

Review

# Theory on the evolutionary history of lamprey metamorphosis: role of reproductive and thyroid axes

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## Abstract

Metamorphosis is a developmental strategy used by only a small number of extant fishes and little is known about its phylogenetic development during the evolution history of this large group of vertebrates. The present report provides a putative evolutionary history of metamorphosis in the lamprey, an extant agnathan with direct descendancy from some of the oldest known vertebrates. The study reviews recent data on the role of the thyroid gland and its hormones in metamorphosis, summarizes some recent views on the evolution of the endostyle/follicular thyroid in lampreys, and provides new data on the content of two gonadotropin-releasing hormones (GnRH-I and -III) in brain during goitrogen-stimulated, precocious metamorphosis. These new data support an earlier viewpoint of a relationship between thyroid and reproductive axes during metamorphosis. It is proposed that the earliest lampreys were paedomorphic larvae and they lived in a marine environment; as such, they resembled in many ways the larvae from which the ancient protochordates, Larvacea, are derived. The iodide-concentrating efficiency of the endostyle was a critical factor in the evolution of metamorphosis and this gland was replaced by a follicular thyroid, for postmetamorphic animals needed to store iodine following their invasion of freshwater. Larval growth and postmetamorphic reproduction in freshwater became fixtures in the lamprey life cycle; a non-parasitic adult life-history type appeared later. The presence among extant lampreys of two different adult life-history types, and examples of the lability of the timing of sexual maturation in some species, imply that there has been a complex interplay between the thyroid and reproductive axes during the evolution of metamorphosis in lampreys. This proposal is consistent with what we know of interplay of these axes in extant adult lampreys and with the long-held viewpoint that thyroid function and sexual maturation are an association with an ancient history. © 2001 Elsevier Science Inc. All rights reserved.

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## 1. Introduction

Lampreys are extant representatives of some of the oldest known vertebrates, the jawless fish or Agnatha. Most recent evidence dates the first lampreys to approximately 350 million years ago and after this time they experienced a rather conserved evolution (Forey and Janvier, 1994). Thus, much of what we see in modern-day lampreys was also present at the earliest time in their recorded evolutionary history. It has not been possible to determine from fossil evidence the nature of the life cycle of the early lampreys, that is, whether there was a larval interval followed by a metamorphosis to a juvenile, or whether, like the other extant agnathan, the hagfish, development to the adult interval was direct from the hatched embryo (Hardisty, 1979). Based on data from extant lampreys, it might be surmised that the earliest lampreys did not have a larval interval, for fossil evidence of lampreys is from marine deposits and modern-day lamprey larvae cannot tolerate even dilute seawater (Morris, 1972). However, in a recent report, Youson (1999) took an alternate view that the first lampreys were larval-like and reproductive, i.e. paedogenetic, in a marine environment and metamorphosis eventually evolved with the movement of lampreys into freshwater. With metamorphosis came a delay in reproductive maturity, a juvenile period, and ultimately two different adult life-history types, based on their postlarval time to enter sexual maturation.

The adult life-history types that are present in extant lampreys are termed parasitic and non-parasitic (brook lampreys) and generally the types are not interchangeable within a species. There is only one well-defined case of polymorphism in an extant, non-parasitic lamprey species (Beamish, 1987). Before commencing sexual maturation, juveniles of parasitic species feed for varying periods, in either marine or freshwater environments, on the blood and tissues of mostly teleost fish. The juvenile period of non-parasitic species is short-lived, for immediately after the completion of metamorphosis there is a commencement of sexual maturation without a feeding interval. Many, but not all, non-parasitic species are genetically related to a parasitic species, and are collectively termed paired or satellite species (Hardisty and Potter, 1971). The members of a paired species were derived from a common parasitic

ancestor. Youson (1999) claimed that adult life-history types in lampreys arose from an alteration in developmental timing, i.e. heterochrony, prior to, but particularly during, lamprey metamorphosis. Consequences of this heterochrony were the lengthening of larval life/delay of metamorphosis in non-parasitic species (Potter, 1980) and the elimination of the adult feeding interval. In the paired species, which includes parasitic *Lampetra fluviatilis* and the non-parasitic *Lampetra planeri*, the total length of life is likely the same in both species (Hardisty, 1979). In the brook lamprey, larval life is protracted over the equivalent period of juvenile feeding in the parasitic species, such that both life history types arrive at sexual maturation at the same time in their identical life span.

The development of an organism requires an intricate set of perfectly timed, integrated signals. Metamorphosis in lampreys, as a late stage of ontogeny, is no exception to this rule (Youson, 1980). The endocrine system plays a part in the developmental signals of lamprey metamorphosis, but the exact mechanism of action is not fully understood (Youson, 1994). To date, much attention has been directed to the thyroid axis, namely the concentration of circulating levels of thyroid hormones (Youson, 1997). The earliest signs of metamorphosis in both parasitic and non-parasitic species coincide with a dramatic drop in serum levels of the thyroid hormones, thyroxine and triiodothyronine. In fact, precocious metamorphosis can be induced in lampreys with the goitrogen,  $KClO_4$ , which alters the synthesis of thyroid hormones (Manzon, 2000) and results in a drop in serum levels of thyroid hormones (Holmes and Youson, 1993; Youson et al., 1995b; Holmes et al., 1999). Induction of metamorphosis with goitrogens has told us much about the role of the thyroid axis in lamprey metamorphosis.

A theory on the importance of the endostyle/thyroid gland to the phylogenetic development of metamorphosis in lampreys has been proposed (Youson, 1999). In this proposed theory, the relationship between the thyroid gland and the reproductive axis was given only passing attention. Earlier, Hardisty (1979) suggested that a physiological explanation for the origin of non-parasitic lamprey species from parasitic forms, through a delayed metamorphosis, can likely be found in the higher centers of the reproductive axis. The possibility of a lability in the timing of sexual

maturation in lampreys has been intriguing ever since the report of mature eggs in the body cavity of larvae of the brook lamprey, *Lampetra zanandreai* (Zanandrea, 1957). Until recently, we lacked the tools to explore the role of any of the higher centers of the reproductive axis in lamprey metamorphosis. Now we have clearly defined stages of metamorphosis and are isolating homologous hormones from the hypothalamus and pituitary. To date, all our data are on the changing concentrations of gonadotropin-releasing hormone (GnRH) in the brain during spontaneous lamprey metamorphosis (Youson and Sower, 1991; Youson et al., 1995a). These data have been supplemented by immunohistochemical studies (Wright et al., 1994; Tobet et al., 1995) showing the distribution during metamorphosis of the two GnRH molecules in lampreys, GnRH-I and -III (Sherwood et al., 1986; Sower et al., 1993). GnRH may have a role in lamprey metamorphosis, since concentrations of brain GnRH-I and a second form increase throughout the stages of spontaneous metamorphosis in anadromous sea lam-

preys (*Petromyzon marinus*), coinciding with accelerations of gonadal growth (Youson and Sower, 1991). Another study of landlocked sea lampreys verified these findings, showing increasing levels of lamprey GnRH-I and -III throughout the metamorphic stages (Fig. 1), and extended this feature of lamprey metamorphosis to include a non-parasitic species (Youson et al., 1995a).

Induced metamorphosis in sea lampreys shows development which is asynchronous compared to each of the clearly defined features of stages 1–7 in spontaneous metamorphosis (Holmes and Youson, 1993; Youson, 1994; Manzon and Youson, 1997). Despite the differences in somatic development between spontaneous and induced metamorphosis, both metamorphoses are characterized by a decline in serum levels of thyroid hormones (Youson et al., 1995b). Since we have previously shown that increased concentration of brain GnRHs is characteristic of successful spontaneous metamorphosis in sea lampreys, we were curious whether  $\text{KClO}_4$ -induced metamorphosis is characterized by changes in brain GnRH con-

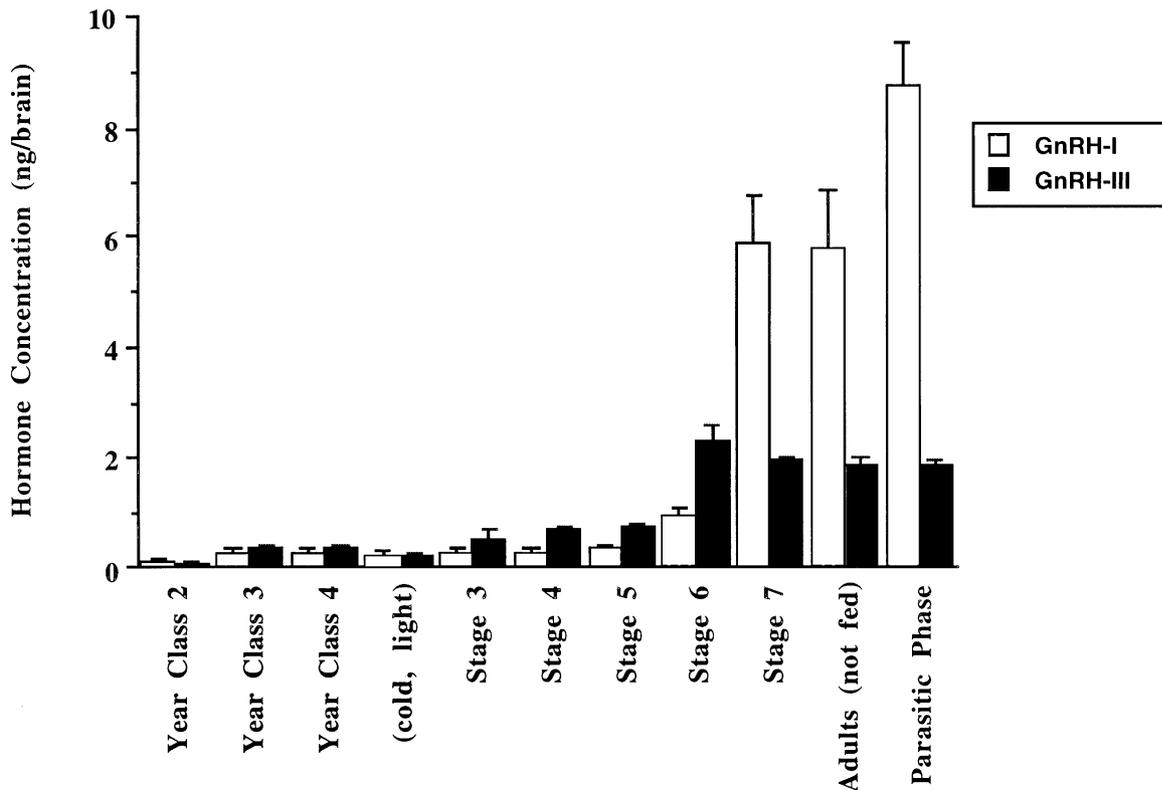


Fig. 1. Concentrations (relative content) of GnRH-I and III (ng/brain) in larvae (year classes 2, 3, and 4; and a group kept in cool water and an ambient photoperiod), in animals of stages of metamorphosis 3 to 7, inclusive, and in both recently metamorphosed (adults not fed) and feeding (parasitic phase) juveniles of *Petromyzon marinus*. Data are from Youson et al. (1995a).

tent. Our hypothesis is that if induced metamorphosis and the subsequent asynchrony of somatic development results in a change in brain content of GnRH, this is further evidence that stimulus to the reproductive axis is a key event in the metamorphic process of lampreys. Furthermore, since we are manipulating the thyroid axis to observe changes in the reproductive axis, then a change in GnRH would reflect the association of these two axes. It has been suggested by many (see Norris, 1997, citation of M. Sage) that the evolution of thyroid function was accompanied by the phylogenetic development of endocrine control of reproduction; the basic (primitive) function of thyroid hormones was associated with gonadal maturation. It is in this context that we ultimately place both the thyroid and the reproductive axes into a putative theory on the phylogenetic development of lamprey metamorphosis.

## 2. GnRH and induced lamprey metamorphosis

Since we have previously shown that increased concentration of brain gonadotropin-releasing hormone (GnRH) is characteristic of successful spontaneous metamorphosis (stages 1–7) in sea lampreys (*Petromyzon marinus*), this study was carried out to determine the effects of  $\text{KClO}_4$ -induced metamorphosis on brain GnRH concentrations (lamprey GnRH-I and -III) in larval sea lampreys. Sea lamprey larvae (ammocoetes, > 130 mm, year class 4) were collected and treated with  $\text{KClO}_4$  as described in Holmes and Youson (1993). Throughout the experiment, larvae were maintained in tanks and provided with aerated, dechlorinated tap water at temperatures of 10–14°C, supplied with a sand substrate (7 cm), exposed to photoperiod of 15L:9D, and fed baker's yeast once a week. Treatments consisted of controls (dechlorinated tap water from 20 November) and continual exposure to 0.01 or 0.05%  $\text{KClO}_4$  from 20 November to 17 March. Between 19 March and 30 March, 148 animals were anaesthetized with tricaine methanesulfonate (MS-222), and examined for stage of metamorphosis (Youson and Potter, 1979); excised brains were placed in microfuge tubes, immediately immersed in liquid  $\text{N}_2$ , and stored at  $-70^\circ\text{C}$ .

GnRH was extracted from brains as described by Yu et al. (1987). Brains from animals of different  $\text{KClO}_4$  concentrations at the same stage of

metamorphosis were pooled (three brains/sample) for the measurement of GnRH-I and -III concentration. Following extraction, the supernatant was stored at  $-80^\circ\text{C}$  until injection on the HPLC. Samples were passed through Gilman Acro LC-13 0.45-mm disposable filters and eluted from a Perkin-Elmer Tri-Det HPLC system as previously described (Sower et al., 1993). Samples were injected into a 20- $\mu\text{l}$  loop and eluted through a Pecosphere 3 CR C18 ( $0.46 \times 8.3 \text{ cm}^2$ ) reverse-phase column using a Perkin-Elmer series 100 pump. The mobile phase consisted of 7.40 g of ammonium acetate and 3.04 g of citric acid in 1 l of 19% acetonitrile/water (final pH adjusted to pH 4.6 with phosphoric acid). A flow rate of 2 ml/min was maintained throughout the experiment. Water blank fractions (20 of approx. 600  $\mu\text{l}$ ) were collected immediately prior to the injection of each sample. Each sample eluted also consisted of 20 fractions (approx. 600  $\mu\text{l}$ ). All samples and blank fractions were lyophilized and stored at  $-80^\circ\text{C}$  until the analysis of GnRH concentration by radioimmunoassay (RIA). GnRH-I and -III concentrations were assayed as described by Stopa et al. (1988), Sower et al. (1993) and Deragon and Sower (1994). Lamprey GnRH-III antibody 3952 was used at a concentration of 1:4800. The lower limit of detection was 9.8 pg/tube, with antibody binding efficiencies ranging from 12.5 to 25.5%. Intra- and inter-assay variations were 2.3 and 6.7%, respectively. Mean and standard error values were calculated for all measured GnRH concentrations. A one-way analysis of variance, 95% multicomparison (Tukey) significance test was used to determine differences between treatment groups ( $P < 0.05$ ).

As expected for the time of the year of the experiment, spontaneous metamorphosis did not occur in any of the control samples. The time of exposure of the animals to  $\text{KClO}_4$  was consistent with the time it would take for normal development to proceed from stages 1 through to stage 7 in spontaneous metamorphosis. Development of various external features in induced metamorphosis was asynchronous relative to spontaneous metamorphosis, but staging attempted to adhere to the criteria of spontaneous metamorphosis (Potter et al., 1978; Youson and Potter, 1979). As was anticipated from previous observations (Holmes and Youson, 1993; Manzon et al., 1998), we could not assign a stage beyond stage 5 in the treatment groups. However, even this staging was

arbitrary compared to a stage 5 in spontaneous metamorphosis, i.e. no direct comparison between the external features of stage 5 of spontaneous and induced metamorphosis is possible. Lamprey GnRH was detected in brains of control samples and in stages 1–5 of  $\text{KClO}_4$ -induced metamorphosis (Fig. 2). Early stages (1 and 2) of metamorphosis had similar GnRH-I and -III content, respectively. GnRH-I showed a significant decrease from stages 4 to 5 ( $P < 0.05$ ), while GnRH-III content fluctuated throughout stages 1–5, with a significant increase ( $P < 0.05$ ) occurring between stages 3 and 4.

The changes in GnRH profiles are very pronounced in spontaneous metamorphosis (Fig. 1), but the picture of the effect of  $\text{KClO}_4$ -induced metamorphosis on GnRH content is far from clear. Despite all precautions to avoid interassay variation, it is not appropriate to directly compare the actual values of these new data from induced metamorphosis with those from a previous study of spontaneous metamorphosis (Fig. 1). Furthermore, it is in the latter stages (6 and 7) of spontaneous metamorphosis and in feeding juveniles where marked changes in concentrations of GnRH-I and III occur. Development from induced metamorphosis does not proceed much beyond a designated stage 5, which is not exactly

equivalent to stage 5 in spontaneous metamorphosis (see Holmes and Youson, 1993; Manzon and Youson, 1997, 1999). It is noteworthy, however, that the profiles of brain GnRH-I and -III are much the same in untreated larvae in both studies. In addition, there is a trend in both studies for an elevation of these two hormones over control levels once metamorphosis has commenced. In particular, GnRH-III content is the first to show a marked change. Whereas in spontaneous metamorphosis a pronounced elevation in GnRH-III occurs between stages 5 and 6 (Fig. 1), a significant elevation in this hormone occurs between designated stages 3 and 4 in induced metamorphosis (Fig. 2). It may be that these periods of GnRH-III elevation are identical intervals from the time of the initiation of metamorphosis, but changes in external characters, which dictate the stage, are asynchronous and retarded relative to hormonal changes following induction. Stage 7 of spontaneous metamorphosis is characterized by a high concentration of GnRH-I relative to GnRH-III, and this former hormone is markedly higher in concentration than at stage 6 (Fig. 1). Stage 5 is the final designated stage following induced metamorphosis, and animals in this stage have been in metamorphosis for a time equivalent for animals to pass from stages 1

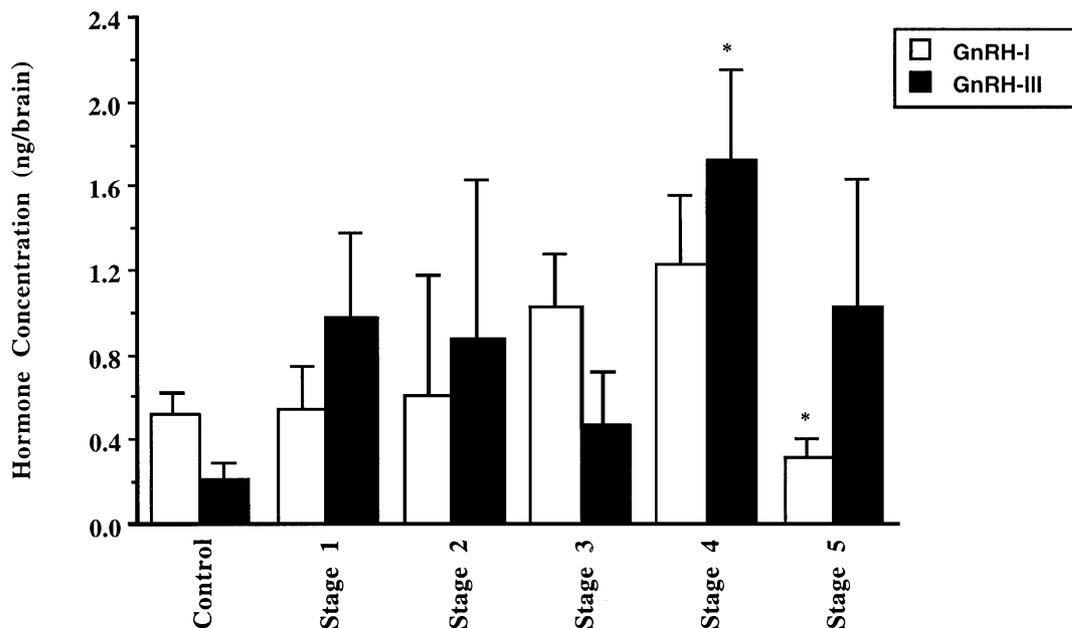


Fig. 2. Concentration (relative content) of brain GnRH-I and GnRH-III (ng/brain) in untreated (control) larval *Petromyzon marinus* and animals in various stages (1–5) of induced metamorphosis following treatment with  $\text{KClO}_4$ . Significant differences ( $P < 0.05$ ) between values from a previous stage are indicated by an asterisk (\*).

through to 7 in spontaneous metamorphosis. In induced metamorphosis, GnRH-I content in the brains of stage-5 animals is significantly lower than the preceding stage.

Spontaneous metamorphosis in sea lampreys is characterized by a significant decline in serum thyroid hormones, changes in lipid metabolism, and elevated hypothalamic GnRH (Youson, 1994, 1997). The aim of this study was to elucidate whether  $\text{KClO}_4$  treatment and the subsequent effect on the thyroid axis and metamorphosis result in changes in the hypothalamic neurosecretory events of the reproductive axis. The GnRH profile did not exactly parallel that appearing in spontaneous metamorphosis (Youson and Sower, 1991; Youson et al., 1995a). We might infer that the failure to show a normal pattern of metamorphosis following goitrogen treatment is at least partly due to insufficient stimulus from one part of the reproductive axis at a critical point in development. Furthermore, the data, when compared to that from spontaneous metamorphosis, also indirectly imply that the upregulation of the hypothalamic segment of the reproductive axis in lampreys may be a major event that is involved in, or coordinated with, the complex changes of a normal metamorphosis in lampreys.

The mode of action of  $\text{KClO}_4$  in the inductive process, or in fact thyroid hormones in spontaneous metamorphosis, are not totally understood (Youson, 1997). Thyroid hormones tend to act like juvenile hormones in larval lampreys (Youson et al., 1997). A previous study on adult sea lampreys has shown that thyroid hormones influence the activity of the reproductive axis (Sower et al., 1984) and there is no reason to doubt that such a response would not exist in larvae. The decline in circulating levels of thyroid hormones at metamorphosis may provide a feedback to hypothalamic neurosecretory centers which facilitates the release of GnRH-I and -III from a hypothalamus in an accelerated state of maturation. Further studies are required to show the direct involvement of the reproductive axis in the initiation, maintenance, and completion of lamprey metamorphosis. In particular, we are waiting with great anticipation for the isolation and sequencing of lamprey gonadotropin.

The conclusion from the induction study is that manipulation of the thyroid axis with  $\text{KClO}_4$  causes some changes in brain GnRH content, but the profile is not identical to the GnRH profile

seen in spontaneous metamorphosis. Although the data are insufficient to draw any definitive conclusions on the role of GnRH in metamorphic development, they do provide additional support for the view that there is some interplay between the thyroid and reproductive axes at this time in the lamprey life cycle (Youson, 1997, 1999). The question now arises as to whether an interaction between the thyroid and reproductive axis played any role in the phylogenetic development of both metamorphosis and the adult life-history strategies in lampreys.

### 3. Phylogenetic development of lamprey metamorphosis

It was mentioned in Section 1 that the nature of the life cycle in the earliest record of lampreys is not known. *Mayomyzon* of 280 million years ago were either fossilized in a stage of metamorphosis, or were adults lacking the feeding apparatus of extant parasitic species (Bardack and Zangerl, 1971). This interpretation implies that if there was a metamorphosis there must have been a larval period, but so far there are no fossils of larval lampreys. The absence of fossils of larval lampreys can be explained by the fact that all fossils to date were in former coastal deltaic regions which would have been subject to changing salinity. Present day larvae cannot survive in such estuarine conditions; this may also have been the case of putative larvae of ancestral species. Another possibility has been proposed that the earliest lampreys were larval-like in phenotype, but they were capable of reproduction in a marine environment (Youson 1997, 1999). Such a lamprey predated *Mayomyzon* and was similar in many ways to extant adult Larvacea, which are free-swimming urochordates of ancient lineage. These early lampreys were paedomorphic larvae, and thus were similar to the larvae from which the extant Larvacea are believed to have been derived (Nielsen, 1995).

The relationship between the need for elemental iodide by all living organisms and the developmental of the thyroid gland has received considerable attention in recent years (e.g. Eales, 1997; Johnson, 1997; Holland and Holland, 1999; Venturi et al., 2000). The parallel in the developmental history between lampreys and many of the protochordates (i.e. that natural selection re-

sulted in metamorphosis) is accompanied by the appearance of the iodide-concentrating, endostyle as a thyroid-like organ in both groups. Whereas, the endostyle persists in adult protochordates, in lampreys this organ transforms into a follicular thyroid gland during metamorphosis. Eales (1997) claimed that the follicular thyroid permitted the movement of animals from the iodine-rich marine environment into iodine-poor freshwater because the organ is efficient in iodine storage. Youson (1999) suggests that the efficiency of the endostyle to produce thyroid hormones in either salt- or freshwater lamprey larvae was a critical step in the appearance of metamorphosis as a developmental strategy. This viewpoint is based on evidence that serum concentrations of thyroid hormones increase over the protracted larva period and metamorphosis is only initiated in animals with peak concentrations (Youson et al., 1994). In the putative ancestral larvae, the follicular thyroid from the endostyle was just one of the many manifestations of an integrated restructuring of the organism in response to the high levels of circulating thyroid hormone. In the extant sea lampreys, peak concentrations of thyroid hormones must coincide with critical metabolic (e.g. lipid) and environmental (primarily temperature) cues before metamorphosis is initiated (Youson, 1994). A decline in serum concentrations of thyroid hormones is an early character of initiated metamorphosis, but we still do not know whether it is the first step (Youson, 1994, 1997). There is one major question that requires an answer to give credence to this interpretation of the evolution of lamprey metamorphosis. If the original lampreys were paedomorphic and larval-like in a marine environment, and the next step in their evolution was the selection of metamorphosis as a developmental strategy, what happened to the timing of reproduction?

The most recent analysis of the subject concluded that paedomorphism or neoteny is not present in any extant lamprey species (Vladykov, 1985); however, there were some earlier claims of precocious development of the ovaries in a few samples of a single species (Zanandrea, 1957). Thus, if early lampreys were larval-like and neotenic, a delay in the timing of reproductive maturation accompanied the evolution of metamorphosis. In fact, in all extant species of lampreys, metamorphosis is critical to maturation of

the reproductive system. During the evolution of lampreys, there must have been a selective advantage to delay reproduction until after metamorphosis. The advantages may have been related to reproductive behavior, egg size, or to fecundity, and revolve around the fact that early lampreys, even *Mayomyzon* (Bardack and Zangerl, 1971), were of small size relative to present-day species. Despite the many hundreds of millions of years that have likely passed since the introduction of metamorphosis into the lamprey life cycle, there still remains a vital intimacy between programmed development and the reproductive system during this phase. The hypothalamic-pituitary portions of the reproductive axis have been addressed as likely being critical in directing the timing of reproductive maturation in closely related non-parasitic and parasitic species (Hardisty, 1979). The failure to reach an appropriate level of regulation in these portions of the reproductive axis during metamorphosis of one population of non-parasitic *L. richardsoni* undoubtedly leads to a delay in reproduction and an opportunity to feed as an adult (Beamish, 1987). The present study has shown that stimulation of precocious metamorphosis in sea lampreys does not result in a pattern of brain concentration of GnRHs that is consistent with the complete development of spontaneous metamorphosis. These types of data emphasize the importance of the timing and the integration of development; in this case the intimacy between the reproductive and thyroid axes in the metamorphosis of lampreys. These ontogenetic data also likely reflect on the intimacy which occurred between these two axes during the phylogenetic development of metamorphosis as a developmental strategy during the long evolutionary history of lampreys. Whether one accepts the view of early lampreys as being larval-like and neotenic, or the view that following hatching there was a direct development to adulthood, there likely was a great deal of interplay between the developmental timing directed by the thyroid axis and that directed by the reproductive axis before the present interaction of these axes was resolved.

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