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Induction of out-of-season spawning in walleye (*Stizostedion vitreum*)

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Abstract

Simple environmental and hormonal treatments were used to induce out-of-season spawning in walleye *Stizostedion vitreum* up to 10 weeks prior to the normal reproductive season. Wild walleye were captured in the autumn, held in earthen ponds, and in late January, February, and March (approximately 10, 6, and 3 weeks prior to natural spawning), 16–20 female and 4–8 male walleye were recaptured and transferred to indoor tanks. Water temperature was raised from 2°C to 10°C over a one week period, and photoperiod held at 12 h light: 12 h dark. The females were injected with either human chorionic gonadotropin (hCG), des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide (LHRHa), hCG and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P), or saline as a control. Each month, at least some females in each treatment group were successfully induced to ovulate. No control fish ovulated at any time. In January, hCG was the most effective treatment at inducing ovulation (3/5 fish). In February and March, all but one hormone-injected fish ovulated. In general, the eggs collected from fish treated with either hCG or LHRHa were of good quality with overall survival highest in hCG-treated fish. Eggs collected from 17,20-P-treated fish were small and had very low survival. In February and March, serum levels of estradiol-17 β and testosterone were different between fish treated with 17,20-P and those treated with either hCG or LHRHa. Out-of-season spawning could be used to provide walleye fry for intensive culture systems at multiple times of the year, thereby facilitating research on indoor fry culture. In addition, walleye fingerling production could be initiated as early as January, allowing public and private hatcheries to produce larger age-0 walleye fingerlings for stocking than would otherwise be possible. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: *Stizostedion vitreum*; Out-of-season spawning; Induction

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1. Introduction

The walleye, *Stizostedion vitreum*, is one of the most important commercial and recreational fish species in the northern United States and Canada. Over one billion walleye fry and fingerlings are produced annually by public hatcheries for stocking into lakes and rivers for resource enhancement (Fenton et al., 1996; Summerfelt, 1996). There is also a growing commercial walleye aquaculture industry which is currently producing fingerlings for stocking public and private lakes, and has the potential to produce larger food-size fish for retail markets and restaurants (e.g., Coolwater Culture Workshop, 1992).

Currently, both public and private walleye hatcheries rely almost exclusively on eggs taken from ovulated fish captured from the wild. For commercial walleye aquaculture to develop and expand, methods are needed for maturing and spawning walleye held in captivity. Furthermore, the development of methods to induce out-of-season spawning in walleye would improve the efficiency of fry culture systems and equipment (e.g., by allowing double- or triple-cropping of systems), and also provide more reliable and increased availability of fingerlings.

Walleye are group-synchronous spawners that reproduce annually in early spring in the midwestern United States (Becker, 1983; Malison et al., 1994). Wild female walleye will not normally spawn if captured more than 1–2 days prior to ovulation, but such fish can be induced to ovulate and spawn using injections of carp pituitary extract (Nelson et al., 1965; Lessman, 1978), human chorionic gonadotropin (hCG, Hearn, 1980; Pankhurst et al., 1986; Heidinger et al., 1990; Barry et al., 1995), or des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide (LHRHa, Pankhurst et al., 1986; Barry et al., 1995). Evidence that 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P) may be the oocyte maturation inducing steroid in walleye (Pankhurst et al., 1986; Barry et al., 1995) suggests that this steroid may also be useful inducing final oocyte maturation in this species.

Recent data from this laboratory on the seasonal changes in sex steroid levels and gonadal morphology in wild adult walleye indicate that vitellogenesis and spermatogenesis are nearly complete by as early as mid-January in this species, and suggest that simple environmental and hormonal manipulations might be used to significantly advance spawning in walleye (Malison et al., 1994). The present study was conducted to evaluate this possibility using selected hormone treatments administered to walleye 3–10 weeks prior to the normal spawning season. Changes in serum concentrations of estradiol-17 β (E₂), testosterone (T), and 17,20-P were also measured to gain insight into the hormonal regulation of spawning in walleye.

2. Materials and methods

2.1. Fish

In autumn, over 100 adult walleye were captured with gill-nets or electrofishing from Lake MacConaughy, Elwood Reservoir and Merritt Reservoir in Nebraska (by the Nebraska Game and Parks Commission), and from the Mississippi River near the Red

Wing Dam (by the Iowa Department of Natural Resources). The Nebraska fish were transported to the Calamus State Fish Hatchery near Burwell, NE, where they were temporarily held in a 0.2-ha pond. In early December, the fish were moved to the Gavins Point National Fish Hatchery near Yankton, SD. Walleye captured from the Mississippi River were transported directly to the Gavins Point hatchery. At Gavins Point, the walleye were weighed, measured, clipped to identify origin, separated by sex, and overwintered in two 0.07-ha ponds at ambient temperatures (2–7°C) stocked with forage fish. The fish averaged 555 mm total length and 1.95 kg weight.

In late January, February, and March (approximately 10, 6, and 3 weeks prior to the time of natural spawning, respectively) 15–20 female and 4–8 male walleye were recaptured from the ponds and transported to the Calamus hatchery. Upon arrival, 3–5 females and 1–2 males were randomly assigned to each of four 750-l indoor tanks. The water temperature was gradually increased over a 7-day period to 10°C, and the photoperiod was kept at LD 12:12. The fish in tanks were not fed.

2.2. *Experimental protocol*

After the acclimation period outlined above (i.e., on day '0'), the fish were removed from each tank, anaesthetized with 60 mg/l tricaine methanesulfonate (MS-222), and individually tagged, weighed, and measured. At this time, oocytes were collected from each female by inserting a polyethylene cannula several centimeters into the genital pore. The oocytes were immediately placed into a clearing solution (ethanol:formalin:glacial acetic acid, 6:3:1, v/v), and the maturational stage of 6 randomly chosen oocytes from each fish were classified as follows: stage 1 = germinal vesicle (GV) central or just off center; stage 2 = GV migrating to periphery; stage 3 = GV peripheral; stage 4 = GV breakdown (GVBD). Additionally, a blood sample (1.0–1.5 ml) was collected from the caudal vasculature of each female using a 22 g needle and 3 ml syringe. The blood was allowed to clot at 4°C for approximately 1 h and the serum was separated by centrifugation for 10 min at 9000 × g. Serum samples were stored at –40°C until assayed for E₂, T, and 17,20-P.

All of the females from each tank were injected in the dorsal musculature with the appropriate vehicle or hormone and returned to the same tank. Treatment regimes were: 'Sal' = physiological saline (0.7 NaCl) as a control on days 0 and 2, 'hCG' = hCG (Sigma Chemical, St. Louis, MO, dissolved directly in physiological saline) at 150 and 500 IU/kg on days 0 and 2, respectively, 'LHRHa' (Sigma Chemical, dissolved directly in physiological saline) at 35 and 100 µg/kg on days 0 and 2, respectively, and '17,20-P' = hCG at 150 IU/kg on day 0 and 17,20-P (Steraloids, Wilton, NH, dissolved in ethanol and diluted 1:10 in physiological saline) at 2 mg/kg on day 2. This strategy of a 'priming' dose of a hormone followed by a larger 'triggering' dose was used because it has frequently proven more effective than a single injection at inducing ovulation in other fish species examined to date (e.g., see Donaldson and Hunter, 1983). Males from each tank were injected with 150 IU/kg hCG on day 0 in an attempt to maximize milt production.

Additional oocyte and blood samples were taken from female fish on days 2, 5, and 8 (oocyte samples only on day 8), and processed as described above. For each fish,

germinal vesicle breakdown was considered to have commenced if one of the oocytes examined on a given day had undergone GVBD, or if the fish had ovulated. The occurrence of ovulation was determined on days 5 through 12 by attempting to strip eggs from individual fish once daily. The eggs from each ovulated female were fertilized with the milt of 3 males (selected from all tanks) using the method described by Malison et al. (1990). Egg size, expressed in the number of eggs/l, was determined after water hardening using a calibrated trough according to the von Bayer method (see Piper et al., 1982). Eggs were incubated in McDonald-type flow-through hatching jars (MPC-300, Midland Plastics, Brookfield, WI). Egg viability was determined 6 days after fertilization (early eyed stage) by counting the percentage of live eggs in 3 samples of 20 eggs each.

2.3. Steroid assays

Levels of E_2 and T were measured in 50 and 20 μ l aliquots of walleye serum, respectively, using commercially available radioimmunoassays (RIAs, Diagnostic Products, Los Angeles, CA). Both assays use iodinated tracer ligands. Standards were prepared in charcoal-stripped walleye serum. Assay characteristics for the E_2 and T RIAs were, respectively: intraassay coefficients of variation (CVs) = 1.8% and 2.3%; interassay CVs = 4.6% and 5.1%; sensitivities = 10 pg/ml and 20 pg/ml; recoveries from spiked serum = 94% and 93% (Malison et al., 1994). The E_2 antiserum cross-reacts with estrone (1.1%) and estriol (0.32%). The T antibody cross-reacts with dihydrotestosterone (34%), androsten-3,17-dione (0.8%) and 11-ketotestosterone (0.4%). All other steroids tested showed less than 0.1% cross reactivity (Diagnostic Products, Los Angeles, CA). Levels of 17,20-P were measured in 20 μ l aliquots using an ELISA validated for use in walleye with assay characteristics as follows: intraassay CV < 3.0%; interassay CV < 4.0%; sensitivity = 50 pg/ml; average recovery = 94% (Barry et al., 1995). The 17,20-P antiserum cross-reacts with $17\alpha,20\beta$ -dihydroxy-5 β -pregnan-3-one (2.45%, Young et al., 1983). For each assay, direct serum aliquots and standards were measured in duplicate.

2.4. Data analyses

For each month of treatment, day-by-day changes in GVBD were compared within each treatment by analysis of variance (ANOVA) followed by a protected LSD test. The percentage of fish which underwent GVBD and ovulation each month was compared between treatments using Chi-square analysis. Egg size and survival were compared by two-way ANOVA with month and treatment as main effects. The steroid data for January were compared by a two-way repeated measures ANOVA with main effects being treatment and ovulatory status (ovulated vs. unovulated), followed by another repeated measures ANOVA with day as the main effect. For February and March, the steroid data were analyzed with two separate ANOVAs (by month and day) to determine treatment effects for hormone-treated fish. Ovulatory status in February and March was ignored because only 1 hormone-treated fish did not ovulate. Percentage data were arc-sin transformed and other data log-transformed prior to analysis. All data are shown as the mean \pm standard error of the mean (SEM), and for all tests, P was set at 0.05.

3. Results

3.1. Egg maturation and ovulation

Within each month, there were no differences in the initial oocyte stage between the fish in the four treatment groups. In January, most oocytes were at stage 1. In February, approximately half of the oocytes were at stage 1 and half at stage 2. In March, almost all eggs were at stage 2.

All three hormone treatments induced oocyte maturation (i.e., change in %GVBD) and ovulation in January, February, and March; no control fish underwent GVBD or ovulation in any month (Table 1). In January, there was no differences in %GVBD or ovulation between the three hormone treatment groups, and fish in all of these groups had a higher incidence of GVBD than the controls eight days after hormone administration (Table 1). In February and March, the percentage of fish which underwent GVBD and ovulated was significantly greater in all three hormone-injected groups compared to the controls by days 5 or 8 (Table 1).

The time between the initial hormone injection and ovulation generally declined from January to March, averaging 9 days in January, 8 days in February, and 7 days in March. Among the fish that spawned, both the average egg size and the mean egg survival on day 6 were significantly less in the 17,20-P treatment groups than in the other treatment groups (Table 1).

Table 1

Percentage of females that underwent germinal vesicle breakdown (GVBD) and ovulation, and egg size and day 6 survival (mean \pm SEM) in wild walleye held in ponds and exposed to selected hormonal treatments at various times

Month	Tmt	GVBD (%) ¹					Ovulation ²	Egg size (number/l) ²	Egg survival (%) ²
		N	d0	d2	d5	d8			
January	Sal ³	5	0	0	0	0	0 ^a	—	—
	hCG ³	5	0	0	60*	60	60 ^a	169,700 \pm 17,290 ^a	41.4 \pm 22.0 ^a
	LHRHa ³	5	0	0	20	20	20 ^a	167,400 ^a	62.8 ^a
	17,20-P ³	5	0	20	40	100*	40 ^a	209,100 \pm 15,908 ^b	1.4 \pm 1.4 ^b
February	Sal	4	0	0	0	0	0 ^a	—	—
	hCG	4	0	0	25	100*	100 ^b	168,400 \pm 12,773 ^a	58.0 \pm 5.1 ^a
	LHRHa	4	0	0	100*	100	100 ^b	184,900 \pm 28,733 ^a	21.7 \pm 13.4 ^a
	17,20-P	4	0	0	75*	100	75 ^b	280,100 \pm 11,706 ^b	0 ^b
March	Sal	4	0	0	0	0	0 ^a	—	—
	hCG	3	0	0	100*	100	100 ^b	170,700 ^a	71.7 ^a
	LHRHa	4	0	0	100*	100	100 ^b	168,700 \pm 21,183 ^a	30.6 \pm 4.7 ^a
	17,20-P	4	0	0	75*	100	100 ^b	233,700 \pm 21,582 ^b	0 ^b

¹Percentages denoted with an asterik (*) are significantly different from the previous day's value.

²Means with the same letter are not significantly different at $P < 0.05$.

³Sal = saline; hCG = human chorionic gonadotropin; LHRHa = des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide; 17,20-P = 17 α ,20 β -dihydroxy-4-pregnen-3-one.

3.2. Changes in steroid hormones

There were no differences in initial E_2 and T levels among fish in the four treatment groups during any month. In the control fish, mean T levels remained constant from day 0 to day 5 (Fig. 1e), and mean E_2 levels decreased slightly between days 0 and 2 (Fig. 1d).

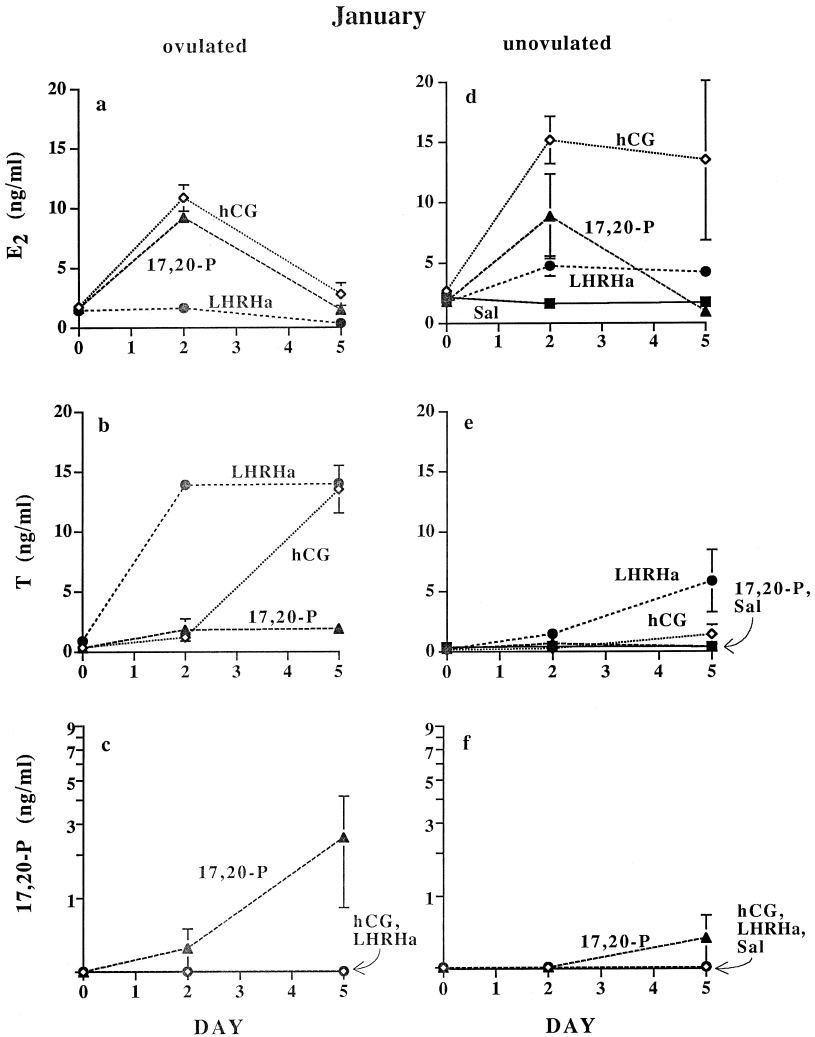


Fig. 1. Serum levels of estradiol-17 β (E_2) and testosterone (T) and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P) in wild female walleye held in ponds, transferred to indoor holding tanks in January, approximately 10 weeks prior to the normal spawning season, and injected with one of four compounds. Data shown are the mean \pm SEM of 1–5 samples/data point. Sal = saline, hCG = human chorionic gonadotropin, LHRHa = des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide, 17,20-P = 17 α ,20 β -dihydroxy-4-pregnen-3-one. See text for results of statistical analyses.

In January, the hCG treatment group had elevated E_2 levels on day 2. Levels of E_2 then decreased by day 5 in fish which eventually ovulated (Fig. 1a), but remained elevated in fish that did not ovulate (Fig. 1d). Testosterone levels were significantly elevated by day 5 in the fish that ovulated (Fig. 1b), but remained near baseline in the fish that did not spawn (Fig. 1e). In the LHRHa injected fish, E_2 levels remained near baseline throughout the sampling period in both ovulated and unovulated fish. In fish that ovulated, T levels were elevated on days 2 and 5, whereas in unovulated fish T levels remained near baseline throughout the 5-day period. In the 17,20-P treatment group, fish that ovulated had E_2 profiles similar to the hCG-treated fish (i.e., elevated on day 2 with a return to baseline on day 5). In contrast to ovulated fish in the other

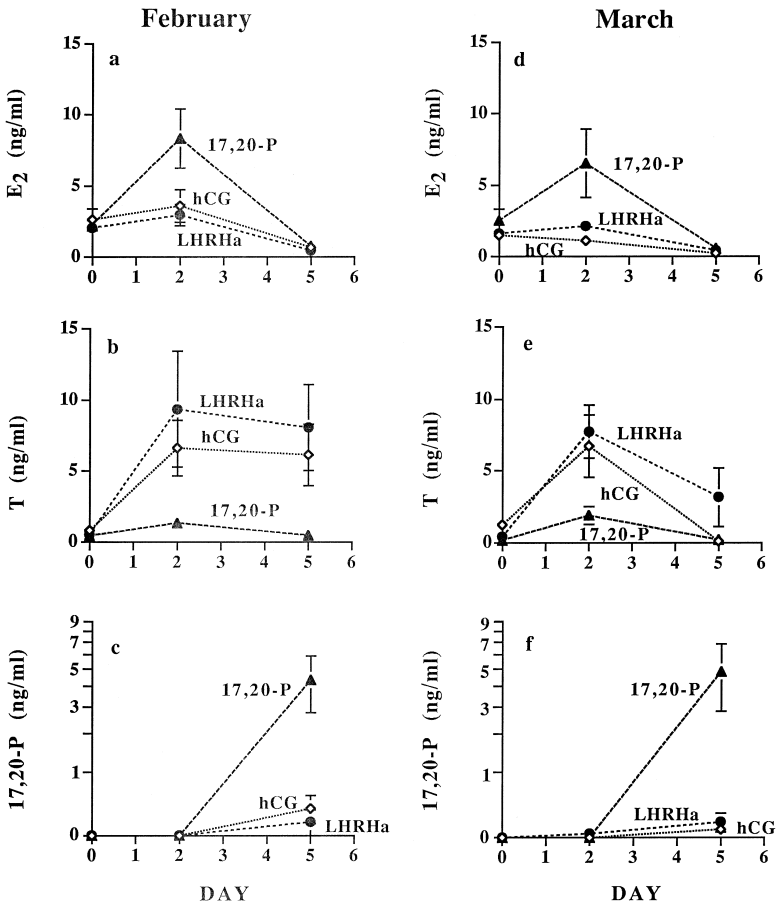


Fig. 2. Serum levels of estradiol-17 β (E_2) and testosterone (T) and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P) in wild female walleye held in ponds, transferred to indoor holding tanks either 6 (February) or 3 (March) weeks prior to the normal spawning season, and injected with one of four compounds. Data shown are the mean \pm SEM of 1–5 samples/data point of fish that had ovulated. hCG = human chorionic gonadotropin, LHRHa = des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide, 17,20-P = 17 α ,20 β -dihydroxy-4-pregnen-3-one. See text for results of statistical analyses.

treatment groups, 17,20-P-treated fish had T levels that remained near baseline through day 5.

In February, all hormone-injected fish ovulated except for 1 fish in the 17,20-P group, and E_2 levels were not different among the treatment groups (Fig. 2a). Testosterone levels, however, were significantly lower in the 17,20-P-injected fish than in the hCG- and the LHRHa-injected fish (Fig. 2b).

In March, all of the hormone-injected fish ovulated. Fish injected with 17,20-P had higher E_2 levels (Fig. 2d) and lower T levels (Fig. 2e) on day 2 than those in the other two treatment groups.

No 17,20-P was detected on days 0 and 2 in any fish except for very low levels (< 0.5 ng/ml) detected in 2 of the 13 17,20-P-injected fish (Fig. 1c) and 1 of the LHRHa-injected fish (Fig. 2f). On day 5, 17,20-P was not detected in the controls but was detected in 15 of the 38 hormone-treated fish, at levels ranging from 0.17 to 9.86 ng/ml. Levels of 17,20-P were significantly higher in 17,20-P-injected fish (both ovulated and unovulated) than in fish injected with hCG or LHRHa (Fig. 1c,f; Fig. 2c,f).

4. Discussion

Our results indicate that wild walleye captured in the autumn and overwintered in ponds can be induced to spawn up to 10 weeks prior to the normal spawning season by using relatively simple environmental and hormonal treatments. This technology can be used to produce walleye fry from late January through March, resulting in several important practical benefits to walleye culturists. Traditionally, large-scale walleye fry culture has been performed in outdoor ponds (e.g., Coolwater Culture Workshop, 1992; Summerfelt, 1996). Recently, however, much research has been conducted on indoor intensive fry culture systems (e.g., Li and Mathias, 1982; Barrows et al., 1993; Bristow and Summerfelt, 1994). The methods described here could be used to provide fry for these systems at multiple times of the year, thereby facilitating research on indoor walleye fry culture (Krise and Meade, 1986; Loadman et al., 1989; Moore et al., 1994).

In addition to research, some large-scale production of walleye is now being conducted using indoor fry culture systems (e.g., Iowa Department of Natural Resources). The availability of fry at multiple times of the year could allow for double- or triple-cropping of these systems and thereby increase their efficiency. Also, the availability of fry as early in the year as January will allow these hatcheries to produce larger age-0 walleye fingerlings for stocking than would otherwise be possible. Such larger fingerlings are desirable because of their greater survival and higher return to the fishery (e.g., Conover, 1986; Coolwater Culture Workshop, 1992).

In general, the eggs collected from fish treated with either hCG or LHRHa were similar to those from walleye spawned during the normal reproductive season. For example, the mean number of eggs obtained/kg of body weight (BW) from hCG- and LHRHa-injected fish in this study was 55,700, ranging from 11,100–125,000 eggs/kg of BW. Comparably, Nickum (1986) reported that walleye normally average 60,000 eggs/kg of BW, and a range of 28,000–120,000 eggs/kg of BW has been reported in other studies (Smith, 1941; Wolfert, 1969). In the present study, the 6-day survival of eggs from hCG- and LHRHa-injected walleye averaged 57 and 38%, respectively,

similar to the survival rates observed during walleye production at the Calamus hatchery during the normal spawning season (Jim Gleim, Nebraska Game and Parks Commission, personal communication). In addition, the Iowa Department of Natural Resources has already successfully used the hCG methodology reported in this paper to induce walleye spawning in February, and obtained survival rates of over 65%, with egg and fry quality comparable to that observed during the normal spawning season (Alan Moore, Iowa Department of Natural Resources, personal communication).

Although both hCG and LHRHa had similar effects on oocyte maturation, ovulation and egg number and quality, we recommend using hCG to induce out-of-season spawning in walleye because it is less expensive than LHRHa, and it will soon be approved by the US Food and Drug Administration for use in aquaculture (Rosalie Schnick, National Aquaculture NADA Coordinator, North Central Regional Aquaculture Center, personal communication).

Eggs from walleye injected with 17,20-P were small, averaging 243,700/1 compared to 173,000/1 from the hCG- and LHRHa-treated fish. Also, egg survival in the 17,20-P treatment groups was very low. The reasons for these findings are unclear, although it is possible that the dose of 17,20-P used in this study may have been too high. Doses of 17,20-P as low as 0.1 ng/ml have recently been reported to induce GVBD in walleye oocytes cultured *in vitro* (Barry et al., 1995), and peak levels of 17,20-P in the serum of spawning walleye are quite low compared to levels reported in other teleosts (Pankhurst et al., 1986; Scott and Canario, 1987; Malison et al., 1994; Barry et al., 1995). An overly high dose of 17,20-P may have had an adverse effect on the maturing oocytes (e.g., resulting in only partial hydration at the time of ovulation). In other teleosts, however, 17,20-P injection levels ranging from 1–3 mg/kg BW did not have an adverse effect on egg survival (see Donaldson and Hunter, 1983).

No attempt was made to separately evaluate the effects of environmental and hormonal treatments for inducing spawning in walleye. Due to constraints of fish availability and tank space, we did not investigate whether environmental treatments (increasing temperature and photoperiod) were essential to induce out-of-season spawning, or whether hormone injections alone would have been sufficient for this purpose. Ovulation did not occur in any control fish suggesting that brief exposure (two weeks) to elevated temperatures and longer photoperiods is insufficient to induce early spawning in walleye. This finding is consistent with the results of Nelson et al. (1965), Lessman (1978), Hearn (1980), Pankhurst et al. (1986), Heidinger et al. (1990) and Barry et al. (1995).

The steroidogenic response of the ovary to gonadotropin (hCG) injection appeared to be dependent on the maturational status of the ovary. In January, for example, a significant rise in E₂ levels was observed 2 days after injection, but such an increase was not seen in February or March. Furthermore, in January, T levels remained constant following hCG injections, but rose by day 2 in both February and March. These phenomena are consistent with the hypothesis that aromatase activity decreases in the 2–3 months prior to the normal spawning season, perhaps in response to photoperiod or other environmental factors. It is also possible that a gradual shift occurs in the steroid secretory response of the ovary to gonadotropins, from androgen and estrogen production to maturational steroid production, as the normal spawning season approaches.

As discussed by Barry et al. (1995), the periovulatory period of the fish which eventually underwent GVBD, ovulated, and produced high quality eggs was generally characterized by low E_2 levels, a rise in T, followed by an increase in 17,20-P. The fish which did not ovulate in response to hormone treatment in January generally showed elevated E_2 levels and/or no rise in T. One interpretation of these data is that fish with high gonadal aromatase activity are resistant to induced spawning. The periovulatory rise in T has been previously reported in walleye as well as in almost all teleosts investigated to date and is thought to be necessary for successful spawning (Pankhurst et al., 1986; Fostier et al., 1983; Kobayashi et al., 1989). In our study, the poor egg quality of fish treated with 17,20-P in February and March may be related to the lack of a periovulatory rise in T (Fig. 2) or to a lack of gonadotropin.

It is unclear why day 2 serum E_2 and T levels were different between fish in the hCG and 17,20-P treatment groups in February and March, as both groups were injected with the same priming dose (150 IU/kg) of hCG on day 0. In January, however, day 2 E_2 and T levels were similar in the two groups. The difference may have been due to the low sample sizes ($n = 5$) used in this study, or some other unknown factor (e.g., a tank effect).

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References

- Barrows, F.T., Zitzow, R.E., Kindschi, G.A., 1993. Effects of surface water spray, diet, and phase feeding on swim bladder inflation, survival, and cost of production of intensively reared larval walleye. *Prog. Fish-Cult.* 55, 224–228.
- Barry, T.P., Malison, J.A., Lapp, A.F., Procarione, L.P., 1995. Effects of selected hormones and male cohorts on final oocyte maturation, ovulation and steroid production in walleye (*Stizostedion vitreum*). *Aquaculture* 138, 331–347.
- Becker, G.C., 1983. *Fishes of Wisconsin*. University of Wisconsin Press, Madison, 1052 pp.
- Bristow, B.T., Summerfelt, R.C., 1994. Performance of larval walleye cultured intensively in clear and turbid water. *J. World Aquacult. Soc.* 25, 454–464.
- Conover, M.C., 1986. Stocking cool-water species to meet management needs. In: Stroud, R.H. (Ed.), *Fish*

- Culture in Fisheries Management, Fish Culture Section and Fisheries Management Section of the American Fisheries Society, Bethesda, MD, pp. 31–39.
- Coolwater Culture Workshop, 1992. Minutes 6–8 January 1992, at Marion, IL, USA.
- Donaldson, E.M., Hunter, G.A., 1983. Induced final maturation, ovulation, and spermiation in cultured fish. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), *Fish Physiology*, Vol. IX. Part B. Behavior and Fertility Control. Academic Press, New York, pp. 351–403.
- Fenton, R., Mathias, J.A., Moodie, G.E.E., 1996. Recent and future demand for walleye in North America. *Fisheries* 21, 6–12.
- Fostier, A., Jalabert, B., Billard, R., Breton, B., Zohar, Y., 1983. The gonadal steroids. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), *Fish Physiology*. Vol. IX, Part A. Academic Press, New York, pp. 351–403.
- Hearn, M.C., 1980. Ovulation of pond-reared walleyes in response to various injection levels of human chorionic gonadotropin. *Prog. Fish-Cult.* 42, 228–230.
- Heidinger, R.C., Tetzlaff, B.L., Brooks, R.C., Bickers, C.A., 1990. Walleye Culture Study. F-60-R. Illinois Department of Conservation, Springfield, IL, USA, 96 pp.
- Kobayashi, M., Aida, K., Hanyu, I., 1989. Induction of gonadotropin surge by steroid hormone implantation in ovariectomized and sexually regressed female goldfish. *Gen. Comp. Endocrinol.* 73, 469–476.
- Krise, W.R., Meade, J.W., 1986. Review of the intensive culture of walleye fry. *Prog. Fish-Cult.* 48, 81–89.
- Lessman, C.A., 1978. Effects of gonadotropin mixtures and two steroids on inducing ovulation in walleyes. *Prog. Fish-Cult.* 40, 3–5.
- Li, S., Mathias, J.A., 1982. Causes of high mortality among cultured larval walleye. *Trans. Am. Fish. Soc.* 111, 710–721.
- Loadman, N.L., Mathias, J.A., Moodie, G.E.E., 1989. Method for the intensive culture of walleye. *Prog. Fish-Cult.* 51, 1–9.
- Malison, J.A., Kayes, T.B., Held, J.A., Amundson, C.H., 1990. Comparative survival, growth, and reproductive development of juvenile walleye and sauger and their hybrids reared under intensive culture conditions. *Prog. Fish-Cult.* 52, 73–82.
- Malison, J.A., Procarione, L.S., Barry, T.P., Kapuscinski, A.R., Kayes, T.B., 1994. Endocrine and gonadal changes during the annual reproductive cycle of the freshwater teleost *Stizostedion vitreum* (walleye). *Fish Physiol. Biochem.* 13, 473–484.
- Moore, A., Prange, M.A., Bristow, B.T., Summerfelt, R.C., 1994. Influences of stocking densities on walleye fry viability in experimental and production tanks. *Prog. Fish-Cult.* 56, 194–201.
- Nelson, W.R., Hines, N.R., Beckman, L.G., 1965. Artificial propagation of saugers and hybridization with walleyes. *Prog. Fish-Cult.* 27, 216–218.
- Nickon, J.G., 1986. In: Stickney, R.R. (Ed.), *Culture of nonsalmonid freshwater fishes*. CRC Press, Boca Raton, pp. 116–126.
- Pankhurst, N.W., van der Kraak, G., Peter, R.E., 1986. Effects of human chorionic gonadotropin, Des-Gly¹⁰ (D-Ala⁶) LHRH-ethylamine and pimozone on oocyte final maturation, ovulation and levels of plasma sex steroids in the walleye (*Stizostedion vitreum*). *Fish Physiol. Biochem.* 1, 45–54.
- Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J.R., 1982. *Fish Hatchery Management*. US Department of the Interior, US Fish and Wildlife Service. Washington, DC, 517 pp.
- Scott, A.P., Canario, A.V.M., 1987. Status of oocyte maturation-inducing steroids in teleosts. In: Idler, D.R., Crim, L.W., Walsh, J.M. (Eds.) *Proceedings of the Third International Symposium on Reproductive Physiology of Fish*. Marine Sciences Research Laboratory, St. John's, Newfoundland, Canada, pp. 224–233.
- Smith, C.G., 1941. Egg production of walleyed pike and sauger. *Prog. Fish-Cult.* 54, 32–34.
- Summerfelt, R.C., 1996. Introduction. In: Summerfelt, R.C. (Ed.) *Walleye Culture Manual*, pp. 1–10. NCRAC Culture Series 101, North Central Regional Aquaculture Center Culture Publications Office, Iowa State University, Ames, IA, USA, 416 pp.
- Wolfert, D.R., 1969. Maturity and fecundity of walleyes from the eastern and western basins of Lake Erie. *J. Fish. Res. Board Can.* 26, 1877–1888.
- Young, G., Crim, L.W., Kagawa, H., Kambegawa, A., Nagahama, Y., 1983. Plasma 17 α ,20 β -dihydroxy-4-pregnen-3-one levels during sexual maturation of amago salmon (*Oncorhynchus rhodurus*): correlation with plasma gonadotropin and in vitro production by ovarian follicles. *Gen. Comp. Endocrinol.* 51, 96–105.