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# The Importance of Size as an Isolating Mechanism in Lampreys

RICHARD J. BEAMISH AND CHRYS-ELLEN M. NEVILLE

**Relatively small differences in size reduced spawning success in crosses of two paired species of lampreys. Typical spawning behavior occurred between the two species in all experiments, but minimal fertilization occurred when females were more than about 20% larger or smaller than males. The results support the hypothesis of Hardisty and Potter (1971) that homogamy is the general mechanism that results in the establishment of paired species of lampreys.**

SPECIES of lampreys that appear similar morphologically but have different life-history types are called paired species (Zanandrea, 1959; Hardisty and Potter, 1971) or satellite species (Vladykov and Kott, 1979) if more than one derivative exists. There are approximately 40 species of lampreys in the world, 20 of which are paired or satellite species (Vladykov and Kott, 1979). In British Columbia, there are two paired species, the river lamprey *Lampetra ayresi*

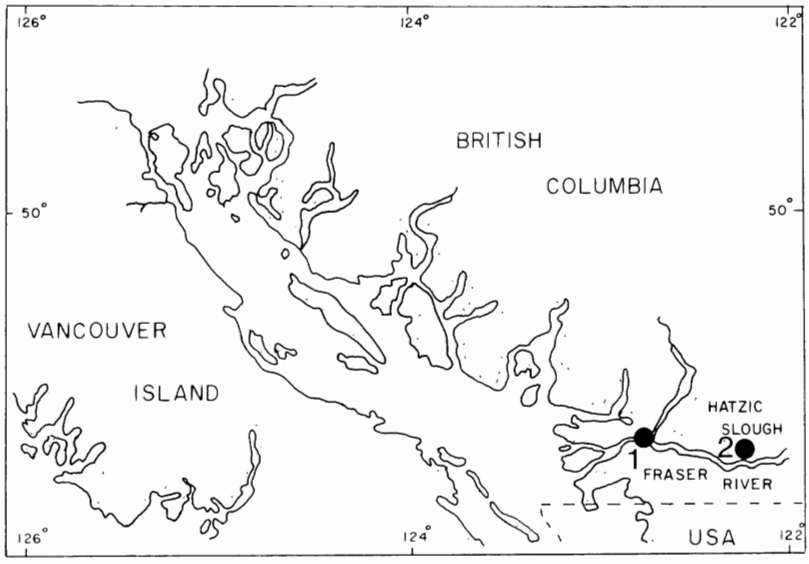


Fig. 1. Collection areas of (1) *L. ayresi* and (2) *L. richardsoni* in British Columbia.

and the brook lamprey *L. richardsoni*. The river lamprey is an anadromous lamprey that is abundant in the Fraser River (Beamish, 1980; Beamish and Youson, 1987) but is found in only one other river in British Columbia (Withler, 1955). Because *L. ayresi* feeds by removing large amounts of tissue (Beamish, 1980), we consider this species to be predatory rather than parasitic. In the Fraser River, river lamprey occur in the main stem of the river, from approximately the city of Hope to the mouth of the Fraser River (Fig. 1). Brook lamprey occur in the slower moving rivers flowing into the Fraser River but have not been found in the same location as river lamprey. Brook lamprey are common in many rivers in British Columbia (Beamish, unpubl.).

Enequist (1937) and Privolnyev (1964) suggested that paired species were not distinct species but ecological races of a single species. The more common belief is that they are distinct but closely related species that occur in most genera. It is believed that the nonparasitic paired species evolved from parasitic species (Hubbs and Trautman, 1937; Zanandrea, 1959; Hardisty and Potter, 1971). This trend to produce nonparasitic species has been repetitive (Hubbs and Potter, 1971) and is considered unique among vertebrates (Vladykov and Kott, 1979).

Hardisty and Potter (1971) have proposed that body size is a barrier to interbreeding. They believe that genetic isolation occurs because of the operation of homogamy. The spawning act is dependent on the application of pressure on the female by the male and a precise positioning of the genital apparatus of the two sexes. Because paired species differ in size, homogamy would be the mechanism that reproductively isolates them. This hypothesis of homogamy is of fundamental importance in the understanding of lamprey phylogeny and the development of life-history types, yet it has not been tested.

## Materials and Methods

Young adult *L. ayresi* were collected from the Fraser River during the downstream migration in 1987. They were raised to maturity in the laboratory because no one has been able to collect upstream migrating river lamprey from the Fraser River.

Metamorphosed *L. richardsoni* were collected from Hatzic Slough (Fig. 1). In mid-May, when the *L. richardsoni* began showing signs of sexual maturity, males and females were separated into different tanks. Sexual maturity in the slower maturing *L. ayresi* was induced by increasing the water temperature two to three degrees above ambient temperature (Richards et al., 1982).

Experimental spawning tanks were designed to simulate the natural stream bed by providing a variety of substrates (approx. 0.5 mm to 5.0 mm) and a slight current over the substrate. Large rocks (approx. 7 cm to 20 cm) were placed

TABLE 1. SPAWNING SUCCESS OF MALE AND FEMALE *L. richardsoni* AND *L. ayresi*.

| Experimental crosses | Date | Female (cm) Length | Female (cm) Mean | Spawning success[a] | Male (cm) Length | Male (cm) Mean | Female/male ratio |
|---|---|---|---|---|---|---|---|
| 1 *L. richardsoni* ♂ × *L. ayresi* ♀ | May 17–June 1 | 18.7 | 18.0 | 3 | 15.5, 14.5, 15.3 | 15.1 | 1.19/1 |
| | | 17.3 | | 1 | | | |
| | | 17.9 | | 1 | | | |
| 2 *L. richardsoni* ♀ × *L. ayresi* ♂ | May 17–May 29 | 15.4 | 15.7 | 1 | 18.6, 18.8, 19.5 | 19.0 | 0.83/1 |
| | | 14.6 | | 2 | | | |
| | | 17.2 | | 1 | | | |
| 3 *L. richardsoni* ♂ × *L. ayresi* ♂ | May 18–June 22 | 20.3 | 20.6 | 3 | 14.5, 14.3, 13.2, 13.4, 13.4, 13.3, 13.2 | 13.6 | 1.51/1 |
| | | 20.5 | | 3 | | | |
| | | 21.0 | | 3 | | | |
| 4 *L. richardsoni* ♀ × *L. ayresi* ♂ | May 17–June 28 | 12.6 | 12.2 | 3 | 20.3, 20.8, 19.7, 18.9 | 19.9 | 0.61/1 |
| | | 12.0 | | 3 | | | |
| | | 12.1 | | 3 | | | |
| 5 *L. ayresi* ♂ × *L. ayresi* ♀ | May 25–June 6 | 18.1 | 18.1 | 1 | 19.0, 20.0, 21.0, 19.6 20.8, 20.4 | 20.1 | 0.90/1 |

[a] 1. Successful spawning, ovary spent or partially spent and squeeze marks; 2. Spawning not successful, ovary mature, no evidence of a loss of eggs but squeeze marks; 3. Spawning not successful, ovary mature, no evidence of a loss of eggs and no squeeze marks.

at random on this substrate to provide areas of cover for the lamprey. The bottom surface areas of the three sizes of experimental tanks used were 1.16 m², 2.39 m², and 2.89 m². Tanks were supplied with dechlorinated City of Nanaimo fresh water at ambient temperature (12–13 C) and an air supply.

Four experimental crosses were conducted; small *L. ayresi* females and large *L. richardsoni* males, large *L. richardsoni* females and small *L. ayresi* males, small *L. richardsoni* females and large *L. ayresi* males, and large *L. ayresi* females and small *L. richardsoni* males (Table 1). A control, containing female and male *L. ayresi*, was handled identically to the experimental crosses. Three to seven lampreys of each sex were placed together in the experimental tanks. Numbers of lampreys in the experiments were restricted because of the difficulty in obtaining mature *L. ayresi*. Observations of spawning activity were made one to two times daily. Any spawning behavior or sign of spawning activity was documented. Dead lampreys were removed from the tank, measured, weighed, and examined for maturity and evidence of spawning activity (squeeze marks). Observations continued until all spawning activity stopped.

After the lampreys were removed from the tanks, the sand and gravel was sieved through a series of screens ranging from 2 mm to 500 μm. Eggs that were retained on the 500 μm screen were counted and transferred by pipette to salmon egg incubation trays suspended in flowing fresh water at ambient temperature. Trays were checked daily, and dead eggs with fungus were removed with pipettes. After a 25-day period, a final count of hatched embryos was made.

Following successful spawning by one female *L. ayresi* in the control, the remaining females were removed, and their eggs were artificially crossed with *L. richardsoni* males. Fertilized eggs were reared to hatching.

In a previous experiment in 1981, larvae from an artificial cross of *L. ayresi* females and *L. richardsoni* males were put into a simulation stream tank (100 cm × 300 cm). Fine sand (7 cm deep) and a few rocks covered the bottom of the tank. The ammocoetes were fed a nutrient solution of green algae combined with Brewer's yeast (Hanson et al., 1974) and yeast extract. The tank was electroshocked periodically to collect lampreys and record their length and weight. High larval mortality rates resulted in the termination of the study in Jan. 1984.

## RESULTS

Experimental crosses started on 17 May. The *L. richardsoni* matured later than *L. ayresi*; however there was sufficient overlap in the spawning period to carry out the experiments.

In experiment 1 (Table 1), the female to male length ratio was 1.19 to 1 (1.8 to 4.2 cm or

<22% difference). Typical nesting and spawning behavior was observed within two days. One female *L. ayresi* (17.9 cm) spawned completely within four days. This female had squeeze marks on her body that typically occur when males attempt to squeeze out eggs. The second and largest female (18.7 cm) died on 21 May without spawning. Spawning activity stopped 30 May, and the last female (17.3) was removed 1 June. This female had squeeze marks on the body; however about 90% of the eggs had not been released. No males died. On 7 June, the substrate was sieved, and approx. 400 eggs were found and placed in incubation trays. Approximately 33% of these hatched by 14 June.

In experiment 2 (Table 1), the female to male length ratio was 0.83 to 1 (1.4 to 4.9 cm or <25% difference). Spawning activity was observed within one day. A partially spawned female (15.4 cm) with squeeze marks died after two days. Within four days, the remaining two females had squeeze marks but showed no evidence of having released eggs. One of these females (14.6 cm) died four days later on 25 May without spawning. On 29 May, the third female (17.2 cm) was dead and partially spawned. The substrate was sieved on 3 June, and approx. 70 eggs were collected and placed in an incubation tray. By mid-June about 33% had hatched.

In experiment 3, the female to male length ratio was 1.52 to 1. The female *L. ayresi* were considerably larger (5.5 to 7.8 cm or >29% difference) than the male *L. richardsoni*. Spawning activity started 29 May, 12 days after the experiment started. By this time, one male had died. Spawning activity and nest building by one male and female continued for three days to 1 June. The male exhibited normal spawning behavior by attaching dorsally to the head or gliding up and down the body of the female with its disc. However, the male was unable to coil its body around the female despite forming coils in its own body. Also, the male frequently could not remain attached to the head of the female, perhaps because its own coiling action reduced suction. On 6 June, one female died without spawning. On 9–10 June, spawning and nest building continued with two males attempting to spawn with one female. By 18 June, the second female died without spawning. The third female died on 22 June without spawning after two males attempted to spawn with her on 21 June. Although no squeeze marks were observed on any of the females in this experiment, there were 24 eggs found in the substrate after the dead females were removed. These eggs were incubated in hatching trays, and approx. 18% hatched.

The female to male length ratio in experiment 4 was 0.61 to 1. Female *L. richardsoni* were considerably smaller than the male *L. ayresi* (6.3 to 8.8 cm or >33% difference). One female and one male began nest building on 17 June. Initial spawning attempts by the male apparently were unsuccessful because the loop made by the male formed distal to the cloacal opening of the female. Males died on 20, 26, and 27 June. Females died between 26 and 28 June without any indication of having spawned. About 115 eggs were retrieved from the tank and were incubated. Approx. 20% of these hatched.

The female to male length ratio in the control was 0.90 to 1 (0.9 to 2.9 cm or <13% difference). The spawning activity in the *L. ayresi* control started immediately when the males and the female were combined on 25 May. The female (18.1 cm) exhibited normal spawning activity and had completely spawned by 30 May. When the substrate was sieved, approx. 400–500 eggs were found, and approx. 45% hatched in the incubation trays.

Approximately 4000 eggs were fertilized in the artificial crosses of *L. ayresi* females and *L. richardsoni* males. One-third of these eggs hatched. Egg mortalities apparently resulted from fungus development. The hatching rate in this experiment was similar to the hatching rate in the 1981 artificial crosses that were reared for 2½ years (Table 2).

The pigmentation patterns of the ammocoete crosses reared in 1981 were intermediate between *L. ayresi* and *L. richardsoni* (Fig. 2). *Lampetra ayresi* are weakly pigmented in the suborbital, prebranchial-, branchial-, and caudal-ridge areas in comparison to *L. richardsoni*. Crosses have an intermediate amount of pigmentation. In particular, the crosses have reduced pigmentation in the vicinity of the eye spot compared to *L. richardsoni*. Crosses also have reduced pigmentation in the prebranchial and suborbital areas compared to *L. richardsoni* but are more pigmented dorsally and branchially than the *L. ayresi*.

## Discussion

In this study, there was an effect of size on reproduction and no effect of size differences or species differences on spawning behavior. Fe-

male to male length ratios of close to 1 to 1 resulted in successful spawning. When length ratios differed by more than 20%, spawning success declined. At length ratio differences larger than approx. 25%, spawning was not successful. We believe this was related to the ability of the male to squeeze eggs from the female.

The most successful spawning, other than the control, occurred when the average female to male length ratio was between 1.19 to 1.00 and 0.83 to 1.00. Spawning success decreased when the individual female to male ratio exceeded these ratios (experiment 1, *L. ayresi* female 18.7 cm; experiment 2, *L. richardsoni* female 14.6 cm; Table 1). When males were much smaller or larger than females (average female to male length ratio of 1.51 to 1.00 and 0.61 to 1.00), none of the males produced squeeze marks on females. Despite this problem, some eggs were released and fertilized possibly from the vigorous movements of the males and females during the spawning attempts.

The size of *L. richardsoni* used in this study varied from 12.0 cm to 17.2 cm. It would be possible for a population with this size range to have spawning pairs with female to male length ratio of 1.43:1. As demonstrated in this study, such ratios reduced spawning success.

TABLE 2. LENGTHS OF *L. ayresi* FEMALES AND *L. richardsoni* MALES FROM ARTIFICIAL CROSSES CONDUCTED IN 1981.

| | October 1981 | June 1982 | May 1983 | January 1984 |
|---|---|---|---|---|
| Mean length (cm) | 1.65 | 3.64 | 4.40 | 4.84 |
| Range (cm) | 1.2–2.4 | 1.1–5.1 | 3.6–5.4 | 4.4–5.3 |

In our study, we examined the spawning success and not fertilization efficiency. Malmqvist (1983) used a narrow range of sizes of males and females of one species to study fertilization efficiency. His results indicated that, even with a large release of eggs, there is a reduction in fertilization related to increased size differences. This indicates that, in addition to a decrease in the numbers of eggs released, there is a declining rate of fertilization as the differences in size increased. These observations of size-assortive mating indicate that relatively small differences in size will affect spawning success and fertilization efficiency both between species and among individuals of the same species. This size difference occurs between the life-history types in paired species. Therefore homogamy,

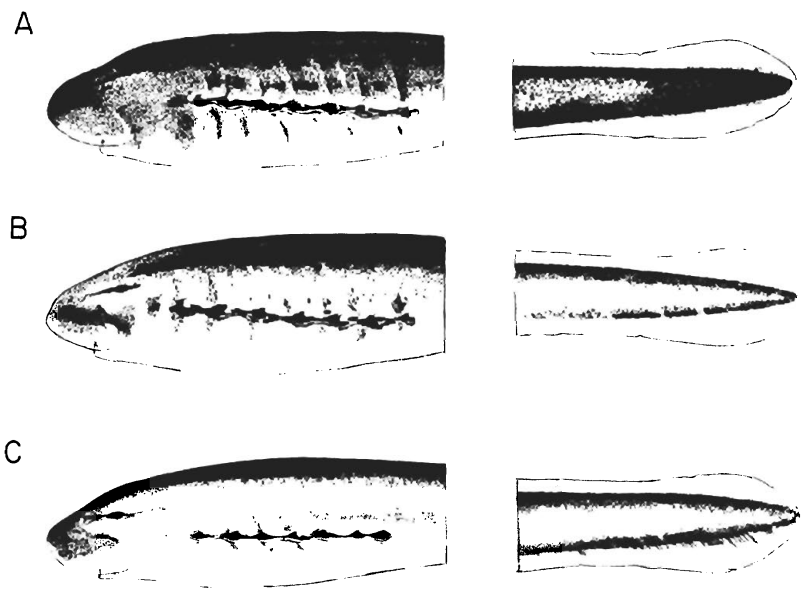


Fig. 2. Head and tail pigmentation of ammocoetes from (A) *L. richardsoni*, (B) artificial crosses, and (C) *L. ayresi*.

based on body size, is an important mechanism for maintaining reproductive isolation between paired species.

There was some successful fertilization in all experiments in this study indicating that other barriers to hybridization probably exist. The pigmentation of ammocoetes of these two species suggests that the habitats are different. *Lampetra ayresi* has much less pigment than does *L. richardsoni* (Fig. 2), possibly because the heavy sediment load of the Fraser River reduces light penetration. In the Fraser River drainage, we have found *L. ayresi* only in the main section of the river. In contrast, *L. richardsoni* is widely distributed, occurring in most river systems but not in the main stem of the Fraser River. Also, we have not found an ammocoete in the Fraser River drainage that was similar to our artificially produced hybrid. It is probable, therefore, that habitat differences as well as size differences maintain the reproductive isolation of these two species. Although the mechanism for producing nonparasitic species is not understood, we believe that this study confirms that the principle of homogamy proposed by Hardisty and Potter (1971) is a powerful isolating mechanism once the new life-history type is formed.

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Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6 Canada. Accepted 23 Jan. 1991.