designated time were released.

Normality of fish weight data was tested with the Shapiro-Wilk test. Median weight values are presented because the data did not follow a normal distribution. Weight distributions of injected and control fish (except at Piyas, where control fish initial weights were not recorded) were compared with the Mann-Whitney U test. The Mann-Whitney test was also used to compare weights of ripe injected and ripe control fish. The percentage of held green females that ripened each day for injected and control fish was determined and daily cumulative percent ripening histograms were constructed for each grouping at each lake. A Chi-square test was used to test the overall percentage ripening for injected and controls at each lake. We used logistic regression to model whether a female Walleye would be ripe or green across the range of water temperatures and Julian days observed in this study for at-large, injected and control fish. The probability of a female Walleye being ripe across the temperatures and Julian days observed was determined using the equation:

$$\text{Probability(ripe)} = \frac{e^{(\beta_0 + \beta(x))}}{1 + e^{(\beta_0 + \beta(x))}}$$

where β_0 = logistic regression constant coefficient, β = logistic regression logic coefficient, and x = either temperature or Julian day. All statistical tests were completed with Systat 13.0 (Systat Software Inc., Richmond, California) with a significance level of $\alpha = 0.05$.

Results

Daily water temperatures ranged from 3.9 to 6.4 ºC at Middle Lynn Lake and 3.3 to 6.1ºC at Piyas Lake. At Middle Lynn Lake, 197 female Walleyes were injected with hCG, and at Piyas Lake, 54 were injected (Table 1). A total of 40 fish at Middle Lynn Lake and 20 fish at Piyas Lake served as controls. The median weight of injected fish at Middle Lynn Lake was 1,701 g (range 879–4,337 g) and the median weight of control fish was 1,644 g (range 1,162–4,339 g). The weights at capture at Middle Lynn Lake did not differ between the injected and control fish ($U = 3{,}237$; $P = 0.485$). At Piyas Lake the median weight of injected fish was 3,246 g (range 1,021–4,678 g), and no initial weights were recorded for control fish.

Median weights of ripe injected and control ripe fish were similar at both lakes. We detected no difference between the weights of ripe injected (median = 1,715 g; range 992–4,224 g) and ripe control fish (median = 1,531 g; range 1162–3,997 g) at Middle Lynn Lake ($U = 1{,}556$; $P = 0.161$). Similarly, no difference in the weights of ripe injected fish (median = 3,303 g; range 624–4,678 g) and ripe control fish (median = 2,821 g; range 1,389–5,046 g) was found at Piyas Lake ($U = 164.0$; $P = 0.906$).

The largest cumulative percentage increase of injected fish ripening occurred the second day after being injected at both lakes (Fig. 1). Overall, at Middle Lynn Lake 58.9% of the injected fish ripened and 77.5% of the control fish ripened (Table 1). The percentage of control fish ripening was higher than the percentage of injected females ripening ($\chi^2 = 4.892$; $P = 0.027$). We detected no difference in the percentage of fish ripening between injected fish (53.7%) and control fish (60.0%) at Piyas Lake ($\chi^2 = 0.234$; $P = 0.628$).

Positive logit coefficients for temperature and Julian day were identified in the logistic regression for at-large, injected, and control females (Table 2). Temperature and Julian day were found to be important predictors of whether or not a female was ripe for all female groupings at Middle Lynn and the at-large females at Piyas Lake (logit $P < 0.05$). Small sample sizes for injected and control fish likely resulted in the observed non-significant logit coefficients at Piyas Lake.

Table 1. The total number, number ripened, and percent (%) ripened of female Walleye either injected with human chorionic gonadotropin (500 IU/kg) or not injected (Control) and held in in-lake net pens at Middle Lynn Lake and Piyas Lake, South Dakota, in 2022. Chi-square (χ^2) test values and P-values for test between percent ripening by grouping for each lake are provided.

	Injected			Control				
Lake	No.	No. ripe	% ripe	No.	No. ripe	% ripe	χ^2	*P*-value
Middle Lynn	197	116	58.9	40	31	77.5	4.892	0.027
Piyas	54	29	53.7	20	12	60.0	0.234	0.628

At-large females were more likely to be ripe (odds ratio = 3.075) for each degree increase in temperature than the injected fish (odds ratio = 1.705) and control fish (odds ratio = 2.211) at Middle Lynn Lake. Similarly, at Piyas Lake the odds ratio was highest for the at-large fish and temperature, but the other female groupings were found to not provide significant logit coefficients reducing our ability to make inferences.

The probability of an at-large female being ripe was higher across all temperatures and Julian days observed in this study than the observed probabilities for injected and control fish (Fig. 2). Injected and control females had similar probabilities of being ripe at Middle Lynn. A similar pattern for control and injected fish was observed at Piyas Lake, but the non-significant logit regression parameters resulting from small sample sizes limit any comparison.

Discussion

Injecting hCG (500 IU/kg fish weight) into wild-caught green female Walleye brood-stock held within the lake did not increase the percentage ripening in Middle Lynn Lake or Piyas Lake. Our results showed non-injected (control) green female Walleyes held within net pens in the lake ripen at a similar or slightly higher rate than those injected with hCG.

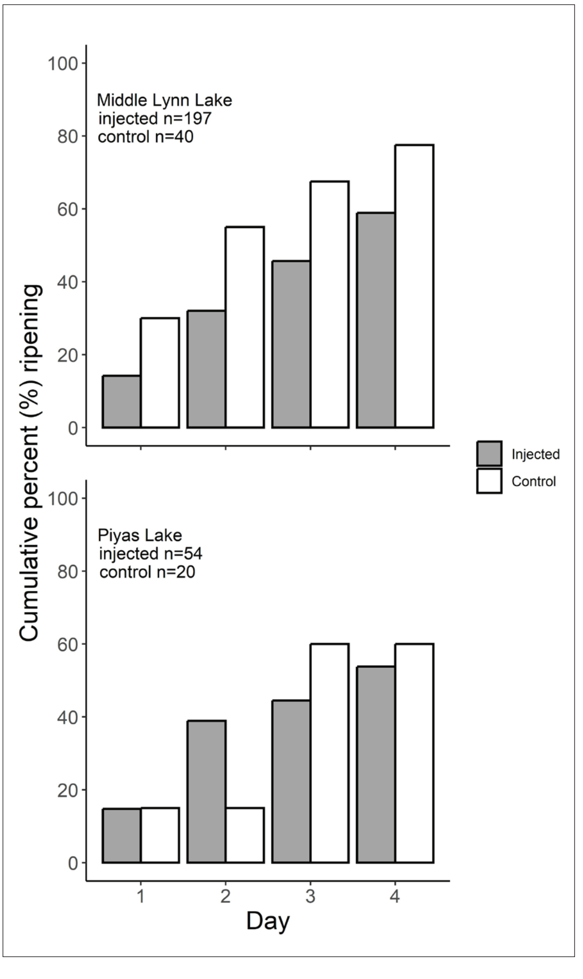

Figure 1. Cumulative percent of female Walleyes ripening in 4 days for females administered human chorionic gonadotropin (500 IU/kg) and held in in-lake net pens (injected), and females not injected (control) and held in in-lake net pens during 2022 spring Walleye artificial spawning operations at Middle Lynn Lake (top panel) and Piyas Lake (bottom panel), South Dakota.

The similar rates of ripening between controls and injected fish indicates that the stress of being injected did not adversely alter those ripening. It does not appear that using hCG in Walleye field spawning operations will increase the number of eggs collected or shorten the amount of time personnel are needed in the field to collect eggs. Because we found the probability of an at-large female being ripe to increase across temperatures and Julian days, it may not be necessary to hold green females in net pens in hopes they ripen. Schneider et al. (2010) indicated that it is a common practice for agencies to halt egg collections before the actual end of Walleye spawning run because egg goals have been met. Concentrating spawning efforts during the peak spawning run and not holding green females could shorten the time staff is in the field because female Walleyes held in net pens would not have to be checked daily.

It is possible that the fish we injected were only in the early stages of oocyte development, resulting in the limited number ripening. We did not check the egg maturity status of the green female Walleyes before injecting hCG or of our control fish. The probability of a female being ripe was similar across water temperatures and days for injected and control fish, but both were lower than at-large females. Malison and Held (1996) indicated that the identification of oocyte maturation can be beneficial when trying to ripen green female Walleyes in a hatchery setting. The authors specified that females with eggs before stage-3 oocyte development will likely not ripen when held in a hatchery setting without the use of hormones. At Jake Wolf Memorial Fish Hatchery, Manito, Illinois, green Walleyes classified as having immature eggs when injected required an average of 4 days to ovulate after injection, and those with nearly mature eggs ovulated in an average of 2.4 days following a hCG injection (Heidinger et al. 1996). Since we were completing our research for use as part of field spawning operations, we chose not to check oocyte development because of the extra time and equipment that would be needed in remote sites and likely would not be included in field spawning operations.

Table 2. Logistic regression coefficients, Z-ratios, *P*-values for coefficients and odds ratios for at-large (at-large), injected green (injected) with human chorionic gonadotropin (500IU/kg), and not-injected green (control) female Walleye ripeness by water temperature and Julian day during 2022 spring Walleye artificial spawning operations at Middle Lynn Lake and Piyas Lake, South Dakota.

			Intercept (β_0)			Logit (β)			Odds
Lake	Type	Parameter	Coefficient	Z-ratio	*P*-value	Coefficient	Z-ratio	*P*-value	ratio
Middle	at-large	temperature	-5.417	-7.013	<0.001	1.123	7.214	<0.001	3.075
Lynn		Julian day	-46.203	-9.104	<0.001	0.384	9.139	<0.001	1.467
	injected	temperature	-4.068	-4.760	<0.001	0.559	3.458	0.001	1.705
		Julian day	-33.918	-4.512	<0.001	0.269	4.366	<0.001	1.309
	control	temperature	-5.131	-3.069	0.002	0.794	2.800	0.005	2.211
		Julian day	-40.305	-2.756	0.006	0.324	2.725	0.006	1.382
Piyas	at-large	temperature	-4.463	-4.595	<0.001	1.206	5.637	<0.001	3.341
		Julian day	-78.731	-7.792	<0.001	0.660	7.897	<0.001	1.934
	injected	temperature	-3.100	-2.107	0.035	0.436	1.446	0.148	1.546
		Julian day	-21.382	-1.593	0.111	0.167	1.520	0.129	1.182
	control	temperature	-7.177	-1.492	0.136	1.405	1.318	0.187	4.077
		Julian day	-77.868	-2.245	0.025	0.633	2.226	0.026	1.884

Cold water temperatures may have reduced the number of green females ripening during our study. In 2022, water temperatures were slow to rise after the Walleye spawn began in our two lakes. The water temperature at Middle Lynn was only 6.4 ºC on May 4, 2022, the last day broodstock were collected, and on the last day of broodstock collection at Piyas Lake the temperature was 6.1 ºC (May 5, 2022). Both of these temperatures are at the lower end of identified peak spawning temperatures (5.6–10.0 ºC; Summerfelt et al. 2011) and are below the reported optimum Walleye spawning temperature range of 7.2–15.5 ºC (Piper et al. 1982). A gradual warming of water temperatures may have changed the percentage of hCG injected fish ripening. Success with ripening green female Walleyes with hCG injections has occurred in hatchery settings where water temperature typically is warmer than lake temperatures and generally is held at a constant level. The percentage of wild-caught

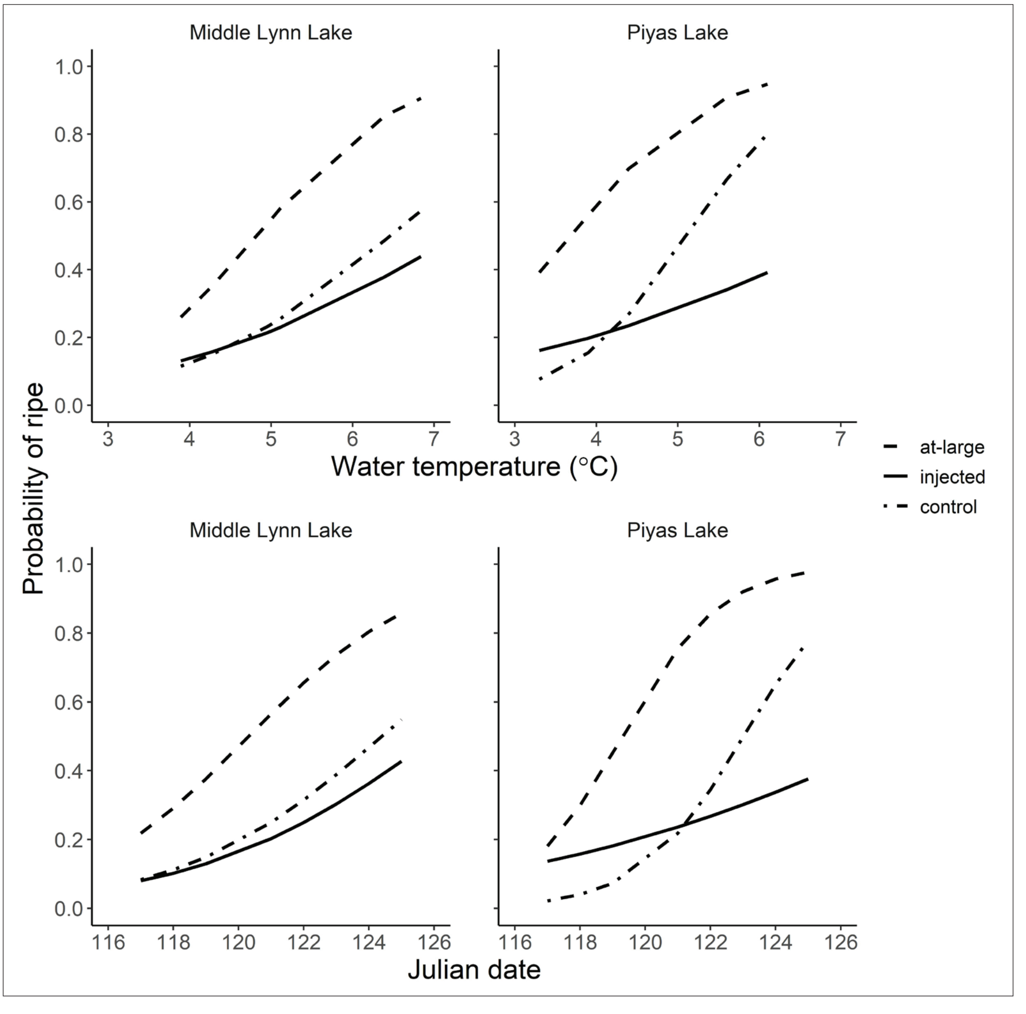

Figure 2. Lines showing the probability of a female Walleye being ripe by water temperature and Julian day for at-large (at-large), injected with human chorionic gonadotropin (500 IU/kg) and held in in-lake net pens (injected), and not injected (control) and held in in-lake net pens during the 2022 spring Walleye artificial spawning operations at Middle Lynn Lake (left column) and Piyas Lake (right column), South Dakota. The lines representing injected, and control fish at Piyas Lake used non-significant ($P > 0.05$) coefficients and should be interpreted with caution.

female Walleyes which ovulated while held at Jake Wolf Memorial Fish Hatchery, Manito, Illinois, was significantly higher for those injected with hCG than those not injected when the water temperature was warmer than the lake from which they were collected and was held at a constant 12.2 ºC (Heidinger et al. 1996).

We did not observe a large increase in the percent of females ripening any of the days they were held. Administering a second hCG injection in green females that failed to ripen may have led to an increase in the number of fish ripening. In three field trials assessing the efficacy of hCG in wild-caught and hatchery pond fish, the spawning rate was 17.5% for green female Walleyes receiving a single hCG injection and 92.1% ripened after a second injection 3-4 days after the first injection (Grizzle and Xu 1996). However, wild-caught females receiving a single injection had a higher percent ripening (30.6%) after a single injection than pond females (8.7%). To limit the amount of time green Walleyes are held and limit the amount of stress the females incur, we chose not to make a second injection of hCG. Daily handling and prolonged captivity can lead to high mortality of held Walleyes (Malison and Held 1996).

Additionally, we did not use a higher hCG dose than 500 IU/kg because previous research has indicated that low doses are as effective as high doses. Heidinger et al. (1996) found significant increases in the percentage of female Walleyes ovulating when hCG was injected regardless of the dosage (110–1100 IU/kg). In a 3-year study, doses as low as 330 IU/kg had similar rates of ripening green female Walleyes as higher doses (3,667 IU/kg; Grizzle and Xu (1996). Similarly, a dosage of 220 IU/kg in green female Walleyes resulted in comparable ovulation rates as doses ranging from 440–1,540 IU/kg (Hearn 1980). However, these studies were completed in a hatchery setting where water temperatures were warmer and held constant. Thus, it is possible a higher hCG dose may have been needed to stimulate ovulation at our water temperatures. Future research could examine the role of temperature and the amount of hCG necessary for ovulation.

The use of hCG did not increase the number of green Walleye females that ripened at Middle Lynn Lake or Piyas Lake. In years when water temperatures are slow to increase, it may be beneficial for the South Dakota Department of Game, Fish and Parks to transport green females to Blue Dog Lake State Fish Hatchery (Waubay, South Dakota), inject them with hCG, and place in a warmer, constant water temperature (10º C well water). However, transporting Walleyes would add financial costs and increase staff time. It is unknown if warmer spring water temperatures, administering a second dose of hCG, or increasing the initial dosage of hCG would have led to a higher percentage of hCG injected fish ripening when compared to those not injected. We do not believe that implementing hCG will increase the number of collected gametes during field-based artificial spawning operations, at least at the water temperatures and the slow warming we experienced in 2022. Additionally, because the probability of collected at-large females being ripe was high when our 2022 Walleye spawning operations concluded, it may not be necessary to hold green females in in-lake net pens in hopes they ripen.

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