

**Comparison of the internal morphology of adults of a population of lampreys that contains a nonparasitic life-history type, *Lampetra richardsoni*, and a potentially parasitic form, *L. richardsoni* var. *marifuga***

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Received, February 15, 1990

YOUSON, J. H., and BEAMISH, R. J. 1991. Comparison of the internal morphology of adults of a population of lampreys that contains a nonparasitic life-history type, *Lampetra richardsoni*, and a potentially parasitic form, *L. richardsoni* var. *marifuga*. *Can. J. Zool.* **69**: 628–637.

Histological techniques were used to study adults of a single population of the brook lamprey, *Lampetra richardsoni*, in Morrison Creek, Vancouver Island, British Columbia, to establish whether there are any differences between the internal morphology of a "silver" variety (*L. r.* var. *marifuga*) and that of the nonparasitic variety (*L. richardsoni*). The results of the study support the view of two life-history types in this creek. When *L. richardsoni* is sexually mature and has an atrophied intestine and sparse haemopoietic tissue, most *L. r.* var. *marifuga* possess a maturing (not totally mature) gonad, a highly developed alimentary canal containing ingested food, and extensive haemopoietic tissue. Although the gills, thyroid gland, and the definitive kidneys are similar in both forms, incomplete regression of the larval kidney and the absence of a cranial pancreas in all *L. r.* var. *marifuga* imply that a slow sexual maturation is accompanied by a slow, and perhaps incomplete, metamorphosis. The incomplete metamorphosis is particularly exemplified in female *L. r.* var. *marifuga* and may account for the abnormal sex ratio of this variety. The retardation of sexual maturation in male *L. r.* var. *marifuga* permits the retention of a functional digestive system and, hence, provides an opportunity for feeding. The significance of these observations to present views on the evolution of lampreys is discussed.

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Des techniques histologiques ont été utilisées au cours d'une étude sur les adultes d'une population de lamproies de ruisseau, *Lampetra richardsoni*, dans le ruisseau Morrison, Île de Vancouver, Colombie-Britannique, afin de déterminer s'il existe des différences entre la morphologie interne d'une variété « argentée » (*L. r.* var. *marifuga*) et celle d'une variété non parasite (*L. richardsoni*). Les résultats de l'étude confirment l'hypothèse de l'existence de deux types de cycles biologiques dans le ruisseau. Alors que *L. richardsoni* atteint sa maturité sexuelle, a un intestin atrophié et peu de tissu hématopoiétique, la plupart *L. r.* var. *marifuga* ont des gonades en voie de maturation (pas encore à maturité), un canal alimentaire bien développé contenant de la nourriture, et une grande abondance de tissu hématopoiétique. Bien que les branchies, la glande thyroïde et les reins adultes soient semblables chez les deux variétés, la régression incomplète du rein larvaire et l'absence d'un pancréas crânien chez tous les *L. r.* var. *marifuga* indiquent que la maturation sexuelle est un processus lent qui s'accompagne d'une métamorphose lente et peut-être incomplète. La métamorphose incomplète est particulièrement évidente chez les femelles de *L. r.* var. *marifuga* et explique sans doute le rapport mâles:femelles anormal chez cette variété. Le retard de la maturation sexuelle chez les mâles de *L. r.* var. *marifuga* permet la rétention d'un système digestif fonctionnel et, conséquemment, de la consommation d'aliments. Ces résultats sont examinés à la lumière des théories récentes sur l'évolution des lamproies.

[Traduit par la rédaction]

### Introduction

Lampreys are extant representatives of an ancient group of vertebrates, the Agnatha, and the limited amount of fossil evidence suggest that they have undergone little change over the past 280 million years (Bardack and Zangerl 1971). Following a protracted larval period believed to range from 3 to 7 years (Hardisty and Potter 1971a; Potter 1980a; Beamish and Northcote 1989), during which they are microphagous filter feeders, the larvae (ammocoetes) undergo metamorphosis into juveniles. Upon completion of metamorphosis, the young adults (juveniles) of parasitic species feed on the flesh or the blood and other body fluids of host fishes, while those of nonparasitic species do not feed but begin sexual maturation (Hardisty and Potter 1971b). After a feeding phase that is of variable length among the parasitic species, adults become non-

trophic during sexual maturation. Advanced sexual maturation in all lamprey species is accompanied by atrophy of some body organs, particularly the intestine (Larsen 1980).

Parasitic and nonparasitic lampreys that show some morphological and genetic similarity and overlap in their distribution are still considered distinct species because they represent two different life-history types. Nevertheless, they are termed paired or satellite species, and it is believed that members of these paired species shared a common evolutionary history (Zanandrea 1959; Hardisty and Potter 1971c; Vladykov and Kott 1979; Potter 1980b). The currently held view is that nonparasitic lampreys evolved from parasitic (probably anadromous) lampreys. However, Beamish (1985) proposed that in some cases nonparasitic species may evolve from an intermediate freshwater, parasitic form. This latter viewpoint is supported by the recent discovery of a population of lampreys in Morrison Creek on Vancouver Island, British Columbia, that produce

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parasitic and nonparasitic life-history types (Beamish and Withler 1986; Beamish 1987). In this stream, identical allelic frequencies are seen in the nonparasitic *Lampetra richardsoni* and the parasitic variety, *Lampetra richardsoni* var. *marifuga* (Beamish and Withler 1986). It is also suggested that gene flow occurs between the two life-history types (Beamish 1987). Morphologically, the parasitic variety is intermediate between *L. richardsoni* and the anadromous, parasitic *Lampetra ayresi*, but the electrophoretic patterns of the Morrison Creek forms are different from those of *L. ayresi* and other populations of *L. richardsoni* (Beamish and Withler 1986). Further evidence that *L. r.* var. *marifuga* is not a recent hybrid of *L. richardsoni* and *L. ayresi* is provided in the fact that no *L. ayresi* have been found in the Morrison Creek watershed and the variety was captured in the population each year from 1978 to 1987 (Beamish and Withler 1986; Beamish 1987).

Taxonomic characterization of lamprey species has relied heavily on morphometric and meristic analyses of external features (Vladykov and Kott 1980; Potter 1980b). The two life-history types in a paired species are frequently distinguished by differences in the stage of gonad development prior to and immediately following metamorphosis (Hardisty et al. 1970; Potter 1980a). However, it has become clear in recent years that various unrelated and paired lamprey species also show differences in morphology of internal tissues and organs, other than the gonads (Youson 1981; Macey et al. 1982; Hilliard et al. 1983; Youson and Beamish 1986; Youson et al. 1988). For instance, some preliminary studies comparing specific organs of *L. richardsoni* and *L. r.* var. *marifuga* in Morrison Creek (Youson and Beamish 1986; Youson et al. 1988) have tended to provide support for the view that they are distinctly different life-history types. In the present study, we use histology to provide a detailed comparative analysis of many tissues and organs of adults of the two forms in Morrison Creek, Vancouver Island. It was our hope that the results would be of assistance in explaining the relationship between these two forms. Although there may be two other examples of populations of lampreys that produce two life-history types (Kux 1965; Cochran 1984), observations on the interrelationship between these two forms in Morrison Creek are to date the most extensive. As lampreys are one of the most successful groups of living fishes, understanding the relationship between paired species, or between different life-history types in the same population, offers an excellent opportunity to study the reasons for this success.

### Materials and methods

Adults of *Lampetra richardsoni* used in this study were captured in Morrison Creek, Vancouver Island, in either trap nets during their downstream migration or by electrofishing during a period extending from April 1984 to July 1987. Animals were classified as to being the nonparasitic form, *L. richardsoni*, or the parasitic variety, *L. richardsoni* var. *marifuga*, using the criteria outlined by Beamish and Withler (1986), but the distinction was mainly based on the silver body coloration of the latter in early spring (April and May). At this time of the year, adults of the nonparasitic form were sexually mature and easily separated from *L. r.* var. *marifuga* (Beamish 1987). The mean length of the "silver" form was 12.4 cm, while that of the sexually mature form was 11.2 cm (Beamish and Withler 1986). The silver form was isolated to freshwater aquaria where they were fed live Pacific herring (*Clupea harengus pallasi*). The silver form was observed to feed on the dying and dead herring by removing large pieces of flesh of this host. Adults of the parasitic variety used in this study were obtained from samples that were sacrificed immediately after capture or after feeding over the period from June to mid-September. No adults that spawned in the

following year were examined because, as noted by Beamish (1987), this form is difficult to keep in the laboratory during and following their feeding period. Owing to cannibalism, they must be maintained separately, and the large numbers of animals required to ensure some survival to maturity would harm the natural population. Attempts were made to examine equal numbers of specimens of both sexes for each form, but this was difficult with *L. r.* var. *marifuga* in which ~80% of the population seems to be male (Beamish 1985; Beamish and Withler 1986). Twenty-six (21 male, 5 female, 1 undetermined sex) *L. r.* var. *marifuga* and 10 (7 male, 3 female) nearly fully mature or spent *L. richardsoni* were examined. No secondary sex characters were present in the variety at the time of their capture in June and July. Three of the *L. richardsoni* were collected at an early stage of metamorphosis and were maintained in the laboratory until they were mature (Beamish 1987). Two ammocoetes (larvae), which had been collected from Morrison Creek by electroshocking and which could not be separated into the two forms, were also observed.

Death was produced through overdose of anaesthetic and then the animals were immediately placed in Bouin's fluid for 24 h, followed by storage in 70% ethanol. Whole-body segments were then embedded in paraffin and the entire animals were serially sectioned at 10  $\mu$ m. The sections on glass slides were stained with either haematoxylin and eosin or with periodic acid - Schiff, acid haemalum, and orange G.

Slides of both *L. richardsoni* and *L. r.* var. *marifuga* were examined in detail for differences in the following structures: the extent of development of the thyroid follicles, the number of gill chloride cells, the state of degeneration of the larval pronephric and opisthonephric kidneys, the extent of haemopoietic tissue in the fat column, the maturity of the gonad, the degree of development of the endocrine pancreas, the number of mucosal folds in the oesophagus and intestines, and the presence or absence of bile ducts.

### Results

The general features of body organs and tissues of ammocoetes from Morrison Creek were the same as those described for other holarctic lamprey species, i.e., there was nothing in the larvae that could be said to be unique to the ammocoete population from this creek.

Although the males that were examined far outnumbered the females for *L. r.* var. *marifuga*, the general impression was that the females were far less developed than the males. In fact, as noted below, two females showed rather limited completion of metamorphosis. As expected, samples of *L. richardsoni* showed more advanced stages of sexual maturity than *L. r.* var. *marifuga* (Figs. 1 and 2). The following is a description of body organs and tissues in var. *marifuga* and a comparison with those of *L. richardsoni*. Particular emphasis is placed on those definitive or adult tissues and organs that arise from Anlagen or through transformation of larval tissues during metamorphosis. Included are the following: the alimentary canal (oesophagus and intestines), pancreas, liver, sites of deposition of haemopoietic tissue, renal tissue, thyroid, and gills. The state of maturity of the gonads was also noted.

#### Gonad

Female *L. r.* var. *marifuga* preserved in June and July had 25-50 eggs in each section of the trunk region (Fig. 3). The eggs were of variable size, with the largest (~0.1 mm diameter) yolky eggs more frequent in the anterior trunk region, and along with the highly dilated anterior intestine, filled the coelomic cavity (Fig. 3). The males of this form were sacrificed between early June and late September and were quite variable as to the degree of maturity of their testes. In a few cases the testes consisted of cysts of only spermatocytes in a June sample, but in early July a few animals had cysts that were full of seemingly mature spermatozoa and no earlier spermatogenic cells (Fig. 4).

However, in the majority of cases the testes over this time period showed a mixture of cysts with late spermatids or spermatozoa (Fig. 1). The anterior trunk was also swollen owing to the large testes and dilated anterior intestine (Figs. 1 and 4). No males possessed spermatozoa that were free within the coelomic cavity. Female *L. richardsoni* preserved in June had ovaries with 1 mm diameter eggs that filled the coelomic cavity. At the same time, all unspent males of this form had spermatozoa that were free within the coelomic cavity (Fig. 2).

#### Alimentary canal

The dorsally located oesophagus extended from the mouth and terminated in the pericardial region at its junction with the anterior intestine. The anterior intestine – oesophagus junction was also the site of an anteriorly directed left diverticulum of the anterior intestine. In *L. r. var. marifuga* the diverticulum was 350–400 µm long, but in *L. richardsoni* it was inconspicuous (Fig. 5). As noted previously (Youson et al. 1988), the oesophageal–intestinal junction was more caudal in *L. r. var. marifuga* compared with *L. richardsoni*. In one female of *L. r. var. marifuga*, which also showed other unusual characteristics noted below, there was no connection between the oesophagus and the anterior intestine. The oesophagus of most *L. r. var. marifuga* had many (10–25) longitudinal mucosal folds, with the smallest numbers noted in females. The oesophagus and the anterior and posterior intestine of *L. richardsoni* had only a few stunted folds, and in most cases the intestine was atrophied (Fig. 2). The anterior intestine of *L. r. var. marifuga* had a patent lumen and possessed 18–40 longitudinal intestinal folds of various lengths (Figs. 1, 3, and 4). One female had either no folds or some that were just developing, as seen later in the posterior intestine (Figs. 6 and 7), and a second female had two additional intestinal diverticula behind the one at the oesophageal–intestinal junction. The second of these diverticula was located within the area of the caudal pancreas and there were islets of pancreatic cells within the epithelium (Fig. 8). The anterior intestine of most males, when they were fed in the laboratory, was often highly dilated owing to the presence of muscle and integument of host fish (Fig. 1). Others had a parasite in the lumen of lower regions of their anterior intestine (Fig. 4), perhaps the digenean *Ophioxenos lampetra*, as described by Beverley-Burton and Margolis (1982). The females were preserved directly from the field and were not fed. The posterior intestine of all but one female *L. r. var. marifuga* had 30–60 longitudinal mucosal folds and a typhlosole, while all *L. richardsoni* showed an atrophied posterior intestine. The posterior intestine of one female *L. r. var. marifuga* resembled that of a larval or early metamorphosing lamprey in having only a single large fold, the typhlosole, and some low folds and much subepithelial haemopoietic tissue (Fig. 7). The epithelium had many mitotic figures (Fig. 7) and the cells were densely packed. Curiously, there was no ovary in the middle-posterior trunk of this female (Fig. 6). In all animals, a short hindgut was continuous with the cloaca.

#### Endocrine pancreas

Most adult holarctic lampreys possess a discontinuous intermediate cord of pancreatic islets extending between separate cranial and caudal pancreatic masses (Youson and Elliott 1989). It had been noted previously that the distribution of pancreatic endocrine tissue varies markedly between *L. r. var. marifuga* and *L. richardsoni* from both Morrison Creek (Youson and Elliott 1989) and from the Puntledge River (Youson et al. 1988). The present study confirmed the previous observation that male *L. r. var. marifuga* is unique among holarctic lampreys in lacking a distinct cranial pancreas, even though *L. richardsoni* from the same creek has a distinct cranial mass near the diverticulum (Fig. 5). However, one male *L. r. var. marifuga* possessed significant numbers of islets around the diverticulum to permit the designation of a small cranial pancreas. All female *L. r. var. marifuga* in the present study showed no cranial pancreas. Both forms from Morrison Creek possessed an extensive intermediate cord that in *L. richardsoni* served to directly connect the cranial pancreas with the caudal mass (Fig. 5). The caudal pancreas in all animals was located in the connective tissue that bridged the liver and the typhlosole of the anterior intestine. The round to oval caudal pancreas was 650–1700 µm long and the majority of the islets resided close to the liver. The only significant difference in this pancreas in the two forms was the presence of numerous isolated intrahepatic islets in *L. r. var. marifuga* (Youson et al. 1988; Youson and Elliott 1989). One female had numerous highly dilated follicles and bile ducts within a caudal pancreas that was associated with what appeared to be an intestinal diverticulum (Fig. 8). The basal epithelium of the latter had clusters of cells that resembled those that, in larvae, give rise to islets of the endocrine pancreas.

#### Liver

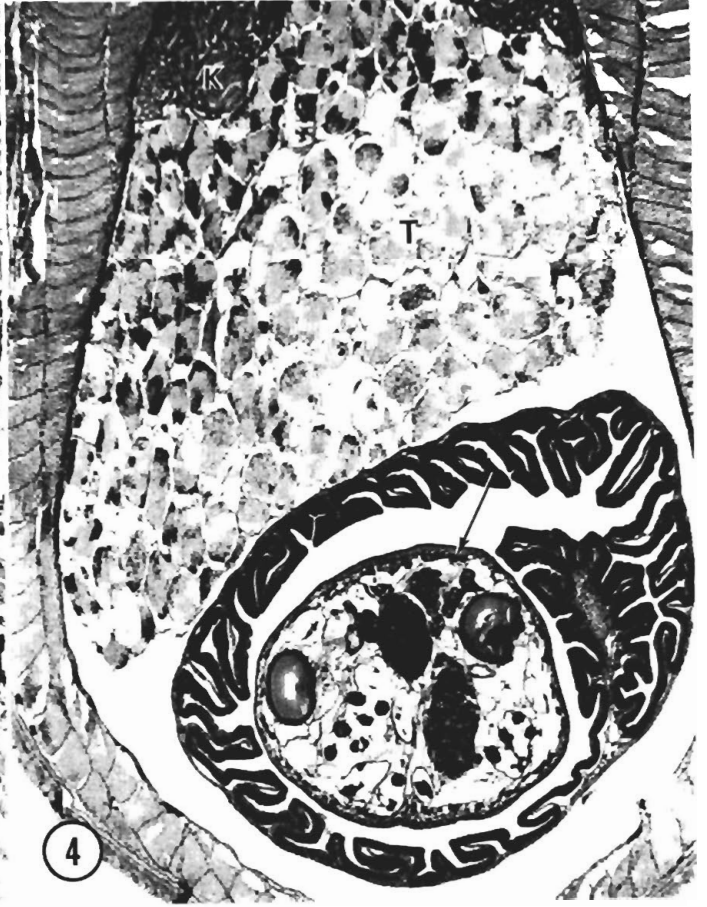
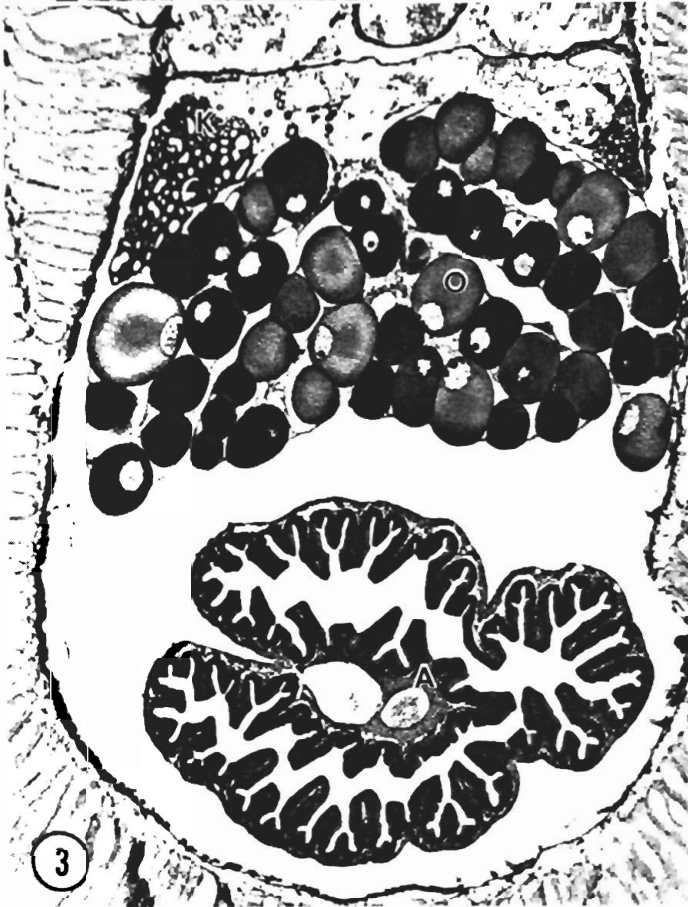
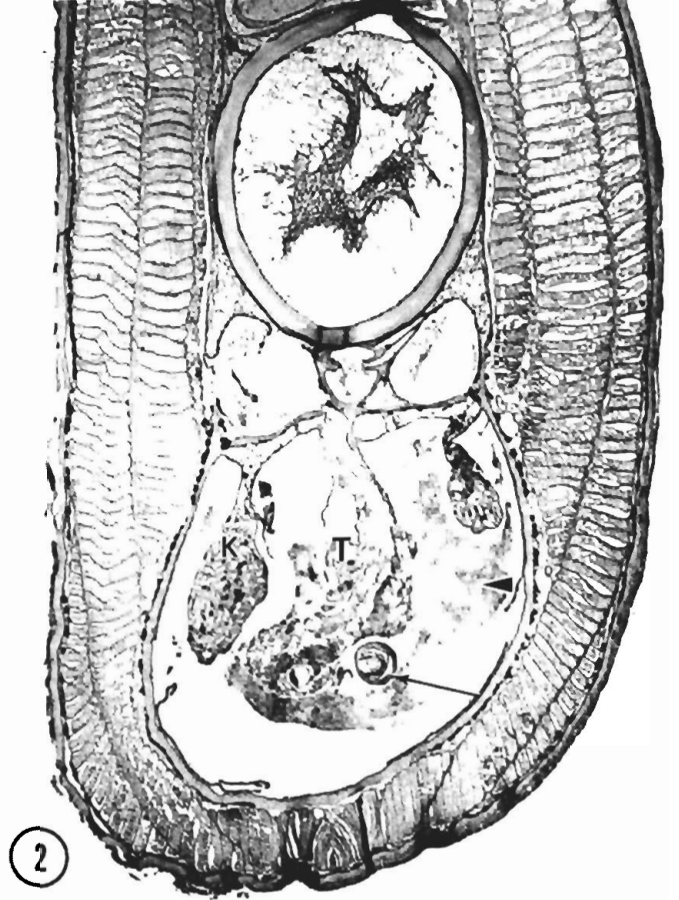
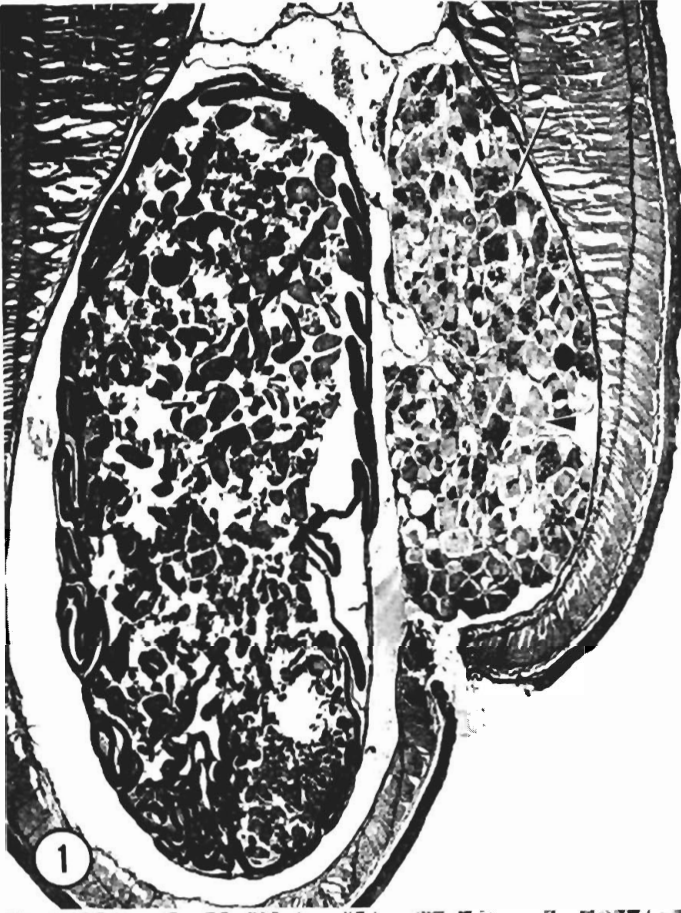
The liver of most larval lampreys loses all bile ducts and the gall bladder during its transformation into the adult liver (Sidon and Youson 1983). A few small intrahepatic bile ducts were noted in both forms in Morrison Creek, but bile canaliculi were absent and the hepatocytes were arranged into cords (Fig. 9). In one female *L. r. var. marifuga*, there were large follicles or ducts within the caudal pancreas in the region occupied in larvae by the extrahepatic common bile duct (Fig. 8).

#### Haemopoietic tissue

The fat column above the spinal cord had extensive concentrations of haemopoietic tissue in all male *L. r. var. marifuga* but was reduced in females, and in all *L. richardsoni* this region contained only sparse amounts (Figs. 10 and 11). The intestinal typhlosole and the larval opisthonephric kidney, sites of haemopoiesis in larvae, were devoid of haemopoietic tissue in all but one female *L. r. var. marifuga*. In the female with the underdeveloped intestine (noted earlier), besides being located in the fat column, the haemopoietic tissue was found in the intestinal typhlosole (Fig. 7), in the nephric fold of the degenerating larval opisthonephros, and in the dorsal region of the newly formed adult kidney (Figs. 6 and 12). The three latter

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FIG. 1. The anterior trunk region of a male *L. r. var. marifuga* showing the lumen of the anterior intestine filled with muscle fibres (large arrow) and the testes with cysts of spermatozoa (arrowhead) and late spermatids (small arrow).  $\times 3.3$ . FIG. 2. The middle trunk region of a male *L. richardsoni* indicating the anterior end of the adult kidneys (K), the atrophied intestine (arrow), the spent testes (T), and spermatozoa (arrowhead) in the coelomic cavity.  $\times 3.3$ . FIG. 3. The anterior trunk region of a female *L. r. var. marifuga* revealing a large anterior intestine (A), eggs of variable sizes in the ovary (O), and a fully formed larval kidney (K).  $\times 5.3$ . FIG. 4. The posterior trunk region of a male *L. r. var. marifuga* demonstrating adult kidneys (K), a large, seemingly mature testes (T), and a parasite (arrow) in the lower anterior intestine.  $\times 5.3$ .



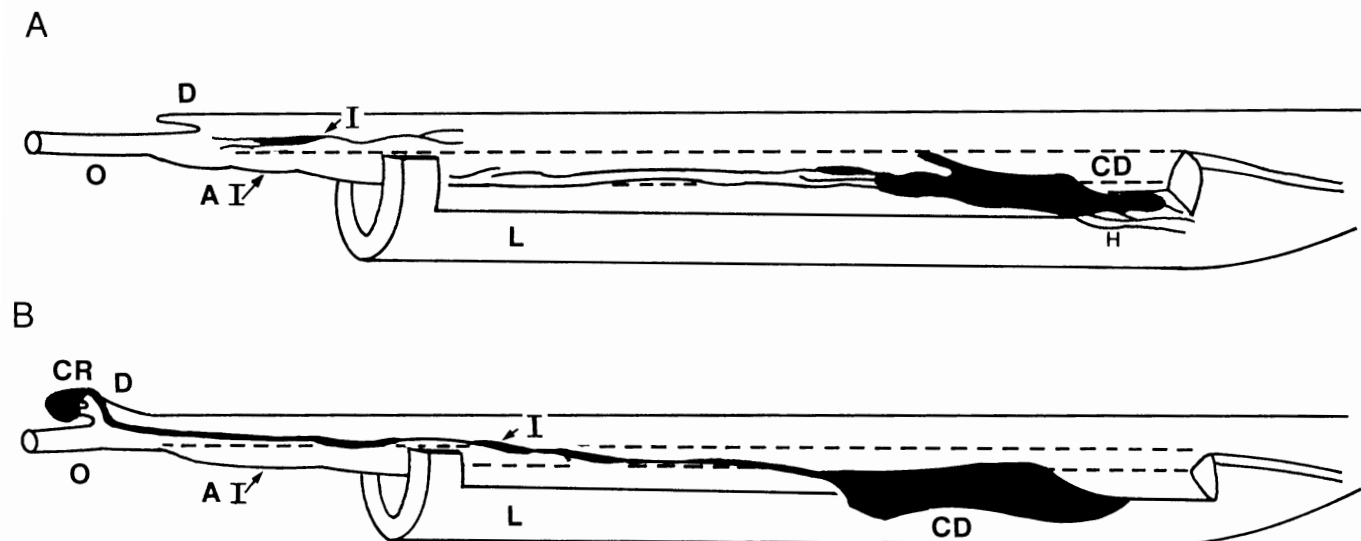


FIG. 5. Diagrammatic representation of the distribution of endocrine pancreatic tissue in (A) *L. r. var. marifuga* and (B) *L. richardsoni*. The latter has a cranial pancreas (CR) near the junction of the oesophagus (O), anterior intestine (AI), and diverticulum (D), both possess an extensive intermediate cord (I) and a caudal pancreas (CD), and the variety has significant intrahepatic islets (H). A portion of the liver (L) has been shown as cut away and the broken line represents the approximate position of the typhlosole (T).

sites of deposition of haemopoietic tissue are usually only found in larval lampreys (Potter et al. 1982).

#### Kidneys

The pronephric kidneys undergo a regression during larval life such that only nephrostomes and renal corpuscles persist to adult life (Youson 1981; Ellis and Youson 1989). All *L. richardsoni* demonstrated the above condition, but the pronephroi of four *L. r. var. marifuga* also had renal tubules (Fig. 13). The larval opisthonephric kidney is located in the anterior portion of the nephric fold and all components of this kidney usually disappear when the adult kidney forms at metamorphosis (Youson 1981). Although only small remnants of the larval opisthonephroi were present in *L. richardsoni*, these kidneys of *L. r. var. marifuga* always showed many viable tubules and the renal corpuscle as well as degenerating components (Fig. 3). One male and one female (Fig. 3) had regions of the larval opisthonephroi that resembled the condition seen in larvae. All *L. richardsoni* and most *L. r. var. marifuga* had adult opisthonephroi that could not be distinguished from one another. That is the kidneys were roughly the same shape and the three main components (renal tubules, renal corpuscle, and archinephric duct) had similar distribution and size in both forms from Morrison Creek (Fig. 6). However, three *L. r. var. marifuga* had kidneys in the region of the adult opisthonephros that more closely resembled the larval opisthonephros. The female with the underdeveloped intestine had a fully developed adult kidney (Fig. 6).

#### Thyroid

The larval lamprey endostyle transforms into a typical

vertebrate thyroid gland with follicles during metamorphosis (Youson 1980). All individuals of both forms from Morrison Creek had complete transformation of the endostyle and their thyroid glands were of similar structure.

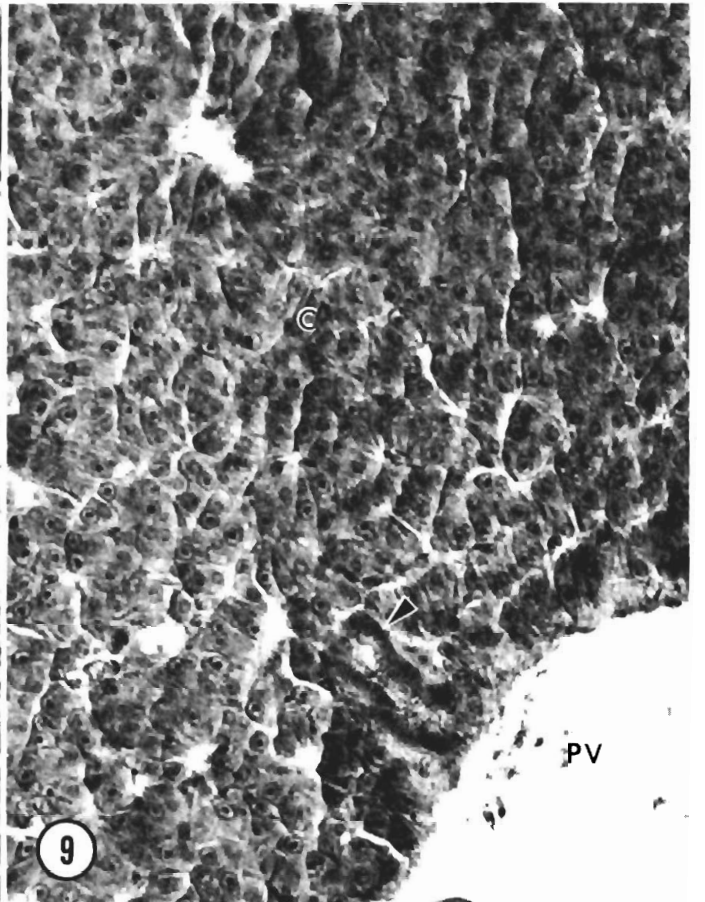
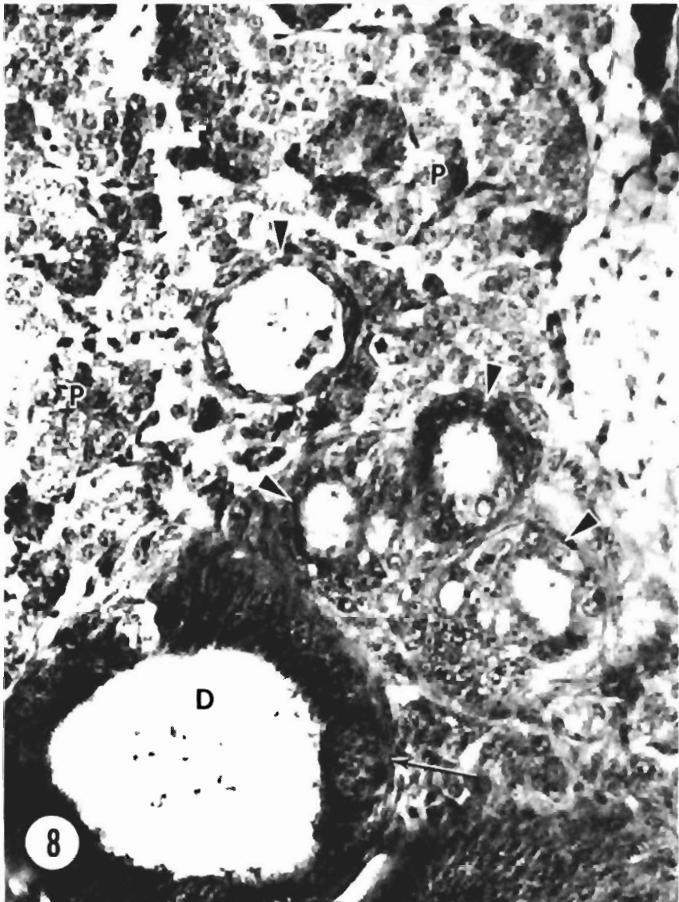
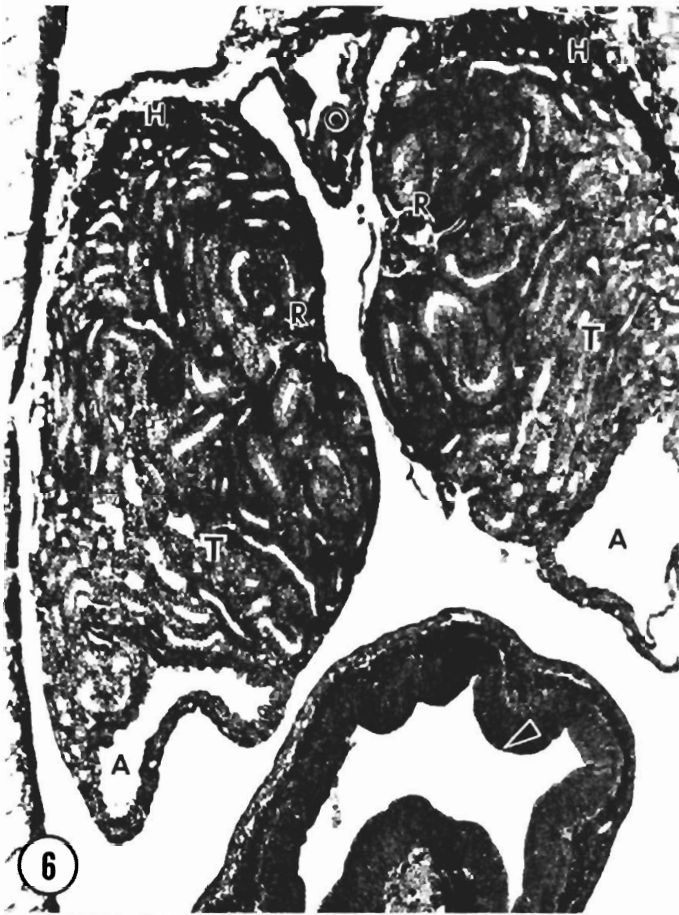
#### Gills

The most obvious change in gills during metamorphosis of lampreys is the development of chloride cells in the interlamellar region (Peek and Youson 1979). In cross-sectional profiles of the gill filaments of both forms, these acidophilic cells were present in groups of two to four in the interlamellar region.

#### Discussion

Zanandrea (1959) was the first to use the term "paired species" to describe closely related parasitic and nonparasitic lamprey species. Meristic and morphometric characters have been used to distinguish between sexually mature adults of each member of the paired species, but at earlier stages the amount of gonadal development, size at metamorphosis, and life-history type are the main distinguishing characters (Hardisty and Potter 1971c; Potter 1980a). Weissenberg (1925, 1926, 1927) made a clear distinction between adults of closely related parasitic *Lampetra fluviatilis* and nonparasitic *Lampetra planeri* by comparing the degree of development of the gonad and the structure of the alimentary canal. In general, it is now accepted that, immediately following metamorphosis, nonparasitic species continue their nontrophic behaviour, they show no growth, and they enter a phase of rapid maturation of gonads and atrophy of the intestine. On the other hand, metamorphosis in parasitic

FIG. 6. The middle-posterior trunk region of a female *L. r. var. marifuga*, showing completely developed adult kidneys with renal corpuscle (R), tubules (T), and archinephric duct (A) but also with a dorsal mass of haemopoietic tissue (H). Also note the absence of eggs in the ovary (O) and the small folds (arrowhead) in the intestine.  $\times 152$ . FIG. 7. Transverse section of the posterior intestine of the same animal as in Fig. 6, showing low folds (arrow), a dense aggregation of epithelial cells with some in mitosis (arrowheads), and haemopoietic tissue (H) in the subepithelial connective tissue of the typhlosole.  $\times 345$ . FIG. 8. The caudal pancreas (P) of a female *L. r. var. marifuga* with many remnants of bile ducts (arrowheads) and also a secondary diverticulum (D) with an epithelium containing a cell cluster of islets (arrow).  $\times 530$ . FIG. 9. The liver of male *L. r. var. marifuga* indicating a remnant of a bile duct (arrowhead) near the hepatic portal vein (PV) but hepatic cords (C) without bile canaliculi throughout the organ.  $\times 530$ .



species is followed by a trophic period in which growth and a relatively slow gonadal maturation are characteristic.

There has been some discussion that some nonparasitic lampreys demonstrate paedomorphism because sexual maturation begins to accelerate in late larval life before metamorphosis (Zanandrea 1956, 1957; Hubbs 1971; Walsh and Burr 1981). However, Vladykov (1985) feels that there is no strong evidence for either neoteny (a more general retention of larval characters in the adult) or paedogenesis in lampreys. In order for there to be a neoteny in lampreys, metamorphosis would have to be incomplete.

In the present study we compared the internal morphology of adults of two forms of nonparasitic *Lampetra richardsoni* from Morrison Creek, with the objective of trying to explain why one form is capable of feeding in the laboratory, even though it is genetically similar to the nonparasitic form (Beamish and Withler 1986). The parasitic form, *L. r.* var. *marifuga*, is clearly different from the nonparasitic form in that many animals have ingested food in a highly developed intestine when the gonads are in a rapid state of maturation. There are also indications that some *L. r.* var. *marifuga* have not undergone a complete internal metamorphosis, despite the fact that external characters are definitely adult. It is clear that the situation in Morrison Creek is not one of paedomorphism because the maturing animals under consideration are adult in character. It is also apparent that *L. r.* var. *marifuga* is not neotenus because internal larval characters are, in most cases, no longer visible in juveniles. Instead, it seems that the various *L. r.* var. *marifuga* described in this study are forms of *L. richardsoni* either in a phase of slow metamorphosis, and hence still have a few larval characters, or are the result of both a delayed sexual maturation and an incomplete metamorphosis, or are an intermediate, completely metamorphosed form with delayed sexual maturation that, given the appropriate conditions, can feed.

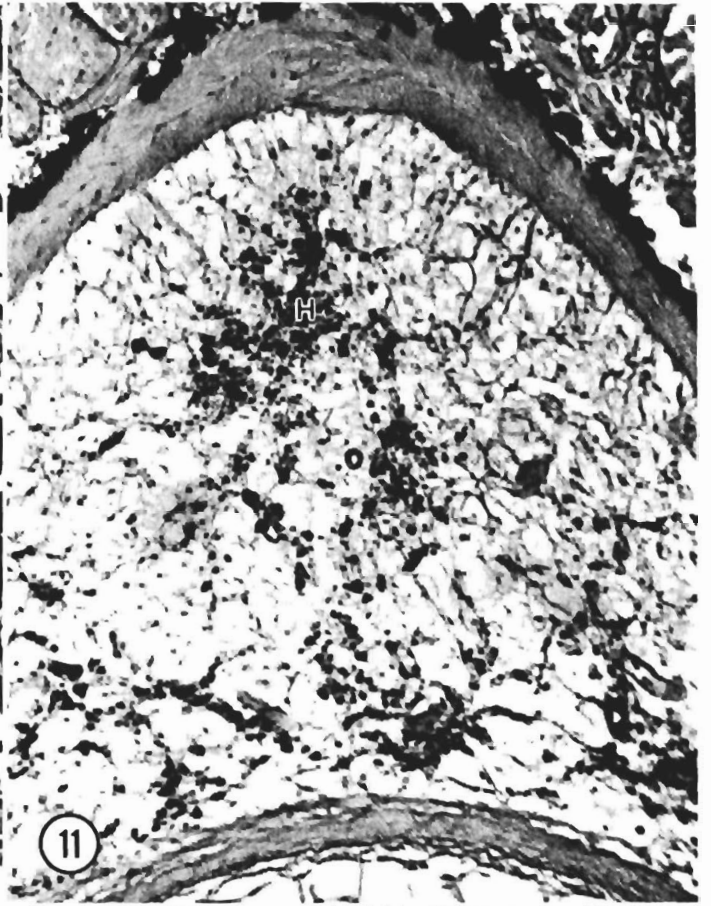
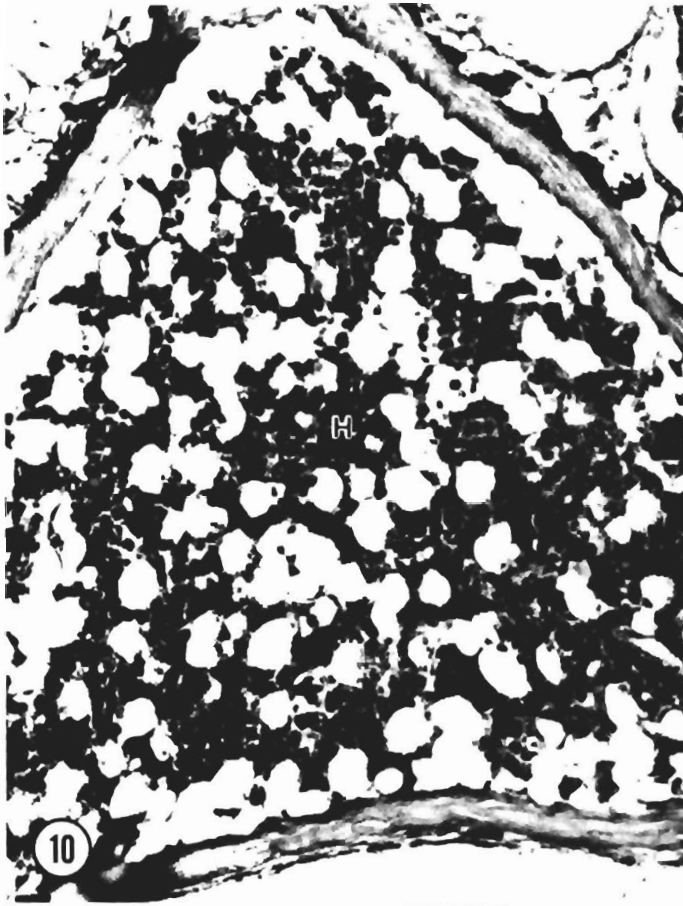
An alternative explanation for the two varieties in Morrison Creek might be expected to be provided from the extensive data that have arisen through comparison of the life histories of nonparasitic *L. planeri* and parasitic *L. fluviatilis* in Europe and of *Mordacia* spp. in Australia (Hardisty and Potter 1971c; Bird and Potter 1979a, 1979b). Arising from these studies is a belief that the age to sexual maturity is a more constant feature of the life cycle than age at metamorphosis (Hardisty 1971; Gould 1977; Hardisty 1979). In this context, *L. r.* var. *marifuga* may metamorphose a year earlier than *L. richardsoni* and their immature gonads would produce insufficient amounts of hormone to elicit intestinal atrophy and hence feeding could be possible. However, Beamish (1985, 1987) demonstrated that metamorphosis begins in Morrison Creek in July–August and that two adult forms of *L. richardsoni* appear from this metamorphosing population in the spring of the following year: a spawning variety with all the signs of advanced sexual maturation and a “silver” variety, *L. r.* var. *marifuga*. Present genetic evidence indicates that these two forms represent two life-history types of a single species (Beamish and Withler 1986). The large intestine and oesophagus, with their numerous longitudinal mucosal folds, coupled with the rapidly maturing, but not totally mature gonad, indicates that *L. r.* var. *marifuga*

could wait another year before spawning. This form began metamorphosis at the same time as the animals about to spawn, but they must have undergone a slow metamorphosis or they were prevented from undergoing sexual maturation by some unknown cue. This delay in sexual maturation, which also includes a retention of a patent lumen in a large alimentary canal, provides a potential period for feeding. In other words, it favours the retention of the parasitic period that, in most nonparasitic species, was given up for the sake of precocious sexual maturation. There is no concrete evidence that *L. r.* var. *marifuga* feeds in its natural environment (R. J. Beamish, unpublished observations), but it is abundantly clear that it can feed and grow if the proper conditions are met (Beamish 1985; Beamish and Withler 1986).

Morphological evidence from the present study shows that the two lamprey forms in Morrison Creek have identical adult gills, thyroid glands, and definitive kidneys. These organs had undergone a complete metamorphosis in all animals. However, there were signs that *L. r.* var. *marifuga* had not undergone a transformation that was exactly identical to that of *L. richardsoni*. For instance, several *L. r.* var. *marifuga* females had an underdeveloped intestine with subepithelial haemopoietic tissue and some intrapancreatic bile ducts. Furthermore, all *L. r.* var. *marifuga* showed significant portions of the larval kidneys that normally undergo extensive degeneration during metamorphosis (Youson 1981). Haemopoietic tissue was present within the definitive kidney, a feature that is not generally seen in adult lampreys (Potter et al. 1982). There were number of morphological differences between *L. r.* var. *marifuga* and *L. richardsoni* that can be attributed to the fact that they are in different periods of the life cycle. These include the amount of haemopoietic tissue of the fat column and the integrity of the intestine. However, a most obvious difference between the two forms was found in the distribution of their endocrine pancreatic tissue, a feature that had been noted earlier (Youson et al. 1988; Youson and Elliott 1989). This difference may be attributed, once again, to the slower metamorphosis in *L. r.* var. *marifuga*. Intrahepatic bile ducts undergo a programmed degeneration during metamorphosis of holarctic lampreys, while part of the intrahepatic and all of the extrahepatic common bile duct transforms into a caudal pancreas. A cranial pancreas forms from expansion of larval pancreatic islets and budding of cells from the intestinal diverticulum (Elliott and Youson 1987; Youson and Elliott 1989; Youson and Cheung 1990). The presence of intrahepatic pancreatic islets and the retention of some small bile ducts and ductules in the liver of all *L. r.* var. *marifuga* suggest that a slow degeneration was followed by transformation of bile duct cells to pancreatic endocrine cells. As noted recently (Youson and Cheung 1990), the timing of the degeneration of lamprey bile ducts is highly synchronized with the transformation of some bile duct cells into pancreatic endocrine cells. Anything that disrupts this pattern will result in an alteration in the distribution of the pancreatic islets in the adult.

The preceding viewpoint may be used to explain the virtual absence of a cranial pancreas in *L. r.* var. *marifuga*, a feature so far unique among holarctic lampreys (Youson and Elliott 1989).

FIG. 10. The fat column of *L. r.* var. *marifuga* contains abundant haemopoietic tissue (H).  $\times 390$ . FIG. 11. The fat column of *L. richardsoni* contains little haemopoietic tissue (H).  $\times 220$ . FIG. 12. The dorsal region of the adult kidney in *L. r.* var. *marifuga* possesses haemopoietic tissue (H) above the tubules (T) and the renal corpuscle (R).  $\times 350$ . FIG. 13. The pronephros of *L. r.* var. *marifuga* has nephrostomes (N), tubules (T), and renal corpuscle. O, oesophagus.  $\times 64$ .





The difference in position of the oesophageal-intestinal junctions and the diverticula of the two forms (Youson et al. 1988) may account for variation in distribution of pancreatic tissue rostral to the caudal pancreas. Instead, there is a large quantity of pancreatic tissue anterior to the caudal pancreas in the so-called intermediate cord. It is of interest to note that an extensive intermediate cord unites a small cranial pancreas with a large caudal pancreas in *L. richardsoni* of Morrison Creek. This distribution is different from that of other *L. richardsoni* (Youson and Elliott 1989; J. H. Youson and R. J. Beamish, unpublished observations). Is it possible that a cranial pancreas may still develop in *L. r. var. marifuga* over the next year? Evidence from *Petromyzon marinus* indicates that islets continue to form from the diverticular epithelium and contribute to the mass of the cranial pancreas into the upstream spawning migration (Youson and Cheung 1990). It has not been possible to distinguish between spawning *L. r. var. marifuga* and *L. richardsoni* in a wild population (Beamish and Withler 1986). If the variety feeds in the stream in the same manner as in the laboratory, then there should be some large spawning lamprey in Morrison Creek. No such lamprey have been found. All that can be concluded at this point is that two varieties exist in the population of *L. richardsoni* of Morrison Creek when spawning commences in May and June.

There has been considerable discussion on the evolutionary relationship between members of a paired species, but the most widely accepted view is that the nonparasitic form evolved from an anadromous parasitic form (Potter 1980b). According to Zanandrea (1959, 1961), a freshwater feeding phase may be an intermediate step in the evolution of freshwater nonparasitic lampreys from anadromous parasitic forms. In support of this viewpoint, there has been a continual search in both fossils and extant lampreys for examples of what might be considered an intermediate step in this evolution. *Lampetra richardsoni* and *L. ayresi* are members of a paired species, with the former being a derivative of the latter anadromous parasitic lamprey or both having originated from a common parasitic ancestral form that had a morphology and life cycle similar to *L. ayresi* (Vladykov and Follett 1958; Vladykov and Kott 1979; Potter 1980b). Beamish (1985) proposed that some nonparasitic species may have evolved from an intermediate freshwater parasitic lamprey and recently, Morris (1989) revealed a freshwater feeding and dwarf form of *L. fluviatilis* that he suggested may be an intermediate stage between anadromous *L. fluviatilis* and nonparasitic *L. planeri*. In the absence of any apparent interaction between *L. richardsoni* and *L. ayresi* in Morrison Creek (Beamish 1985, 1987), it was suggested that the stable population of lampreys in this creek was capable of producing the two life-history types, the nonparasitic form, *L. richardsoni*, and the potentially parasitic form *L. r. var. marifuga*. The latter form is the intermediate freshwater parasitic stage.

The present morphological study adds credence to the view of the existence of a single population of lampreys in Morrison Creek that is capable of yielding two life-history types and that one of these is an intermediate freshwater parasitic variety (Beamish 1985, 1987). This study also provides evidence for morphological differences in internal organs and tissue that account for the nonparasitic and parasitic life histories. The intermediate freshwater parasitic life style is due to a slower sexual maturation and, possibly, a slower and incomplete metamorphosis. It is of interest, and perhaps of some significance, that *L. ayresi*, the anadromous parasitic lamprey from which *L. richardsoni* is supposedly derived, also undergoes

a rather slow metamorphosis and a short feeding period before commencing sexual maturation (Beamish and Youson 1987). That nonparasitic lampreys evolved directly from an anadromous parasitic form requires further analysis. The situation of *L. richardsoni* in Morrison Creek, and perhaps *L. danfordi* and *L. gracilis* in Europe (for discussion see Beamish 1987) and *Ichthyomyzon castaneus* in the Namekagon River in Wisconsin (Cochran 1984) may reflect the fact that an intermediate freshwater parasitic stage may be more common than was previously believed and is representative of a key phase of lamprey evolution.

### Acknowledgements

This study was supported through a research contract with Supply and Services on behalf of the Department of Fisheries and Oceans, Government of Canada, and through a grant from the Natural Sciences and Engineering Research Council of Canada to J.H.Y. D. Chong, A. Kawamura, and S. Hall provided excellent technical assistance.

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