GREAT LAKES FISHERY COMMISSION

1986 Project Completion Report[1]

The Use of Hormone and their Analogues to Control Reproduction in the Sea Lampreys

by:

Stacia A. Sower[2]

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

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(with attached manuscripts)

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PRINCIPAL INVESTIGATOR:

Stacia A. Sower
Assistant Professor
University of New Hampshire
Durham, NH 03824

ACKNOWLEDGEMENTS:

Supplied the peptides: Dr. Dan Marshak (NIH)
Summer technician: Clyde Barr (Michigan)
Technicians: Cindy Burne and Sue Charpentier (UNH)
Graduate student: Jane Linville (UNH)

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SUMMARY (a)

1. The biological activities of lamprey GnRH, a mammalian GnRH superagonist ([D-Ala^6, Pro^9 NEt] GnRH) and a lamprey GnRH antagonist ([D-Phe^{2,6}, Pro^3] lamprey GnRH were determined in male and female lamprey in two different reproductive stages. Lamprey GnRH is biologically active in stimulating the pituitary-gonadal axis as determined by steroidogenesis and an ovulatory response and is dependent on the stage of maturation.

2. The mammalian GnRH superagonist ([D-Ala^6, Pro^9 NEt] GnRH) stimulated the occurrence of the spawning behavior in male and female lampreys. The mammalian GnRH antagonist ([Ac^3-Pro, HFD Phe^3, D-Trp^{3,6}] GnRH) inhibited the occurrence of the spawning behavior in male and female lampreys.

SUMMARY (b)

The information from these studies support the concept of brain control over reproductive activity in the lamprey. However, further experiments are needed to determine the proper dosage, timing of treatments, and the role of temperature, to develop methods for possible control of the population of the sea lamprey.

Possible methods for lamprey control based on these data include:

1. The lamprey GnRH decapeptide has only 50% homology with mammalian and chicken I GnRH, and 60% homology with salmon and chicken II GnRH. Due to the striking structural differences of lamprey GnRH compared to the other vertebrate GnRH, the administration of a GnRH antagonist to lampreys in streams to inhibit their reproductive processes without affecting other vertebrates may be possible. The use of a GnRH antagonist would be feasible in streams since it is a small peptide and would degrade rapidly.

2. Alternatively, male lampreys could be injected with lamprey GnRH to induce early maturation and then these lampreys could be irradiated for the sterilization program (Lee Hanson). Lee Hanson has indicated if the lampreys are irradiated too early in their reproductive cycle, these males do not seem to be completely sterilized. Thus, this method could be complementary to the proposed sterilization program, which includes the release of sterilized males into streams where they would spawn with females but the eggs would not be properly fertilized.

3. Or, early in the season, a small number of male and female lampreys could be injected with lamprey GnRH to accelerate their maturational processes. These matured lampreys could then be placed in a river to attract other lampreys which could aid in increasing trapping efficiency (Jim Seeyle).

Our overall research program has been to identify the structure of lamprey gonadotropin-releasing hormone (GnRH, 1984-1985), and then to investigate lamprey GnRH and its analogues (1985-1986) to determine if they could inhibit or control reproduction and metamorphosis in the sea lamprey as a complimentary and/or alternative method in regulating the population of sea lampreys in the Great Lakes.

Specifically, the objectives included:

1985-1986:

1) To test the synthetic lamprey GnRH and its antagonist in adult lampreys to determine biological activity and to determine if the antagonist effectively inhibits reproduction.

2) To test the synthetic lamprey GnRH and its antagonist in metamorphosing lampreys to determine biological activity and to determine the effects of the antagonist on the metamorphosis of the sea lamprey.

3) To test the effects of GnRH antagonist on spawning behavior of adult lampreys.

OBJECTIVES 1 AND 2

A) See enclosed manuscript (I)

B) Ovulatory response to lamprey GnRH and a lamprey GnRH antagonist

The purpose of this study was to determine the effects of the lamprey GnRH and a potential lamprey GnRH antagonist (D-Phe^{2,6}-Pro^{3} lamprey GnRH) on ovulation in female lampreys that were approximately 1 month from their normal ovulatory period. The lampreys were treated with two injections or a single injection to determine if there is a potential priming action by GnRH in the lamprey as demonstrated in teleosts (Peter, 1980; Sower et al., 1984b) or in mammals (Chappel et al., 1983).

The treatment regimes are summarized in Table 1.

The female lampreys were checked every other day for a period of 30 days to determine if they had ovulated as judged by external physical characteristics as described in my earlier studies in Sower et al., 1983. The lampreys were killed on the day they had ovulated. At day 12, ovulation had occurred in 80% of the lampreys treated with either two single injections or single injections of lamprey GnRH at 200 or 100 (Table 2). Accumulative ovulation in the lampreys decreased in a dose-related manner. The control lampreys had ovulated by day 20 and the lampreys treated with all the various doses except at the lowest dose (1.5 ug/kg) of antagonist did not ovulate by day 29 or 30 when all lampreys were killed.

The lamprey GnRH analogue (D-Phe^{2,6} Pro^{3} lamprey GnRH) used in this experiment clearly inhibited the ovulatory process demonstrating GnRH's regulatory influence in reproductive processes. These data provide further

evidence that receptors for GnRH are specific and can distinguish between molecular variants of this peptide.

C) Immersion experiment

The purpose of this study was to determine if lamprey GnRH and the lamprey GnRH antagonist can effect steroid production by immersing the adult females in water containing the peptide(s). Four groups of 3 lampreys each were immersed in aerated lake water daily for 3 days for 1 hr. The water temperature was 60°. Each group was immersed in 3L of water in battery jars. Immediately before the immersion, the peptides were added to the water. The lampreys were sampled for plasma on the third day immediately following the last immersion treatment. The results were not significant due to the few animals tested. However, plasma estradiol was higher in those females treated with the lamprey GnRH and lower in those females treated with the lamprey GnRH antagonist.

Table 3. Plasma estradiol of female lamprey immersed in water containing one of three peptides.

Treatment (ug/3L H_2O)	Plasma Estradiol ng/ml	(n)
Control	1.4	(1)
GnRHa (1500)	1.46 ± 0.30	(3)
lGnRH (2500)	1.60 ± 0.02	(2)
Ant GnRH (2500)	1.07	(1)

More experiments are needed to examine the dosage of peptide and immersion time utilizing higher numbers of lampreys to determine if indeed the peptides can be absorbed by the gill pouches as indicated in this experiment.

OBJECTIVE 3

A) Effects of mammalian GnRH superagonist and antagonist on a specific spawning behavior

The purpose of this study was to test the effects of GnRH antagonists on spawning behvior of adults. These experiments were conducted at the AFAIRS Building (UNH). Three groups of 12 sea run lampreys each were injected two times with saline; the mammalian GnRH superagonist ([D-Ala^6, Pro^9 NEt] GnRH) at 50 ug/kg; or the mammalian GnRH antagonist ([Ac^3 Pro, HFD

Phe^{3}, D-Trp^{3,6}] GnRH) at 50 ug/kg. After the second injection, the lampreys were introduced into the artificial stream channel and behaviors monitored similar to the methods described in Linville, Hansen, and Sower (submitted, appendix II). The lampreys were observed for 10 minute periods over 1/2 hour for 2 hr periods 3 to 4 times daily. The treatment of GnRH superagonist to male and female lampreys significantly increased the occurrence of the spawning act compared to controls (Figs. 1 and 2). The lampreys (both males and females) injected with the mammalian GnRH antagonist showed no occurrence of spawning act compared to slight activity in the controls. These data indicate that there is brain control over spawning behaviors.

These studies confirm the use of behavioral studies as bioassays in testing lamprey GnRH antagonists which have potential in controlling the reproductive processes.

Table 1. Treatment regime and schedule.

No. of Lampreys	First Injection Day 0 (ug/kg Lamprey)	Second Injection Day 2 (ug/kg Lamprey)
10	saline (control)	saline (control)
10	[a]GnRHa (50)	GnRHa (50)
10	lamprey GnRH (200)	lamprey GnRH (200)
10	lamprey GnRH (200)	no injection
10	lamprey GnRH (100)	lamprey GnRH (100)
10	lamprey GnRH (100)	no injection
10	lamprey GnRH (50)	lamprey GnRH (50)
10	lamprey GnRH (50)	no injections
10	lamprey GnRH (5)	lamprey GnRH (5)
10	[b]Ant GnRH (300)	Ant GnRH (300)
10	Ant GnRH (150)	Ant GnRH (150)
10	Ant GnRH (75)	Ant GnRH (75)
10	Ant GnRH (1.5)	Ant GnRH (1.5)

[a]GnRHa: mammalian superagonist D-Ala^{6}-Des Gly^{10} ethylamide

[b]Ant GnRH: lamprey GnRH antagonist: D-Phe^{2,6}-Pro^{3} lamprey GnRH

Table 2. Accumulative percent ovulations at day 12 following the injections of female sea lamprey injected with saline on day 0 and 2, lamprey GnRH (lGnRH at 200, 100, 50, or 5 ug/kg) on day 0 and 2; lamprey GnRH (lGnRH at 200, 100, or 50 ug/kg) on day 0; D-Ala^{6}-Des Gly^{10} ethylamide (gnRHa; 50 ug/kg) on day 0 and 2; or Ant GnRH (D-Phe^{2,6} Pro^{3} lamprey GnRH at 300, 150, 75, or 1.5) on day 0 and 2.

1985 SEA LAMPREYS	
	% Ovulation Day 12
Control	0
GnRHa 50/ GnRHa 50	100
1 GnRH 200/ 1 GnRH 200	80
1 GnRH 200/ no injection	100
1 GnRH 100/ 1 GnRH 100	100
1 GnRH 100/ no injection	100
1 GnRH 50/ 1 GnRH 50	50
1 GnRH 50/ no injection	38
1 GnRH 5/ 1 GnRH 5	20
Ant GnRH 300/ Ant GnRH 300	0
Ant GnRH 150/ Ant GnRH 150	0
Ant GnRH 75/ Ant GnRH 75	0
Ant GnRH 1.5/ Ant GnRH 1.5	0

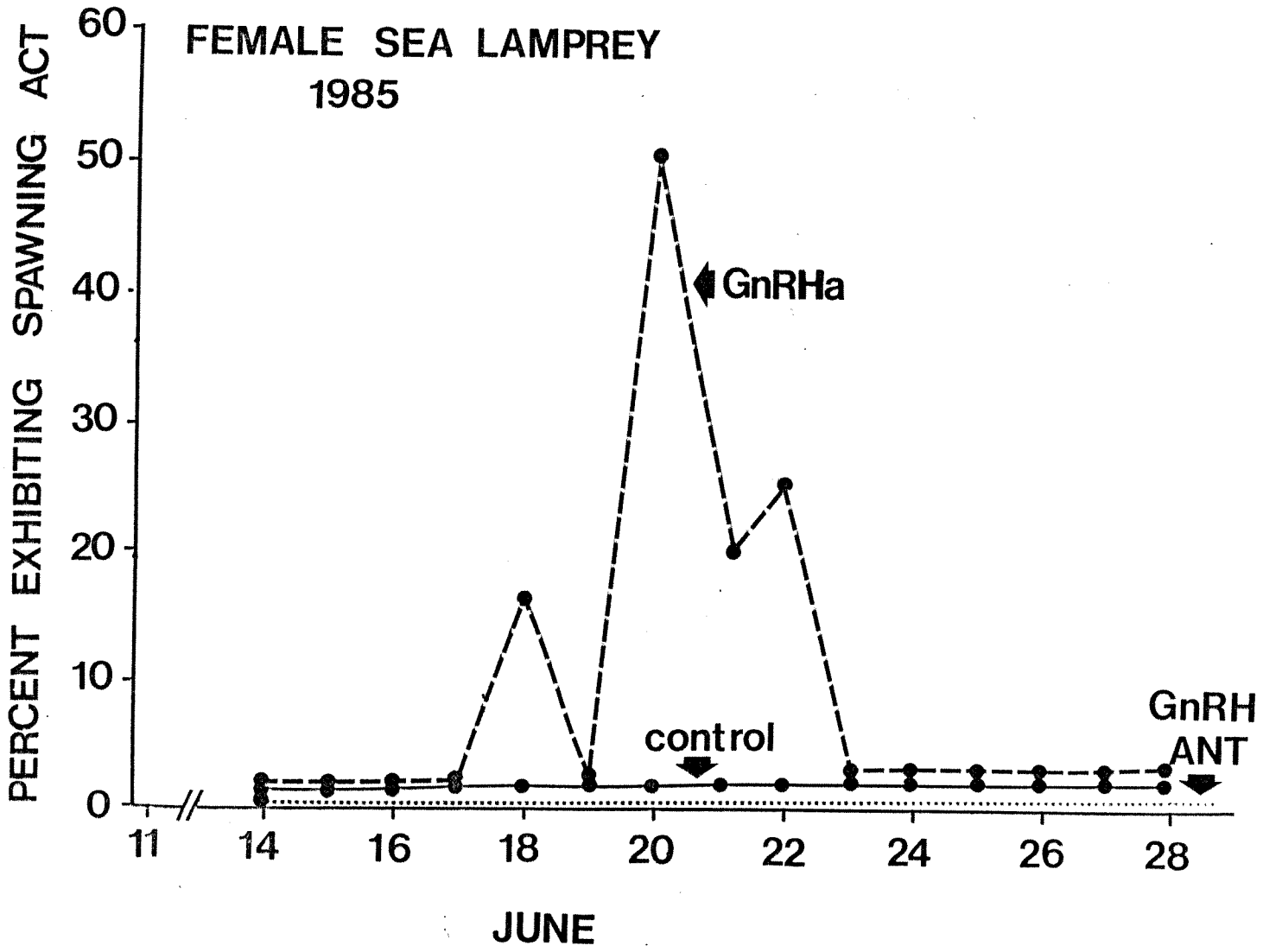
FEMALE SEA LAMPREY
1985
PERCENT EXHIBITING SPAWNING ACT
60
50
40
30
20
10
0
GnRHa
control
GnRH
ANT
11
14
16
18
20
22
24
26
28
JUNE

FIGURE 1

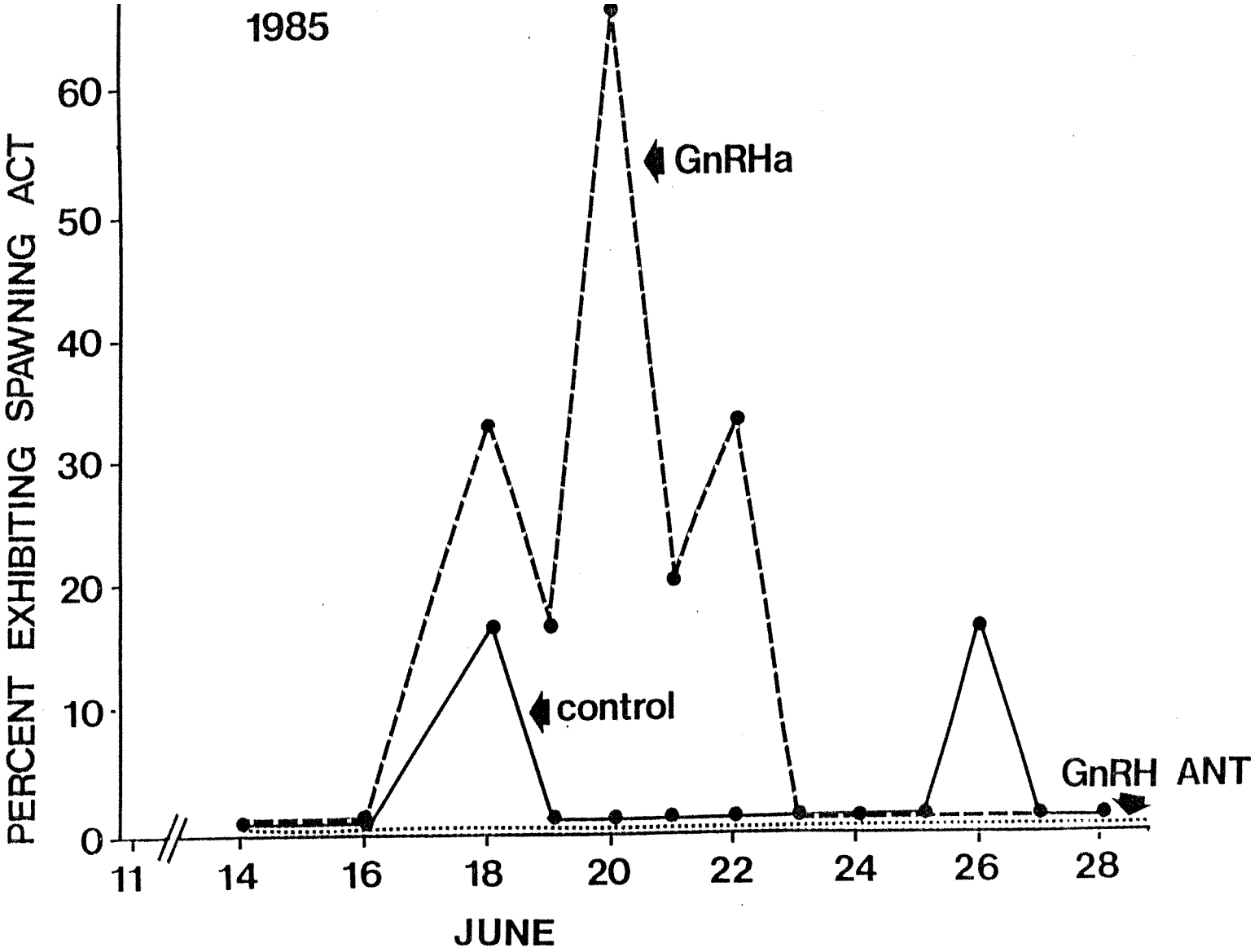
1985
PERCENT EXHIBITING SPAWNING ACT
60
50
40
30
20
10
0
GnRHa
control
GnRH ANT
11
14
16
18
20
22
24
26
28
JUNE

FIGURE 2

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