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Morphometric Changes during Growth of the Brook Lamprey *Lampetra reissneri*

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Abstract The brook lamprey (*Lampetra reissneri*) was collected at the Hosshoji River, Tottori Prefecture, Japan, once a month for a year. Metamorphosing individuals were collected in autumn and immature adults in winter. Ammocoetes larvae were collected throughout the year. From size-frequency histograms, the larval period was estimated to be more than three and one-half years. Ammocoetes larger than 160 mm in total length were always female. Metamorphosing and adult females were larger than corresponding males. This may mean that at least some females metamorphose one year later than males. In ammocoetes, the relative prebranchial and branchial length decreased, and the relative tail length increased during growth. Egg diameter increased rapidly during metamorphosis.

The cyclostomes, hagfishes and lampreys, are extant agnathans and supply critical material for evaluation of lower vertebrate phylogeny (Janvier and Blieck, 1979; Novitskaya, 1981). To persons who work in this field, the natural history of cyclostomes should be well-documented to ensure a constant supply of animals and for general background knowledge. In a previous paper, we reported seasonal migration and annual gonadal changes of the hagfish, *Eptatretus burgeri* (Tsuneki et al., 1983). In this and a following paper (Tsuneki and Ouji, in press), we will report the life history of the non-parasitic brook lamprey, *Lampetra reissneri* (Dybowski), with special reference to changes in the external morphology and in some internal organs. The life history of non-parasitic lampreys has been extensively studied in European and North American rivers (Hardisty, 1944, 1961a; Hardisty and Potter, 1971a, b; Rohde et al., 1976; Malmqvist, 1978; Seagle and Nagel, 1982), but only a few reports (Yamada, 1951) are available in Far Eastern rivers. Morphometrical studies will be dealt with in this paper, and histological studies in the following paper.

Materials and methods

Lampetra reissneri was collected at the Hosshoji River, Tottori Prefecture, Japan. This is a tributary of the Hino River which empties into the south-western part of the Japan Sea. The lamprey were distributed along at least 6 km of the mid-stream of the Hosshoji River. The materials for this study were collected exclusively at "Point G" (35°20′20″N, 133°16′52″E). Collections were made on about the 10th of each month from January through December, 1982. A shovel and net were used for the capture.

The lamprey were carried to the laboratory, and anesthetized on the day after collection. Ammocoetes became motionless about 1 to 2 min after immersion in 0.05% MS 222 at about 15°C. Adult lampreys became motionless after about 30 sec in the same conditions.

After anesthesia, the following measurements were made: body weight, total length (TL), prebranchial length (from the tip of the snout to the anterior rim of the first branchial pore), branchial length (from the anterior rim of the first branchial pore to the posterior rim of the last branchial pore), trunk length (from the posterior rim of the last branchial pore to the anterior rim of the cloaca) and tail length (from the anterior rim of the cloaca to the tip of the tail). Lengths were measured to the nearest 0.1 mm with vernier calipers. If the sum of prebranchial, branchial, trunk and tail length was different from TL by more than 2%, such measurements were not used for further calculations (4 out of 252 individuals). Trunk myomeres were counted with the naked eye. Such counting was possible only in larger individuals (usually more than 100 mm in TL).

Metamorphosis stages were determined according to criteria given for *Lampetra planeri* (see Bird and Potter, 1979a) with slight modifications as described below. Stage 1^- individuals are those in which the eye is hardly visible externally. Stage 1 individuals were not collected. Stage 2 individuals are characterized by the distinctly visible eye. Stage 3 individuals possess a crescent-shaped white area in front of and behind the dark eye. Stage 4 individuals are characterized by the white eye totally circumscribing the dark eye. At this stage, indistinct teeth are observable. Stage 5 individuals are characterized by distinct teeth. The external appearance of the branchial region of stages 4 and 5 individuals was slightly advanced compared to equivalent stages for *L. planeri*. Stage 6 (macrophthalmia stage) and stage 7 (adults without secondary sex characters) were treated together as immature adult stage. When the lamprey were kept overnight in a bucket of sand, adults remained in water, but ammocoetes and metamorphosing animals earlier than stage 5 concealed themselves in sand.

In adults, gonadal weight (fresh) and diameter of eggs (histological preparations) were measured. Gonado-somatic index was calculated as follows: (gonadal weight/body weight) × 100.

The results were statistically analyzed. To ascertain homogeneity of variance, a *F*-test was performed prior to a *t*-test, and Bartlett's test prior to analysis of variance.

Results

Collection site. The river was about 12 m wide at the collection site. The center of the river was about 60 cm in depth and the bottom consisted of coarse sand. The margin of the river was about 10 to 30 cm in depth and the bottom consisted of fine sand and was covered with detritus. Lampreys were collected only at the margin. The flow was slow, especially at the margin, because there was a weir several meters downstream from the collection site. The water temperature and pH at the collection site are given in Table 1. The high water temperature on May 13 is due to unusually warm weather in early May of this year (1982).

The fishes captured together with the lamprey were *Zacco temmincki, Zacco platypus, Pseudogobio esocinus, Carassius carassius langsdorfi, Misgurnus anguillicaudatus, Cobitis biwae, Silurus asotus* and *Odontobutis obscura*. These are fishes known to live in the mid- and/or down-stream of the river.

Growth and metamorphosis. A size-frequency histogram of the individuals collected in each month is shown in Fig. 1. Small ammocoetes which probably hatched in this year were first collected in August. From September to June, newly hatched ammocoetes could be easily differentiated from older individuals because of a distinct gap in TL. However, separation of older individuals into further groups was impossible probably because of small numbers of individuals available and actual overlaps in TL. The largest ammocoetes measured 180.1 mm in TL.

The TL of the new hatchlings is given in Table 2. From this table, it is clear that they grew rapidly in warmer seasons, and grew slowly or even did not grow in colder seasons.

Correlation between body weight and TL of ammocoetes is shown in Fig. 2. This figure is based on 31 ammocoetes which were randomly chosen from each 5 mm class of TL, respectively. The regression line was: $\log Y = 2.7408 \log X - 5.3101$.

Metamorphosing individuals were first collected in September. In this month, six females were identified as being stage 1^-, and three males in stage 2. In October, one individual

Table 1. Environmental features at the collection site.

Collection date	Water temperature (°C) at 3:00 p.m.	pH of water
Jan. 11	9.0	7.20
Feb. 15	8.3	7.17
Mar. 11	12.7	7.21
Apr. 8	16.5	7.38
May 13	26.0	7.84
Jun. 10	24.0	7.23
Jul. 8	27.0	7.29
Aug. 10	26.8	7.27
Sep. 10	21.2	7.08
Oct. 13	20.5	7.60
Nov. 12	13.0	7.21
Dec. 10	11.2	7.25

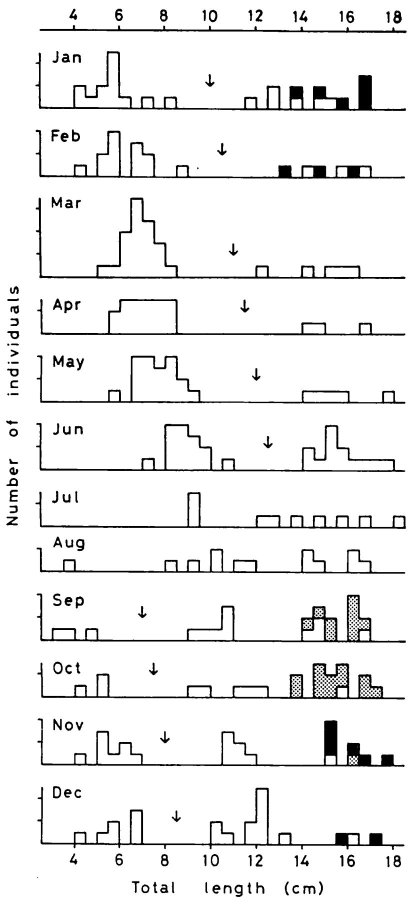

Fig. 1. Size-frequency histogram of the lamprey caught in each month. Each bar in the ordinate represents two individuals. Arrows indicate possible gaps between two successive ages. Blank area represents ammocoetes, dotted area metamorphosing individuals, and solid area adults.

Table 2. Growth of new hatchlings.

Month (n)	Total length (mean±S.E.M., mm)	Differences of the means between two successive months (mm)
Sep. (3)	39.5±3.7	
		10.4
Oct. (3)	49.9±2.8	
		6.4
Nov. (8)	56.3±2.7	
		2.9
Dec. (7)	59.2±3.1	
		−1.8
Jan. (13)	57.4±2.9	
		5.2
Feb. (13)	62.6±3.2	
		5.9
Mar. (22)	68.5±1.5	
		2.1
Apr. (17)	70.6±2.0	
		4.9
May (19)	75.5±1.8	
		13.7**
Jun. (15)	89.2±2.1	

** $P<0.001$

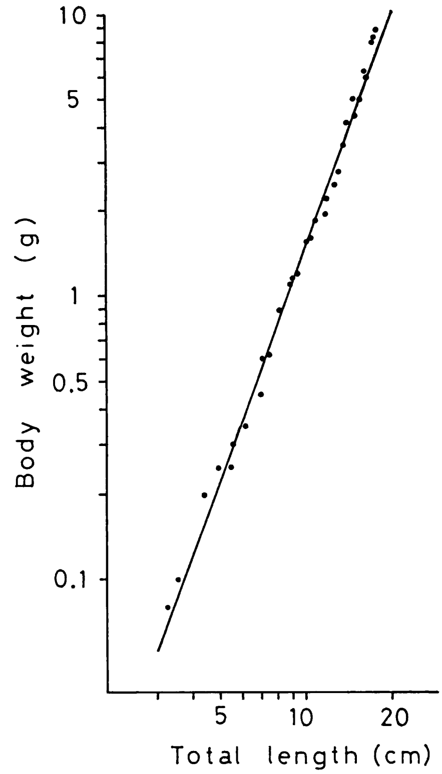

Fig. 2. Correlation between total length and body weight of 31 ammocoetes.

(female) was in stage 3, seven individuals (three males and four females) were in stage 4, and four individuals (two males and two females) were in stage 5. In November, one individual (female) was in stage 5, and six individuals (one male and five females) were immature adults (macrophthalmia). In December, January and February, five immature adult males and six immature adult females were collected.

Among metamorphosis stages, body weight and TL did not differ markedly either in males or females, although a statistical test could not be applied because of small numbers of individuals available. Body weight and TL of metamorphosing males, adult males, metamorphosing females, and adult females are shown in Table 3. Although there were no differences in weight and length of metamorphosing animals versus adults of each sex, females were heavier and longer than males both as metamorphosing individuals and adults.

Changes of proportional length during growth

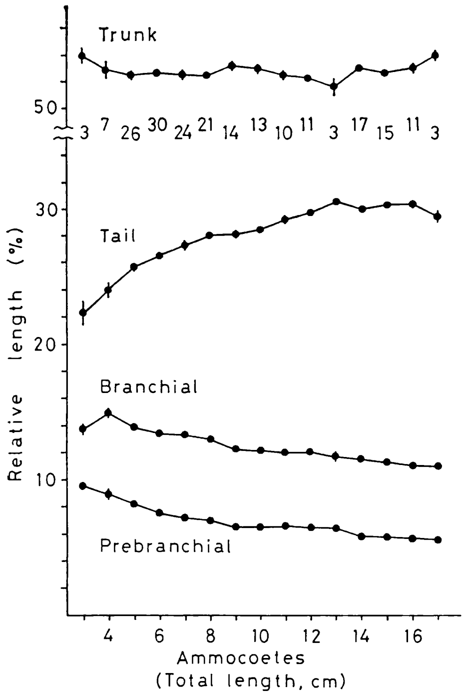

Fig. 3. Proportional changes of lengths of four body regions in ammocoetes. Numbers studied in each class are shown under trunk length. Standard errors were frequently so small that they are included in the size of mean data points.

of ammocoetes are shown in Fig. 3. It is apparent that both prebranchial and branchial length decreased and tail length increased in the course of development. The change of trunk length was not significant ($F_{cal}=1.5770$). Difference of proportional length between two sexes of metamorphosing animals or adults is shown in Table 4. In metamorphosing males,

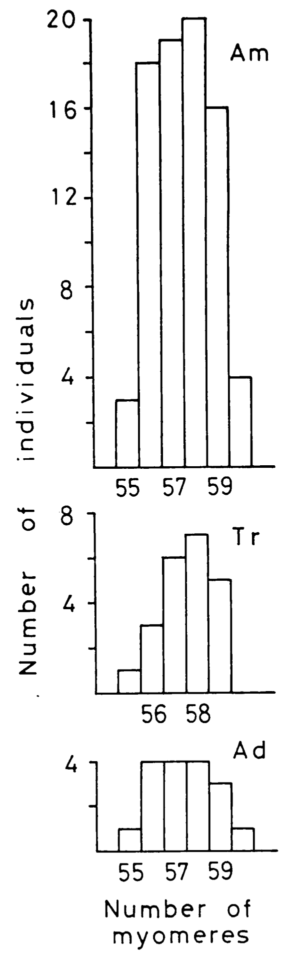

Fig. 4. Number of trunk myomeres in ammocoetes (Am), metamorphosing animals (transformers, Tr) and adults (Ad).

Table 3. Comparison of body weight and total length between males and females. Stage I^- females were excluded.

	Body weight (mean±S.E.M., g)	Total length (mean±S.E.M., mm)
Metamorphosing animals		
male	5.0±0.3 (n=8)	146.0±2.5 (n=8)
female	6.2±0.3*(n=8)	160.2±2.6**(n=8)
Immature adults		
male	4.7±0.3 (n=6)	150.7±3.0 (n=6)
female	5.6±0.3*(n=11)	162.0±3.4* (n=11)

* Significantly different ($P<0.05$).
** Significantly different ($P<0.005$).

branchial length was longer than in females. In adults, prebranchial length was longer in males and trunk length was longer in females. Both in males and females, prebranchial length was longer in adults and trunk length was longer in metamorphosing animals.

Teeth and myomeres. The number of teeth was counted in nine immature adults collected in January and February. Teeth in the anterior field varied from 5 to 14 in number. In addition, several pits were found in the most anterior field. These pits probably foreshadow future tooth development. The supraoral tooth consistently had 2 cusps. Lateral circumorals were 3 in number on each side, and any lateral circumoral consistently had 2 cusps. The infraoral had varying numbers of cusps: six individuals had 6 cusps, two individuals had 7 cusps, and one individual had 8 cusps. Posteior circumorals could not be seen, but their positions were marked by tiny pits in a few individuals. The longitudinal lingual and transverse lingual had cusps in some individuals, but not in others. From these observations, it is apparent that the individuals studied had not fully differentiated their teeth. The teeth pattern is definitely that of the subgenus *Lethenteron* (Hubbs and Potter, 1971).

The number of trunk myomeres is given in Fig. 4. The mode was 58. The myomere number did not change during growth of ammocoetes (Kruskal-Wallis test applied to groups separated into each sequential 10 mm length class, $H_{cor}=3.8956$). There was no sex difference in the number of trunk myomeres in adult lampreys (Mann-Whitney *U* test, $U_{cal}=32.5$).

Reproduction. Upon histological inspection of the gonad, the sex could be determined in individuals larger than 120 mm in TL (see Tsuneki and Ouji, in press). The sex ratio of ammocoetes from 120 to 159 mm in TL, metamorphosing individuals, and immature adults did not depart from unity (Table 5). However, ammocoetes larger than 160 mm in TL were all females except for one sterile individual.

The number of eggs in a transverse section about the mid-trunk region did not change as the animals grew (Table 6). The mean and standard errors of all female ammocoetes larger than 120 mm in TL were 21.9±0.8, and those of all metamorphosing females were 21.0±1.2. These two values were not significantly different ($t_{cal}=0.5833$, $0.5<P<0.8$). The diameter of eggs (oocytes) slightly increased during growth of ammocoetes, but it drastically increased during metamorphosis (Fig. 5). Gonado-somatic index did not differ between sexes, and increased from November to February (Fig. 6).

In addition to 252 lampreys systematically collected at the margin during the year, about 20 adult lampreys were collected in late March and early April at the weir where they tried to climb the wall. They were not used in the present morphometric study, but kept in a tank with circulating aerated water of about 16 to 19°C. Secondary sex characters became prominent by the end of April and artificial insemination was successfully carried out.

Table 4. Comparison of proportional lengths[1] between males and females.

Sex (n)	Prebranchial length	Branchial length	Trunk length	Tail length
Metamorphosing animals[2]				
male (8)	6.5±0.2	9.8±0.1	53.2±0.4	30.7±0.4
female (8)	6.6±0.2	9.2±0.1 *	53.9±0.2	30.4±0.3
Immature adults	*** ¦ ***		*** ¦ ***	
male (6)	8.9±.02	9.6±0.1	50.8±0.3	31.3±0.4
female (11)	8.0±0.2 **	9.4±0.1	52.5±0.3 ***	30.6±0.3

[1] Expressed in percentage of total length (mean ± S.E.M.).
[2] Stage 1⁻ females were excluded.
* Significantly different ($P<0.05$).
** Significantly different ($P<0.01$).
*** Significantly different ($P<0.005$).

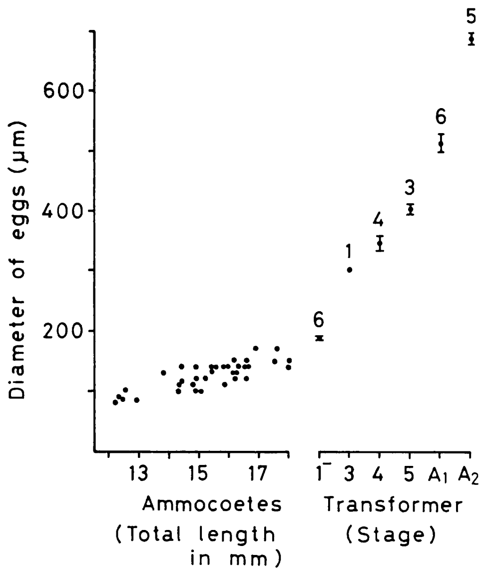

Fig. 5. Growth of eggs. In ammocoetes, original measurements are given. In metamorphosing individuals (transformer) and adults (A), mean and standard errors are shown. Stage 2 females were not collected. A_1 means adults caught in November and December, and A_2 in January and February.

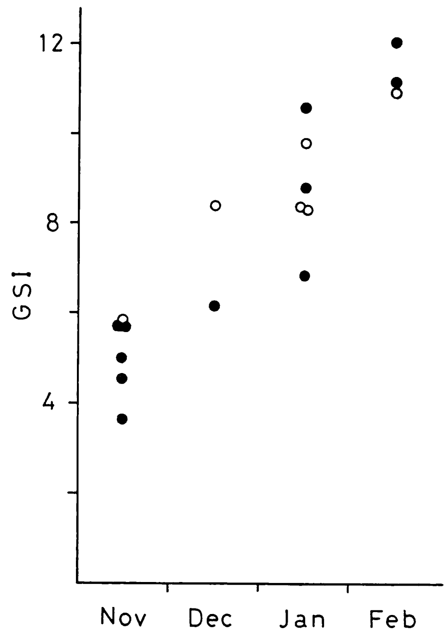

Fig. 6. Gonado-somatic index of immature adults caught in late autumn and winter. Open circles represent male and solid circles represent female.

Table 5. Results of binomial test on sex ratio.

	Male	Female	P
Smaller ammocoetes[1]	23	23	
Larger ammocoetes[2]	0	14	
Metamorphosing animal	8	14	0.2862
Immature adult	6	11	0.3324

[1] Individuals from 120 to 159 mm in total length.
[2] Individuals larger than 160 mm in total length. One individual (169 mm) was sterile and was not included.

Table 6. Number of eggs in each developmental stage[1].

Class (n)	Number of eggs (mean ±S.E.M.)	F_{cal}
Ammocoetes[2]		
120 mm (6)	23.5±1.4	
140 mm (8)	21.8±2.0	
150 mm (8)	21.5±1.8	0.4508
160 mm (10)	23.0±1.9	(n.s.)
170 mm (3)	19.3±0.7	
Metamorphosing animal[2]		
stage 1⁻ (6)	20.2±2.6	
stage 4 (4)	20.8±2.1	0.2416
stage 5 (3)	22.7±1.3	(n.s.)

[1] Counted in a transverse section at the middle of the trunk.
[2] There were not enough 130 mm- and 180 mm-class ammocoetes and stage 2 and stage 3 animals available for analysis.

Discussion

In Japan, four lamprey species occur, but *Lampetra* (*Entosphenus*) *tridentata* and *Lampetra* (*Lethenteron*) *matsubarai* are probably rare species, and not recorded so far in the San-in district including Tottori Prefecture. The common species are *Lampetra japonica* and *Lampetra reissneri* which constitute a paired species. The ammocoetes studied in this work were identified as *L. reissneri* because of the following. (1) The number of trunk myomeres was about 58, and this is much smaller than the number claimed for ammocoetes of *L. japonica* (about 73) (Okada, 1937). Although Okada counted trunk myomeres between the posterior rim of the last branchial pore and the posterior rim of the cloaca, the difference from the present method may be a few in number. However, more extensive study of trunk myomeres may be necessary, since the European paired species (*Lampetra fluviatilis* and *Lampetra planeri*) could not be separated by the number of trunk myomeres (Hardisty, 1961b; Potter and Osborne, 1975). (2) The tail fin was translucent or only slightly pigmented. The tail fin of ammocoetes of *L. japonica* is claimed to be densely pigmented (Okada, 1937). (3) The San-in district borders the southern limit of the distribution of *L. japonica*, and the population of this species seems to be small here. Although we collected a few upstream migrants of *L. japonica* in Nakaumi Lake, we have never found either macrophthalmia or upstream migrants of *L. japonica* at the Hosshoji River. (4) The number of oocytes in a transverse section about the mid-trunk region was about 19 to 23. This is a number comparable with that for *L. planeri* (about 20 to 40), but not for *L. fluviatilis* (more than 60) (Hardisty, 1961b; Malmqvist, 1978). According to Hardisty (1963), low fecundity is a characteristics of non-parasitic species.

Although we could not find the spawning bed in the river, the lamprey might spawn in late April or early May, judging from laboratory data. The failure to find any adult lamprey in March and April samplings may be due to their more or less swimming habit, which obviously enable them to escape from nets or shovel trapping at the margin. The failure to collect very small individuals in June and July may be attributable either to inadequate methods (net and shovel) to collect smaller individuals or to a possibility that the newly hatched individuals had not yet been washed out from the nest to the collection site.

According to Abakumov (1960, cited in Hardisty and Potter, 1971a), larval duration of *L. reissneri* is four years. Naive interpretation of the present size-frequency histograms may lead to the conclusion that the larval period covers three and one-half years. This interpretation is based mainly on autumn samplings where three peaks of ammocoetes TL were evident. The first peak probably represents individuals hatched out this year. They may be about one-half year old, since the lamprey probably spawn in April or May. The TL of individuals of the third peak is similar to that of metamorphosing and adult animals, and the individuals of this peak may survive as ammocoetes for one more year to enter metamorphosis. Thus, the larval period can be estimated as three and one-half years. However, this estimation should be considered with some reservations. The larger ammocoetes were collected in relatively small numbers and they apparently grew slowly as compared with younger ammocoetes, which may not produce a sharp valley in the size-frequency histogram. Much more detailed analysis based on large numbers of individuals may be necessary to draw a definite conclusion on the larval duration of this species. However, it may be safe to say that the larval period of *L. reissneri* is not less than three and one-half years.

The estimation of larval duration is further complicated by the fact that all ammocoetes larger than 160 mm in TL were females. This may suggest that some females survive as ammocoetes at least one year longer than males. Females were heavier and longer than males both in metamorphosing individuals and adults. This also supports the estimation that the larval life of females is longer than males. In September, females were at the very beginning of metamorphic stage (stage 1^-), but males were already at stage 2. Therefore, males may metamorphose not only one year earlier, but also about one month earlier than females. The possibility that the larval duration of at least some females is longer than that of males has been suggested also in the European brook lamprey, *L. planeri* (Bird and Potter, 1979b; Malmqvist, 1978).

During larval development, relative tail length increased at the expense of prebranchial length and branchial length. The tail may be relatively more important in larger ammocoetes, because they must swim in a more controlled fashion to settle in a new environment after each flooding which washes out ammocoetes to lower reaches. The relatively long prebranchial region of the immature adults may be related to the developing funnel-shaped mouth. Although non-parasitic lampreys do not feed after the onset of metamorphosis, the toothed mouth is important for upstream migration and nest building. The fact that the prebranchial length of adult males is longer than adult females may be related to the usage of the mouth by males in an additional purpose, namely, suction on females to facilitate spawning. The proportional change of the body regions has also been studied in *L. planeri* (Lohnisky, 1968; Potter and Osborne, 1975). In accordance with our results, these authors reported that prebranchial length and branchial length decrease, and tail length increases during larval development. However, a discrepancy exists in the trunk length, which decreased during larval development in *L. planeri* (Lohnisky, 1968), but did not change in *L. reissneri,* at least within the size range studied.

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Literature cited

Bird, D. J. and I. C. Potter. 1979a. Metamorphosis in the paired species of lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). 1. A description of the timing and stages. Zool. J. Linn. Soc., 65: 127–143.

Bird, D. J. and I. C. Potter. 1979b. Metamorphosis in the paired species of lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). 2. Quantitative data for body proportions, weights, lengths and sex ratios. Zool. J. Linn. Soc., 65: 145–160.

Hardisty, M. W. 1944. The life history and growth of the brook lamprey (*Lampetra planeri*). J. Anim. Ecol., 13: 110–122.

Hardisty, M. W. 1961a. The growth of larval lampreys. J. Anim. Ecol., 30: 357–371.

Hardisty, M. W. 1961b. Oocyte numbers as a diagnostic character for the identification of ammocoete species. Nature, 191: 1215–1216.

Hardisty, M. W. 1963. Fecundity and speciation in lampreys. Evolution, 17: 17–22.

Hardisty, M. W. and I. C. Potter. 1971a. The behaviour, ecology and growth of larval lampreys, pp. 85–125. *In* M. W. Hardisty and I. C. Potter, eds., The biology of lampreys, Vol. 1. Academic Press, London.

Hardisty, M. W. and I. C. Potter. 1971b. The general biology of adult lampreys, pp. 127–206. *In* M. W. Hardisty and I. C. Potter, eds., The biology of lampreys, Vol. 1. Academic Press, London.

Hubbs, C. L. and I. C. Potter. 1971. Distribution, phylogeny and taxonomy, pp. 1–65. *In* M. W. Hardisty and I. C. Potter, eds., The biology of lampreys, Vol. 1. Academic Press, London.

Janvier, P. and A. Blieck. 1979. New data on the internal anatomy of the Heterostraci (Agnatha), with general remarks on the phylogeny of the Craniota. Zool. Scr., 8: 287–296.

Lohnisky, K. 1968. Proportionate characters of ammocoetes used in taxonomy and their variability during growth and development in the brook lamprey, *Lampetra planeri* (Bloch, 1784). Acta Soc. Zool. Bohem., 32: 350–364.

Malmqvist, B. 1978. Population structure and biometry of *Lampetra planeri* (Bloch) from three different watersheds in South Sweden. Arch. Hydrobiol., 84: 65–86.

Novitskaya, L. I. 1981. The problem of the relationship between agnathan and gnathostome vertebrates. Acta Palaeontol. Pol., 26: 9–18.

Okada, S. 1937. On ammocoetes of two lamprey species found in Hokkaido. Shokubutsu oyobi Doubutsu, 5: 957–966. (In Japanese).

Potter, I. C. and T. S. Osborne. 1975. The systematics of British larval lampreys. J. Zool., Lond., 176: 311–329.

Rohde, F. C., R. G. Arndt and J. C. S. Wang. 1976. Life history of the freshwater lampreys, *Okkelbergia aepyptera* and *Lampetra lamottenii* (Pisces: Petromyzonidae), on the Delmarva Peninsula (East Coast, United States). Bull. South. Calif. Acad. Sci., 75: 99–111.

Seagle, H. H., Jr. and J. W. Nagel. 1982. Life cycle and fecundity of the American brook lamprey, *Lampetra appendix*, in Tennessee. Copeia, 1982: 362–366.

Tsuneki, K., M. Ouji and H. Saito. 1983. Seasonal migration and gonadal changes in the hagfish *Eptatretus burgeri*. Japan. J. Ichthyol., 29: 429–440.

Tsuneki, K. and M. Ouji. (In press). Histological changes of several organs during growth of the brook lamprey *Lampetra reissneri*. Japan. J. Ichthyol., 31(2).

Yamada, H. 1951. Life-history of brook lamprey. Saishu to Shiiku, 13: 121–127. (In Japanese).

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スナヤツメの成長に伴う計量的変化

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鳥取県の法勝寺川で各月1回，年間にわたり，スナヤツメの採集を行った．幼生は毎月採集できたが，変態中の個体は9月から 11 月まで，成体は 12 月以降翌春までの間でしか採集されなかった．全長と頻度のヒストグラムから，幼生期間は3年半以上であると考えられた．ただし全長 16 cm 以上の幼生はすべて雌であり，成体でも雌の方が大きかったので，少なくとも一部の雌の幼生期間は，雄よりも長いものと考えられる．幼生では鰓前部および鰓部の相対長は成長につれ減少するが，尾部の相対長は増加した．卵は変態期の間に急激に大きくなった．

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