

GREAT LAKES FISHERY COMMISSION

1988 Project Completion Report¹

The Use of Lamprey Gonadotropin-Releasing Hormone and its Analogues to Control Reproduction in the Sea Lampreys

by:

S. A. Sower²

²Department of Zoology
University of New Hampshire
Durham, New Hampshire 03824

August 1988

¹Project completion reports of Commission-sponsored research are made available to the Commission's Cooperators in the interest of rapid dissemination of information that may be useful in Great Lakes fishery management, research, or administration. The reader should be aware that project completion reports have not been through a peer review process and that sponsorship of the project by the Commission does not necessarily imply that the findings or conclusions are endorsed by the Commission.

Also see the following peer-reviewed publications associated with this research:

Sower, S. A. 1989. Effects of lamprey gonadotropin-releasing hormone and analogues on steroidogenesis and spermiation in male sea lampreys. *Fish Physiol. Biochem.* 7:101-107.

Fahien, C. M., and S. A. Sower. 1990. Relationship between brain gonadotropin-releasing hormone and final reproductive period of the adult male sea lamprey, *Petromyzon marinus*. *Gen. Com. Endocrinol.* 80:427-437.

GREAT LAKES FISHERY COMMISSION
Research Completion Report *

THE USE OF LAMPREY GONADOTROPIN-RELEASING HORMONE AND ITS
ANALOGS TO CONTROL REPRODUCTION IN THE SEA LAMPREYS

by

Dr. Stacia A. Sower
Department of Zoology
University of New Hampshire
Durham, New Hampshire 03824

August, 1988

* Project completion reports of Commission-sponsored general research are made available to the Commission's cooperators in the interests of rapid dissemination of information which may be useful in Great Lakes fishery management, research or administration. The reader should be aware that project completion reports have not been through a peer review process and that sponsorship of the project by the Commission does not necessarily imply that the findings or conclusions contained in the report are endorsed by the Commission.

SUMMARY

OVERALL SUMMARY:

The overall research program has been to investigate lamprey gonadotropin-releasing hormone and its analogs to determine if these hormones could be used to inhibit or control reproduction in lampreys as a complimentary method in regulating the population of sea lampreys in the Great Lakes Region. During the past few years, my laboratory has made substantial and significant progress in understanding the neuroendocrine control of reproduction in lampreys. This information and further studies have indicated a potential complementary method for control of sea lampreys by sterilization of male sea lampreys using a lamprey GnRH antagonist. The antagonist would provide a cost effective (less than 3 cents per lamprey) and efficient method (one injection of GnRH attached to a carrier molecule which would allow slow release of this molecule in the lamprey for approximately 2 months) for sterilizing male lampreys and releasing them for a sterile release program. Additional advantages include that this compound is non-toxic to humans and other organisms and is easy to synthesize. The clinical and practical use of GnRH analogs in mammals has expanded very rapidly in the past few years such that GnRH analogs are being used for conceptive, contraceptive measures and for chemotherapy for some cancers. It would take an additional two-three years to develop the optimal GnRH antagonist that would prove to be the most effective compound as a sterilant in lampreys. The potential use of a lamprey GnRH antagonist as a sterilant for a lamprey release program is feasible and further study is recommended.

1. **OBJECTIVE:** To determine the effects of administered lamprey GnRH and its analogs on steroidogenesis, spermatogenesis, and spawning behavior in adult male lampreys.

RESULTS:

a. INDUCED SPERMATION AND STEROIDOGENESIS EXPERIMENT: Two successive injections of lamprey GnRH or [D-Ala⁶,Pro⁹-NH₂] mammalian GnRH stimulated plasma estradiol and progesterone levels in adult male lampreys undergoing final maturation. In these experiments, a lamprey GnRH putative antagonist ([D-Phe^{2,6},Pro³]lamprey GnRH)

stimulated plasma estradiol but not progesterone levels. Four successive injections of lamprey GnRH stimulated plasma progesterone and spermiation. Lamprey GnRH antagonist at 0.1 or 0.05 $\mu\text{g/g}$ depressed plasma progesterone levels and inhibited spermiation. In contrast, a lamprey GnRH analog, [D-Ala⁶,Pro⁹-OH] lamprey GnRH, at 0.1 or 0.05 $\mu\text{g/g}$ stimulated plasma progesterone levels and inhibited spermiation. In summary, lamprey GnRH is biologically active in stimulating the pituitary-gonadal axis in male adult lampreys as determined by steroidogenesis and spermiation. No differences in percent survival of larvae to stage 15 were noted among treatment groups. However, this part of the study needs to be repeated using control temperature incubation channels which were not available for the 1987 studies.

b. STEROIDOGENESIS, GNRH AND SPERMATOGENESIS EXPERIMENT:

Plasma levels of estradiol, progesterone and total brain GnRH were determined in relation to the reproductive state of male sea lampreys during their upstream spawning migration from the sea to coastal rivers and spawning grounds. Samples were collected at fish ladders on coastal rivers, spawning grounds, and an artificial spawning channel at the University of New Hampshire, which contained lampreys that were transferred from the fish ladders. In lampreys from the natural streams, GnRH was elevated at the beginning of the sampling period and increased when the testes contained 1⁰ and dividing 1⁰ spermatocytes or spermatids and immature sperm. Estradiol significantly increased when the lampreys were spawning and increased when the testes contained 1⁰ spermatocytes through spermatids or immature sperm. Progesterone levels were low or undetectable in most lampreys, however an increase was detected when the testes contained mature sperm.

c. SPAWNING BEHAVIOR EXPERIMENT: The lamprey GnRH antagonist stimulated earlier spawning activity in male lampreys compared to controls. The lamprey agonist inhibited spawning activity and lamprey GnRH delayed spawning activity in adult male lampreys. Sperm from some of the treated lampreys were tested for viability using eggs from control females. Survival of the larvae to stage 15 was less than 17% in the GnRH antagonist group compared to over 35% for controls. Again, however, this part of the study needs to be repeated using control temperature incubation channels.

2. OBJECTIVE: To determine the effects of lamprey GnRH and a lamprey GnRH antibody in parasitic phase lampreys to determine biological activity and induction of potential immunoneutralization.

RESULTS: Similar to the results of the previous year, lamprey GnRH significantly reduced plasma estradiol compared to controls. This is in sharp contrast to the response noted in adult spawning lampreys. In contrast to the results of the previous year, the lamprey GnRH antibody increased plasma progesterone while lamprey GnRH reduced plasma progesterone compared to controls. These studies add further evidence that the hypothalamus appears to have different mechanisms of control of the pituitary-gonadal axis in the parasitic phase lampreys compared to the adult lampreys.

EXPERIMENTS

1. Effects of lamprey gonadotropin-releasing hormone and analogs on steroidogenesis and spermiation in male sea lampreys. See enclosed manuscript. Not included in the manuscript is information on the fertilization and survival of larvae from the treated male lampreys from the spermiation experiment. Eggs from control females were fertilized with sperm from the treated males and incubated in incubation jars in ambient temperature tanks to determine survival of the larvae to stage 10s and 15s. There were no significant differences between the percent survival of the larvae from the treated males versus the controls (Fig 1a). Survival of all larvae from treated groups by stage 15 was below 30%. There were many difficulties in maintaining the larvae in the facilities provided. This part of the study needs to be repeated using control temperature incubation channels which were not available for the 1987 studies.

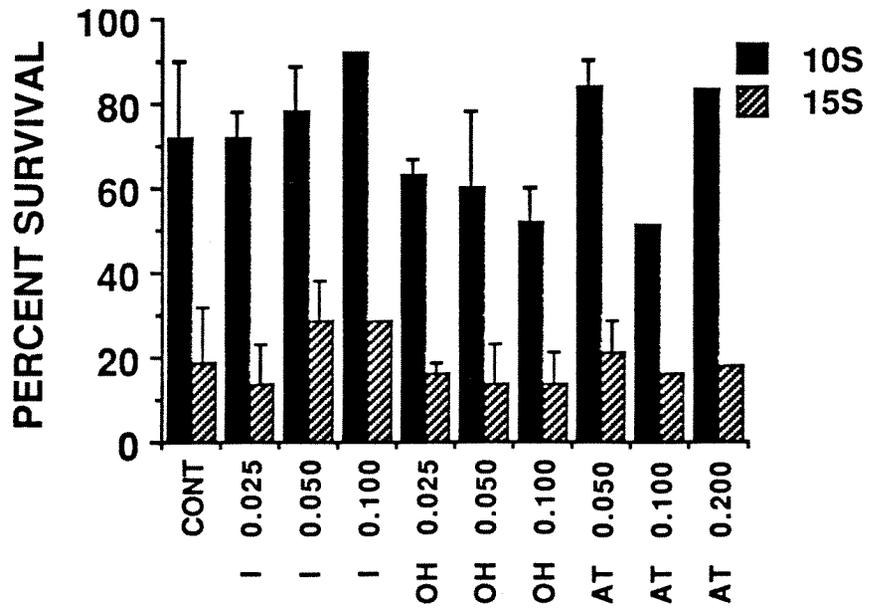
2. Changes in brain GnRH, and plasma estradiol, and progesterone of male sea lampreys, *Petromyzon marinus*, during their upstream spawning migration. See enclosed manuscript.

3. Lamprey Spawning Behavior Experiment:

The purpose of this study was to test the effects of lamprey GnRH and analogs on spawning behavior male lampreys. These experiments were conducted at Hammond Bay Biological Station. Four groups of 12 sea run lampreys each were injected on June 2, 5, 23, and 25 with saline; lamprey GnRH (0.1 $\mu\text{g/g}$ lamprey), lamprey GnRH agonist (0.1 $\mu\text{g/g}$) or lamprey GnRH antagonist (0.1 $\mu\text{g/g}$). After the second injection, the lampreys were introduced into the stream channel and behaviors of spawning activity, nest building and fanning were monitored. The lampreys were observed for 5-6 hrs daily on a continuous basis until July 11. Males treated with lGnRH antagonist showed earlier spawning activity compared to controls with little or no spawning activity after the initial surge of activity (Fig.

2). Lamprey GnRH agonist inhibited the spawning activity compared to controls and lamprey GnRH appeared to delay spawning activity compared to controls (Figs. 2 and 3). Two of the male lampreys each of a different treatment were used for a small fertilization study. Survival of the larvae to stage 15 was less than 17% in the GnRH antagonist group compared to over 35% for controls. ^(Fig 1b) Again, however, this part of the study needs to be repeated using control temperature incubation channels. These data indicate that lamprey GnRH and analogs influence behavior, either directly or indirectly, in lampreys. Additionally, these data indicate that GnRH antagonist stimulated earlier spawning activity in males compared to controls and that this antagonist induced lower survival rates of the larvae compared to controls. In mammals, it has become very clear that GnRH analogs are being used for sterilization, conception and other therapeutic and clinical applications. Further information is needed on the basic biological actions of lamprey GnRH as well as further studies on the actions of variant lamprey GnRH antagonist molecules as potential sterilants. Further studies on other lamprey GnRH antagonist molecules with various amino acid substitutions show promise for the eventual use of sterilizing the lampreys as a potential management tool. The potential of using GnRH analogs is exciting since these various antagonists like GnRH are proteins, easily degraded within the body, nontoxic to humans and easy to synthesize.

FIG 1a



1b

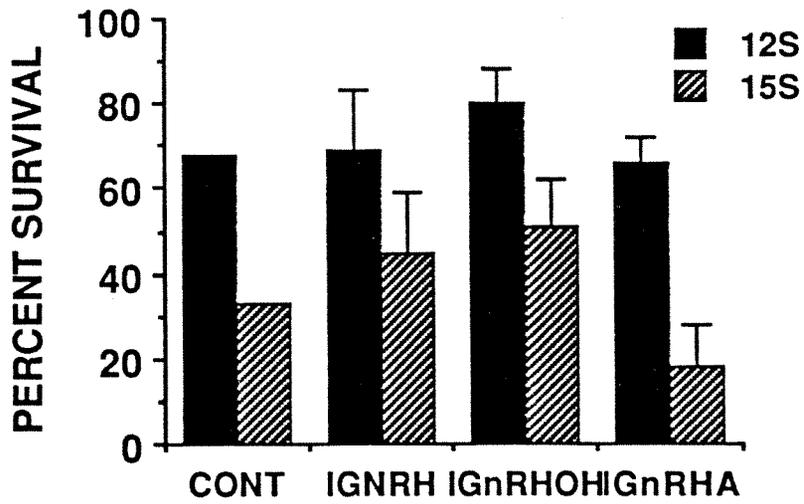


Fig 3

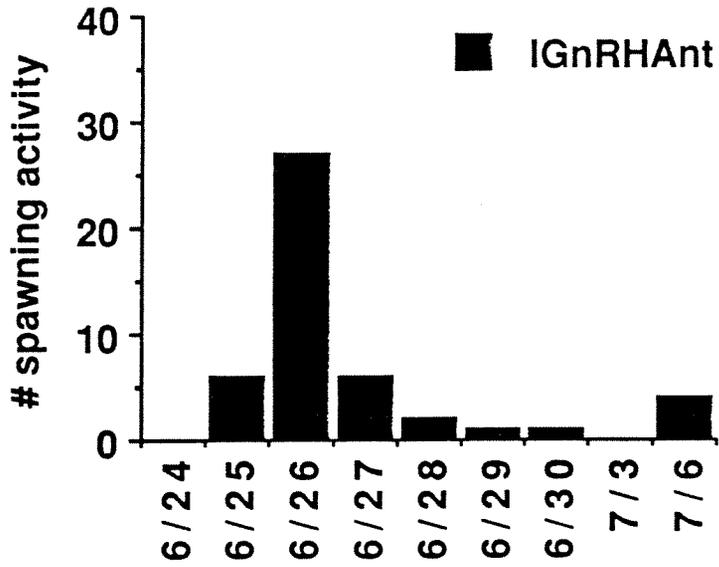
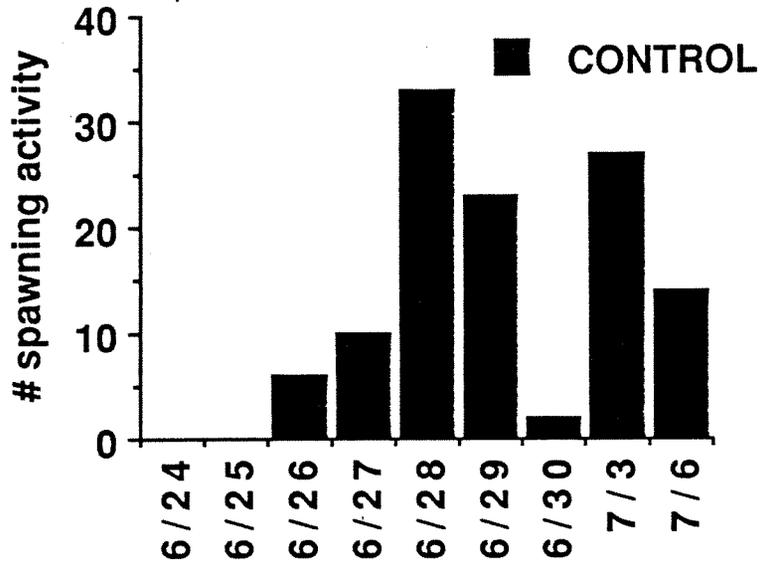
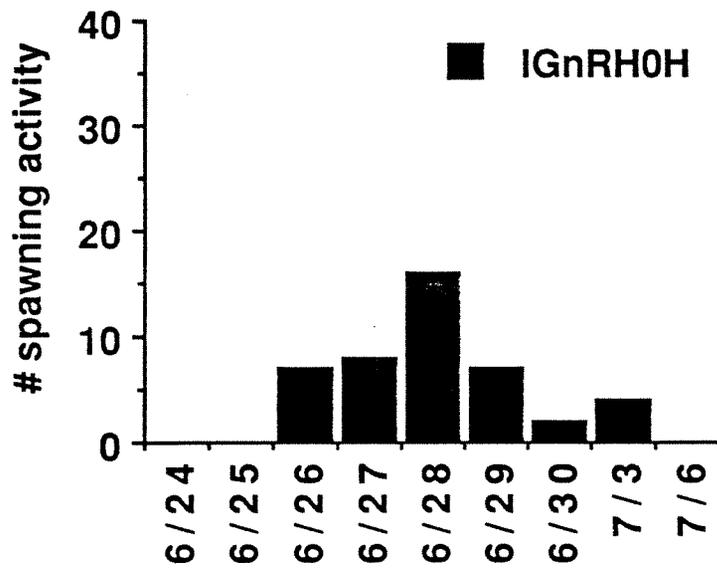
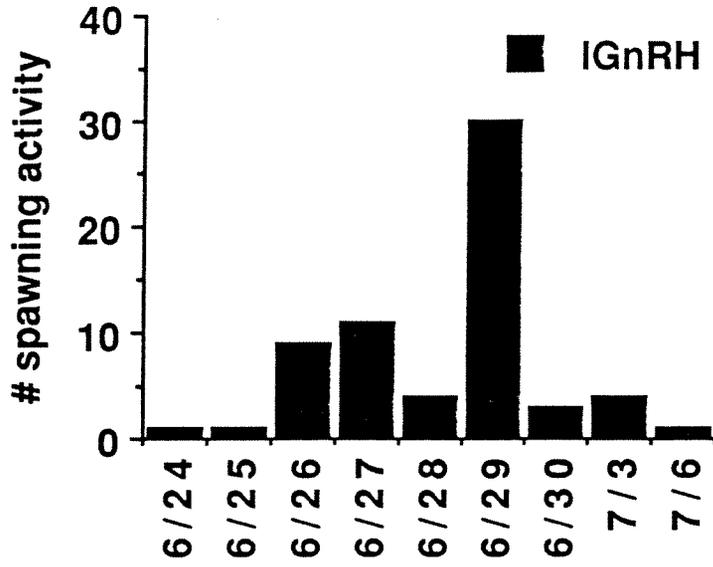


Fig 4



4. Parasitic Phase Lamprey Experiments

The purpose of this study is to determine the role of the hypothalamic-pituitary-gonadal axis in parasitic phase lampreys. It is very important to understand the role of neuroendocrine system in other phases of lampreys particularly the earlier stages to fully understand the development and role of the hypothalamus during the life cycle of the sea lamprey.

Three groups of nine parasitic phase lampreys were each injected with Normal rabbit serum (control), lamprey GnRH (5 µg/lamprey) or with lamprey GnRH antibody. The objective was to determine if the lamprey GnRH antibody can effectively immunoneutralize the reproductive system. These experiments were done at Hammond Bay Biological Station. Lampreys were injected June 30, 1987 at 8:00 am and 8:00 pm. Blood samples were taken the following morning at 8:00 am by cardiac puncture. The blood was centrifuged and the plasma collected until analyzed for progesterone and estradiol. The results are as follows:

Table 1: Progesterone and estradiol levels of parasitic phase lampreys treated with normal rabbit serum (control), lamprey GnRH or lamprey GnRH antibody.

TREATMENT	Estradiol (pg/ml; $\bar{X} \pm SE$)	Progesterone (pg/ml; $\bar{X} \pm SE$)
Control (NRS)	12 \pm 4	98 \pm 24
lamprey GnRH	8 \pm 1	54 \pm 24
GnRH antibody	ND	129 \pm 18

Similar to the results of the previous year (Annual Report, 1987), lamprey GnRH significantly reduced plasma estradiol compared to controls. This is in sharp contrast to the response noted in adult spawning lampreys. In contrast to the results of the previous year, the lamprey GnRH antibody increased plasma progesterone while lamprey GnRH reduced plasma progesterone compared to controls. These studies add further evidence that the hypothalamus appears to have different mechanisms of control of the pituitary-gonadal axis in the parasitic phase lampreys compared to adult lampreys.

ACKNOWLEDGEMENTS:

Summer technicians: Clyde Barr, Lisa Gleason
Laboratory technicians: Linda Penney Jane Calvin
Graduate Student: Cathy Fahien

EFFECTS OF LAMPREY GONADOTROPIN-RELEASING HORMONE
AND ANALOGS ON STEROIDOGENESIS AND
SPERMIATION IN MALE SEA LAMPREYS

Stacia A. Sower

Department of Zoology
University of New Hampshire
Durham, NH 03824

Key Words: lamprey; GnRH; spermiation; steroidogenesis; reproduction

Address all correspondence and requests for reprints to:

Dr. Stacia A. Sower
Department of Zoology
University of New Hampshire
Durham, NH 03824

ABSTRACT

EFFECTS OF LAMPREY GONADOTROPIN-RELEASING HORMONE AND ANALOGS ON STEROIDOGENESIS AND SPERMATION IN MALE SEA LAMPREYS

Stacia A. Sower

Department of Zoology, University of New Hampshire, Durham, NH 03824 USA

The biological activities of lamprey GnRH and analogs were determined in the adult male lamprey, Petromyzon marinus. Two successive injections of lamprey GnRH or mammalian (D-Ala⁶, Pro⁹-NEt) GnRH stimulated plasma estradiol and progesterone levels in adult male lampreys undergoing final maturation. In these experiments, a lamprey GnRH putative antagonist ([D-Phe^{2,6}, Pro³] GnRH) stimulated plasma estradiol but not progesterone levels. Four successive injections of lamprey GnRH stimulated plasma progesterone and spermiation. Lamprey GnRH antagonist at 0.1 or 0.05 µg/g depressed plasma progesterone levels and inhibited spermiation. In contrast, lamprey GnRH analog ([D-Ala⁶, Pro⁹-OH] lamprey GnRH) at 0.1 or 0.05 µg/g stimulated plasma progesterone levels and inhibited spermiation. In summary, lamprey GnRH is biologically active in stimulating the pituitary-gonadal axis in male adult lampreys as determined by steroidogenesis and spermiation.

CHANGES IN BRAIN GNRH, AND PLASMA ESTRADIOL-17 β ,
AND PROGESTERONE OF MALE SEA LAMPREYS, PETROMYZON MARINUS,
DURING THEIR UPSTREAM SPAWNING MIGRATION

Catherine M. Fahien and Stacia A. Sower¹

Department of Zoology
University of New Hampshire
Durham, New Hampshire 03824

Running Title: GnRH and Steroids in Male Lampreys

¹Present Address: Department of Zoology, University of New Hampshire,
Durham, NH 03824

Send Proofs and All Correspondence to: Dr. Stacia A. Sower
Department of Zoology
University of New Hampshire
Durham, NH 03824

ABSTRACT

Plasma levels of estradiol-17 β , progesterone, and total brain GnRH were determined in relation to the reproductive state of male sea lampreys, Petromyzon marinus, during their upstream spawning migration from the sea to coastal rivers and spawning grounds. Samples were collected at fish ladders on coastal rivers, spawning grounds, and an artificial spawning channel at the University of New Hampshire, which contained lampreys that were transferred from the fish ladders. In this study, concentrations of brain GnRH and plasma steroids changed significantly through time and as spermatogenesis progressed. In lampreys from the natural streams, GnRH was elevated at the beginning of the sampling period, and increased when the testes contained 1 $^{\circ}$ and dividing 1 $^{\circ}$ spermatocytes or spermatids and immature sperm. Estradiol significantly increased when the lampreys were spawning and increased when the testes contained 1 $^{\circ}$ spermatocytes through spermatids or immature sperm. Progesterone levels were low or undetectable in most lampreys, however an increase was detected when the testes contained mature sperm. In lampreys from the artificial spawning channel, GnRH significantly increased at the end of the sampling period and was slightly elevated when the testes contained immature sperm. Estradiol significantly increased when the lampreys were spawning and was elevated when the testes contained immature and/or mature sperm. Progesterone was again low or undetectable in most of the lampreys, however a slight increase was detected when the testes contained a mixture of immature and mature sperm. In lampreys from the natural streams and the artificial spawning channel the elevation of brain GnRH preceded the elevation of plasma steroids, which provides further evidence for the role of a hypothalamo-hypophysial-gonadal axis in adult sea lampreys.