A nonparasitic lamprey produces a parasitic life history type: the Morrison Creek Lamprey enigma

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4 authors, including:

R.J. Beamish
Fisheries and Oceans Canada

Joy Wade
Fundy Aqua Services Inc.

Terry D. Beacham
Fisheries and Oceans Canada

CHAPTER EIGHT

A NONPARASITIC LAMPREY PRODUCES A PARASITIC LIFE HISTORY TYPE: THE MORRISON CREEK LAMPREY ENIGMA

RICHARD BEAMISH, RUTH WITHLER, JOY WADE AND TERRY BEACHAM

Introduction

Very little was known about lampreys in British Columbia, Canada in the 1970s. As part of a study of the survival of juvenile Pacific salmon (*Oncorhynchus* spp.) in the Strait of Georgia (Fig. 8-1), it was discovered that river lamprey (*Lampetra ayresii*) were a major predator of juvenile Pacific salmon (Beamish & Youson 1987; Beamish & Neville 1995). It was known that river lamprey were in the Fraser River (Hart 1973) and the fish collection at the University of British Columbia had several specimens identified as *L. ayresii* that were collected from Morrison Creek on Vancouver Island (Fig. 8-1). As part of a study to determine the impact of river lamprey predation on juvenile Pacific salmon and herring (*Clupea pallasii*), specimens for a laboratory feeding study were collected from Morrison Creek in the late 1970s because it was more accessible than the Fraser River. Soon, it was discovered that the lamprey in Morrison Creek was not *L. ayresii*.

Nonparasitic lamprey are considered as paired species (Hardisty & Potter, 1971) or satellite species (Vladykov & Kott 1979) of parasitic lamprey. In Morrison Creek, it was discovered that there was a population that produced both parasitic and nonparasitic lamprey (Beamish 1987). Both forms had two supraoral cusps, three inner lateral teeth (endolaterals) on each side of the mouth and an average of seven cusps on the infraoral lamina. One form was in spawning condition with uniformly dark colour, obsolete teeth typical of nonparasitic lamprey and typical secondary sexual characteristics of a shortened tail and large dorsal fins. This form was considered to be *L. richardsoni* (Fig. 8-2). The second form that occurred at the same time, had prominent dentition, sharp cornified teeth, an absence of secondary sexual characters and silver pigmentation dorsally with white countershading ventrally (Beamish 1985; Beamish & Withler 1986; Fig. 8-2). Subsequently this form was found to not be able to osmoregulate in saltwater (Beamish 1985; Beamish & Withler 1986). It was classified as a variety with the name of *L. richardsoni* var. *marifuga* and a common name of the Morrison Creek lamprey. The name *mari* from the Latin for "sea" and *fuga* from the Latin for "flight" meaning that the fish avoids or cannot live in the sea.

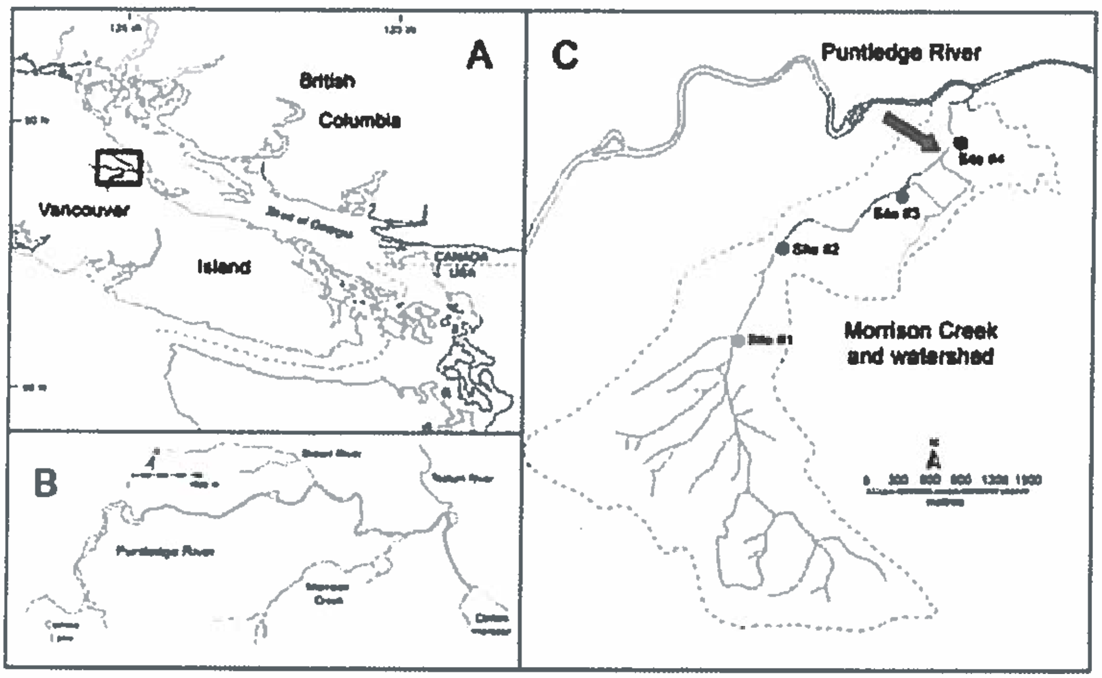


Figure 8-1. (A) Location of Morrison Creek on Vancouver Island, (B) Showing Morrison Creek flowing into the Puntledge River, (C) The drainage area showing the trapping sites. Largest catches occurred at Site 3. Area upstream of Site 1 is broad, swamp-like spring fed area with intermittent areas of flowing water.

Some of the Morrison Creek lamprey would feed and grow in fresh water when brought back to the laboratory (Beamish 1985, 1987). Feeding continued through to the early fall and it was possible to keep some individuals through to the next spring when they spawned and produced viable eggs that hatched into ammocoetes (Fig. 8-3). In the early years of the study, some of the spawning L. *richardsoni* were much smaller than

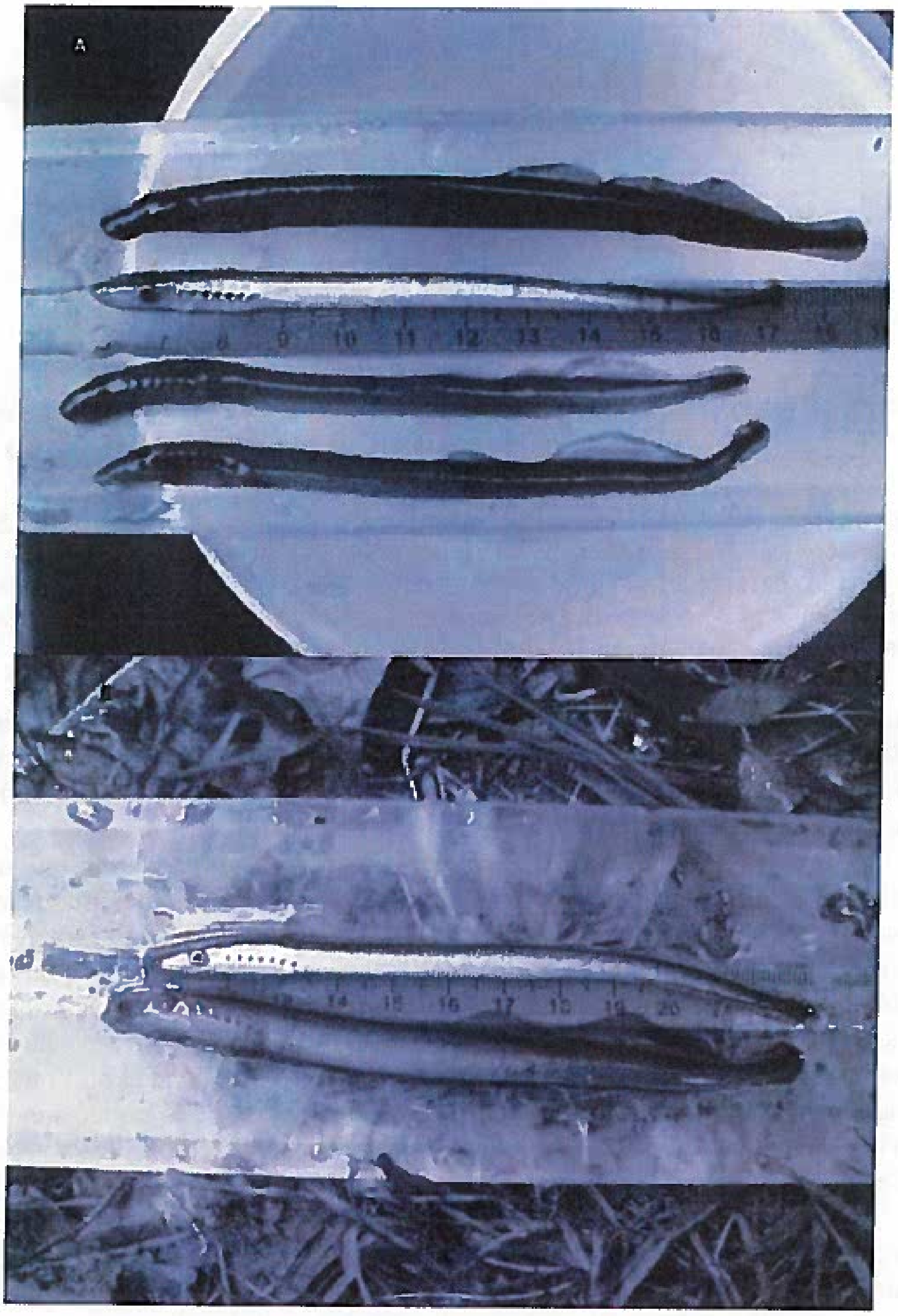


Figure 8-2. (A) Examples of metamorphosed lamprey captured in Morrison Creek in July 2011. The silver coloured lamprey is the Morrison Creek lamprey, and the others were considered to be *L. richardsoni*. (B) An example of a larger brown coloured mature lamprey that appears to have fed and grown and is larger than the silver Morrison Creek lamprey.

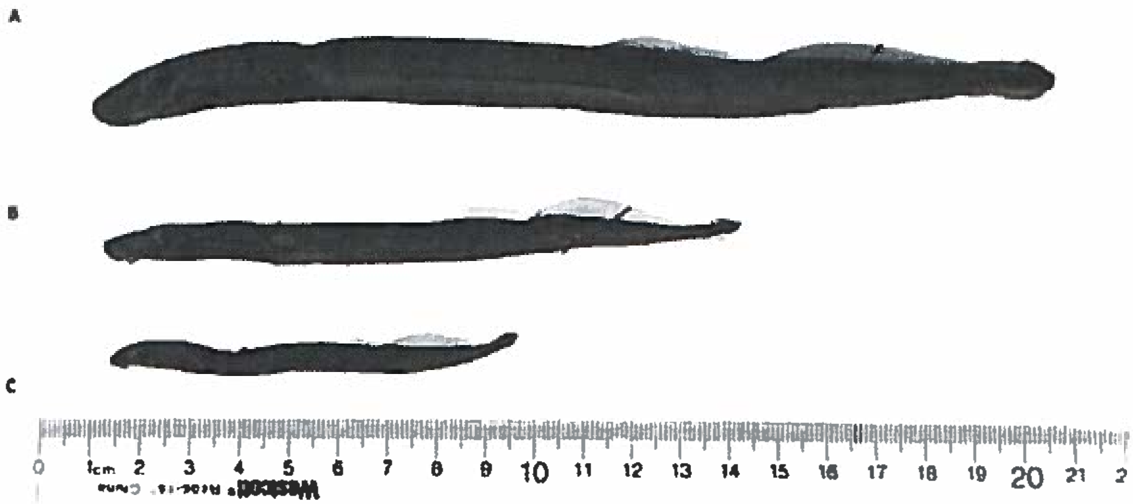


Figure 8-3. (A) A Morrison Creek lamprey that was fed in freshwater in the laboratory and was kept over the winter of 1983 until the spring of 1984. (B) A preserved specimen of a typical silver Morrison Creek lamprey when it was first brought into the laboratory. (C) An example of some of the smallest *L. richardsoni* that were captured in the traps in the late 1970s and 1980s.

the Morrison Creek lamprey (Fig. 8-3). Following the realization that the Morrison Creek lamprey was not *L. ayresii*, a series of studies (Table 8-1) identified a number of anomalous biological developments that were unique to the Morrison Creek lamprey.

In 1995 the Morrison Creek lamprey was recognized as being unique and was designated as Endangered by the Committee on the Status of Endangered Wildlife in Canada, (COSEWIC). In 2003, the Morrison Creek lamprey was protected under the Canadian Government's Species at Risk Act (SARA) even though it was known that the lamprey was not a distinct species. Wisely, it was recognized that the variety was a unique example of a process that could ultimately help us understand evolutionary relationships between parasitic and nonparasitic species and perhaps even an understanding of speciation in lampreys over the past 360 million years (Gess et al. 2006).

This paper summarizes the results of studies of the Morrison Creek lamprey (Table 8-1) and relates results to recent studies in 2011 and 2012 that show that the abundances are greatly diminished. The taxonomy is reviewed and reassessed based on new DNA-based genetic information. An interpretation is provided of how the Morrison Creek lamprey developed along with a recommendation for the protection of the population and future research. The Morrison Creek lamprey will also be referred to as the "silver form".

Table 8-1: Morrison Creek lamprey publications.

Beamish, R.J., 1985. Freshwater parasitic lamprey on Vancouver Island and a theory of the evolution of the freshwater parasitic and nonparasitic life history types. In Evolutionary Biology of Primitive Fishes. Edited by R.E. Foreman, A. Gorbman, J.M. Dodd and R. Olsson. Plenum, New York, pp. 123-140.

Beamish, R.J., 1987. Evidence that parasitic and non-parasitic life history types are produced by one population of lamprey. Canadian Journal of Fisheries and Aquatic Sciences 44:1779-1782.

Beamish, R.J., and R. E. Withler, 1986. A polymorphic population of lampreys that may produce parasitic and nonparasitic varieties. Indo-Pacific Fish Biology: Proceedings of the Second International conference on Indo-Pacific Fishes. Edited by T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, 1986, pp.31-49, Ichthyological Society of Japan, Tokyo.

Beamish, R.J., J. H. Youson, and L.A. Chapman. 1999. COSEWIC status report on the Morrison Creek lamprey, *Lampetra richardsoni*, in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-14 pp.

Beamish, R.J., J. H. Youson, and L.A. Chapman. 2001. Status of the Morrison Creek Western Brook lamprey, *Lampetra richardsoni*, in Canada. Canadian Field-Naturalist 115(4):573-578.

Docker, M.F., J.H. Youson, R.J. Beamish, and R.H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome b and ND3 gene sequences. Canadian Journal of Fisheries and Aquatic Sciences 56: 2340-2349.

Youson, J. H. 2004. The impact of environmental and hormonal cues on the evolution of fish metamorphosis. Environment, development, and evolution. Cambridge, MA: MIT Press. p, 239-77.

Youson, J.H., and R.J. Beamish. 1986. Morphological comparison of organs in several species of lampreys on the west coast of Canada. Page 926 *in* T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura (eds.) Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. The Ichthyological Society of Japan, Tokyo.

Youson, J.H., and R.J. Beamish. 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*. Canadian Journal of Zoology 69: 628-637.

Youson, J.H., W.M. Elliott, R.J. Beamish, and D.W. Wang. 1988. A comparison of endocrine pancreatic tissue in adults of four species of lampreys in British Columbia: a morphological and immunohistochemical study. General and Comparative Endocrinology 70: 247-261.

Study area and sampling

Morrison Creek on Vancouver Island is a small creek that flows into the Puntledge River (Fig. 8-1). The creek is about 35 km long, averages 2-3m wide and originates in a spring-fed wetland and now mostly winds its' way through the backyards of a residential area (Fig. 8-4). Fish can access Morrison Creek from the Puntledge River and the ocean (Strait of Georgia) as indicated by the presence of numerous juvenile Pacific salmon of several species.

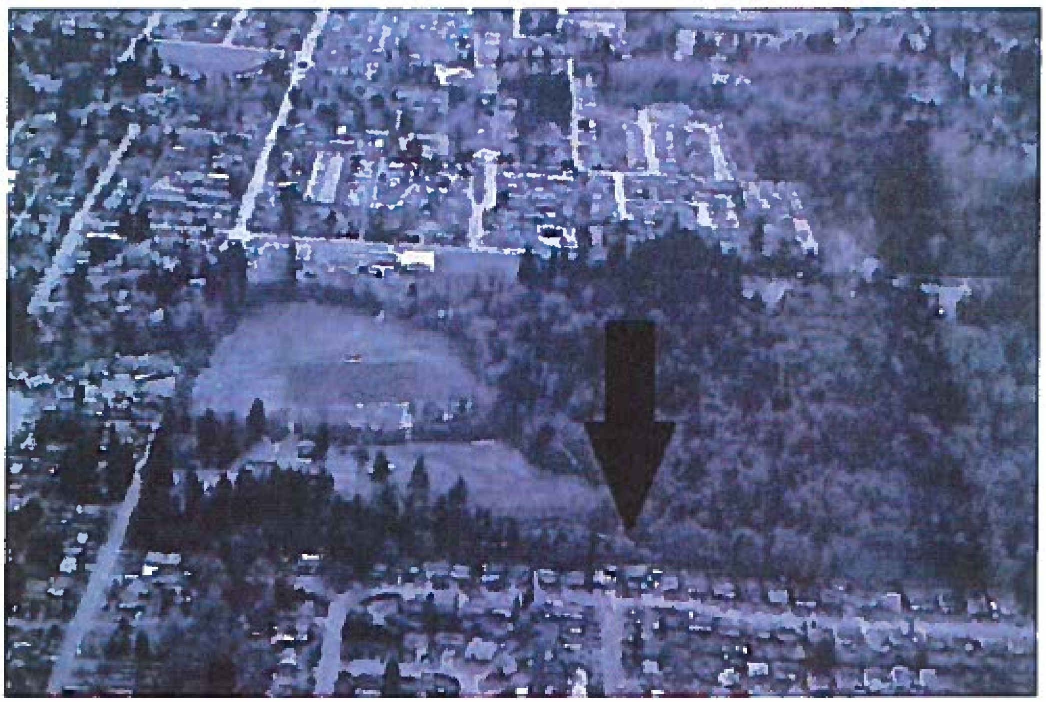


Figure 8-4. Photograph of Morrison Creek in 2009. Arrow shows Morrison Creek in a highly developed area between trapping Sites 3 and 4 shown in Fig. 8-1.

Studies of lampreys in Morrison Creek from 1978 to 1987

Data collections were opportunistic, in part, because funding was minimal and Morrison Creek was about 110 km from where the investigators worked at the Pacific Biological Station in Nanaimo. Because of the opportunistic nature of the study and because interpretations of the biology and life history relationships evolved during the study, data and measurements may also be less than ideal. The initial studies terminated in 1987 when it was realized that the population of the Morrison Creek lamprey should be protected. However, in 2011 and 2012, a trap and release study was undertaken as part of the Department of Fisheries and Ocean's Species at Risk Assessment (SARA) program to determine the status of the Morrison Creek lamprey relative to the 1980s. All spawning lamprey in Morrison Creek were considered to be *L. richardsoni* as there was no method of identifying Morrison Creek lamprey that might be spawning. A summary of the initial field studies is available in Beamish (2013) and the recent studies in Wade and MacConnachie (2014).

Evidence that metamorphosing lampreys produce two life history types

In February 1978, 18 metamorphosed lampreys were collected in Morrison Creek using an electroshocker and kept at the Pacific Biological Station in tanks that had a mud substrate (Beamish 1987). By June, 1978, 16 had matured and were in spawning condition and, two had the silver appearance of the Morrison Creek lamprey. One of the silver forms fed in fresh water. Attempts were made to convert the two silver lampreys to salt water, but both died. The remaining 16 were considered to be *L. richardsoni*.

The study was repeated in 1985 when four metamorphosed lampreys were collected in Morrison Creek in August and transported to the Pacific Biological Station (Beamish 1987). By early October 1985, metamorphosis appeared complete. By March 1986 one of the four lamprey started to develop a silver appearance and counter shading. In mid-May, the three other lampreys were mature. In early June the three were in spawning condition. The silver form and these three lampreys in spawning condition were preserved and the body proportions measured. There were no differences in the body proportions of the two forms in this small sample (Table 8-2).

Table 8-2. Comparison of the morphology of one Morrison Creek lamprey and three specimens of *L. richardsoni* from Morrison Creek that were captured during metamorphosis in August 1995 and matured in the laboratory through to June 1986.

Species	Sample Size	Total Length (TL) in mm	Disc Length / TL	Branchial Length / TL	Prebranchial Length / TL	Eye Diameter / TL	Postorbital Length / TL
Morrison Creek lamprey	1	106	0.06	0.10	0.01	0.03	0.02
L. richardsoni	3	99	0.05	0.11	0.11	0.02	0.03
		95	0.06	0.11	0.12	0.02	0.03
		111	0.06	0.10	0.12	0.03	0.03

Feeding and spawning

In the early 1980s, Morrison Creek lamprey were kept in fresh water in tanks at the Pacific Biological Station in Nanaimo and fed (Beamish 1985). The lamprey were difficult to keep alive and the majority did not survive. Those that survived started to feed in July and fed most actively in September. Feeding continued until mid-November, but most feeding stopped by mid-October. From 1982 to 1983, 24 fish fed and lived through the winter until the next spring. After feeding, these fish ranged in length from 10.0 to 20.6 cm with an average length of 18.7 cm and weight ranging from 0.8 to 12.0g and an average of 9.2 g. The lamprey fed on a variety of living and dead fishes, but preferred live Pacific herring (*C. pallasii*) that were added to fresh water. It was of interest that Pacific herring is the preferred prey of *L. ayresii* in the Strait of Georgia (Beamish 1980). Most fish that survived to November lived through the winter and were in spawning condition in the spring of the next year. Assuming that the males were mostly mature when brought into the laboratory, (as discussed later in this report), these males maintained their maturity through the feeding period and over winter. On June 12, 1984, one female spawned in the tanks with four males. The eggs hatched and a sample of the larval lamprey was preserved.

Studies of lampreys in Morrison Creek from 1978 to 1987

Our trapping studies covered all of the creek that had flowing water. There is a large spring-fed area in the headwater area that contains numerous pools and some flowing water. There are lamprey in this area but electroshocking studies showed that the lamprey are not abundant compared to the main creek. Over the period 1978 to 1987, fish traps were placed in the river at sites where local residents could monitor the catches each day (Fig. 8-1). The traps were modified trap net designs that had two wings facing into the current and forming a tunnel that directed fish into a 200 L tank that was weighted to remain on the stream bottom. The sides of the tank had openings covered by 5 mm square mesh screens that allowed water to flow out of the tank, but retained small organisms. Water levels in the tank were about half way up the sides. The tank was covered to prevent predators from feeding on the catch. Catches of ammocoetes were counted in 1978, 1981 and 1984. Counts were available for some sites in other years, but the reliability of the counts was uncertain. Ammocoetes were preserved in 1978 and 1981 and lengths were measured in later years. Lengths were taken from the total catch, except for a small number of samples that were missed for various reasons that occur in field studies. In other years, ammocoetes were returned to the creek. No ammocoete was identified with a pigmentation pattern that approximated the pattern that is characteristic of *L. ayresii* (Richards et al. 1982). Several mature *Entosphenus tridentatus* were captured each year, but they were rarely observed. Because *E. tridentatus* was rarely observed and because of the difficulties of identifying smaller ammocoetes, the species of ammocoetes in this paper are not considered to be *E. tridentatus* even though it is possible that a very small percentage of the ammocoetes in this study would be *E. tridentatus*.

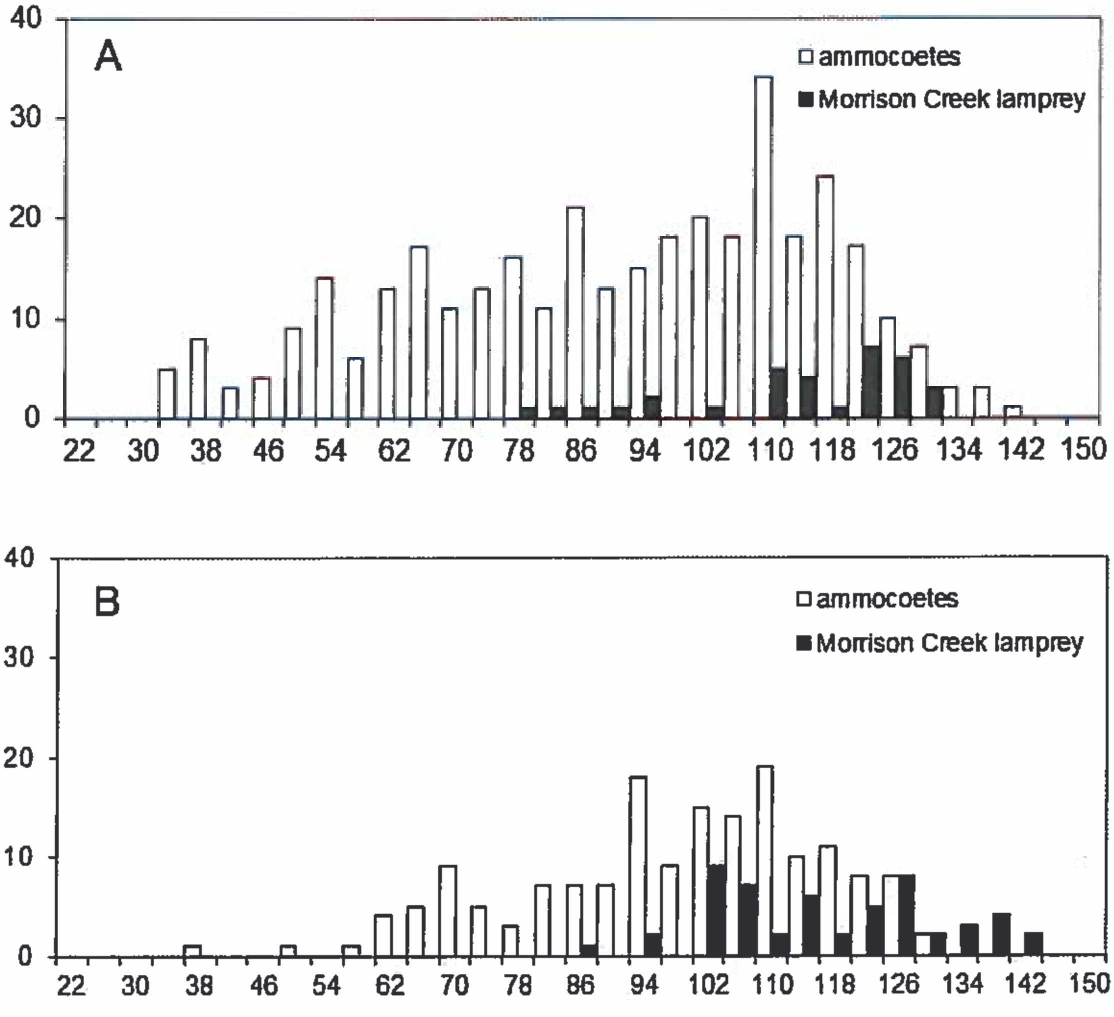


Figure 8-5: Length frequency of ammocoetes and Morrison Creek lamprey from trapping studies in (A) 1978 (ammocoetes, n=352 and Morrison Creek lamprey, n=33) and (B) 1981 (ammocoetes, n=164 and Morrison Creek lamprey, n=53).

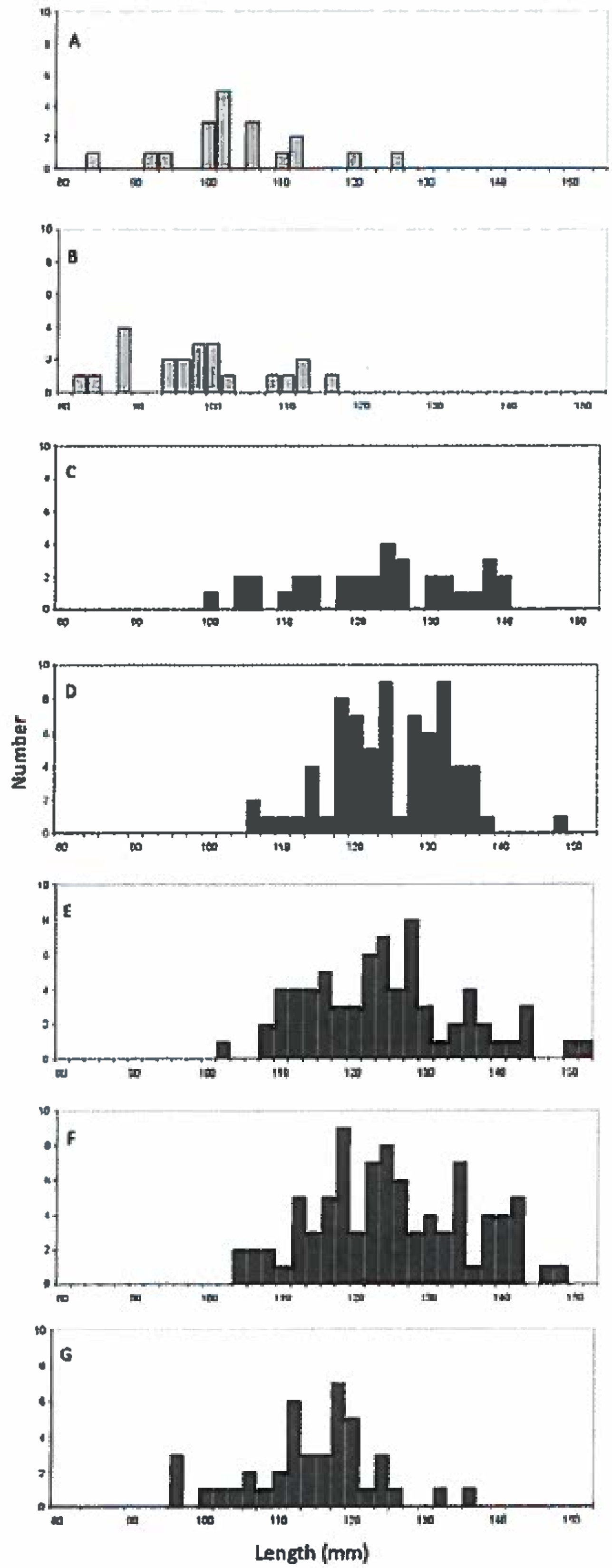
All *L. richardsoni* captured were counted in 1981, 1984 and 1987 and total lengths recorded only in 1981 and 1987. The sex of *L. richardsoni* was determined for the total catch in 1987 by examining the gonad. Counts of the total catch of the Morrison Creek lamprey and their lengths were obtained in 1981, 1982, 1983, 1984 and 1987. The sex ratio of the total catch of the Morrison Creek lamprey was determined in 1987. The sex ratio of a small sample of 24 lamprey was also examined in 1984.

In 1978, there were 352 ammocoetes measured, ranging from 31 mm to 140 mm with an average length of 90 (SD = 25.6; Fig. 8-5A). In 1981, 164 ammocoetes lengths, ranged from 36 mm to 129 mm, with an average length of 97 (SD =18.5; Fig. 8-5B). Seven (4%) of the Morrison Creek lamprey captured in 1981 were longer than any of the ammocoetes (Fig. 8-5B).

In 1981 and 1987 the catches of the Morrison Creek lamprey (Fig. 8-6C, G) were larger than the catches of *L. richardsoni* (Fig. 8-6A, B). In 1981 and 1987, the mean lengths of *L. richardsoni* were 104 mm (SD = 9.5) and 97 mm (SD = 9.5), respectively, and were significantly smaller than the mean length of the Morrison Creek lampreys of 122 mm (SD = 11.3) in 1981 and 114 mm (SD = 8.8) in 1987 (t test, $P \leq 0.05$). There was no difference in the mean length of the Morrison Creek lampreys (Fig. 8-6) in 1982, 1983 and 1984 (124 mm (SD = 8.1), 124 mm (SD = 10.7), 124 mm (SD = 10.7), respectively; ANOVA $P > 0.05$). However, the mean length of the Morrison Creek lamprey in 1987, (114 mm (SD = 8.8)), was significantly smaller than in 1982, 1983 and 1984 (Fig. 8-6). A linear regression fit to the lengths of the Morrison Creek lamprey for each of the years produced R^2 values that ranged from 0.00 to 0.07 (Fig. 8-7).

In 1987, there were 22 *L. richardsoni* captured from May 1 to July 9, consisting of 11 females and 11 males. All were in spawning condition or had spawned. Males averaged 102 mm (SD = 7.4) and females averaged 93 mm (SD = 9.8) in length. There were 42 Morrison Creek lamprey captured from May 1 until July 9. Five (12%) were female with an average length of 118 mm (SD = 14.3) and 37 (88%) were male with an average length of 113 mm (SD = 8.0; Fig. 8-8).

Figure 8-6 (next page). Length frequency of lamprey captured in traps. (A) *L. richardsoni* 1981; n=19; mean length=104 mm (SD=9.5). (B) *L. richardsoni* 1987; n=22; mean length= 97 mm (SD=9.5). (C) Morrison Creek lamprey 1981; n=34; mean length= 122 mm (SD=11.2). (D) Morrison Creek lamprey 1982; n=72; mean length= 124 mm (8.1). (E) Morrison Creek lamprey 1983; n=70; mean length = 124mm (SD=10.7). (F) Morrison Creek lamprey 1984; n=86; mean length = 124 mm (SD=10.7). (G) Morrison Creek lamprey 1987; n=42; mean length= 114 mm (SD=8.8).


A
B
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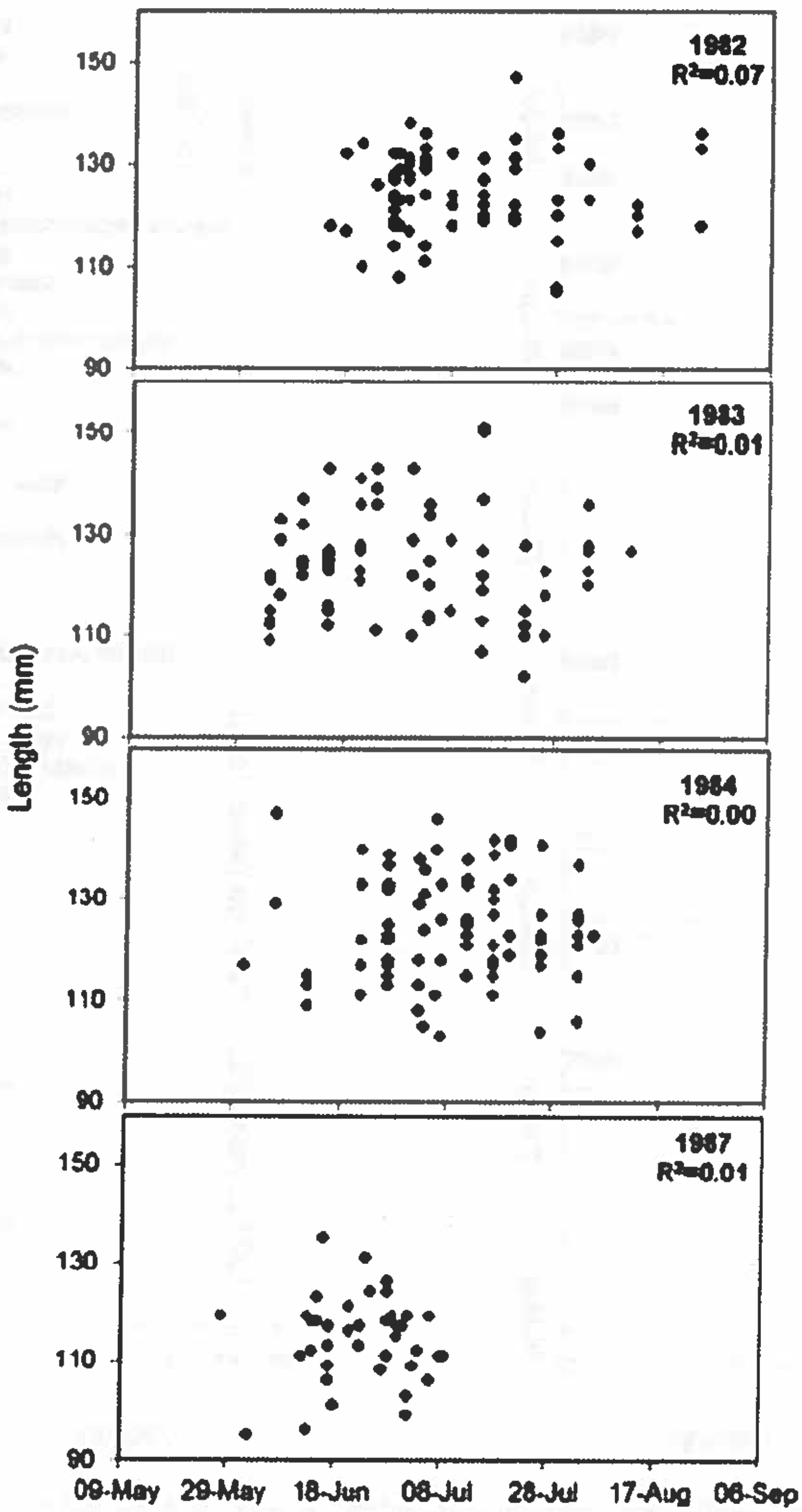

Figure 8-7. Lengths of the Morrison Creek lamprey captured during the trapping studies in 1982, n=72; 1983, n=70; 1984, n=86 and 1987, n=42. A linear regression fitted to the data for each year showed no trend (not shown).

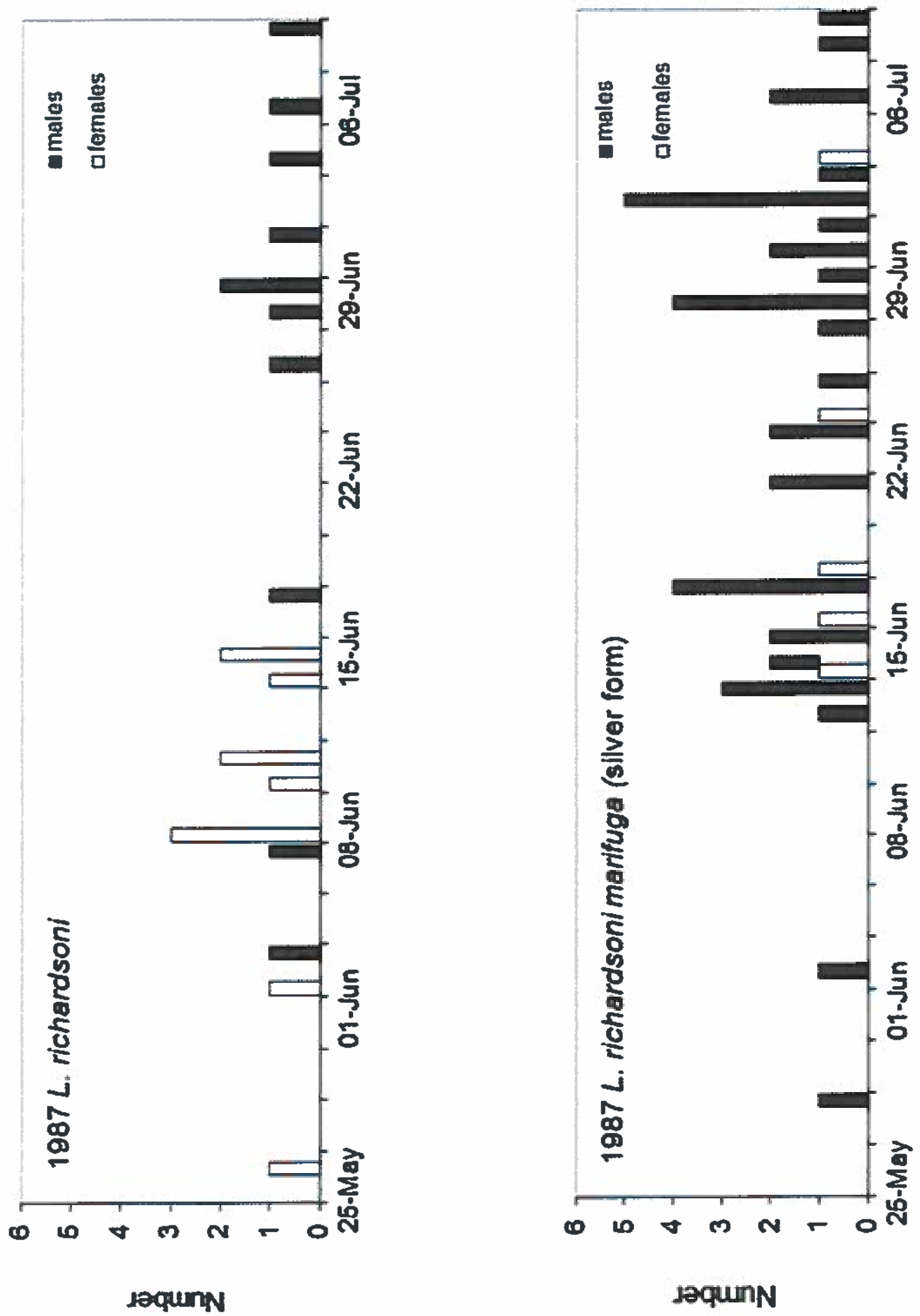

Figure 8-8. Number, sex and timing of capture of (A) *L. richardsoni* (male, n=11; female, n=11) and (B) the Morrison Creek lamprey (male, n=37; female, n=5) from the trapping study in Morrison Creek in 1987.

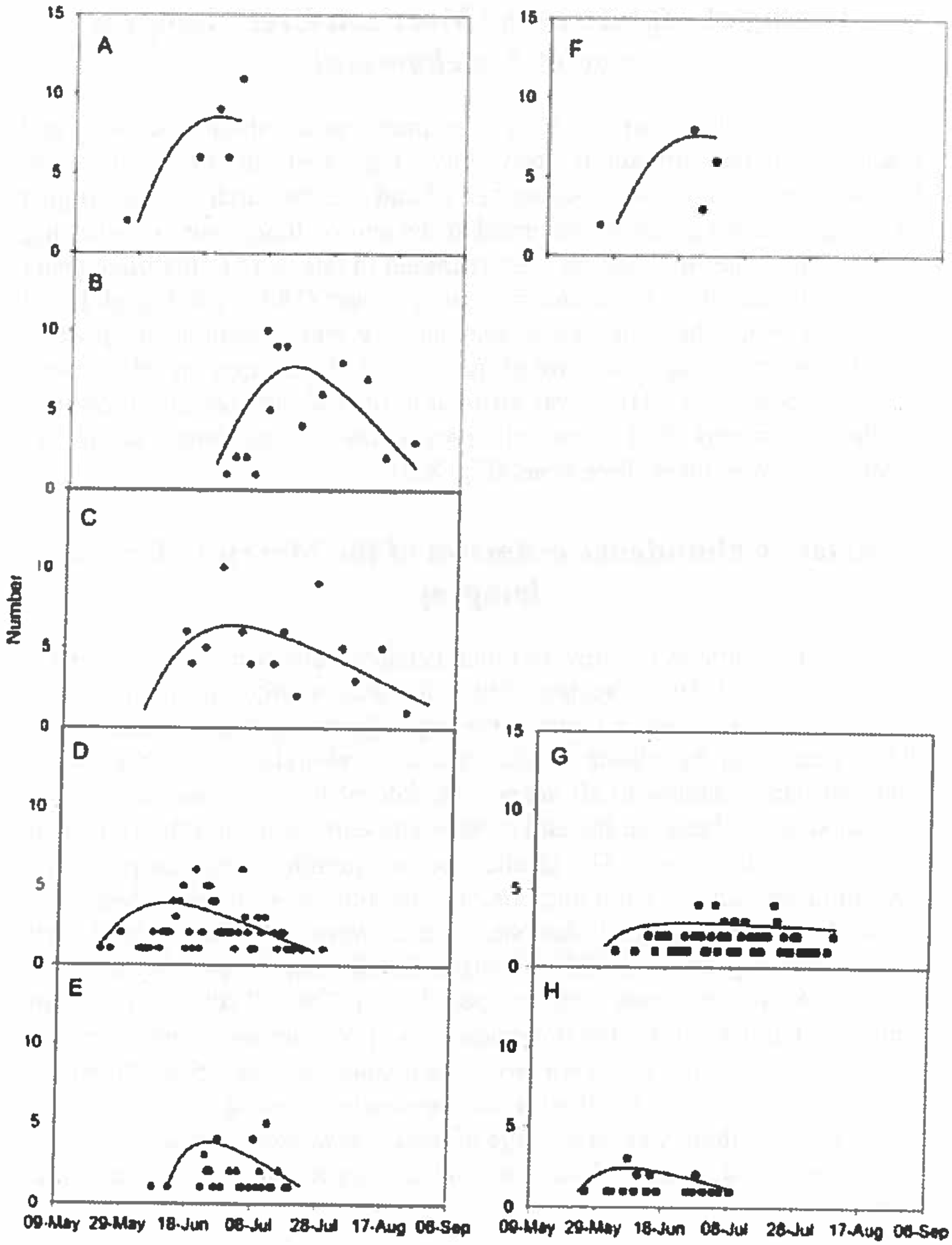

Figure 8-9. The number of Morrison Creek lamprey captured in the trapping study in (A) 1981, n=34; (B) 1982, n=72; (C) 1983, n=70; (D) 1984, n=111; (E) 1987, n=42; and *L. richardsoni* in (F) 1981, n=19; (G) 1984, n=86; and (H) 1987, n=22. The curves are approximations of the possible trends.

Timing of capture of the Morrison Creek lamprey and of *L. richardsoni*

Catches of the Morrison Creek lamprey started about late May and reached a maximum about early July (Fig. 8-9). In 1982, the traps remained in the creek until September 17 and the last catch was on August 24. Large catches generally occurred at the end of June, usually following a heavy rain. The first catches were recorded in late May in the three years that all catches of *L. richardsoni* were recorded (1981, 1984, and 1987) (Fig. 8-9). Both the Morrison Creek lamprey and spawning or spent *L. richardsoni* were captured through to the end of the trapping effort each year (Fig. 8-9D, E, G, H). It was difficult to identify any pattern of catches of the specimens that were all provisionally considered to be *L. richardsoni* from these three years (Fig. 8-9).

Relative abundance estimates of the Morrison Creek lamprey

Reliable estimates of daily and total catches were available at site 3 for 1983, 1984, and 1987 catches. This site was monitored by the same observers who allowed us to place the trap adjacent to their property. This site appeared to be closer to the centre of abundance as there were relatively large catches in all years. The date of the first catch at the site was consistently between the end of May and early June and the last catch was around mid-August. The catches for one month during the period of maximum abundance, from mid-June to mid-July, were used to determine an average daily catch at this site. There were 1.1 Morrison Creek lamprey per day in 1983, 2.2 Morrison Creek lamprey per day in 1984 and 1.4 Morrison Creek lamprey per day in 1987 (Table 8-3) and an average catch per day for the three years was 1.5 Morrison Creek lamprey. The catches of adult *L. richardsoni* were much smaller than Morrison Creek lamprey catches at all sites, as previously reported. Over the same period at site 3, there was an average of 0.4 *L. richardsoni* caught each day or between 3 to 4 more Morrison Creek lamprey than *L. richardsoni* (Table 8-3).

Table 8-3. Average daily catch of Morrison Creek lamprey and adult *L. richardsoni* in the area of maximum abundance of the silver form (Site #3) and during the period of maximum abundance.

Year	Date	Number of days	Total catch of the silver form	Average daily catch of the silver form	Total catch *L. richardsoni*	Ratio of the silver form to *L. richardsoni*
1983	June 11 - July 15	35	39	1.1	14	2.8 to 1
1984	June 15 - July 15	31	67	2.2	14	4.8 to 1
1987	June 12 - July 9	29	40	1.4	12	3.3 to 1

Summary of field studies up to 1987

For the two years that ammocoetes were measured, the lengths of the larger ammocoetes were similar to the lengths of the Morrison Creek lamprey. This indicated that the ammocoetes in the creek most likely produced both the Morrison creek lamprey and *L. richardsoni* as was also observed in the two ammocoetes rearing studies. Because no ammocoetes were found with the pigment pattern of *L. ayresii*, it was concluded that there were no *L. ayresii* in the population.

Sampling from late May through to mid or late July, and in one year until mid-August, resulted in consistent catches of Morrison Creek lamprey with a possible maximum catch about early July. At the same time, a smaller number of mature or spent lamprey were also consistently captured and identified as *L. richardsoni.* This indicated that mature *L. richardsoni* were not as abundant in the sampling area as the Morrison Creek lamprey and that *L. richardsoni* on average were smaller. Thus, because the catches of the Morrison Creek lamprey each year were larger than the catches of *L. richardsoni* some of the lamprey identified as *L. richardsoni* could be mature Morrison Creek lamprey. There was no indication of growth in length in the Morrison Creek lamprey in any of the years, which indicated that if feeding occurred, it was minimal. However, because it is possible that some of the adult lamprey identified as *L. richardsoni* would be spawning Morrison Creek lamprey that were silver coloured in the previous year, it is likely that some feeding occurred. The DNA studies that will be discussed later in this paper showed that there was no genetic difference among the lamprey of the genus *Lampetra* that were spawning in Morrison Creek.

Morphological studies

The morphologies of the two forms in Morrison Creek were compared to *L. richardsoni* from other rivers, to *L. ayresii* and to the closely related *L. planeri* and *L. fluviatilis*. The comparison with *L. planeri* and *L. fluviatilis* provides a reference for differences which have previously been accepted as being taxonomically diagnostic.

L. richardsoni was separated from *L. planeri* by Vladykov and Follett (1965) because of a longer branchial region in *L. richardsoni* and a bicuspid middle inner lateral tooth (endolaterals). Other differences are a pigmented caudal fin in *L. richardsoni* and a smaller number of cusps (5-11) on the transverse lingual lamina in *L. richardsoni* compared to 9-13 in *L. planeri* (Renaud 2011). Also, the gill pore papillae (Beamish 2010) in *L. planeri* are much less numerous (less than 20) than in *L. richardsoni* (about 30) (Fig. 8-10A, B). Gill pore papillae in *L. richardsoni* are also clumped dorsally and ventrally along the posterior margin of the pore (Fig. 5-10 A, B). Renaud (2011) reports that *L. richardsoni* has a smaller eye than *L. ayresii*, one less cusp on the middle tooth in the endolaterals (inner laterals) and fewer cusps on the transvers lingual lamina.

L. ayresii was separated from *L. fluviatilis* by Vladivkov and Follett (1958) because *L. ayresii* had a larger eye, longer pre-branchial, shorter branchial and a higher number of trunk myomers. *L. ayresii* has a smaller number of velar tentacles according to Renaud (2011). There also are differences in the gill pore papillae between *L. fluviatilis* and *L. ayresii* (Fig. 8-10 C, D). There are fewer papillae in *L. fluviatilis* (average 19 for 13 specimens from museum collections, including paratypes, R. Beamish, unpublished data), than in *L. ayresii* (average 32 in Beamish 2010).

Renaud (2011) reported that *L. richardsoni* had a smaller eye than *L. ayresii*, one less cusp on the inner laterals (endolaterals) and fewer cusps on the transvers lingual lamina. In addition, Beamish and Withler (1986) showed that disc length and prebranchial length also differ between the two species. Furthermore, Beamish and Withler (1986) showed that the mean number of cusps on the transverse and longitudinal lingual laminae were significantly different between the two species (Table 8-4A, B). This level of taxonomic separation between *L. richardsoni* and *L. ayresii* is similar to the amount of separation used to distinguish other paired species.

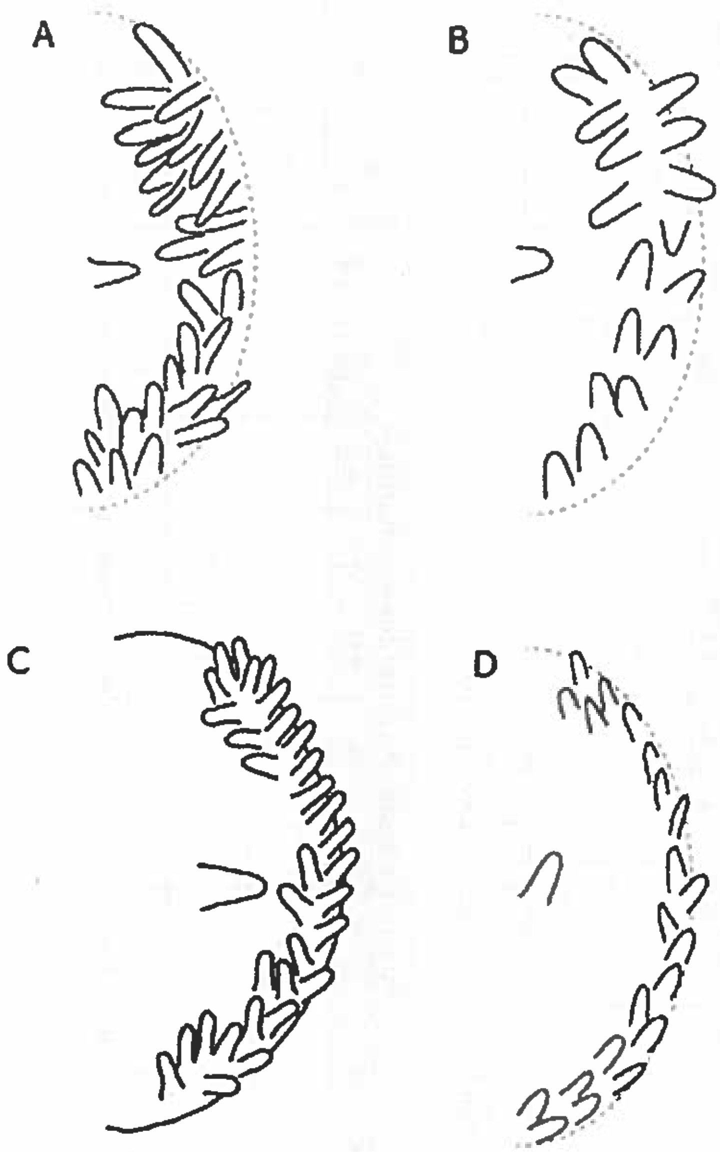

Figure 8-10: Interpretive drawing of the structures in the gill pore cup (A) *L. richardsoni* (B) *L. pleneri* (C) *L.* ayresii (D) *L. fluviatilis*. There is a central process in the middle of the cup and marginal papillae along the posterior margin (Beamish 2010).

Table 8-4. Number of cusps on the (A) transverse and (B) longitudinal lamina of the Morrison Creek lamprey, *L. ayresii* and *L. richardsoni* from five locations in British Columbia.

A	Number on transverse lingual lamina												N	Ave	SD
Sample	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**			
Morrison Creek lamprey	2	3	4	25	19	21	2	2	1	-	-	-	79	9.8[1]	1.43
L. ayresii	-	-	-	-	-	8	6	25	8	13	2	4	65	13.5[1]	1.56
L. richardsoni	-	-	-	3	13	13	2	1	-	-	-	-	32	10.5[1]	0.88

[1]All means are significantly different from each other (ANOVA $p \leq 0.05$)

B	Number on longitudinal lingual lamina										N	Ave	SD
Sample	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
Morrison Creek lamprey	3	3	25	23	17	7	1	-	-	-	79	7.9[2]	1.23
L. ayresii	-	1	-	-	5	8	19	9	9	1	52	11.1	1.44
L. richardsoni	-	-	8	10	2	-	4	1	1	-	26	8.6[2]	1.77

[2]There is no difference in the mean number of cusps on the teeth of the Morrison Creek lamprey and *L. richardsoni* (ANOVA $p \geq 0.05$). All other means are significantly different (ANOVA $P \leq 0.05$).

Morphological studies of the Morrison Creek lamprey

Beamish and Withler (1986) compared the number of cusps on the teeth on the tongue of the Morrison Creek lamprey, *L. ayresii* from the Strait of Georgia and from *L. richardsoni* from five rivers in British Columbia (Table 8-4A, B). The metamorphosing *L. richardsoni* were held in the laboratory until mid-September to early October when the dentition was prominent. *L. richardsoni* from Morrison Creek could not be used as the cusps were blunt. The number of cusps on the longitudinal lingual lamina of the Morrison Creek lamprey was significantly smaller than for *L. ayresii* (Table 8-4; ANOVA, $P \leq 0.05$). There was no difference in the mean number of cusps on the longitudinal lingual lamina between the Morrison Creek lamprey and *L. richardsoni* from the other locations (Table 8-4B). For both the transverse lingual lamina and the longitudinal lingual lamina the number of cusps is more closely matched to *L. richardsoni* from the other rivers than to *L. ayresii* (Table 8-4A, B). Despite the significant difference in the mean number of cusps on the transverse lingual lamina of the Morrison Creek lamprey and *L. richardsoni* from other rivers, there was an overlap in counts indicating a similarity with the Morrison Creek lamprey (Table 8-4A).

Body proportions reported in Beamish & Withler (1986) were measured using the criteria of Vladykov & Follett (1965). All measurements were percentages of the total length. *L. ayresii* were from the Strait of Georgia and *L. richardsoni* were from 10 rivers that flowed directly or indirectly into the Strait of Georgia. Specimens of the *L. richardsoni* in Morison Creek were separated into individuals that were collected before mid-June and individuals collected in July and August (Table 8-5A). There were many significant differences among the groups (Table 8-5B) as well as some similarities. Of importance to this study is that the *L. richardsoni* in Morrison Creek were more similar to the Morrison Creek lamprey than to other *L. richardsoni* in all measurements except for the branchial length of the July and August spawning sample (Table 8-5A, B). It is also relevant that the small sample of one Morrison Creek lamprey and three *L. richardsoni* collected in 1985 and kept until 1986 (Table 8-2), were similar in their body proportions. It is of interest that *L. richardsoni* from the 10 rivers can be distinguished from the *L. ayresii* by their smaller disc and eye and a shorter prebranchial length.

Table 8-5. (A) Morphometric measurements for the species or populations used in this study. (B) Results of Analysis of Variance (ANOVA) on morphometric measurements of groups used in this study. The * indicates a significant difference (ANOVA $P \leq 0.05$). NS indicates that there is not a significant difference (ANOVA $P \geq 0.05$).

A

Group	Species	Sample Size	Average Total Length (TL) in mm (SD)	Disc Length / TL (SD)	Branchial Length / TL (SD)	Prebranchial Length / TL (SD)	Eye Diameter / TL (SD)	Postorbital Length / TL (SD)
(1)	Morrison Creek lamprey	91	128.2 (±17.45)	0.062 (±0.0051)	0.101 (±0.0057)	0.134 (±0.0079)	0.026 (±0.0033)	0.028 (±0.0020)
(2)	*L. ayresii*	73	138.7 (±38.97)	0.067 (±0.0062)	0.100 (±0.0058)	0.138 (±0.0124)	0.030 (±0.0054)	0.026 (±0.0021)
(3)	*L. richardsoni* from 10 locations	95	136.6 (±22.83)	0.046 (±0.0065)	0.096 (±0.0059)	0.105 (±0.0094)	0.021 (±0.0026)	0.026 (±0.0021)
(4)	*L. richardsoni* spawning before mid June	16	92.6 (±0.96)	0.061 (±0.0068)	0.106 (±0.0115)	0.134 (±0.0139)	0.031 (±0.0038)	0.031 (±0.0039)
(5)	Morrison Creek lamprey spawning in July and August	12	110.8 (±1.17)	0.055 (±0.0074)	0.093 (±0.0089)	0.113 (±0.0114)	0.024 (±0.0039)	0.026 (±0.0032)

B

Group	AverageTotal Length (TL)	Disc Length / TL	Branchial Length / TL	Prebranchial Length / TL	Eye Diameter / TL	Postorbital Length / TL
1 vs. 2	NS	*	NS	NS	*	*
1 vs. 3	NS	*	*	*	*	*
1 vs. 4	*	NS	*	NS	*	*
1 vs. 5	NS	*	*	*	NS	NS
2 vs. 3	NS	*	*	*	*	NS
2 vs. 4	*	*	*	NS	NS	*
2 vs. 5	*	*	*	*	*	NS
3 vs. 4	*	*	*	*	*	*
3 vs. 5	*	*	NS	*	*	NS
4 vs. 5	NS	NS	*	*	*	*

Developmental biology studies

In one sample of 24 out of 111 Morrison Creek lampreys collected in 1984, there were 79% males and 21% females (Beamish 1985). The gonad of these males was almost mature and the intestine was fully functional. The gonad contained mature spermatozoa, but they were not free within the coelomic cavity (Youson & Beamish 1991). In contrast, female Morrison Creek lampreys and some other male Morrison Creek lamprey were not mature and they had a less developed intestine and liver (Youson & Beamish 1991). The gonad development of the Morrison Creek lamprey also differed from *L. ayresii* from the Strait of Georgia, as juvenile male *L. ayresii* did not show any gonad development (Youson & Beamish 1991). The gonad development of most male Morrison Creek lamprey was more typical of the development found in nonparasitic lampreys (Hardisty & Potter 1971; Potter 1980a, b) and like *L. richardsoni*. The *L. richardsoni* from Morrison Creek had an atrophied intestine and a distinct cranial mass. They also had sparse amounts of haemopoietic tissue compared to all male Morrison Creek lamprey that had extensive concentrations of haemopoietic tissue (Youson & Beamish 1991). The Morrison Creek lamprey retained significant portions of the larval kidney which normally degenerates during metamorphosis. In fact, the Morrison Creek lamprey was found to be unique among holarctic lampreys because it lacked a cranial pancreas (Youson et al. 1988; Youson & Elliott 1989). It appeared that some Morrison Creek lamprey at the time of capture had not completed internal metamorphosis despite having fully developed external characteristics that were typical of parasitic forms.

New studies in 2011 and 2012

Trapping was repeated in Morrison Creek in June and July 2011, 2012 near previous sites (Fig. 8-1). In 2011, 17 Morrison Creek lamprey were captured in 101 trapping days from five areas between June 8 and July 23. In 2012, there were four Morrison Creek lamprey captured in 165 days of trapping between June 1 and July 26 at four trapping sites. The area where the largest catches occurred was close to the area in the 1980s where the largest catches occurred. If catches in this site were at the centre of abundance, there were 9 Morrison Creek lamprey captured in 24 days in 2011 and 2 in 55 days in 2012 for a total of 11 in 79 days or an average of 0.14 lamprey/day for the two years of sampling. The total catch of 0.14 Morrison Creek lamprey/day in the two years is a major decline (92%) from the average catch of 1.5 lamprey/day in 1983, 1984, and 1987. The

Table 8-6. Polymerase chain reaction annealing temperature (T_m), number of alleles (A), allele size range in base pairs, primer sequences and GenBank accession number for lamprey loci. The type of repeat (imperfect or interrupted) and repeat length (dinucleotide, trinucleotide or both) for each microsatellite locus are also indicated.

Locus	Tm (°C)	A	Size range	Forward primer	Reverse primer	Repeat type	Repeat length	Accession No.
Lri-2	54	7	142-170	GGCTCTTACCGAACACCTG	CAGCGTGCTAACTGCTATCC	Imperfect	Di	HM594255
Lri-3DFO*	58	14	369-406			Imperfect	Di	HM594256
Lri-5DFO*	54	13	394-430			Imperfect	Tri	HM594258
Lri-7DFO*	49	6	183-198			Imperfect	Tri	HM594260
Lri-8	40	7	273-283	GCGAACGCCTATTAAGGC	TCTCCCTTGGGTCGATTC	Imperfect	Di	HM594261
Etr-2DFO*	62	10	196-241			Interrupted	Di	HM594249
Etr-3DFO*	60	7	246-267			Interrupted	Di/Tri	HM594250
Etr-5DFO*	60	22	276-356			Interrupted	Tri	HM594252
Lspn088	50	14	168-221	GGATAATCGTCAGCAGTGTT	TCCATCTCTCTCGTTACCAT	Imperfect	Di	AB209985
Lspn094	53	15	215-388	GGTGTTGACTGAATCGAACT	GTTCTCTAGAGCTGTCGCAC	Interrupted	Di	AB209986

*The initials DFO after the locus name indicate that the primer sequences have been modified from those originally published.

catch of 240 *L. richardsoni* compared to 21 Morrison Creek lamprey is a major increase in the catch of *L. richardsoni* compared to 1981 and 1987 when the total catch for the two years was 76 Morrison Creek lamprey and 41 *L. richardsoni* (Fig. 8-6A,B). Thus, the sampling in 2011 and 2012 identified a major change in the population of both life history types in the creek since 1987. In about 25 years, the catches of *L. richardsoni* relative to the Morrison Creek lamprey had increased approximately 21 times.

The average length of the 21 Morrison Creek lamprey was 125 mm (+/- 9.0) and the average length of all 269 *L. richardsoni* measured in the two years was 116 mm (+/- 10.4), Fig. 8-11). The length of the Morrison Creek lamprey was virtually unchanged (t test P>0.05), but the average length of the *L. richardsoni* was significantly larger (t test P<0.01), with an increase of 15 mm. In 2011, some of the spawning lamprey identified as *L. richardsoni* were large bodied (Fig. 8-2B) relative to other Morrison Creek lamprey and ammocoetes that were found in the creek. It is possible that the brown coloured, mature specimen in Fig. 8-2B is a Morrison Creek lamprey that had fed in the creek in the summer of 2010.

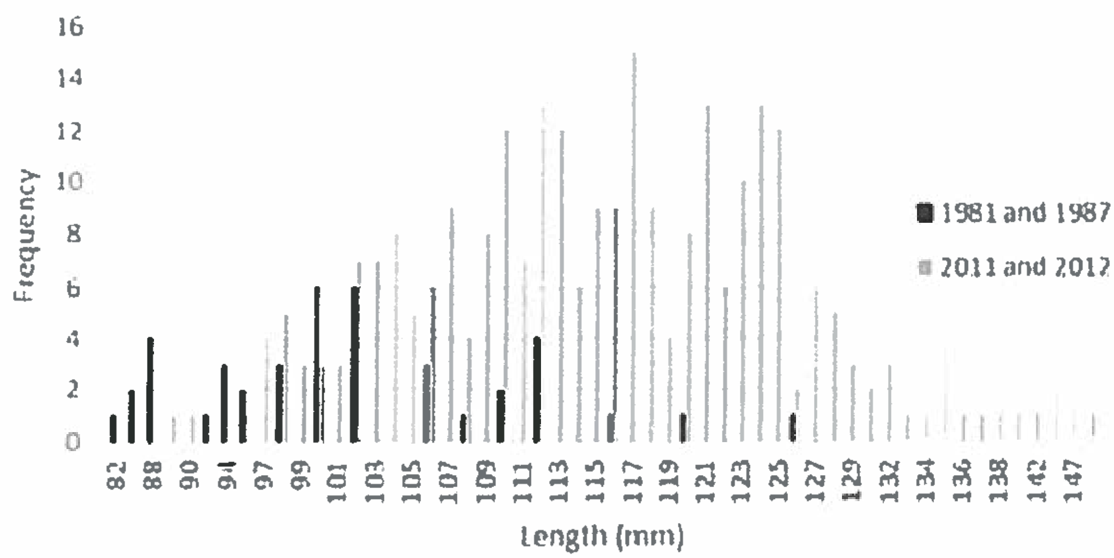

Figure 8-11. Length frequencies of adult *L. richardsoni* captured in1981 and 1987 (n= 41) and in 2011 and 2012 (n=269) show the large increase in length between the two sampling periods.

Genetic relationships

Sample Collection and DNA extraction

Lamprey tissue samples for genetic analysis were collected from three freshwater and one marine location in British Columbia between 2010 and 2012. Freshwater samples consisted of adult *L. richardsoni* from Morrison Creek (n=173) and Zolzap Creek (n=25) in northern British Columbia and, ammocoetes from the Cowichan River (n=85) on Vancouver Island. Ammocoetes were identified using the pigmentation pattern in the caudal fin (Richards et al. 1982). Samples of adult Morrison Creek lamprey (n=19) were from the trapping studies in Morrison Creek in 2011 and 2012. Samples (n=125) of *L. ayresii* were obtained from the Strait of Georgia using both seine and trawl nets. Tissue samples consisting of fin clips from the dorsal or caudal fins were preserved in individual tubes of 95% undenatured ethanol. Genomic DNA was extracted from tissue samples using a standard Qiagen 96-well Dneasy® procedure.

PCR amplification of microsatellite loci

For each sample, DNA amplification was conducted for 10 microsatellite loci (Table 8-6A) with the original or modified primer sequences developed for *L. richardsoni* (Lri-2,-3,-5-,-7, and -8) (Luzier et al. 2010), *E. tridentatus* (Etr-2,-3, and -5) (Spice et al. 2011) and *Lethenteron* (Lspn088 and 094) (Takeshima et.al. 2005).

In general, PCR DNA amplifications were conducted using a DNA Engine Tetrad2 thermal cycler (BioRad, Hercules, California) in 5-μL volumes consisting of 0.14 units of HotStar Taq DNA polymerase (Qiagen), 1 μL of undiluted DNeasy extracted DNA, 1× PCR buffer, 84 μM of each nucleotide, 0.50 μM of fluorescently labelled M13 forward primer (TGTAAAACGACGGCCAGT), 0.13 μM of M13 tailed forward primer, 0.50 μM of reverse primer with 5' GTTT consensus, and deionized water. The thermal cycling profile involved one cycle of Taq activation for 15 min at 95°C followed by cycles of denaturation for 30 s at 94°C, annealing for 30 s, and extension for 30 s at 72°C; and a final extension for 10 min at 72°C. Annealing temperatures for each locus are outlined in Table 8-6. Microsatellite alleles were size fractionated on an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software (v 4.0) using an internal lane sizing standard.

Genetic data analyses

Microsatellite diversity within and among lamprey samples was assessed using FSTAT (Goudet 2001). Within sample variation was measured as allelic richness (A_R) standardized to a sample size of 15 individuals, and gene diversity (H_E; expected heterozygosity). Significant deviations from Hardy Weinberg equilibrium were examined by sample and locus, with correction for conducting multiple tests. Among sample diversity was examined on a pairwise basis between all samples by estimation of significant differentiation in allele frequencies and F_{ST} values.

Contemporary effective population size (N_e) values for lamprey population samples were estimated from linkage disequilibrium (LD) between loci in the microsatellite data using NEEstimator 2.0 (Do et al., 2014). The LD method provides N_e values with good precision for small populations (those with N_e values $\leq$ 100) but for populations of larger size, less precise estimates are obtained (Waples & Do 2010). In NEEstimator software, rare alleles which may bias N_e estimation can be screened out with a P_{CRIT} parameter. The small sample size of some lamprey populations in this study (<30) necessitated the application of a relatively high P_{CRIT} value (0.10) to limit the effect of rare alleles in N_e estimation for lamprey samples.

Genetic variation within populations

All ten microsatellite loci surveyed in the lamprey of this study were polymorphic, with total allele numbers ranging from 6 to 22 among loci (Table 8-6). All five lamprey samples were in Hardy-Weinberg equilibrium at all loci except as follows. Significant shortages of observed heterozygotes compared with those expected occurred at Etr-2 in the Morrison Creek *L. richardsoni* and *L. ayresii* and at Lri-5 in the Cowichan River *L. richardsoni*, after correction for multiple comparisons (all $P < 0.05$). Gene diversity and allelic richness were greatest in the *L. ayresii* samples and lower but variable among all the *L. richardsoni* samples and Morrison Creek lamprey samples (Table 8-7).

Table 8-7. Sample size (N), expected heterozygosity (H_E) and allelic richness (A_R) standardized to a sample size of 15 individuals for ten microsatellite loci surveyed in lamprey from British Columbia, Canada.

Sample	Date	N	H_E	A_R
Cowichan River *L. richardsoni*	2012	85	0.52	3.6
Morrison Creek *L. richardsoni*	2011	173	0.58	4.2
Morrison Creek lamprey	2011-12	19	0.51	4.1
Strait of Georgia *L. ayresii*	2010	125	0.65	5.2
Zolzap Creek *L. richardsoni*	2012	25	0.62	4.7

Contemporary effective population size

Contemporary Ne estimates based on linkage disequilibrium in the microsatellite data ranged from 111 (Zolzap Creek *L. richardsoni*) to infinity (Cowichan River *L. richardsoni*). The confidence intervals of estimates were large, with an upper value limit of infinity in all cases. The lack of a finite N_e estimate for Cowichan River *L. richardsoni* reflected an absence of linkage disequilibrium in the sample, precluding an estimation of effective size for this population based on the sample included in the current study. The estimates of N_e provided by the Morrison Creek *L. richardsoni* and the Morrison Creek lamprey samples were similar (Table 8-8).

Table 8-8. Estimated contemporary effective population and jackknife 95% confidence interval size for lamprey populations sampled in this study.

Sample	N_e	Confidence Int.
Cowichan River *L.richardsoni*	∞	137 - ∞
Morrison Creek *L. richardsoni*	582	148 - ∞
Morrison Creek lamprey	544	17 - ∞
Strait of Georgia *L. ayresii*	208	100 - ∞
Zolzap Creek *L. richardsoni*	111	15 - ∞

Genetic diversity among populations

Allele frequencies differed significantly in all pairwise comparisons of lamprey samples ($P < 0.05$ after correction for multiple comparisons), except for the Morrison Creek *L. richardsoni*-Morrison Creek lamprey comparison. Pairwise F_{ST} values between samples ranged from 0.001 between the Morrison Creek *L. richardsoni* and the Morrison Creek lamprey samples to 0.154 between the Morrison Creek *L. richardsoni*, Morrison Creek lamprey and Cowichan River *L. richardsoni* samples. All F_{ST} values in pairwise comparisons of *L. richardsoni* samples from the three different watersheds (range 0.090 - 0.113) were greater than the values generated between the samples from the three populations of *L. richardsoni* and *L. ayresii* (range 0.036 - 0.059) (Table 8-9).

Table 8-9. Pairwise F_{ST} values (above diagonal) and significance of microsatellite allele frequency differentiation (below diagonal) for lamprey samples. Significance of allele frequency differentiation of $P < 0.05$ is denoted by an asterisk and $P > 0.05$ is denoted NS (nonsignificant).

Sample	Cowichan River *L. richardsoni*	Morrison Creek *L. richardsoni*	Morrison Creek lamprey	Strait of Georgia *L. ayresii*	Zolzap Creek *L. richardsoni*
Cowichan River *L.richardsoni*	-	0.113	0.154	0.059	0.090
Morrison Creek *L. richardsoni*	*	-	0.001	0.046	0.094
Morrison Creek lamprey	*	NS	-	0.074	0.119
Strait of Georgia *L. ayresii*	*	*	*	-	0.036
Zolzap Creek *L. richardsoni*	*	*	*	*	-

The genetic data support the interpretation that the Morrison Creek lamprey is closely related and still reproductively connected to the Morrison Creek *L. richardsoni*. Samples of the two life history types in Morrison Creek display no differentiation in allele frequencies and provide the same estimates of effective population size. If the Morrison Creek lamprey were reproductively isolated from *L. richardsoni* within the creek, the current low level of Morrison Creek lamprey abundance in the watershed would typically be reflected in a small N_e for the population. There are no estimates of N_e based on historical Morrison Creek lamprey samples, but the current estimate of an N_e in the hundreds or greater is discordant with the scarcity of individuals encountered through trapping efforts.

In contrast, the Morrison Creek life history type is not closely related to *L. ayresii*. The F_{ST} value between *L. ayresii* and Morrison Creek lamprey was greater than between *L. ayresii* and the Morrison Creek *L. richardsoni* refuting any likelihood that the parasitic Morrison Creek lamprey arose directly from *L. ayresii* or from hybridization between *L. ayresii* and *L. richardsoni*. The Morrison Creek life history type instead, appears to be an example of the ability of individuals within a nonparasitic species to revert back to the parasitic feeding type. Each of the three *L. richardsoni* populations sampled in the study was more closely related to the single *L. ayresii* population sampled than any of them were to each other (Fig. 8-12). This indicates that *L. richardsoni* are polyphyletic, having arisen independently from *L. ayresii* multiple times, consistent with the interpretation of the evolution of paired species by some authors (Docker 2009; Yamazaki et al. 2011). The degree of reproductive isolation between the anadromous and freshwater resident life history types is not well documented for many lamprey species pairs, but is apparently variable among species pairs (Docker 2009). For the *L. ayresii* and *L. richardsoni* in this study, the average pairwise distances between the three *L. richardsoni* populations were twice as great as those of the *L. richardsoni* and *L. ayresii* comparisons. This indicates that the establishment of the *L. richardsoni* populations of this region is relatively recent and/or that *L. ayresii* may not be reproductively isolated from some or all *L. richardsoni* populations. Nevertheless, this study clearly demonstrated that in Morrison Creek lamprey did not arise from hybridization between *L. richardsoni* and *L. ayresii*. Instead, our results indicated that at least some *L. richardsoni* populations retain the capacity for facultative parasitism, consistent with a relatively recent derivation of *L. richardsoni* populations from *L. ayresii* in this geographic area.

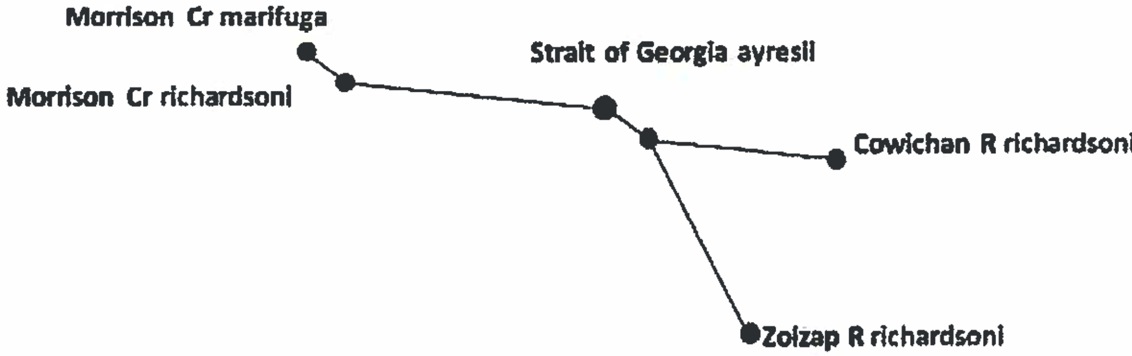

Figure 8-12. Dendrogram of genetic relationships among three *L. richardsoni* and a single *L. ayresii* population in British Columbia, Canada with branch lengths based on pairwise F_{ST} values at microsatellite loci as listed in Table 8-9. The very close genetic relationship of the Morrison Creek lamprey to the Morrison Creek *L. richardsoni* population and its differentiation from *L. ayresii* (and both other *L. richardsoni* populations) are also depicted.

A recent genomic study of limited samples of the paired European species *L. fluviatilis* and *L. planeri* revealed strong differentiation at loci throughout the genome between the sympatric anadromous parasitic and freshwater nonparasitic life history types from a single watershed (Mateus et al. 2013). Candidate loci for some strongly differentiated SNPs (single nucleotide polymorphisms) were associated with presumptive adaptive functions that expected to be associated with life history and morphometric differentiation. A genomic comparison of adaptive loci in *L. ayresii* and *L. richardsoni* would likely reveal similar differentiation, and provide tools with which to study the re-emergence of parasitism (e.g. Morrison Creek lamprey) in nonparasitic *L. richardsoni* lamprey populations.

Discussion

The genetic data provided convincing evidence that the nonparasitic *L. richardsoni* in Morrison Creek produced the parasitic Morrison Creek lamprey. The genetic data and the morphometric data showed that the parasitic life history type is of *L. richardsoni* origin. It is also clear that the Morrison Creek lamprey is not a freshwater resident form of *L. ayresii* as has been reported in some publications (Renaud 2011; Potter et al. 2015).

There is indirect evidence that the nonparasitic lamprey, *Lethenteron. appendix* produces a parasitic life history type (Manion and Purvis 1971; Cochran 2008). But the examples are rare and the interpretation disputed (Vladykov and Kott 1980). Thus, the Morrison Creek lamprey population remains as the only documented example of a population of nonparasitic lampreys that produce a parasitic life history type.

The parasitic life history type in Morrison Creek was originally only known to feed and grow in the laboratory, but specimens captured in 2011 and 2012 in spawning condition were larger than the silver coloured Morrison Creek lamprey. The size increases were small, suggesting that feeding might be limited to scavenging, but it is an indication that despite developmental issues in some specimens after metamorphosis (Youson & Beamish 1991; Youson 2004), the Morrison Creek lamprey could mature and reproduce successfully.

There has been a change in the population structure over the past 25 years. The data we collected would be comparative but not usable to make absolute abundance estimates. Recognizing these constraints, we found that the trapping in 2011 and 2012 showed that after 25 years, there was a 91% decline in the catch of the Morrison Creek lamprey and a 21 times increase in the catch of *L. richardsoni*. In addition, there was a large increase in the average length of the *L. richardsoni*, but no change in the average length of the Morrison Creek lamprey.

We can only speculate that over a 25 year period, Morrison Creek had become more productive. If this occurred, it might have benefited the ammocoetes that became the nonparasitic *L. richardsoni* and it may suggest that growth rates in the early life of the ammocoetes affected the production of a life history type as proposed by Enequist (1937) and Kux (1967). Youson (2004) also discusses the possibility that environmental conditions early in the growth of an ammocoete can influence the genetically controlled metabolism later in life. Accordingly, poor feeding conditions in the early ammocoete stage would result in reduced growth and reduced energy (lipid) reserves that would be needed for a metamorphosed nonparasitic lamprey. The reduced growth according to Youson (2004), could result in an "unsynchronized metamorphosis" relative to the ammocoetes that had rapid growth because rapid growth was associated with an accumulation of sufficient energy reserves needed for normal metamorphosis and rapid sexual maturation typical of the nonparasitic life history type. The very small size of the *L. richardsoni* in the early 1980s and the much larger size in 2011 and 2012 seems to fit this possible explanation for the Morrison Creek lamprey to result from a population that had the genetic capability of being influenced by environmental conditions. However, we suspect that the stimulus to produce the Morrison Creek lamprey is more complex. In the early years of the study, a sample of 18 and a second sample of 4 recently metamorphosed lamprey were held in the laboratory until they were in spawning condition. During this development, only three of the 21 lamprey developed the silver colour of the Morrison Creek lamprey, yet all were about the same size during metamorphosis although their age was unknown. Kucheryavyi et al. (2007) describe a population of the Arctic lamprey, *Lethenteron camtschaticum* that produces anadromous parasitic forms and a resident form that apparently does not feed. A small anadromous "praecox" form was reported to be 92% males which is similar to the high percentage of Morrison Creek lamprey that were males. Yamazaki and Nagai (2013) also document genetic changes in an anadromous lamprey population that could lead to speciation. However, these are examples of a population of a parasitic lamprey that produced a nonparasitic derivative in contrast to the Morrison Creek population. Most importantly, these examples remain extremely rare. We suggest that there is more than an environmental stimulus that affects the selection of life history type and it may be that the mechanism is not found within the genotype of all nonparasitic populations. An increase in the productivity in Morrison Creek may have directly or indirectly decreased the survival of the Morrison Creek lamprey as well as altered the stimulus to produce the parasitic life history type. A decreased survival would greatly reduce spawning events for the Morrison Creek lamprey and these events may be a necessary part of maintaining the population as might be occurring in the Kucheryavyi et al. (2007) study.

There is evidence that the structure and genetics of lampreys is more complex than previously thought. Boguski et al. (2012) have identified molecular differences in four populations of ammocoetes that were sufficient for them to consider the populations as distinct species. Mateus et al. (2013) considered the genetic and morphometric differences they found in populations of adult *L. planeri* to distinguish three new species. At the same time, other investigators using molecular studies are proposing that recognized paired species are one species with two ecotypes (Espanhol et al. 2007; Docker et al. 2012). In our study, we show that there are taxonomic differences between *L. richardsoni* from rivers other than Morrison Creek and samples of feeding *L. ayresii*. In addition to morphological differences, the number of cusps on the teeth on the tongue clearly distinguishes the two species. These differences are consistent with distinctions used to separate *L. richardsoni* from *L. planeri* and *L. ayresii* from *L. fluviatilis*.

Within the Morrison Creek population, the morphometric and genetic analyses place the population closer to *L. richardsoni* than to *L. ayresii*. The enigma is how to recognize the lamprey in Morrison Creek as the only documented example of a population of nonparasitic lamprey that produces a parasitic derivative. Thus, the labelling of the Morrison Creek lamprey is now part of a rapidly increasing issue of how to find a taxonomy that recognizes the complexity that is being discovered within lamprey populations. Until these larger issues are resolved, we propose that all lamprey in Morrison Creek, other than *E. tridentatus,* be recognized either as *L. richardsoni* var. *marifuga* or as a subspecies *L. richardsoni marifuga* with the common name, Morrison Creek lamprey. The subspecies designation would ensure that the Morrison Creek lamprey is recognized in the taxonomic literature as.an unique population.

The absence of prior observations of a freshwater resident nonparastic lamprey giving rise to a parasitic form (Docker 2009; Docker et al. 2012) indicates that either the Morrison Creek environment or the *L. richardsoni* population it houses, or both, have highly unusual characteristics. If the population of both life history types are protected, there will be an opportunity for future study. Units below the species level have been recognized for protection if they are discrete and significant (Waples 1991; COSEWIC 2010). Significance is interpreted to be a population that is an important component of the evolutionary legacy of a species (Waples 1991). The discovery of a discrete population of nonparasitic lamprey that produces the parasitic derivative of the species, but most closely related to the nonparasitic species is unique and significant. Thus there should be little doubt that the Morrison Creek lamprey and the lamprey considered to be *L. richardsoni* in Morrison Creek should receive the protection previously given only to the parasitic form recognized as *L. richardsoni* var. *marifuga*. It is possible that hidden within the genetic structure of this population are the keys to understanding the production of paired species (Zanandrea 1959, Vladykov & Kott 1979; Potter 1980; Docker 2009) and possibly even to the success of lampreys on the planet over the past 360 millions of years (Gess et al. 2006).

Future research

Genomic characterization of adaptive variation in the Morrison Creek population and comparison with other *L. richardsoni* populations will be required to provide an understanding of the origin of the parasitic life history type in the population. For this reason, the entire Morrison Creek population, including both life history types, warrants some level of taxonomic recognition and enhanced conservation measures. Monitoring of Morrison Creek lamprey needs to continue about every two or three years, but all specimens should be returned to the creek unharmed. An immediate need is to ensure that the habitat that is critical to the survival of both life history types in Morrison Creek lamprey is protected. Spawning areas for lamprey in Morrison Creek should be identified and protected.

Summary

In Morrison Creek, on Vancouver Island, Canada, there is a parasitic lamprey that was thought to be *L. ayresii*. Studies showed that it was unable to osmoregulate in salt water and differed from *L. ayresii* in body proportions and the number of cusps on the tongue. Because of these differences, the parasitic life history type was given the name *Lampetra richardsoni* var. *marifuga* and a common name of the Morrison Creek lamprey. Subsequently, a DNA analysis showed that the Morrison Creek lamprey is of *L. richardsoni* origin and that it originated within the Morrison Creek *L. richardsoni* population. The Morrison Creek lamprey was not a freshwater resident form of *L. ayresii* as had been proposed by several authors. Despite the developmental anomalies that occur in the metamorphosed Morrison Creek lamprey, it was feeding, growing and probably reproducing. The population becomes the first example of the derivation of a parasitic life history type from an established nonparasitic life history type.

A recent survey to determine the status of the Morrison Creek lamprey identified a decline in catch of 91% over 25 years while the catches of the nonparasitic life history type increased to be 21 times larger than earlier catches relative to the Morrison Creek lamprey. At the same time, the nonparasitic lamprey increased in size while the parasitic life history type remained at the same average size. The decline in abundance of the Morrison Creek lamprey may relate to an increase in the productivity of the creek that may have reduced the production of the Morrison Creek lamprey or its survival or both.

Because it is now known that one population produces both life histories, and this is the only documented example of a nonparasitic species producing a parasitic life history type, it is proposed that both life history types in the population be given the designation of either *L. richardsoni* var. *marifuga* or recognized as a subspecies *L. richardsoni marifuga*. This population should be given the protection already assigned to the Morrison Creek lamprey because of the importance in understanding the speciation process in lampreys.

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