WALLEYE[9]

Project Component Termination Report for the Period
September 1, 1989 to August 31, 1993

NCRAC FUNDING LEVEL: $321,740 (May 1, 1989 to August 31, 1993)

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Iowa DNR	Rathbun State Fish Hatchery	Iowa
Iowa DNR	Spirit Lake State Fish Hatchery	Iowa
Minnesota DNR	Devil's Track Hatchery	Minnesota
Nebraska Game & Parks Commission	Calamus State Fish Hatchery, Burwell	Nebraska
Ohio DNR	London State Fish Hatchery	Ohio
U.S. Fish & Wildlife Service	Garrison Dam National Fish Hatchery	North Dakota
U.S. Fish & Wildlife Service	Valley City National Fish Hatchery	North Dakota
U.S. Fish & Wildlife Service	Gavins Point National Fish Hatchery	South Dakota
U.S. Fish & Wildlife Service	Genoa National Fish Hatchery	Wisconsin

REASON FOR TERMINATION

The objectives for this work on Walleye were completed.

PROJECT OBJECTIVES

(1) Develop baseline information on the mechanisms regulating the natural reproductive cycle of wild and pond-held walleye by characterizing seasonal changes in hormone titers and gonadal histology.

(2) Develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning.

(3) Evaluate zooplankton seeding for pond culture of fingerling walleye.

(4) Evaluate strategies for control of clam shrimp in ponds used for culture of fingerling walleye.

(5) Determine the etiology of noninflation of the gas bladder of intensively cultured walleye fry.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

The endocrine and gonadal changes during the annual reproductive cycle of walleye were described for the first time. No differences were observed between the developmental patterns of wild walleye (captured primarily from the Mississippi River near Minneapolis, Minnesota) and walleye held in ponds in Carbondale, Illinois. Gonadal growth in wild male walleye began in August/September, and testes contained mature spermatozoa by late fall (October/November). Mature spermatozoa could be expressed from males collected from January through April. Testosterone levels were low (<0.5 ng/mL) throughout the summer, rose in October to a plateau of >1.0 ng/mL from November through January, and peaked in March at 2.8 ng/mL. Serum levels of 11-ketotestosterone were <10 ng/mL from late April through January, and rose in March and early April to >35 ng/mL.

In wild female walleyes, oocytes increased in diameter from 184.0 $\pm$ 18.6 μm in October to 998.7 $\pm$ 39.8 μm in November. This increase occurred coincident with a marked rise in gonadosomatic indices (GSIs) and circulating levels of serum estradiol-17β (from 0.2 ng/mL to 3.7 ng/mL), the steroid responsible for stimulating hepatic vitellogenin synthesis in teleosts. Just prior to spawning in March, oocytes were approximately 1,500 μm in diameter. Following spawning, average GSIs in females declined from 15.3% to 1.5%. Data from *in vitro* cultures of walleye oocytes conducted at the University of Wisconsin-Madison (UW-Madison) suggested that 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P) may be the steroid responsible for inducing final oocyte maturation in walleye. *In vivo* studies showed that 17,20-P levels rose very transiently to approximately 2 ng/mL coincident with final oocyte maturation. Taken together, these results suggest that vitellogenesis and spermatogenesis are at or near completion as early as mid-January, and that simple environmental and/or hormonal manipulations could be used to induce spawning from mid-January to late March.

OBJECTIVE 2

University of Nebraska-Lincoln (UNL) and UW-Madison investigators developed methods to manipulate the annual reproductive cycle and induce out-of-season spawning of walleye. Wild adult walleye were captured in autumn by the Nebraska Game and Parks Commission from Lake McConaughy, Elwood Reservoir, and Merritt Reservoir in Nebraska, and by the Iowa Department of Natural Resources (DNR) from the Mississippi River. The Nebraska fish were transported to the Calamus State Fish Hatchery near Burwell, Nebraska, where they were maintained in a lined pond. In early December, these fish were transferred to earthen ponds at the Gavins Point National Fish Hatchery near Yankton, South Dakota. Walleye captured from the Mississippi River were transported directly to the Gavins Point hatchery. All fish were fin-clipped to identify their origin, and transported by UNL personnel. At the Gavins Point hatchery, the walleye were separated by sex and overwintered in two 0.07-ha (0.17-acre) ponds stocked with forage fish.

In January, February and March, 16-20 females and 4-5 males were recaptured from the Gavins Point hatchery ponds and transferred to the Calamus State Fish Hatchery, where they were placed in indoor tanks. There, the water temperature was gradually increased over a 10-d period to 10°C (50°F) and the photoperiod was set at 12- h light/12-h dark. Females were subject to one of four injection regimes: (1) saline on days 0 and 2; (2) human chorionic gonadotropin (hCG) on day 0 and day 2; (3) a synthetic luteinizing hormone-releasing hormone analogue (LHRHa) on day 0 and day 2; or (4) hCG on day 0 and 17,20-P on day 2. No difference in response patterns to these treatments was observed in walleye of different origins.

At each month, all hormone injection regimes successfully induced GVBD (germinal vesicle breakdown) and ovulation in at least some of the females, whereas none of the saline-injected fish underwent GVBD or spawned. In January, hCG was the most effective treatment for inducing ovulation. In February and March, egg survival was the highest in hCG-treated fish. At all times, the 17,20-P treatment resulted in very low egg survival and small egg size. The results demonstrate that appropriate hormone and environmental treatments can be successfully used to induce spawning in walleye from late January through March. The most effective hormone treatment in this regard was hCG. Regardless of when it was used, hCG at 150 and 500 IU/kg (days 0 and 2, respectively) (68 IU/lb and 227 IU/lb) induced spawning 6-8 days after the last injection.

OBJECTIVE 3

Investigators at Iowa State University (ISU) conducted experiments in 1989 and 1990 to evaluate zooplankton seeding (inoculation) for pond culture of fingerling walleye at the Valley City National Fish Hatchery (VCNFH), Valley City, North Dakota, and Garrison Dam National Fish Hatchery (GDNFH), Riverdale, North Dakota. Zooplankton for the inoculation experiments were collected with an air-lift pump and cage system or by filtering inflowing water to raceways. In both cases, the zooplankton that were used for seeding the ponds were those large enough to be retained by a 0.5-mm (0.02-in) screen. All of the inocula consisted of cladocera (*Bosmina* and *Daphnia*) as well as cyclopoid and calanoid copepods.

The zooplankton inoculation was done during the first week after fry stocking. The zooplankton inocula ranged from 0.4 to 28.8 kg/ha, or 70 to 990 organisms per m^3 based on pond volume. In 1989, 95 to 100% of the inoculum was *Daphnia pulex*; in 1990, 77% of the inoculum used at Valley City was *Daphnia pulex*, but at Garrison Dam, only 13.5% were *Daphnia* and 67% were copepods. Over two years, 22 ponds were inoculated with zooplankton, and 22 ponds served as the controls (i.e., without zooplankton inoculation).

In each year and at both hatcheries, the average yield (kg/ha, and number/ha) of walleye fingerlings from ponds that had received the zooplankton inoculation was lower than the control ponds. The yield of walleye fingerlings (number per acre) from ponds receiving a zooplankton inoculation at the VCNFH in 1989 was 21.8% less than the control ponds, and 22.4% less in 1990; at GDNFH, the yield from ponds receiving the zooplankton inoculation was 50.9% less in 1989 and 66.9% in 1990 than the control ponds. Also, data combined over both years and both hatcheries showed that smaller biomass yield of fingerlings (42.7 kg/ha; 38.1 lb/acre) was obtained from ponds that were seeded with zooplankton than the control ponds that were not inoculated (54.9 kg/ha; 49.0 lb/acre).

Overall, the findings indicate that zooplankton inoculation of culture ponds during the week fry are stocked had reduced fish production. These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. Ponds should not be inoculated with large cladocera such as *Daphnia pulex* shortly after stocking walleye fry because the larger zooplankton in the inoculum have a competitive advantage over the smaller zooplankton copepoda nauplii and other smaller zooplankton that serve as important prey for first feeding larval walleye. This does not mean that an inoculation with smaller zooplankton or use of larger zooplankton will not be desirable, however, the findings demonstrate that the timing of such methods for biomanipulation need to be carefully evaluated. At these hatcheries, the normal inflowing water carried an abundance of zooplankton, but if zooplankton populations are not abundant in the water used to fill the ponds (i.e., when ground water is used) or if zooplankton numbers decline during the culture interval, inoculation may be used to initiate or to sustain zooplankton populations. However, prior research on the effects and benefits of zooplankton inoculation is limited, and it has not been systematically studied in walleye fingerling culture.

OBJECTIVE 4

ISU investigators carried out studies on the ecology of clam shrimp at the GDNFH. In 1992, the studies were carried out on 23, 0.64-ha (1.58-acre) ponds during the culture season for northern pike, and 19 of the same ponds during the season for walleye. Ponds at the GDNFH were first used to raise northern pike. They were drained after 3 to 4 weeks to harvest the pike, then refilled to raise walleye. Adult clam shrimp were observed in 12 of the 23 ponds during northern pike culture, and 10 of the 19 ponds during walleye culture. Northern pike were cultured up to 29 days in ponds with clam shrimp, while pike were cultured a maximum of 22 days in ponds without clam shrimp. Survival and yield (number/ha, and number/pond) of northern pike was significantly lower in ponds with clam shrimp compared to ponds without clam shrimp. Similar differences, although not significant, were seen in walleye culture ponds. Northern pike and walleye were cultured longer in ponds with clam shrimp, implying that fish growth is reduced in culture ponds with clam shrimp. The majority of large clam shrimp found during the walleye culture season were most likely hatched during northern pike culture. When ponds are used in tandem to raise northern pike and walleye, to prevent development of clam shrimp during the walleye culture the ponds should be thoroughly dried between culture periods.

OBJECTIVE 5

Studies on the etiology (cause for) of noninflation of the gas bladder (NGB) were carried out as a collaborative effort among ISU, Michigan State University (MSU), and University of California-Davis (UCD). The study objectives included development of methods for intensive rearing of larval walleye on formulated feeds (ISU), identification of pathological lesions that will indicate the etiology of non-inflation of the gas bladder (MSU), and a description of developmental histology of the gas bladder, pneumatic duct, and other tissues and glands (UCD).

Each year, researchers at ISU obtained 1- to 5-day posthatch walleye fry or eyed-eggs from at least three cooperating state and federal fisheries agencies. ISU personnel reared walleye fry in an intensive culture environment and fed the fish a formulated feed, "fry feed Kyowa" (BioKyowa, Inc.), sizes B-400 through B-700, for 21-30 days. Each lot of fish obtained each year was used to evaluate different intensive culture treatments. Culture conditions involved different tank design (cylindrical and square), single-pass and recycle water systems, and pH. These different culture systems have aspects of them that may influence feed particle density (i.e., feeding success affects survival) and water quality (i.e., surface films or pH) which, in turn, may affect gas bladder inflation.

The fry samples provided a progression in age and size of fish, some collected before and others after feeding, with and without the yolk sac, and fish with and without gas bladder inflation, from a variety of experiments in which environmental variables differed substantially. MSU investigators found degenerative changes in the gas bladders (i.e., hyperplasia and abundance of macrophages) which were indicative of an inflammatory disease, and preliminary evidence to suggest a microbial infection as a specific initiating process. The observation of bacteria in the macrophages suggested a bacterial infection, at least as a secondary invader.

UCD investigators found that inflation of the swimbladder began on the 6th day posthatch, coinciding with the time of yolk sac depletion and initiation of feeding. In larvae with noninflated swimbladders, the pneumatic duct was obvious and its diameter remained fairly constant (25-45 μm) through the 19th day posthatch, but the pneumatic duct atrophied in larvae with inflated swimbladders. During the interval of swimbladder inflation, from the 6th to the 12th day posthatch, the common bile duct and pneumatic duct both opened to undifferentiated foregut where surfactant-like secretions from the common bile duct could affect fragmentation of large ingested air bubbles for transfer into the relative small-diameter pneumatic duct. After the 12th day posthatch, however, the pyloric sphincter developed and separated the common bile duct in the intestine from the pneumatic duct in the dorsal wall of the stomach. Thus, this finding indicates that differentiation of the foregut prevents inflation of larvae after 12th day posthatch. The day for these events, however, will vary depending on water temperature.

IMPACTS

OBJECTIVES 1 AND 2

These studies initially generated the basic knowledge of the reproductive cycle of walleye that was needed to begin efforts at controlling reproduction in walleye. This information was subsequently used to develop methods for inducing out-of-season spawning in walleye from late January through March.

The investigators also detailed techniques useful for synchronizing spawning in walleye, resulting in greater predictability of gamete production, and reduced incidence of failed spawning, gamete resorption and subsequent brood fish losses. These techniques can be used to increase hatchery efficiency and reliability.

Recently, UW-Madison personnel successfully led an effort to gain FDA-INAD approval to use hCG to induce spawning in walleye and yellow perch. This approval involves three regional private sector producers, and was done with the help of and in conjunction with the Iowa DNR.

Walleye producers (including the Iowa DNR) have used the technologies developed from these studies to produce walleye fry 9-12 weeks prior to the normal spawning season, and thereby greatly extended the period of time during which larval walleye can be reared intensively. This, in turn, has greatly increased the efficiency of existing intensive fry culture systems, facilitated research on the intensive culture of walleye fry, and aided hatcheries in their efforts to produce larger walleyes for stocking.

OBJECTIVE 3

Research on the use of zooplankton inoculation for pond culture of fish has not been systematically studied in walleye fingerling culture. In the present study, zooplankton inoculation of walleye culture ponds during the week walleye fry were first stocked reduced survival and yield. It was surprising to find lower survival and production in ponds supplemented with zooplankton because the method is believed to increase forage for fingerlings. However, basic studies by aquatic ecologists has long demonstrated the difficulties of precise prediction of zooplankton dynamics in ponds and lakes. Moreover, the use of zooplankton inoculation is an unproven method for biomanipulation of aquatic ecosystems, even as small as fish culture ponds. Zooplankton inoculation may be beneficial in ponds filled with water which is devoid of planktonic life (e.g., well water) but at hatcheries which fill ponds with surface water there may be no benefit of adding zooplankton and it may actually be detrimental to production.

These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. It seems, however, that ponds should not be inoculated with large cladocera shortly after stocking walleye fry because seeding ponds with zooplankton that are too large to be eaten by first feeding walleye may encourage a competitive advantage for the larger zooplankton over the smaller cladocera and copepods that are essential prey for first feeding larval walleye. In this study, the organisms used for seeding were generally larger than 2 mm (0.08 in) which is larger than first feeding walleye (about 9 mm; 0.35 in) can consume. It has been reported by others that the mean length of zooplankters in gut contents of first feeding walleye was 1.1 mm (0.04 in) at one study site and 0.8 mm (0.03 in) at another study site.

OBJECTIVE 4

Basic studies on the ecology of clam shrimp in culture ponds demonstrate that strategies for control of clam shrimp in culture ponds need to consider both the life history characteristics of clam shrimp and fish cultural practices. Clam shrimp life history information provided insight into pond management strategies to reduce the impacts of clam shrimp on fish production. The typical habitat of most North American clam shrimp species is small, ephemeral ponds. The key to clam shrimp survival in this habitat is their ability to produce eggs that are highly resistant to drying, mechanical injury, and freezing. Clam shrimp problems in fish culture ponds are persistent because the resting eggs are resistant to mechanical injury, sunlight, and desiccation. Clam shrimp resting eggs can survive long periods of direct sunlight and wind, which they encounter when culture ponds are drained for harvest. Control measures for clam shrimp include interruption of the wet-dry cycle in fish culture ponds, a fill-drain-and-fill strategy, biological control, and chemical control. A fill-drain-and-fill strategy would involve partial pond filling in the spring long enough for clam shrimp eggs to hatch, then drained to flush out the newly hatched clam shrimp nauplii. The current tandem culture system at the GDNFH is a type of fill-and-drain strategy. At GDNFH, the northern pike culture season seems to end before clam shrimp reach sexual maturity, and many juvenile clam shrimp are flushed out before they were able to produce either summer or resting eggs. Also, many, but not all juveniles stranded on the pond bottom die before the ponds are refilled. These practices reduced the abundance of clam shrimp during the walleye culture season because clam shrimp that are hatched during the first week of northern pike season were unable to reproduce before they were washed out when the ponds were drained to harvest the northern pike. Although many of the clam shrimp were washed out, as observed in the catch basin when the ponds were drained, some clam shrimp are carried-over to the walleye culture season by surviving in the kettle and on the wet pond bottom. Although these clam shrimp would be killed with a longer drying period, it is not possible to delay refilling (mean of 1.6 d in 1992) because hatching of these walleye has already been delayed to facilitate the double-cropping strategy. Biological control of clam shrimp with a predaceous fish does not seem to be effective because neither northern pike nor walleye culture feed on clam shrimp. Chemical control may be possible. Quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, $Ca(OH)_2$) is generally recognized as safe as a pond sterilant by FDA and can be applied at the rate of 1,500 kg/ha (1,338 lb/acre) as quick lime or 2,000 kg/ha (1,784 lb/acre) as slaked lime. Lime is often used as a pond disinfectant to kill infectious organisms and parasites, including fish, tadpoles, and insects. The toxicity of lime to clam shrimp resting eggs has not been evaluated, but it is a potential treatment for killing clam shrimp eggs if the lime is applied to the moist pond bottom after it is drained at the end of each production season. In a hatchery such as the GDNFH, the best time to make a lime application would follow the walleye harvest. Previously, trichlorfon (commercially sold as Masoten™ or Dylox™) was widely used for control of clam shrimp, but is not registered for use in fish culture ponds. Other studies show that trichlorfon treatment may be detrimental to zooplankton and invertebrates.

OBJECTIVE 5
Culture tanks equipped with a surface spray, using about 10% of the inflow directed through a 90° nozzle to the water surface, removed the oil film, and feed and bacterial growth from the tank surface, thereby greatly enhancing gas bladder inflation. Gas bladder inflation, which was 20-40% without a surface spray, was 80 to 100% with a spray. Circular tanks (cylindrical tanks) with black-painted side walls were found to be more effective culture vessels than cuboidal tanks or tanks with blue-colored side walls. A near neutral pH is a healthier environment for larval fish than one supplied with water with high pH ($\geq$8.5).

Development of a successful intensive culture system is essential for use of out-of-season spawning of walleye when ponds are not available for stocking. The successful development of techniques for out-of-season spawning and intensive culture system for rearing larval walleye represent a major breakthrough in walleye culture, opening new opportunities of research and commercial culture.

RECOMMENDED FOLLOW-UP ACTIVITIES
OBJECTIVES 1 AND 2
Further efforts should be directed at developing techniques to induce out-of-season spawning in walleye throughout the year.

OBJECTIVE 3
The findings of negative effects from zooplankton inoculation suggest the need for further research to provide further explanation, and the need to define how and when (i.e., the timing) zooplankton inoculation may be used in pond culture of walleye. Distinction needs to be made between ponds receiving zooplankton from the water supply and those filled with well-water and devoid of zooplankton. Likewise, little attention has been given to measuring the quantity and impact of zooplankton inoculation from the water supply of fish hatcheries using surface water sources.

OBJECTIVE 4
Strategies for control of clam shrimp in culture ponds with quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, $Ca(OH)_2$) need evaluation because these chemicals are approved for use in aquaculture.

OBJECTIVE 5
Noninflation of the gas bladder has been a major constraint to successful mass culture of walleye. The development of tank design and a spray—system to remove surface contaminants was a

major breakthrough, however, survival is still typically less than 50% and further research would be beneficial to improve commercial feasibility. Research is especially needed on use of turbid water, optimizing light intensity, and feeding strategies for enhancing survival and growth of larval walleye.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Walleye activities.

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL [a]	OTHER [b]	TOTAL	
1989-91	$177,517	$127,535		$17,511		$145,046	$322,563
1991-92	$109,223	$73,242		$8,935		$82,177	$191,400
1992-93	$35,000	$26,475		$9,424	$40,990	$76,889	$111,889
TOTAL	$321,740	$227,252		$35,870	$40,990	$304,112	$625,852

[a]University of Wisconsin Sea Grant Program/USDC/NOAA
[b]Nebraska Game and Parks Commission

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