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Endocrine Control of Lamprey Reproduction

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FINAL REPORT

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SUMMARY

1. Our results provide the first direct experimental evidence of hypothalamic control over pituitary and gonadal function in lampreys as determined by steroidogenesis and induced ovulation following injections of salmon gonadotropin or gonadotropin-releasing hormone analogue.
2. Changes in plasma estradiol and androgens during the final spawning are similar in males and females. As in higher vertebrates, there is in female lampreys a general increase in plasma estradiol prior to ovulation followed by a drop at ovulation. The profile of estradiol changes in males and females during the final maturational period are possibly related, at least in part, to their spawning behavior.
3. The noted increase of plasma estradiol levels following administration of an anti-estrogen compound can be taken as evidence of an estrogen feedback mechanism on the hypothalamo-pituitary axis.
4. Plasma estradiol in both male and female ammocoetes were in some cases as much as five-fold higher than levels noted in the spawning adults. These unusual data can be considered preliminary and in need of confirmation and explanation.
5. The information produced from these studies provide new evidence of endocrine control in the final maturational processes in the reproduction of the sea lamprey. Accordingly, these studies provide a rational basis for development of procedures for endocrine control of metamorphosis of ammocoetes and of reproduction of adults.

GENERAL INTRODUCTION

The goal of our studies has been to increase our understanding of the little explored area of the endocrine regulation of reproduction of lampreys. Information derived from such study promises a basis for future manipulative control of lamprey reproduction as an alternative or supplemental method to those now in use for regulating the population of sea lampreys in the field.

Experiments conducted over the last three years are summarized here and presented as individual experiments (in terms of materials, methods, and results section) described in the final report. The report is followed by an overall discussion section. The experiments were as follows:

Experiments

1. Determination of ovulatory and steroidal responses in the lamprey following administration of salmon gonadotropin and agonistic and antagonistic analogues of GnRH.
2. Observation of changes in plasma steroid and thyroid hormones, IR insulin, PFA, and protein during final maturation and spawning of the sea lamprey.
3. Characterization of the steroid hormone profile following a single injection of salmon gonadotropin and agonistic and antagonistic analogues of GnRH.
4. Determination of the effects of steroid agonists and antagonists, prostaglandin, indomethacin, and benserazide on plasma estradiol and gonads in adult Pacific lamprey and sea lamprey.
5. Conduct of preliminary experiments with ammocoetes.

BACKGROUND

One of the major goals of the Great Lakes Fisheries Commission is the development of an integrated lamprey control program that will continue the use of larvicides, hopefully to a diminishing degree, plus new and supplemental methods that may also prove effective in regulating the population of sea lampreys to desired levels.

Selective toxicants have been used very successfully in reducing lamprey abundance by about 80% in the Great Lakes. The resulting lowered populations of sea lampreys, concurrent with fish stocking programs, have vastly increased the fishery of the Great Lakes from the greatly depleted levels of the 1950′s. However, recommendations made at the Sea Lamprey International Symposium in 1979 presented a reduction in the use of the chemical treatments despite the success of lampricides in reducing lamprey populations. Concerns expressed about long-term chemical treatments included the following: (1) potential developed resistance to the chemicals could reduce the effectiveness of these treatments and/or (2) future regulations and/or societal pressure could prevent the use of the selective toxicants (Lamsa et al., 1980). Thus, continued attempts to seek acceptable, efficient and cost-effective ways to control the sea lamprey in the Great Lakes are now ongoing.

An obvious area of research for alternative methods for lamprey control is the field of endocrinology, particularly in the realm of reproductive endocrinology. Little concrete information exists concerning the actual hormonal processes involved during the reproductive cycles of lampreys. There is almost nothing known of the complex interplay of the hypothalamo-pituitary-gonad axis with external factors, i.e., food, temperature and photoperiod. The success of an alternate lamprey control procedure depends,

at least in part, on understanding of the endocrine mechanisms that regulate phases of the sea lamprey life cycle.

The parasitic sea lamprey, like Pacific salmon, reproduces only once in its lifetime. Thus, gametogenesis progresses synchronously as a single event during the lamprey's life cycle, similar to the Pacific salmon. The Great Lakes form of Petromyzon marinus spawns from mid-May through July, depending on ambient temperature. The eggs hatch and the young ammocoetes leave the nest after several days and bury in the sand. They remain as ammocoetes in the sand approximately 5 1/2 ± 2 years, though exceptions to this timing are known. During the first two larval years, the gonads are in an undifferentiated state. The gonads in all ammocoetes go through an "ovarian" phase. Sexual differentiation then occurs 1-2 years before metamorphosis. The ammocoetes after about 5 years undergo the drastic physiological and morphological changes that are referred to as metamorphosis or transformation. At this time, ammocoetes become parasitic organisms which migrate downstream into the lake or ocean. After 2 years of the parasitic phase, the lamprey migrates upstream in a non-eating phase coinciding with the final sexual maturation process, followed by spawning, and finally death.

The lampreys comprise one of two surviving Agnathan groups that are representatives of the most ancient vertebrates; they are unique and unlike other vertebrates in lacking a hypothalamo-hypophysial portal vascular or innervation system (Gorbman, 1965). There is at this time only limited evidence that the Agnathan hypothalamus exerts any regulatory influence on the adenohypophysis as has been demonstrated in teleosts (Crim et al., 1976; van der Kraak et al., 1983); in elasmobranchs (Deery and Jones, 1974); in amphibians (Mazzi et al., 1974; Thornton and Geschwind, 1974; McCreery et

al., 1982); in birds (van Tienhoven and Schally, 1972; Burke and Cogger, 1977); and, of course, in mammals (Schally et al., 1973). However, Crim et al. (1979) have demonstrated that immunoreactive gonadotropin-releasing hormone (GnRH) is in the preoptico-neurohypophysial system of the Pacific lamprey (_Lampetra tridentata_). This substance, in higher vertebrates, in response to environmental seasonal cues (photoperiod, temperature, etc.), stimulate release of pituitary gonadotropin and thus, in turn, stimulates gonadal development and sex hormone secretion . Since, in petromyzontids reproduction is seasonal (occurring in early summer) and therefore seems to be responsive to environmental cues, hypothalamic control over gonadotropic and gonadal function has seemed to be a real possibility. The adenohypophysis of lampreys has been shown in older limited research to exert some influence on gonadal tissues at various reproductive phases (reviewed by Larsen, 1980). Although there may be regulation of sexual development mediated through the hypothalamo-hypophysial-gonadal axis, such regulation is now only inferred and has yet to be established in the lampreys.

One phase of our study was planned to evaluate the effects of exogenous administration of chemical analogues of GnRH and of salmon gonadotropin on steroidogenesis and ovulation in anadromous lampreys to determine if the completion of the reproductive processes is mediated by elements of the hypothalamo-hypophysial-gonadal axis as in other vertebrates. In this first study of the actions of two synthetic analogues of mammalian GnRH, the biological actions ofGnRHa (gonadotropin-releasing hormone analogue; D-ala^{6}, des Gly^{10}-LH-RH ethylamide), GnRH antagonist (Ac-^{3}Pro, 4 FD Phe^{2}, DTrp^{3,6}-LRF; GnRH ant) or a partly purified coho salmon gonadotropin in the sea lamprey, _Petromyzon marinus_ were tested. Following the administrations of

these compounds, singly or in combination, at different dosages, we monitored the occurrence of ovulatory responses during the lamprey's final spawning phase. In addition, plasma estradiol levels were measured as another indicator of pituitary responsiveness. Gonadotropin(s) have not been isolated from the lamprey pituitary gland, so that no radioimmunoassay of secreted gonadotropins is possible at this time.

Plasma steroids have been identified in the plasma of sea lampreys (Weisbart et al., 1980) and in gonadal tissue (Botticelli et al., 1963; Callard et al., 1980). However, there has been relatively little of the biological actions of sex steroids in lampreys. Replacement with testosterone or estradiol has been found to normalize development in gonadectomized lampreys and this has suggested strongly steroid involvement (Evenett and Dodd, 1963). However, these steroids have not been shown to induce precocious development in intact lampreys. Furthermore, until this year, there have been no studies correlating plasma sex steroid concentrations with the different reproductive stages. We sampled *P. marinus* during the spawning season in 1982 for plasma and gonadal tissue in order to establish the normal sequence of hormone levels during this phase of reproduction. The data from this experiment will provide much needed information concerning the normal cycle of reproductive endocrine processes. This information provides the basis for a better understanding and evaluation of the effects of hormonal inhibitors that were tested in two experiments.

It is clear that the endocrine influences that regulate sexual differentiation are even more complex than those that are involved in adult reproduction. As mentioned, the early stages of gonadal development and sex differentiation are completed during the larval stages of the lamprey

(Hardisty, 1979). Cytoplasmic growth is then slow, and it is only after metamorphosis that final growth of the oocyte and vitellogenesis occurs (Hardisty and Potter, 1971). Normal differentiation of the male gonad involves atresia of the primitive oocytes, with nests of cells remaining. It has not been shown whether the pituitary and/or steroids have any involvement in this phase of sex differentiation of the ammocoete. Hardisty and Taylor (1965) failed to observe sex reversal of undifferentiated ammocoete larvae when they were treated by immersion in estradiol or testosterone solutions.

Sex hormones have been used successfully to induce sex reversal in many fish species (reviewed by Yamamoto, 1969; Harrington, 1974; Yamazaki, 1976). However, in other experiments improper timing or dosages have produced no apparent changes in differentiating gonadal tissue, impairment of gonadal maturation, or sterility. Thus, we studied the actions of steroids and GnRHa in ammocoetes to determine the feasibility and possibility of sex reversal in these animals.

Overall, our experiments were designed to determine various endocrine influences on the final reproductive processes in adult lampreys and on metamorphosis in ammocoetes. These studies would then be the basis for future studies on endocrine control and development of an alternate/additional method in regulating the population of sea lampreys in the field.

Ovulatory and Steroidogenic Responses in the Lamprey Following Administration of Salmon Gonadotropin and Agonistic and Antagonistic Analogues of GnRH

MATERIALS AND METHODS

Hormonal Preparations

Frozen coho salmon pituitaries collected in 1978 at the Fall Creek Hatchery, Oregon, were used to prepare the partly purified salmon gonadotropin (GTH) by ethanol extractions and gel filtration (Sower et al., 1982).

Gonadotropin-releasing hormone analogue, D-Ala^{6}, des Gly^{10}-LH-RH ethylamide, GnRHa, a high potency analogue (reviewed by Donaldson et al., 1981), was otained from Sigma Chemical Company. The GnRH antagonist used was Ac-^{3}Pro^{1}, 4 FD Phe^{2}, DTrp^{3,6}-LRF-(GnRH ant) Rivier et al. (1981a). During the morning of the day when the fish were injected, all peptides were dissolved in 0.6% NaCl in distilled water. They were injected intraperitoneally into each fish. Blood samples (40-600 µl) were collected in heparinized syringes by cardiac puncture (1982) or from the caudal veins (1981). Plasma samples were kept frozen at -20°C until assayed.

1981 Studies

Twenty-two adult landlocked sea lampreys, _Petromyzon marinus_, were sent by air from the Hammond Bay Biological Station, Millersburg, Michigan to Seattle, on 2 June and 6 June, 1981. These animals had been captured in a trap in the Cheboygan River, Michigan on their anadromous spawning migration after their parasitic phase in Lake Huron, about 1 1/2 months before their presumed normal spawning time. The lampreys were maintained in two 0.6 m diameter cylindrical tanks (114 L) supplied with flowing lake (Lake Washington) water. Nineteen of the lampreys were females. The temperature

ranged between 16.6^{o} and 22°C. The specimens used averaged 165 g in body weight.

On 15 June, 1981, the lampreys were anesthetized by immersion in 0.2 g/L of ethyl m-aminobenzoate methanesulfonate (MS-222), and injected in the morning either with a saline solution (control) followed 3 days later by saline or by GnRHa at a dosage of 0.01 mg/fish (day 3). Lampreys were reinjected on day 11 with either saline or GTH. Plasma samples were taken on day 4, 24 hrs after the GnRHa injection. The female lampreys were checked every other day to determine if they had ovulated as judged by external physical characteristics such as softness of the abdominal region and eggs free flowing from the cloaca on application of gentle pressure on the anterior abdominal wall.

The lampreys were killed on the day they were determined to have ovulated. An ovulatory response was considered to have occurred if an individual female had more than 50% of her eggs loose in the body cavity.

1982 Studies

Landlocked sea lampreys were captured in a trap in the Cheboygan River in May, 1982 on their anadromous spawning migration following completion of their parasitic lake phase. They were retained in raceways at the Hammond Bay Biological Station, supplied with flow-through lake water ranging in ambient temperature from 5.5^{o}C to 20°C. Two weeks prior to injection, the lampreys were divided into groups by transfer into five 1.5 m diameter fiberglass cylindrical tanks. The lampreys averaged 230 g in body weight and 41.9 cm in body length. One week before injections, all lampreys were individually identified with Floy tags and, in addition, the dorsal fins were clipped to designate the mode of treatment.

The lampreys were treated as follows:

Number of lampreys	First injection Day 0 Hormone Dosage (µg/kg lamprey)	Second injection Day 2 or 3 Hormone Dosage (µg/kg lamprey)
12	saline (control)	saline
12	GTH (100)	GTH (100)
12	GTH (100)	no injection
12	GTH (100)	GnRHa (50)
12	GTH (100)	GnRHa (5)
12	GTH (100)	GNRHa (0.5)
12	GnRHa (50)	GnRHa (50)
12	GnRHa (50)	no injection
12	GnRHa (5)	GnRHa (5)
12	GnRH ant (50)	GnRH ant (50)
12	GnRH ant (5)	GnRH ant (5)
12	GnRH ant (0.5)	GnRH ant (0.5)
12	GnRH ant (50)	no injection
12	GnRH ant (5)	no injection

The scheduling of the treatment regimes outlined above was as follows:

Phase I	Day 0	22 June	injections
	Day 1	23 June	plasma samples taken
(temp 13°C)	Day 3	25 June	injections
	Day 4	26 June	plasma samples taken
Phase II	Day 0	7 July	injections
(temp 13°C)	Day 2	9 July	injections
Phase III	Day 0	19 July	injections
(temp 18°C)	Day 2	21 July	injections

Each treatment group contained at least 10 females and 2 males. Thus, 168 lampreys were subjected to the designated injection treatments 3 times over a two-month period. Plasma samples were taken 24 hrs after the first injections, as indicated. Plasma samples were also taken when individual fish ovulated or had died. Ovulatory responses were monitored in all groups twice per week by direct examination.

Radioimmunoassay

Plasma estradiol-17β level in blood plasma was measured by radioimmunoassay (RIA) as described by Sower and Schreck (1982). The radioimmunoassay was validated for adult sea lamprey plasma. Comparisons were made of plasma subjected to extraction and partition chromatography on Celite columns (Abraham, 1973) and plasma was extracted twice with diethyl ether (100 μl plasma: 1.5 ml diethyl ether). Since similar values were obtained from both procedures, either extraction was used exclusively. Serial dilutions of extracted lamprey plasma were parallel with the curve of the estradiol-17 β standards. Briefly, 100 μl of plasma samples were extracted twice with diethyl ether; the extract was evaporated to dryness under a nitrogen gas atmosphere and assayed directly for estradiol. Antiestradiol-17β antibody (S-244) was obtained from Dr. G. Niswender (Colorado State University, Fort Collins, CO) and diluted 1:8500 in phosphate buffered saline-gelatin (PG). This assay is highly specific for estradiol-17β . The lower limit of detection was about 6 pg/ml. The antibody binding efficiency ranged between 47 and 57% in the assays. The intraassay and interassay coefficients of variation for the estradiol RIA of lamprey plasma were 3.2% (n = 9) and 13% (n = 10), respectively.

Statistics

The percent ovulation data were analyzed by use of a 2 x 2 contigency table followed by the Bonferonni approach (Neter and Wasserman, 1974). Data for hormone concentrations were analyzed by a Student-Newman-Keuls test after preliminary analysis of variance. In all tests the level of significance for differing groups P was less than 0.05.

RESULTS

1981

By day 11 after the initial injection, 22% of treated lampreys had ovulated; at this time none of the controls had ovulated (Fig. 1). The first spontaneous ovulation of the controls was not until day 21. By day 28, 56% of hormone treated lampreys had ovulated, compared with 22% of the controls. Mortality was about 30%, possibly a consequence of shipment and frequent handling.

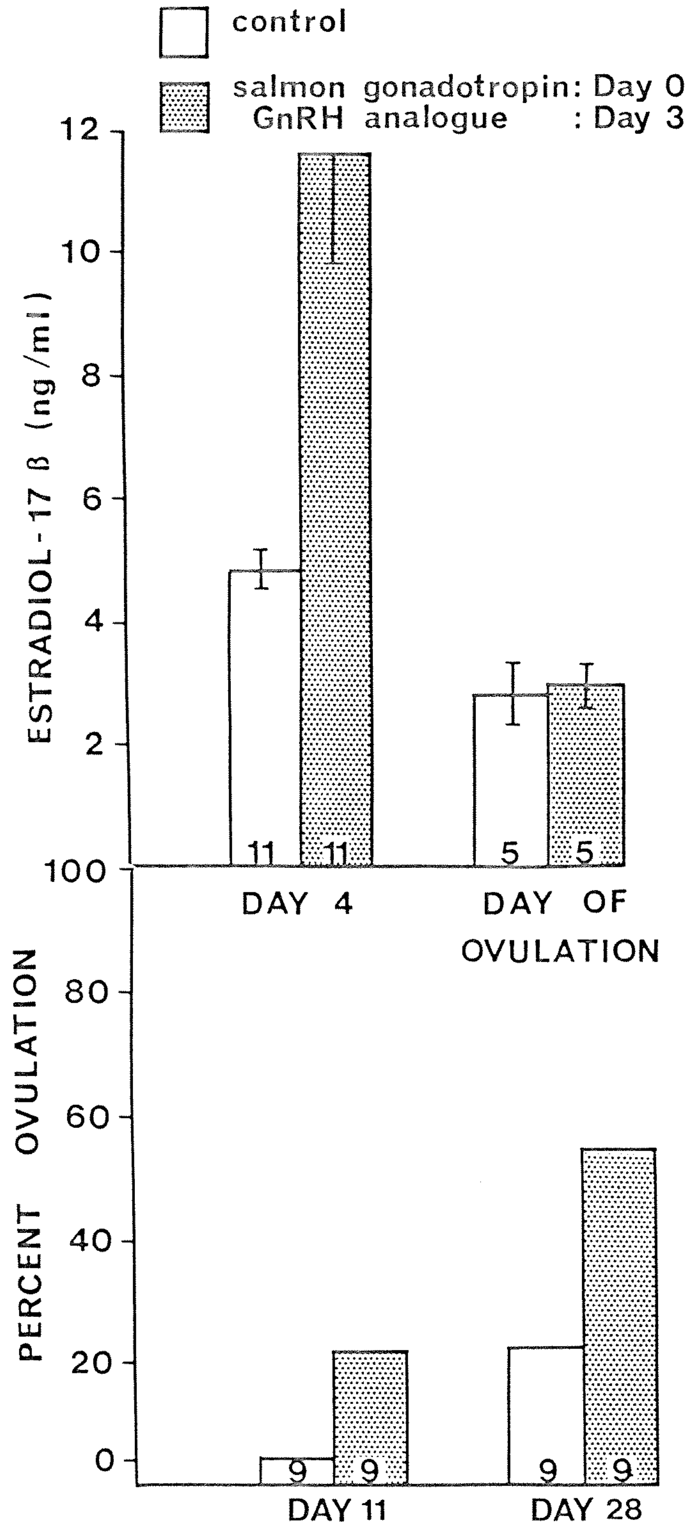
Mean plasma estradiol was significantly elevated in the treated fish, 11.54 ± 1.70 ng/ml, compared to the controls, 4.76 ± 0.29 ng/ml on day 4 (Fig. 1). Plasma estradiol significantly decreased from this level in the controls, and in treated lampreys, but did not differ between groups on the day of ovulation.

1982 Steroid Responses

Plasma estradiol during the first phase of treatment was significantly elevated 24 hrs (day 1) after lampreys were treated twice with salmon gonadotropin compared to controls (Fig. 2). Plasma estradiol remained elevated after a second injection of gonadotropin (4.69 ± 1.08 ng/ml). With no second injection of salmon gonadotropin, plasma estradiol diminished (1.36 ± 0.16 ng/ml) and was not significantly different from controls

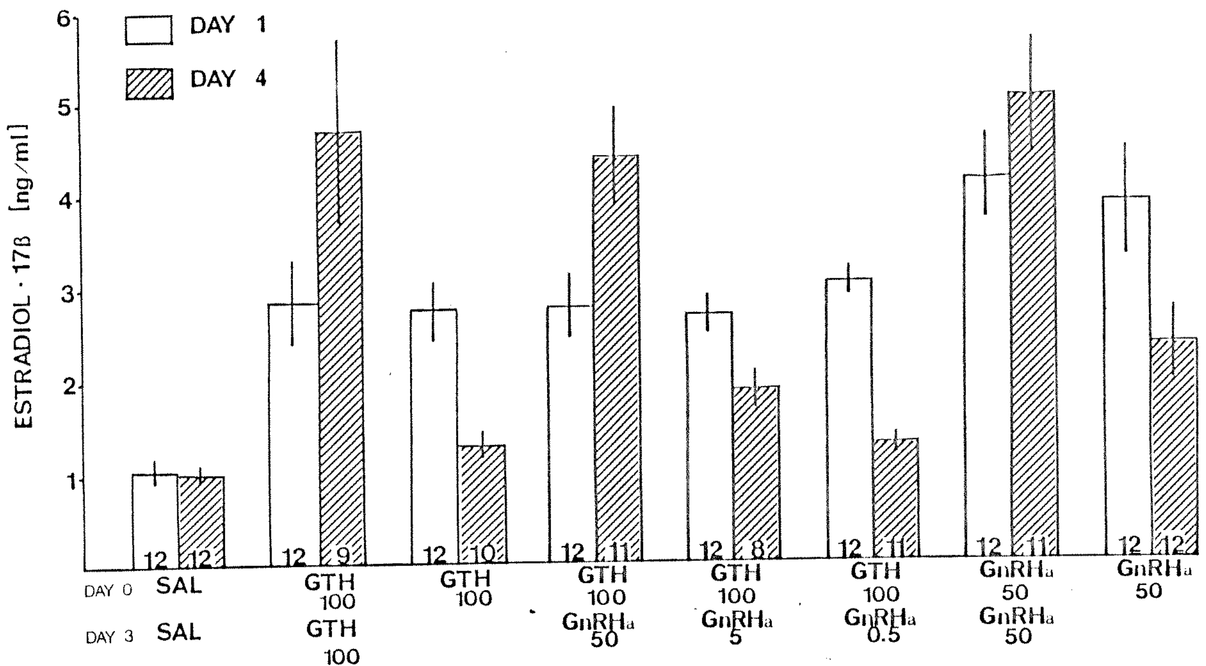
Figure 1. Accumulative percent ovulation at days 11 and 28 of female sea lampreys injected (1981 experiment) with saline (control) or GTH followed at 3 d by GnRHa and at 11 d with GTH. Plasma estradiol-17β (ng/ml) at days 4 and on the day of ovulation injected with saline (control) or GTH followed at 3 d by GnRHa.

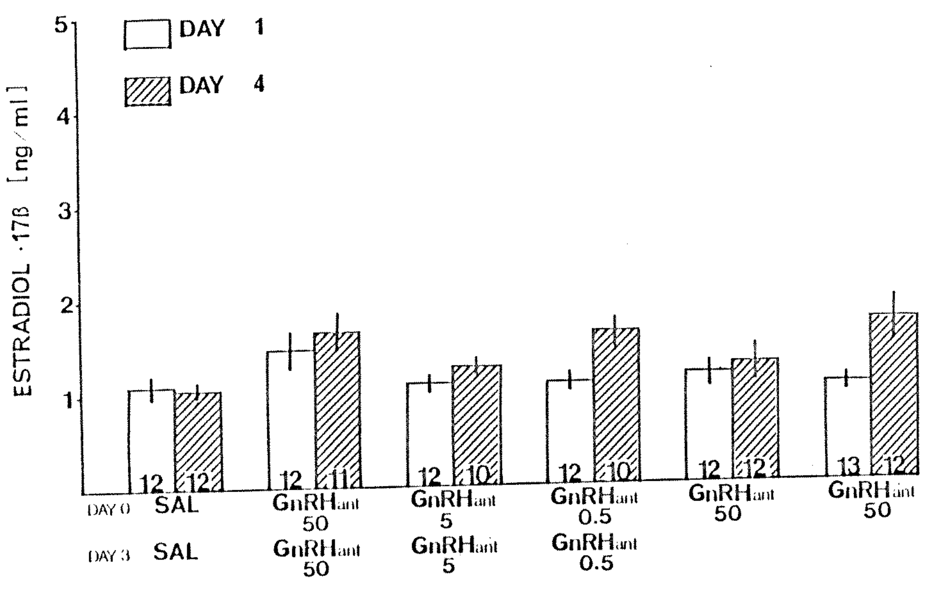
Figure 2. Plasma estradiol-17β of female sea lampreys, (ng/ml) at days 1 and 4 injected in 1982 with saline on day 0 and 3; GTH (100 ug/kg lamprey) on day 0 and 3; GTH (100) on day 0; GTH (100) followed at 3 d by GnRHa (50); GTH followed at 3 d by GnRHa (5); GTH followed at 3 d by GnRHa (0.5); GnRHa followed at 3 d by GnRHa; GnRHa on day 0; GnRHant (50) followed at 3 d by GnRHant (50); GnRHant (5) followed at 3 d by GnRHant (5); GnRHant (0.5) followed at 3 d by GnRHant (0.5); GnRHant (50) on day 0; or GnRHant (5) on day 0.



1981 SEA LAMPREY
control
salmon gonadotropin: Day 0
GnRH analogue : Day 3
ESTRADIOL-17 β (ng/ml)
12
10
8
6
4
2
11
11
5
5
DAY 4
DAY OF OVULATION
PERCENT OVULATION
100
80
60
40
20
0
9
9
9
9
DAY 11
DAY 28

1982
SEA LAMPREY





(1.06 ± 0.12 ng/ml). High plasma estradiol levels were found at 24 hrs (day 3) after an injection of GnRHa which followed a gonadotropin injection on day 0 (Fig. 2). Plasma estradiol responded in a significant dose-related manner (4.38 ± 0.50; 1.83 ± 0.23; or 1.28 ± 0.11 ng/ml) 24 hrs after injections of GnRHa (50, 5, or 0.5 μg/kg) which had followed a gonadotropin injection on day 0.

Following two injections of GnRHa (50 μg/kg), plasma estradiol also was significantly elevated compared to controls (Fig. 2). GnRHa treatment yielded higher values of estradiol (3.82 ± 0.61 ng/ml) 24 hrs following 50 ug/kg of GnRHa compared to controls (1.07 ± 0.15 ng/ml); however, with no second GnRHa injection, plasma estradiol diminished by day 4, but it was still significantly higher (2.30 ± 0.40 ng/ml) compared to controls (1.06 ± 0.12 ng/ml).

In most cases, plasma estradiol was not significantly different from controls after injections of GnRHant at different dosages (Fig. 2). On day 4 plasma estradiol was higher in lampreys treated with two injections of GnRHant (50 μg/kg), two injections of GnRHant (0.5 μg/kg) or one injection of GnRHant (5 μg/kg) on day 0, but only significantly higher in lampreys treated with two injections of GnRHant (0.5 μg/kg), 1.5 ± 0.18 ng/ml, compared to controls, 1.06 ± 0.12 ng/ml.

1982 Ovulation Responses

There were no ovulatory responses following the first two phases of injections. It is probably significant that the ambient water temperature (13°C) was unusually low for this date at this place. *P. marinus* at this location have not been known to spawn at temperatures below 15.5°C (Louis King, personal communication). Accordingly, heaters were introduced into the tanks and the water was warmed to 21°C. At this temperature, the same

animals were injected a third time with the same combinations of hormones. The ovulations observed all followed the third set of injections.

Thus, at day 12 after the third sequence of injections was begun, ovulation had occurred in 78% of the lampreys treated with GTH followed by 2 d by GnRHa (50 µg/kg), in 64% of those treated with a single injection of GnRHa (50 µg/kg), in 57% of those treated with GTH followed 2 d later by GTH and in 56% of those treated with GnRHa (50 µg/kg) followed after 2 d by GnRHa (50 µg/kg) (Fig. 3). These incidences may be contrasted with ovulation in 18% of control lampreys (Fig. 3). In lampreys treated with GnRHant at different dosages and combinations, a similar low level of ovulation was observed (18%). A single injection of GTH, or GTH followed after 2 d by a low dose of GnRHa (5 µg/kg), or by GnRHa (0.5 µg/kg) also was ineffective in accelerating ovulation. The experiment was terminated on day 18 due to high mortality.

Figure 3. Cumulative percent ovulations at day 12 after the third sequence of injections of female sea lamprey injected in 1982 with saline on day 0 and 3, GTH (μg/kg lamprey) on day 0 and 3, GTH on day 0, GTH followed at 3 d by GnRHa (50 μg/kg), GTH followed at 3 d by GnRHa (5 μg/kg), GTH followed at 3 d by GnRHa (0.5 μg/kg), GnRHa followed at 3 d by GnRHa, GnRHa on day 0, GnRHant (50 μg/kg) followed at 3 d by GnRHant (50 μg/kg), GnRHant (5) followed at 3 d by GnRHant (5), GnRHant (0.5 μg/kg) followed at 3 d by GnRHant (0.5 μg/kg), GnRHant (50 μg/kg) on day 0, or GnRHant (5 μg/kg) on day 0.

1982
SEA LAMPREY
DAY 12
PERCENT OVULATION
80
60
40
20
11
7
8
9
7
0 %
8
9
11
0%
8
8
0%
9
0%
8
9
SAL
SAL
GTH
100
GTH
100
GTH
100
GTH
100
GnRHa
50
GTH
100
GnRHa
5
GTH
100
GnRHa
0.5
GnRHa
50
GnRHa
50
GnRHa
50
GnRHant
50
GnRHant
50
GnRHant
5
GnRHant
5
GnRHant
0.5
GnRHant
0.5
GnRHant
50
GnRHa
5

Changes in Plasma Steroid and Thyroid Hormones, IR Insulin and Protein During Final Maturation and Spawning of the Sea Lamprey

MATERIALS AND METHODS

Landlocked adult sea lampreys were captured by trap in the Cheboygan River in May, 1982, on their anadromous spawning migration following completion of their parasitic lake phase. They were retained in raceways at Hammond Bay Biological Station supplied with flow through lake water ranging in ambient temperature from 5.5°C to 20°C.

Twenty lampreys (10 males and 10 females) were sampled for gonadal tissue (small anterior section) and blood by anesthetizing with ethyl m-aminobenzoate methanesulfonate (MS 222) bi-weekly at the same hour (1:00 p.m.) beginning 18 May to 2 August 1982. Individual lengths and weights were recorded for every lamprey at each sampling. Blood was collected by cardiac puncture with heparinized syringes and centrifuged. The plasma was drawn off and stored frozen at -20°C until assayed for estradiol-17β, total androgens, thyroxine, triiodothyronine, ir insulin, protein, and free fatty acids.

The gonadal tissues were dehydrated in a series of alcohols, embedded in paraplast, sectioned at 8-10 μm and stained with hematoxylin and eosin.

Plasma estradiol and total androgens were determined in 100 μl of plasma by radioimmunoassay as described by Sower and Schreck (1982). The radioimmunoassays were validated for adult sea lamprey plasma. For estradiol RIA validation, see Sower et al. (in press). Antitestosterone (11-BSA) was obtained from Dr. G. Niswender. This antibody cross-reacts with testosterone (100%), 11-ketotestosterone (112%), and dihydrotestosterone (69%). The antibody binding efficiency ranged between

38 and 49% in the assays. Each plasma sample was extracted twice with hexane:benzene (2:1). The intraassay and interassay coefficients of variation for the androgen RIA of lamprey plasma were 4.6% (n=11) and 9.3% (n=9), respectively.

Plasma thyroxine (T4) and triiodothyronine (T3) were determined by radioimmunoassay validated for lamprey plasma as previously described (Plisetskaya et al., 1983b). ^{125}I labeled T3 and T4 (specific activity 750 mCi/mg) were purchased from Industrial Nuclear Company, St. Louis, Missouri. Ethanol-extracted plasma was used for all analyses. 200 µl of absolute ethanol was added to 100 µl extracted plasma and to the standard, placed in an ice bath for 30 min, centrifuged for 10 min at 3000 rpm, and the extract was stored in a freezer until assayed. Aliquots of 50 µl of extracted plasma were measured in duplicate. Extraction efficiencies determined in some of the samples were 75.5 $\pm$ 0.45% (n=10) for T3 and 84.4 $\pm$ 0.69% (n=10) for T4.

Plasma immunoreactive insulin (IRI) was measured by use of a mammalian RIA system at the Diabetes Research Center, Seattle, according to the double antibody methods of Morgan and Lazarow (1963).

Plasma protein was measured as described by Hartree (1972) by pooling the samples from 10 lampreys of each sex at each sampling time. Free (plasma) fatty acids (PFA) were measured according to the method of Noma et al., 1973.

Data for length, weight, and hormone concentrations were evaluated by analyses of variance. In all tests the level of significance for mean data differences among the animal groups, P was less than 0.05.

RESULTS

Lengths and weights did not change or vary significantly between male and female lampreys through the time of observation (Table 1).

Sexual development, fairly uniform in both males and females, changed through time as outlined in Table 2. In May, at the start of the lampreys' upstream migration, vitellogenesis was completed in the ovaries with the oocyte nuclei peripheral in location; and the testes consisted primarily of primary and secondary spermatocytes. By the first of June, over half of the testes examined contained spermatocytes with some spermatids and two of the testes contained mature sperm. At this same time, the oocytes contained granular yolk, and almost every ovary tissue sample contained at least one or two small eggs which had thickened enveloping follicular layers. Also, microscope fields of about half of the ovarian samples had at least one or two eggs that displayed an elevation of the follicular layer at one pole. On June 22, in a majority of ovaries studied there were a few large eggs that had invaginations or concave zona pellicidae. In one ovary, there were oocytes whose yolk had started to liquify (vacuoles present and granular margins becoming indistinct). The testes at this time consisted mainly of spermatids and some mature sperm. By July 7, most ovaries displayed yolk that was mostly liquified with some possible polar development. The eggs containing liquified-yolk were distinctly smaller in diameter compared to eggs in May and June. It is not clear whether the pole development is a positive process or whether it is a passive result of the liquifaction of the yolk. A majority of testes contained late spermatid stages and mature sperm. During July, oocytes contained either granular or mainly liquified yolk with some polar development. The testes until spermiation were fairly uniform displaying late stages of spermiogenesis and/or mature sperm.

Generally, the testes consisting of spermatids and/or mature sperm (the later half of the spawning period) tended to have moderately to well-developed interstitial cells.

TABLE 1. Weights and lenghts of male and female lampreys during their final spawning period. $\bar{X} \pm$ SE (n=10)

Date	Weight (g) Female	Weight (g) Male	Length (cm) Female	Length (cm) Male
18 May	248 ± 21	213 ± 20	49.1 ± 1.6	47.4 ± 6.6
21 May	212 ± 16	189 ± 14	46.8 ± 1.0	46.1 ± 1.4
25 May	231 ± 19	211 ± 16	46.9 ± 1.6	47.3 ± 1.4
28 May	226 ± 18	161 ± 11	48.1 ± 1.3	44.3 ± 0.9
1 June	233 ± 25	162 ± 19	47.2 ± 1.4	44.2 ± 1.4
4 June	212 ± 16	192 ± 20	44.7 ± 1.3	45.3 ± 1.4
11 June	252 ± 18	239 ± 33	50.3 ± 1.5	47.3 ± 1.6
15 June	236 ± 15	240 ± 10	47.9 ± 1.2	46.8 ± 1.1
22 June	217 ± 13	234 ± 22	46.4 ± 0.7	45.9 ± 1.5
25 June	211 ± 19	218 ± 19	43.3 ± 1.5	44.5 ± 1.2
29 June	207 ± 20	223 ± 16	46.7 ± 1.5	46.3 ± 1.1
2 July	173 ± 17	216 ± 9	44.5 ± 1.3	47.7 ± 0.8
7 July	206 ± 23	176 ± 18	41.9 ± 2.0	40.4 ± 2.0
9 July	182 ± 13	196 ± 16	45.1 ± 1.2	46.3 ± 1.2
14 July	217 ± 21	194 ± 18	45.2 ± 2.0	44.3 ± 2.1
16 July	171 ± 22	192 ± 23	43.4 ± 1.3	48.2 ± 2.3
20 July	221 ± 9	233 ± 45	46.9 ± 0.7	45.9 ± 1.4
23 July	223 ± 18	225 ± 18	48.4 ± 1.5	49.5 ± 1.3
27 July				
30 July	196 ± 18	227 ± 20	42.4 ± 1.3	46.1 ± 1.1
2 August	260 ± 25		46.3 ± 1.7	

TABLE 2. Gonadal development of sea lampreys before and during spawning.

Date	MALE		FEMALE	
18 May	5/10:	consist of primary and secondary spermatocytes	9/9:	peripheral germinal vesicle; granular yolk
	4/10:	consist of primary spermatocytes		
	1/10:	consist mainly of primary and secondary spermatocytes with some spermatids		
	1/10:	testis contained 1 "oogonium"		
21 May	3/10:	consist of primary and secondary spermatocytes	8/8:	granular yolk; peripheral germinal vesicle small atretic-like eggs have thick follicular layer
	4/10:	consist mainly of primary and secondary spermatocytes with some spermatids		
	3/10:	consist primarily of primary spermatocytes		
25 May	1/9:	consist primarily of primary spermatocytes	10/10:	granular yolk; 5/10 contain small atretic-like eggs that have thick follicular layer
	3/9:	consist of primary and secondary spermatocytes		
	4/9:	consist mainly of primary and secondary spermatocytes with some spermatids		
	1/9:	consist mainly of spermatids		
1 June	1/8:	mainly primary spermatocytes	5/10:	granular yolk; some contain small atretic-like eggs that have thick follicular layer
	1/8:	primary and secondary spermatocytes		
	4/8:	primary and secondary spermatocytes and some spermatids	5/10:	granular yolk; one or two of the eggs each section contained striking elevation of the theca of the animal pole; some thickening of follicle cells in the vegetative pole
	2/8:	spermatids and mature sperm (tails)		

Date		MALE		FEMALE
11 June	4/10:	primary and secondary spermatocytes, spermatids	7/9:	granular yolk; some contain small atretic-like eggs that have thick follicular layer
	4/10:	mostly spermatids and some mature sperm (tails)	2/9:	granular yolk; elevation of theca of animal pole
	2/10:	mostly mature sperm		
22 June	2/7:	spermatocytes, spermatids, and mature sperm	6/9:	granular yolk, a few atretic-like eggs with cuboidal follicular layer; a few large eggs have invaginations or concaved zona pellucida
	2/7:	spermatids and mature sperm	2/9:	granular yolk; early developing poles; atretic-like eggs with thickened follicular layer
	1/7:	mainly early spermatids, few spermatocytes	1/9:	yolk beginning to liquify/atretic-like eggs present
	2/7:	mostly late spermatids		
29 June	7/8:	late stages of spermatids and tailed sperm	3/11:	granular yolk; a few large concaved eggs
	1/8:	uniform late stages of spermiogenesis tailed sperm	3/11:	granular yolk; a few atretic-like eggs with thick follicular layer
			5/11:	granular yolk (starting) to liquify); beginning stages of poles *one group of eggs contains one or two large concaved eggs
7 July	1/9:	uniform early spermatids	6/10:	yolk mostly liquified (2/10 have concaved eggs)
	8/9:	late stages of spermatids	4/10:	yolk mostly liquified; beginning pole development (may not be true pole dev.) (2/10 have concaved eggs)

Date		MALE		FEMALE
17 July	4/9:	mainly spermatids	8/10:	yolk mostly liquified; some beginning possible pole development (6/10 have concaved eggs)
	1/9:	few spermatocytes; mainly spermatids; very few mature tailed sperm	2/10:	yolk mostly liquified (1/10- center of possibly two eggs have small central space surrounded by membrane)
	4/9:	later stages; tailed sperm		
20 July	1/8:	spermatocytes and spermatids	4/11:	granular yolk (1 has 1 egg- possible pole dev.)
	1/8:	late spermatids and early spermatocytes (short tail)	5/11:	yolk liquified; (2 have beginning possible pole development)
	4/8:	late spermatids and tailed sperm	2/11:	partly liquified yolk (1 has possible pole dev. in most of the eggs; thick follicle layer)
	2/8:	mainly uniform late spermatids (no mature sperm)		
27 July	6/9:	mostly packed spermatids, few tailed sperm	3/1:	granular yolk (1 has 1 egg--possible early pole development)
	2/9:	mainly early packed spermatids	7/9:	yolk liquified; (4 have possible early pole dev.
	1/9:	mature sperm	1/9:	partly liquified yolk
		**two testes had "oogonia"		
30 July	5/9:	packed late spermatids, mature sperm	3/10:	liquified yolk; (some ovulated eggs)
	4/9:	mature sperm	1/10:	liquified yolk; poles formed
		*one testis has possible "oogonia" (slide was badly prepared)	6/10:	liquified yolk

Plasma estradiol levels fluctuated significantly and generally covaried in males and females through time (Fig. 4). In females, mean plasma estradiol levels peaked four times during the final spawning period. The greatest increase occurred between 2 July to 7 July from 0.85 ± 0.31 ng/ml to 2.86 ± 0.42 ng/ml. Plasma estradiol then decreased to 1.53 ± 0.19 ng/ml on 9 July, followed by an increase of 2.47 ± 0.41 ng/ml with a very sharp, significant decrease to 0.60 ± 0.06 ng/ml on 2 August, the time of ovulation. In males, mean plasma estradiol values peaked 7 times during the final spawning period. Similar to females, the greatest increase in plasma estradiol in males occurred between 2 July to 7 July from 0.91 ± 0.15 ng/ml to 3.03 ± 0.79 ng/ml; it then decreased on 9 July to 2.14 ± 0.25 ng/ml. Plasma estradiol again increased to 2.97 ± 0.46 ng/ml followed by a very significant decrease to 1.18 ± 0.09 ng/ml on 23 July with a final pre-spawning peak of 2.69 ± 0.46 ng/ml occurring on 30 July, the time of spermiation.

Plasma androgens also significantly covaried in males and females through time (Fig. 5). Plasma androgens were low during the entire final spawning period ranging from 0.099 ± 0.009 ng/ml to 0.211 ± 0.058 ng/ml in females and from 0.096 ± 0.009 ng/ml to 0.223 ± 0.050 ng/ml in males. The small fluctuations of plasma androgen levels occurred at times that generally coincided with the peaks of estradiol that occurred in both males and females.

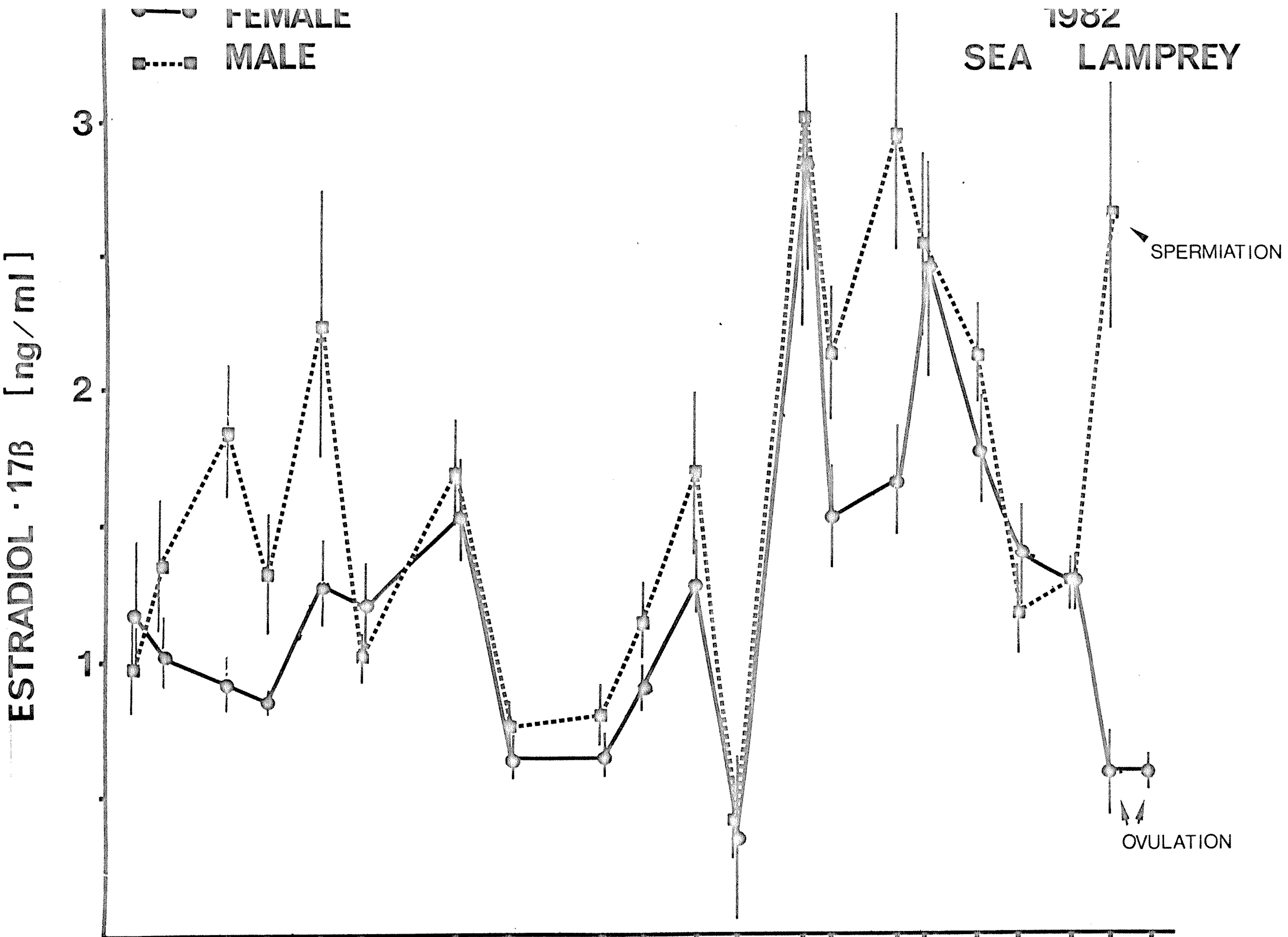
Like plasma steroid profiles, there were coordinated changes in plasma triiodothyronine and thyroxine levels in males and females through time (Figs. 6 and 7). In females, mean plasma triiodothyronine increased markedly between 18 May to 15 June from 1.00 ± 0.13 ng/ml to 3.00 ± 0.09 ng/ml. Plasma triiodothyronine then decreased to 1.11 ± 0.02 ng/ml on 22

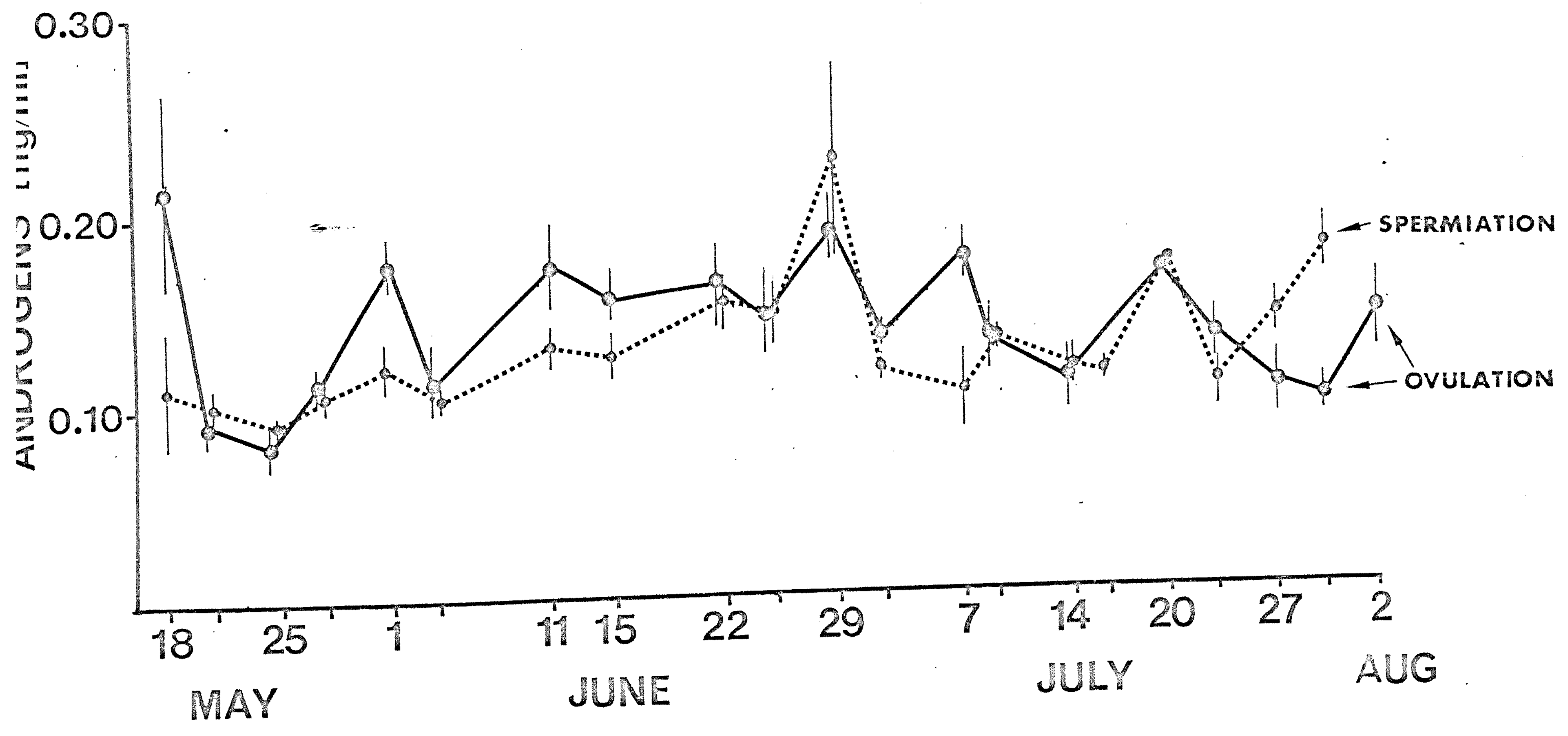
Figure 4. Plasma estradiol of male and female sea lamprey during final maturation and spawning in 1982.

Figure 5. Plasma androgens of male and female sea lamprey during final maturation and spawning in 1982.

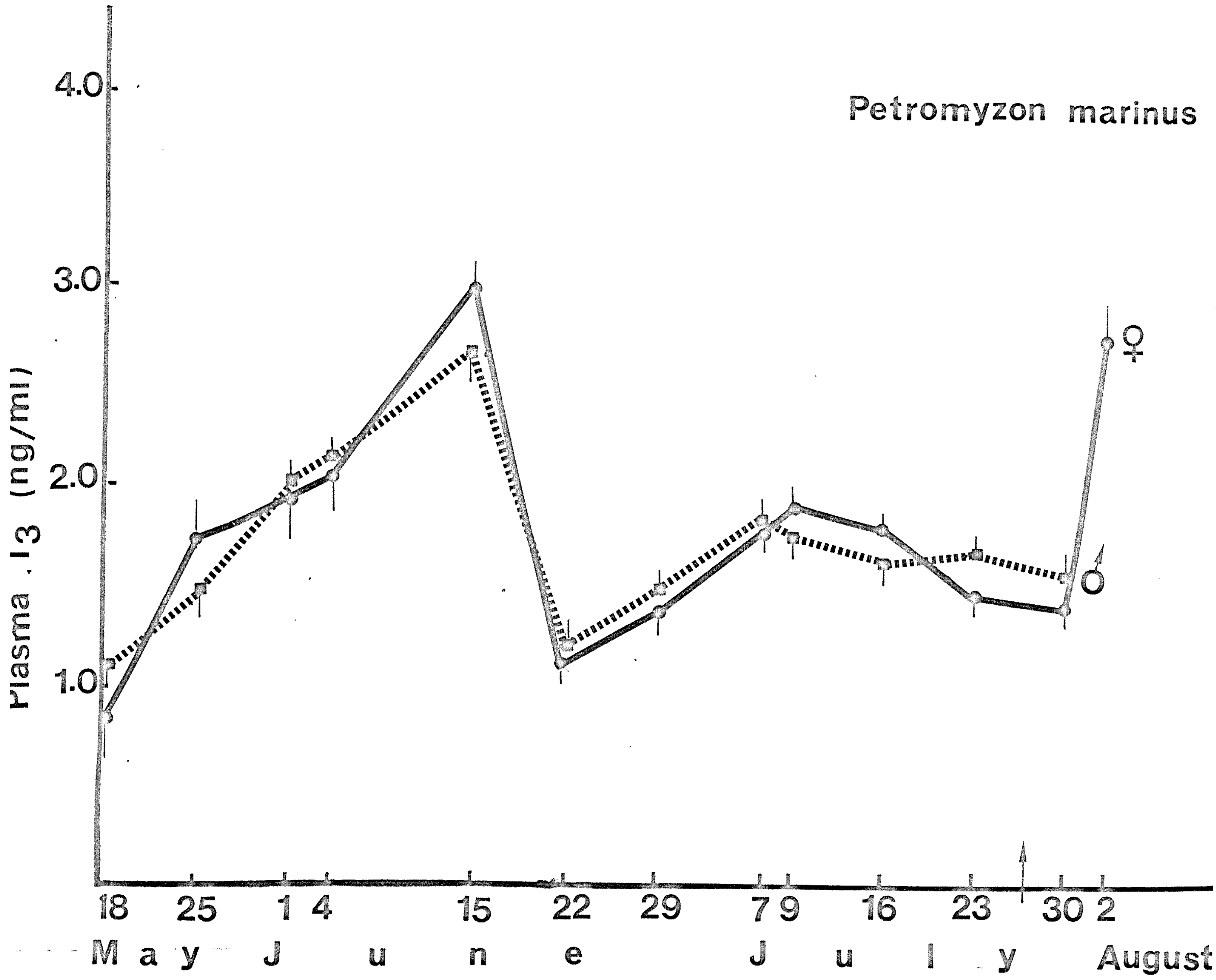
Figure 6. Plasma triiodothyronine of male and female sea lamprey during final maturation and spawning in 1982.

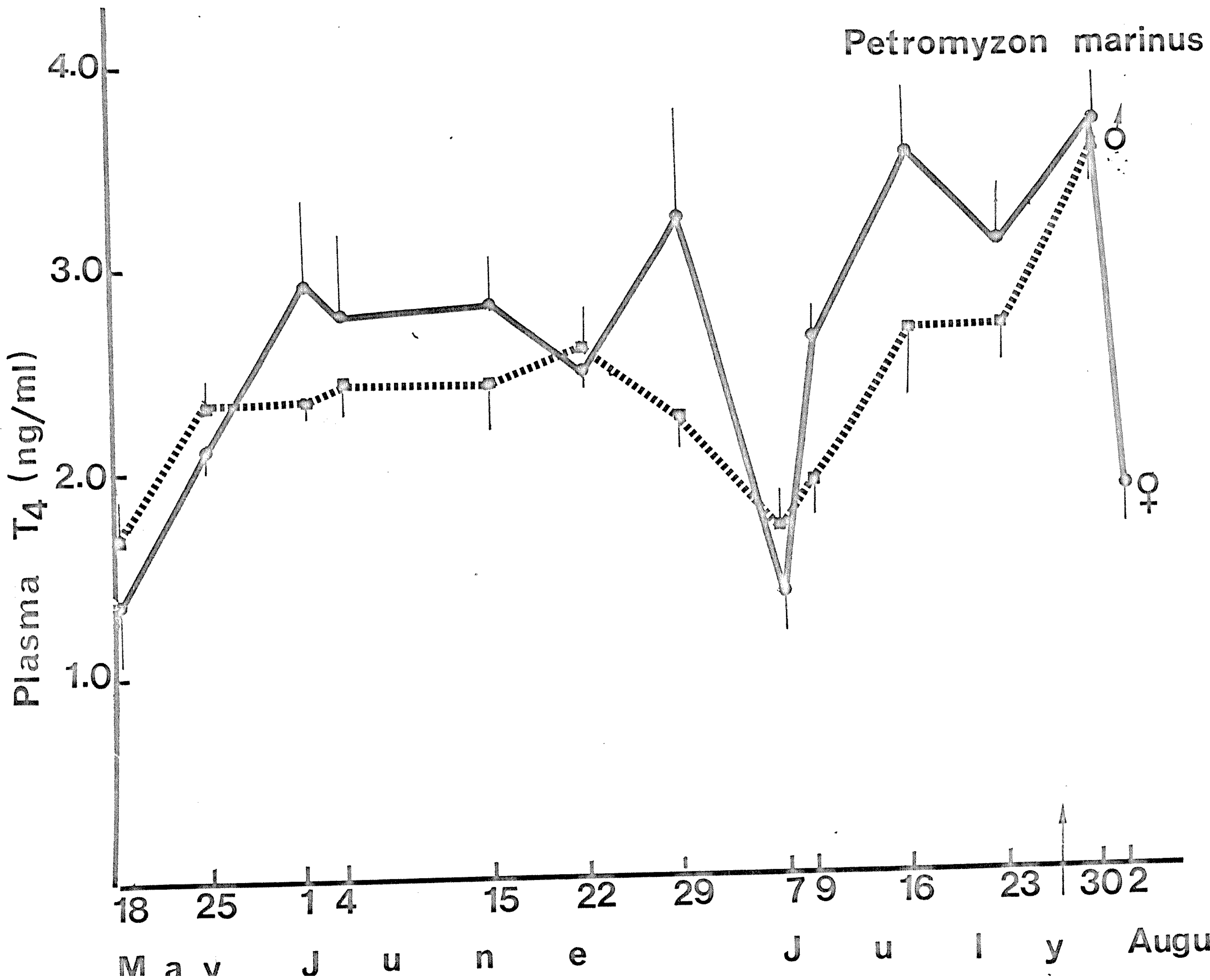
Figure 7. Plasma thyroxine of male and female sea lamprey during final maturation and spawning in 1982.


FEMALE
MALE
1982
SEA LAMPREY
3
2
1
ESTRADIOL · 17β [ng/ml]
SPERMIATION
OVULATION
18
21
25
28
1
4
11
15
22
25
29
2
7
9
14
16
20
23
27
30
2
MAY
JUNE
JULY
AUG
SOWER and GORBMAN



FEMALE
MALE
1982
SEA LAMPREY
0.30
0.20
0.10
SPERMIATION
OVULATION
18
25
1
11
15
22
29
7
14
20
27
2
MAY
JUNE
JULY
AUG


Petromyzon marinus
Plasma T3 (ng/ml)
4.0
3.0
2.0
1.0
♀
♂
18
25
1 4
15
22
29
7 9
16
23
30 2
May
June
July
August


Petromyzon marinus
Plasma T_4 (ng/ml)
4.0
3.0
2.0
1.0
♂
♀
18
25
1
4
15
22
29
7
9
16
23
30
2
May
June
July
August

June followed by two increases on 9 July and 2 August (time of ovulation) of 1.94 ± 0.04 ng/ml and 2.69 ± 0.11 ng/ml, respectively. In males, plasma triiodothyronine generally followed a similar pattern. In females, plasma thyroxine significantly increased between 18 May to 1 June from 1.31 ± 0.22 ng/ml to 2.91 ± 0.45 ng/ml. Thyroxine levels remained elevated until 7 July and then decreased to 1.35 ± 0.14 ng/ml; this was succeeded by increases occurring on 16 and 30 July; there was an abrupt decrease to 1.90 ± 0.18 ng/ml on 2 August. In males, fluctuations in plasma thyroxine levels were less pronounced. Generally, plasma thyroxine in males was slightly elevated between 18 May to 22 June from 1.58 ± 0.16 ng/ml to 2.63 ± 0.21 ng/ml. Plasma thyroxine then decreased to 1.70 ± 0.16 ng/ml on 7 July followed by a significant increase of 3.66 ± 0.29 ng/ml at spermiation on 30 August.

The temperature profile during this final maturation and spawning period is depicted in Figure 8. No significant correlations were noted between hormone concentrations and ambient water temperature.

There was a very slight increase in plasma irIRI during sexual maturation of the lampreys. However, at ovulation, the irIRI levels abruptly decreased in females, whereas in males, they remained unchanged (Fig. 9). Plasma protein and FFA levels gradually decreased until ovulation/spermiation. At ovulation, plasma FFA increased (Table 3). Plasma protein gradually decreased in females, and remained essentially unchanged in males (Table 4).

Figure 8. The temperature profile at Hammond Bay Biological Station in 1982.

Figure 9. Plasma irIRI of male and female sea lamprey during final maturation and spawning in 1982.

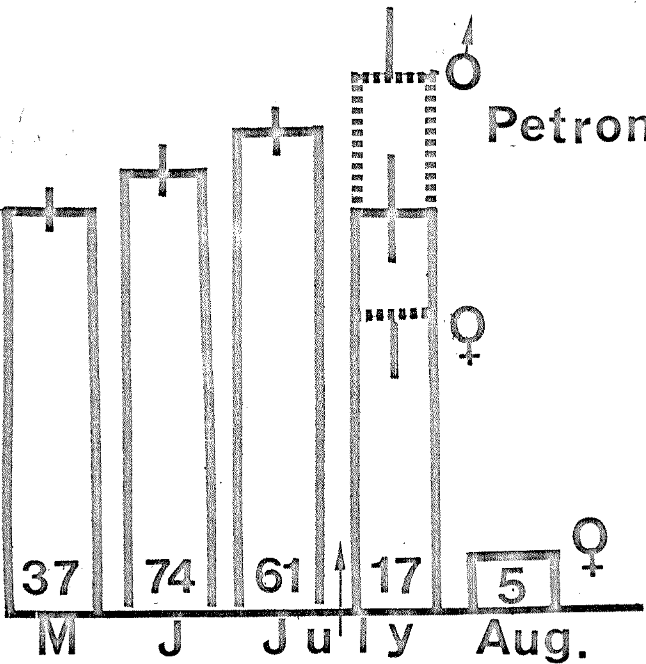
1982

TEMPERATURE · °C

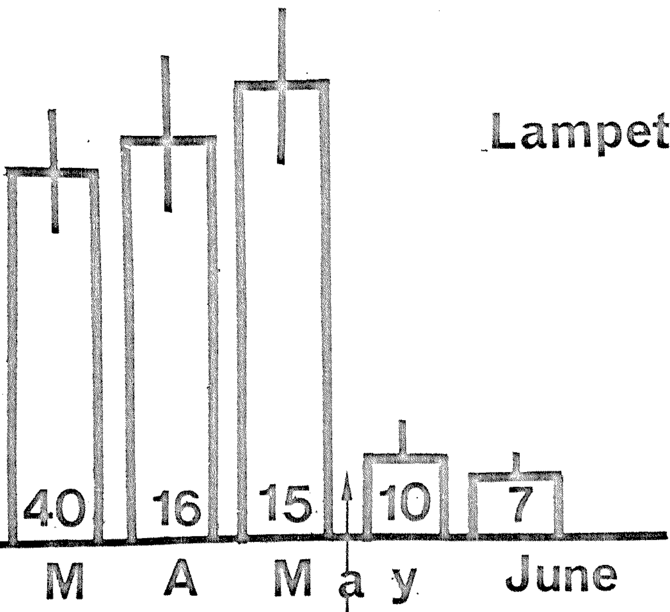
22
16
14
12
10
8
6

18 21 25 1 4 11 15 22 29 2 7 11 16 20 27 2

AUG



Petromyzon marinus
37
74
61
17
5
M
J
July
Aug.



Plasma IRI
Lampetra fluviatilis
25
22
8
18
10
40
16
15
10
7
O
N
D
J
F
M
A
May
June

TABLE 3. The concentration of plasma fatty acids during upstream migration of *Petromyzon marinus*.

Date	t (°C)	PFA (μEg)	
		Female	Male
May 18	5.0	0.85 ± 0.03 (7)	0.88 ± 0.03 (9)
May 25	5.6	0.58 ± 0.04 (11)	0.76 ± 0.07 (8)
June 1	10.5	0.76 ± 0.05 (6)	0.73 ± 0.01 (6)
June 4	8.9	0.77 ± 0.08 (6)	0.73 ± 0.05 (6)
June 15	9.4	0.75 ± 0.07 (6)	0.75 ± 0.09 (6)
June 22	12.8	0.58 ± 0.06 (10)	0.54 ± 0.06 (9)
June 29	15.6	0.64 ± 0.02 (10)	0.66 ± 0.04 (5)
July 9	10.0	0.62 ± 0.04 (5)	0.64 ± 0.03 (6)
July 16	11.1	0.59 ± 0.03 (9)	0.71 ± 0.03 (10)
July 23	14.4	0.59 ± 0.06 (9)	0.35 ± 0.09 (7)
July 30[a]	18.9	0.42 ± 0.08 (10)	0.36 ± 0.07 (10)
August 2[a]		0.68 ± 0.02 (5)	---

[a]Spawning

Number of samples in brackets

TABLE 4. The concentration of plasma protein during upstream spawning migration of *Petromyzon marinus*.

Date	Protein (mg/ml) Female	Male
May 18	22.6	26.3
May 25	27.5	26.3
June 1	26.6	25.4
June 4	26.3	27.6
June 15	27.2	27.2
June 22	25.9	29.5
June 29	27.9	29.2
July 9	20.7	23.0
July 16	18.0	22.5
July 23	18.0	24.8
July 30	9.6	27.5
August 2	9.9	23.0

Values represent pooled samples from 9-10 lampreys.

Steroid Hormone Profile Following a Single Injection of Salmon Gonadotropin and Agonistic and Antagonistic Analogues of GnRH

MATERIALS AND METHODS

Landlocked adult sea lampreys were captured by trap in the Cheboygan River in May, 1982, during their anadromous spawning migration following completion of their parasitic lake phase. They were retained in raceways at Hammond Bay Biological Station supplied with flow-through lake water ranging in ambient temperature from 5.5°C to 20°C. One week prior to injection, the lampreys were divided into groups in the raceways which were divided into sections by screens. In experiment A and B, dorsal fins of all lampreys were clipped so as to designate mode of treatment. In experiment C, all lampreys were identified individually with floy tags in addition to the clipped dorsal fins. The lampreys averaged 200 g in body weight and 44 cm in length.

In each of three experiments, groups of 150 lampreys were tested (either all males or all females) at different temperatures with single injections of various doses of GnRHa (D-ala^{6}, des Gly^{10} ethyl amide), GnRHant (antagonist, Ac-^{3}Pro^{1}, 4 FD Phe^{2}, DTrp^{3,6}-LRF) or partly purified coho salmon gonadotropin (Sower et al., 1982). The GnRH antagonist was generously donated by J. E. Rivier and W. W. Vale, the Salk Institute, La Jolla, California. During the morning of the day when the fish were injected, all peptides were dissolved in 0.6% NaCl in distilled water. They were injected intraperitoneally into each fish. The animals were serially bled at 8, 24, and 48 hrs from the initial injection at 0 hr by anesthetizing with ethyl m-aminobenzoate methanesulfonate (MS 222). Blood samples (approx. 400 µl) were collected in heparinized syringes by cardiac

puncture. The plasma was drawn off and stored frozen at -20°C until assayed for estradiol and androgens. Plasma estradiol and androgens were measured by radioimmunoassay as described by Sower and Schreck (1982) and validated for lampreys (Sower et al., in press; Sower et al., in preparation).

Experiment A included only female lampreys from 25 May to 27 May, 1982. The temperature ranged between 5 and 6°C. The treatments of 12 lampreys each were: control (no injection, plasma samples only at 0 and 48 hr); control (saline); GnRHa, 10 μg/lamprey; GnRHa, 1 μg/lamprey; GnRHant, 10 ug/lamprey; GnRHant, 1 μg/lamprey; or salmon GTH, 100 μg/kg.

Experiment B included only male lampreys from 2 June to 4 June, 1982. The temperature ranged between 8.3 and 8.8°C. The treatments of 12 male lampreys each were: control (no injection, plasma samples only at 0 and 48 hr); control (saline); GnRHa, 10 μg/lamprey; GnRHant, 10 μg/lamprey; or salmon GTH, 20 μg/lamprey.

Experiment C included only female lampreys from 15 July to 17 July, 1982. The temperature was 11°C. The treatment schedules of 12 lampreys each were identical to the treatments of Experiment A.

RESULTS

Experiment A: Plasma estradiol in females was significantly elevated at 8, 24, and 48 hrs after lamprey were treated with GnRHa (10 μg/lamprey or 50 μg/kg) (5.16 ± 0.51, 5.51 ± 0.61, and 6.16 ± 0.82 ng/ml), GnRHa (5) (4.66 ± 0.48, 5.00 ± 0.49, and 3.80 ± 0.56 ng/ml), or GTH (2.84 ± 0.48, 3.45 ± 0.66, and 3.01 ± 0.44 ng/ml), compared to controls (1.59 ± 0.31, 1.36 ± 0.23, and 1.15 ± 0.10 ng/ml) (Fig. 10). Plasma estradiol was not significantly different from controls after injections of GnRHant at 50 or 5 μg/kg. However, plasma estradiol significantly decreased from 8 to 48 hr

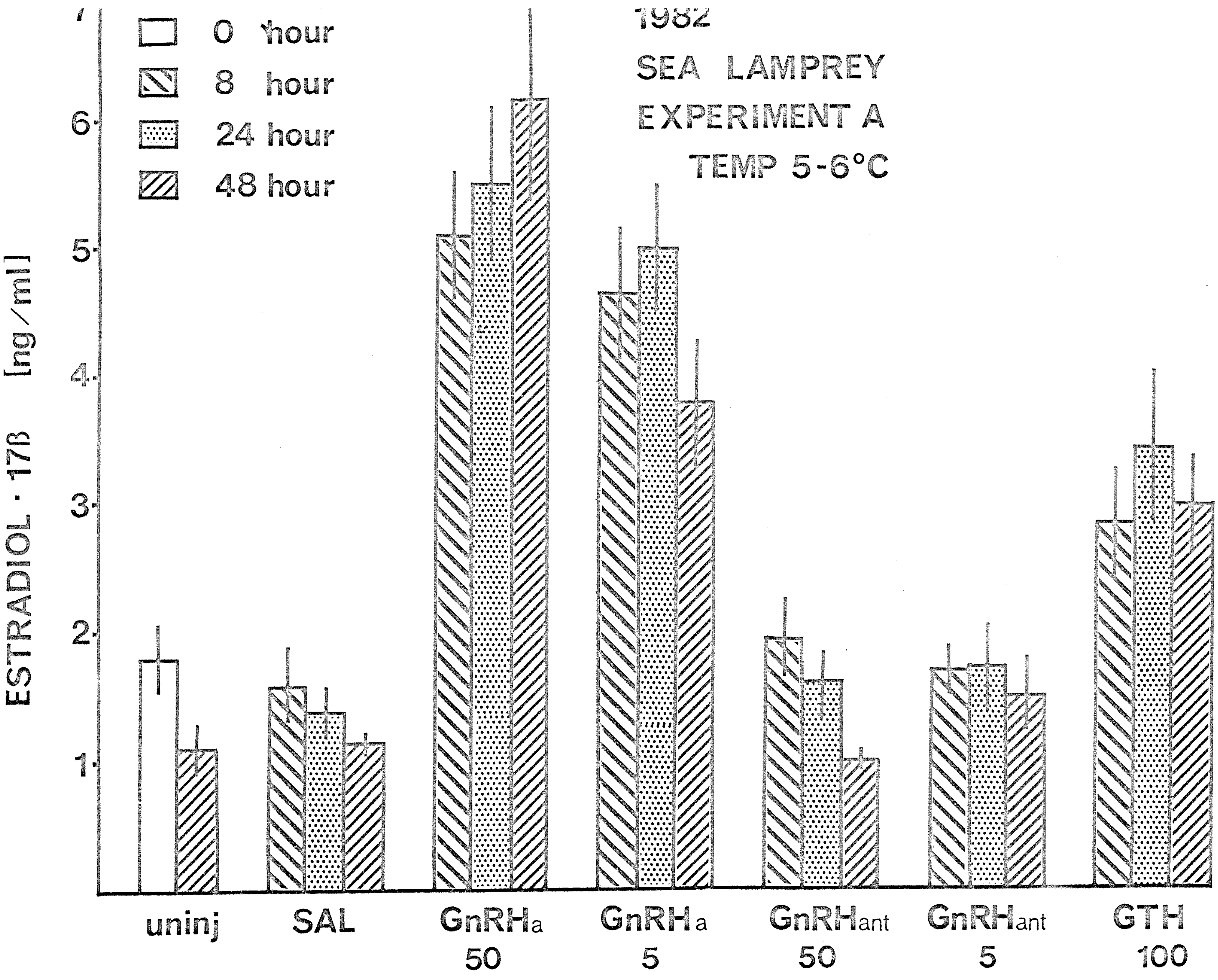
following injection of GnRH at 50 μg/kg. Estradiol did not vary significantly between the different sampling times in all treatment groups except for GnRHant (50 μg/kg).

Experiment B: Plasma estradiol in males was significantly elevated at 8, 24, and 48 hrs after lampreys were treated with GnRHa (50) (1.71 ± 0.26, 1.35 ± 0.18, and 1.11 ± 0.18 ng/ml) compared to controls (0.75 ± 0.10, 0.70 ± 0.07, and 0.65 ± 0.10 ng/ml) (Fig. 11). Estradiol was not significantly different from controls after a single injection of GnRHant at 50 μg/kg. Plasma estradiol did not vary significantly between the different sampling times in all treatment groups. Plasma androgens were only significantly higher at 24 hr after lampreys were treated with GnRHa (50 μg/kg) compared to controls. Following injection of GnRHa (50 μg/kg), plasma androgens decreased significantly from 8 to 48 hr. In the saline controls, plasma androgens (0.145 ± 0.018 ng/ml) at 8 hr were significantly different compared to uninjected controls (0.101 ± 0.005 ng/ml) at 0 hr and saline controls (0.099 ± 0.014 ng/ml and 0.093 ± 0.005 ng/ml) at 24 and 48 hr. Plasma estradiol or androgens were not significantly different from controls after injections of GnRHant.

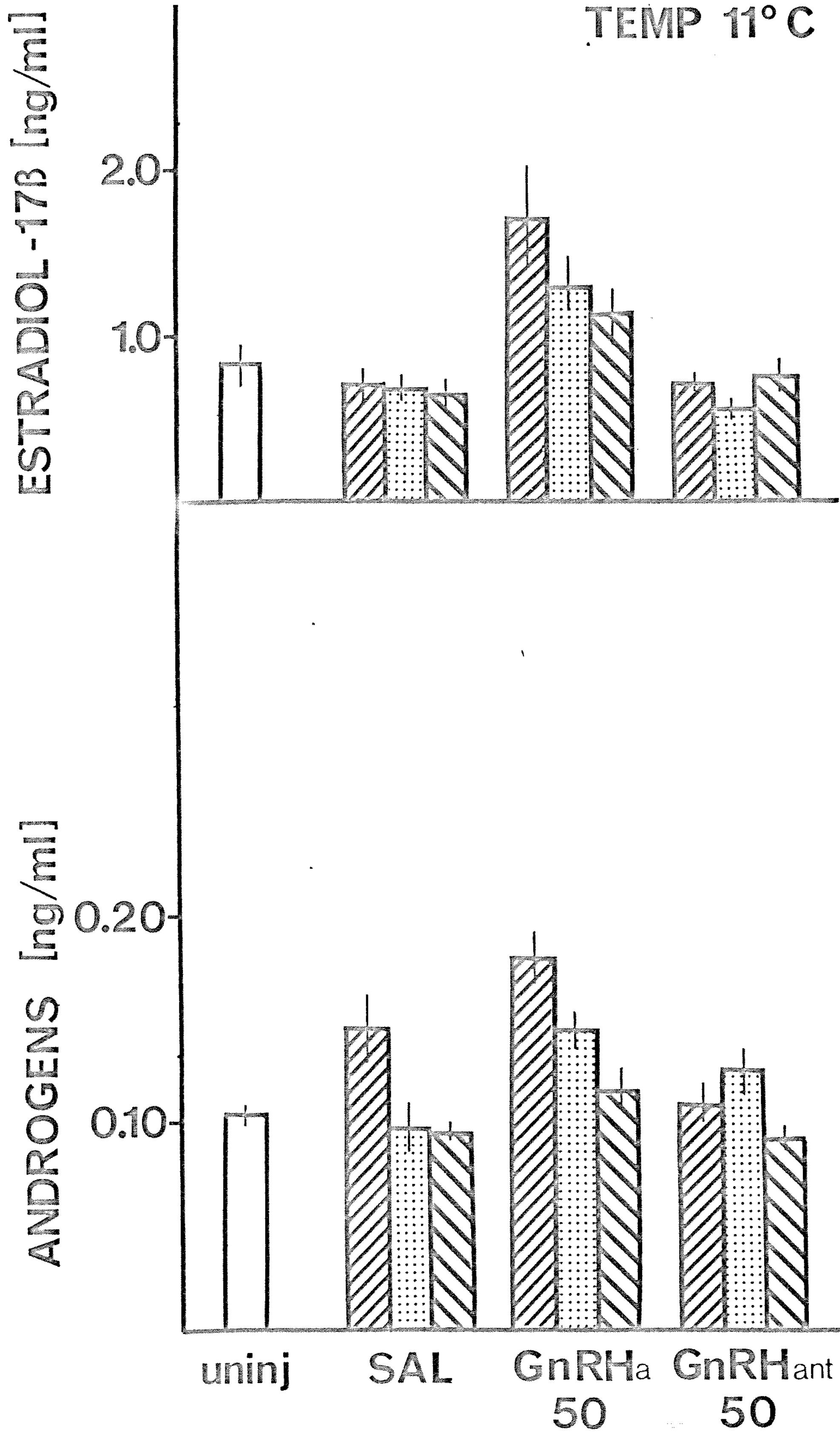
Experiment C: Plasma estradiol in females was significantly elevated at 8, 24, and 48 hrs after lampreys were treated with GnRHa (50), GnRH (5), or GTH (100) compared to saline controls and at 8 and 48 hrs after lampreys

Figure 10. Plasma estradiol (ng/ml) at 0, 8, 24, and 48 hrs of female lampreys uninjected or injected at 0 hr with saline, GnRHa (50 μg/kg), GnRHa (5 μg/kg), GnRHant (50 μg/kg), GnRHant (5 μg/kg), or GTH (100 μg/kg).

Figure 11. Plasma estradiol (ng/ml) and total androgens (ng/ml) at 0, 8, 24, and 48 hrs of male lampreys uninjected or injected at 0 hr with saline, GnRHa (50 μg/kg) or GnRHant (50 μg/kg).

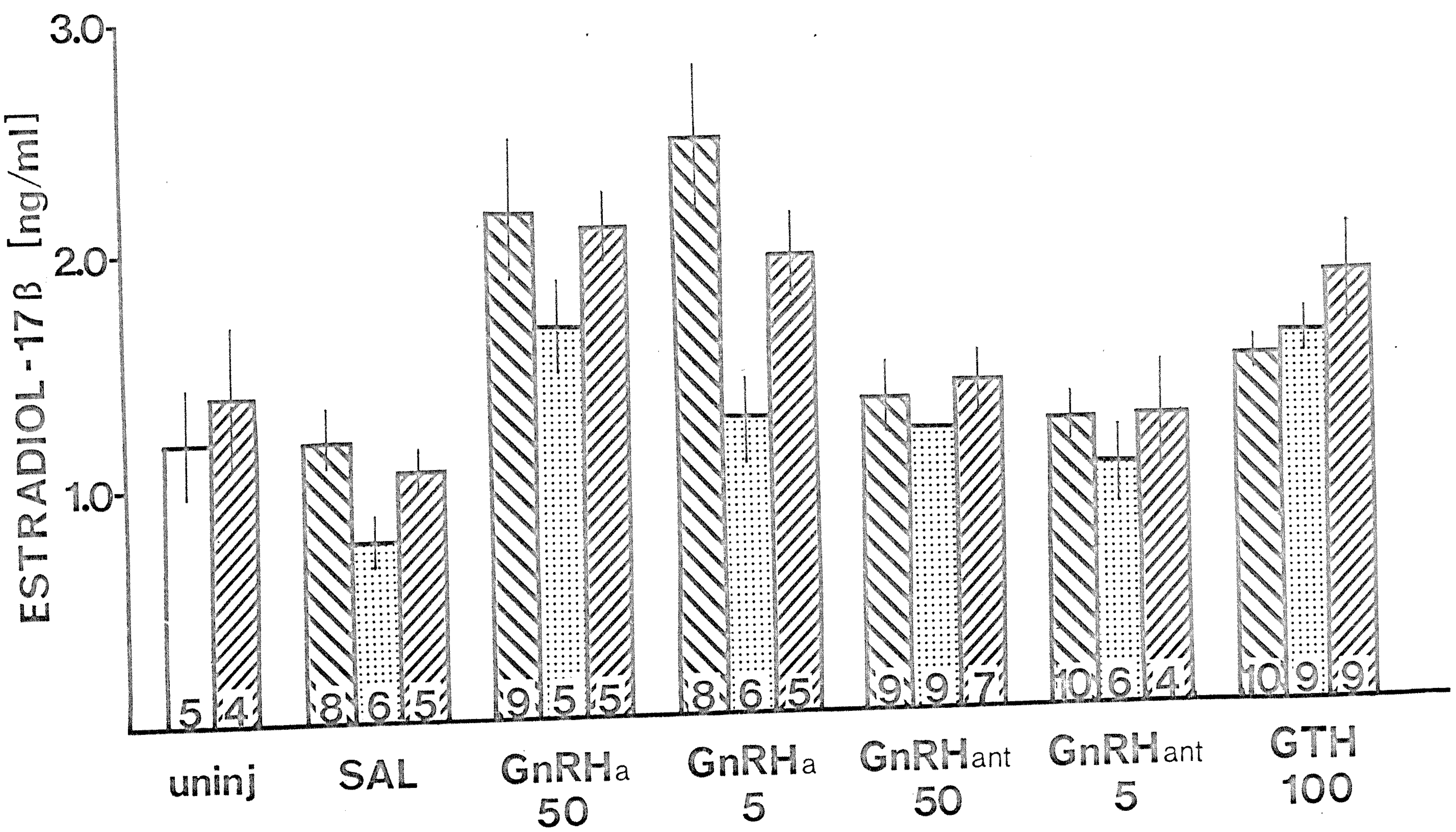


1982
SEA LAMPREY
EXPERIMENT A
TEMP 5-6°C
0 hour
8 hour
24 hour
48 hour
ESTRADIOL · 17β [ng/ml]
1
2
3
4
5
6
7
uninj
SAL
GnRHa 50
GnRHa 5
GnRHant 50
GnRHant 5
GTH 100


0 HOUR
8 HOUR
24 HOUR
48 HOUR
MALES
SEA LAMPREY
1982
EXPERIMENT B
TEMP 11° C
ESTRADIOL-17β [ng/ml]
2.0
1.0
ANDROGENS [ng/ml]
0.20
0.10
uninj
SAL
GnRHa 50
GnRHant 50

were treated with GnRHa (50 μg/kg) and GTH (100 μg/kg) compared to uninjected controls (Fig. 12). Following injection of GnRHa at 5 μg/kg, plasma estradiol decreased significantly from 8 hr to 24 hr from 2.47 ± 0.32 to 1.25 ± 0.19 ng/ml and then increased at 48 hr to 1.94 ± 0.18 ng/ml. Saline injected lamprey showed a similar trend from 8, 24, to 48 hrs of 1.18 ± 0.15 ng/ml, 0.74 ± 0.09 ng/ml, to 1.06 ± 0.08 ng/ml. Plasma estradiol was not significantly different from controls after injections of GnRHant at 50 or 5 μg/kg, except at 24 hr where estradiol was significantly higher in lampreys treated with GnRHant at 50 μg/kg (1.19 ± 0.08 ng/ml) compared to saline control (0.74 ± 0.09 ng/ml).

Figure 12. Plasma estradiol (ng/ml) at 0, 8, 24, and 48 hrs in female lampreys uninjected or injected at 0 hr with saline, GnRHa (50 µg/kg), GnRHa (5), GnRHant (50), GnRHant (5), or GTH (100).


0 hour
8 hour
24 hour
48 hour
1982
SEA LAMPREY
EXPERIMENT C
TEMP 11°C
ESTRADIOL-17β [ng/ml]
3.0
2.0
1.0
5 4
8 6 5
9 5 5
8 6 5
9 9 7
10 6 4
10 9 9
uninj
SAL
GnRHa 50
GnRHa 5
GnRHant 50
GnRHant 5
GTH 100

Effects of Steroid Agonists and Antagonists, Prostaglandin, Indomethacin, Benserazide on Plasma Estradiol and Gonads in Adult Pacific Lamprey, Entosphenus tridentatus and Petromyzon marinus

MATERIALS AND METHODS

Pacific Lampreys

Ocean-going adult Pacific lampreys were captured in the Stamp River, Vancouver Island, Canada in May, 1981 on their anadromous upstream spawning migration following completion of their parasitic ocean phase. They were transported to the National Marine Fisheries Service, Montlake Facility, Seattle and were kept in tanks supplied with flowing water, ranging in ambient temperature from 5.0^{o} to $14.0^{o}C$. The temperature ranged from $5.6^{o}C$ to $6.8^{o}C$ during the experiment. Generally, Pacific lampreys spawn in August, about one year, after return to freshwater, and thus they were approximately seven months from spawning.

The forty-five male and female lampreys used in these experiments averaged 38 g in body weight. Starting 6 January, 1982, (Day 0), the lampreys were treated every other day as follows:

Number of Fish	Treatment
10	Saline
10	Saline & tween 80 (1 ml /10 ml saline)
12	Clomiphene citrate (0.45 mg/fish)
13	Methallibure (0.45 mg/fish)

Plasma and gonadal tissue samples were taken following anesthetization with ethyl m-aminobenzoate methanesulfonate (MS 222) on days 0, 8, and 15, 24 hrs after the last injection. Individual weights and lengths were recorded for every lamprey at each sampling. Blood was collected in heparinized syringes from the caudal veins.

<u>Sea Lampreys</u>

Landlocked adult sea lampreys were captured in a trap in the Cheboygan River in May, 1982, on their anadromous spawning migration following completion of their parasitic lake phase. They were retained in raceways at the Hammond Bay Biological Station supplied with flowing lake water, ranging in ambient temperature during the experiment between $11.6^{\circ}C$ to $15^{\circ}C$. On 24 June, 240 lampreys were identified with floy tags and, in addition, the dorsal fins were clipped to designate mode of treatment.

Twelve different groups of lampreys, each consisting of 10 males and 10 females, were injected as follows: <u>saline</u> (0.6%; CS), <u>tween in saline</u> (tween; CT), ethamoxytriphetol (M2), medroxyprogesterone acetate (MA), clomiphene citrate (CC), flutamide (FL), methallibure (MT), benserazide (B), dexamethasone (DEX), metopirone (MET), prostaglandin F2α (Pg F2), and indomethacin (IND). The dose used for all treated lampreys (except those treated with prostaglandin F2α) was 0.45 mg/lamprey. Prostaglandin was given in a dose of 25 μg/lamprey. The lampreys used averaged 200 g in body weight. The injection volume given to each fish was 0.1 ml, intraperitoneally. The lampreys were injected with the appropriate compound on June 29, July 1, 3, and 5. On June 29, blood was taken from 10 males and females by collecting in heparinized syringes by cardiac puncture; at this time, gonad tissue was fixed in Bouin's solution. On July 6, all remaining lampreys were sampled for blood and gonad tissue.

Histology and Radioimmunoassay

The blood was centrifuged and plasma drawn off and stored frozen at -20°C until assayed for estradiol and androgens by radioimmunoassay (Sower et al., 1984; Sower and Gorbman, see preceding section).

The gonadal tissues were dehydrated in a series of alcohols, embedded in paraplast, sectioned at 8-10 μm, and stained with haematoxylin and eosin.

RESULTS

Pacific Lamprey

There were no significant sex differences in plasma estradiol levels of the various treatment groups. Therefore, the data from the males and females were combined for each treatment group. Plasma estradiol was significantly higher in lampreys treated with clomiphene citrate only on day 15 (3.63 ± 1.37 ng/ml) but not on day 7 (2.80 ± 1.06 ng/ml), compared to controls on day 0 (1.66 ± 0.23 ng/ml) (Fig. 13). Plasma estradiol significantly decreased from day 0 (1.66 ± 0.23 ng/ml) to day 7 and 15 (0.57 ± 0.12 and 0.93 ± 0.13 ng/ml) in the controls. Whether this decrease is the result of confinement or handling stress or whether it is part of a normal process is not clear. Plasma estradiol was not significantly different from controls after injections of methallibure. Plasma androgens were extremely low (< 0.01 ng/ml) or undetectable in most samples in our assays and showed no variation among the several treatment groups (Table 5). However, plasma androgens were slightly higher in lampreys treated with clomiphene citrate on day 15 compared to controls.

Figure 13. Plasma estradiol of Pacific lamprey (ng/ml) at days 0, 7, and 15 after injection with saline (S-control), tween-saline, (t-control), clomiphere citrate or methallibure.

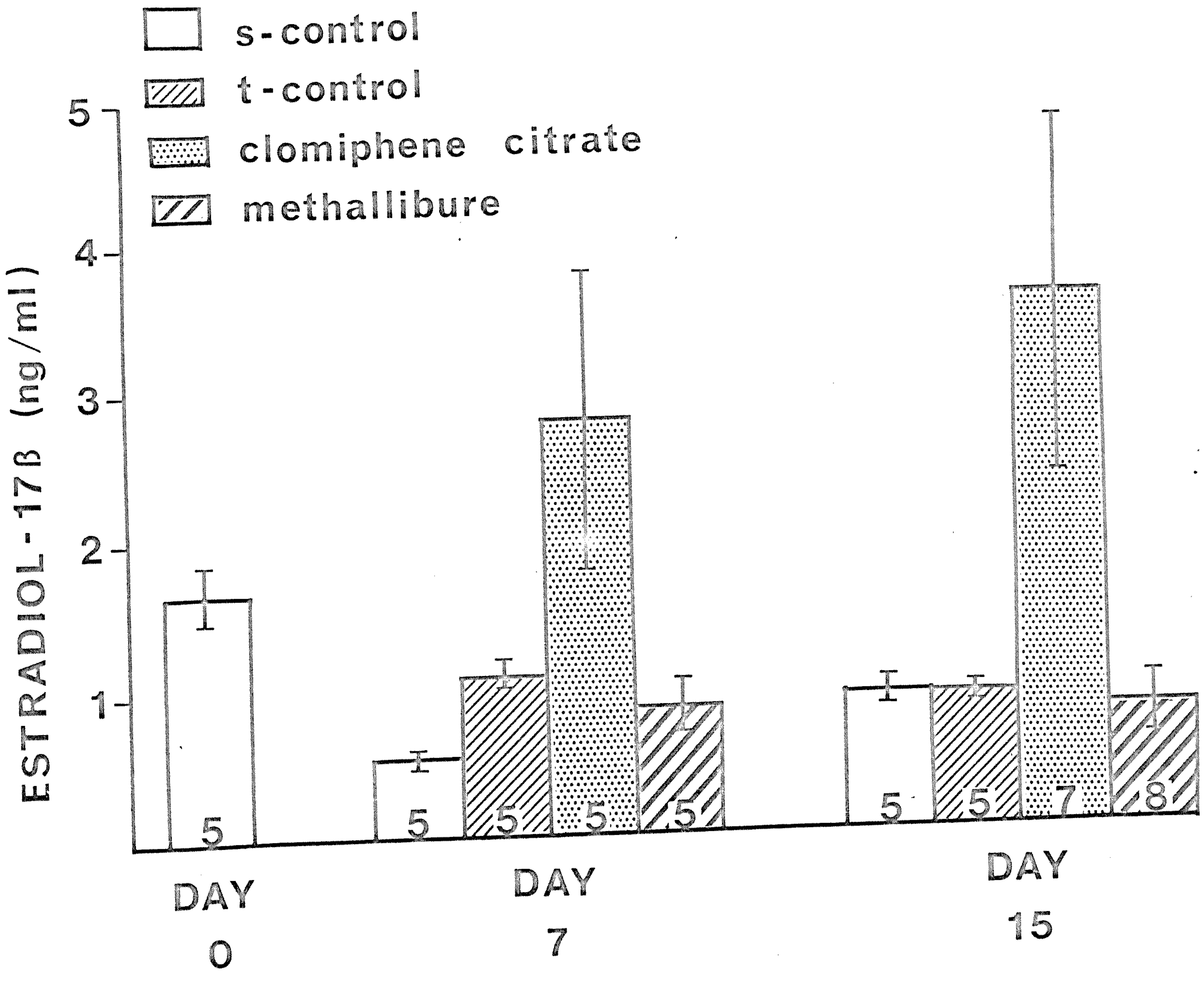

1982 PACIFIC LAMPREY
s-control
t-control
clomiphene citrate
methallibure
ESTRADIOL-17β (ng/ml)
5
4
3
2
1
5
5
5
5
5
5
5
7
8
DAY 0
DAY 7
DAY 15

TABLE 5. Total plasma androgens (ng/ml) of individual Pacific lamprey treated with clomiphere citrate, methallibure, saline (control) or tween-saline (control). *N.D., non-detectable

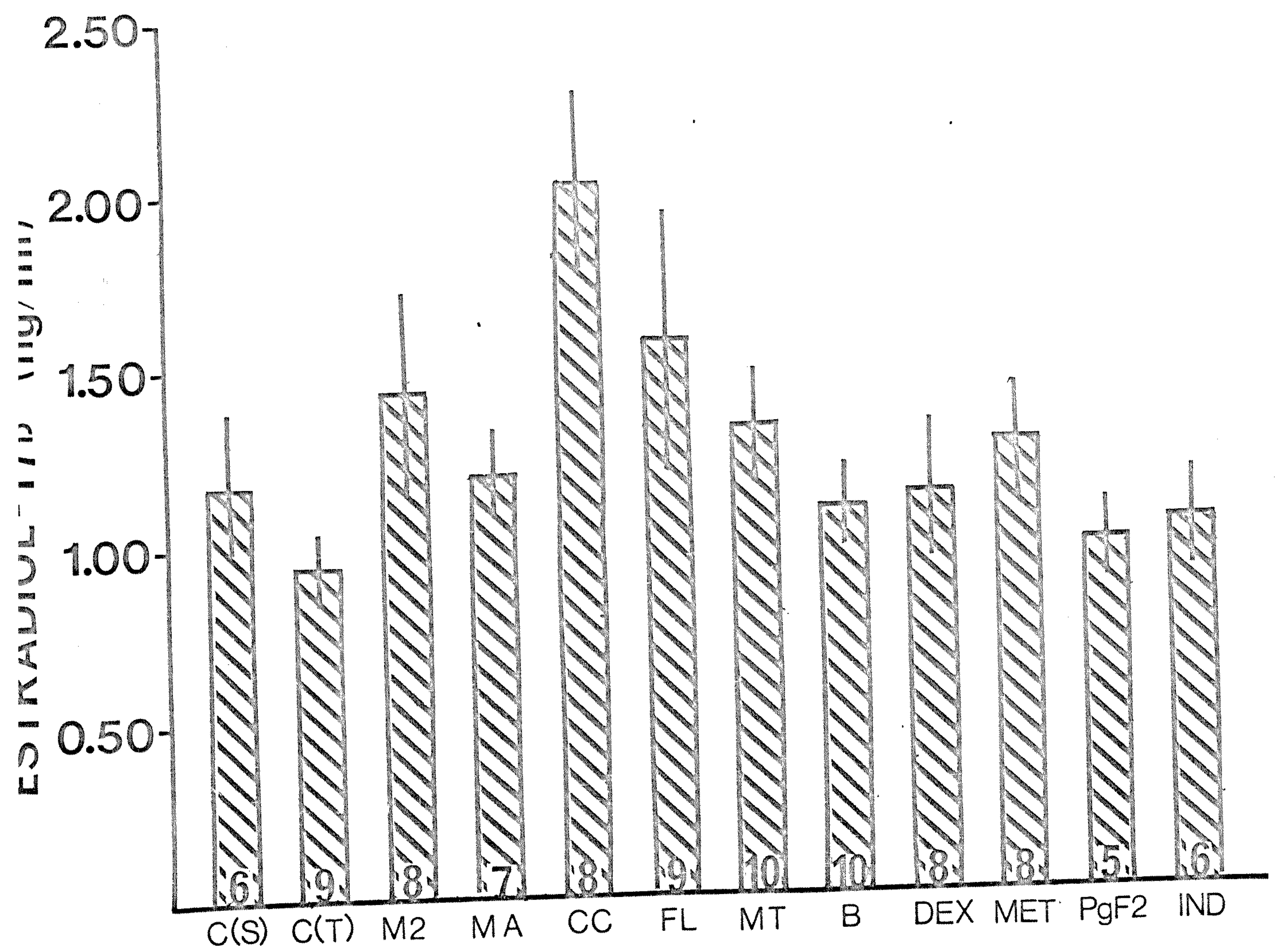
Day 0	Androgens (ng/ml)
Saline control	0.01
	*N.D.
	0.10
Day 7	
Saline control	0.02
Tween-saline control	0.10
	0.12
	0.03
Clomiphene citrate	0.08
	0.10
Methallibure	0.05
	N.D.
Day 15	
Saline control	0.02
	N.D.
	N.D.
	N.D.
Saline-tween	N.D.
	N.D.
	N.D.
	N.D.
	N.D.
Clomiphene citrate	0.07
	0.18
	0.30
	0.19
Methallibure	0.01
	N.D.
	N.D.
	N.D.
	N.D.

The testes of the control fish consisted of small defined cysts containing mainly primary spermatocytes. The ovaries of the control fish contained oocytes that were stained deeply by eosin and their germinal vesicles were at the periphery, indicative that vitellogenesis was completed. The larger oocytes had a thin follicular epithelial layer, while the smaller oocytes had a relatively thicker granulosa layer. There were no apparent changes in microscopic structures in the gonads treated with clomiphene citrate or methallibure that differentiated them from the controls.

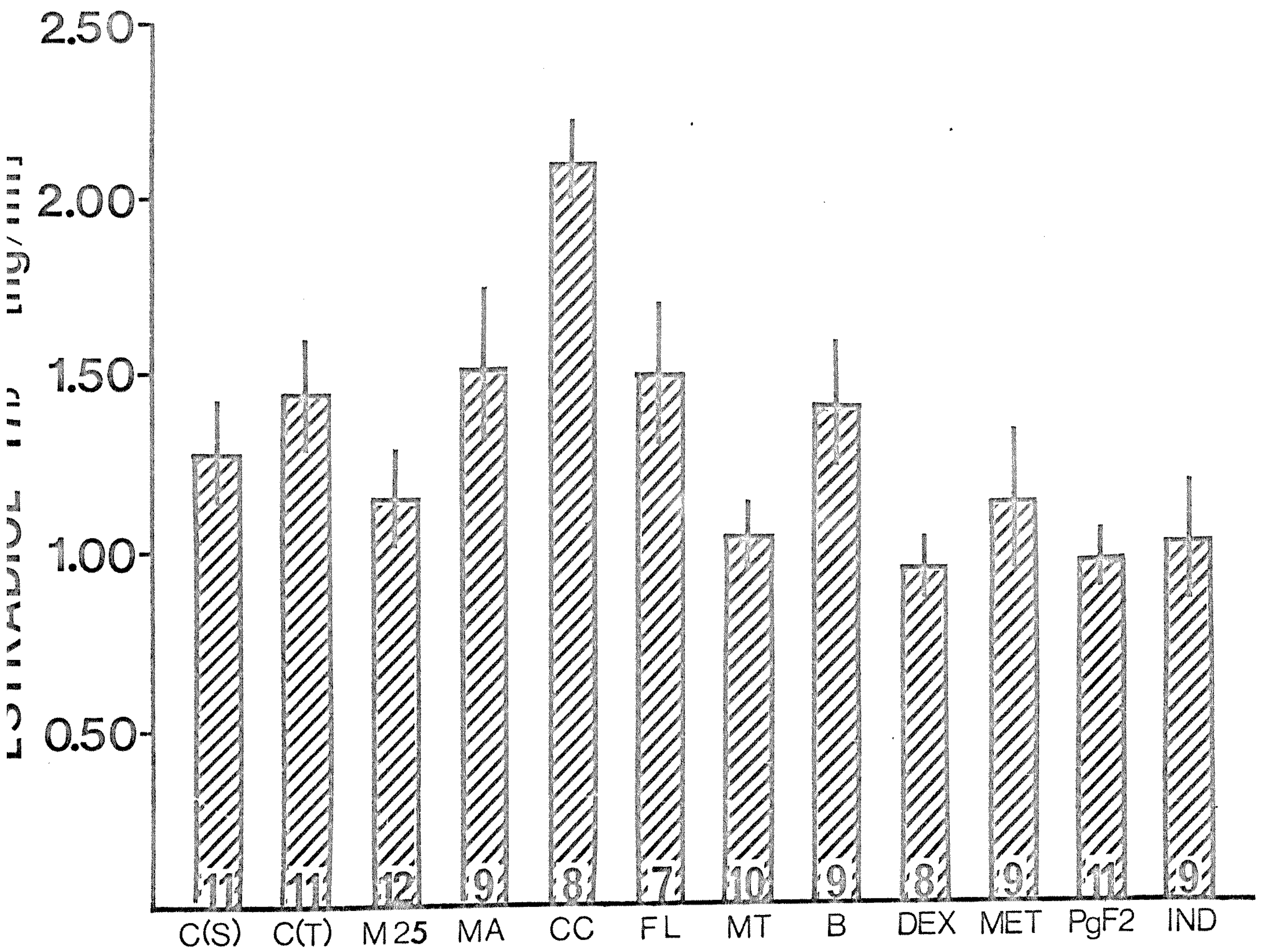
Sea Lamprey

There were no significant differences in plasma estradiol in the males and females of the different groups, but the data from the males and females are plotted separately in Figs. 14 and 15. Plasma estradiol was significantly higher in both male and female lampreys treated only with clomiphene citrate compared to controls. Plasma estradiol was not significantly different from controls after injections of any of the other substances.

Figure 14 and 15. Plasma estradiol of male and female sea lampreys (ng/ml) at day 8 after injection with saline (CS), saline-tween (CT), ethamoxytriphetol (MZ), medroxyprogesterone acetate (MA), clomiphene citrate (CC), flutamide (FL), methallibure (MT), benserazide (B), dexamethasone (PEX), metopirone (MET), prostaglandin F2α (Pg2F), and indomethacin (IND).



FEMALE
1982
SEA LAMPREY
2.50
2.00
1.50
1.00
0.50
6
9
8
7
8
9
10
10
8
8
5
6
C(S)
C(T)
M2
M A
CC
FL
MT
B
DEX
MET
PgF2
IND



MALE
1982
SEA LAMPREY
2.50
2.00
1.50
1.00
0.50
11
11
12
9
8
7
10
9
8
9
11
9
C(S)
C(T)
M25
MA
CC
FL
MT
B
DEX
MET
PgF2
IND

Preliminary Experiments with Ammocoetes

MATERIALS AND METHODS

Experiment 1, Sea Lamprey

Three treatment groups, each consisting of 20 ammocoetes (avg length = 125 mm), were injected intraperitoneally in 1981 with estradiol-17β (10 mg/fish), 17α -methyltestosterone (10 mg/fish), or solvent control (cocoa butter). The steroids were initially dissolved in warm cocoa butter and injected warm into each ammocoete. After cooling, the warm cocoa butter formed a pellet in the coelom, but was not well tolerated; in consequence, many of the ammocoetes died. The experiment was terminated.

Experiment 2

Ammocoetes were treated by immersion in estradiol or 17α - methyltestosterone solution in June, 1981. Again, three treatment groups consisted each of 20 ammocoetes (mean length, 125 mm). Dosages of estradiol and 17α -methyltestosterone used were 24.9 ug/ml water and 24.9 ug/ml water, respectively. In this experiment, a fungal infection not manageable by salt treatments terminated the experiments.

Experiment 3

In June, 1981, ammocoetes were transferred from a large freshwater tank with a sand bottom to 5-gallon plastic containers without sand, one week before they were injected. There were five treatment groups, each consisting of 10 ammocoetes. The fish were injected intraperitoneally as follows:

Treatment	(Dosage µg/10 g fish) June 9, 12
Control	--
GnRHa*	5
GnRHa	0.5
GnRHa	0.05
GnRHa	0.005

*GnRHa: D-Ala^{6}, des Gly^{10} ethyl amide gonadotropin-releasing hormone

Injection volume on June 9 (day 0) was 0.1 ml and on June 12, 0.05 ml. On day 0 and 8, ammocoetes were sampled for blood and gonadal tissue. The blood was centrifuged and plasma drawn off and stored frozen at -20°C until assayed for estradiol and androgens by radioimmunoassay (Sower et al., in press). The gonadal tissues were dehydrated in a series of alcohols, embedded in paraplast, sectioned at 8-10 µm, and stained with hematoxylin and eosin. Mortalities occurred in all groups.

RESULTS

Experiment 3

The gonads were relatively well differentiated in all groups. The ovaries contained oocytes that were in the first and second growth phase. The testes had nests of cells and atresia of oocytes, with about 2 or 3 oocytes remaining in a given tissue sample.

There were no significant differences of plasma estradiol in the males and females of the different groups. Therefore, the data from the males and females were combined for each treatment group. Plasma estradiol was not

significantly different in any of the groups on day 8, compared to controls (Table 6).

TABLE 6.

Treatment (μg/10 g fish)	Plasma estradiol) (ng/ml + SE(n))
Control	6.69 ± 2.23 (9)
GnRHa (5)	5.08 ± 2.80 (6)
GnRHa (0.5)	11.37 ± 3.47 (6)
GnRHa (0.05)	7.26 ± 4.97 (2)
GnRHa (0.005)	9.93 ± 2.51 (6)

DISCUSSION

Part I

The principal question addressed by our experiments 1 and 3 is whether there is hypothalamic control over reproduction in lampreys. This question in lampreys has special significance since these animals, with the hagfishes, are modern descendants of the most primitive vertebrates available for study. Proof that there is hypothalamic regulation of adenohypophysial function in this group would imply that evolution of this mechanism most likely antedated the origin of all known vertebrates.

There has been surprisingly little prior research on control over reproduction in Agnatha despite its significance. Characterization of this endocrine phenomenon is important in devising any practical procedures for regulating or deterring reproduction in lampreys. At our present level of knowledge it would not be possible to conceive in a rational way any kind of program aimed at field control of reproduction of lampreys.

The earlier experiments of Evennet and Dodd (1963) and Larsen (1973) showed by hypophysectomy and mammalian gonadotropin injections that although there is a relationship between the lamprey adenohypophysis and gonadal function this relationship is not the same as in higher vertebrates. In hagfishes there seems to be no demonstrable pituitary-gonad relationship (Matty et al., 1976; Gorbman, 1983). The hypophysial gonadotropic activity of lampreys appears to be directed more toward regulation of gonadal steroidogenesis than gametogenesis (see review by Gorbman, 1983). A further difference between petromyzontids and myxinoids is in the detectability of immunoreactive GnRH in the hypothalamus. Crim et al. (1979) found in lampreys such immunoreactivity in discrete preoptic neurones and in axonal

projections toward the neurohypophysis. They found no such immunoreactivity in the brains of hagfish (Eptatretus stouti).

Immunoreactive lamprey GnRH occurs in a brain region which in higher vertebrates also contains GnRH localization and forms part of a neuroendocrine mechanism for gonadotropic regulation. We now can add to this evidence the findings reported here showing that exogenous GnRH stimulates directly or indirectly (through the adenohypophysis) two phases of ovarian function in Petromyzon. Structurally and functionally the evidence strongly indicates that there is hypothalamic control over lamprey reproduction. Even without such experimental evidence, there has been circumstantial evidence of hypothalamic control in that breeding activity and completion of gonadal development in lampreys is synchronized with season, and all of a population of lampreys will mature and breed simultaneously.

It remains unclear which physical environmental clue(s) (photoperiod, temperature) normally can trigger gonadal maturation and function since appropriate experiments have not been done. Our own experiments have shown that endocrine stimulation alone by injection of doses of fish gonadotropin or GnRH sufficient to elevate plasma estradiol will not evoke ovulation at lower environmental temperatures (mean: 13°C). When the temperature was increased to 18°C by use of electric heaters, ovulation followed hormon injection. Unfortunately, Experiment 1 was not adequately designed to critically test the effect of temperature since no control animals were retained at 13°C. Practical experience at the Hammond Bay Biological Station is that adult P. marinus kept at temperatures below 15.5°C will not ovulate for up to 8 months and will generally die before that time without ovulation (Louis King, personal communication).

However, in Experiment 3, the lampreys were tested under different temperature and reproductive status; in addition, one of the studies included male lampreys. Lampreys that were tested in May at low temperatures and at times when normal circulating plasma estradiol was about 1 ng/ml were relatively more responsive to fish gonadotropin or GnRH in terms of elevated plasma estradiol (4-6 ng/ml), compared to lampreys tested later in the season. In July, the temperature averaged 11°C and normal circulatory levels of estrogen in the females were similar (mean, 1 ng/ml) and yet their response was less (2-3 ng/ml) than by females treated in May. In both experiments, plasma estradiol remained elevated for 48 hrs. Plasma estradiol in males treated with salmon gonadotropin GnRHa responded in a pattern similar to the second group of females at 11°C, however, plasma estradiol had started to decline by 48 hrs in the treated fish.

It is clear from our experiments that receptors in the lamprey adenohypophysis and/or ovary recognize and respond to a nonapeptide GnRHa analogue that has been found active in mammals and teleost fish. It is also clear that the GnRHant analogue, which in mammalian tests (Rivier et al., 1981a; Rivier et al., 1981b) is a competitive inhibitor of GnRH, has little or no activity in _Petromyzon_. Thus, receptors for GnRH in _Petromyzon_ are apparently specific and can distinguish between molecular variants of this peptide.

The fact that the lamprey gonad responds specifically by steroid secretion and ovulation to injections of exogenous heterospecific gonadotropic preparations indicates that a reasonably typical pituitary-gonadal relationship exists in this group. The possibility that the gonad may respond directly to some degree to GnRH cannot be ruled out by our experimental design. If this evidence now can be accepted as reasonably

well established then it is clear that in this Agnathan, and presumably in its extinct ancestors nearer the primitive vertebrate evolutionary line, there already was an evolved brain-pituitary-gonadal regulatory mechanism for control of reproduction. Thus, the origin of this mechanism appears lost in the unknown pre-vertebrate forms about which no clear information is available. The apparent absence of such a regulatory mechanism for gonadal function in hagfishes must be a secondary degenerative evolutionary phenomenon. If this were not so, it would be difficult to explain why in hagfishes the adenohypophysis separates off from the adjacent pharyngeal epithelium and becomes apposed to the neurohypophysis.

In neither the hagfishes nor lampreys is there a vascular portal system which could carry GnRH from the brain to the adenohypophysis (Gorbman, 1965; Jasinski, 1969). If this is a primitive pattern, then in the lampreys we must assume that GnRH must reach the adenohypophysis by diffusion from the adjacent and co-extensive neurohypophysis. If these several presumptions concerning the functional route for GnRH in lampreys are correct, then evolution of the portal vascular connection between neuro- and adenohypophysis is the only major anatomical feature of the hypothalamo-hypophysial system that evolves within the vertebrates (Gorbman, 1980).

Part II

In Agnathans, the cellular site of steroid synthesis and the physiological role of the sex steroids have yet to be clarified. Results from recent research suggest that steroid secretions and functions in lampreys differ from those of teleosts and higher vertebrates. Katz et al. (1982) demonstrated in adult sea lampreys the presence of androstenedione, estradiol, and estrone in similar quantities in both males and females; this may indicate a lack of sex-specific steroids and similarities in their

biological activities in these animals. Further evidence provided by Belvedere and Colombo (1983) showed that testicular and ovarian steroidogenesis were similar in quantity and type of steroid produced *in vitro* from two precursors, pregnenolone and testosterone, in the Brook lamprey, *Lampetra zanandreai Vladykov*.

Our findings support the conclusions derived from the research of Katz et al. and Belvedere and Colombo. Plasma estradiol levels fluctuated significantly and generally covaried in males and females through final maturation. However, a striking contrast occurred at ovulation and at spermiation: plasma estradiol significantly decreased in females but it increased in males. Plasma androgens, which were very low, also significantly covaried in males and females through final maturation and also at spermiation and ovulation. As Katz et al. (1982) have suggested, the estrogens in the sea lamprey, at least during final maturation, may not be sex-specific in their physiological roles; however, further work with steroid receptors at tissue sites would be needed to verify this conclusion.

Thus the role of estradiol in reproduction during this period is unclear. It is possible that certain "sex" steroids which are similar in quantity in both males and females may be involved in the complex spawning behaviour processes as suggested by Hardisty and Baker (1983). Certain areas of the brain have been demonstrated to accumulate ^{3}H-labelled estradiol in the river lamprey (Kim et al., 1980) indicating a potential influence of estrogens on brain receptors in influencing sex behaviour, as has been shown in higher vertebrates (Hardisty and Baker, 1983).

In our studies, there is in female lampreys a general increase in plasma estradiol prior to ovulation followed by a fall in the levels of estradiol at ovulation. This general increase in plasma estradiol before

ovulation coincides with the time of liquifaction of the yolk in oocytes. Furthermore, plasma estradiol increases following gonadotropin or GnRH injections, which induce ovulation. In salmonids, temporal interrelationships of gonadotropin and estradiol during gonadal development have been established (Fostier et al., 1978; Whitehead et al., 1978a,b). An inverse relation in time occurs between estradiol and gonadotropin as ovulation approaches: elevated estradiol levels and low gonadotropin levels exist prior to ovulation; subsequently, there is a decrease of estradiol just before and at ovulation followed and an elevation of gonadotropic levels. This negative feedback relationship reflects a similar and "classic" relationship seen in higher vertebrates and it appears in part to be present in lampreys. As stated earlier, gonadotropin(s) have not been identified in the lamprey, nor is it clear that they have a role in final maturation and ovulation. This is an area that is only partly addressed by our data as reported here, and it will require more research for fuller elucidation.

Katz et al. (1982) demonstrated the apparent absence of 5α-dihydrotestosterone (DHT) and testosterone in the sea lamprey blood during final maturation. The levels of total androgens which reflect DHT, testosterone, and 11 keto testosterone in the sea lamprey in the present study were very low and showed no distinct pattern over time. Testosterone in testes of _L. fluviatilis_ has been shown to be rapidly converted _in vitro_ into its 15-hydroxy derivatives (Kime and Rafter, 1981). 15-hydroxy steroids have not been identified in the endocrine glands of other vertebrates, nor have they been shown to have any biological activity (reviewed by Kime and Rafter, 1981). Seiler et al. (1983) reported considerable hydroxysteroid dehydrogenase (HSD) activity in studies of

testosterone metabolism which indicated that $3\beta,17\beta$-dihydroxy-5α androstane is formed as a 5α-reduced metabolite of testosterone. These authors suggest the possibility that the testosterone metabolites may have an active physiological role in lampreys. The functional role of various sex steroids will not be resolved until we have identified the steroids in lampreys and correlated these steroids with biological activity during reproductive processes.

Part III

The levels of thyroid hormones at the time of spawning observed by us do not coincide with the levels reported by Hornsey (1977) for the same species of lamprey. In a single fully mature female, Hornsey (1977) measured 80 ng/ml of T_4. In our previous investigation on Pacific lamprey (Entosphenus tridentata), we measured T_4 levels as high as 37 ng/ml in specimens maintained for more than two months in the laboratory (Plisetskaya et al., 1983a). This elevation from lower levels could be considered as a part of the maturation process. The more natural conditions of the anadromous adult lampreys kept at Hammond Bay Station may possibly account for the fact that T_4 did not increase.

It has been shown previously by Sower et al. (1984) that during smoltification of salmon there are surges of both plasma E_2 and T_4 and these surges clearly coincide. A similar tendency of these two hormones to vary together can be observed in lampreys during late prespawning and spawning periods. There are no clear correlations between levels of estradiol and T_3.

There have been recent reports concerning the stimulatory action of thyroid hormones on insulin secretion in fish (Plisetskaya et al., 1983a,

1983b). The non-homologous RIA system used in this recent study does not permit any conclusions about the absolute levels of circulating hormones. Nevertheless, the relative changes in IRI circulating levels in both species of lampreys are very similar in pattern. The Baltic lampreys enter the fresh water in Leningrad area in October-November and stay in the rivers over winter before spawning. Spawning begins in May-June at particular limiting water temperatures. At the time when the lampreys enter the river mouth the growth of the oocytes and the very beginning of vitellogenesis can be observed histologically in the ovary. Vitellogenesis proceeds slowly during the autumn and early winter, and completed in March (Plisetskaya et al., 1976). At the same time, the IRI levels which have been decreasing gradually during the winter begin to increase in the fish during the spawning period.

Histological examination of the ovaries of _P. marinus_ in these studies (Table 2) indicated that vitellogenesis was already completed at the time when we began plasma sampling for hormone levels. Therefore, we can recognize that there is similarity in the circulating IRI profiles in both lamprey species, probably reflecting the similar metabolic requirement patterns of regulation of metabolic processes during this period of life cycle. Since we measured the plasma concentrations of only a few metabolites like protein and PFA, we are not able to generalize about the metabolic events in these anadromous fish but some parallels between _L. fluviatilis_ and _P. marinus_ can be suggested. The plasma protein concentrations in Baltic lampreys when they enter the river is about 50-55 mg/ml (Ivanova-Berg and Sokolova, 1959; Larsen, 1980). By the time of spawning the values drop to 19-20 mg/ml (Ivanova-Berg and Sokolova, 1959). Sex differences have not been investigated. The same trends in circulating

protein profiles appeared in P. marinus, but in addition, we found substantial differences in plasma protein concentrations between male and female lampreys. About two weeks before spawning, plasma protein concentration began to decline in females, the most abrupt drop occurred at ovulation. At the same time in males, no substantial changes took place. We can speculate that the sexual differences in plasma protein concentration may reflect the decline in E_2, insulin, and T_4 levels in female fish. Whereas in males, neither E_2 nor insulin levels, nor protein concentrations appear to be changed.

Recent studies using in vitro salmon hepatocytes provide evidence that insulin as well as E_2 stimulate protein secretion from hepatocytes, whereas T_3 has no such stimulating effect (Bhattacharya et al., 1984; Plisetskaya et al., 1983). We do not know whether this phenomenon occurs in lampreys and to what we can ascribe the large changes in plasma protein in lampreys at this stage. Furthermore, it is not even clear whether the degenerating lamprey liver can be the source of plasma proteins. Future investigations are needed to answer this question.

Plasma fatty acids seem to provide an important energetic substrate during the spawning migration (Plisetskaya, 1980). Along with the already described depletion of carcass fat (Bentley and Follett, 1965; Beamish, 1979), the circulating levels of plasma fatty acids (PFA) gradually decline, but they remain comparatively high until the very end of spawning. In view of the known lipolytic action of T_3 in cyclostomes (Plisetskaya et al., 1983c), both the decline of PFA levels in June and their elevation in females at the last day of our investigation on August 2 (when T_3 levels were accordingly low and high), are understandable. The low level of IRI in

female lamprey observed on August 2 may enhance lipolysis since insulin in lampreys has an obvious lipogenic effect (Plisetskaya, 1980).

Part IV

Experiment 3 was designed to investigate the actions of 10 different compounds on plasma steroid levels and on gonadal histology. Gonadal histology in the study has not yet been fully evaluated. The ten compounds tested are all pharmacologic agents with known gonadal actions in mammals. The different compounds and their essential actions in higher vertebrates are as follows:

	Known actions in vertebrates other than cyclostomes
ethamoxytriphetol	antiestrogen
medroxyprogesterone acetate	antiestrogen
clomiphene citrate	antiestrogen
flutamide	androgen antagonist
methallibure	antigonadotropin
benserazide	inhibitor of dopa decarboxylase; stimualtes prolactin release
dexamethasone	corticosteroid agonist
metopirone	11-β hydroxylase inhibitor
prostaglandin FZ	
indomethacin	blocks prostaglandin synthesis

These compounds, excepting methallibure and clomiphene citrate, were tested only at one dose and in a single experiment, and yielded no differences compared to controls. Methallibure and clomiphene citrate were tested both in Pacific lamprey and the sea lamprey. Clomiphene citrate in

these limited experiments was the only compound that changed plasma estradiol levels in both species of lampreys.

Clomiphene citrate (Clomid, trademark Merrell-National Labs) is presently used as a ferility drug in anovlatory women. Its suggested mode of action in these women is competitive inhibition of estradiol which is followed by negative feedback release of pituitary luteinizing hormone (LH) (Boyar, 1970; Nagel et al., 1970). In rats, clomiphene citrate acts as an antifertility agent, suppressing LH (Schally et al., 1970). Clomiphene citrate is considered to inhibit the full expression of the effects of estrogen on a variety of target cells. The primary site of action of clomiphene citrate is considered the pituitary, with a second possible site being the hypothalamus (Roy et al., 1963).

The observed increase of estradiol following clomiphene citrate in the lampreys undergoing final maturation indicates a possible gonadotropic stimulation by release from negative feedback of estrogens on the hypothalamo-pituitary axis. A study by Kim et al. (1981) provides further evidence of potential feedback of estrogens by the demonstrated presence of estrogen target neurons in brain of both the ammocoete and adult lamprey. These studies are only preliminary and further research must be done to verify the mode of action of clomiphene citrate in lampreys. Negative type feedback in lampreys has yet to be characterized. However, the clear response to clomiphene argues for a type of negative feedback by estrogen at the hypothalamic and/or adenohypophysial level. The demonstrated presence of such a feedback relationship is most encouraging since it indicates that this mechanism can be attacked in future experiments aimed at control of lamprey reproduction.

Part V

The initial and preliminary experiments with ammocoetes following injections of GnRHa at different doses did not yield any different results compared with controls. However, the fact that ammocoetes had higher plasma estradiol levels compared to those of adults is interesting and suggests some possible role of estrogens perhaps in the metamorphosis of these animals. Further experiments are certainly warranted in the process of endocrine control of sexual differentiation and metamorphosis in ammocoetes.

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3. Ethamoxytriphetol and clomiphene citrate

   Merrel Dow Pharmaceuticals, Inc., Cincinnati, OH
4. Medroxyprogesterone acetate -- Diosynth, Inc., Chicago, IL
5. Flutamide -- Schering Corporation, CA
6. Benserazide -- Hoffman-LaRoche, Inc., CA
7. Metopirone -- CIBA-GEIGY Corporation, Summit, NJ

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