TARGET ANIMAL SAFETY AND DOSAGE EFFICACY STUDIES FOR WALLEYE FED 17α-METHYLTESTOSTERONE

Chairperson: Christopher C. Kohler, Southern Illinois University-Carbondale

Industry Advisory Council Liaison: Rosalie Schnick, National Aquaculture NADA Coordinator

Extension Liaison: Joseph E. Morris, Iowa State University

Funding Request: $27,000

Duration: 1 Year (July 1, 1996 - June 30, 1997)

Objectives:

1. To conduct a Target Animal Safety Study under Good Laboratory Practice (GLP) compliance for walleye fingerlings fed 17α-methyltestosterone.

2. To evaluate selected dosages of 17α-methyltestosterone for efficacy at inducing sex inversion in walleye.

Proposed Budgets:

Institution	Principal Investigator	Objective	Year 1	Total
Southern Illinois University-Carbondale	Christopher C. Kohler	1	$25,000	$25,000
University of Wisconsin-Madison	Jeffrey A. Malison	2	$2,000	$2,000
	TOTALS		$27,000	$27,000

TABLE OF CONTENTS

JUSTIFICATION

Sexual differentiation in teleosts is diverse and labile (Francis 1992) allowing for endocrine sex inversion in many species (Pandian and Sheela 1995). Hormonal induction of sex inversion is a valuable tool to not only understand sex differentiation, but also to produce monosex populations for the aquaculture industry (Guerrero 1975; Hunter and Donaldson 1983; Shelton 1986; Pandian and Sheela 1995). Treatment protocols for 47 species using one of 31 natural or synthetic steroids are reviewed by Pandian and Sheela (1995).

In most teleosts, males grow faster than females or are more colorful in the case of ornamental fishes. Accordingly, protocols for masculinization are more numerous than for feminization. However, in species such as walleye, *Stizostedion vitreum*, and yellow perch, *Perca flavescens*, females grow faster than males (Scott and Crossman 1973; Schott 1980). In a recently completed (unpublished) study, Malison and Held found that intensively reared female walleye begin to outgrow males at a size of 250 mm total length (TL), far before they reach a marketable size. In yellow perch, this sexually related dimorphic growth pattern becomes apparent in even smaller fish, i.e., when yellow perch reach 100 mm TL (Malison et al. 1986). Masculinization protocols still have utility in producing all-female populations in that sex-reversed homogametic females crossed with normal homogametic females result in all-female offspring. The procedure of using such sex-reversed brood stock offers the additional advantage of not having to treat with hormones fish destined for human consumption.

Among androgens, 17α-methyltestosterone (MT) is the most widely used hormone for sex inversion and has been tested on more than 25 species belonging to Salmonidae, Cichlidae, Cyprinidae, Anbantidae, Poecilidae, Cyprinodontidae (Pandian and Sheela 1995) and Percidae (Malison et al. 1986; Malison and Held Manuscript). It is most often provided to fish in the diet (Hunter and Donaldson 1983) either by homogeneous mixing of the steroid with ingredients (Yamamoto 1953) or more often by the alcohol evaporation method (Guerrero 1975).

Protocols to sexually reverse yellow perch and walleye with MT are described by Malison et al. (1986) and Malison and Held (Manuscript), respectively. A dosage of 1.5 mg/kg was >90% effective in yellow perch, whereas the only dosage listed for walleye was 15 mg/kg, and this dosage was ~50% effective. Both percids were shown to be homogametic in that offspring were 100% female when sperm collected from (genetic) females treated with MT was used to fertilized normal eggs.

Walleye and yellow perch are highly valued food fishes in the United States and Canada (Becker 1983), and have been designated as priority species by the Industry Advisory Council of the North Central Regional Aquaculture Center. The use of monosex female populations could significantly benefit the aquaculture potential of these species. In the case of walleye, public stocking programs for recreational fishing may also benefit in that only females have trophy-size potential.

Because female walleye and yellow perch are homogametic and males are heterogametic (XY), monosex female populations could be produced as follows: (1) direct estrogen treatment of juveniles; (2) gynogenesis; or (3) indirect use of hormones in which juveniles are treated with androgens to induce phenotypic sex inversion of genetic females and the sperm from these masculinized females are then used to fertilize normal eggs (Malison and Garcia-Abiado In press). The latter technique is preferable because it is inherently 100% effective and does not require that fish destined for human consumption be treated with hormones. In addition to the previously cited percid studies, this technique has been applied to various commercially important species, including grass carp, *Ctenopharyngodon idella* (Boney et al. 1984), chinook salmon, *Oncorhyncus tshawytscha* (Hunter et al. 1983), and Nile tilapia, *Oreochromis niloticus* (Mair et al. 1991).

Congress has charged the U.S. Food and Drug Administration (FDA) with the control of the use of drugs. Although extensive testing is required for both food fishes and for nonfood fishes, additional data on metabolites, residues, and residue persistence must be submitted for the establishment of a minimum tolerance for chemicals applied to food fish (Schnick et al. 1989). The Food, Drug, and Cosmetic Act and corresponding regulations (21 CFR 511) do not permit the use of an unapproved new animal drug unless an exemption, i.e., an Investigational New Animal Drug (INAD) exemption, has been granted. Currently, Auburn University holds an INAD for MT for sex reversing tilapia. The INAD is associated with the generation of data to support approval of a New Animal Drug Application (NADA). A data base, apparently sufficient for a NADA on MT for tilapia, is currently in the final stages of promulgation (R. Schnick, National Aquaculture NADA Coordinator, LaCrosse, Wisconsin, personal communication). Because MT would only be utilized to sex-reverse percids <u>not</u> destined as food, a pivotal study needed to add this group to the application is a Target Animal Safety Study. Target Animal Safety Studies address concerns regarding the safety of the drug to the animal to which it is being applied. Simply stated, does MT have any adverse effects on the fish (percids in this case)? Recently, FDA has shown a willingness to group fishes rather than requiring specific studies on each species (R. Schnick, National Aquaculture NADA Coordinator, LaCrosse, Wisconsin, personal communication). Accordingly, a Target Animal Safety Study conducted on walleye should suffice for sauger, walleye-sauger hybrids, yellow perch, and possibly other coolwater fishes. In addition to a Target Animal Safety Study, an additional efficacy study needs to be conducted in walleye to demonstrate a highly effective treatment, and to test whether the dose of MT needed in walleye can be lowered to a range known to be effective in yellow perch.

A Target Animal Safety Study must be conducted under Good Laboratory Practice (GLP) regulations (21 CFR 58) which are minimum requirements for conducting a study to assure the quality and integrity of data generated. Briefly, the GLP regulations require that each and every procedure is fully documented as a Standard Operating Procedure (SOP), all facilities and equipment have written procedures and maintenance documented, the use and distribution of the test article is controlled and documented, the procedures are monitored by quality assurance personnel, and that personnel are properly trained for their assigned duties and responsibilities (Beleau 1991). Documentation of all procedures followed, reasons for any deviations from SOPs, the protocol, all correspondence pertaining to the study, quality assurance audit reports and findings, and the final report of the study with all data generated, must be kept in a designated, separate archive area in a fireproof cabinet for a minimum of two years, and usually for the duration of the new animal drug registration. In addition to the facility quality assurance personnel, the sponsor also monitors the study for compliance with the GLP regulations and adherence to the written, approved (by FDA) protocol. Lastly, a final report is written by the testing facility personnel, reviewed by the sponsor, and submitted to the FDA as a part of the registration package.

RELATED CURRENT AND PREVIOUS WORK

Partial or complete sex inversion in numerous teleost fishes has been achieved by administration of sex steroids before or during sexual differentiation (see reviews by Hunter and Donaldson 1983 and Pandian and Sheela 1995). University of Wisconsin-Madison (UW-Madison) researchers (Malison et al. 1986; Malison and Held Manuscript) have developed techniques to partially sex-reverse walleye and yellow perch using MT. Partial sex-inverted female walleye and sauger were produced by feeding 50 mm TL walleye or 45 mm TL sauger a diet containing MT at a rate of 15 mg/kg feed for 60 consecutive days. This treatment was ~50% effective at inducing partial or complete sex inversion. At age 2, mature spermatozoa were collected from the walleye and used to fertilize eggs collected from normal females. The resultant progeny were reared to 145 mm TL and were determined by morphological and histological examination to be 100% female (Malison and Held Manuscript). Similar results had previously been found for yellow perch (Malison et al. 1986) except that partial or complete sex inversion was induced in >90% of the yellow perch when 20-35 mm TL yellow perch were fed MT at 1.5 to 60 mg/kg feed for 84 days. The much greater effectiveness of the MT treatment of yellow perch compared to walleye (despite the lower effective dosages used in yellow perch) probably resulted because sexual differentiation had probably progressed beyond a certain critical stage in walleye. Accordingly, an efficacy study using smaller, less developed walleye needs to be conducted.

Partial sex inversion, resulting in an ovotestes, is preferable in this case because it requires no genotypic analysis to assure that collected spermatozoa are coming from sex inverted females and thus contain only X chromosomes. The disadvantage is that these intersex fishes must be sacrificed. The testicular tissues are separated from the ovicular and thoroughly teased in an ice-chilled Petri dish. The sperm from several specimens are then diluted 1:5 with an extender solution (Moore 1987) for later use.

A Target Animal Safety Study for human chorionic gonadotropin was recently completed at Southern Illinois University-Carbondale (SIUC) under the direction of C. Kohler. This study was sponsored by Intervet, Inc., conducted under GLP compliance, and results were recently submitted to FDA. The study required approximately 50 SOPs, a housing facility fully accredited by the American Association of Laboratory Animal Care, specialized training of key personnel, an American College of Veterinary Medicine (ACVM) board-certified veterinarian for necropsies, and a American College of Veterinary Pathologists (ACVP) board-certified veterinarian pathologist for histological examinations.

ANTICIPATED BENEFITS

The results of this study would allow percids to be added in a timely manner to the package being promulgated by Auburn University to gain FDA approval for use of MT for sex reversing tilapia. The production of all-female populations of walleye and yellow perch would greatly enhance their aquaculture potential since females of both species grow significantly faster and to a larger size. Accordingly, this study has the potential to significantly benefit the aquaculture industry in the North Central Region where yellow perch culture is rapidly emerging, and where interest in walleye commercial production remains high.

PROGRESS TO DATE

New project.

OBJECTIVES

1. To conduct a Target Animal Safety Study under Good Laboratory Practice (GLP) compliance for walleye fingerlings fed 17α-methyltestosterone.

2. To evaluate selected dosages of 17α-methyltestosterone for efficacy at inducing sex inversion in walleye.

PROCEDURES

Target Animal Safety Study (Objective 1)

The procedures that follow are written in the format required as a protocol for FDA approval for a Target Animal Safety Study. Accordingly, there is some redundancy with other sections of this proposal namely **RELATED CURRENT AND PREVIOUS WORK**, **FACILITIES**, and **REFERENCES**. The appendices are part of the protocol and are thus also included in this section. Also included is an index of SOPs (Appendix VIII) that will be employed during this study.

1 Title: **SAFETY OF 17α-METHYLTESTOSTERONE FOR INDUCTION OF SEX INVERSION IN WALLEYE**.

2 Purpose and Objective: To assess the safety of 17α-methyltestosterone (MT) fed to walleye (family Percidae) fry for induction of sex inversion to produce genotypic females that produce viable sperm. When the treated females reach sexual maturity, they must be sacrificed in order to obtain sperm from the ovotestes. When this sperm is used to fertilize untreated females the offspring are all females. No walleye destined for human consumption are treated with MT. The results of the safety study will be used to support a New Animal Drug Application to the U.S. Food and Drug Administration to approve the use of MT for this indication for the family Percidae.

3 GLP Compliance: The study will be conducted in compliance with the Good Laboratory Practices (GLP) for nonclinical laboratory studies at 21 CFR Part 58.

4 Sponsor

Ronald P. Phelps, Ph.D.
Auburn University
Department of Fisheries
and Allied Aquacultures
Auburn, AL 36849
Phone: (334) 844-9317

5 Study Director

Christopher C. Kohler, Ph.D.
Fisheries Research Laboratory

Mailcode 6511
Southern Illinois University-Carbondale
Carbondale, IL 62901-6511
Phone: (618) 453-2890
FAX: (618) 536-7761

5.1 Investigators

5.1.1 Anita M. Kelly, Ph.D.
Fisheries Research Laboratory
Mailcode 6511
Southern Illinois University-Carbondale
Carbondale, IL 62901-6511
Phone: (618) 453-2890
FAX: (618) 536-7761

5.1.2 Elaine M. Carnevale, DVM, Ph.D.
Department of Animal Science
Mailcode 4417
Southern Illinois University-Carbondale
Carbondale, IL 62901-4417

5.1.3 Marcos DeJesus
Fisheries Research Laboratory
Mailcode 6511
Southern Illinois University-Carbondale
Carbondale, IL 62901-6511

6 <u>GLP Monitor</u>

6.1 William L. Muhlach, Ph.D.
Department of Zoology
Mailcode 6501
Southern Illinois University-Carbondale
Carbondale, IL 62901-6501

7 <u>Test Facilities</u>

7.1 Fisheries Research Laboratory
Mailcode 6511
Southern Illinois University-Carbondale
Carbondale, IL 62901-6501

7.2 Vivarium
Mailcode 6506
Southern Illinois University-Carbondale
Carbondale, IL 62901

7.2.1 All fish will be maintained in a single room of the Vivarium in 208-L glass tanks attached to a single biological filter for maintenance of water quality. The room will be solely dedicated to this study.

7.2.2 The Southern Illinois University-Carbondale facilities above are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

8 <u>Test Animals</u>

Walleye *(Stizostedion vitreum)* fry 35-55 mm total length (TL) from the family Percidae.

8.1 Justification

The species selected for this study are important sportfish and have potential as an aquaculture species. MT has been utilized to induce phenotypic males containing only X chromosomes. These sex inverted males are then crossed with normal females to produce all-female offspring.

8.2 Procurement

Fish will be obtained from Steve Krueger, Jake Wolfe Memorial Fish Hatchery, Manito, Illinois.

9 <u>Test Article</u>

MT will be incorporated into #1 salmon starter feed, Batch Number #tba. This size feed is not recognizable as food by advanced juvenile and adult fish.

9.1 Source: Range, Inc., Buhl, Idaho. Certification of MT content in feed will be provided by Range, Inc.

9.2 Dosage

Feeds will contain 0, 15, 45, and 75 mg/kg MT. Fish will be fed 3-5% of their wet body weight daily utilizing automatic feeders.

9.2.1 Justification

The walleye, *Stizostedion vitreum*, is a highly valued sport fish in the United States and Canada (Becker 1983). Walleye fingerlings are produced commercially, and the culture of walleye as a food fish has promise (Malison and Garcia-Abiado In press). Sexual dimorphism exists in walleye, with the female demonstrating faster growth and reaching a much larger size than males (Colby et al. 1979; Scott and Crossman 1973). Therefore, the development of monosex female populations of walleye would be beneficial to commercial walleye production by increasing production efficiency. The development of all female populations would, therefore, benefit management techniques by developing a trophy fishery since only female have trophy-size potential, and would benefit culturists due to the rapid growth of females which would increase production efficiency.

One method of producing all female populations is to treat juvenile fish with androgens to induce phenotypic sex inversion of genetic females and then utilizing the sperm from these masculinized females to fertilize normal eggs, resulting in 100% female offspring. Moreover, the fish destined for human consumption are not treated with hormones. This technique has been successfully applied to various aquaculture species including the grass carp, *Ctenopharyngodon idella* (Boney et al. 1984), chinook salmon, *Oncorhynchus tshawytscha* (Hunter et al. 1983), tilapia, *Oreochromis niloticus* (Mair et al. 1991), yellow perch, *Perca flavescens* (Malison et al. 1986), and most recently walleye and sauger, *Stizostedion canadense* (Malison and Held 1996).

All female populations of walleye have been produced by fertilizing eggs with the sperm of partially sex-inverted genotypic females. The sex-inverted females were produced by feeding 50-mm TL walleye a diet containing MT at a rate of 15 mg/kg of food for 60 consecutive days (Malison and Held Manuscript). However, this concentration and timing of MT was only about 50% effective in inducing partial or complete sex inversion (Malison and Held Manuscript). In yellow perch, sex inversion at >90% was accomplished utilizing MT at concentration of 1.5 to 60 mg/kg diet, when fed to yellow perch initially 20-35 mm TL (Malison et al. 1986). A concentration of 15 mg/kg for 60 days is speculated to produce 100% sex-inverted females in walleye if treatment is begun in fish at 35-40 mm TL (J. Malison, UW-Madison, personal communication).

The dosages to be used in this safety study are 0, 1, 3, and 5x the proposed standard dosage. Because efficacious dosages for walleye are the same for sauger and are considerably higher than for yellow perch, a Target Animal Safety Study conducted for walleye will be representative of the family Percidae.

9.3 Inventory: Appendix I.

10 Placebo: #1 salmon starter which does not contain MT.

10.1 Source: Rangen, Inc.

10.2 Dosage

Fish will be fed 3-5% of wet body weight daily utilizing automatic feeders.

10.2.1 Justification: The feeding regime for placebo is consistent with feeding regime for test article.

10.3 Inventory: Appendix II

11 Experimental design

Twelve 208-L glass tanks with each containing 25 fish will be utilized in this study.

12 Procedure

12.1 Twenty-five fish will be randomly assigned to each of the twelve experimental tanks.

12.2 Each tank will be randomly assigned to the placebo or test article groups. Fish will be fed the assigned diets for 60 days. This duration is 1x the standard duration. A 3x duration will not be tested because the fish would reach a size where they would not be able to recognize the starter feed as food.

12.3 An analysis of starting water in the tanks will be obtained from the Carbondale Water District. Thereafter, water quality and temperature will be maintained at optimum levels according to standard aquaculture practices.

12.4 All fish will be fully acclimated to the facilities for a minimum of seven days before treatments are administered.

12.5 On day 0, each tank of fish will be weighed and measured. Feed calculations will be determined from the initial weights.

12.6 After weighing, each tank of fish will be fed their assigned feeds and fish will be observed twice daily for 60 days (Days 1-60) for any unusual clinical signs. Fish that die during the period will be subjected to complete gross and histopathological examination (see below).

12.7 On day 61, all fish fed the test article will be weighed and given the placebo diet for a 30 day withdrawal period.

12.8 On day 61, all fish fed the placebo diet will continue to be weighed and receive the placebo diet for an additional 30 day period.

12.9 Starting on day 61, each tank of fish will be observed twice daily for 30 days (Days 61-90).

12.10 On Day 91, five randomly selected fish from each tank will be euthanized with a lethal dose of anesthetic (MS-222) and subjected to a thorough postmortem examination. Each major organ/organ system will be weighed and examined grossly by a veterinarian. Samples of any tissue that appear abnormal will be submitted for histopathological examination by an ACVP board-certified pathologist.

13 Data Collection and Recording

The following data will be collected on each fish and recorded on the data record forms in Appendices III-VII:

13.1 Tank no., temperature, and amount fed

13.2 Twice-daily observation Days 1-60 for any unusual clinical signs

13.3 Weights and lengths of fish per tank on day 0, day 61, and day 91

13.4 Twice-daily observation Days 61-90 for any unusual clinical signs

13.5 Date of euthanization, body weight, length, and general condition of fish at time of euthanization

13.6 Weight and gross appearance of major organ/organ systems:

13.6.1 Viscera

13.6.2 Heart

13.6.3 Gastrointestinal tract

13.6.4 Liver

13.6.5 Spleen

13.6.6 Kidney (appearance only)

13.6.7 Urogenital tract

13.7 Visceral somatic index and liver somatic index

13.8 Complete histopathology report on any tissue that appears abnormal

14 <u>Data Analysis</u>

14.1 Primary response variables:

Unanticipated clinical signs during Days 1-60
Unanticipated clinical signs during Days 61-90
Body weight change during Days 1-60
Body length change during Days 1-60
Body weight change during Days 61-90
Body length change during Days 61-90
Gross appearance of organs/organ systems
Organ/organ system weight
Visceral-somatic index and liver-somatic index
Histopathological findings

14.1.1 Methods of analysis

Differences between treated and control fish for the following variables will be analyzed in analyses of variance with treatment as main effect:

Body weight change during Days 1-60
Body length change during Days 1-60
Body weight change during Days 61-90
Body length change during Days 61-90
Organ/organ system weight
Visceral-somatic index and liver-somatic index

14.2 Other data: Tabulated in final report

15 <u>Anticipated Starting Date</u>: July 1, 1996

16 <u>Anticipated Completion Date</u>: June 30, 1997

17 <u>Approval Signatures</u>

17.1 Sponsor

____ Date

17.1.1 Sponsor's Monitor

____ Date

17.2 Study Director

____

Christopher C. Kohler, Ph.D. Date
Fisheries Research Laboratory
Southern Illinois University-Carbondale

17.2.1 Investigators

____

Anita M. Kelly, Ph.D. Date
Fisheries Research Laboratory
Southern Illinois University-Carbondale

____

Elaine M. Carnevale, DVM, Ph.D. Date
Department of Animal Sciences
Southern Illinois University-Carbondale

____

Marcos DeJesus Date
Fisheries Research Laboratory
Southern Illinois University-Carbondale

17.3 GLP Monitor

____

William L. Muhlach, Ph.D. Date
Department of Zoology
Southern Illinois University-Carbondale

18 <u>Citations</u>

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.

Boney, S.E., W.L. Shelton, S.-L. Yang, and L.O. Wilken. 1984. Sex reversal and breeding of grass carp. Transactions of the American Fisheries Society 113:348-353.

Colby, P.J., R.E. McNicol, and R.A. Ryder. 1979. Synopsis of biological data on the walleye *Stizostedion v. vitreum* (Mitchill 1918). FAO (Food and Agricultural Organization of the United Nations) Fisheries Synopsis 119.

Hunter, G.A., E.M. Donaldson, J. Stoss, and I. Baker. 1983. Production of monosex female groups of chinook salmon *(Oncorhynchus tshawytscha)* by the fertilization of normal ova with sperm from sex-reversed females. Aquaculture 33:335-364.

Mair, G.C., A.G. Scoot, D.J. Penman, J.A. Beardmore, and D.O.F. Skibinski. 1991. Sex determination in the genus *Oreochromis* 1. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theoretical and Applied Genetics 82:144-152.

Malison, J.A., T.B. Kayes, C.D. Best, C.H. Amundson, and B.C. Wentworth. 1986. Sexual differentiation and the use of hormones to control sex in yellow perch *(Perca flavescens)*. Canadian Journal of Fisheries and Aquatic Sciences 43:25-35.

Malison, J.A., and M.A.R. Garcia-Abiado. In press. Sex control and ploidy manipulations in yellow perch *(Perca flavescens)* and walleye *(Stizostedion vitreum)*. Journal of Applied Ichthyology.

Malison, J.A., and J.A. Held. Manuscript. The production of all-female populations of walleye *Stizostedion vitreum* and sauger *Stizostedion canadense* using partially sex-inverted broodstock. University of Wisconsin Aquaculture Program, Department of Food Sciences, Madison.

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

Appendix I

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

TEST ARTICLE INVENTORY

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

17α-methyltestosterone feed 1, 3, 5x Standard Dose ___________________ Expir. Date __________

Batch No. ______________

Date	No. Grams Received	From	No. Grams Used	By	No. Grams Remaining

Date	No. Grams Received	From	No. Grams Used	By	No. Grams Remaining

Date	No. Grams Received	From	No. Grams Used	By	No. Grams Remaining

Remainder (check one): Destroyed ☐ Returned ☐ Date ______________

Investigator signature ___________________________ Date ______________

Appendix II

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

PLACEBO INVENTORY

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Placebo Feed Batch No. ____________________________ Expir. Date ________________________

Date	No. Grams Received	From	No. Grams Used	By	No. Grams Remaining

Date	No. Grams Received	From	No. Grams Used	By	No. Grams Remaining

Remainder (check one): Destroyed ☐ Returned ☐ Date ______________

Investigator signature ___________________________ Date ______________

Appendix III

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

PROCUREMENT AND TREATMENT RECORD

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Tank No. ______________________________ Fish Qty ____________________________________

ACQUISITION

Date ________________ Origin ____

Transport Time __________________hr Transport Temp __________________________˚C

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
18			
19			
20			
21			
22			
23			
24			
25			

Holding Temp ____________________ ˚C Ration ______________________________

Additional Comments ____

____

Received By (signature) ____ Date _______________

FEED TREATMENT

Date ______________ Tank No. ____

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
21			
22			
23			
24			
25			

Holding Temp ___________________ ˚C Ration _____________________________

Additional Comments ____

____

Received By (signature) _______________________________________ Date _______________

WITHDRAWAL PERIOD

Date _____________ Tank No. ____

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Fish No.	Weight (gm)	Length (mm)	Gen. Condition
21			
22			
23			
24			
25			

Holding Temp ___________________ ˚C Ration _____________________________

Additional Comments ____

____

Received By (signature) ______________________________________ Date _______________

Investigator signature ____ Date _______________

Appendix IV

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

ACCLIMATION OBSERVATION RECORD

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Tank No. ________ Treatment: ☐ MT 1, 3, 5x ☐ PLACEBO

Day	Date	Time	Temp, °C	Observation	By
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					

Appendix V

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

TREATMENT OBSERVATION RECORD

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Tank No. ________ Treatment: ☐ MT 1, 3, 5x ☐ PLACEBO

Day	Date	Time	Temp, °C	Observation	By
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

Day	Date	Time	Temp, °C	Observation	By
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					

Day	Date	Time	Temp, °C	Observation	By
32					
33					
34					
35					
36					
37					
38					
39					
40					
41					
42					
43					
44					
45					
46					
47					
48					

Day	Date	Time	Temp, °C	Observation	By
49					
50					
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					

Investigator signature ____ Date _________________

Appendix VI

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

WITHDRAWAL OBSERVATION RECORD

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Tank No. ________ Treatment: ☐ MT 1, 3, 5x ☐ PLACEBO

Day	Date	Time	Temp, °C	Observation	By
61					
62					
63					
64					
65					
66					
67					
68					
69					
70					
71					
72					
73					
74					

Day	Date	Time	Temp, °C	Observation	By
75					
76					
77					
787					
79					
80					
81					
82					
83					
84					
85					
86					
87					
88					
89					
90					

Investigator signature ____ Date _________________

Appendix VII

Safety of 17α-methyltestosterone For Induction Of Sex-Inversion In Walleye:

POSTMORTEM RECORD

INSTRUCTIONS: Complete one form for each batch of test article received - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Christopher Kohler, Ph.D., Fisheries Research Laboratory, Mailcode 6511, Southern Illinois University-Carbondale, Carbondale, IL 62901-6511.

Tank No. ________________ Fish No. ____

Date _______________ Wet Weight ____g

General Condition ____

Organ	Observations
VISCERA: Wt g	Visceral-Somatic Index: Histo: Y ☐ N ☐
HEART: Wt g	Histo: Y ☐ N ☐
GI TRACT: Wt g	Histo: Y ☐ N ☐
LIVER: Wt g	Histo: Y ☐ N ☐
SPLEEN: Wt g	Histo: Y ☐ N ☐
KIDNEY: N/A	Histo: Y ☐ N ☐
UG TRACT: Wt g	Histo: Y ☐ N ☐

Additional Comments ____

____

Signature ____ Date ______________

Investigator signature _________________________________ Date ______________

Appendix VIII

Standard Operating Procedures Index:

1. Definition of Standard Operating Procedure (SOP)
2. Development, Revision, Deletion and Review of SOPs
3. Numbering and Format Systems for SOPs
4. Duties and Responsibilities Testing Facility Management
5. Study Director Criteria
6. Study Director Duties and Responsibilities
7. Preparation and Management of Resumes
8. Employee Training
9. Signature Sheet
10. Research Room(s) Security
11. Receipt and Distribution of Test and Control Substances
12. Storage of Test and Control Substances
13. Random Sampling
14. Test Facility Maintenance and Preparation
15. Test Organism Identification, Care, Transfer and Handling
16. Balance Calibration and Basic Use
17. Anesthetizing Fish
18. Weighing and Measuring of Fish
19. Test Organism Observation Methods
20. Feeding 17α-methyltestosterone to Test Fish
21. Water Quality Monitoring
22. Standard Procedure for Explaining Notebook Entry Errors
23. Significant Figures and Number Rounding
24. Telephone, Conversation, and Meeting Documentation
25. Archive
26. Submission and Retrieval of Material into the Archive
27. Visitors
28. Test Organism Necropsy Methods
29. Quality Assurance Unit (QAU) Responsibilities
30. Documentation of Personnel Training
31. Inventory of Chemicals
32. Maintenance of Charcoal Filters
33. Computer Entry of Data
34. Disease Treatment of Fish

Efficacy Studies for Inducing Sex Inversion (Objective 2)

Efficacy studies will be conducted by UW-Madison investigators, using standard laboratory procedures rather than GLP procedures. Walleye brood fish will be captured from Rock Lake, Jefferson County, Wisconsin. The fish will be transported to the main wet laboratory at Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. There, the fish will be spawned and their offspring initially reared in fertilized 0.2-0.5 ha production ponds. When the fingerlings reach approximately 30-35 mm TL (6-8 weeks of age), they will be harvested and habituated to intensive culture conditions and formulated feeds using the following procedures. The fish will be stocked into indoor 750-L flow-through fiberglass tanks provided with heated water (21 ± 1°C) and airstone aeration. During the first three weeks indoors, the fish will be exposed to constant lighting, after which the lighting will be set to a 14-h light/10-h dark photoperiod. The fish will be trained to consume formulated feeds using Silver Cup salmon starter feeds (Nelson and Sons, Inc., Murray, Utah).

When the walleye reach a mean size of 35-40 mm TL, three groups of 100 fish each will be stocked into three 220-L tanks, and the fish in each tank will be fed a diet containing MT at 0, 6, or 15 mg/kg of feed for 60 consecutive days. The diets will be prepared by dissolving the MT in ethanol, immersing the food in the ethanol and evaporating the ethanol as described previously (Malison et al. 1986). The fish will be returned to a normal diet after the androgen treatment, and then reared for an additional 4-6 months until they reach approximately 150-200 mm TL.

The walleye will then be sacrificed and their sex identified by macroscopic and histological examination as previously described (Malison et al. 1986; Malison and Held Manuscript). Briefly, after macroscopic examination, the walleye gonads will be fixed in Bouin's fluid, and 6-8 µm paraffin sections will be stained with alum hematoxylin and eosin. The number of phenotypic males, females, and intersex (partially sex-inverted) genetic females will be determined, the last group characterized by the presence of both spermatogenic and ovarian gonadal tissues.

FACILITIES

Target Animal Safety Study (Objective 1)

All fish will be maintained in Room 39 of the SIUC Vivarium in 208-L glass tanks attached to a single biological filter for maintenance of water quality. The room will be solely dedicated to this study and will have restricted access. A freezer in the room will be used for storing feed and any dead fish. The SIUC Vivarium is under the direction of an ACVM board-certified veterinarian, and is fully accredited by the American Association of Laboratory Animal Care.

Water temperature will be maintained at 18 ± 2°C by a 1 hp Delta Star Model CH9A chiller. Feed will be administered twice daily using automatic feeders. Water quality will be monitored using a LaMotte water quality test kit (model L363302).

Necropsies will be conducted in Room 41 of the SIUC Vivarium. This room will be designated solely to this study and will have restricted access. The room is designed as a dry lab and contains stainless steel countertops. A Fisher Model 1200D electronic balance will be used for weighing fish (± 0.1 g). A fireproof file cabinet is in place and will be used for archiving project documents.

Efficacy Studies for Inducing Sex Inversion (Objective 2)

Studies conducted by the UW-Madison will use ponds located at UW-Madison Aquaculture Program's main research facility at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. The Lake Mills Hatchery has 32 ponds ranging in size from 0.2-0.5 ha, all of which have high-volume lake water inputs. At the Lake Mills Hatchery the UW-Madison Aquaculture Program has all of the facilities and equipment needed to rear fish in tanks and ponds. These facilities include over 100 flow-through fiberglass rearing tanks and over 600 L/min of temperature-regulated (10 to 30 ± 0.5°C) well or carbon-filtered city water. In addition, the UW-Madison Aquaculture Program has all of the equipment needed to conduct the macroscopic and histological examinations described under Objective 2.

REFERENCES

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.

Beleau, M.H. 1991. Aquaculture drug development: a pharmaceutical industry perspective. Veterinary and Human Toxicology 33:11-13.

Boney, S.E., W.L. Shelton, S.-L. Yang, and L.O. Wilken. 1984. Sex reversal and breeding of grass carp. Transactions of the American Fisheries Society 113:348-353.

Colby, P.J., R.E. McNicol, and R.A. Ryder. 1979. Synopsis of biological data on the walleye *Stizostedion v. vitreum* (Mitchill 1918). FAO (Food and Agricultural Organization of the United Nations) Fisheries Synopsis 119.

Francis, C.R. 1992. Sexual lability in teleost: developmental factors. Quarterly Review in Biology 67:1-17.

Guerrero, R.D. 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). Transactions of the American Fisheries Society 104:342-348.

Hunter, G.A., E.M. Donaldson, J. Stoss, and I. Baker. 1983. Production of monosex female groups of chinook salmon *(Oncorhynchus tshawytscha)* by the fertilization of normal ova with sperm from sex-reversed females. Aquaculture 33:335-364.

Hunter, G.A., and E.M. Donaldson. 1983. Hormonal sex control and its application to fish culture. Pages 223-291 *in* W.S. Hoar, R.J. Randall, and E.M. Donaldson, editors. Fish physiology, volume 9. Academic Press, New York.

Mair, G.C., A.G. Scoot, D.J. Penman, J.A. Beardmore, and D.O.F. Skibinski. 1991. Sex determination in the genus *Oreochromis* 1. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theoretical and Applied Genetics 82:144-152.

Malison, J.A., T.B. Kayes, C.D. Best, C.H. Amundson, and B.C. Wentworth. 1986. Sexual differentiation and the use of hormones to control sex in yellow perch *(Perca flavescens)*. Canadian Journal of Fisheries and Aquatic Sciences 43:25-35.

Malison, J.A., and M.A.R. Garcia-Abiado. In press. Sex control and ploidy manipulations in yellow perch *(Perca flavescens)* and walleye *(Stizostedion vitreum)*. Journal of Applied Ichthyology.

Malison, J.A., and J.A. Held. Manuscript. The production of all-female populations of walleye *Stizostedion vitreum* and sauger *Stizostedion canadense* using partially sex-inverted broodstock. University of Wisconsin Aquaculture Program, Department of Food Sciences, Madison.

Moore, A.A. 1987. Short-term storage and cryopreservation of walleye semen. Progressive Fish-Culturist 49:40-43.

Pandian, T.J., and S.G. Sheela. 1995. Hormonal induction of sex reversal in fish. Aquaculture 138:1-22.

Schnick, R.A., F.P. Meyer, and D.L. Gray. 1989. A guide to approved chemicals in fish production and fishery resource management. Publication No. MP241-5M-3-89RV. United States Fish and Wildlife Service and University of Arkansas Cooperative Extension Service, Little Rock, Arkansas.

Schott, E.F. 1980. Sexually dimorphic growth in young-of-the-year yellow perch *(Perca flavescens)* under controlled environmental conditions. Master's thesis. University of Wisconsin-Madison.

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

Shelton, W.L. 1986. Broodstock development for monosex production of grass carp. Aquaculture 57:311-319.

Yamamoto, T. 1953. Artificially induced sex reversal in genotypic males of the medaka *(Oryzias latipes)* with special reference to YY male. Journal of Experimental Zoology 123:571-594.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Christopher C. Kohler Southern Illinois University-Carbondale	Aquaculture
Wisconsin	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture, Reproductive Physiology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Southern Illinois University-Carbondale (SIUC)
Christopher C. Kohler

University of Wisconsin-Madison (UW-Madison)
Jeffrey A. Malison

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Southern Illinois University-Carbondale Fisheries Research Laboratory Carbondale, IL 62901-6511				**USDA AWARD NO.** Year 1 - Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christopher C. Kohler				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	9			$16,686	
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$16,686	
B. Fringe Benefits (If charged as Direct Costs)				$5,151	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$21,837	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,163	
F. Travel 1. Domestic (Including Canada)					
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Postage ($100), Report preparation ($200), Pathology services ($500), Telephone ($150), FAX ($50)				$1,000	
J. Total Direct Costs (C through I) →				$25,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$25,000	
M. Other →					
N. Total Amount of This Request →				$25,000	$
O. Cost Sharing (If Required Provide Details)	$ 19,455				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

(Kohler)

Objective 1

A. **Wages and Salaries.** One Researcher III (post-doc) (0.7s FTE) will be hired to assist in conducting the study. Due to the nature of the study it is not practical to use graduate students.

B. **Fringe Benefits.** 10.3% of wages and salaries and $381/mo. for medical insurance.

E. **Materials and Supplies.** These funds will be used for acquisition of fish, feeds, maintenance of experimental holding systems, glassware, and chemicals.

I. **Other Direct Costs.** These costs are specified on the budget form and include: postage ($200), report preparation ($200), pathology services ($500), telephone ($150), and FAX ($50).

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS
University of Wisconsin-Madison Aquaculture Program
Department of Food Science, 123 Babcock Hall
Madison, WI 53706

USDA AWARD NO. Year 1 - Objective 2

Duration Proposed Months: 12 | Duration Awarded Months: ________

PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S)
Jeffrey A. Malison

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages					$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	0.6			$1,000	
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$1,000	
B. Fringe Benefits (If charged as Direct Costs)				$310	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$1,310	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$690	
F. Travel 1. Domestic (Including Canada)					
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$2,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$2,000	
M. Other →					
N. Total Amount of This Request →				$2,000	$
O. Cost Sharing (If Required Provide Details)	$ 1,920				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison)

Objective 2

A. Wages and Salaries. A professional research person will be hired to assist in conducting the study.

B Fringe Benefits. UW-Madison fringe benefit rate is 31%.

E. Materials and Supplies. These funds will be used for acquisition of fish, feeds, maintenance of experimental holding systems, glassware, and chemicals.

WALLEYE MT PROJECT

Budget Summary for Each Participating Institution

	SIUC	UW-MADISON	TOTALS
Salaries and Wages	$16,686	$1,000	$17,686
Fringe Benefits	$5,151	$310	$5,461
Total Salaries, Wages and Benefits	$21,837	$1,310	$23,147
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$2,163	$690	$2,853
Travel	$0	$0	$0
Other Direct Costs	$1,000	$0	$1,000
TOTAL PROJECT COSTS	**$25,000**	**$2,000**	**$27,000**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

State/Institution		Year 1
Southern Illinois University-Carbondale		
Salaries and Benefits: SY @ 0.10		$8,955
Waiver of Overhead		$10,500
	Total	$19,455
University of Wisconsin-Madison		
Salaries and Benefits: SY @ 0.02 FTE		$1,060
Waiver of Overhead		$860
	Total	$1,920
	GRAND TOTAL	$21,375

[1]Because cost sharing is not a legal requirement universities are not required to provide or maintain documentation of such a commitment.

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objectives 1 and 2: Initiated in Year 1 and completed in Year 1.

LIST OF PRINCIPAL INVESTIGATORS

Christopher C. Kohler, Southern Illinois University-Carbondale

Jeffrey A. Malison, University of Wisconsin-Madison

VITA

Christopher C. Kohler
Department of Zoology/Fisheries Research Laboratory
Southern Illinois University-Carbondale
Carbondale, IL 62901-6511

Phone: (618) 453-2890
FAX: (618) 536-7761
E-Mail: ckohler@siu.edu

EDUCATION

B.S. St. Mary's College of Maryland, 1973
M.S. University of Puerto Rico, 1975
Ph.D. Virginia Polytechnic Institute and State University, 1980

POSITIONS

Professor (1993-present), Associate Professor (1989-1993), Assistant Professor (1982-1988), and Research Associate, (1980-1981), Department of Zoology, Southern Illinois University-Carbondale

Associate Director (1991-present) and Assistant Director (1988-1991), Fisheries Research Laboratory, Southern Illinois University-Carbondale

Assistant Professor (1980), Department of Fisheries and Wildlife Science, Virginia Polytechnic Institute and State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Culture, Management, Introduced, Education and International Sections
World Aquaculture Society (USA Chapter)
Sigma Xi, Phi Kappa Phi

SELECTED PUBLICATIONS

Kelly, A.M., and C.C. Kohler. 1994. Human chorionic gonadotropin injected in fish degrades metabolically and by cooking. World Aquaculture 25(4):55-57.

Kohler, C.C., R.J. Sheehan, C. Habicht, J.A. Malison, and T.B. Kayes. 1994. Habituation to captivity and controlled spawning of white bass. Transactions of the American Fisheries Society 123:964-974.

Ayala, C.E., C.C. Kohler, and R.R. Stickney. 1993. Protein digestibility and amino acid availability of fish meal in largemouth bass infected with Acanthocephala. Progressive Fish-Culturist 55:275-279.

Killian, H.S., and C.C. Kohler. 1991. Influence of 17-α-methyltestosterone on red tilapia under two thermal regimes. Journal of the World Aquaculture Society 22:83-94.

Phillips, P.C., and C.C. Kohler. 1991. Establishment of tilapia spawning families providing a continuous supply of eggs for *in vitro* fertilization. Journal of the World Aquaculture Society 22:217-223.

Stickney, R.R., and C.C. Kohler. 1990. Maintaining fishes for research and teaching. Pages 633-663 *in* C. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

VITA

Jeffrey A. Malison
University of Wisconsin Aquaculture Program
Department of Food Science, 123 Babcock Hall
University of Wisconsin-Madison
Madison, WI 53706

Phone: (608)263-1242
FAX: (608)262-6872
E-mail: jmalison@facstaff.wisc.edu

EDUCATION

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

POSITIONS

Director (1995-present) and Assistant Director (1990-1995), University of Wisconsin Aquaculture Program, University of Wisconsin-Madison

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

Barry, T.P., J.J. Parrish, and J.A. Malison. 1995. Ontogeny of the cortisol stress response in rainbow trout. General and Comparative Endocrinology 97:57-65.

Malison, J.A., L.S. Procarione, A.R. Kapuscinski, and T.B. Kayes. 1994. Endocrine and gonadal changes during the reproductive cycle of walleye. Fish Physiology and Biochemistry 13:473-484.

Kohler, C.C., R. J. Sheehan, C. Habicht, J.A. Malison, and T.B. Kayes. 1994. Habituation to captivity and controlled spawning of white bass. Transactions of the American Fisheries Society 123:964-974.

Barry, T.P., A.F. Lapp, T.B. Kayes, and J.A. Malison. 1993. Validation of a microtitre plate ELISA for measuring cortisol in fish and comparison of stress responses of rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture 117:351-363.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Measurement of resting and stress-elevated serum cortisol in rainbow trout *Oncorhynchus mykiss* in experimental net-pens. Journal of the World Aquaculture Society 23:83-88.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health 4:1-6.