GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

Hormonal control of metamorphosis in sea lampreys: examination of the potential for alteration of life history type

by:

Dr. John H. Youson[a], Fred W. Keeley[b], and John A. Holmes[a]

[a]Division of Life Sciences
University of Toronto at Scarborough
1265 Military Trail
Scarborough, Ontario Canada M1C 1A4

[b]Cardiovascular Research
Research Institute
Hospital for Sick Children and the Departments of Biochemistry and Clinical Biochemistry
University of Toronto
Toronto, Ontario Canada M5S 1A1

February 1998

---

[1]Project 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.

Hormonal control of metamorphosis in sea lampreys: examination of the potential for alteration of life history type

Year 3 Report - 1997

Great Lakes Fishery Commission
2100 Commonwealth Blvd
Suite 209
Ann Arbor, MI 48105-1563

John H. Youson[1], Fred W. Keeley[2], and John A. Holmes[1]

[1] Division of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ontario, Canada M1C 1A4;

[2] Cardiovascular Research, Research Institute, Hospital for Sick Children and the Departments of Biochemistry and Clinical Biochemistry, University of Toronto, Toronto M5S 1A1

Address correspondence to J. H. Youson[1]

Phone: (416) 287-7436
FAX: (416)-287-7642
e-mail: youson@scar.utoronto.ca

February, 1998

Introduction

Metamorphosis is an interval in the life cycle of lampreys when the larval growth phase is terminated and the larvae enter a new developmental phase of immense changes to tissues and organs which prepare the organism for life as an adult. The same basic changes take place during metamorphosis in all lamprey species, including both parasitic and nonparasitic species. These two life history types are designated as such because metamorphosis in the former results in a juvenile which will immediately commence feeding as a parasite on teleost fishes, whereas recently transformed juveniles of nonparasitic species start to undergo sexual maturation immediately without ever feeding. There is no information on the factors (hormonal, environmental or genetic) which direct the two adult life history types in lampreys. However, a comparison of these factors during metamorphosis and early adult life in nonparasitic and parasitic species could provide very useful data for redirecting adult behaviour in one or both of these two life history types. Furthermore, developmental events in all animals utilize energy which is redirected from normal metabolic processes and these events may result in a dramatic modification of an essential body organ. Thus, like other vertebrates, lampreys undergoing developmental changes during metamorphosis are particularly vulnerable to external factors which might influence the critical synchrony of events and lead to either death or to aberrant essential structures or physiological processes.

The introduction of the parasitic sea lamprey (*Petromyzon marinus*) into the Great Lakes had pronounced effects on both sport and commercial fisheries. The nonnative sea lamprey caused such a disruption in the benthic fish community various measures had to be introduced to control the abundance of this predator. One of the most successful methods of control has been the chemical treatment of streams to kill the harmless larvae before and during their metamorphosis.

An unfortunate consequence of this treatment, is that the chemicals are specific to all lampreys, including native lamprey species (almost all are nonparasitic), which are part of the ecosystem of their natal streams. Recently, however, there has been a call from the Great Lakes Fishery Commission (GLFC) for alternate means of controlling lamprey abundance to meet the goal of reducing the use of chemical control with the present larvicide.

When responding to this call from the GLFC we were directed by the following facts: (1) larval lampreys and adult nonparasitic lampreys are not a problem in the Great Lakes, (2) metamorphosis is a vulnerable interval of the lamprey life cycle, (3) we know very little about metamorphosis in nonparasitic species, and (4) our laboratory had just recently been able to induce metamorphosis in sea lampreys (Holmes and Youson 1993). Given these findings, we believed that it might be possible over the long-term to develop a method to redirect the life history of immediately postmetamorphic sea lampreys. We supported our claim that this might prove to be a novel and viable alternate control with proof of some major advances in lamprey biology that have been made in our laboratories in the past several years. Some of these advances are concerned with environmental and physiological cues which are important to metamorphosis and these have been summarized in the Completion Report of our companion contract (Holmes and Youson, Dec. 31, 1996). Of particular relevance to the present report is that following induction, metamorphosis in sea lampreys (Holmes and Youson 1993) does not follow a normal pattern of development (Youson 1994) in that the animals are similar in appearance to nonparasitic adults. However, the serum levels of thyroid hormones, which are drastically reduced during spontaneous metamorphosis of Great Lakes sea lampreys (Youson et al. 1994), were similarly reduced in the induced animals (Youson et al. 1995a; reported in 1995 annual report). The idea that plasticity exists during metamorphosis, such that adult life history can follow either a parasitic or a nonparasitic mode, was previously noted in a natural population of nonparasitic lampreys in British Columbia (Youson and Beamish 1991). In addition, we showed that metamorphosis in sea

lampreys is a critical phase in larval life when a major stimulus to the reproductive system can be found (Youson and Sower 1991). A subsequent study, described substantial differences in the timing of increases in concentrations of the two gonadotropin-releasing hormones (GnRHs) between the metamorphoses of a nonparasitic and a parasitic lamprey (Youson et al. 1995b, reported in 1995 annual report). These data support our hypothesis that adult life history may be redirected. Therefore, in addition to continuing our exploration of the hormone control of metamorphosis in sea lampreys, we believed that we should seek basic information on the metamorphosis of nonparasitic lampreys from both spontaneous metamorphosis and those subjected to experimental manipulation.

Statement of Objectives

The above findings lead us to make the following statements regarding metamorphosis and sea lamprey control: "We believe that we have provided a new and fresh approach to the question of control by examining all aspects of metamorphosis and have yielded data which could lead to the reduction in use of TFM and other data which indicate that we may be able to manipulate development at this vulnerable period in the life cycle."; and "There are existing data on metamorphosis which provide support for the potential of this phase of the life cycle as a future control measure."

Our primary long-range objectives are:

1. To identify all hormones taking part in the metamorphic process in lampreys; and
2. To examine methods that might be used to induce, delay or alter metamorphosis in sea lampreys as a future technique for control, including elimination of the adult feeding phase or advancing/retarding sexual (reproduction) maturation.

Our short-term objectives are:

1. To further characterize our method of inducing precocious metamorphosis in sea lampreys.
2. To clone and sequence lamprey pituitary hormones and establish their involvement and development in both parasitic and nonparasitic species during metamorphosis.

Work Plan for 1997 (Year 3)

1. Minimum exposure to goitrogens necessary for induction of metamorphosis in sea lampreys,
2. Test the efficacy of other goitrogens for inducing metamorphosis in sea lampreys, and
3. Expression of pituitary hormones during metamorphosis in parasitic (sea lamprey) and nonparasitic (brook lampreys) species.

Results

The results described below include the descriptions of the three items listed in the work plan for 1997. Also included are additional research, which we began in response to our results from 1995 and 1996.

1. Induced Metamorphosis

A. Minimum exposure time needed to induce metamorphosis

Our earlier studies demonstrated that the goitrogen $KClO_4$ induces precocious metamorphosis in sea lampreys at a time of the year and at a size when a larva would not normally

initiate metamorphosis (Holmes and Youson 1993). Furthermore, treatment with $KClO_4$ resulted in reductions in of serum thyroid hormone (TH) levels comparable to those seen in animals in the early stages of spontaneous metamorphosis (Youson et al. 1995a). Both of these findings were confirmed by subsequent studies of $KClO_4$ (see 1995 and 1996 annual reports). Although larvae were continuously exposed to $KClO_4$ for 2-3 months in these studies, we do not know if continuous exposure for this length of time is a necessary prerequisite for inducing precocious metamorphosis in sea lampreys. Thus, we conducted a study designed to determine the minimum length of $KClO_4$ treatment needed to induce precocious metamorphosis in sea lampreys. We briefly reported on this study in our Year 2 (1996) annual report. Here we add the serum TH data which wasn't available for the previous report.

Larval sea lampreys ($\geq$ 120 in length) were collected from Putnam Creek (New York) in June 1995 and were transported to our laboratory at the University of Toronto. Larvae were kept in a large fibreglass tank with 6-8 cm of sand for burrowing and continuous flow of aerated, dechlorinated tap water until the start of the experiment. Sea lampreys were fed a suspension of bakers yeast once weekly throughout the holding and experimental periods. One week prior to the start of the study larval lengths and weights were recorded, and 10 individuals were randomly assigned to each of the five experimental tanks (21 L). All experimental tanks contained 6-8 cm of sand for burrowing and 12 L of standing, aerated, dechlorinated tap water. Tanks were cleaned, their water changed, and fresh treatments were added every two weeks.

Following a one week acclimation period, each tank was randomly assigned a 0.05% $KClO_4$ treatment length of 0 (control), 2, 4, 8 or 16 weeks exposure. After the exposure period, sea lampreys were removed from their treatment tank, examined for external signs of metamorphosis, and placed in a clean tank containing untreated water for the reminder of the study.

When the last group of lamprey reached the completion of its treatment length (16 weeks) all animals were examined for external signs of metamorphosis and returned to their untreated aquaria for 9 weeks. Twenty-five weeks from the start of the study all sea lampreys were measured for length and weight, examined for external signs of metamorphosis, bled by caudal severance, and sacrificed. The experiment began Oct 17, 1995, and was terminated on Mar 26, 1996. Serum thyroxine (T_4) and triiodothyronine (T_3) concentrations were determined using radioimmunoassays (Manzon and Youson 1997). Length, weight, serum T_4 and T_3 data were examined for statistically significant differences between treatment groups using analysis of variance (ANOVA) and Tukey-Kramer's post hoc test. Means were accepted as significantly different if $P \leq 0.05$.

The incidence of metamorphosis increased significantly with an increase in the length of exposure to 0.05% $KClO_4$ (Fig. 1). The highest incidence of metamorphosis (100%) and the latest stage of development (stage 6) were both observed in sea lamprey treated with $KClO_4$ for 16 weeks. Sea lamprey treated with $KClO_4$ for less than 16 weeks showed a lower incidence of metamorphosis, but after only 4 weeks of treatment 30% of the animals had been induced into metamorphosis (Fig. 1B). The incidence of metamorphosis among animals exposed for 4 and 8 weeks was 60% and 80%, respectively, 16 weeks from the onset of the study (Fig. 1A). However, at 25 weeks from the onset of the study only 30% and 33% of the same sea lamprey, respectively, showed any signs of metamorphosis (Fig. 1B). Additionally, sea lampreys treated with $KClO_4$ for 4, and 8 weeks were predominately in stages 1 and 2 of metamorphosis, in comparison to stages 5 and 6 for sea lamprey receiving 16 weeks of $KClO_4$ treatment. Since there were no statistically significant differences in larval size (length and weight) between treatment tanks at the beginning of the study, size is unlikely to be a factor in explaining the above differences in the incidence of metamorphosis.

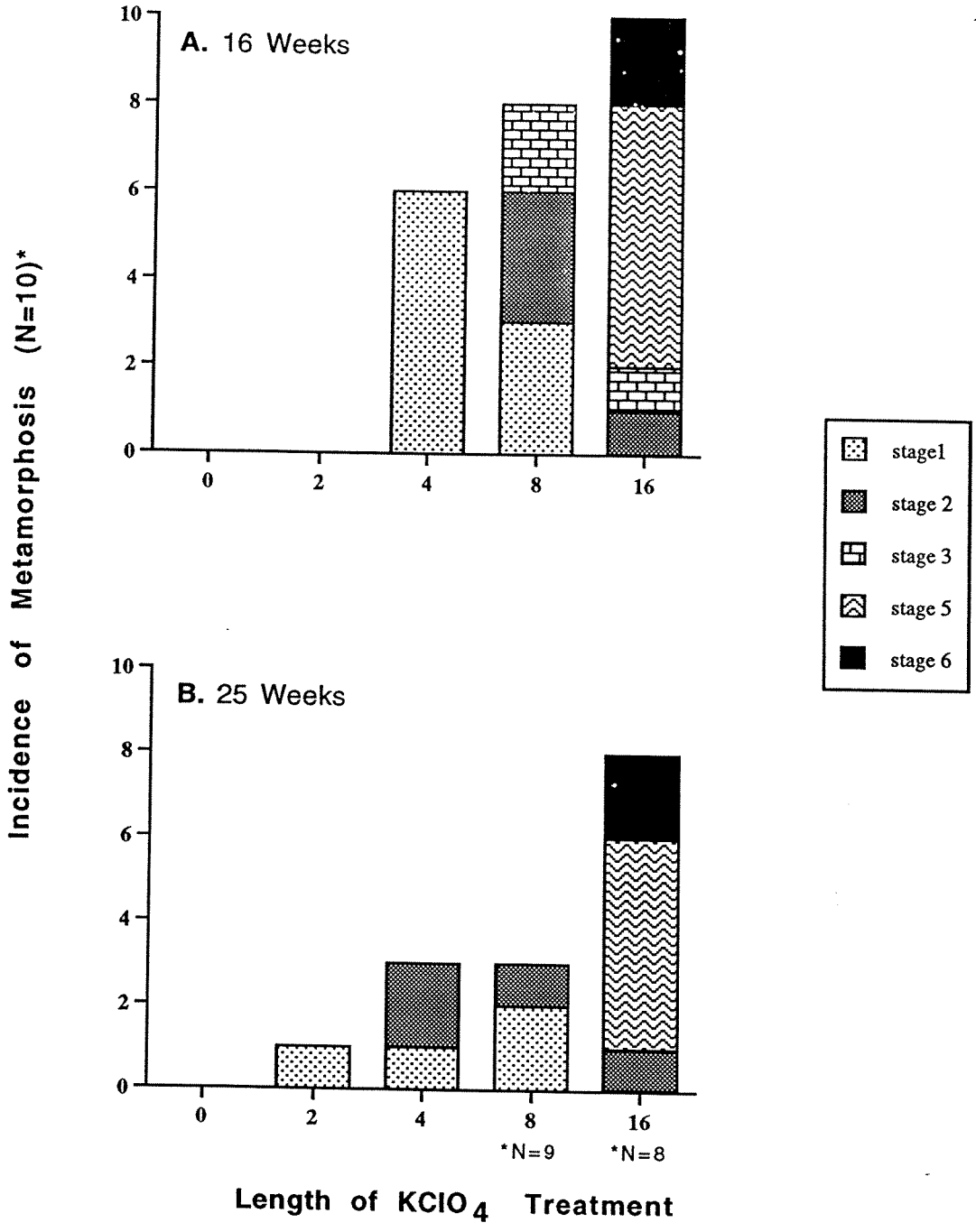

Figure 1. Stage and incidence of metamorphosis in larval sea lamprey treated with $KClO_4$ for 0, 2, 4, 8, and 16 weeks (**A**) 16 weeks from the onset of the study and (**B**) 25 weeks from the onset of the study.

Serum T_4 concentrations were significantly depressed relative to control values in sea lampreys treated with $KClO_4$ regardless of treatment length (Fig. 2). Even sea lamprey exposed to $KClO_4$ for only 2 weeks maintained serum T_4 concentrations significantly lower than control values 23 weeks after the removal of $KClO_4$ treatment.

Serum T_3 concentrations in sea lamprey treated with $KClO_4$ for 2 weeks did not differ significantly from control values. However, sea lamprey receiving 4 or 8 weeks of $KClO_4$ treatment had serum T_3 concentrations which were significantly lower than values for control sea lamprey (Fig. 2). Sea lamprey in the 16 week $KClO_4$ treatment group had serum T_3 concentrations which were significantly lower than values in all other treatment groups.

The results of this study indicate that long-term ($\geq$ 16 weeks) treatment with $KClO_4$ results in the highest incidence of induced metamorphosis in larval sea lamprey (Fig. 1). Although treatment of larvae with $KClO_4$ for shorter lengths did induce metamorphosis, both the incidence and stage of metamorphosis were greatest in the 16 week treatment group. A comparison of the metamorphosis data collected at 16 and 25 weeks from the onset of the study (Figs. 1A and 1B) also suggested that the animals may have some plasticity in reversing the early changes of induced metamorphosis. This suggestion was based on the observation of 60% and 80% induced metamorphosis 16 weeks from the onset of the study in 4 and 8 week $KClO_4$ treatment groups, respectively, but only 30 and 33% induced metamorphosis in the same group of animals 9 weeks later.

Treatment of larval sea lamprey with $KClO_4$ for as little as 2 weeks (followed by 23 weeks of untreated water) resulted in significantly depressed serum T_4, but not serum T_3 concentrations. These data suggest that $KClO_4$ treatment has some long-term effects on the ability of the larval

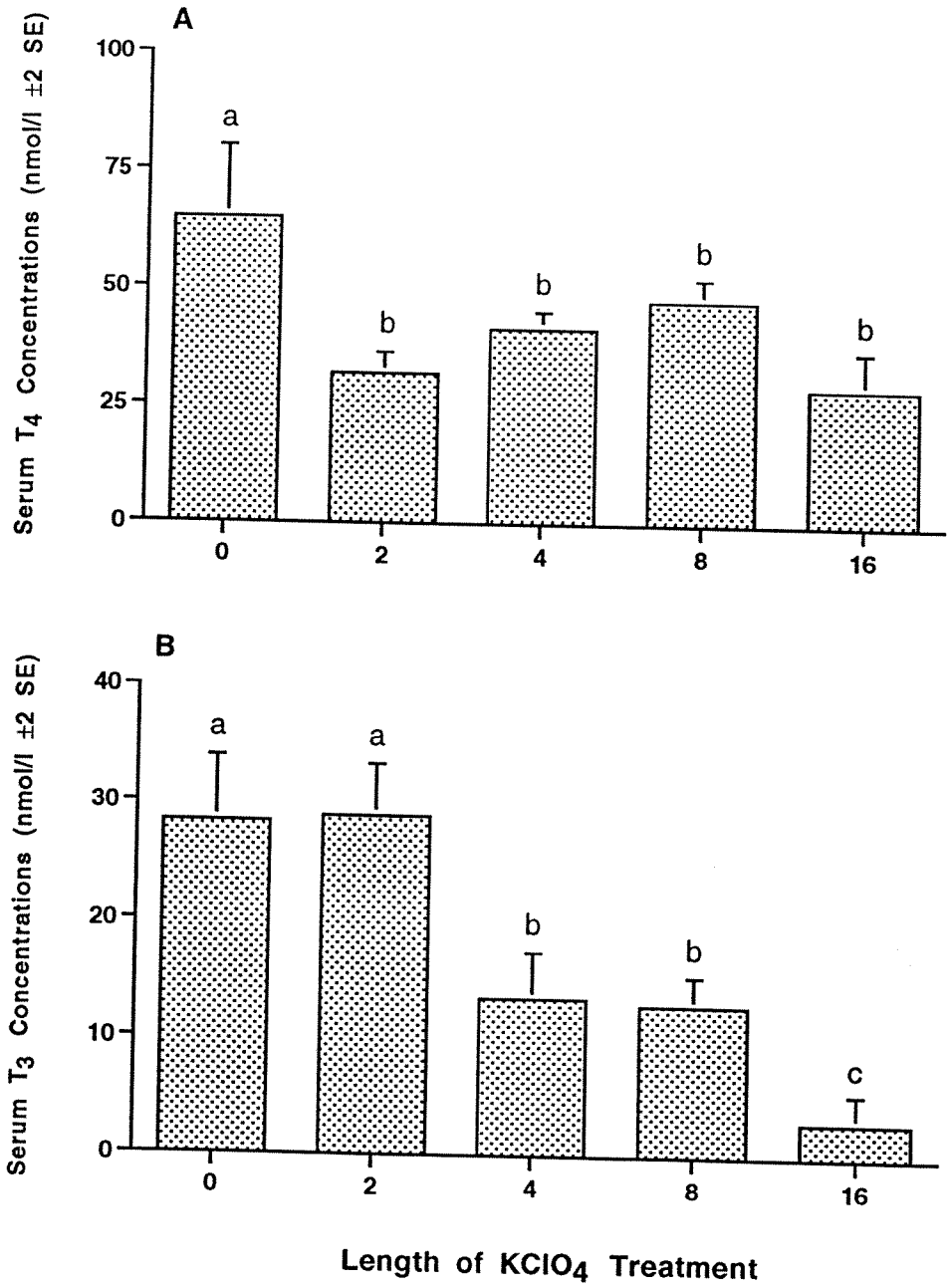

Figure 2. Mean serum thyroxine (T_4; **A**) and triiodothyronine (T_3; **B**) concentrations in larval sea lamprey exposed to $KClO_4$ for 0, 2, 4, 8, and 16 weeks, at 25 week from the onset of the study. Within a plot values are statistically different if letters are different ($P \leq 0.05$).

endostyle to synthesize T_4, and that serum T_3 concentrations recover more rapidly than serum T_4 concentrations (Fig. 2). This concurs with the suggestions that T_3 is more biologically active than T_4, and thus should be regulated more stringently (Jameson and DeGroot 1995). We infer from the fact that the incidence of induced metamorphosis was low when the serum T_3 concentrations were not fully depressed (2, 4, and 8 week treatments) suggests that a decline in serum T_3 is particularly important for induced metamorphosis. The importance of depressed serum T_3 concentrations is supported by the observation that treatment with exogenous T_3, but not T_4, will inhibit spontaneous metamorphosis (Youson et al. 1997). Furthermore, the observed decline in serum T_4 but not serum T_3 concentrations in $KClO_4$ treated larvae, which did not metamorphose, also supports the suggestion that declines in serum T_3 concentrations are critical to induced metamorphosis, and concur with the notion that T_3 is more biologically active than T_4 (Jameson and DeGroot 1995). We conclude that $KClO_4$ as an inducer of precocious metamorphosis exhibits some characteristics of a typical dose-response relationship in that the incidence of metamorphosis increases with the length of exposure period. A period of 16 weeks is required to induce metamorphosis in all sea lampreys $\geq$ 120 mm in size exposed to 0.05% $KClO_4$. The effect of $KClO_4$ on serum TH concentrations, particularly T_3, the more biologically active TH, is important to the induction of metamorphosis. We do not know the mode of action of $KClO_4$ at present.

B. Potential of other goitrogen treatments

Studies of precocious metamorphosis in lampreys using the goitrogen propylthiouracil (PTU) have produced contradictory results. Leatherland et al. (1990) exposed larval *Geotria australis* to 10 mg PTU/L for 70 days, but did not find any animals induced into metamorphosis, despite significant reductions in serum TH levels relative to untreated control larvae. These findings were confirmed by our own work on sea lampreys in which exposure of three size-classes (65-95 mm, 110-119 mm, > 130 mm) to 10 mg PTU/L for up to 127 days failed to induce

metamorphosis, even though TH levels were significantly depressed relative to levels in untreated control animals (see 1995 and 1996 annual reports). In contrast, Suzuki (1986, 1989) reported that PTU induced complete metamorphosis in some Lampetra reissneri. Although some of these reports might be explained by species differences, we believe the fact that PTU has a presumed mode of action that differs from that of $KClO_4$ (Cooper 1990), offers a more logical explanation for the differing abilities of these goitrogens to induce metamorphosis in lampreys. Accordingly, we examined the potential of other goitrogens, with presumed modes of action similar to either $KClO_4$ or PTU, as inducers of precocious metamorphosis in lampreys.

Larval sea lampreys (≥ 120 m in length) were collected from the Platte River (Michigan) in September 1996 and transported to our laboratory at the University of Toronto. Details of the holding procedures prior to and during the experiment are the same as described in 1A above. Thirty larval sea lamprey were exposed to the following goitrogens: $KClO_4$, potassium thiocyanate (KSCN), or methimazole (MMI) as shown below:

Goitrogen Treatment	Concentration (%)
Controls	Untreated water
MMI	0.01, 0.001
KSCN	0.005, 0.0005
$KClO_4$	0.01, 0.001

at one of two possible concentrations for 16 weeks. The experiment was initially designed to last 16 weeks beginning January 28, 1997. However, 6 weeks after the onset of goitrogen treatment mortality was greater than 50% in the MMI tanks. This prompted the immediate sacrifice and sampling of all remaining MMI treated sea lamprey. Sampling involved the determination of metamorphic stage, the recording of length and weight, the collection of serum, and the sacrifice and fixation of the sea lamprey in Bouin's fluid. At the time the MMI animals were sampled, sea

lamprey in one aquarium from each of the control, $KClO_4$, and KSCN groups were examined for external signs of metamorphosis, staged, and returned to their tanks. All control, $KClO_4$- and KSCN-treated sea lamprey were sampled 16 weeks after the study began.

Treatment of larval sea lamprey ≥ 120 mm in length with 0.001% MMI or 0.01% MMI for less than 6 weeks results in 57% and 63% mortality, respectively, however, metamorphosis was induced in 31% and 55% of the surviving animals in the above treatment groups, respectively (Fig. 3A). This is in contrast to the much lower incidence of metamorphosis (20%) in sea lamprey treated with 0.01% $KClO_4$, and the absence of metamorphosis in the 0.005% KSCN treatment group at 6 weeks. By 16 weeks of treatment sea lamprey in the later stages of metamorphosis (stages 5 - 7) were observed among both $KClO_4$ and KSCN treated larvae. However, the incidence of metamorphosis varied depending on the goitrogen and treatment concentration. The highest incidence of metamorphosis (67%) was observed in sea lamprey treated with 0.01% $KClO_4$ followed by 47% metamorphosis in the 0.005% KSCN treatment , 20% in the 0.0005% KSCN, and 10% in the 0.001% $KClO_4$ treatments (Fig. 3B).

Sea lamprey serum T_4 concentrations were depressed relative to control values following exposure to all goitrogen treatments and concentrations except the 0.0005% KSCN treatment (Fig. 4A). On the other hand the serum T_3 concentrations varied considerably, depending on the goitrogens and treatment concentrations. In both the $KClO_4$ and KSCN treatment groups the higher treatment concentrations (0.01% and 0.005%, respectively) significantly depressed serum T_3 concentrations relative to levels in untreated control larvae, but the lower treatment concentrations (0.001% and 0.0005%, respectively) did not have the same effect. However, following 6 weeks of treatment with MMI, serum T_3 concentrations were significantly lower than levels in all other treatments except 0.01% $KClO_4$ (Fig. 4B). Additionally, although serum T_3

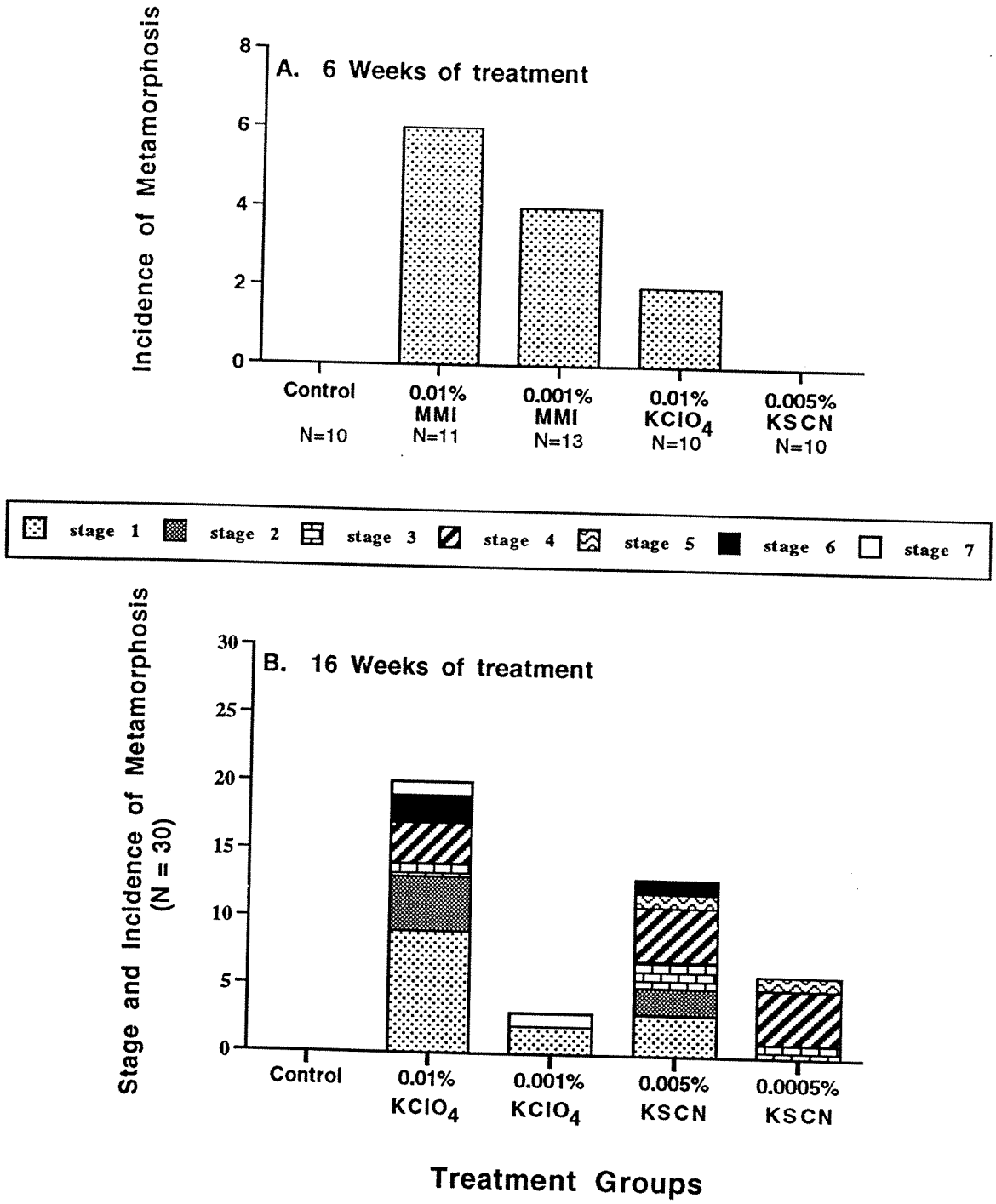

Figure 3. Stage and incidence in larval sea lamprey exposed to various goitrogens and concentrations following 6 weeks (**A**) and 16 weeks (**B**) of treatment.

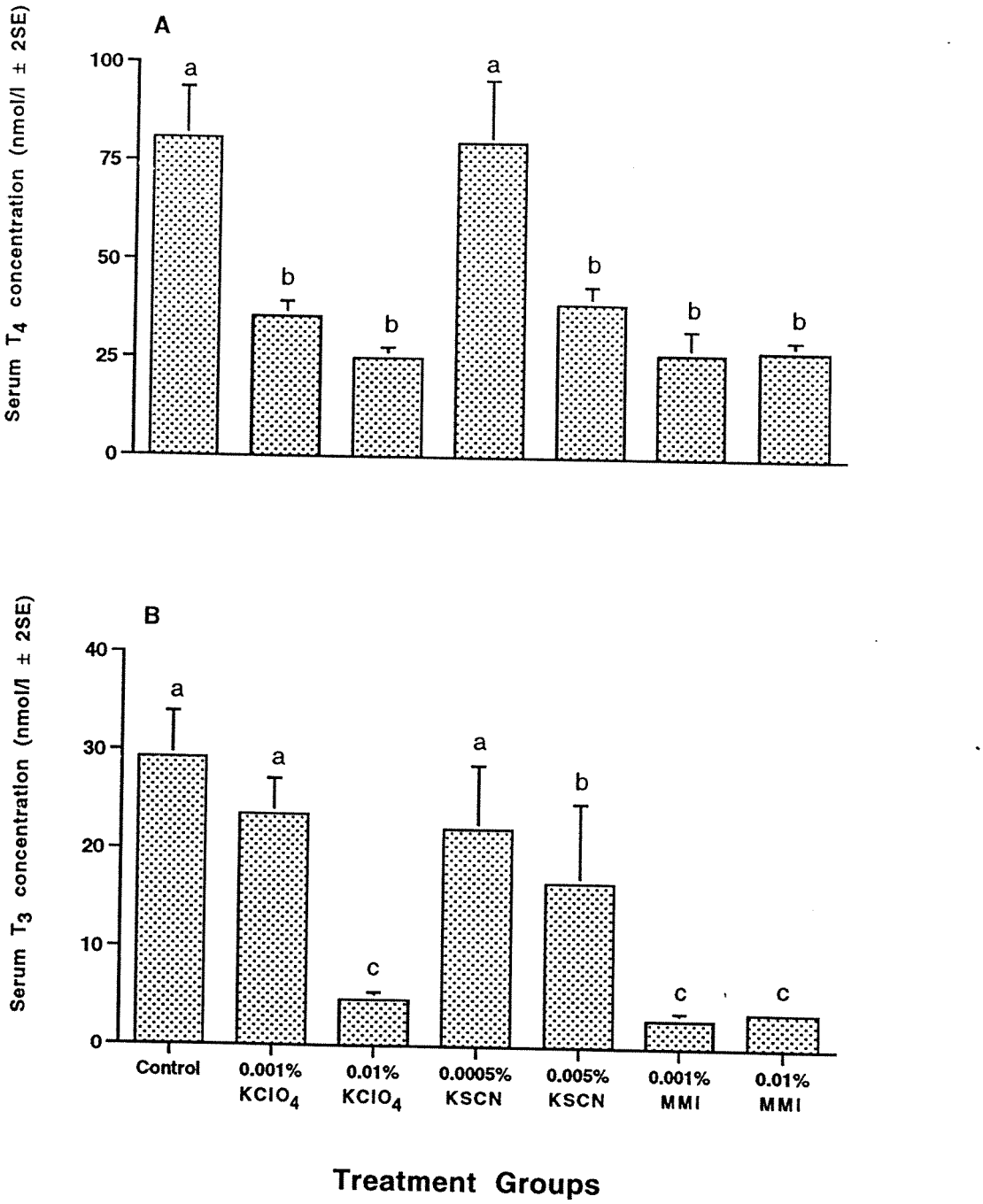

Figure 4. Mean serum thyroxine (T_4; **A**) and triiodothyronine (T_3; **B**) concentrations in larval sea lamprey exposed to various goitrogen and concentrations for 6 (MMI treatment groups) or 16 weeks (controls, $KClO_4$, and KSCN treatment groups). Within a plot values are statistically different if letters are different ($P \leq 0.05$).

concentrations in the 0.005% KSCN treatment group were significantly lower than control values, they were significantly greater then values measured in the 0.01% $KClO_4$, and both MMI treatment groups.

The results of this study clearly indicate goitrogens other than $KClO_4$, namely KSCN and MMI, can induce precocious metamorphosis in larval sea lampreys. Furthermore, our data suggest that MMI may be even more efficient at inducing metamorphosis than $KClO_4$, as was indicated by the higher incidence of metamorphosis in sea lamprey treated with MMI in comparison to the other goitrogens at 6 weeks (Fig. 3A). The ability of MMI to induce metamorphosis in larval sea lamprey is particularly interesting because its presumed mode of anti-thyroid action is the same as PTU (Cooper 1990), which does not induce metamorphosis in sea lampreys (see our 1995 and 1996 annual reports) or *G. australis* (Leatherland et al. 1990). MMI like PTU inhibits TH synthesis in a follicular thyroid gland by the inhibition of thyroid peroxidase, the enzyme responsible for iodinating tyrosine residues on the thyroglobulin molecule (Cooper 1990). $KClO_4$ and KSCN on the other hand inhibit thyroid hormone synthesis in a follicular thyroid gland by inhibiting the uptake of iodide into the gland (Cooper 1990). However, the mechanism of action of the various goitrogens on the lamprey endostyle has yet to be elucidated, but is presently under investigation in our laboratory by Richard Manzon, a Ph.D. student in JHY's lab. Additionally, to confirm if MMI is in fact a more efficient inducer of metamorphosis than $KClO_4$ we will attempt to induce metamorphosis in sea lamprey using MMI concentrations which are less toxic than the concentrations used in the present study.

Corresponding to the ability of MMI to induce metamorphosis, we have also shown that it is an extremely potent anti-thyroid agent. The results of this study indicate that treatment with either 0.01% or 0.001% MMI for 6 weeks was as effective at depressing serum TH concentrations

as treatment with 0.01% $KClO_4$ for 16 weeks, and more effective than the other treatments. MMI's potent anti-thyroid activity and its potential efficiency at inducing metamorphosis may mean it is an excellent goitrogen for the induction of metamorphosis in larval sea lampreys.

As was the case in the minimum $KClO_4$ treatment length study, the serum TH data in the current study also suggest that serum T_3 concentrations may be of greater importance in induced metamorphosis than serum T_4 concentrations. This is supported by the fact that the incidence of metamorphosis was highest in treatment groups with the greatest depression in serum T_3 concentrations (0.01% $KClO_4$ and MMI, although through differing lengths of exposure) and that a much lower incidence of induced metamorphosis occurred in treatment groups in which serum T_3 concentrations did not differ significantly from control levels (0.001% $KClO_4$, 0.0005% KSCN) or were depressed but significantly greater those in the MMI groups (0.005% KSCN) (Figs. 3 and 4). The results of the current study have also shown that to obtain a high incidence of $KClO_4$-induced metamorphosis, the $KClO_4$ treatment concentrations should be a minimum of 0.01%. However, these data also suggest goitrogens such as MMI may be as effective at lower concentrations.

In summary the results of this study indicate: (1) that goitrogens other than $KClO_4$ can successfully induce precocious metamorphosis in larval sea lamprey at time of year metamorphosis does not occur; (2) that the minimum $KClO_4$ treatment concentration to induce metamorphosis is 0.01%; (3) that MMI may be a more efficient inducer of metamorphosis in larval sea lamprey than $KClO_4$; and (4) that a decline in serum T_3 appears to be particularly important to the induction of precocious metamorphosis in sea lampreys.

2. Expression of pituitary hormones during metamorphosis

A. Expression of POMC genes

There are two alternate life history types among lampreys following metamorphosis: parasitic and nonparasitic. Juveniles of parasitic species commence an interval of feeding and somatic growth with little gonadal maturation for 1-1.5 years following metamorphosis. In contrast, nonparasitic species begin sexual maturation upon the termination of metamorphosis. Adult feeding seems to be important for both somatic growth and gonadal growth prior to sexual maturation and reproduction in parasitic species such as sea lampreys. If the animals can be stimulated to bypass feeding and begin sexual maturation, it is possible that they would not survive because of the lack of opportunity to prepare physiologically for reproduction. We hypothesize that lamprey metamorphosis involves hormones of the anterior pituitary, which are important in the initiation and developmental processes of metamorphosis and are important for sexual maturation and reproduction.

One of the primary short-term objectives of our 3-year contract was to clone and sequence lamprey pituitary hormones in order to investigate their involvement in the metamorphosis of both parasitic and nonparasitic species. In 1995 (Year-1 annual report, 1995) we isolated a clone from a pituitary cDNA library which contained one of the two genes for proopiomelanocorticotropin (POMC). The prohormone we isolated was proopiocortin (POC) (Heinig et al. 1995) which codes for nasohypophysial factor (NHF), adrenocorticotropin hormone (ACTH), β-endorphin, and α-MSH (melanin stimulating hormone). A gene for the other prohormone, proopiomelanotropin (POM), which has the remaining MSHs, was subsequently found by a group in Japan (Takahashi et al. 1995a). We obtained the second cDNA from the Japanese group and, along with our own cDNA, utilized these probes to screen Northern blots with pituitary mRNA throughout the life cycle of sea lampreys for the expression of these two genes (Heinig et al., submitted manuscript).

Low but detectable levels of POC and POM expression occur in larvae and metamorphic animals. The levels of POC and POM expression increased in immediately post-metamorphic lampreys that hadn't fed and in larger juveniles which had been fed on teleosts. The highest levels of expression for both prohormones were observed in animals from the prespawning period. Individual northern blots suggested that the level of POM expression increases through stages 4, 5, and 6 of metamorphosis, which is prior to the increased level of POC expression.

During 1997 we compared the expression of POC and POM using northern blots at different stages in the life cycle of the sea lamprey and nonparasitic brook lampreys *Lampetra appendix*. Analysis of our northern blots showed that sea lamprey cDNAs of POC and POM used as probes cross hybridize with brook lamprey pituitary RNA at very high strigencies (Fig. 5) and that the size of the message for POC and POM from brook lamprey pituitary tissue is approximately the same size as the message from sea lamprey pituitaries. When we compared northern blots of various tissues from a brook lamprey at stage 7 of metamorphosis probed with sea lamprey POC and POM, we found that these genes were only expressed in the pituitary gland (Fig. 6). As with our initial northern blots of sea lampreys described above, POM showed higher levels of expression than POC in sea lampreys at stages 5 and 7 of metamorphosis, but both POC and POM were highly expressed in unfed postmetamorphic juveniles (dissected in Feb 1997) and fed juveniles, and both reached their highest levels of expression in upstream-migrant adults. POC expression in brook lampreys was consistent with the pattern exhibited by sea lampreys: low in the larval and early metamorphic phases. However, in contrast to sea lampreys, the level of POC expression rose significantly in brook lampreys at stage 7 of metamorphosis. Furthermore, POC levels were much higher in sexually maturing brook lampreys sampled in Mar 1997 compared with unfed postmetamorphic sea lampreys sampled in Feb 1997. The pattern of POM expression in brook lampreys also differed from the pattern observed in sea lampreys: POM expression in brook lampreys began increasing at stages 3 to 5 of metamorphosis, which is earlier than in sea lampreys,

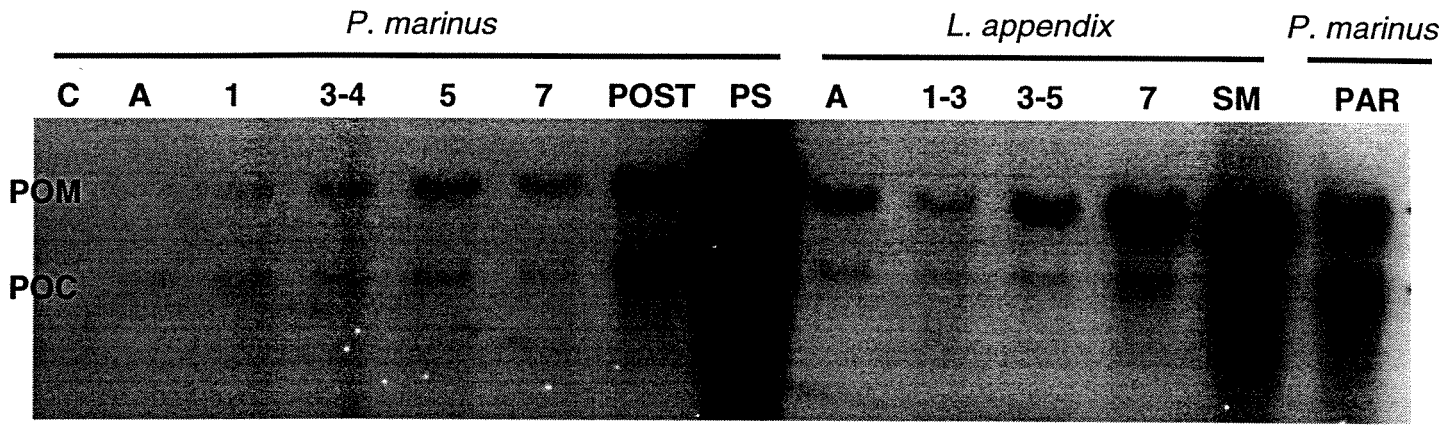

Figure 5. Northern blots of POC and POM mRNA levels in total RNA isolated from parasitic (Petromyzon marinus) and nonparasitic (Lampetra appendix) lamprey pituitaries. The different stages of development and maturation included are ammocoetes (A), stages 1 to 7 of metamorphosis, postmetamorphic sea lampreys (POST), prespawning sea lampreys (PS), parasitic sea lamprey (PAR), and sexually maturing brook lamprey (SM). Total RNA from prespawning muscle (C) was used as a negative control. Loading of lanes was confirmed by staining with ethidium bromide prior to hybridization. The membrane was first probed with a cDNA corresponding to POC, then re-probed with a cDNA corresponding to POM.

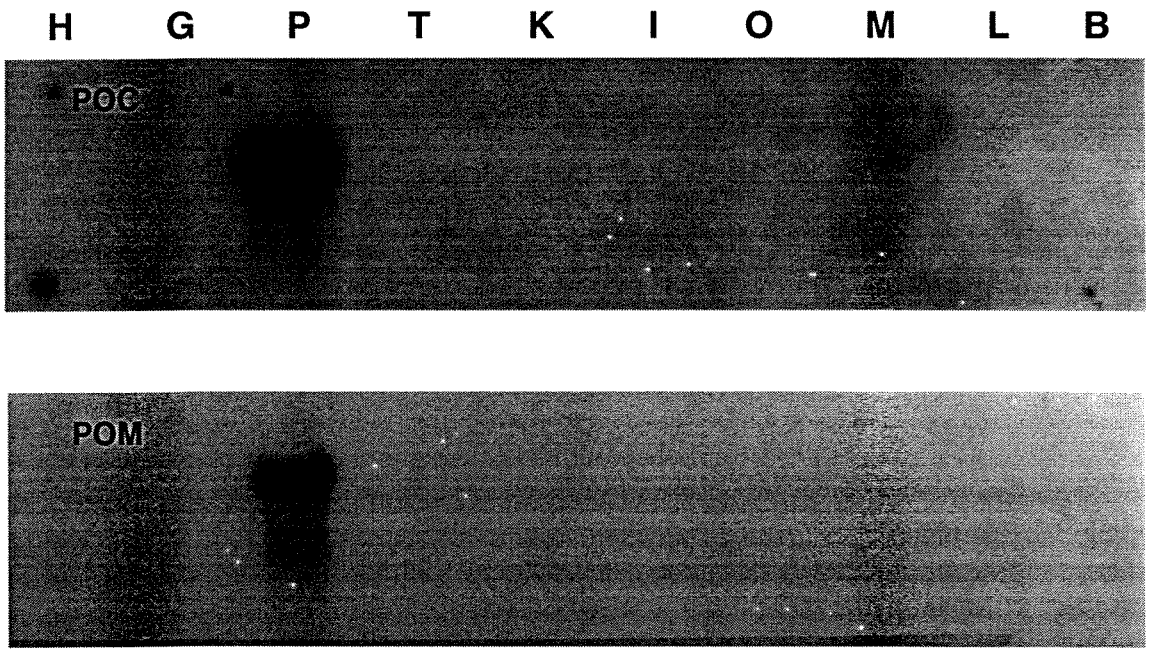

Figure 6. Northern blots of total RNA isolated from heart (H), gill (G), pituitary (P), teste (T), kidney (K), intestine (I), ovary (O), muscle (M), liver (L), and brain (B) of stage 7 metamorphic brook lamprey. Equal loading of lanes was confirmed by staining with ethidium bromide. The membrane was first probed with a cDNA corresponding to POC, then stripped and re-probed with a cDNA corresponding to POM.

and the level of expression in brook lampreys was generally greater than observed in sea lampreys throughout the metamorphic, postmetamorphic, and juvenile periods of the life cycle. We did not have RNA samples from sexually mature brook lampreys so we were unable to compare POC and POM expression at this phase of the life cycle.

In summary, we found that both POC and POM expression levels increased earlier during metamorphic development of brook lampreys than in sea lampreys. We believe the expression of POC and POM, which encode for different sets of hormones, may be related to developmental and maturation events over the life cycle of lampreys and that they may be involved in directing the juvenile and adult life history, i.e., parasitic or nonparasitic.

A complementary study to the above was carried out using *in situ* hybridization techniques and riboprobes generated from the two POMC cDNAs by the graduate student of our co-operator, G.M. Wright, of the Atlantic Veterinary College, University of Prince Edward Island. This study, primarily supported by an NSERC Strategic Grant (endorsed by the GLFC), showed temporal and spatial expression of these genes in the pituitaries during metamorphosis and during the rest of the life cycle. This work has recently been accepted for publication in General Comparative Endocrinology (Ficele et al. 1998).

The above studies show that, although expression of the two POMC genes is not implicated in the initiation of metamorphosis, they are important to specific developmental events during this process. There is a very strong correlation between the onset of sexual reproduction and the highest level of expression of the two genes.

B. Distribution of the products of the two POMC genes

We expected that the significance of the above observations would be clear when we

examined, through immunohistochemistry, the time of appearance of the various post-translational products from this prohormone, POMC. During year 1 and 2 we collected and fixed pituitaries from the various stages of metamorphosis and sent them to a collaborator, Masumi Nozaki, on Sado Island (Niigata University), Japan for immunohistochemical analysis. This immunohistochemical analysis was to provide an additional dimension to our pituitary studies, since we hoped to be able to recognize, through increased immunoreactivity, those hormones playing a prominent role at the time of metamorphosis. As noted in a previous annual report (1995), the post-translational products of this prohormone all play some role in adult life and one of these, ACTH, has been implicated in metamorphosis (Youson 1994). JHY travelled to Japan (Fellowship from the Japan Society for Promotion of Science) in November-December 1997 to help complete this analysis. The following are results of this analysis for the POMC products. We also took the opportunity to examine the distribution of immunoreactivity to antisera against ovine luteinizing hormone. The latter data appear under (c).

Nozaki et al. (1995) used antisera directed against the melanotropins (Takahashi et al. 1995b) and NHF (Sower et al. 1995) to describe the distribution of MSH-A, MSH-B (melanin stimulating hormone - A, -B), ACTH (adrenocorticotropin hormone), and NHF (nasohypophysial factor) in the pituitaries of early larval and prespawning adult sea lampreys. We used these antisera to show the distribution of the same hormones during later larval life (immediately premetamorphic) and throughout the seven stages of metamorphosis. The heads of three larvae (130-133 mm, 3.3-3.6 g) and 3 individuals at each stage (1 to 7) of metamorphosis (113-150 mm, 2.2-6.0 g) were fixed in Bouin-Hollande fluid for 24 hr and then preserved in 70% ethanol. Serial sections were prepared so that immunoreactivity to different antisera could be visualized in adjacent sections. The antisera used were made in rabbit and were directed against synthetic lamprey MSH-A, MSH-B, NHF, and ACTH. A relative, subjective assessment of the immunoreactivity was made with the degree of staining ranked from - (absent), $\pm$ (faint to absent), +(weak), ++

(moderate), +++ (strong) within the pars intermedia (PI), rostral pars distalis (RPD), and the proximal pars distalis (PPD) of the pituitary gland. We also included an evaluation of the same regions in small (young) larvae 45-85 mm in length. The latter evaluation would allow us to examine developmental changes in the pituitary during larval life and to see whether there is any change in the pituitary immediately prior to metamorphosis.

The results are provided in Table 1 and are summarized below.

i) Immunoreactivity to MSH-B is highest in the PI of larvae and the earliest stage of metamorphosis. It disappears to being faint from stages 2-3 to 5. At stages 6 and 7 the immunoreactivity returns to that seen at stage 1-2. It is noteworthy that external pigment changes occur in the animal at these late stages. We infer from this that MSH-B may be the pituitary hormone stimulating melanin production at this time.

ii) MSH-A immunoreactivity is consistently high in the PI and RPD throughout larval life and metamorphosis. This MSH does not seem to play a major role in the process of metamorphosis.

iii) Immunoreactivity to ACTH antiserum was consistently high in the RPD throughout all intervals examined. The PI had moderate immunoreactivity to this antiserum in young larvae, but was not a significant reactive site in other stages. The most interesting finding was the presence of immunoreactivity in the PPD. This region of the pituitary showed weak to moderate staining in cells with long processes during early stages of metamorphosis but thereafter the immunoreactivity was weak. The significance of this finding is that presumptive adrenocortical cells have been shown to be hyperactive in early metamorphosis, presumably in the production of corticosteroids (for review see Youson 1994). The hormone which stimulates corticosteroid synthesis in other vertebrates is ACTH. This finding correlates well

Table 2.1. Immunoreactivity against various hormones in the pituitaries of larval and metamorphosing sea lampreys, *Petromyzon marinus*.

	lMSH-B	lMSH-A	lACTH			lNHF			oLH
Animal	PI	RPD+PI	RPD	PPD	PI	RPD	PPD	PI	
Young larvae (45-85 mm; N=4)									
#9	NA	+++	+++	–	++	+++	–	++	
#10	NA	+++	+++	–	++	+++	+	++	
#11	NA	+++	++	+	++	+++	++	++	
#12	++ to +++	+++	+++	+ to ±	++	+++	+	++	
Premetamorphic larvae (130-133 mm; N=3)									
#7-9	+	+++	+++	+	– to ±	+++	+ to ++	–	± to +
Metamorphosing (N=3 per stage)									
late 1 - early 2	+ to ++	+++	+++	+ to ++	– to +	++	+ to ++	– to ±	± to +
late 2 - early 3	+	+++	+++	++	– to +	+++	+ to ++	– to +	± to +
2	–	+++	+++	+ to ++	– to +	+++	++	– to ±	–
3	–	+++	+++	+	– to +	+++	+ to ++	–	–
4	– to ±	+++	+++	± to +	± to +	+++	++	– to ±	–
5	– to ±	+++	+++	± to +	± to +	+++	+ to ++	– to ±	–
6	+ to ++	+++	+++	±	±	+++	+ to ++	– to ±	– to ±
7	+ to ++	+++	+++	±	±	+++	+ to ++	– to ±	– to ±

Pituitary regions are: PI - pars intermedia; RPD - rostral pars distalis; PPD - proximal pars distalis.

Antisera made in rabbits against the following hormones: lMSH - lamprey melanin stimulating hormone; lACTH - lamprey adrenocorticotropin hormone; lNHF - lamprey nasohypophysial factor; oLH - ovine lutenizing hormone.

Immunoreactivity rankings are: – no activity; ± absent to barely detectable; + weak; ++ moderate; +++ strong.

with the observation from partial hypophysectomy of *Geotria australis* that the RPD is required for the initiation of metamorphosis (Joss 1985). Thus, our finding may be the first direct implication of the pituitary-adrenal axis in lamprey metamorphosis.

iv) There was a strong immunoreactivity to the NHF antiserum in the RPD throughout all of the stages. In young larvae (stained at a different time and reported in Sower et al. 1995) there was a moderate immunoreactivity in the PI, but PI staining with this antisera was absent or faint in all other stages. It is curious that this same stage-specific result was found with the ACTH antiserum and the PI. This is a result which needs further clarification. On the other hand, the immunoreactivity to the NHF antiserum in the PPD was weak to moderate throughout the intervals we examined and thus did not show the strong early metamorphic immunoreactivity of ACTH. NHF immunoreactivity in the pituitary during these developmental stages did not provide us with any new ideas on pituitary control of metamorphosis. However, as described below the reward came with the study of immunoreactivity with this antibody in other tissues.

We noted that the NHF antiserum showed a positive reaction with sensory cells in the integument of the head of all stages and in the cirrhi of the larval buccal funnel. Furthermore, in the metamorphosing stages the immunostaining was ubiquitous and found in cells of many developing tissues of the head (cartilage, muscle, buccal glands etc.). This is a finding which should not be overlooked. Although the function of NHF is not known, it has been suggested that it may be a type of growth factor (Sower et al. 1995). The localization of NHF in these developing tissues may be of important relevance to our studies of factors regulating development during lamprey metamorphosis.

C. Screening for other pituitary hormones

i) Differential Display (reported in the 2nd year report but ongoing in year 3)

To identify other genes that are expressed during metamorphosis we have been using mRNA differential display. This technique is particularly useful for identifying and cloning genes that are expressed (upregulated or turned-on) at different times in the life cycle. mRNA samples are analyzed side by side on the same gel, which allows for the differentially expressed genes to be identified and probes for them to be recovered and used to clone their cDNAs or genomic DNA. Thus, the expression of genes should be identical between two different stages (for example, larval lamprey and metamorphosing individual) except for those genes which initiate change, and especially where the genes code for major products of the tissue.

Gene expression in the pituitaries of larvae and stage 4 metamorphosing sea lampreys were compared on Northern blots. We selected 10 cDNAs which were highly expressed genes of larvae and not seen at stage 4 and 10 highly expressed genes in stage 4 and not seen in larvae, i.e., 20 cDNAs in total. The 20 bands, ranging in size from 150 to 700 base pairs (bp), were excised from the gel, reamplified by PCR, cloned, and sequenced. At present, our sequencing data show no nucleotide sequence homologies to pituitary hormones in the databases we have checked because the majority of the cDNAs we obtained are at the extreme 3' end of the mRNA, i.e., the untranslated region of the gene. This part of the mRNA is frequently not included in gene databases and varies greatly with organisms.

In order to confirm that these 20 fragments were differentially expressed between larvae and metamorphosing animals, and therefore of interest to us, we probed Northern blots with their cDNAs. Using this method we identified 5 cDNAs that were differentially expressed between the larval and metamorphic stages in sea lampreys. The majority of cDNA fragments that we identified were too small to remain on the membrane when rinsed. Larger PCR fragments ($\approx$ 1,000 bp) of

the 5 cDNAs of interest, which are easier to work with, have been obtained using a spawning-phase pituitary cDNA library as a template. We are now developing a vector for insertion and cloning, afterwhich we will begin sequencing these fragments.

The use of differential display to look at gene expression throughout the life cycle of sea lampreys provides assurance that we are examining all possibilities of novel gene expression. We anticipate that it will provide us with new information on the endocrine control of metamorphosis and we expect to have sequence information on the 5 cDNAs that we can compare with gene databases for sequence homologies, by Apr-May 1998.

ii. Search for specific hormones in sea lampreys (reported in year 2 but ongoing in year 3)

Thyroid stimulating hormone (TSH), growth hormone (GH), and prolactin (PR) have been implicated in the metamorphic processes of other vertebrates. These are not posttranslational products of the POMC genes that we have discovered nor have they ever been identified in lampreys. However, there is some morphological and experimental evidence to suggest that putative thyrotrophs (TSH-secreting cells) and somatotrophs (GH/PR-secreting cells) either significantly change or are important to the metamorphic process (for review, see Youson 1994). Thus, we are continuing throughout the course of the 3-year contract to search for these hormones of the anterior pituitary. The identification of either one of these hormones has implications not only for our study of lamprey metamorphosis but also for other applied studies presently being funded by the GLFC. All three of these hormones have a direct relationship with animal growth and metabolism and these are important factors which ultimately determine the time to metamorphosis and the time of feeding during the parasitic period of the life cycle of sea lampreys. Since there is variation in these intervals between comparable life cycle periods of nonparasitic lampreys and sea lampreys, a comparison of these hormones is important to our long-range objective, i.e., alteration of life history.

Because we have implicated thyroid hormones in lamprey metamorphosis, we have directed our attention to identifying TSH in the sea lamprey, for this hormone stimulates the lamprey thyroid-gland equivalent, the endostyle, to secrete thyroid hormones. Although we are personally involved in the search for this hormone, we are also co-operating/collaborating with other laboratories in their search for this gene. It is likely that when TSH is found that gonadotrophic hormone (GTH) will also be discovered, for the may be products of the same gene. Dr. Stacia Sower (U. New Hampshire) and her colleague, Dr. Hiroshi Kawauchi (Japan) are both directly searching for GTH in sea lamprey pituitary. In addition, we have also sent our pituitary cDNA library to a group in France (Querat) who are also looking for GTH, with the understanding that they will provide us with TSH should they beat us to the discovery.

In our search we have been using PCR and an adult lamprey pituitary cDNA library as the template. A degenerate primer was created which corresponded to a region of TSH-β subunit conserved among mammals, amphibians and the eel. To date we have generated three PCR fragments (900 bp, 1600 bp and 2500 bp) and they have been subcloned into vector PCR-script and have been prepared for sequencing. We are presently selecting primers for GH and PR. Our first attempt at isolating GH utilizing a conserved sequence as a primer lead to the discovery of POMC.

These studies are the first to directly show changes in pituitary hormone expression during lamprey metamorphosis. At present, we do not know the exact hormonal mechanisms of lamprey metamorphosis. Since pituitary hormones have the potential for both direct and indirect stimulation of physiological and developmental processes, our findings represent a giant step forward to reaching both short- and long-range goals and providing the projected deliverables. In addition, the identification, sequencing, and characterization of lamprey pituitary hormones from cDNA

libraries is pertinent to all studies of development, metamorphosis, sexual maturation, and migration now being funded by the GLFC.

iii) Immunohistochemistry of luteinizing hormone

An unexpected bonus of the study of immunohistochemistry of POMC products in the Nozaki laboratory (see 2b above), was the opportunity to examine the pituitary sections of various aged larvae and all stages of metamorphosis with antibody directed against ovine luteinizing hormone (anti-oLH). Drs. Sower, Kawauchi, and Nozaki have been searching for a sea lamprey gonadotropin (GTH) for close to 10 years. The latest approach was to try various antisera against LH on tissue sections to see whether one of these antisera might be useful in their isolation procedures for a lamprey GTH. The most promising to date is anti-oLH. When this antiserum was applied to pituitary sections of the developing stages listed above, faint to weak immunoreactivity was found consistently in cells of the PPD of immediately premetamorphic larvae and animals in the first 3 stages of metamorphosis only (Table 1). An absent to faint immunoreactivity was present again in stages 6 and 7. <u>These results are very exciting from two points of view</u>:

1. The data are support for involvement of the hypothalamic-pituitary-gonadal axis at a time when metamorphosis is initiated, and
2. The immunoreactivity in late metamorphosis implies that there is a stimulation/upregulation of the reproductive system in late metamorphosis. This finding is consistent with our earlier studies in which the activity of gonadotropin-releasing hormones (GnRHs) increased during late metamorphosis (Youson and Sower 1991; Youson et al. 1995). Since GnRHs act directly on the pituitary, the increase in immunoreactivity to anti-oLH at the end of metamorphosis implies that GTH-containing cells are responding to stimulation by GnRH.

Summary

The primary goals for the third year of our contract were: to determine the minimum exposure to goitrogens needed to induced metamorphosis in sea lampreys; to assess the efficacy of other goitrogen treatments (e.g., methimazole, thiocyanate) for inducing metamorphosis; to compare the expression of pituitary hormones during metamorphosis in parasitic (sea lamprey) and nonparasitic (brook lamprey) lamprey species to determine the role of these hormones in stimulating and directing the events of metamorphosis ; and to organize our data from year 2 for publication. All of these goals were met and we can now state that continued exposure to 0.05% $KClO_4$ for at least 8 weeks is required to induce metamorphosis in all sea lampreys $\geq$ 120 mm in size and that a $KClO_4$ concentration of at least 0.01% is required to successfully induce metamorphosis in sea lampreys.

A second goal was to test the efficacy of other goitrogens for inducing metamorphosis in sea lampreys. We found that KSCN and methimazole (MMI) induce precocious metamorphosis in larval sea lampreys and that MMI may be a more efficient inducer of metamorphosis in sea lampreys than $KClO_4$, in that the incidence of MMI-induced metamorphosis after 6 weeks of treatment was much higher compared with the other goitrogen treatments. The fact that MMI can successfully induce metamorphosis in sea lampreys is particularly interesting since its presumed mode of action is believed to be the same as PTU, which does not induce metamorphosis in sea lampreys or *G. australis*.

Our induction studies with different goitrogens and different periods of exposure clearly show that the depression of serum T_3 levels appears to be particularly important to the induction of precocious metamorphosis in sea lampreys. However, although a decline in serum TH levels by

itself does not trigger metamorphosis in lampreys, we believe that those goitrogens that successfully induce precocious metamorphosis in lampreys ($KClO_4$, KSCN, MMI) have other effects either on the putative thyroid gland itself, on other tissues outside of the thyroid axis, on the peripheral metabolism of the thyroid hormones, or on general body metabolism. We are currently attempting to determine what these effects are and which ones are important to lamprey metamorphosis.

Our third goal was to compare the expression of pituitary hormones in parasitic and nonparasitic lamprey species. Both POC and POM expression levels increase earlier in the metamorphic development of brook lampreys than in sea lampreys. Although expression of the two POMC genes is not implicated in the initiation of metamorphosis, they are important to specific developmental events during this process. There is a very strong correlation between the onset of sexual reproduction and the highest level of expression of the two genes.

The hormonal products of the two POMC genes exhibited important differences in the timing of their expression in the pituitary during sea lamprey metamorphosis. MSH-A immunoreactivity was high throughout larval life and metamorphosis while MSH-B immunoreactivity is present in the larval period and during the early stages of metamorphosis, disappears in the middle stages, and reappears at stages 6 and 7. We infer from these findings that MSH-A does not play a major role in lamprey metamorphosis and that MSH-B may be the hormone stimulating melanin production in the later stages of metamorphosis when an external color change occurs. The expression of ACTH during metamorphosis (immunoreactivity in the PPD) may be the first direct evidence implicating the pituitary-adrenal axis in lamprey metamorphosis. Immunoreactivity to NHF was found in cells of many developing head tissues, including cartilage, muscle, and the buccal glands. If NHF is a growth factor as hypothesized, then the localization of NHF in these growing tissues is an important consideration for studies of

factors regulating metamorphic development in lampreys.

Although our search for the expression of other pituitary hormones during metamorphosis has not yet provided tangible results, we have identified 5 cDNAs of interest using differential expression and we expect to have sequence information on them shortly. We are presently attempting to clone them into vector PCR-scripts for amplification.

Perhaps our most exciting result, and an unexpected bonus, was the finding of weak immunoreactivity to lutenizing hormone (LH) in the pituitary of premetamorphic larvae and animals in the early and later stages of metamorphosis. If these results are confirmed in additional specimens, then we believe that they provide support for the hypothesis that the hypothalamic-pituitary-gonadal axis is involved in the initiation of metamorphosis is initiated (immunoreactivity in stages 1-3), and they also support the hypothesis that there is a stimulation/upregulation of the reproductive system in late metamorphosis.

Deliverables

The third year of our 3-yr research plan was highly successful. Our ultimate deliverable after a minimum of three years was to provide some means of stimulating immediate, postmetamorphic development of the reproductive system and precocity in the sea lamprey in a laboratory setting. This precocity can be achieved through induction by three goitrogens: $KClO_4$, KSCN, and MMI. We are more advanced in our knowledge of the method of induction in terms of exposure time (at least 8 weeks), minimum concentration (0.01% $KClO_4$), and the importance of the goitrogens on the regulation serum T_3 levels. The data generated from the spontaneously metamorphosing nonparasitic species allows us to examine an interval of the lamprey life cycle,

which has never been followed in any lamprey species. In particular, the events of progressive sexual maturation in sea lampreys has only been indirectly implied through observations of the two ends of the spectrum, i.e., recently metamorphosed animals and animals in their upstream migration. In the latter situation, sexual maturation is near completion. We have delivered on our objective to show the involvement of the pituitary hormones in metamorphosis of parasitic and nonparasitic species by demonstrating differences in expression of two POMC genes and we are very close to being able to identify other highly expressed genes which are related to metamorphosis.

Publications based on contract research

Published or in press

Eales, J. G., J. A. Holmes, J. M. McLeese, and J. H. Youson. 1997. Thyroid hormone deiodination in various tissues of larval and upstream-migrant sea lampreys, *Petromyzon marinus*. Gen. Comp. Endocrinol. 106:202-210.

Ficele, G., J.A. Heinig, H. Kawauchi, J.H. Youson, F.W. Keeley, and G.M. Wright. 1998. Spatial and temporal distribution of proopiomelanotropin and proopiocortin mRNA during the life cycle of the sea lamprey: a qualitative and quantitative in situ hybridization study. Gen. Comp. Endocrinol. (in press)

Kao, Y-h., J. H. Youson, J. A. Holmes, and M. A. Sheridan. 1997. Changes in lipolysis and lipogenesis in selected tissues of the landlocked lamprey, *Petromyzon marinus*, during metamorphosis. J. Exp. Zool. 277:301-312.

Kao, Y-h., J. H. Youson, and M. A. Sheridan. 1997. Differences in total lipid and lipid class composition of larvae and metamorphosing sea lamprey, *Petromyzon marinus*. Fish Physiol. Biochem. 16:281-290.

Manzon, R.G. and J. H. Youson. 1997. The effects of exogenous thyroxine (T_4) or triiodothyronine (T_3), in the presence and absence of potassium perchlorate, on the incidence of metamorphosis and on serum T_4 and T_3 concentrations in larval sea lampreys (*Petromyzon marinus* L.). Gen. Comp. Endocrinol. 106:211-220.

Youson, J. H. 1997. Is lamprey metamorphosis regulated by thyroid hormones? Am. Zool. 37:441-460.

Youson, J.H., R.G. Manzon, B.J. Peck, and J. A. Holmes. 1997. Effects of exogenous thyroxine (T_4) and triiodothyronine (T_3) on spontaneous metamorphosis and serum T_4 and T_3 levels in immediately premetamorphic sea lampreys, *Petromyzon marinus*. J. Exp. Zool. 279:145-155.

Submitted Manuscripts

Heinig, J.A., F.W. Keeley, H. Kawauchi, and J.H. Youson. Expression of proopiocortin and proopiomelanotropin during the life cycle of the sea lamprey (*Petromyzon marinus*). J. Exp. Zool.

Holmes, J.A., H. Chu, R.G. Manzon, and J.H. Youson. Spontaneous and induced metamorphosis in the American brook lamprey, *Lampetra appendix*. Can. J. Zool.

Manzon, R.G., J.G. Eales, and J.H. Youson. Blocking of $KClO_4$-induced metamorphosis in immediately premetamorphic sea lampreys by exogenous thyroid hormones (TH): effect of $KClO_4$ and TH on serum TH concentrations and thyroxine outer-ring deiodination. Gen. Comp. Endocrinol. (accepted upon revision).

Other related papers:

Filosa, M.F., I. Adam, P. Robson, J.A. Heinig, K. Smith, F.W. Keeley, and J.H. Youson. A partial clone of the gene for AS protein of the lamprey *Petromyzon marinus*: a member of the albumin supergene family whose expression is restricted to the larval and metamorphic phases of the life cycle. J. Exp. Zool. (submitted).

Kao, Y.-h., J.H. Youson, J.A. Holmes, and M.A. Sheridan. Effects of somatostatin on lipid metabolism of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. (submitted).

Kao, Y.-h., J.H. Youson, J.A. Holmes, and M.A. Sheridan. Effects of insulin on lipid metabolism of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. (submitted).

Robson, P., F.Li, J.H. Youson, and F.W. Keeley. Identification and characterization of serpin with differential expression during the life cycle of the sea lamprey. Comp. Biochem. Physiol. (accepted upon revision).

References cited in Contract Report - Year 3

Cooper, D.S. 1990. Substances that affect thyroid function or thyroid hormone metabolism. *In* The Thyroid Gland. M.A. Greer (ed.). Raven Press, New York, pp. 323-344.

Ficele, G., J.A. Heinig, H. Kawauchi, J.H. Youson, F.W. Keeley, and G.M. Wright. 1998. Spatial and temporal distribution of proopiomelanotropin and proopiocortin mRNA during the life cycle of the sea lamprey: a qualitative and quantitative in situ hybridization study. Gen. Comp. Endocrinol. (in press)

Heinig, J.A., F.W. Keeley, H. Kawauchi, and J.H. Youson. Expression of proopiocortin and proopiomelanotropin during the life cycle of the sea lamprey (*Petromyzon marinus*). J. Exp. Zool. (submitted).

Heinig, J.A., F.W. Keeley, P. Robson, S.A. Sower, and J.H. Youson. 1995. The appearance of proopiomelanocortin early in vertebrate evolution: cloning and sequencing of POMC from a lamprey pituitary cDNA library. Gen. Comp. Endocrinol. 99:137-144.

Holmes, J. A., and J. H. Youson. 1993. Induction of metamorphosis in landlocked sea lampreys, *Petromyzon marinus*. J. Exp. Zool. 267: 598-604.

Jameson, J.L., and L.J. DeGroot. 1995. Mechanisms of thyroid hormone action. *In* Endocrinology. Third edition. L.J. DeGroot, M. Besser, H.G. Burger, J.L. Jameson, D.L. Loriaux, J.C. Marshall, W.D. Odell, J.T. Potts, Jr., and A.H. Rubenstein (eds.). W.B. Saunders Co., Philadephia, PA, Volume 1, pp. 583-601.

Joss, J.M.P. 1985. Pituitary control of southern hemisphere lamprey, *Geotria australis*. Gen. Comp. Endocrinol. 60:58-62.

Leatherland, J. F., Hilliard R. W., Macey D. J., and I. C. Potter. 1990. Changes in serum thyroxine and triiodothyronine concentrations during metamorphosis of the Southern Hemisphere lamprey *Geotria australis*, and the effect of propylthiouracil, triiodothyronine and environmental temperature on serum thyroid hormone concentrations of ammocoetes.

Fish Physiol. Biochem. 8:167-177.

Manzon, R.G. and J. H. Youson. 1997. The effects of exogenous thyroxine (T_4) or triiodothyronine (T_3), in the presence and absence of potassium perchlorate, on the incidence of metamorphosis and on serum T_4 and T_3 concentrations in larval sea lampreys (*Petromyzon marinus* L.). Gen. Comp. Endocrinol. 106:211-220.

Nozaki, M., A. Takahashi, Y. Amemiya, H. Kawauchi, and S.A. Sower. 1995. Distribution of lamprey adrenocorticotropin and melanotropins in the pituitary of the adult sea lamprey, *Petromyzon marinus* . Gen. Comp. Endocrinol. 98:147-156.

Sower, S.A., A. Takahashi, M. Nozaki, A. Gorbman, J.H. Youson, J. Joss, and H. Kawauchi. 1995. A novel glycoprotein in the olfactory and pituitary systems of larval and adult lampreys. Endocrinol. 136:349-356.

Suzuki, S. 1986. Induction of metamorphosis and thyroid function in the larval lamprey. *In* Frontiers in thyroidology. G. Mederios-Neto and E. Gaitan (eds.). Plenum Press, New York, Vol. 1, pp. 667-670.

Suzuki, S. 1989. Why are goitrogens are chemical triggers of metamorphosis in the lamprey? Relationship between thyroid function and metamorphosis. Gen. Comp. Endocrinol. 74:277.

Takahashi, A., Y. Ameniya, M. Sarashi, S. A. Sower, and H. Kawauchi. 1995a. Melanotropin and corticotropin are encoded on two distinct genes in the lamprey, the earliest evolved extant vertebrate. Biochem. Biophys. Res. Comm. 213:490-498.

Takahashi, A., Y. Ameniya, M. Nozaki, S. A. Sower, J. Joss and H. Kawauchi. 1995b. Isolation and characterization of melanotropins from lamprey pituitary glands. Int. J. Pep. Protein Res. 46:197-204.

Youson J. H. 1994. Environmental and hormonal cues and endocrine glands during lamprey metamorphosis. *In* Perspectives in Comparative Endocrinology. K. Davey, R. Peter, and S. Tobe (eds.). National Research Council of Canada Research Journals, Ottawa,

pp.400-407.

Youson, J. H. 1997. Is lamprey metamorphosis regulated by thyroid hormones? Am. Zool. 37:441-460.

Youson, J.H. and R.J. Beamish. 1991. Comparison of the internal morphology of adults of a population of lampreys which produces two life history types: nonparasitic *Lampetra richardsoni* and potentially parasitic, *Lampetra richardsoni* variety *marifuga.* Can. J. Zool. 69:628-637.

Youson, J. H., and S.A. Sower. 1991. Concentration of gonadotropin-releasing hormone in the brain during metamorphosis in the lamprey, *Petromyzon marinus.* J. Exp. Zool. 259:399-404.

Youson, J.H., E.M. Plisetskaya, and J.F. Leatherland. 1994. Concentrations of insulin and thyroid hormones in the serum of landlocked sea lampreys (*Petromyzon marinus*) of three larval year classes, larvae exposed to two temperature regimes, and both during and after metamorphosis. Gen. Comp. Endocrinol. 94:294-304.

Youson, J.H., J. A. Holmes, and J.F. Leatherland. 1995a. Serum concentrations of thyroid hormones in $KClO_4$-treated larval sea lampreys (*Petromyzon marinus* L.). Comp. Biochem. Physiol. 111C:265-270.

Youson, J. H., M. Docker, and S. A. Sower. 1995b. Concentration of gonadotropin-releasing hormones in brain of larval and metamorphosing lampreys of two species with different adult life histories. *In* Proceedings of the 5th International Symposium of the Reproductive Physiology of Fishes. P. Thomas and F. Goetz (eds.). University of Texas Press, Austin, p. 83

Youson, J.H., R.G. Manzon, B.J. Peck, and J. A. Holmes. 1997. Effects of exogenous thyroxine (T_4) and triiodothyronine (T_3) on spontaneous metamorphosis and serum T_4 and T_3 levels in immediately premetamorphic sea lampreys, *Petromyzon marinus*. J. Exp. Zool. 279:145-155.