GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

Hormonal Sterilization of Early Lamprey Larvae

by

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RESEARCH PROPOSAL FINAL REPORT

TO:

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Great Lakes Fishery Commission
2100 Commonwealth Blvd., Suite 209
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PROJECT TITLE: Hormonal Sterilization of Early Lamprey Larvae

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TIME PERIOD: Feb. 1 1994 to July 31, 1996

FINAL REPORT: Hormonal Sterilization of Early Lamprey Larvae

OVERALL SUMMARY:

This was a project that was first proposed by Drs. Margaret Docker and Aubrey Gorbman. I "inherited" the project upon request from Aubrey Gorbman. This project was first proposed to use a nonparasitic species of lampreys. We did not have access to a nonparasitic species, so we attempted to use the searun sea lamprey, *Petromyzon marinus*, for the proposed experiments.

During the summer of 1994, we attempted to treat embryos with various doses of steroids using a newly constructed incubation stream. Treatments were on day 6 following fertilization and were as follows: (1) control; (2) 0.01 mg/L 17ß-estradiol; (3) 0.10 mg/L 17ß-estradiol; (4) 0.005 mg/L 17α-methyltestosterone and (5) 0.05 mg/L 17α-methyltestosterone. Each treatment was replicated. The eggs were incubated with steroid solutions for two hours with airstones. After this time the water was removed and replaced with fresh well water. The eggs were checked for any mortalities daily. Within three days, almost all lampreys treated with methyltestosterone had died. By day 10, one entire treatment of estradiol-treated embryos had died. Based on Dr. Docker's earlier work, we suspect that the embryos died from exposure to the steroids. By mid-fall, only control larvae lampreys had survived and the experiment was terminated. Gonadal histology was attempted but the lampreys were too young to identify gonadal sex.

Overall, we would propose that these types of sterilization experiments are not viable in lampreys for the following reasons:

1. The problems with the containment of steroid and steroid wastes. The use of steroids in a hatchery situation requires specialized holding and disposal units at a high cost.
2. The high mortalities of embryos that occurred when exposed to steroids.
3. The safety of the people has to be considered when handling the steroids.
4. The eventual permits that will have to be obtained for use of steroids in hatchery situations which would be an expensive and very time consuming process.

RATIONALE:

Competition among lamprey ammocoetes for environmental resources and among adults for mating opportunities is an attractive strategy for ecologically-benign control of sea lamprey populations. For example, interspecific competition between larvae of a native or introduced nonparasitic lamprey and the sea lamprey has been proposed as a means of reducing the latter's growth rate and consequently, decreasing its adult fecundity and/or increasing its generation time (Murdoch et al., 1991). In particular, if the nonparasitic species were introduced into a stream shortly after its treatment with the lampricide 3-trifluoromethyl-4-ntirophenol (TFM), the non parasitic lampreys could outnumber the residual sea lampreys and would themselves escape the effects of the TFM. Competition could then result in less frequent applications of the lampricide. An alternative approach involves the introduction of sterile male sea lampreys at the adult stage to compete for mating opportunities with their conspecifics. Chemosterilization of spawning run sea lampreys has been shown to reduce the reproductive capacity of the unsterilized individuals, and the actual reduction in reproductive success is directly related to the ratio of sterile to normal males in the population (Hanson and Manion, 1980). A third option, a combination of the above alternatives, could involve the introduction of sterile sea lampreys at the larval, rather than the adult, stage. As with the introduction of non parasitic larvae, the resulting increase in density would decrease growth rate and, given that sex differentiation in lampreys may be influenced by population density (Purvis, 1979; Docker, 1992), could result in a male-biased sex ratio in the residual, unsterilized larvae. Additional advantages, however, would be elimination of the concern that a nonparasitic competitor may not share the sea lamprey's habitat preference (Schuldt and Goold, 1980) and, if the induced sterility did not have serious adverse effects on adult mating behavior, that there would be additional competition at spawning.

RELEVANT LITERATURE:

The principles for altering the sex differentiation of lower vertebrates are well established. Most pertinent is the extensive experience in hormonal control of sex differentiation in salmonids in which, by choice of steroids and their dosages, sex differentiation can be altered in either male or female direction, or can be completely suppressed to produce sterile individuals (see review by Hunter and Donaldson, 1983; Baker et al., 1988; Pifferer and Donaldson, 1989). Effective treatment protocols have been established following years of experimentation.

In salmonids and other economically important species, hormonal sex control has a number of practical applications. For example, monosex stocks may be desirable where one sex has a faster growth rate than the other, or where there is a preference for other sex-related morphological or physiological characteristics (Hunter and Donaldson, 1983). In particular, all-female salmonid stocks are highly desirable since they increase the number of adults harvested by eliminating precocious male development, increase the egg production from a given number of broodstock or hatchery escapement, and increase the value of the harvest where the roe is highly valued (Hunter et al., 1983). Monosex stocks have also been used to prevent unwanted spawning. Since tilapias reproduce at relatively small sizes, monosex stocks prevent reproduction and thereby increase the yield of harvestable-sized fish (Guerrero, 1975). Likewise, non-reproducing populations of grass carp, which are often stocked in waters for aquatic weed control, are also desirable since the establishment of a breeding population in these waters may be detrimental to the native fish species (Boney et al., 1984). Deliberate hormonal sterilization also has several management applications. Unwanted spawning in tilapia and grass carp could also be prevented by sterilization, and the

introduction of sterile individuals is preferable to that of monosex stocks where bisexual populations already exist. Sterile salmonids are produced to maximize growth by diverting resources which would otherwise be used for gonadal development, to permit year-round marketing in species where sexual maturation reduces the commercial value, and to produce trophy-sized individuals by extending the life cycle beyond the normal time of spawning and death (Donaldson and Hunter, 1982).

Note: Steroids have not been approved for use in fish aquaculture in the United States. Drs. Donaldson and Hunter are from Canada where some steroids have been approved on a limited basis.

Hormonal sex control in lampreys has received comparatively little attention. Studies of sex differentiation in lampreys (Okkelberg, 1921; Busson-Mabillot, 1965; Hardisty, 1965a,b) reveal a prolonged period of sexual indeterminacy, during which future testes and ovaries both possess oocytes. Despite this apparent sexual lability, hormonal sex reversal in lampreys has been unsuccessful to date (Hardisty and Taylor, 1965; Docker, 1992; L.H. Hanson, unpublished data). Nevertheless, in each of these studies, although the normal course of sex differentiation was not completely redirected, the exogenous steroids often caused gonadal abnormalities. L.H. Hanson (unpublished study) found very thin, unusual-looking gonads in sea lamprey larvae following prolonged immersion in a variety of steroids. Estradiol benzoate appeared to cause oocyte degeneration in undifferentiated *Lampetra planeri* larvae (Hardisty and Taylor, 1965), and this inadvertent sterilization by estradiol was very pronounced in female sea lamprey larvae (Docker, 1992).

OBJECTIVE:

The overall objective of this research program was to investigate the ability of gonadal hormones (steroids) to sterilize lamprey embryos.

Specific Objective

1. To determine the effects of estradiol or 17α-methyltestosterone on gonadal development in lamprey eggs, embryos and larval lampreys.

Materials and Methods

During the summer of 1994, an incubation stream was setup with battery jars and airstones (see enclosed diagram-Page 6). This in addition to the aquarium system was a major cost expenditure of the funds. The incubation stream served as a temperature control unit with recirculating water.

Adult sea lampreys were collected during their upstream migration from the sea from a trap located at the end of the salmon ladder on the Cocheco River in Dover, New Hampshire in May and June of 1994. When lampreys was observed spawning, they were removed from the spawning channel and artificially spawned. Eggs were placed in 8-L glass battery jars (225 mm in diameter) containing 3 L of well water and immersed in a constant temperature water bath at 18°C, the optimum temperature for development of sea lamprey embryos. Treatments were on day 6 following fertilization and were as follows: (1) control; (2) 0.01 mg/L 17ß-estradiol; (3) 0.10 mg/L 17ß-estradiol; (4) 0.005 mg/L 17α-methyltestosterone and (5) 0.05 mg/L 17α- methyl-testosterone. Each treatment was replicated. Lamprey eggs were sorted and counted to give 250 eggs in each of eight battery jars. Several large rocks were also placed in the battery jars to prevent

the eggs from clumping. The eggs were incubated with steroid solutions for two hours with airstones. Stock solutions of the hormones were prepared in absolute ethanol and aliquots of these solutions were added to the battery jars. After this time the water was removed and placed into designated waster containers and replaced with fresh well water. The eggs were checked for any mortalities daily. Within three days, almost all lampreys treated with methyltestosterone had died. By day 10, one entire treatment of estradiol-treated embryos had died. Based on Dr. Docker's earlier work, we suspect that the embryos died from exposure to the steroids. By mid-fall, only control larvae lampreys had survived and the experiment was terminated. Gonadal histology was attempted but the lampreys were too young to identify gonadal sex.

CONCLUSIONS:

In summary, the experiments did not work. By mid-fall, as already explained, only control larvae lampreys had survived and the experiment was terminated.

Overall, we would propose that these types of sterilization experiments are not viable in lampreys for the following reasons:

1. The problems with the containment of steroid and steroid wastes. The use of steroids in a hatchery situation requires specialized holding and disposal units at a high cost. This was an unanticipated problem--trying to contain these steroid wastes in specialized drums at a fish hatchery is not easy. The costs of disposal at a regular fish hatchery if the steroids were used would be very high.

2. The high mortalities of embryos that occurred when exposed to steroids. Dr. Docker (1992) has also noted high mortalities in lamprey larvae when she treated with 17α-methyltestosterone. Further work would have to be done on doses and length of exposure to minimize the mortalities that could occurred when the embryos are exposed to steroids.

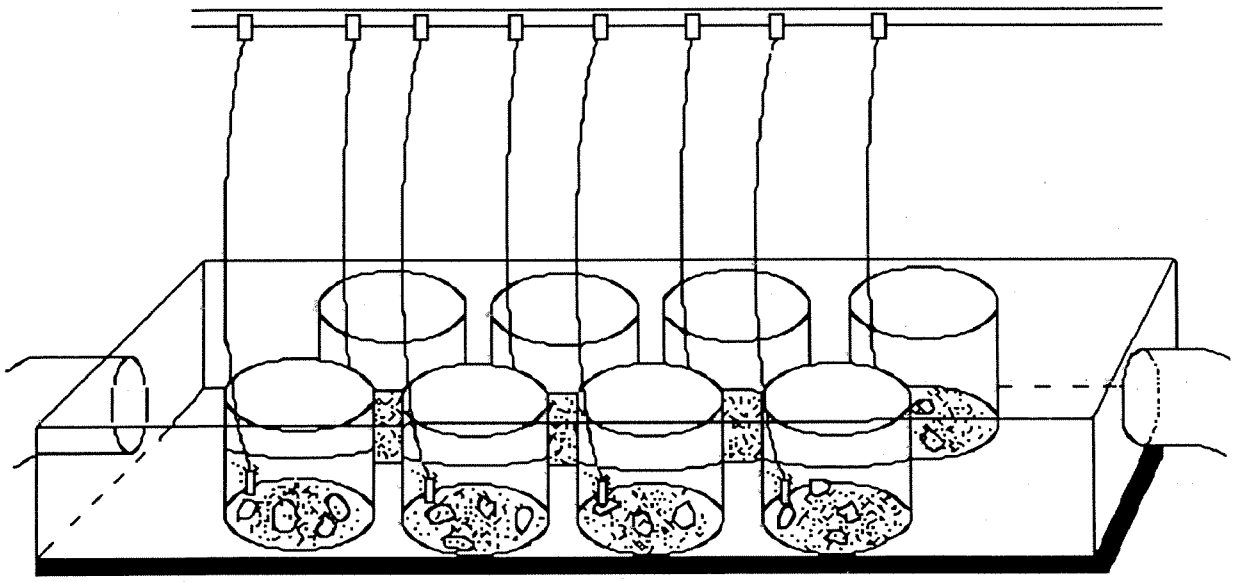
3. The safety of the people has to be considered when handling the steroids. Employees handling the steroids need to be in specialized clothing, need to wear two to three layers of gloves as well as use of face masks.

4. The eventual permits that will have to be obtained for use of steroids in hatchery situations which would be an expensive and very time consuming process. Currently, steroids have not been approved for use in fish aquaculture in the United States. Some steroids have been approved on a limited basis in Canada.

BUDGET:

Most of the funds were used. The major costs included the battery jars, the incubation stream setup, the aquarium system setup, transport of adult lampreys to the Fish Hatchery (included rental of a van and student labor) and student labor to maintain the adult lampreys and the embryos at the hatchery. Other costs included the steroids, histology supplies and disposable glassware, gloves and pipettes for handling the steroids.

EXPERIMENTAL DESIGN



Treatments:

(1) control

(2) 0.01 mg/L 17ß-estradiol

(1) 0.10 mg/L 17ß-estradiol

(2) 0.005 mg/L 17α-methyltestosterone

(2) 0.05 mg/L 17α-methyltestosterone

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