ATTACHMENT A

PROJECT NAME: Cultural Technology of Walleye

FUNDING LEVEL: $109,223

DURATION: 1 Year (91-92)

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JUSTIFICATION

The coolwater percid fishes (Family Percidae) have been designated a priority candidate group for development of commercial aquaculture in the North Central Region (Joint meeting of the Industry Advisory Council and the Technical Committee for Extension and Research, May 1988, East Lansing, Michigan). The coolwater fishes that have significant potential for commercial aquaculture are the yellow perch (*Perca flavescens*), walleye (*Stizostedion vitreum vitreum*) and the walleye-sauger *(S. v. vitreum X S. canadense)* hybrid. Research planning for the percids, under the auspices of the North Central Regional Aquaculture Center (NCRAC), has been divided into one work group for yellow perch and a second for walleye. This proposal concerns the walleye.

The yellow perch and walleye are the most exploited percid species in North American commercial and recreational fisheries (Kendall 1978). Although a commercial fishery for yellow perch still exists on the Great Lakes, commercial harvest of walleye in the U. S., except for a few tribal fisheries, has been eliminated in favor of sport fishing. The walleye has been recognized by the National Aquaculture Development Plan (Joint Subcommittee on Aquaculture 1983) as an important recreational and commercial fish with substantial aquaculture potential. In 1983 and 1984, state, federal and provincial fisheries management agencies in North America stocked more than one billion walleye fry and fingerlings (Conover 1986).

Given the numbers of walleye fry and fingerlings reared for maintenance stocking, many fisheries agencies in the U.S. and Canada have conducted applied research on various phases of walleye aquaculture for many years (Coolwater Culture Workshop 1984, 1985, 1986, 1987, 1988, 1989, 1990). The fish culture activities traditionally associated with producing walleye include spawning of wild broodstock, hatching fry, and rearing small fingerlings in ponds. Numerically, fry make up 98% of walleye stockings in the U.S. and Canada (Conover 1986). However, the relative survival of fingerling walleye after stocking is 16 to 60 times greater than with fry (Heidinger et al. 1985). Large numbers of fingerlings can be produced to a size of 35-50 mm total length (TL) by traditional pond-culture methods (Beyerle 1979b; Fox 1989). Increasingly, the focus of research by government agencies is being directed to training pond-reared fingerling to formulated feeds in intensive culture in order to meet the demand from recreational fisheries management for a larger (100-150 mm TL) fingerling (Cheshire and Steele 1972; Nagel 1974, 1976; Beyerle 1975; Nickum 1986). A variety of factors have been studied, including stock density, temperature, light, diet, and feeding frequency (Nickum 1986, Summerfelt 1990b).

At this time, commercial walleye aquaculture has been geared to the production of eggs, fry and pond-reared fingerlings. Commercial walleye producers are selling fry and fingerlings to lake associations, sportsman clubs and individual lake and pond owners for maintenance stocking. Given the incentive of excellent market prices, commercial walleye production has expanded rapidly in the past 5 years. Newly hatched fry sell for 1 to 1.5 cents, and fish of 35 to 100 mm are sold by the producer for $0.25 to $0.75, respectively. The growth of private-sector pond production has been particularly marked in Minnesota, Nebraska, Wisconsin, Iowa and Michigan. Experience with rearing walleye to food-size has been largely limited to a few researchers in the region, including the principle participants of this proposal, who have the expertise to train pond-reared fingerlings to formulated feed. Most food-size walleye are imported from Canada, and the only U.S. supply is from a few Indian tribal lakes. The limited supply has produced exceptional high retail prices, walleye fillets range from $16.09-25.35/kg, which has been a strong stimulus to private sector interest in the production of food-size fish. Although the contribution of aquaculture to production of food-size fish has been limited, in 1989 a commercial venture to rear walleye to food-size was started by Aquaculture Inc., Rolla, Missouri (NCRAC Journal 1990).

For commercial walleye aquaculture to expand throughout the region in the direction of food-fish production, a sustained, collaborative, interdisciplinary research effort needs to be focused on critical bottlenecks, including: (1) the lack of procedures for manipulating reproduction and inducing spawning in walleye broodstock; (2) the lack of captive,

domesticated broodstock; (3) the unreliability of pond management and harvesting strategies for fingerling production; (4) disease identification and FDA-approved therapeutics to control disease problems; and (5) the lack of commercially-produced diets for rearing advanced fingerlings to food-sized fish. Many aspects of the production process need further study to facilitate the development of a commercial aquaculture industry based on sound scientific principles.

This proposal describes an ongoing cooperative regional research project by the North Central Regional Aquaculture Center Walleye Work Group. The principal goal of the Work Group is to address key problems that pertain to the development of commercial walleye culture in the North Central Region. The focus of the project is on: (1) characterization of the natural reproductive cycle of walleye; (2) the evaluation of various zooplankton seeding and clam shrimp control strategies for their effects on the pond production of walleye fingerlings; and (3) to determine the cause of non-inflation of the gas bladder in intensively cultured walleye fry. Significant progress on these problem areas has been made during the first two years of the project. The proposal constitutes a request for a third year of funding which is needed for these three lines of research to reach full fruition and thus yield maximum benefits.

This research effort is a closely integrated interdisciplinary project which could not be completed by a single institution. It involves cooperation with federal and state agencies and investigators with six institutions in the North Central Region (Southern Illinois University at Carbondale, Iowa State University, Michigan State University, the University of Minnesota at St. Paul, the University of Nebraska-Lincoln, the University of Wisconsin-Madison) and the University of California-Davis. We believe this is the first example of inter-regional collaboration within the Regional Aquacultural program of U.S.D.A. The University of California-Davis was invited to draw upon the expertise of Dr. D. E. Hinton to conduct a histological study of walleye gas bladder development. A basic study of organogenesis and histology is needed to determine the cause for non-inflation of the gas bladder of larval walleye.

1. Characterization of the Natural Reproductive Cycle

Strategies for manipulating reproduction are needed to insure the availability of "seed stock" for commercial aquaculture and for selective breeding and other types of genetic manipulations such as gene implantation (see Donaldson and Hunter 1983; Idler et al. 1987). In turn, the development of efficacious procedures to manipulate sexual maturation and induce out-of-season spawning is an important component of optimal broodstock management. The benefits of such procedures include: (1) greater predictability of gamete production; (2) reduced incidents of failed spawnings, gamete resorption and subsequent broodfish losses (e.g., due to toxemia); and (3) the production of fertilized eggs and fry at multiple and predetermined times during the year.

The availability of fertilized eggs outside the normal spawning season would greatly facilitate research on the intensive culture of walleye fry. On a larger scale, the production of fertilized eggs out-of-season could facilitate a fuller, more efficient use of culture facilities and equipment, and might allow such innovative techniques as the double- or triple-cropping of fry in rearing ponds.

Considering the walleye's importance as a food and game fish, remarkably little is known about its reproductive physiology. Baseline information on the physiological mechanisms regulating the natural reproductive cycle of walleye is essential to the development of efficacious procedures for managing captive broodstock and manipulating walleye reproduction (Donaldson and Hunter 1983; Idler et al. 1987). A starting point for obtaining such information is to characterize changes in specific circulating hormone titers and gonadal development during the annual reproductive cycle. Thereafter, with appropriate experimentation, precise practical methods of controlling reproduction and inducing out-of-season spawning can be developed.

2. Zooplankton Seeding and Clam Shrimp Control Strategies

Large numbers of 35-50 mm walleye fingerlings are needed by the public and private sectors for direct stocking of lakes for recreational fishing, and as stock to be trained to formulated feed for further rearing to an advanced size. Advanced fingerlings (i.e., fish >150 mm) are desired for stocking and for rearing to food-fish. At this time, pond culture is the only practical way to produce large numbers of 35-50 mm fingerlings. Given the state-of-the art of pond culture, it is difficult, however, to direct the productive process of ponds to specific end points. In spite of the widespread use of ponds for fish culture, the success of pond culture of walleye and many other species is still unpredictable (Beyerle 1979b; Coolwater Culture Workshop 1984, 1985, 1986, 1987, 1988, 1989, 1990). The ability to predictably produce walleye fingerlings in large quantities is one of the most important problems in the culture of coolwater fishes today (National Task Force for Public Fish Hatchery Policy 1974; Nickum 1978; Joint Subcommittee on Aquaculture 1983). In 1989, the North Central Regional Aquaculture Center (NCRAC) recognized the need to solve this problem and identified development of effective culture procedures for the walleye as one of its nine research priorities (Announcement, Culture Technology of Walleye and its Hybrids, NCRAC, 26 July 1988).

Basic pond management strategies involve well-timed filling of ponds, pond fertilization, zooplankton seeding, and control of predaceous insects (Kuss 1988; Buttner and Kirby 1986; Richard and Hynes 1986). However, the specific approach to these activities varies considerably because they have evolved from trial-and-error practice rather than controlled experimentation (Richard and Hynes 1986). A driving force in pond management is the need to optimize capital costs for pond construction and to meet the increasing demand for fingerlings. These needs are translated into high stocking densities of fry and heavy fertilization, usually with some type of organic fertilizer, to develop and sustain large populations of microcrustaceans and chironomids used by the fingerling walleye as their principle food supply (Mathias and Li 1982; Buttner and Kirby 1986). Higher fish densities, however, cannot be accommodated by increased fertilization without causing an adverse decline in water quality (e.g., adequate dissolved oxygen).

Organic fertilizers are routinely applied to walleye fingerling production ponds to increase the invertebrate forage (Dobie 1956; Buttner and Kirby 1986; Richard and Hynes 1986). But a diversity of complex factors affect the abundance and composition of the zooplankton community in ways that are still unpredictable. Although organic fertilization is recommended for production of striped bass (Bonn et al. 1976) and walleye (Richard and Hynes 1986) fingerlings, the selection of specific kinds and sizes of organic fertilizers and application schedules to specifically regulate the abundance of particulate organic matter, its associated microbes, and zooplankton composition is a new direction in pond culture derived from experimental studies such as that of Barkoh and Rabeni (1990).

As a starting point for development of reliable pond-cultural practice and to overcome a clam shrimp problem commonplace at several walleye culture hatcheries in the North Central Region, a study was designed to evaluate alternate types of organic matter (alfalfa hay, pellets, and meal, and soybean meal) for pond fertilization, and zooplankton inoculation. The purpose of this two-factor experimental design was to examine the performance of different kinds and particle sizes of organic fertilizer and zooplankton seeding as methods to enhance the production (no/hectare) and quality (final mean size and condition factor) of fingerlings, and to overcome competitive interactions between clam shrimp and other zooplanktors. The kind and particle size of organic fertilizers is important because it affects the rates of decomposition and development of microbial populations (Barkoh and Rabeni 1990). Zooplankton seeding is seen as serving a special need in walleye production in northern climates where the interval between pond filling and stocking of walleye fry is limited, water temperatures are low and there is less than the optimum length of time available to maximize zooplankton populations. We also regarded zooplankton seeding as a potential form of biomanipulation to overcome community dominance by clam shrimp.

3. Etiology of Non-inflated Gas-Bladder Inflation

As an alternative to pond rearing fingerlings, considerable interest exists in rearing walleye from hatch (first feeding larvae) to fingerling size in intensive culture (Nickum 1978; Colesante et al. 1986; Krise and Meade 1986; Loadman et al. 1989). Research on this phase of walleye aquaculture has included comparisons of live foods and formulated feeds, design of cultural systems (flow pattern, light intensity, and tank color), and studies of food density, fish density, feeding frequency, and temperature (Nickum 1978; Li and Mathias 1982; Nickum 1986; Barrows et al. 1988; Kindschi and MacConnell 1989; Loadman et al. 1989). A major finding is that walleye can be reared successfully on formulated (dry) diets alone (Barrows et al. 1988; Kindschi and MacConnell 1989; Loadman et al. 1989). In some experiments survival of walleye fry reared exclusively on formulated feed has been better than fish fed live brine shrimp (Kindschi and MacConnell 1989).

Examining a series of experimental studies over the last 20 years indicates a progressive improvement in survival of fry reared in intensive culture. In the formative years, survival to 30 days was typically less than 1% (Nickum 1978), but survival in recent research is typically closer to 10% (Kindschi and MacConnell 1989), and in some studies survival has been as high as 29 percent in groups of fish fed only formulated feed (Loadman et al. 1989). Thus, results from intensive culture are reaching or exceeding typical survival rates of pond culture. In spite of the advances in nearly all of the studies, non-inflation of the gas bladder has been a major problem, invariably regarded as the key factor in poor survival (Nickum 1978; Colesante et al. 1986; Barrows et al. 1988; Kindschi and MacConnell 1989; Loadman et al. 1989).

Fry without an inflated gas bladder struggle to maintain position, and eventually, the high energy cost of swimming and the difficulty in capturing food results in starvation. Intuitively, the problem seems to be an important contributing factor to the incidence of cannibalism because the erratic behavior of these fry would be attractive to a sibling, and their poor swimming ability makes them vulnerable prey. Failure of gas-bladder inflation is also a major problem in the intensive culture of striped bass (Bulak and Heidinger 1980; Cornacchia 1981; Chapman et al. 1988). Noninflation of the gas bladder in striped bass leads to decreased growth rates and increased susceptibility to stress (Lewis et al. 1977). Generally, the same appears to be the case for walleye, fry lacking an inflated gas bladder are typically smaller than siblings which have inflated their gas bladders (Kindschi and McConnell 1989).

Although it has been speculated that there is something about the intensive culture environment which caused the problem, however, noninflation of the gas bladder has also been reported in 73% of a group of 13,000 pond-reared walleye

fingerlings (Fish Technology Center 1989). Thus, the problem is not unique to fish reared in tanks, yet, the etiology of this problem has not been identified. Many hypothesis have been proposed, including nutritional, environmental and genetic factors (Barrows et al. 1988; Kindschi and MacConnell 1989; Loadman et al. 1989).

As a starting point to discover the cause for noninflation of the gas bladder, a collaborative effort between Iowa State and Michigan State Universities was initiated in May 1989 under the auspices of the North Central Regional Aquaculture Center. ISU arranged to acquire 1- to 2-day posthatch fry and reared them in state-of-the-art facilities. The experimental approach was to rear the fry from several sources under controlled environmental conditions and to collect samples of the developing fry for Michigan State University veterinary pathologists. Their job was to determine the presence of indicating lesions in the gas bladder and related tissues. In this study, the two universities combined expertise in fry culture of walleye (Iowa State University) and histopathology to determine the cause (etiology) of the problem.

As our studies progressed over the past two years, it was evident that the search for pathological lesions required more basic information. The gas bladder epithelium was easy enough to characterize in sagittal sections, but not the pneumatic duct or rete mirabile. Clearly, the lack of prior anatomical studies of the development of these structures in walleye was limiting progress. In hindsight, an anatomical atlas to developmental morphogenesis, such as that described in the turbot (Cousin and Laurencin 1985), was needed. Thus, it was obvious that an additional team member was needed to investigate this problem. Because Dr. Hinton, University of California-Davis, has expertise in descriptive histology and morphogenesis of fish, he was invited to carryout research on this topic as a subcontract from Iowa State University. His component of the project is to provide details of normal development patterns of critical structures such as the gas gland, pneumatic duct, and rete mirabile. In addition, Dr. Hinton will evaluate some alternative causes for the gas bladder problem. These include localization of intracellular energy sources, a cellular source of surfactant, and evaluating the potential for an internal source of gas for filling the gas bladder. Because gas gland epithelial cells in postlarval, and likely larval fish, require energy (glycogen), a surfactant, and carbonic anhydrase to fully function in inflating the gas bladder and/or maintaining inflation (Morris and Albright 1975; Fange 1983), measurement of the levels of these three substances can provide insight into the etiology problem. It is important to bring this study to fruition and to identify the causative agent and/or factor(s) in order to develop effective strategies to overcome this imposing problem.

RELATED CURRENT AND PREVIOUS WORK

1. Mechanisms Regulating the Natural Reproductive Cycle

a. Background

To develop maximally effective methods of managing broodstock, baseline information on the physiological mechanisms regulating reproduction is needed (Donaldson and Hunter 1983; Idler et al. 1987). A starting point for obtaining such information is to characterize changes in specific circulating hormone titers and gonadal development during the annual reproductive cycle. Thereafter, with appropriate experimentation, precise practical methods of controlling reproduction and inducing out-of-season spawning can be developed.

At the functional level, reproduction in fish is controlled by the brain and mediated by the endocrine system. The hypothalamus produces gonadotropin-releasing and (apparently at least in some species) release-inhibiting hormones that regulate the secretion of one or more gonadotropic hormones (gonadotropins) from the pituitary glands (Peter 1983; Peter et al. 1986; Sherwood 1987). At least one gonadotropin (GTH), in turn, stimulates the production and release of sex steroid hormones from the gonads and possibly other steroidogenic tissues, such as the interrenal (Idler and Ng 1983; Fostier et al. 1983, 1987; Fontaine and Dufour 1987; Kunimasa et al. 1988). Estradiol-17ß (E_2) appears to be the primary steroid hormone responsible for ovarian growth and development in female fishes (Fostier et al. 1983; Lazier et al. 1987). Testosterone (T) and 11-ketotestosterone (11-KT) appear to be the primary steroid hormones responsible for testicular growth and development in male fishes (Fostier et al. 1983; 1987).

Typically, the reproductive cycles of annually spawning fishes, such as the walleye, are divided sequentially into periods of spawning, gonadal involution and quiescence, gonadal growth and recrudescence, final gonadal maturation and gamete release (ovulation or spermiation), which again leads to spawning (Billard and Breton 1978, Lam 1983; Lam and Munro 1987). During the period of gonadal quiescence, circulating levels of sex steroids are usually quite low (Idler and Ng 1983; Fostier et al. 1983, 1987; Fontaine and Dufour 1987). During the period of gonadal growth and recrudescence in females, increasing concentrations of E_2 stimulate vitellogenesis (yolk protein formation and deposition) and oocyte growth (Fostier et al. 1983; Nagahama 1983; Idler and Ng 1983; Lazier et al. 1987; Wallace et al. 1987). In males, increasing levels of T and/or 11-KT stimulate spermatogenesis (Idler and Ng 1983; Fostier et al. 1983, 1987).

Sometime after completion of the gonadal growth phase in most species examined to date, a GTH rise (or surge) triggers final maturation and subsequent gamete release (Billard and Breton 1978; Idler and Ng 1983; Goetz, 1983; Fostier et al. 1987). In females, final maturation typically involves migration of the oocyte nucleus (termed the germinal vesicle) to the cell periphery, followed by dissolution of the nuclear membrane and dispersal of the chromosomes (collectively termed germinal vesicle breakdown or GVBD) and by a resumption of meiosis. Concurrently, during final maturation, yolk globules and oil droplets in the cytoplasm of the oocyte coalesce, the degree of coalescence depending on species (Goetz 1983).

The release of ova (ovulation) and sperm (spermiation) from their investing tissues in the ovaries and testes, respectively, are typically preceded and/or accompanied by a fluid shift (hydration) into these organs (Nagahama 1983; Goetz 1983; Fostier et al. 1987; Wallace et al. 1987). A growing body of experimental evidence indicates that the stimulatory effects of the GTH surge on final maturation and ovulation in the females of many species is partially mediated by C-21 steroids produced in the ovaries (Goetz 1983). The principal steroids identified as functioning in this role are 17a,20b-dihydroxy-4-pregnen-3-one (17,20-DHP) and 17a,20b,21-trihydroxy-4-pregnen-3-one (17,20,21-THP) (Goetz 1983; Trent et al. 1986; Goetz et al. 1987; Scott and Canario 1987; Patiño and Thomas 1990). In some species, both 17,20-DHP and 11-KT have been implicated as playing major roles in spermiation in males (Fostier et al. 1983, 1987).

Numerous life history studies have reported on the reproductive habits and fecundity of walleye (see Scott and Crossman 1973; Colby 1977; Colby et al. 1979; Becker 1983). Hokanson (1977) and Colby et al. (1979) have discussed circumstantial evidence that female walleye, at least of certain "races", may require an over-winter period of cold temperatures to successfully reproduce.

Hearn (1980) and Nagel (1985) have reported successfully raising walleye broodfish in ponds; the Ohio Department of Natural Resources presently has F_2-generation broodstock at its London Fish Hatchery (T. Nagel, personal communication). Iowa State University and the Fisheries Research Laboratory at Southern Illinois University at Carbondale have raised walleye to reproductive size/age in tanks on formulated feed, but these fish have not yet yielded progeny.

To date, endocrine studies on walleye reproduction have focused almost entirely on the pharmacological induction of final oocyte maturation and ovulation, generally during the normal spawning season. Agents, such as carp pituitary extracts, mammalian luteinizing hormones and human chorionic gonadotropin (HCG), have been used to induce final maturation and ovulation, both in vivo (Nelson et al. 1965; Lessman 1978; Hearn 1980) and in vitro (Goetz and Bergman 1978). Walleye hatcheries in Illinois have experienced difficulties in obtaining female walleye broodfish with completely maturated gonads (Heidinger et al. 1989), and several attempts have been made to induce maturation of these fish at the hatcheries. Heidinger et al. (1989) found that although injections of HCG at levels of 550 and 1100 IU/kg of prespawning walleye females would induce ovulation, final maturation of the ova could not be induced. Ova were released prior to GVBD, resulting in final hatch rates of only 38 percent as compared to 68 percent for fish captured at ovulation. Multiple injections of HCG at levels of 110 and 330 IU/kg also had no effect on maturation of the ova.

In another study, Pankhurst et al. (1986) observed that HCG, "LHRH-A" (a synthetic luteinizing hormone-releasing hormone analogue) and "pimozide" (a dopamine antagonist and presumptive blocker of endogenous GTH release-inhibiting hormone) all stimulated final oocyte maturation and ovulation in prespawning female walleye. An examination of plasma steroid dynamics in relation to changes in oocyte development in fish treated with LHRH-A or pimozide (either alone or in combination) revealed that E_2 and T declined prior to final maturation, but that 17,20-DHP levels increased coincident with GVBD. The latter finding suggests that 17,20-DHP is a maturation-inducing steroid in walleye.

To our knowledge, no definitive investigations characterizing hormonal events and gonadal development during the natural reproductive cycle of walleye have been published. Also not known is whether, and the extent to which, captivity or holding adult walleye in ponds alters their reproductive endocrinology and annual cycle of gonadal development.

b. Progress

Significant progress was made on Objective 1 during Year 1, and in general, our research is being conducted according to the time frame described in the original proposal. In the early months of the project, University of Wisconsin-Madison researchers developed a detailed set of protocols for sampling adult-sized walleye in the field. These protocols, which were forwarded to collaborating investigators at Southern Illinois University and the University of Minnesota, outlined specific instructions on "least stress" fish capture methods, sampling frequency and intervals, methods for collecting, processing and shipping blood and tissue samples, and the implementation of a uniform sample labelling system. Subsequently, Dr. Terrence B. Kayes of the University of Wisconsin-Madison distributed a first-year sampling schedule to all other principal investigators participating in this objective.

Also in the first year of the project, UW-Madison researchers developed and validated accurate and precise methods for directly measuring levels of estradiol-17β (E_2) and testosterone (T) in white bass serum obtained from SIU. For E_2, a commercially available solid-phase antibody radioimmunoassay (RIA) with an iodinated tracer ligand (Coat-a-Count Estradiol, Diagnostic Products Corporation [DPC], Los Angeles, CA) has been adapted for use on white bass, using standards prepared from charcoal-stripped white bass serum. This assay employs duplicate 25 μL serum aliquots, and has minimal cross-reactivity with other naturally occurring steroids. Quality control procedures are routinely being carried out, and quality control data generated for the E_2 RIA to date are as follows:

Coefficient of Variation (CV) of intraassay standards = 1.28%
CV of interassay standards = 11.50% (and has been <10%, except for one assay)
Sensitivity = 10 pg/mL
Recovery = 80-92%

To measure T, both a solid-phase antibody RIA (Coat-A-Count, DPC) and a double antibody RIA (DPC) have been adapted for use on white bass in a manner similar to that described for E_2 above. Like the RIA for E_2, these assays have low cross-reactivity with other steroids (except that the double antibody RIA exhibits 34% cross-reactivity with 5α-dihydrotestosterone). Quality control data generated to date for the T RIAs are as follows:

	Solid-phase antibody	Double antibody
CV of intraassay standards	2.06%	2.25%
CV of interassay standards	4.80%	6.30%
Sensitivity	0.15 ng/mL	0.02 ng/mL
Recovery	80-100%	80-100%

University of Wisconsin-Madison researchers have also examined various histological procedures and developed protocols that produce acceptable results in evaluating adult white bass gonads at all (seasonal) stages of development. One common problem with ovarian histotechnique in animals having relatively large eggs (e.g., many fishes, reptiles and birds) is that many routine procedures result in tissue hardening, or incomplete fixation, clearing and/or infiltration of large, maturing oocytes. This problem has been resolved by using Bouin's fluid as a fixative, xylene as a clearing agent and a two-part infiltration and embedding system available from Surgipath Medical Industries,Inc., Grayslake, IL.

University of Minnesota researchers have determined that Mille Lacs, a large inland lake in central Minnesota, will serve as the best primary source for the Minnesota walleyes needed for this objective. Mille Lacs has a large walleye population, walleye can be reliably sampled from this lake on a year-round basis, using minimal stress procedures.

Adult walleye are being held in drainable ponds at Southern Illinois University-Carbondale and are being maintained on forage fish, which are supplied at regular intervals. Initial sampling of these walleye for hormone analysis was by drag seining. However, stress induced by this technique made obtaining adequate amounts of blood difficult. Therefore, the use of frame nets was evaluated. This sampling technique appears to be adequate, with little or no apparent stress on the fish. A large volume of pooled serum and two standard monthly samples were thus obtained, to be analyzed by the University of Wisconsin-Madison.

To date, samples received by University of Wisconsin-Madison investigators and currently being analyzed consist of the following:

Source	Number of Fish	Sex	Month collected	Presumed state of gonadal maturation
Minnesota	2	M	April	Spawning
Minnesota	2	M	April	Post-spawning
Minnesota	2	F	April	Spawning
Minnesota	2	F	April	Post-spawning
Southern Illinois	4	M	May	Quiescent
Southern Illinois	4	F	May	Quiescent
Minnesota	4	M	May	Quiescent
Minnesota	4	F	May	Quiescent
Southern Illinois	4	M	July	Quiescent-recrudescent
Southern Illinois	4	F	July	Quiescent-recrudescent
Minnesota	4	M	July	Quiescent-recrudescent

2. Zooplankton Seeding and Clam Shrimp Control Strategies

a. Background

Survival and growth of walleye at high density (e.g., 50,000-400,000 fish/hectare) is dependent on pond management strategies that result in: (1) maintenance of good water quality (e.g., adequate dissolved oxygen); (2) large populations of the principle foods of young walleye; and (3) minimization of undesirable animal and plant species that compete for food or which are predators on the fry.

Organic matter rather than inorganic fertilizer is recommended for production of many kinds of fingerling fish because zooplankton response to a detrital food chain started by the addition of organic matter is faster in freshly filled ponds than using inorganic fertilizers (Huner and Dupree 1984). Organic fertilizers, including animal manures, seed meals and hays, provide nutrients and substrates for detrital bacterial and protozoa, which in turn are consumed by zooplankton (Geiger 1983b; Buttner and Kirby 1986). Organic fertilizers have been recommended for production of fingerling striped bass (Bonn et al. 1976) and walleye (Richard and Hynes 1986). Fertilization to increase fish production, however, has its limits, generally sharply defined by oxygen depletion and fish kills, and excess nutrients can cause problems from excessive algae and macrophytes.

Other troublesome problems encountered in pond-culture of fingerling fish is the occurrence of invertebrate predators (both air-breathing and gill-breathing insects) and competitors such as clam shrimp. Clam shrimp are common in ephemeral ponds in the Great Plains, and they are also found in small intermittent streams (Horne 1967; Frank 1988). They have been reported to be a competitor of fish in hatcheries in the Great Plains (Dexter et al. 1967; Call 1990). Clam shrimp problems have been reported from the Tishomingo National Fish Hatchery, Tishomingo, Oklahoma, (J. P. McCraren, personal communication), Minor E. Clark Hatchery, Morehead, Kentucky (Michael Hearn, personal communication), the Wolf Lake Hatchery, Michigan (Don Waile, personal communication), and Blind Pony Hatchery, Missouri (Terry Hamilton, personal communication).

When abundant, clam shrimp have reduced the production of fingerling northern pike (Dexter et al. 1967; Call 1988), walleye (Call 1988) and goldfish (Dexter et al. 1967). A clam shrimp infestation reduced goldfish (*Carassius auratus*) production by 60 to 80% and ponds with clam shrimp required more fertilization (Dexter et al. 1967). Hatchery personnel have reported they can detect heavy infestations of clam shrimp by the conspicuous increase in pond turbidity (J. Call, personal communication) caused by their burrowing activities. Turbidity can reduce fish production because it reduces the effectiveness of visual feeding fish, and, more importantly, the production of green algae which is food for desirable zooplankton. In addition to the negative indirect effect clam shrimp have on fish production (Dexter et al. 1967), clam shrimp also consume detritus, diatoms and green algae (Strenth and Sisson 1975; Royan 1976), competing directly for the same food resources used by copepods and cladocerans. Because clam shrimp are not consumed by young walleye or northern pike (Dexter et al. 1967), they do not contribute to fish production. Lacking predators to control their abundance in ponds used for fry culture, clam shrimp populations continue to expand during the culture season.

A major complaint of fish hatchery personnel is that clam shrimp become so numerous that they clog the outlet screens in the kettle during harvest, causing delays in harvest and extra effort to keep the screens clean.

It has been common practice to control air breathing invertebrate insect predators in ponds by application of diesel fuel to the water surface, and it is reported that methyl parathion or Baytex have been used by gill breathing insects (Dupree and Huner 1984). However, control of microcrustacean predators (copepods) and competitors (clam shrimp) is more difficult because candidate chemical to control these organisms (copper sulfate or dylox) are also toxic to the microcrustaceans used as food by walleye.

Another approach to clam shrimp control is to use ecological strategies, or biomanipulation, derived from understanding resource partitioning; i.e., ways to perturbate resource competition between clam shrimp and zooplankton in favor of zooplankton. In 1989 and 1990, our search for tools to enhance zooplankton abundance was to use various pond fertilization schemes (kinds, amounts and balance between inorganic and organic fertilizers) and zooplankton inoculation. Alfalfa, for example, is used as ground hay, pellets or meal, which vary in particle size from large to small; these factors affect decomposition rate and the detritus/microbial particles available for zooplankton consumption (Barkoh and Rabeni 1990). In this case, the zooplankton inoculation was used to give the desirable zooplankton an overwhelming numerical advantage in an effort to limit survival of newly hatched clam shrimp nauplii.

b. Progress

This research has been a collaborative effort between Iowa State University and the U.S. Fish and Wildlife Service National Fish Hatcheries in North Dakota (Garrison Dam NFH at Riverdale and the Valley City NFH at Valley City).

These hatcheries produced 28% of all fry and fingerlings reared by the U.S. Fish and Wildlife Service in 1990 (Fish and WIldlife Service 1990). Even though funds were not available until May 1, 1989, the contract was signed in time to initiate experiments at both hatcheries in 1989, and a second field season was completed in 1990. The successful completion of the two field seasons is due to the close cooperation between hatchery personnel of the U.S. Fish and Wildlife Service and Iowa State University. Both hatcheries provided living accommodations for university staff and space for ad hoc laboratory that was used for chemical analyses and to support the collection, sorting and storage of specimens. Each year, field work began at the Valley City NFH; there was a week of overlap in activity at both hatcheries, then university staff moved operations to the Garrison Dam NFH to complete the field season.

These two hatcheries provide contrast in edaphic factors, clam shrimp problems, and pond management strategies. In 1989, the ponds used for walleye culture at the Garrison Dam NFH had a history of clam shrimp problems, whereas clam shrimp were never a problem at the Baldhill Unit of the Valley City NFH. The ponds were 0.30 hectare at the Valley City NFH and 0.61 hectare at the Garrison Dam NFH. The ponds at the Garrison Dam NFH used in 1989 were supplied with water of low fertility from a deep intake from Lake Sakakawea, but the new ponds used in 1990 were supplied with water from the Spillway Pond. The ponds at the Baldhill Unit of the Valley City NFH are supplied with highly fertile water from the Sheyenne River. Alfalfa pellets have been the principle form of fertilizer at the Valley City NFH, and in the recent past, the Garrison Dam NFH has been using only chopped alfalfa hay.

Both hatcheries provided 12 ponds for experimental manipuation in 1989, and Valley City NFH provided the same 12 ponds again in 1990. At Garrison Dam NFH, however, the ponds used in 1989 were not available in 1990 because a reduction in egg take of walleye from Lake Sakakawea altered their pond management plans and the old ponds used in 1989 were not available. In 1990, the ponds used at the Garrison Dam NFH were new, constructed in 1989 and used for the first time in 1990. Because 15 ponds were available, two application rates of soybean meal were evaluated at the Garrison Dam NFH, while only one application rate was used at the Valley City hatchery.

In 1989, a 2 x 3 factorial experimental design was used at both hatcheries, where the factors were zooplankton inoculation (with and without zooplankton inoculation) and three forms of alfalfa fertilizer (chopped hay, meal, and pellets). In 1989, the application rate of organic fertilizer at Valley City was 637 kg/hectare of either alfalfa meal, pellets, or hay (4 ponds each), with or without zooplankton inoculation (6 ponds each). This treatment plan provided 2 replicates of each combination treatment type. At Garrison Dam NFH, the same kinds of organic fertilizer were used but the application was 1338 kg/hectare. The difference in the application rates used by the two hatcheries reflects differences in fertility of the water supplies (see comment above).

In 1990, a 2 x 2 factorial was used at the Valley City NFH, where the factors were zooplankton inoculation (with and without zooplankton) and two kinds of organic fertilizer. The fertilizers were applied at 299 kg/ha of soybean meal and 793 kg/hectare of alfalfa pellets, the rates were calculated to provide approximately equal quantities of protein (N). There were 3 replicates of the four treatment combinations: (1) zooplankton inoculation-soybean fertilizer, (2) without zooplankton inoculation-soybean fertilizer, (3) with zooplankton inoculation-alfalfa fertilizer, and (4) without zooplankton inoculation-alfalfa fertilizer. A similar experimental design was used at the Garrison Dam NFH, where the factors were zooplankton inoculation (with and without) and fertilizer type (ground alfalfa hay and soybean meal). The additional 3 ponds

available there (15 total) gave us a high and low soybean application. Alfalfa hay was applied to six ponds at 1519 kg/hectare, and soybean meal to 9 ponds—6 ponds for the low rate (578), 3 ponds with and 3 without zooplankton inoculation, and 3 ponds at the high rate (1181 kg/hectare) without zooplankton inoculation.

Fertilization was done by hatchery personnel, university personnel or both. University personnel collected zooplankton for inoculation from a separate pond using a pump and cage system as described by Graves and Morrow (1988). University personnel made collections of zooplankton, benthos (1990), and fish, as well as several physical-chemical variables (oxygen, temperature, pH, conductivity, ammonia, secchi disk, alkalinity). The process of identification and counting of zooplankton and benthos was done on the ISU campus. All of the zooplankton and benthos samples have been enumerated. The fish stomach contents have been identified and counted in the 1989 collections, and lab work on the 1990 collections is nearly completed. Data are being entered for computer analysis.

In 1989, clam shrimp were not collected with the Schindler-Patalas plankton trap in the 12 ponds sampled during mid-morning hours at the Garrison Dam NFH even though clam shrimp were so abundant as to clog the screens of the catch-basin when the ponds were drained. Obviously, the Schindler-Patalas trap was not an effective sampling device for clam shrimp, at least not when sampled during the day. Therefore, in 1990 the sampling techniques were expanded, including diurnal sampling with the Schindler-Patalas trap, and use of Ekman dredge samples and seining (3 mm mesh). However, in 1990, a different set of ponds were used as explained above, these 15 ponds were new, first used for fish culture in 1990. Clam shrimp were not collected by any of our sampling and they were not present at the time of draining in any of these ponds. In both 1989 and 1990, however, clam shrimp were present in the old ponds. Although the water supplies for the two groups (old and new ponds) were different, our hypothesis is that clam shrimp were not found in the new ponds because the new ponds were excavated from subsoil which lacked a "seed" bank of clam shrimp eggs to populate the ponds. Because clam shrimp eggs are highly resistant to drying, mechanical injury, and freezing (Mattox 1950; Mattox and Velardo 1950; Belk 1969; Frank 1999), the resistant egg stage can accumulate and develop a large "seed bed" in the pond basin after a few years of pond culture.

A detailed literature review of clam shrimp life history and ecology is underway. An experiment conducted on clam shrimp in aquaria at Garrison Dam NFH provides further evidence of the clam shrimp on pond turbidity. Clam shrimp (collected from the old ponds) were stocked in glass containers with a bottom layer of soil at a density of 0, 2, 4, 8 and 16 per aquarium. After 24 and 48 hours, there was a rectilinear relationship between turbidity (FTU) and clam shrimp density. This verified the observations reported by the hatchery personnel that clam shrimp abundance in ponds can be easily spotted by the high turbidity.

3. Development and Etiology of Gas-Bladder Inflation Problems

a. Background

The gas bladder of fish, frequently called the swim bladder because of its hydrostatic function, develops as an outpocketing of the alimentary tract (Jones and Marshall 1953). Even physoclistous fishes, which as juveniles and adults lack a direct connection between the gut and the gas bladder, are initially physostomous. The hydrostatic function of the bladder is important to maintenance of body position in the water column and in feeding. Observations suggest that absence of an inflated gas bladder causes the fish to struggle to maintain position in the water column, and eventually, the high energy cost for swimming and the difficulty in capturing food results in death. Failure of gas bladder inflation is also a major problem in culture of striped bass (Bulak and Heidinger 1980; Chapman et al. 1988; Cornacchia 1981).

The age at which larval walleye develop a gas bladder is a small window of opportunity in the early posthatch interval, and if not developed at that time, it will probably not develop at all. In striped bass, it is said that gas-bladder inflation must occur within the first 2 weeks of larval life if it is to occur at all (Bennett et al. 1987; Doroshev and Van Eenennaam 1987). A common finding is that gas bladder inflation occurs soon after yolk is depleted: Chapman et al. (1988) reported that larval striped bass first inflated their gas bladder on day 7 posthatch; Cornacchia (1981) reported initial inflation in striped bass occurred 5-7 days posthatch; the yolk of the logperch, *Percina caprodes*, is depleted 5-days posthatch and the gas bladder is first seen 7-days posthatch (Grizzle and Curd 1978).

Larval walleye absorb the yolk sac 5 days after hatch (Li and Mathias 1982), and first feeding begins at 100-127 TU (degree-days above 0 °C) (Krise and Meade 1986), an age of 5-6 days when reared at 20 °C. Krise and Meade (1986) stated that gas bladder inflation happened at 214 TU, about the 11-12 days posthatch when reared at 18-20 °C. Barrows et al. (1988) reported that swim bladder inflation in walleye started by day 5, peaked between days 6 and 9, and seemed to be complete by day 11 at 21 °C (i.e., 105-231 temperature units). As a working hypothesis, it is assumed that gas bladder inflation in walleye occurs between the 4th and 12th day posthatch (Summerfelt 1990a). This interval approximately coincides with the disappearance of the yolk sac (5th day) and it is completed ~2-days after the disappearance of oil globule (Li and Mathias 1982). The interval of gas bladder inflation coincides with the transition from endogenous to exogenous feeding and a transition from yolk sac respiration to gill respiration (Krise and Meade 1986).

The critical factors in gas bladder inflation in walleye are not known, but environmental, nutritional and genetic hypotheses have been proposed. The most prominent environmental factor, with strong conceptual and experimental support, involves the role of a surface film or a surface tension problem. This hypothesis presumes that the larval fish must gulp air at the air-water interface in order to inflate their gas bladder. Experimental evidence for this hypothesis has been reported: access to the water surface is necessary for medaka (*Oryzias latipes*) (Marty et al. 1990a), and striped bass and striped bass x whitebass hybrids (Chapman et al. 1988). Because walleye are physoclistous fishes they may also require contact with the air to effect initial inflation (Chapman et al. 1988). The strongly phototactic response of larval walleye seems to support an air-water relationship; in tanks it is common to see them push up against the surface so strongly that dimples can be seen on the water surface. Catches of walleye fry in lakes indicates a close association of walleye with the lake surface until they are juveniles (Forney 1980).

It has been suggested that an oil film on the water surface (originating from the feed or oil globule of dead larvae) in intensive culture systems may prevent the larvae from breaking the surface film to inflate their gas bladders (Colesante et al. 1986; Barrows et al. 1988; Kindschi and MacConnel 1989). Indeed, the W-16 feed commonly used for training pond-reared walleye fingerlings to formulated (Coolwater Culture Workshop 1984, 1985, 1986, 1987, 1988, 1989, 1990), produces an oily sheen when it is distributed on the water surface; half of the total fish oil content of the W-16 feed is applied to the surface of the feed rather than incorporated in the mixture in order to maintain pellet quality (personal communication, Dewey Klaustermeier, Glencoe Mills, Minnesota). Paradoxically, diesel fuel has been spread on the surface of ponds to control air-breathing insects in walleye culture ponds. Perhaps, this accounts some of the observed incidence of non-inflation of the gas bladder observed in pond-reared walleye fingerlings (Fish Technology Center 1989, 1990).

A feeding ring, which retains the feed within a prescribed area, has been suggested as a mechanism for reducing the spread of an oil film from oily formulated feeds (Barrows et al. 1988). Barrows et al. (1988) enclosed the feeder with a ring of plastic extending into the water as a means to retained the feed within a prescribed area of the surface. However, Kindschi and MacConnell (1989) stated that use of a surface-feeding ring did not improve survival or swim bladder inflation of walleye. Friedmann and Bates (1988) reported an improvement in gas bladder inflation in striped bass culture by using a floating oil-sorbent cloth of spun-polypropylene to remove oil film from the culture tanks. However, gas bladder inflation of walleye fry reared in tanks with the oil sorbent cloth was not significantly different than fry reared in tanks without the cloth (Summerfelt 1990a).

An oil film can be distrupted and surface tension reduced by release of air bubbles from the bottom of the tank. Chapman et al. (1988) found that turbulence in the water in aerated aquaria produced consistently higher inflation rates in larval striped bass compared to fish reared in unaerated aquaria. It is also possible for young fish to swallow a gas bubble captured from within the water column (Hadley et al. 1987). The release of air or oxygen at the bottom of the culture vessel is a way to provide an abundance of small air or oxygen bubbles. In rivers, air bubbles may be available to fish larvae below water falls and in pool areas downstream of riffles; in lakes, surface turbulence from toppling of waves (white-caps) provide opportunities for gas bubble formation near the surface.

Chapman et al. (1988) acknowledged that substantial variation in inflation rate is from "extraneous" variables. Other potential environmental factors for noninflation of the gas bladder include gas exchange problems (partial pressure of oxygen or gas bladder trauma) and infection when the fry swallow an air bubble contaminated by bacteria growing on the surface film (Kindschi and MacConnell 1989). It is well known fact that hyperbaric total dissolved gas pressure in the water column. (i.e., gas supersaturation) produced by natural and man-induced factors may produce gas-bubble disease (GBD). One clinical sign of GBD is swimbladder hyperinflation (see review by Cornacchia and Colt 1984). Cornacchia and Colt (1984) reported overinflation of the gas bladder of striped bass at total gas pressure as low as 102.9% and mortality at total gas pressure of 105.6-106.0%. However, experimental efforts to utilize high total gas pressure, oxygen supersaturated water, or oxygen enriched water as a mechanism to enhance gas bladder inflation of larval fish has been unsuccessful (Kindschi and MacConnell 1989; Summerfelt 1990a).

Recent studies demonstrate that non-inflation of the gas bladder may be the product of events that occur during early organogenesis, not in larval rearing. In medaka (*Oryzias latipes*), another physoclistous species, Marty et al. (1990b) reported that a 2-h pulse exposure to a toxicant 8 days before hatching resulted in up to 70% failure of gas bladder inflation among eggs that hatched (compared with 6% failure in controls). Thus, if the non-inflation problem is a result of exposure of the embryos to an environmental contaminant, there is limited potential to increase gas bladder inflation by optimizing larval care. It is likewise possible that some environmental contaminants accumulated in the maternal parent are transmitted through the yolk to the developing embryo, producing an effect similar to that reported by Marty et al. (1990b). This problem is further complicated by genetic effects.

In using formulated feed, it is obviously necessary to consider a nutritional hypothesis, with the implication that there is a basic nutrient deficiency in the formulated feed which affects gas bladder development and filling. However,

Barrows et al. (1988) reported up to 83% gas bladder inflation for walleye reared on Biotrainer, a commercially prepared, closed formula feed (Bioproducts Incorporated, Warrenton, Oregon), and Kindschi and MacConnell (1989) had up to 54.1% inflated swim bladders on formulated feed (fry feed Kyowa), which was substantially better than the 24.7% gas bladder inflation rate of walleye fry reared on brine shrimp in the same study. In a national survey to evaluate the incidence of non-inflation of the gas bladder in pond-reared walleye fingerlings, non-inflation of the gas bladder was found in walleye fingerlings from 37% of 56 ponds examined from 8 of 9 hatcheries, from Pennsylvania, Illinois, South Dakota, North Dakota, and Montana (Fish Technology Center 1990). The Fish Technology Center reports (1989, 1990) clearly establish that this problem is not unique to fish reared on formulated feeds in an intensive culture environment.

An infectious disease etiology for noninflation of the gas bladder has not received much attention, however, Kindschi (1988) stated that heavy bacterial levels produced by overfeeding from 5-11 day posthatch when the walleye fry are inflating the gas bladder may physically prevent gas bladder inflation by closing off the pneumatic duct. Kindschi and MacConnell (1989) found bacterial infection in fish with uninflated and partially inflated gas bladders, but not in fully inflated gas bladders. They conjectured that bacteria and feed can enter the gas bladder when fry gulped air from the surface to first fill the gas bladder. However, bacteria may enter the gas bladder from the gut as a result of normal feedings.

Physiological functions may be involved in gas bladder inflation and/or in sustaining the gas pressure. The gas gland epithelial cells have been shown to contain a surfactant within cytoplasmic osmiophilic bodies (Morris and Albright 1975), similar to type II alveolar pneumocytes in mammals. The surfactant minimizes surface tension, thus whatever factor affects surfactant secretion will prevent initial inflation of the gas bladder. Another alternative concerns the role of the gas gland to initially fill or maintain gas pressure in the gas bladder. Some physoclistous fish (e.g., *Tilapia mossambica*) never develop a pneumatic duct, but can inflate their gas bladder without access to air (Doroshev and Cornacchia 1979). In these species a functioning rete mirabile and gas gland is able to concentrate sufficient quantities of gases in the gas bladder for initial inflation. If the walleye functioned in the same manner (option 1), or if after gas bladder inflation by gulping air, the gas gland is unable to supply gas to maintain inflation (option 2), the gas bladder would deflate. If a substantial fraction of the gas pressure in the gas bladder is from the partial pressure of carbon dioxide, control over carbon dioxide secretion into the gas gland could be significant. The exact mechanism of carbon dioxide release into the gas bladder has not been determined, but it seems related to relatively high levels of carbonic anhydrase in the gas gland cells, to carbonic anhydrase in erythrocytes (Dimberg et al. 1981), or perhaps in the vascular walls of the countercurrent vessels (Fange 1976). Radiolabelling studies with ^{14}C have shown that essentially all of the CO_2 in the gas bladder arises from bicarbonate (D'Aoust 1970). In summary, localization of glycogen (energy storage) and carbonic anhydrase (essential for CO_2emission) is expected to be an important contribution to finding the cause for a serious constraint to fry culture.

b. Progress

Samples of fry reared in an intensive culture facility at Iowa State University (ISU) were collected in 1989 and 1990, preserved in neutral formalin, and transported to Michigan State University (MSU) for examination to determine presence of histopathological characteristics in the gas bladder that can indicate the etiology of the non-inflation problem. The fry were reared on a commercial formulated fry feed (fry feed Kyowa, BioKyowa, Inc., St. Louis, Missouri) in a carefully controlled environment. Fry were collected daily from each of two cultural tanks from two groups of walleye in 1989 and three groups in 1990. These preserved fry were transported to MSU 7/31/89 and 7/16/90.

At MSU, three fry were selected at random from each sample bottle containing five fry and the remainder retained for any required re-examinations that should become necessary. Each fish, already fixed in 10% formalin was dehydrated, permeated with paraffin, sectioned sagittally (central longitudinal plane), stained with hematoxylin and eosin, and examined by light microscopy. Examinations were done both macroscopically and microscopically.

Macroscopic examination-—Each specimen was examined under a dissection microscope at magnifications from 10 to 40x and the presence or absence of the gas bladder was noted as well as the occurrence of pathological lesions. Microscope examination-—Each specimen was examined under various magnifications to determine gas bladder inflation and to note presence of pathological lesions. Microscopic observations were recorded on dictaphone as they were accomplished and the recordings were transcribed. Analysis of the transcriptions are in progress.

Some fish with a partially inflated (developed) gas bladder had a proliferative and necrotizing aerocystitis. These gas bladders contained detached cells, half degenerate epithelial cells and half macrophages. The macrophages contained small coccobacilli. The inner surface of gas bladder epithelium was characterized by hyperplasia and a necrosis characterized by vacuolization, with eosinophilic intranuclear inclusions. The affected cells had pyknotic nuclei with karyorrhexis.

The degenerative changes in the gas bladders (i.e., hyperplasia and abundance of macrophages) indicate an inflammatory disease, and preliminary evidence is present to suggest a microbial infection as a specific initiating process. The observation of bacteria in the macrophages supports the findings of Kindschi and MacConnell (1989) of a bacterial inflection, at least as a secondary invader, and the degenerative changes of the nuclei and presence of eosinophilic inclusions suggests a possible viral etiology.

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--characterization of the natural reproductive cycle.

2. Develop an index to the abundance of clam shrimp eggs (seed) in pond soil, describe effects of clam shrimp abundance on the ecology of culture ponds (turbidity, algae, zooplankton), and evaluate clam shrimp-zooplankton-fish production interrelationships.

3. Determine the etiology of non-inflation of the gas bladder in intensively cultured walleye fry.

PROCEDURES

Objective 1

Research to characterize the natural reproductive cycle of walleye is being done collaboratively by investigators from Southern Illinois University (SIU), the University of Minnesota (UM), University of Wisconsin-Madison (UW-Madison) and the University of Nebraska-Lincoln (UNL). In general, SIU is responsible for collecting blood and tissue samples from (brood-size and -age) adult walleye captured from the wild in Illinois and maintained (by SIU) in large holding ponds on forage fish. The UW-Madison is responsible for the analysis of blood and tissue samples and interpretation of endocrinological data. The UM is responsible for the collection of blood and tissue samples from wild walleye in Minnesota.

During the course of the project, Dr. Terrence B. Kayes, a principal investigator and author of much of the research proposed under Objective 1, moved from the UW-Madison to the UNL. By mutual agreement, Dr. Jeffrey A. Malison is assuming the lead for the research being done at the UW-Madison. Dr. Kayes, from the UNL, will continue to assist UW-Madison investigators and the group with project coordination, the analysis and interpretation of research findings, and the preparation of reports and any subsequent publications. This will be accomplished through close communications and several working visits by Dr. Kayes to the UW-Madison, and possibly other participating institutions (SIU and the UM).

The principal (null) hypothesis of Objective 1 is that the annual reproductive cycles of walleye held in ponds in Illinois and walleye in the wild in Minnesota will not differ significantly. As an alternative hypothesis, we suggest that holding walleye in ponds alters hormonal dynamics and gonadal development (e.g., changes in hormone levels will not be as great in pond held fish as in wild fish, at the time of final maturation in spring). Regardless of hypothesis, this comparison will provide valuable insights on the mechanisms regulating walleye reproduction and on the extent to which captivity and pond holding (which includes the possible effects of latitudinal temperature differences) influence the reproductive cycle of walleye.

Present plans are to sample fish over a 25-month period, through three spawning seasons. For the first 12 months, sampling is being done about every 8 to 10 weeks from mid-May to mid-November, once in mid-winter (January-February), and shortly before, during and shortly after the spawning season. In the final 13 months of sampling, critical or weak data sets will be replicated as needed, and more frequent sampling will be done during key periods of the reproductive cycle (e.g., the prespawning and spawning periods). At each sampling time and location, blood and tissue samples will be collected from a minimum of four fish of each sex. Additional blood samples from up to ten fish of each sex will be collected, when and if they are available, using nonlethal procedures (e.g., see Wingo and Muncy 1984).

Sampling procedures include measurement of total fish length, total body weight, liver and eviscerated carcass weights, and collection of the gonads and blood. Representative portions of the gonads are being fixed in Bouin's fluid, embedded in paraffin, sectioned and stained with alum hematoxylin and eosin according to standard methods. Maturational state of the gonads is being assessed gravimetrically and by routine histological, histometric and cytometric procedures (employing light microscopy). Levels of E_2 and T in the blood samples are being measured by the radioimmunoassay (RIA)

procedures described under "Related Current and Previous Work;" enzyme-linked immunosorbent assay (ELISA) procedures (e.g., Cochran et al. 1988, Mao 1988) may also be evaluated. Present plans are to develop ELISAs to measure 17,20-DHP, 17,20,21-THP and 11-KT in samples collected during the second and third spawning seasons. As a backup, if such procedures cannot be developed, Dr. Peter Thomas of the University of Texas Marine Science Institute has agreed to measure 17,20-DHP and 17,20,21-THP using existing RIAs.

All immunoassay procedures have been or will be validated, and intra- and interassay coefficients of variation calculated. Whenever possible, parametric statistical methods will be used to analyze numerical data. Nonparmetric statistics will be employed when the application of parametric methods is found to be inappropriate or unfeasible.

Data collected by investigators of the participating institutions will be collated on an ongoing basis, and the findings published in a timely manner in appropriate peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

Objective 2

In 1992, the experimental design will focus on the ecology of the clam shrimp at the Garrison Dam NFH. This research will be done collaboratively with staff of the U.S. Fish and Wildlife Service's Garrison Dam National Fish Hatchery. Our 1992 studies will focus of measures of the clam shrimp seed bed, the relationship between physicochemical variables and clam shrimp abundance, and the relationship between clam shrimp abundance and abundance of zooplankton and algae. The availability of large numbers of ponds, both old and new, is an optimum situation for the proposed ecological study.

The null hypothesis is that clam shrimp abundance, measured by Ekman dredge and seining, and verified by observing their presence in the catch basin on draining is the same in all ponds. The alternate hypothesis is that clam shrimp abundance varies between old and new ponds, and is related to the "seed" density in the pond basin. If clam shrimp seed density can be used to predict with a reasonable degree of accuracy the abundance of clam shrimp during the season, then this assessment should be of value to hatchery managers and researchers. Obviously, new ponds cannot be constructed each year to avoid clam shrimp problems, however, if clam shrimp abundance can be quantified by sampling the pond basin before filling, this measurement will be a useful predictor of an impending problem and it can serve as a practical means to evaluated treatments of the basin with pond disinfectants such as slaked lime (CaOH) or calcium hypochlorite (Dupree and Huner 1984).

The clam shrimp seed bank will be sampled from a random sample of 6 of the old ponds and 6 of the ponds which were new in 1990. Clam shrimp seed bank density will be determined by measuring the abundance of clam shrimp naupli which develop from a composite sample of pond soil collected before pond filling and after draining. Seed density can be measured by enumerating the hatch of naupli in aquarium water (Mattox et al. 1950). Clam shrimp "seed" density will be sampled in the pond bottoms before filling and through both the northern pike and walleye culture seasons, and again after draining. Pond fertilization will be according to the traditional schedule, using chopped alfalfa, sometimes supplemented with applications of inorganic fertilizer. The hay is purchased in the fall, stored over winter in bales and chopped before application. Due to the difficulty in handling, alfalfa hay is applied to only one spot, and distributed about the pond by the wind. A proximate analysis will be done on moisture, total nitrogen, and energy content of each feed type by taking random samples of the feed when it is applied.

Physicochemical and biological variables will be used for correlation and regression analysis. Combinations of variables and clam shrimp abundance that are significant at the 0.05 level will be used in stepwise multiple regression analysis in search for significant prediction formulas to clam shrimp abundance, and relationships to turbidity.

The findings will be described in a completion report and important components prepared for publication in appropriate peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

Objective 3

As a starting point to discover the cause for noninflation of the gas bladder, a collaborative effort between Iowa State and Michigan State Universities was initiated in May 1989 under the auspices of the North Central Regional Aquaculture Center. The experimental approach was to rear the fry from several sources under controlled environmental conditions and to examine samples of the developing fry to determine the presence of indicating lesions in the gas bladder and related tissues. In this study, the two universities combined expertise in fry culture of walleye (Iowa State University) and experience in the histopathology of fish gas-bladders (Machado et al. 1989 and 1990). The collaboration between Iowa State and Michigan State will continue, but in addition, Dr. Hinton, University of California-Davis, was invited to carry out a subcontract related to this objective to provide details of normal development patterns of critical structures such as the gas

gland, pneumatic duct, and rete mirabile. There are no prior anatomical studies of the development of these structures in walleye, but an anatomical atlas to developmental morphogenesis is needed. In addition, he will evaluate, by use of special histochemical stains, some alternative causes for the gas bladder problem. Because gas gland epithelial cells in postlarval, and likely larval fish, require energy (glycogen), a surfactant, and carbonic anhydrase to fully function in inflating the gas bladder and/or maintaining inflation (Morris and Albright 1975; Fange 1983), measurement of the levels of these three substances can provide valuable insight into the etiology problem.

Research to determine the etiology of noninflation of the gas bladder of walleye will involved rearing of walleye fry under precisely controlled conditions and sampling of known-aged fish at prescribed intervals during the first 21 days posthatch, the interval when gas bladder inflation will take place if it takes place at all. Eggs or newly hatched fry will be obtained from one to three different geographical sources (e.g., hatcheries in Ohio, Kansas, Iowa, Minnesota, South Dakota and North Dakota). The number of 21-day rearing trials which can be completed during a spring season depends on the time the fry are available from the different sources, but at least two sources are anticipated. The goal will be to stock 20-30 fry per L with at least two replicate tanks of each strain. Initial fry stock numbers are estimated by a gravimetric technique to obtain an average weight. In 1990, mean weight of fry, 1 to 2 days posthatch, was 7.95±0.51 mg. Wiggins et al. (1981) reported 3.2 as the weight of fry at first feeding, about 5-days posthatch, after the fry had lost most of their yolk sac. The mean weight of the fry is used to calculate the numbers of fry from weighed samples.

Fry will be reared in square, 150 L fiberglass tanks of the same design as that described by Loadman et al. (1989). The tank shape and inflow create a circular, upwelling water flow. Feed is swept along the bottom of the tank, up the front wall, across the surface toward the back and then down the outflow screen at the back. Inflow will be maintained at 2.5 L per minute (1.0 exchanges/h) before feeding, then at 5 L per hour (2.0 exchanges/h) from the commencement of feeding to the termination of the rearing interval. Although the flow does not maintain all of the food particles in suspension, eddy areas exist where food settling occurs but a sufficient portion of the feed is kept in suspension to obtain the recommended goal of 100 food particles per L (Li and Mathias 1982). The flow pattern also forces the fish to orient into the current, a factor which has said to reduce cannibalism (Loadman et al. 1989). The sidewalls are painted a flat black 152 mm down from the top of the tank, 102 mm below the water surface to deter the tendency of the fry to cling to shiny surfaces. The water temperature of the tanks when the fry are stocked will be 15ºC, then raised to 18ºC by the time the fry are 5-days posthatch when feeding is begun. Physical-chemical parameters of temperature, oxygen, oxygen partial pressures, total gas pressure, pH, alkalinity, ammonia, and nitrite are monitored (APHA et al. 1989) frequently and maintained within recommended limits for continuous exposure (Piper et al. 1982).

Fry will be fed "Fry Feed Kyowa," Series B (Kyowa Hakko Kogyo Company, Ltd., Japan). This feed has been used by many investigators who have reported walleye fry survival equal to or better than obtained using live feed (Loadman et al. 1989; Kindschi and MacConnell 1989). The Kyowa B-400, 250-400 µm, is used to initiating feeding as the size distribution of this feed is within the 0.3-0.4 µm suggested by Merna (1977) and McElman and Balon (1979) for first-feeding walleye, and close to the recommendation of 0.2-0.25 µm by Nickum (1978). After feeding begins, feed size is increased as walleye consume prey of increasing size. The B-700 (400-700 µm) feed is started on the 9th day posthatch at 25% of the daily ration, then increased by 25% each day until the 12th day posthatch when it is 100% of the daily ration. Feed will be dispensed every 3.0 minutes for 18 h/d. Vibrator feeders (Sweeney Enterpises, Boerne, Texas, Model AF-6) used previously to dispense the feed have been replaced because the amount of food these feeders dispense at each feeding is not accurate or consistent (Loadman et al. 1989; Kindschi and MacConnell 1989). An auger feeder will be used to dispense the feed by electronic time-clock. Feeding will begin when the fry are 5-days posthatch. A feeding ring, 7.1 cm inside diameter, which extends 2 cm into the water, will be used to contain the feed and to direct it into the water column. The feeding ring restricts the area of surface film produced by the feed (Barrows et al. 1988), and in our application of this ring, it also functions to input the feed into the circulating pattern of water of the tank below the surface, which prevents the surface flow from driving the feed into the screen.

During the rearing interval, waste feed and dead fish will be siphoned from the bottom of the tanks daily and counts of dead fish will be made to relate daily mortality to fish age and environmental conditions. Waste feed will be separated from the dead fish using a number 30 standard sieve with 600 µm openings (the sieve retains the fish but passes the waste feed). Survival over the fry rearing phase (first 21 days) will be determined from a final count of fish in the tank, hand counted if less than 1000, or estimated from the total weight and using the mean weight of individuals. At the termination of each group, a sample of 100 fish from each tank will be used to determine the occurrence of an inflated gas bladder. Gas bladder inflation will be observed under a dissecting microscope with transmitted light.

Although the basic and most widely accepted working hypothesis for non-inflation of the gas bladder is that of a surface tension phenomena prevents the fry from gupping air, no measurements of surface tension have been reported to actually relate surface tension to gas bladder inflation rates. A Du Nouy type fluid tensiometer for measuring static surface tension will be leased to measure surface tension from grab samples from the surface of the culture tank.

At Michigan State University, analysis of the H&E sections cut from fish collected in 1990 and 1991 will be completed and a characterization of the cytological lesions examined by electron microscopy. The full range of lesions occurring in the gas bladder epithelium can be fully described and quantified and the cytological changes in the epithelial nuclei can be evaluated by electron microscopy to determine potential for a viral etiology.

At the University of California-Davis, the focus will be to describe developmental patterns of the pneumatic duct, gas gland, rete mirabile, and gas bladder epithelium and to describe glycogen (energy) stores and carbon anhydrase activity. Samples of 20 larvae from the ISU larval culture facility will be fixed every 24 hours (10% neutral buffered formalin) until day 20 posthatch, then every 48 hours until day 28 posthatch. As soon as gas bladder inflation can be determined from macroscopic examination, 12 fish with and without inflated gas bladders will be fixed separately on each sampling day. All fish will be processed routinely in paraffin, and selected fish will be serially sectioned at 5 μm to determine details of development.

Because previous ISU studies (Summerfelt 1990a) have shown that walleye inflate their gas bladder between 4 and 12 days posthatch, sampling rates during this interval will be augmented. First, five fish each form the inflated and noninflated groups will be fixed in 1/2-strength Karnovsky's and selected fish will be processed for electron microscopy (EM) as described by Hinton and Poole (1976). The EM studies will be used to demonstrate osmiophilic granules (surfactant) in gas gland epithelial cells. Second, five fish each from the inflated and noninflated groups will be rapidly frozen in isopentane immersed in liquid nitrogen, shipped to Dr. Hinton on dry ice, freeze-dried to preserve maximum enzyme activity, and embedded in glycol methacrylate (GMA, Hinton et al. 1988). Serial sections will be cut at 2 to 4 μm and alternatively stained for morphological (H&E), glycogen (periodic acid-Schiff), and carbonic anhydrase (Lacy 1983).

The findings will be described in a completion report prepared collaboratively by all PIs; significant findings will be prepared for publication in appropriate peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

FACILITIES

Objective 1

Blood and tissue samples relevant to Objective 1 will be collected, preliminarily processed, and shipped or transported to the UW-Madison. All hormone and tissue analyses will be done by the UW Aquaculture Program, which has its main research facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. These facilities include an analytical laboratory that is well equipped for histological, cytological and endocrinological research. The UW Aquaculture Program also has additional analytical and wet laboratory facilities on the main UW-Madison campus, and has access to much of the laboratory facilities, equipment and instrumentation of the UW-Madison Endocrinology-Reproductive Physiology Program, Department of Poultry Science and Center for Limnology.

Continued participation in Objective 1 by Dr. T.B. Kayes (now) of the UNL will be accomplished through close communications and working visits to the UW-Madison (and SIU and the UM, as needed). Development of aquaculture research facilities at the UNL will be initiated in 1990-91. In addition, UNL researchers will have access to the Nebraska Game and Park Commission's new Calamus State Fish Hatchery, which is scheduled for completion in 1991. This facility with its 50-plus rearing ponds, outdoor raceways, 900-plus m^2 of floor space equipped for indoor rearing, three separate water sources (a river reservoir and two different aquifers), water-temperature control and pure oxygen aeration systems should provide ample opportunities for future research, pilot-scale studies and demonstration projects on the culture technology for walleye.

The Fisheries Research Laboratory on the SIU campus has several 4.3-plus m electrofishing and net boats available for collecting broodfish. The Laboratory also has a number of gill nets, trap nets and other collection gears suitable for capturing walleye. Three pickup truck hauling tanks are available for transport. These are equipped with surface agitator/aerators and compressed oxygen diffusers. Two of the hauling tanks are insulated. Four pickup trucks are operated by the Laboratory which can be used to transport boats, collection gear, and hauling tanks. Equipment, boats, vehicles and facilities at the Laboratory's two satellite research stations in northern and central Illinois parallel those of the SIU Campus laboratory. The two field stations, the campus facilities, and a good working relationship with the Illinois Department of Conservation permit ready access to walleye broodfish populations anywhere in the state.

Two 0.6 hectare ponds on the SIU campus will be used to hold walleye broodfish. A third, larger pond will also be used if broodfish are captured in numbers sufficient to warrant its use. A number of other ponds will be used to provide supplemental forage fish for the walleye if necessary. However, threadfin shad and rosy red minnow broodfish will be

stocked into the walleye ponds, and we believe natural reproduction will provide enough forage. Forage fish populations and broodfish condition will be routinely monitored in both ponds to ascertain whether additional forage organisms are needed.

Objective 2

The field component of this research will be done at the U.S. Fish and Wildlife Service, Garrison Dam National Fish Hatchery, Riverdale, North Dakota. Hatchery personnel will be in charge of pond stocking, fertilization and fish harvest. University personnel, a graduate student and assistant, will monitor water quality and carryout sampling for zooplankton, benthos, and clam shrimp. The hatchery will make available a trailer for housing university personnel and space for laboratory work at the hatchery. Campus facilities at Iowa State University will be used to make estimate of clam shrimp abundance from sediment samples. Facilities on campus include lab space; dissecting and compound microscopes; plankton splitter and counting cells to enumerate zooplankton; and computer facilities for data analysis.

Objective 3

Fry Culture Facilities

The Iowa State University fry culture facilities consist of 12, 150 L fiberglass tanks of a design that provides an upwelling water flow to maintain a high food particle density (Loadman et al. 1989). Each tank is equipped with individual flowmeter to control exchange rate, an auger feeder, and light intensity controller. Temperature, oxygen, and pH are controllable to provide options for experimental evaluations. Water quality is monitored for temperature, pH, dissolved oxygen, gas pressures, ammonia, nitrite, alkalinity, and residual chlorine. Many other analyses can be conducted to meet special needs or sample analysis carried out in the laboratory of the Engineering Research Institute.

Histopathology--Michigan State University

The College of Veterinary Medicine, Michigan State University has extensive facilities for preparation of tissues for histopathology, as well as research-grade microscopes and apparatus for photomicrography. The histopathology studies will be conducted at facilities of the Animal Health Diagnostic Laboratory of Michigan State University. The equipment includes microtomes, stainers, blade sharpeners, heaters, microscopes, photomicroscopes and photocopiers.

Histopathology--University of California-Davis

Dr. Hinton has in his laboratories research microscopes with photomicrography and phase (Nomarski) capabilities; a Pease tissue freeze drier; FTS tissue-dry; 2-meter hood; pH meter; balances; computer-assisted morphometry workstation; LKB historange microtone; two ultramicrotomes. In the same building, a Phillips 410 transmission electron microscope, and completely equipped dark rooms are available on a time-share basis. These facilities are staffed by a full-time technologist.

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Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada 184, Ottawa.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
California	David E. Hinton	Fish anatomy
	University of California	Toxicologic pathology
Illinois	Robert J. Sheehan	Aquaculture/environmental
	Southern Illinois University	Physiology of fishes
	Bruce L. Tetzlaff	Finfish aquaculture
	Southern Illinois University	
Iowa	Robert C. Summerfelt	Finfish aquaculture: General,
	Iowa State University	larviculture, and fish health
Minnesota	Anne R. Kapuscinski	Aquaculture
	University of Minnesota	Quantitative genetics
Michigan	Thomas G. Bell	Veterinary pathology
	Michigan State University	
	Allan L. Trapp	Veterinary pathology
	Michigan State University	
Wisconsin	Jeffrey A. Malison	Aquaculture
	Univ. of Wisconsin-Madison	Physiology/endocrinology

ATTACHMENT A

INDIVIDUAL BUDGETS FOR PARTICIPATING INSTITUTIONS

Illinois

Southern Illinois University
Robert J. Sheehan
Bruce L. Tetzlaff

Iowa (includes subcontract to University of California)

Iowa State University
Robert C. Summerfelt

University of California-Davis
David E. Hinton

Michigan

Michigan State University
Thomas G. Bell
Allan L. Trapp

Minnesota

University of Minnesota
Anne R. Kapuscinski

Wisconsin

University of Wisconsin-Madison
Jeffrey A. Malison

PROPOSED WALLEYE BUDGET SHEET FOR SOUTHERN ILLINOIS UNIVERSITY

(Sheehan and Tetzlaff)

Objective 1. Mechanisms Regulating the Natural Reproductive Cycle

		No.	FTEs	Year 1
A.	Salaries and Wages			
	1. No. of Senior Personnel & FTEs[1]			
	a. (Co)-PI(s)	1	0.10	$0
	b. Senior Associates	0	0	$0
	2. No. of Other Personnel (Non-Faculty) & FTEs			
	a. Research Assoc./Postdoc			$0
	b. Other Professionals			$0
	c. Graduate Students			$10,850
	d. Prebaccalaureate Students			$800
	e. Secretarial-Clerical			$0
	f. Technical, Shop, and Other			$0
	Total Salaries and Wages			$11,650
B.	Fringe Benefits			$0
C.	**Total Salaries, Wages and Fringe Benefits**			$11,650
D.	Nonexpendable Equipment			$0
E.	Materials and Supplies			$2,300
F.	Travel - Domestic (*Including Canada*)			$1,000
G.	Other Direct Costs			$2,000
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$16,950

[1]FTEs = Full Time Equivalents based on 12 months.

PROPOSED WALLEYE BUDGET SHEET FOR
IOWA STATE UNIVERSITY

(Summerfelt)

Objective 2. Zooplankton seeding and clam shrimp control

		No.	FTEs	Year 1
A.	Salaries and Wages			
	1. No. of Senior Personnel & FTEs[1]			
	a. (Co)-PI(s)	1	0.10	$0
	b. Senior Associates	0	0	$0
	2. No. of Other Personnel (Non-Faculty) & FTEs			
	a. Research Assoc./Postdoc			$0
	b. Other Professionals			$0
	c. Graduate Students	1	0.50	$12,400
	d. Prebaccalaureate Students	1	0.25	$3,200
	e. Secretarial-Clerical			$0
	f. Technical, Shop, and Other			$0
	Total Salaries and Wages			$15,600
B.	Fringe Benefits			$650
C.	**Total Salaries, Wages and Fringe Benefits**			$16,250
D.	Nonexpendable Equipment			$3,000
E.	Materials and Supplies			$2,000
F.	Travel - Domestic (*Including Canada*)			$2,000
G.	Other Direct Costs			$300
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$23,550

[1]FTEs = Full Time Equivalents based on 12 months.

PROPOSED WALLEYE BUDGET SHEET FOR
IOWA STATE UNIVERSITY

(Summerfelt)

Objective 3. Etiology of Non-Inflation of the Gas Bladder

		No.	FTEs	Year 1
A.	Salaries and Wages			
	1. No. of Senior Personnel & FTEs[1]			
	a. (Co)-PI(s)	1	0.10	$0
	b. Senior Associates	0	0	$0
	2. No. of Other Personnel (Non-Faculty) & FTEs			
	a. Research Assoc./Postdoc			$0
	b. Other Professionals			$0
	c. Graduate Students	1	0.25	$6,200
	d. Prebaccalaureate Students	1	0.25	$3,200
	e. Secretarial-Clerical			$0
	f. Technical, Shop, and Other			$0
	Total Salaries and Wages			$9,400
B.	Fringe Benefits			$500
C.	**Total Salaries, Wages and Fringe Benefits**			$9,900
D.	Nonexpendable Equipment			$0
E.	Materials and Supplies			$2,300
F.	Travel - Domestic (*Including Canada*)			$1,100
G.	Other Direct Costs			$11,482
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$24,782

[1]FTEs = Full Time Equivalents based on 12 months.

PROPOSED WALLEYE BUDGET SHEET FOR MICHIGAN STATE UNIVERSITY

(Bell and Trapp)

Objective 3. Etiology of Non-Inflation of the Gas Bladder

		No.	FTEs	Year 1
A.	Salaries and Wages			
	1. No. of Senior Personnel & FTEs[1]			
	a. (Co)-PI(s)	2	0.10	$0
	b. Senior Associates	0	0	$0
	2. No. of Other Personnel (Non-Faculty) & FTEs			
	a. Research Assoc./Postdoc			$0
	b. Other Professionals			$0
	c. Graduate Students	1	0.25	$5,217
	d. Prebaccalaureate Students	1	0.25	$0
	e. Secretarial-Clerical			$0
	f. Technical, Shop, and Other			$0
	Total Salaries and Wages			$5,217
B.	Fringe Benefits			$0
C.	**Total Salaries, Wages and Fringe Benefits**			$5,217
D.	Nonexpendable Equipment			$0
E.	Materials and Supplies			$640
F.	Travel - Domestic (*Including Canada*)			$730
G.	Other Direct Costs			$857
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$7,444

[1]FTEs = Full Time Equivalents based on 12 months.

PROPOSED WALLEYE BUDGET SHEET FOR UNIVERSITY OF MINNESOTA

(Kapuscinki)

Objective 1. Mechanisms Regulating the Natural Reproductive Cycle

		No.	FTEs	Year 1
A.	Salaries and Wages			
	1. No. of Senior Personnel & FTEs[1]			
	a. (Co)-PI(s)	1	0.02	$0
	b. Senior Associates	0	0	$0
	2. No. of Other Personnel (Non-Faculty) & FTEs			
	a. Research Assoc./Postdoc			$0
	b. Other Professionals			$0
	c. Graduate Students			$0
	d. Prebaccalaureate Students			$0
	e. Secretarial-Clerical			$0
	f. Technical, Shop, and Other	1	0.19	$4,142
	Total Salaries and Wages			$4,142
B.	Fringe Benefits (26% of 2f)			$1,077
C.	**Total Salaries, Wages and Fringe Benefits**			$5,219
D.	Nonexpendable Equipment			$0
E.	Materials and Supplies			$400
F.	Travel - Domestic (*Including Canada*)			$1,880
G.	Other Direct Costs			$500
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$7,999

[1]FTEs = Full Time Equivalents based on 12 months.

PROPOSED WALLEYE BUDGET SHEET FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison)

Objective 1. Mechanisms Regulating the Natural Reproductive Cycle

		No.	FTEs	Year 1
A.	Salaries and Wages			
1.	No. of Senior Personnel & FTEs[1]			
a.	(Co)-PI(s)	1	0.07	$0
b.	Senior Associates	1	0.07	$0
2.	No. of Other Personnel (Non-Faculty) & FTEs			
a.	Research Assoc./Postdoc			$0
b.	Other Professionals	1	0.50	$12,700
c.	Graduate Students			$0
d.	Prebaccalaureate Students	1	0.25	$3,150
e.	Secretarial-Clerical			$0
f.	Technical, Shop, and Other	1	0.19	$0
	Total Salaries and Wages			$15,850
B.	Fringe Benefits (24% of 2b)			$3,048
C.	**Total Salaries, Wages and Fringe Benefits**			$18,898
D.	Nonexpendable Equipment			$0
E.	Materials and Supplies			$5,000
F.	Travel - Domestic (*Including Canada*)			$1,800
G.	Other Direct Costs			$2,800
TOTAL PROJECT COSTS PER YEAR (*C through G*)				$28,498

[1]FTEs = Full Time Equivalents based on 12 months.

CULTURAL TECHNOLOGY OF WALLEYE

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION AT 109.2K FOR THE THIRD YEAR

	SIU	ISU[a]	MSU	U MINN.	UNL	UW MAD.	TOTALS
Salaries and Wages	$11,650	$25,000	$5,217	$4,142	$0	$15,850	$61,859
Fringe Benefits	$0	$1,150	$0	$1,077	$0	$3,048	$5,275
Total Salaries, Wages and Benefits	$11,650	$26,150	$5,217	$5,219	$0	$18,898	$67,134
Nonexpendable Equipment	$0	$3,000	$0	$0	$0	$0	$3,000
Materials and Supplies	$2,300	$4,300	$640	$400	$0	$5,000	$12,640
Travel	$1,000	$3,100	$730	$1,880	$0	$1,800	$8430
Other Direct Costs	$2,000	$11,782[b]	$857	$500	$0	$2,800	$17,939
TOTAL PROJECT COSTS	$16,950	$48,332	$7,444	$7999	$0	$28,498	$109,223

[a] The ISU budget combines objectives 2 and 3.

[b] $9,982 of this amount is a subcontract to the University of California-Davis for collaboration on objective 3.

ATTACHMENT A

RESOURCE COMMITMENT FROM INSTITUTIONS

(Salaries, Supplies, Expenses and Equipment)

Institution/Item	Year 1
University of California-Davis	
Salaries and Benefits:	
PI @ SY @ 0.05 FTE	$3,150
Technician @ SY @ 0.10 FTE	$3,685
TOTAL PER YEAR	**$6,835**
Southern Illinois University	
Salaries and Benefits:	
SY @ 0.10 FTE	$3,624
TY @ 0.10 FTE	$800
Supplies, Expenses and Equipment	$18,100
TOTAL PER YEAR	$22,524
Iowa State University	
Salaries and Benefits:	
SY @ 0.10 FTE	$9,497
Supplies, Expenses and Equipment	$0
TOTAL PER YEAR	$9,497
Michigan State University	
Salaries and Benefits:	
SY @ 0.10 FTE	$10,440
Supplies, Expenses and Equipment	$0
TOTAL PER YEAR	$10,440

ATTACHMENT A

RESOURCE COMMITMENT FROM INSTITUTIONS

(Salaries, Supplies, Expenses and Equipment)

Institution/Item	Year 1
University of Minnesota	
Salaries and Benefits:	
SY @ 0.03 FTE	$1,378
TY @ 0.03 FTE	$763
Supplies, Expenses and Equipment	$1,700
TOTAL PER YEAR	$3,841
University of Wisconsin-Madison	
Salaries and Benefits:	
SY @ 0.14 FTE	$4,948
TY @ 0.04 FTE	$1,300
Supplies, Expenses and Equipment	$14,857
TOTAL PER YEAR	$20,105
GRAND TOTAL, ALL INSTITUTIONS	$73,242

ATTACHMENT A

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: To be completed in Year 1.

Objective 2: To be completed in Year 1.

Objective 3: To be completed in Year 1.

VITAE LIST OF PRINCIPAL INVESTIGATORS

Thomas G. Bell, Michigan State University

David E. Hinton, University of California-Davis

Anne R. Kapuscinski, University of Minnesota

Terrence B. Kayes, University of Nebraska-Lincoln

Jeffrey A. Malison, University of Wisconsin-Madison

Robert J. Sheehan, Southern Illinois University

Robert C. Summerfelt, Iowa State University

Bruce L. Tetzlaff, Southern Illinois University

Allan L. Trapp, Michigan State University

ATTACHMENT A

VITA

Thomas G. Bell
Professor of Pathology
Project Leader
College of Veterinary Medicine
Michigan State University
East Lansing, MI 48824-1316
Phone: (517) 353-5275

EDUCATION

B.A.	University of Arizona, 1964
D.V.M.	Washington State University, 1969
Internship	University of Minnesota, 1970
Ph.D.	Washington State University, 1976

POSITIONS

Professor, Michigan State University, 1986-present.
Associate Professor, Michigan State University, 1980-1986.
Assistant Professor, Michigan State University, 1976-1980.
Assistant Professor, Washington State University, 1971-1974.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Sigma Xi, National Research Honorary, Associate Member, 1976.
Phi Zeta, National Veterinary Honorary, Member, 1981.
American Association of Veterinary Laboratory Diagnosticians, 1985.
American Association of Pathologist, Member, 1985.

SELECTED PUBLICATIONS

Bell, T. G., A. L. Trapp, J. Machado, D. L. Garling. A method for rapid fixation for preservation of tissue emphysema: Diagnosis of gas bubble disease in hatchery reared rainbow trout. Proceedings of the American Association of Veterinary Laboratory Diagnosis. 28th Annual Proceedings, 81-88, 1985.

Bell, T. G., W. L. Smith, W. D. Oxender. 1986. Biologic interaction of prostaglandin, thromboxane and prostacyclin. In: Advances in Human Nutrition, Vol. 3, Kabara, J. J. (ed.), Pathrox Publication, Chicago.

Machado, J. P., D. L. Garling, Jr., N. R. Kevern, A. L. Trapp, T. G. Bell. 1987. Histopathology of the pathogenesis of embolism (gas bubble disease) in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences. 44(11): 1985-1994.

Machado, J. P., T. G. Bell, D. L. Garling Jr., N. R. Kevern, A. L. Trapp. 1988. Effect of carbon monoxide exposure on gas bubble trauma in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences. In Press.

Schultze, A. E., I Sonea, T. G. Bell. 1988. Primary malignant pulmonary neoplasia in two horses. Journal American Veterinary Medical Association. 193(4):477-480.

VITA

David E. Hinton
Professor
Department of Medicine
School of Veterinary Medicine
University of California
Davis, California 95616

Phone: 916-752-1383

EDUCATION

Ph.D.	Anatomy/Pathology, University of Mississippi, 1969
M.S.	Anatomy/Biomedical Sciences, University of Mississippi, 1967
B.S.	Zoology/chemistry, Mississippi College, 1965

POSITIONS

Professor, Department of Medicine, School of Veterinary Medicine, University of California, David, 1986-present.

Associate Professor through Professor, Department of Anatomy and Pathology, West Virginia University, Medical Center, Morgantown, WV, 1977-1986.

Instructor through Assistant Professor, Department of Pathology, University of Maryland, School of Medicine, Baltimore, MD, 1974-1976.

Research Associate, Department of Pathology, University of Maryland, School of Medicine, Baltimore, MD, 1973-1974.

Instructor through Assistant Professor, Department of Anatomy, University of Louisville, School of Medicine, Louisville, KY, 1969-1973.

ELECTED PUBLICATIONS

Hinton, D. E., and D. J. Lauren. Liver structural alternations accompanying chronic toxicity in fishes: Potential biomarkers of exposure. Pages 17-57 *in* J. McCarthy and L. R. Shugart, editors. Biological markers of environmental contamination. Lewis Publishing Inc., Chelsea, Michigan.

Marty, G. D., J. J. Cech, Jr., and D. E. Hinton. 1990. Effect of incubation temperature on oxygen consumption and ammonia production by Japanese medaka, *Oryzias latipes*, eggs and newly hatched larvae. Environmental Toxicology and Chemistry 9:1399-1405.

Lauren, D.J., P. P. Halarnkar, B. D. Hammock, and D. E. Hinton. 1989. Microsomal and cytosolic epoxide hydrolase, and glutathione S-transferase activities in the gill, liver and kidney of the rainbow trout, Salmo gairdneri: Baseline levels and optimization of assay conditions. Biochemical Pharmacology 38:881-887.

Hampton, J. A., R. C. Lantz, and D. E. Hinton. 1989. Functional units in rainbow trout (*Salmo gairdneri* Richardson) liver: III. Morphometrical analysis of parenchyma, stroma, and component cell types. American Journal of Anatomy 185:58-73.

Miller, M. R., D. E. Hinton, and J. J. Stegeman. 1989. Cytochrome P-450E induction and localization in gill pillar (endothelial) cells of scup and rainbow trout. Aquatic Toxicology 14:307-322.

Hinton, D. E., J. A. Couch, S. J. Teh, and L. A. Courtney. 1988. Cytological changes during progression of neoplasia in selected fish species. Aquatic Toxicology 11:77-112.

Hampton, J. A., R. C. Latnz, and D. E. Hinton. 1988. Functional units in rainbow trout (*Salmo gairdneri* Richardson) liver. II. The biliary system. Anatomical Record 221:619-634.

VITA

Anne R. D. Kapuscinski
Associate Professor
Dept. of Fisheries and Wildlife
130 Hodson Hall
University of Minnesota, St. Paul, MN 55108
Phone: 612-624-3019

EDUCATION

B.A.	Biology, Swarthmore College, 1976
M.S.	Fisheries, Oregon State University, 1980
Ph.D.	Fisheries, Oregon State University, 1984

POSITIONS

Associate Professor/Extension Specialist (Aquaculture), University of Minnesota, 1989 - present.
Assistant Professor/Extension Specialist (Aquaculture), University of Minnesota, 1984 - 1989.
Instructor/Project Leader/Research Assistant Oregon State University, 1980-1984.
Research Assistant, Oregon State University, 1977-1980.
Aquaculture Research Technician, Weyerhaeuser Company, 1976-77.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture Section, Genetics Section, NCD Fish Genetics Technical Committee
Genetics Society of America
International Association of Genetics in Aquaculture (Charter Member)
Society for the Study of Evolution
World Aquaculture Society
Sigma Xi, Phi Kappa Phi, Phi Sigma, Gamma Sigma Delta

SELECTED PUBLICATIONS

Kapuscinski, A. R. D., and J. E. Lannan. 1986. A conceptual genetic fitness model for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences. 43:1606-1616.

Lannan, J. E., and A. R. D. Kapuscinski. 1986. Application of a genetic fitness model to extensive aquaculture. Aquaculture 57:81-87.

Kapuscinski, A. R., and L. D. Jacobson. 1987. Genetic guidelines for fisheries management. Minnesota Sea Grant, St. Paul, MN.

Hallerman, E. M., J. F. Schneider, M. L. Gross, A. J. Faras, P. B. Hackett, K. S. Guise, and A. R. Kapuscinski. 1989. Enzymatic dechorionation of goldfish, walleye and northern pike eggs. Transactions of the American Fisheries Society 117:456-460.

Kapuscinski, A. R. 1990. Integration of Transgenic Fish into Aquaculture. Food Reviews International 6(3):373-378. (Invited Paper).

Yoon, S. J., E. M. Hallerman, M. L. Gross, Z. Liu, J. F. Schneider, A. J.Faras., P. B. Hackett, A. R. Kapuscinski, and K. S. Guise. 1990. Transfer of the gene for neomycin resistance into goldfish, *Carrassius auratus*. Aquaculture 85:21-33.

Kapuscinski, A. R., and E. M. Hallerman. 1990. Transgenic fish and public policy: I. Anticipating environmental impacts of transgenic fish. Fisheries 15(1):2-11.

VITA

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Department of Forestry, Fisheries and Wildlife
12 Plant Industry Building
Eat Campus Mall
University of Nebraska-Lincoln
Lincoln, NE 68583-0814
Phone: (402) 472-8183

EDUCATION

B.A.	Biological Sciences, Chico State College, 1968
M.A.	Biological Sciences, California State University at Chico, 1972
Ph.D.	Zoology Biochemistry, University of Wisconsin-Madison, 1978

POSITIONS

Associate Professor, Department of Forestry, Fisheries and Wildlife, University of Nebraska-Lincoln, 1990-present.
Assistant Director and Associate Scientist, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison, 1979-1990.
Project Biologist, Aquaculture Research Laboratory, University of Wisconsin-Madison, 1974-1979.
Teaching Assistant, Department of Zoology, University of Wisconsin-Madison, 1972-1974.
EPA Trainee, Laboratory of Limnology, University of Wisconsin-Madison, 1970-72.
Instructor, Department of Biological Sciences, Chico State College, 1968-70.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Society of Zoologists: Divisions of Comparative Endocrinology, Comparative Physiology and Biochemistry, Ecology and Comparative Immunology.
American Fisheries Society: Fish Culture, Bioengineering, Fish Health, Water Quality and Early Life History Sections.
World Aquaculture Society.

SELECTED PUBLICATIONS

Malison, J.A., T.B. Kayes, J.A. Held, and C.H. Amundson. 1990. Comparative survival, growth and reproductive development of juvenile walleye (*Stizostedion vitreum*), sauger (*S. canadense*) and their hybrids reared under intensive culture conditions. Progressive Fish-Culturist 52:73-82.

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Heidinger, R. C., and T. B. Kayes. 1986. Yellow perch. Pages 103-113 *in* R. R. Stickney, editor. Culture of nonsalmonid freshwater fishes. CRC Press, Boca Raton, Florida.

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EDUCATION

B.S.	Biology, University of Wisconsin-Stevens Point, 1976
M.S.	Endocrinology-Reproductive Physiology, University of Wisconsin-Madison, 1980
Ph.D.	Endocrinology-Reproductive Physiology, University of Wisconsin-Madison, 1985

POSITIONS

Assistant Director, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison, 1990-present.
Associate Researcher, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison, 1987-1990.
Project Associate, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison, 1985-1987.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Sciences
American Fisheries Society
American Society of Zoologists
World Aquaculture Society

SELECTED PUBLICATIONS

Malison, J.A., T.B. Kayes, J.A. Held, and C.H. Amundson. 1990. Comparative survival, growth and reproductive development of juvenile walleye (*Stizostedionvitreum*), sauger (*S. canadense*) and their hybrids reared under intensive culture conditions. Progressive Fish-Culturist 52:73-82.

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Malison, J. A. 1985. Growth promotion and the influence of sex-steroids on sexually related dimorphic growth and differentiation in yellow perch (*Perca flavescens*). Ph.D. Thesis, University of Wisconsin-Madison, Madison, WI.

Malison, J.A., C. D. Best, and T. B. Kayes. 1983. Hormonal control of growth and size dimorphism in yellow perch (*Perca flavescens*). American Zoologist 22:955.

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ATTACHMENT A

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EDUCATION

B.S.	Biology, Northeastern Illinois University
M.A.	Zoology, Fisheries Specialty, Southern Illinois University
Ph.D.	Zoology, Fisheries Specialty, Southern Illinois University

POSITIONS

Assistant Professor, Department of Zoology, Southern Illinois University-Carbondale 1986-present.
Assistant Professor, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University. 1985-1986.
Researcher, Fisheries Research Laboratory, Southern Illinois University-Carbondale. 1981-1983.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society:
Fish Culture Section, Water Quality Section, Early Life History Section,
Fisheries Educators Section, and Exotic Fishes Section
Illinois Chapter

SELECTED PUBLICATIONS

Nielsen, L. A., R. J. Sheehan, and D. J. Orth. 1987. Impacts of navigation on riverine fish production in the United States. Proceedings of the International symposium on Fish Production in Rivers. Polish Archives of Hydrobiology 33(3/4):277-294.

Sheehan, R. J. and W. M. Lewis. 1986. Relationships between the toxicity of aqueous ammonia solutions, pH, ammonia salt formulations, and water balance in channel catfish fingerlings. Transactions of the American Fisheries Society. 115:891-899.

Helfrich, L. A., R. J. Sheehan, and J. S. Odenkirk. 1986. Fishing for sale: fee-fishing opportunities in Virginia. Virginia cooperative Extensive Service Publication 420-898.

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Lewis, W. M. and R. J. Sheehan. 1977. Channel catfish culture: state of the art 1976. Proceedings, Southeastern Conference of Game and Fish Commissioners 31 (1976):234-238.

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EDUCATION

B.S.	University of Wisconsin-Stevens Point, Biology, 1957
M.S.	Southern Illinois University, Zoology, 1959
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Ph.D.	Southern Illinois University, Zoology, 1964

POSITIONS

Professor, Dept. of Animal Ecology, Iowa State University, 1990-Present.
Professor, Dept. of Animal Ecology, and Associate Director of the North Central Regional Aquaculture Center, Iowa State University, 1988-1990.
Professor, Department of Animal Ecology, Iowa State University, 1985-88.
Professor and Chairman, Department of Animal Ecology, Iowa State University, 1976-85.
Leader (Fishery Research Biologist, U.S. Fish and Wildlife Service, GS-13), Oklahoma Cooperative Fishery Research Unit, Oklahoma State University, 1966-76.
Assistant Professor, Department of Zoology, Kansas State University, 1964-66.
Lecturer, Department of Zoology, Southern Illinois University, Carbondale, 1962-64.
Visiting Professor: Utah State Univ. (1983), Oregon Inst. of Marine Biology (1975), and Southern Illinois Univ. (1965).

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society:
Fish Culture, Fish Health (Charter member), Education (Charter member), Bioengineering, Computer User, Fisheries Management Sections (Iowa Chapter).
American Institute of Fishery Research Biologists (Fellow)
Fisheries Society of the British Isles
Iowa Academy of Sciences
North American Lake Management Society
Societas Internationalis Limnologiae
World Aquaculture Society
Honorary: Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta

SELECTED PUBLICATIONS

Siegwarth, G. L., and R. C. Summerfelt. 1990. Growth comparison between fingerling walleyes and walleye x sauger hybrids reared in intensive culture. Progressive Fish-Culturist 52:100-104.

Marty, G. D., and R. C. Summerfelt. 1990. Wound healing in channel catfish by epithelization and contraction of granulation tissue. Transactions of the American Fisheries Society 119:145-150.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia and surgery. Chapter 8 *in* C. B. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.
Summerfelt, R. C., and G. E. Hall, editors. 1987. Age and growth of fish. Iowa State University Press, Ames, Iowa.

Summerfelt, R. C. 1981. Practice and prospects of fish farming for food production. Pages 81-120 *in* D. C. Beitz, editor. Proceedings of the International Symposium on Animal Products in Human Nutrition. Nutrition Foundation Monograph Series, Academic Press, New York.

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EDUCATION

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M.A. Zoology, Southern Illinois University, Carbondale, Illinois

POSITIONS

Research Project Director, Fisheries Research Laboratory, Southern Illinois University, Carbondale, Illinois, 1980-present.
Researcher, Fisheries Research Laboratory, Southern Illinois University, 1976-1980.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society:
Fish Culture Section, Bioengineering Section, and Fish Management Section.

SELECTED PUBLICATIONS

Heidinger, R. C., J. H. Waddell, and B. L. Tetzlaff. 1985. Relative survival of walleye fry versus fingerlings in two Illinois reservoirs. Proceedings of the Annual Conference Southeast Association of Fisheries and Wildlife Agencies 39:306-311.

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Heidinger, R. C., L. J. Wawronwicz, and B. L. Tetzlaff. 1983. Applications of water reuse technology for overwintering threadfin shad (*Dorosoma petenense*) at northern latitudes. Aquaculture Engineering 2:153-162.

Lewis, W. M., R. C. Heidinger, and B. L. Tetzlaff. 1981. Tank culture of striped bass production manual. Southern Illinois University, Fisheries Research Laboratory, Carbondale, IL.

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EDUCATION

B.S.	Michigan State University, 1954
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Ph.D.	Iowa State University, 1960

POSITIONS

Professor, Michigan State University, 1970-present.
Associate Professor, Michigan State University, 1960-1970.
Associate Professor, Ohio Research and Development Center, 1965-1966.
Assistant Professor, Ohio Research and Development Center, 1960-1965.
Research Associate, Iowa State University, 1957-1960.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Wildlife Disease Association
American Veterinary Medical Association
American Associate of Veterinary Laboratory Diagnosticians
Phi Zeta
Sigma Xi

SELECTED PUBLICATIONS

Bartkiewicz, S. E., D. E. Ullrey, A. L. Trapp and P. K. Ku. 1982. A preliminary study of niacin needs of the bull snake (*Pituophis malanoleucus saqi)*. Journal of Zoo Animal Medicine. 13:55-58.

Bell, T. G., A. L. Trapp, J. Machado, D. L. Garling. A method of rapid fixation for preservation of tissue emphysema: Diagnosis of gas bubble disease in hatchery reared rainbow trout. Proceedings of the American Association Veterinary Laboratory Diagnosis: 28th Annual Proceedings, 81-88, 1985.

Lass, R. E., D. E., Ullrey and A. L. Trapp. 1982. A study of calcium requirements of the red-eared slider turtle (*Pseudemys scripta elegans*). Journal of Zoo Animal Medicine. 3:62-65.

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