Spontaneous and induced metamorphosis in the American brook lamprey, *Lampetra appendix*

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Abstract: We described the seven stages of spontaneous metamorphosis in the American brook lamprey (*Lampetra appendix*) and assessed the importance of size as a determinant of spontaneous and induced metamorphosis. Serum concentrations of the thyroid hormones (TH) thyroxine (T_4) and triiodothyronine (T_3) were measured in metamorphosing and nonmetamorphosing *L. appendix*. The sequence of stages in metamorphosis and changes in the relative lengths of most body regions were consistent with data reported for other lamprey species. However, premetamorphic and metamorphosing *L. appendix* in the early stages of metamorphosis (1–3) were much larger in size (at least 155 mm and 5.40 g) than has been observed for other lamprey species. Serum concentrations of T_4 and T_3 were high by the end of the larval period and declined significantly by stage 2 of metamorphosis. Larvae ≥ 130 mm in length were treated with either potassium perchlorate ($KClO_4$; 0.01 and 0.05%) or 10 mg/L propylthiouracil (PTU; 0.0001%) for 117 days from September to January to determine if metamorphosis could be induced by these goitrogens. Both concentrations of $KClO_4$ successfully induced metamorphosis in *L. appendix*, but the incidence of metamorphosis (62%) was much lower than reported in sea lampreys (98%) of comparable size. Serum concentrations of T_4 and T_3 declined by 64–76 and 93–96% relative to control values, respectively, in metamorphosing and nonmetamorphosing *L. appendix* treated with $KClO_4$. PTU elicited declines of 55% for T_4 and 80% for T_3 , but only one animal metamorphosed. Based on these data, we conclude that a decline in serum TH levels is necessary for metamorphosis in *L. appendix*, but not sufficient by itself to trigger the process.

Résumé : On trouvera ici la description des sept stades de métamorphose spontanée chez la lamproie (*Lampetra appendix*); nous avons évalué l'importance de la taille comme facteur déterminant de la métamorphose spontanée et de la métamorphose provoquée. Les concentrations sériques des hormones thyroïdiennes (TH) thyroxine (T_4) et triiodothyronine (T_3) ont été mesurées chez des lamproies en métamorphose et chez d'autres lamproies non en métamorphose. La séquence des stades de métamorphose et les modifications de la longueur relative de la plupart des zones du corps correspondent aux données signalées pour d'autres espèces. Cependant, chez cette espèce, les lamproies pré-métamorphiques et les lamproies en métamorphose au cours des premiers stades (1–3) sont de taille bien supérieure (au moins 155 mm et 5,40 g) à celle mesurée chez d'autres espèces. Les concentrations sériques de T_4 et de T_3 sont élevées à la fin de la période larvaire et diminuent considérablement jusqu'au stade 2 de la métamorphose. Nous avons administré du perchlorate de potassium ($KClO_4$; 0,01 et 0,05%) ou 10 mg/L de propylthiouracil (PTU; 0,0001%) à des larves de longueur ≥ 130 mm durant 117 jours, de septembre à janvier, afin de vérifier si ces goitrogènes peuvent provoquer la métamorphose chez les poissons. Les deux concentrations de $KClO_4$ ont provoqué la métamorphose, mais la fréquence des métamorphoses (62%) a été de beaucoup inférieure à celle enregistrée chez des Lamproies marines (98%) de tailles semblables. Les concentrations sériques de T_4 et de T_3 ont subi des chutes de 64–76 et de 93–96% par rapport aux valeurs témoins enregistrées chez les lamproies en métamorphose ou non traitées au $KClO_4$. L'administration de PTU a entraîné une baisse de 55% de T_4 et de 80% de T_3 , mais un seul animal s'est métamorphosé. D'après ces données, il faut que les concentrations sériques des hormones thyroïdiennes diminuent pour que la métamorphose ait lieu chez la lamproie, *L. appendix*, mais cette diminution ne suffit pas par elle-même à provoquer la métamorphose.

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Introduction

All species of lampreys undergo a metamorphosis in which extensive morphological, physiological, and behavioural changes occur (Youson 1988), followed by a species-specific parasitic or nonparasitic adult life. Most parasitic

species migrate from their natal streams into large bodies of water, where they feed on the blood, body fluids, and (or) tissues of teleosts for 12–18 months, resulting in a rapid increase in size of juveniles. Sexual maturation is coincident with the cessation of feeding (1.5–2 years after metamorphosis) and an upstream migration and is followed by spawning

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(Hardisty and Potter 1971). In contrast, nonparasitic species begin sexual maturation immediately after the completion of metamorphosis, foregoing a feeding interval, with spawning and death usually occurring the following spring. Thus, non-parasitic adults are smaller and less fecund than their parasitic counterparts and do not exhibit their migratory behaviour (Hardisty and Potter 1971).

Larval size (length in combination with mass) is an important determinant of whether sea lamprey larvae (*Petromyzon marinus*, a parasitic species) are likely to enter metamorphosis. Metamorphosis is a nontrophic interval in the lamprey life cycle (Potter 1980) and, in *P. marinus*, the catabolism of lipid is the primary energy source (Youson et al. 1979). In nonmetamorphosing sea lamprey larvae, lipids comprise, on average, about 4% of the live body mass, increasing to about 14% of the live body mass during the last year of larval life after the animal has reached a length comparable with that of metamorphosing animals (Lowe et al. 1973; O'Boyle and Beamish 1977). Although sea lamprey larvae in an anadromous population in New Brunswick, Canada, must be at least 110 mm (length) and 2.0 g (mass) in size prior to beginning metamorphosis (Potter et al. 1978), larvae in land-locked populations from the Great Lakes and Lake Champlain must be at least 120 mm (length) and 3.0 g (mass) in size (Youson et al. 1993). Because both metamorphosis and the subsequent period of sexual maturation are nontrophic in nonparasitic species, the larvae presumably have greater energy reserves prior to entering metamorphosis than those of parasitic species that feed between metamorphosis and spawning. If, as in sea lampreys, lipid is the primary energy store (Lowe et al. 1973; Kao et al. 1997), then animal size and condition might also be important requirements for metamorphosis in nonparasitic lampreys.

Thyroid hormones (TH) influence the events of metamorphosis in those teleost groups (Anguilliformes, Elopiformes, Notocanthiformes, and Pleuronectiformes) and amphibians (anuran and urodele) that exhibit a true metamorphosis. Thyroxine (T_4) and triiodothyronine (T_3) concentrations increase during spontaneous metamorphosis of amphibians and flounders, peaking at metamorphic climax (Inui and Miwa 1985; Galton 1988; Galton et al. 1994; Inui et al. 1994), and precocious metamorphosis can be induced in larvae with exogenous T_4 and (or) T_3 treatments (Inui and Miwa 1985; Miwa and Inui 1987; Galton 1988). In contrast, the initial stages of spontaneous metamorphosis in lampreys, particularly sea lampreys, are accompanied by a substantial decline in serum levels of both T_4 and T_3 (Wright and Youson 1977; Lintlop and Youson 1983; Leatherland et al. 1990; Youson et al. 1994). Precocious metamorphosis can be induced in some lamprey species with exogenous treatments of potassium perchlorate ($KClO_4$; Hoheisel and Sterba 1963; Suzuki 1986, 1989; Holmes and Youson 1993). $KClO_4$ also depresses serum levels of both T_4 and T_3 (Suzuki 1986, 1989; Youson et al. 1995; Manzon and Youson 1997; Manzon et al. 1998), mimicking the situation in the early stages of spontaneous metamorphosis. However, treatment with propylthiouracil (PTU) produces mixed results; Suzuki (1986) reported complete metamorphosis in *Lampetra reissneri*, while Leatherland et al. (1990) were not able to induce metamorphosis in *Geotria australis*, despite the fact that larvae of the latter species exposed to PTU had significantly lower serum TH levels than

untreated control animals. Thus, although TH probably do not regulate the initiation of lamprey metamorphosis in the manner described for teleosts and amphibians, they may be important for the synchrony of developmental processes during metamorphosis (Youson 1997).

In the present study, we compare spontaneous and induced precocious metamorphosis in the nonparasitic American brook lamprey, *Lampetra appendix*, in terms of the incidence of metamorphosis, the importance of size as a determinant of metamorphosis, and serum T_4 and T_3 concentrations in metamorphosing and nonmetamorphosing individuals. Although induced metamorphosis has previously been reported in brook lampreys (e.g., *Lampetra planeri*, Hoheisel and Sterba 1963; *Lampetra reissneri*, Suzuki 1986, 1989), there is no large-scale study of induced metamorphosis in any nonparasitic species where size and time of year have been carefully controlled and both serum T_4 and T_3 concentrations have been measured. We also provide the first descriptions of the stages of the normal metamorphic development (which we call spontaneous) in *L. appendix*.

Materials and methods

Spontaneous metamorphosis

In June 1995, *L. appendix* larvae were collected from Bowmanville Creek (43°53'N, 78°40'W), a Lake Ontario tributary about 50 km east of Metropolitan Toronto, using DC backpack electrofishing gear, and transported to the University of Toronto at Scarborough. The larvae were maintained in a large fibreglass tank (152 (length) × 55 (width) × 35 (height) cm) and provided with 7–8 cm of sand for burrowing and aerated dechlorinated tap water, the temperature of which was changed seasonally. Photoperiod was held constant at 15 h light : 9 h dark before and during the experiment, because previous studies on sea lampreys did not detect a short-term photoperiod effect on metamorphosis (Cole and Youson 1981; Youson et al. 1993; Holmes et al. 1994), and larvae were fed baker's yeast once each week at the rate of 1 g/animal per week. Those animals in which spontaneous metamorphosis was initiated by July 1995 ($N = 20$) were measured for length and mass in the early stages of metamorphosis (stages 1, 2, and 3), to estimate the minimum size (length and mass) required for premetamorphic larvae to begin spontaneous metamorphosis.

We sampled some of the metamorphosing *L. appendix* periodically throughout the summer and fall of 1995 to describe the stages of metamorphosis ($N = 1$ or 2 per stage). Descriptions of stages 1–5 are based on animals collected from Bowmanville Creek. Stages 6–7 and adult specimens were collected from Duffins Creek, a Lake Ontario tributary about 10 km east of Metropolitan Toronto, in early September 1995, transported to our laboratory, and held under the conditions described above. Although the separation of metamorphosing animals into stages (stage 1 (earliest) to stage 7 (latest)) was based on live animals, using the criteria in Youson and Potter (1979) as a guide, our descriptions of the key characters are based on preserved specimens. We anaesthetized animals in 0.05% MS-222 (tricaine methanesulfonate, Syndel Laboratories, Vancouver, B.C.), recorded total length (± 1 mm) and mass (± 0.01 g), and then fixed and preserved examples of premetamorphic larvae, each metamorphic stage, and maturing adults in 5% formalin. The following body regions (Potter et al. 1980) were measured (± 0.01 mm) 29–36 months after preservation: length of the oral disc, prebranchial length, branchial length, eye diameter, trunk length, trunk depth, cloaca length, tail length, and the heights of the first and second dorsal fins ($N = 1$ or 2 per stage). These measurements were converted to relative lengths (percent), using the

total length of the preserved animal. Sagnes (1997) investigated the effects of 4% formalin preservation on larval Arctic grayling (*Thymallus thymallus*) and found that shrinkage of body regions measured along the axis of total length were proportionately comparable with shrinkage in total length, and that changes in body depth due to preservation (similar to our measurement of lamprey trunk diameter) were insignificant. We assume that changes due to preservation are equivalent in all body regions, so we did not attempt to correct our data to represent fresh-specimen lengths.

Larvae and spontaneously metamorphosing *L. appendix* were collected from Duffins Creek between 21 June and 22 August 1996 and transported to our laboratory at the University of Toronto at Scarborough for blood collection. Water temperatures (based on instantaneous measurements) varied between 14 and 17°C at the time of collection. The stage 1 animals were collected 1 year earlier, on 18 July 1995, from the same location. On the same day that they were collected, the animals were anaesthetized in 0.05% MS-222, length (± 1 mm) and mass (± 0.01 g) were measured, stage of metamorphosis was assessed (Youson and Potter 1979), and blood was collected in heparinized microcapillary tubes following caudal severance. The blood was clotted overnight at 4°C and centrifuged the next morning, and the serum was separated and stored at -70°C for later determination of serum TH concentrations.

Induced metamorphosis

Lampetra appendix larvae ≥ 130 mm long ($N = 150$) from Bowmanville Creek that did not enter spontaneous metamorphosis by August 1995 were randomly sorted into small experimental tanks ($40 \times 20 \times 25$ cm) at a density of 10 animals/tank (125 animals/m²) on 13 September 1995. Each tank was supplied with 10 L of static aerated dechlorinated tap water at room temperature and 6–7 cm of sand for burrowing, and was kept at a photoperiod of 15 h light : 9 h dark throughout the experiment. Experimental treatments (three replicate tanks per treatment) consisted of 0.01 and 0.05% KClO₄ (Aldrich Chemical Co., Milwaukee, Wis.), 10 mg/L PTU (0.0001%; Sigma Chemical Co., St. Louis, Mo.), a dechlorinated tap water control, and a 0.1 M NaOH (50 mL/10 L) control. The NaOH control was necessary, because PTU is only soluble in an alkaline solution; Manzon and Youson (1997) subsequently demonstrated that NaOH has no effect on the incidence of metamorphosis in sea lampreys. These treatments and their concentrations were similar to those used in previous studies of induced metamorphosis in lampreys (e.g., Leatherland et al. 1990; Holmes and Youson 1993; Manzon and Youson 1997).

The experiment began 13 September 1995 and was terminated 8 January 1996. Since the tanks were static, the water was changed and the goitrogens refreshed with the addition of 50 mL of stock solutions every 2 weeks. Water temperature (instantaneous measurement) was checked and recorded twice a day, and observations of mortality and external signs of metamorphosis in animals visible above the sand were recorded at the same time. To maintain animal density in the tanks, dead animals were replaced with animals marked with a subcutaneous injection of yellow latex dye in the caudal sinus. At the end of the experiment, the animals were anaesthetized in 0.05% MS-222, length (± 1 mm) and weight (± 0.01 g) were measured, stage of metamorphosis was assessed (Youson and Potter 1979), and blood was collected; the serum was separated and stored for later determination of serum TH concentrations as previously described.

Water temperature in the experimental tanks was held between 16 and 21°C during the study, averaging 18.6°C ($SE = \pm 0.05^\circ C$). These temperatures (16–21°C) were higher than seasonal temperatures in Toronto area streams during the study period, but were within the normal temperature range for spontaneous metamorphosis (Youson et al. 1993) and the most effective for inducing metamorphosis by KClO₄ in sea lampreys (R.G. Manzon and J.H. Youson, unpublished data). Despite the high temperatures, mortal-

ity was low during the induction experiment, consisting of two larvae in control tanks (6.7%).

Serum TH measurement

TH concentrations were measured using Amersham Amerlex commercial radioimmunoassay (RIAs) kits for T₄ and T₃ that were modified as described by Leatherland et al. (1990). Serum samples for both T₄ and T₃ were thawed and dispensed into duplicate aliquots of 2.5 μ L for measurement. When the volume of serum collected from a single animal was not sufficient for both assays, samples from 2–3 animals were pooled to obtain the necessary volume. Thus, sample sizes (N) correspond to the number of samples assayed rather than the number of animals from which serum was collected. Duplicate measurements were accepted if the difference between the gamma count of each aliquot was $\leq 10\%$ of the count for the zero standard (which contains ¹²⁵I and antibody but no T₄ or T₃). Serum samples from the induction experiment and from spontaneously metamorphosing animals were analyzed more than 1 year apart by different individuals. Assay sensitivities for sera collected from the induction experiment were 14.4 nmol/L for T₄ and 0.26 nmol/L for T₃, and intra- and inter-assay variation was $<12\%$ for T₄ and $<12\%$ for T₃. Assay sensitivities for sera collected from spontaneously metamorphosing *L. appendix* were 12 nmol/L for T₄ and 0.25 nmol/L for T₃, and the inter- and intra-assay variation was $<9.5\%$ for both T₄ and T₃. Serum T₄ concentrations in sea lampreys exposed to KClO₄ or PTU or in spontaneously metamorphosing lampreys at stages 4 and 5 were below the detection limits of the T₄ assay. For these samples, we increased the serum volume to 10 μ L and divided the measured values by the concentration factor (4) to obtain the concentrations reported.

Data analysis

We used the length and mass data from fresh specimens of *L. appendix* to document gross body changes during metamorphosis.

Predictions of the incidence of metamorphosis in *L. appendix* were based on the minimum size (length and mass) and condition factor (CF) of spontaneously metamorphosing *L. appendix* in the summer of 1995. This approach has been used to successfully predict sea lamprey metamorphosis in the laboratory (Holmes and Youson 1993, 1997; Youson et al. 1993; Holmes et al. 1994). CF was calculated as $(\text{mass (g)})/(\text{length (mm)})^3 \times 10^6$. An animal that met the size and CF criteria at the beginning of the experiment was expected to initiate metamorphosis when induced by the goitrogen treatments. We compared these predictions with the observed incidence of metamorphosis, to determine if a minimum size and physiological conditioning were requirements of induced metamorphosis in *L. appendix*.

The incidence of metamorphosis among the different treatments was assessed by one-way ANOVA. Multiple comparisons of treatment means were made using Scheffé's S test, and these data are reported as the mean ± 1 SE. Chi-squared analysis was used to compare the expected number of metamorphosing animals with the observed number of metamorphosing animals in each tank. Because the larvae used in our study ranged in length from 130 to 178 mm, we used linear regression to determine if TH concentration was a function of animal mass for a treatment. When significant linear relationships were found, we used analysis of covariance (ANCOVA) to adjust the mean TH levels, removing the effect of mass prior to analyzing the T₃ data by one-way ANOVA. The T₄ data were also analyzed by one-way ANOVA, but the data from the KClO₄ treatments were omitted from this analysis, because they were below the detection limits of the assay and so we are uncertain of the real values. Results are accepted as significant if $P \leq 0.05$. Since the RIAs for serum TH concentrations in spontaneously metamorphosing animals and those from the induction ex-

Fig. 1. The anterior region of *Lampetra appendix* in premetamorphic larvae (A and B), metamorphosing stages 1 (C), 3 (D), 5 (E), and 7 (F), and sexually maturing adults (G and H). All lateral (A and C–G) and ventral (B and H) views are of specimens preserved in 5% formalin for 34–36 months.

periment were conducted more than 1 year apart by different individuals, we did not directly compare T_4 and T_3 values.

Results

Spontaneous metamorphosis

Description of stages

Larva (Figs. 1A and 1B)—The anterior end of a larva is dominated by the oral hood, which consists of a pair of thin

lateral lips and a thicker ventral transverse lip. When viewed ventrally, large branched cirrhi can be seen prominently in the posterior portion of the hood around the buccal funnel (posterior cirrhi). The dorsal surface of the oral hood is covered with much smaller unbranched projections that are not easily seen, the anterior cirrhi. The incompletely developed eyes are small, irregularly shaped, and located dorsally at the level of the transverse lip. The branchiopores are triangular in shape, with the apex pointing anteriorly, and are connected by a longitudinal groove. The dorsal half of the body is pigmented, but pigmentation is absent from the gill

region and, laterally, from both sides of the head and the lower half of the lateral lips.

Stage 1 (Fig. 1C)—Metamorphosis of *L. appendix* begins in early July with the appearance of stage 1, which is characterized by two features: (i) an enlargement of the eye to a more regular shape and (ii) a dorsoventral enlargement of the branchial region. In this stage, the oral hood is shorter than in the larva and is beginning to fold under, so that the opening is subterminal. These changes are accompanied by a thickening of the lateral lips forming the oral hood of the larva and a reduction in the width of the transverse lip. The posterior cirrhi in the oral cavity are more clearly visible than in the larva, particularly those lateral to the buccal funnel, and the anterior cirrhi seem to be fewer in number but larger than in the larva. Other features, such as the branchiopores, body pigmentation, and the presence of oral cirrhi, retain their larval configurations.

Stage 2—The second stage of metamorphosis appears toward the end of July. In this stage, the lateral lips are thicker and the oral opening is smaller than in the larva, and the transverse lip has become trapezoidal in shape. The anterior oral cirrhi are larger than in stage 1, and some appear to be clumping together to form larger buds. The posterior cirrhi are less prominent in size and the branching appears to be reduced. The snout has a rounded triangular shape in both dorsal and lateral views. The eye is more clearly developed and larger than in stage 1, and the beginning of a notch is evident at the cloaca (cloacal notch).

Stage 3 (Fig. 1D)—The eye is circular rather than oval as in earlier stages and is differentiated into the iris and pupil for the first time. The lips are thick and completely encircle the presumptive oral disc, but grooves are present between the transverse and lateral lips, indicating that they have not fused. The anterior cirrhi are again larger and by this stage appear as large papillae. In contrast, the posterior cirrhi are greatly reduced in size and number relative to the larval condition. The branchial region remains enlarged, but the branchiopores start to lose their triangular larval appearance and acquire an oval shape, and the longitudinal groove connecting them is less prominent. Stage 3 animals appear by early August.

Stage 4—Animals in stage 4 of metamorphosis appear between early and mid-August. The most prominent feature of these animals is that the lateral and transverse lips are fused and form a complete ring of tissue supporting an oval-shaped oral disc that is narrower than the head and snout of the animal. A tongue-like piston has begun to develop deep in the oral cavity, and only 2 or 3 posterior cirrhi are visible lateral to the buccal funnel. The anterior papillae seem to be fewer in number and more rounded in shape than at earlier stages of metamorphosis. In stage 4, the eye is larger than in stage 3, being about one-third the depth of the head. The branchiopores are now narrow oval slits, and there is little evidence of the longitudinal groove. However, the branchial region continues to exhibit the dorsoventral enlargement. Body pigmentation has increased on the dorsal and lateral surfaces of the head and snout.

Stage 5 (Fig. 1E)—The snout is strongly triangular in shape in both dorsal and lateral views. The ring supporting the oral disc is smoothly fused with no grooves present, although there is a triangular notch distal to the oral disc on the ventral surface. The piston is clearly visible in the oral disc and consists of two lateral lingual laminae and a transverse lingual lamina. The supraoral and infraoral laminae are visible as smooth white structures surrounding the buccal funnel. Although none of the laminae have obvious tooth development, papillae on the oral disc have increased in size and points have begun to develop on them. The edge of the oral disc has a fringed appearance, owing to the development of fimbriae. The cloacal notch is well-developed and both dorsal fins are greatly enlarged vertically. Late August to early September is the time when animals at this stage of metamorphosis can be captured.

Stage 6—The branchiopores are clearly oval in shape and the longitudinal groove connecting them has disappeared. Dorsoventral expansion of the branchial region is not as prominent as in earlier stages of development. Prominent fimbriae encircle the outer ring of the oral disc, and tooth development is clearly visible in the oral cavity. The supraoral lamina is prominent and two lateral cusps are visible; the infraoral lamina possesses six raised points. The teeth developing closest to the buccal funnel (circumoral teeth) are bicuspid, while the other teeth, which are smaller, are unicuspid. The posterior portion of the oral disc folds into the ventral triangular notch, so that the oral disc remains narrower than the triangular head. Body pigmentation has continued to increase. Metamorphosing *L. appendix* reach stage 6 by mid-September.

Stage 7 (Fig. 1F)—Stage 7 of metamorphosis begins in October. The bicuspid circumoral teeth are about twice the size of the other teeth, which are unicuspid. The infraoral lamina, although difficult to see ventrally, has six cusps. Neither of the laminae on the piston appears to have cusps by stage 7. Although the oral disc is somewhat open, it remains narrower than the head and oval in shape. The branchial openings are oval and the enlargement of the branchial region is no longer obvious. The eye has enlarged to about one-half the depth of the head. Body colouration has continued to darken but has begun to appear blotchy, particularly laterally near the ventral surface. The cloacal notch is quite prominent, owing to anterior swelling along the midline and a sharp ridge running posteriorly from the cloaca to the caudal fin.

Maturing adult (Figs. 1G and 1H)—Immature adults, which began to appear in December, have a fully developed circular oral disc. Prominent fimbriae fringe the outer edge of the disc and tooth cusps are prominent: in preserved specimens they are orange-brown in colour and curve inwards toward the buccal funnel. The inner surfaces of the lateral lingual lamina have conspicuous spines, which give a serrated appearance to the piston, and three cusps are visible on the transverse lingual lamina. The infraoral lamina has six cusps, while the lateral cusps of the supraoral lamina are more prominent than in earlier stages of metamorphic development. The eye is large and well-developed, accounting for about one-third of the depth of the head. The gill openings are oval and body pigmentation is darker and blotchy. The

Fig. 2. Changes in total length (A) and mass (B) of metamorphosing *Lampetra appendix*. Stage L indicates untransformed larvae exceeding the minimum size for metamorphosis (155 mm and 5.40 g) in June 1995, and stages 1–6 are as described in the text. Sample sizes (*N*) for stages L, 1, 2, 3, 4, 5, and 6 are 22, 5, 6, 3, 3, 18, and 18, respectively.

cloacal notch is extremely pronounced, with the anterior lips swollen and the posterior ridge more prominent than in previous stages. The dorsal fins are large and erect.

Size at metamorphosis

In the early stages of metamorphosis in July and August 1995 (stages 1–3), *L. appendix* ranged from 155 to 189 mm in length and from 5.40 to 9.74 g in mass. Although there were no significant changes in length during metamorphosis

(Fig. 2A), stage 3 animals were significantly heavier than nonmetamorphic larvae at the beginning of metamorphosis or animals in stages 1 and 2 of metamorphosis (Fig. 2B). By stages 5 and 6, metamorphosing animals were significantly lighter than those in stage 3. For *L. appendix* in stages 1, 2, or 3 of metamorphosis, the average values for size were 176 ± 2.26 mm (length $\pm$ SE) and 7.56 ± 0.25 g (mass $\pm$ SE) and the average CF was 1.39 ± 0.023 . For spontaneously metamorphosing *L. appendix*, the minimum values for size were 155 mm (length) and 5.40 g (mass) at stage 1 and the minimum CF was 1.25 at stage 3. These minima were used to predict metamorphosis in the induction study.

Morphological evaluation

Although caution is necessary because of the small sample sizes ($N = 1$ or 2 at each stage), the most obvious external morphological changes during metamorphosis in *L. appendix* are the development of the eye and the oral disc (Fig. 1). The eye doubled in diameter (given as a percentage of total length) from about 0.6% in the larva to 1.2% at stage 1, and doubled again from stage 1 to the adult (about 2%) (Fig. 3A). The initial stages of development of the oral disc involved a reduction in the size of the opening followed by a rapid increase in length after stage 5 (Fig. 3A). These changes in the eye and oral disc were accompanied by an increase in the length of the prebranchial region (Fig. 3B). At the same time, a slight decrease in the length of the branchial region was observed between the larval phase and stage 5 of metamorphosis (Fig. 3B). In *L. appendix*, trunk depth increased throughout metamorphosis, from the larval to the adult stage (Fig. 3D), while trunk length exhibited no consistent pattern of change (Fig. 3C). The length of both the tail and the cloacal slit decreased during metamorphosis (Figs. 3C and 3D). Another prominent morphological change during metamorphosis was a doubling in height of the first and second dorsal fins (Fig. 3E). The second dorsal fin was usually at least twice the height of the first dorsal fin at all stages.

Serum TH hormone concentrations

Concentrations of both T_4 and T_3 in the serum of *L. appendix* increased significantly between nonmetamorphic (i.e., larvae <155 mm long) and premetamorphic larvae (those ≥ 155 mm long; Fig. 4). Concentrations of both T_4 and T_3 declined significantly (about 90%) by stage 2 and then remained relatively constant up to stage 6 of metamorphosis, the last stage for which we have data.

Since serum for stage 1 was collected 1 year earlier than serum for the other stages, the mean T_4 and T_3 concentrations in stage 1 animals are treated separately from those of the other stages (shown in Fig. 4). Mean T_4 concentration peaked in animals in stage 1; in contrast, mean T_3 levels peaked in premetamorphic larvae. This high T_4 level in stage 1 animals represents a doubling of the average levels found in the oldest premetamorphic larvae of similar size.

Induced metamorphosis

Size at metamorphosis

The size of larval *L. appendix* did not vary significantly between treatments in September 1995 (length: $F_{[4,145]} = 0.317$, $P = 0.87$; mass: $F_{[4,145]} = 0.464$, $P = 0.76$), with aver-

Fig. 3. The relative sizes of (A) the eye and oral disc, (B) the prebranchial and branchial regions, (C) the trunk and tail, (D) the cloaca and trunk (depth), and (E) the first and second dorsal fin (height) in the larva (L) and through metamorphosis (stages 1–7) to sexually immature adult (A) in *Lamprologus* appendix. Values in these figures are expressed as a percentage of the total length of formalin-preserved specimens. Sample sizes (N) for stages L, 1, 2, 3, 4, 5, 6, 7, and A are 2, 1, 2, 2, 1, 2, 4, 2, and 1, respectively.

Fig. 4. Serum concentrations of (A) thyroxine (T_4) and (B) triiodothyronine (T_3) in spontaneously metamorphosing *Lampetra appendix*; NM, nonmetamorphosing larvae (<155 mm long); PM, premetamorphic larvae (≥ 155 mm long); stages 1–5, see text. Sample sizes (N) for NM and PM are 5 and 26, respectively, and for stages 1, 2, 3, 4, and 5 are 5, 6, 3, 3, and 18, respectively.

age values for length and mass of 151 ± 0.897 mm and 4.73 ± 0.083 g, respectively. In September 1995, values for CF ranged from 1.34 to 1.37, with an average of 1.36 ± 0.005 . By January, metamorphosing animals were significantly larger than nonmetamorphosing larvae ($P < 0.05$), averaging 153 ± 1.55 mm and 4.19 ± 0.12 g in length and mass, respectively, with an average CF of 1.17 ± 0.01 . Average values for size for nonmetamorphosing larvae were 143 ± 1.16 mm (length) and 3.63 ± 0.09 g (mass) and average CF was 1.20 ± 0.007 . The smallest metamorphosing animal appeared to be in stage 1, was 132 mm long, weighed 2.46 g, and had a CF of 1.06.

Fig. 5. Number of individuals metamorphosing among *Lampetra appendix* exposed to different goitrogen treatments (present study) and among sea lampreys (*Petromyzon marinus*) exposed to two concentrations of $KClO_4$ (results from 0.01 and 0.05% combined; from Holmes and Youson 1993). Means for *L. appendix* with different numbers of asterisks (*) differ significantly from each other ($P \leq 0.05$).

Incidence of metamorphosis

The incidence of induced metamorphosis was strongly influenced by the treatment that *L. appendix* larvae received ($F_{[4,10]} = 53.2$, $P = 0.0001$). Significantly more animals initiated metamorphosis when treated with $KClO_4$ (60% or 36/60 exposed larvae) than when treated with PTU, NaOH, or untreated water (Fig. 5). Although more larvae tended to enter metamorphosis when exposed to 0.05% $KClO_4$ (6.67 ± 0.88 larvae/tank or 67%) than when exposed to 0.01% $KClO_4$ (5.33 ± 0.33 larvae/tank or 53%), the difference was not statistically significant ($P > 0.05$). Only one animal exposed to PTU initiated metamorphosis (3.3%), and none of the animals in either control treatment (NaOH or untreated water) initiated metamorphosis.

From the minimum size and CF criteria from spontaneously metamorphosing animals, we predicted that 5 animals among the untreated controls (17%) would enter metamorphosis; that 8, 9, and 10 animals in the NaOH (27%), 0.05% $KClO_4$ (30%), and PTU (33%) treatments, respectively, would enter metamorphosis; and that 11 animals (37%) in the 0.01% $KClO_4$ treatment would enter metamorphosis (Fig. 6). Significantly more larvae metamorphosed (67%) than expected when treated with 0.05% $KClO_4$ ($P < 0.05$), but there was no significant difference between predicted (53%) and observed rates of metamorphosis when larvae were treated with 0.01% $KClO_4$ ($P > 0.05$). The number of animals expected to metamorphose in the PTU and NaOH- and untreated-control treatments was significantly greater

Fig. 6. Comparison of the number of observed and predicted metamorphosing *Lampetra appendix*. Predictions are based on minimum values for size of 155 mm (length) and 5.40 g (mass) and for CF of 1.25 at the beginning of the study. Observed values differing significantly from predicted values are indicated by an asterisk (*).

than the number observed ($P < 0.05$), but metamorphosis was not expected in the control treatments (NaOH and untreated water) given the timing of the study.

Development

External morphological changes appeared to be uncoordinated in *L. appendix* induced into metamorphosis compared with the features of each stage in spontaneous metamorphosis. For example, some animals had eye development characteristic of stage 5 in metamorphosis, while oral disc development lagged behind at approximately stage 3. Some specimens exhibited greater differences, with features characteristic of stages 1 and 4 of spontaneous metamorphosis appearing in the same individual at the same time. When we terminated this study, individuals with metamorphosing features characteristic of stages 1–6 were present within the same treatments and even within the same replicate tanks.

Serum concentrations of TH

Significant positive relationships between mass and serum T_4 and T_3 concentrations in *L. appendix* were found in the NaOH- and untreated-control treatments for both hormones. These data were adjusted to the mean mass of all animals in a treatment (untreated control, 3.47 g; NaOH control, 3.73 g) using ANCOVA, and these adjusted values were used in subsequent analyses.

Treatment with the goitrogens PTU and KClO₄ for 117 days significantly depressed serum T_3 levels ($F_{[4,135]} = 19.53$, $P = 0.0001$) in both metamorphosing (by 95–96%) and nonmetamorphosing (by 93–95%) *L. appendix*, relative

to levels in animals from the NaOH and untreated controls (Table 1). Average serum T_3 concentrations were significantly lower in animals treated with 0.05% KClO₄ than in those treated with 0.01% KClO₄ ($F_{[1,51]} = 9.39$, $P = 0.0035$). Metamorphosing animals had significantly lower serum T_3 concentrations than treated larvae that did not metamorphose, regardless of the KClO₄ treatment ($F_{[1,51]} = 15.62$, $P = 0.0002$). Nonmetamorphosing larvae and metamorphosing animals exposed to PTU had significantly higher serum T_3 levels than those exposed to KClO₄, but these levels were depressed significantly (by 80%) relative to serum levels in untreated control larvae.

Treatment with KClO₄ for 117 days depressed serum T_4 levels in both nonmetamorphosing larvae and metamorphosing animals (Table 1) to below the detection limits of the assay (14.4 nmol/L). Thus, the estimates of T_4 concentrations from animals in these treatments are less reliable than values for the controls, which were well above the detection limit. The 95% confidence interval for the mean T_4 value in the NaOH control was 22.8–34.1 nmol/L and in the untreated control was 25.0–39.5 nmol/L. Since the lower limit of the assay was well outside these confidence intervals, we infer that reductions in serum T_4 concentrations in *L. appendix* exposed to KClO₄ relative to the control values were probably statistically significant. The 55% reduction in serum T_4 levels in nonmetamorphosing larvae exposed to PTU was significantly less than the reduction in larvae from the NaOH or untreated controls ($F_{[2,77]} = 10.72$, $P = 0.0001$).

Discussion

Although we described separate and distinct stages of metamorphosis in *L. appendix*, we recognize that each stage represents a phase on a continuum of development. The seven stages of metamorphosis are based on external morphological features and have been applied to both parasitic and nonparasitic lamprey species (e.g., Youson and Potter 1979; Bird and Potter 1979a; Potter et al. 1980). Bird and Potter (1979a) described the timing and stages of metamorphosis in the paired species *Lampetra fluviatilis* (parasitic) and *L. planeri* (nonparasitic) based on a sequence of nine stages, two of which are clearly postmetamorphic (i.e., the animals were distinguished on the basis of gonadal development and spawning status). We found that there were marked similarities between *L. appendix* (a nonparasitic species), *L. planeri* (Bird and Potter 1979a), and other lamprey species representing genera from all three extant families of lampreys in all but a few features of development during metamorphosis. For example, by stage 3 the eye is circular and clearly differentiated into the iris and pupil in all lamprey species examined (e.g., Potter et al. 1978, 1980; Bird and Potter 1979a; Youson and Potter 1979). These changes are coincident with internal changes in the eye (Meyer-Rochow and Stewart 1996). During stages 4–6, the body colouration becomes more polar, with the dorsal surface appearing darker and the ventral surface appearing lighter. There were only minor differences in the morphology of metamorphosis between *L. planeri* and *L. appendix* and other lampreys. The piston appears by stage 3 in *G. australis* and *L. fluviatilis* (Bird and Potter 1979a; Potter et al. 1980) and by stage 4 in *P. marinus*, *L. planeri*, and *L. appendix*.

Table 1. Concentrations (nmol/L) of thyroxine (T₄) and triiodothyronine (T₃) in serum from *Lampetra appendix* after 117 days in five treatments.

Treatment	Larval				Metamorphosing			
	[T ₄]		[T ₃]		[T ₄]		[T ₃]	
	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N
Control	32.82±3.52a	27	28.48±5.18d	30				
NaOH	28.45±2.77a	29	26.60±3.92d	30				
KClO ₄								
0.01%	10.24±1.03b	9	2.05±0.23e	13	9.24±0.34c	12	1.33±0.08h	16
0.05%	11.85±0.61b	7	1.44±0.04f	8	8.01±0.66c	18	1.18±0.07i	18
PTU	15.00±0.81b	23	5.80±0.91g	27	9.48	1	0.94	1

Note: Treatment sample sizes (N) differ because of pooling to obtain sufficient serum for RIAs. Mean [T₄] values followed by a common letter are not significantly different at $P \leq 0.05$. Mean [T₃] values followed by a common letter are not significantly different at $P \leq 0.05$ for either treatment or stage of development.

(Bird and Potter 1979a; Youson and Potter 1979; this study). Thus, the staging criteria for stages 1 through 7 first proposed by Youson and Potter (1979) for lamprey metamorphosis appear to be applicable to both parasitic and non-parasitic species.

The consistency in the patterns of change shown by our measurements of certain body regions (Figs. 3A–3E), particularly the eye, the prebranchial region, and both dorsal fins, provides some validity for the criteria used to separate the metamorphosing animals into the different stages, despite the fact that they represent proportional values from only 1 or 2 specimens per stage. We note that the pattern of change for many measurements is similar to that reported in a study of *L. planeri* (Bird and Potter 1979b), which used larger sample sizes at each stage than were used in the present investigation. Our data show that the oral disc and prebranchial region undergo rapid increases in relative length in the latter stages of metamorphosis compared with the eye and dorsal fins, which, in *L. appendix*, increase steadily in size throughout metamorphosis. Trunk depth increases between stage 7 and the adult, presumably reflecting sexual maturation in the animal. In contrast, the functional significance, if any, of the decrease in cloacal and tail lengths is not clear.

Nonparasitic lamprey species tend to metamorphose at a greater age than parasitic species, following an extended larval period (Hardisty and Potter 1971). Statolith aging of individuals demonstrated that *L. appendix* from the Great Lakes tend to metamorphose when they are longer and at least 1 year older than sea lampreys from the same region (Beamish and Medland 1988). We found that *L. appendix* larvae must be at least 155 mm (length) and 5.40 g (mass) in size to initiate spontaneous metamorphosis. This size is greater than the minimum for premetamorphic sea lamprey larvae from the Great Lakes (≥ 120 mm (length) and ≥ 3.0 g (mass); Youson et al. 1993), premetamorphic *L. fluviatilis* and *L. planeri* (Bird and Potter 1979b), and premetamorphic *G. australis* (Potter et al. 1980). If, as in sea lampreys, lipid is the primary source of energy during the nontrophic phase of metamorphosis (Lowe et al. 1973; Kao et al. 1997), then the large size of premetamorphic *L. appendix* larvae may reflect a requirement for larger energy reserves to sustain individuals during the nontrophic periods of metamorphosis and subsequent sexual maturation. However, it is not clear why *L. appendix* is so much larger (length and mass) than some

other nonparasitic species (e.g., *L. planeri*) before, during, and after metamorphosis.

We began the present study in September, when spontaneous metamorphosis of *L. appendix* is nearly complete, and finished the following January, 6 months prior to the normal initiation of spontaneous metamorphosis, and found that treatment with KClO₄ during this period resulted in changes in external morphological features consistent with metamorphosis. The fact that these changes occurred when spontaneous metamorphosis is not normally initiated, and that none of the untreated control animals exhibited similar changes, point to KClO₄ as the causative (inducing) agent. This KClO₄-induced metamorphosis is consistent with other studies on KClO₄ induction both in nonparasitic lampreys, such as *L. planeri* (Hoheisel and Sterba 1963) and *L. reissneri* (Suzuki 1986), and in the parasitic lamprey *P. marinus* (Holmes and Youson 1993). Among larvae of comparable size (≥ 130 mm), exposure to KClO₄ induced metamorphosis in 98% of sea lampreys (Holmes and Youson 1993) but in only 62% of *L. appendix* (present study), although the concentration of KClO₄ (0.01 and 0.05%) did not significantly influence the incidence of metamorphosis in either *L. appendix* (Fig. 4) or sea lampreys (Holmes and Youson 1993). This difference in the incidence of metamorphosis may be related to fundamental differences in the minimum size required for spontaneous metamorphosis in the two species (e.g., see Fig. 6; Manzon and Youson 1997; Manzon et al. 1998).

Attempts to induce metamorphosis in lampreys using exogenous treatments of PTU have not produced the clear results obtained using KClO₄, despite the use of the same concentration of PTU in all studies (10 mg/L). Suzuki (1989) reported complete metamorphosis in some *L. reissneri* individuals treated with PTU for approximately 50 days, but this finding was not replicated in *L. appendix* (this study) or *G. australis* (Leatherland et al. 1990). The absence of metamorphosis in these latter studies may not be related to the length of treatment, since we used an exposure period for *L. appendix* (117 days) that was 67% longer than the 70 days used by Leatherland et al. (1990) and 134% longer than the period used by Suzuki (1989), however, we were still unable to find clear evidence of metamorphosis in all but one *L. appendix*. Furthermore, about one-third of the larvae assigned to the PTU treatment in the present study met the minimum size requirements of presumptive metamor-

phosis, length 155 mm and mass 5.40 g, so the absence of induced metamorphosis is probably not related to the size or physiological preparation of the animals.

Because metamorphosing *L. appendix* were significantly larger than nonmetamorphosing larvae exposed to the goitrogens or larvae in the control treatments, we hypothesize that the variable response to KClO_4 among animals of different size is related to differences in metabolic condition, particularly with respect to lipid metabolism. Kao et al. (1997) found that spontaneous metamorphosis in sea lampreys was dominated by lipogenesis up to stage 3, and that the later stages were characterized by enhanced lipolysis and reduced lipogenesis and oxidative metabolism, relative to nonmetamorphosing larvae. If similar mechanisms are present in *L. appendix*, then metamorphosing animals may be better able than nonmetamorphosing larvae to cope with the energetic demands of metamorphosis and the higher than normal temperatures used in the present study. However, we did not design our study to examine the effects of goitrogens on size, so a definitive conclusion awaits further investigation.

Our interpretation of the serum TH data (Fig. 4) is cautious, because serum samples were collected from stage 1 animals 1 year prior to collecting them from animals in other stages of metamorphosis. Nevertheless, we are confident that our findings that serum levels of both T_4 and T_3 decline by stage 2 and remain low during the subsequent stages of metamorphosis are real, because this pattern is generally similar to the changes in T_4 and T_3 serum levels observed during spontaneous metamorphosis of sea lampreys (Wright and Youson 1977; Lintlop and Youson 1983; Youson et al. 1994). However, we are unable to explain why serum concentrations of T_4 peak in stage 1 animals rather than in premetamorphic larvae. Concentrations of T_3 for these same animals were significantly depressed compared with those in premetamorphic larvae, a situation consistent with that observed in sea lampreys. We are certain that our *L. appendix* data are not an artifact of the RIA procedure, since several early analyses of the same serum samples using differing dilutions produced similar results. We are also confident that incorrect staging is not a factor, because the stage 1 animals were sampled at a time of year well after spontaneous metamorphosis has begun (18 July 1995), and the range of their lengths overlapped that of premetamorphic larvae. Although the sample size for stage 1 is small ($N = 5$), the T_4 data are consistently high for all animals in the sample, so we believe individual variation is minimal. If this finding is subsequently confirmed using data from animals sampled at consecutive stages during a single metamorphic period, then it could have considerable impact on our understanding of the role of TH in the metamorphosis of this lamprey species.

Lampreys exposed to the goitrogens KClO_4 and PTU have significantly lower serum TH levels than untreated control animals (Suzuki 1986; Youson et al. 1995; Manzon and Youson 1997), but only exposure to KClO_4 consistently induces metamorphosis in all lamprey species tested (Hoheisel and Sterba 1963; Suzuki 1986; Holmes and Youson 1993; present study). Although the reductions in serum TH values in animals exposed to either goitrogen (Youson et al. 1995; Table 1) are proportionately similar to those observed during spontaneous metamorphosis, a decline in serum TH levels by itself does not trigger metamorphosis in lampreys. Thus,

the hypothesis that these goitrogens act on the endostyle (larval precursor to the follicular thyroid gland in juveniles and adults) to suppress production of the TH (Youson 1997) triggering metamorphosis does not fully explain our findings, particularly those for PTU. The induction of metamorphosis, particularly with KClO_4 , probably involves other unknown effects either on the putative thyroid gland itself, on other tissues outside the thyroid axis, on the peripheral metabolism of the TH, or on general body metabolism. There is no available evidence of hypothalamic-pituitary control involving TH during spontaneous metamorphosis in lampreys, nor have TH receptors in TH-targeted tissues been identified in lampreys (Youson 1997). However, morphological studies have shown that gut degeneration in the nonparasitic *L. planeri* begins around the time when the rate of gonadal development increases, and may be at least partially related to the secretion of gonadal hormones (Hilliard et al. 1983). In amphibians such as the bullfrog *Rana catesbeiana*, some of the events in metamorphosis are regulated by the expression of type I and type III deiodinase genes in specific tadpole tissues (Becker et al. 1997). Although Eales et al. (1997) suggested that monodeiodinase pathways might have an important role in lamprey metamorphosis, recent data indicate that KClO_4 does not affect the T_4 outer ring deiodination pathway to T_3 in sea lampreys (Manzon et al. 1998). Kao et al. (1997) noted that a switch in lipid metabolism from lipogenesis to lipolysis, which occurs at stage 3, is a key event in sea lamprey metamorphosis. We suggest that KClO_4 may act directly on this pathway to induce metamorphosis in lampreys.

In summary, the sequence of stages in the metamorphosis of *L. appendix* is similar to that given for *Lampetra* spp. (Bird and Potter 1979a), *P. marinus* (Youson and Potter 1979), and *G. australis* (Potter et al. 1980). The most diagnostic changes in the early stages are the enlargement of the eye and the differentiation into iris and pupil, followed by the modification of the oral hood into an oral disc and changes in body pigmentation. The changes in the relative lengths of various body regions are consistent with the more extensive data reported for *L. planeri* (another nonparasitic species). Premetamorphic and metamorphosing larvae of *L. appendix* are much larger (minimum length, 155 mm; minimum mass, 5.40 g) than those of most parasitic or nonparasitic species of lamprey. Average serum TH levels increase during the latter part of larval life and decline approximately 90% by stage 2 of metamorphosis, remaining at the reduced levels throughout the later stages. We are not certain of serum TH levels at stage 1 of metamorphosis, because our data for this stage are from animals collected 1 year earlier than those in all other stages.

Exposure to KClO_4 appears to induce metamorphosis in larvae of *L. appendix* more readily than exposure to PTU (62 and 3%, for KClO_4 and PTU, respectively), and also results in larger declines in serum TH levels. These findings with KClO_4 are consistent with those reported for sea lampreys (Holmes and Youson 1993; Youson et al. 1995). The highest concentration of KClO_4 used in the present study seemed to overcome the size and CF criteria that may be important in spontaneous metamorphosis of *L. appendix*. Lamprey metamorphosis, either spontaneous or induced, is accompanied by a significant decrease in serum TH levels, but other

regulatory pathways are likely involved in this process, since a decline in serum TH levels caused by exposure to a goitrogen is not sufficient by itself to initiate metamorphosis.

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