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Source: Zoological Science, 20(7) : 907-918

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.907

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ZOOLOGICAL SCIENCE **20**: 907–918 (2003) © 2003 Zoological Society of Japan

Biology of the Amphioxus, *Branchiostoma belcheri* in the Ariake Sea, Japan II. Reproduction

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ABSTRACT—We investigated the reproduction of the amphioxus *Branchiostoma belcheri* in the southern Ariake Sea, Japan, from 1999 to 2002. Gonads were very small or almost absent from September to December and began to develop in January. Changes in the gonad length index indicated that spawning began in mid June, after the gonads had attained maximum size. Although most gametes were extruded during the first spawning, some remained in the gonads. Shrunken gonads were much smaller but contained gametes, and the second spawning occurred around 10 July. It was unclear whether all adults spawned twice, but a large proportion of individuals did. After the first spawning, both males and females lost 30% of their body weight. Most one-year-old individuals did not spawn, and the minimum size at maturity was ca. 20 mm. We found no termination of reproduction among very large individuals. The average number of gonads was significantly larger on the right side of the body (26.2 in males and 26.1 in females) than on the left side (24.0 in males and 23.5 in females). We found two hermaphrodites in a total of 11,184 specimens examined. Each had four or three ovaries among a total of 45 or 54 gonads.

Key words: amphioxus, Ariake Sea, *Branchiostoma belcheri*, gonad number, reproduction

INTRODUCTION

Nakagawa (1897) first reported the occurrence of amphioxus in Amakusa suggested that the period of active egg lying was between mid June and late July. Oyama and Yoshii (1940) studied the reproductive period of amphioxus collected from a sandy tidal flat located in the northern Ariake Sea. They suggested that spawning was restricted to a short period between late May and early June. Kubokawa *et al*. (1998) conducted a monthly collection of *Branchiostoma belcheri* in Enshu-Nada Sea, Japan from 16 June to 14 November 1995 and reported that the specimens obtained in June had fully developed gonads and suggested that breeding of most individuals lasts from June to August. Chin (1941) studied changes in gonad size of *B. belcheri* in Amoy, China, and concluded that spawning occurred twice a year, from May to July and again in December.

Futch and Dwinell (1977) reported that *B. virginiae* spawned not only in summer but also in winter; however, they suggested that in winter, larvae did not survive to reach maturity. Stokes and Holland (1996) conducted a very detailed study of the reproduction of *B. floridae*. The breeding season of the Florida amphioxus lasts from early May through early September and spawning may be repeated in as little as one to two weeks. In the present study, we determine the exact breeding period of *B. belcheri* and suggest the occurrence of a second spawning. Moreover, we report the seasonal changes of gonad condition and the reproductive effort in each spawning.

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MATERIALS AND METHODS

Number and size of gonads

From 1999 to 2002, specimens were obtained by dredging at a site in the southern Ariake Sea during an investigation of the population structure and annual growth of *B. belcheri* (see Part I for detailed methods). In *B. belcheri*, a row of whitish testes or yellowish ovaries is located on each side of the body. From the specimens collected just before the breeding season (8 June 1999, 7 May 2000, 1 & 17 May 2001), we chose healthy individuals with well-developed gonads without consideration of body size and counted the number of gonads on each side of the body under a stereomicroscope.

After measuring the body length (BL: from the tip of the snout to the end of the tail), specimens were placed under a stereomicroscope equipped with an ocular micrometer to measure the maximum gonad length (Fig. 1). Three of the longest gonads were measured and the average of these values was used to describe gonad length. The longest gonads were typically located in the fourth to seventh positions from the anterior end. The gonad length index (GLI) was defined as follows:

GLI = maximum gonad length (mm) $\times$ 100 / body length (mm). The GLI was determined for 5,944 males and 5,238 females

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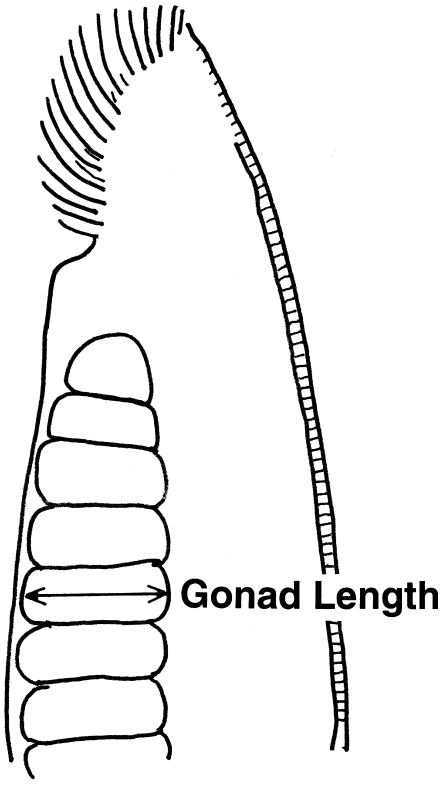


Fig. 1. Measuring gonad length in *B. belcheri*. The three longest gonads were measured and their average length was used to describe gonad length.

between 25 June 1999 and 29 July 2002.

We initially defined five developmental stages of gonad condition as specified by Fang *et al.* (1991), but we eventually realized that a quantitative measurement was more useful to describe changes in gonad condition. Stokes and Holland (1996) measured the total live weight of whole gonads and defined the ratio of gonad weight to whole-body weight as the gonad index. Measuring this index was impractical for us because the process was too complicated and too time-consuming. We therefore adopted a simpler and quicker method (GLI). By the change of GLI we could know how the amphioxus develops gonads and extrudes eggs and sperm. Although measuring the length of gonads was not difficult, it was too late to switch to the new method in 1999. We therefore do not have a clear understanding of seasonal changes in gonad condition in 1999.

Body weight

Only healthy individuals were chosen for this measurement. After measuring BL, each specimen was blotted dry on a paper towel and placed in a small aluminum-foil container weighing ca. 150 mg. Specimens were then oven-dried for 24 hr at 70°C, and dried specimens were weighed to the nearest 1 mg using an electric balance.

RESULTS

Number of gonads

Fig. 2 summarizes the results of the enumeration of gonads in 221 males and 221 females. Both sexes had more gonads on the right side than on the left side. In some individuals, one to several gonads were missing, leaving spaces where the gonads should have been. Absence of several gonads was observed not only in damaged specimens but also in healthy, intact individuals, suggesting that this is a normal phenomenon; therefore, we did not exclude these individuals from the counts. The number of gonads on the right side was always larger than on the left side. The number of gonads on the left side was 24.0±1.8 (mean±SD, range: 16–28) in males and 23.5±2.0 (12–27) in females, whereas that on the right side was 26.2±1.7 (20–29) in males and 26.1±1.5 (21–29) in females. The average number was significantly larger on the right side than on the left side in both sexes (each $P<0.001$, $t=13.21$ in males, $t=15.46$ in females, *t*-test) and significantly larger in males than in females on the left side ($P<0.01$, $t=2.76$, *t*-test), but was not significantly different between sexes on the right side ($P>0.05$, $t=0.66$, *t*-test).

Fig. 3 shows the relationship between body length and the number of gonads on both sides of the body. Although there is a slight tendency of increasing gonad number with increasing body size and the relationships were significant (each $P<0.001$, $t=5.069$ in males and $t=4.739$ in females on the left side, $t=3.359$ in males and $t=7.399$ in females on the right side, respectively, *t*-test), but the r-values were very low.

Seasonal change of GLI

Fig. 4 shows changes in the average values of the GLI, and the photographs in Fig. 5 illustrate the morphological changes before and after spawning. The values for males and females are shown separately, and seasonal changes in the percentage of unsexed individuals are presented below. From September to December, gonads were very small, making it impossible to determine sex. After January, gonad size increased, and the number of unsexable individuals decreased. On 3 February 2000, 86.3% of collected animals were unsexable, but this ratio dropped to 43.4% on 31 March. On 7 May 2000, only 1.7% of the specimens were unsexable. The percentage of unsexable individuals remained low from May to mid July (0–2.0%), and increased again after mid July to reach 32.8% on 11 August 2000.

On 3 February 2000, the GLI of males was ca. 1.6 and increased to 2.90 on 23 May, and the GLI of females indicated the similar tendency. We expected further increase of the value, but engine trouble on our ship prevented us from obtaining samples in June. On 2 July 2000, the GLI was 3.57 (males) and 3.72 (females); by 28 July 2000, this value had decreased to 2.36 (males) and 2.18 (females). In 2001 we were able to measure changes in the GLI in more detail. Two peaks were evident in the GLI curves of 2001. The first peak occurred on 12 June, when the average GLI was 4.24 (males) and 3.85 (females). After dropping to 3.51 (males) and 2.72 (females) on 2 July, a second peak appeared on 9 July, with value of 3.85 (males) and 3.28 (females).

Fig. 6 shows changes in the percentage distribution of the GLI in June and July 2001. The distribution pattern of

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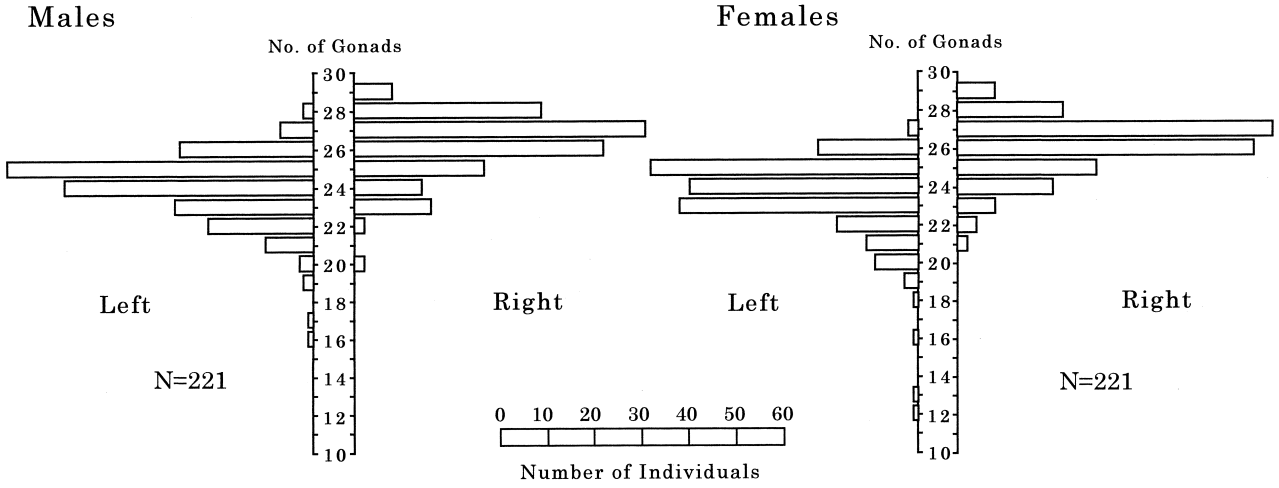
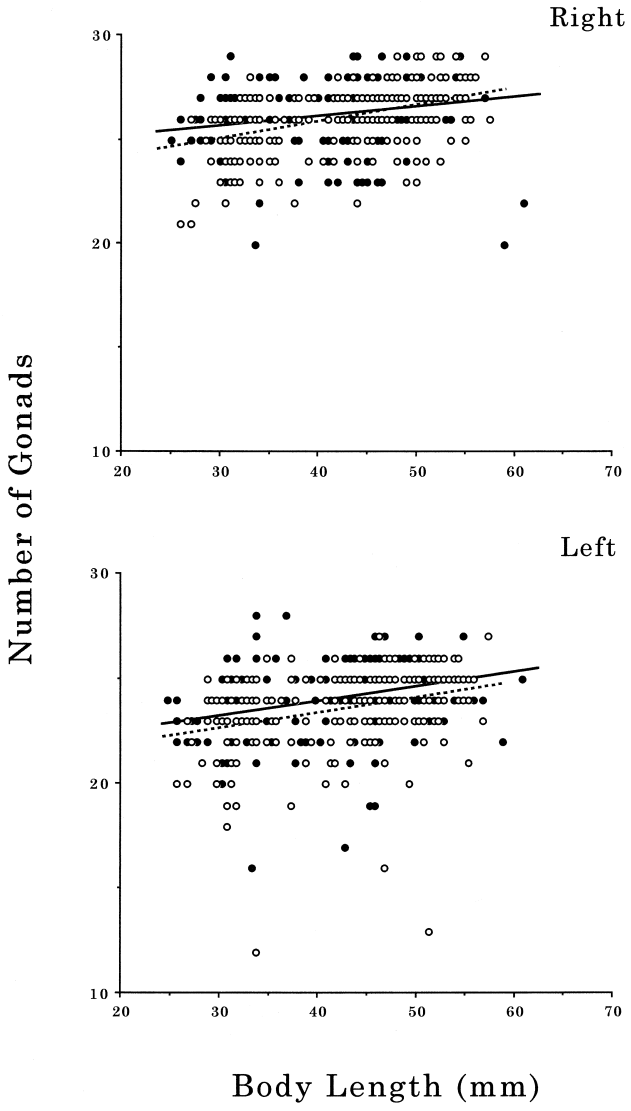


Fig. 2. Frequency distribution of the number of gonads on the left or right side of the body in *B. belcheri*.



the GLI differed markedly between samples from 12 and 26 June. On 12 June, most individuals had well-developed gonads and a higher GLI value; however, by 26 June, many individuals, especially males, had finished spawning, resulting in a lower GLI. On 26 June, the GLI distribution was greatly different between sexes. In females, we observed two groups. The group with a low GLI consisted of individuals that had already spawned, whereas the other group had a higher GLI and had not yet completed spawning. On 2 July, females were in a single group with a low GLI, and it was therefore clear that most of them had finished spawning. A slight increase in the GLI occurred in males, which was clearer in the sample of 9 July; this increase was also observed in females. Although the gonads of individuals that had finished spawning shrank, they were not empty; some gametes remained inside (Fig. 5 [3]). Several large oocytes remained in the ovaries, but they were separated from each other by large spaces. After spawning, the testes contained less concentrated sperm, and groove-like spaces appeared (Fig. 5 [4]); however, these were not so conspicuous as the spaces inside the ovaries. Therefore, morphological changes following spawning were less obvious in testis.

On 26 June 2001, 54.1% of females had spaces inside the ovaries. We expected a subsequent increase in the percentage of spawned females, but in the following sample almost all females had ovaries filled with oocytes. Only three of 86 females (3.5%) had spaces in their ovaries. The percentage of such females remained low through 9 July, when

Fig. 3. Relationship between body length and number of gonads on the right (above) or left side (below) of male (solid lines) and female (dotted lines) *B. belcheri*.
Right: ○ — male Y = 0.046X + 24.29 (r=0.221, n=221)
● — female Y = 0.082X + 22.63 (r=0.447, n=221)
Left: ○ — male Y = 0.071X + 21.09 (r=0.324, n=221)
● — female Y = 0.074X + 20.45 (r=0.305, n=221)

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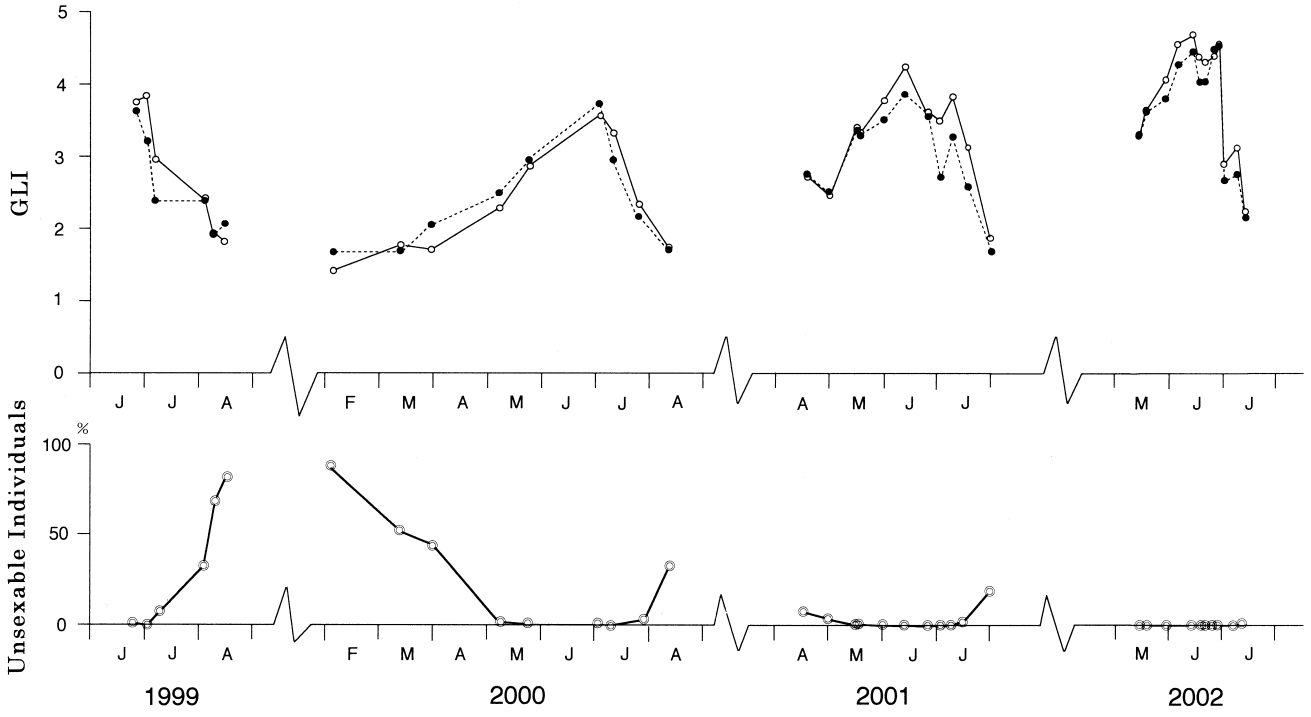


Fig. 4. Seasonal changes in GLI (gonad length index; above) and percentage of unsexable individuals (below) in *B. belcheri* from 1999 to 2002. Open circles: males; solid circles: females.

only two of 98 specimens (2.0%) showed spaces. On 16 July, this ratio increased to 25.4% (17/71), and the percentage of the individuals with a low GLI increased in both sexes. From these changes of the GLI distribution and gonad condition, we assumed that amphioxus spawned a second time, ten to 14 days after the first spawning.

To confirm our assumption, we collected 15 samples between 13 May and 29 July 2002. In June, two peaks appeared in the GLI curve (Fig. 4). The first peak was recorded on 13 June, with an average GLI of 4.70 (males) and 4.46 (females). In the subsequent two samples, GLI values dropped, although this decrease was small compared to the decrease observed in 2001. The second peak appeared on 28 June, with an average GLI of 4.56 (males) and 4.53 (females). This peak was followed by a sudden decline in the GLI on 1 July, and it averaged 2.91 (males) and 2.68 (females).

To analyze changes in the GLI that occurred in 2002, histograms of the percentage distribution of the GLI are presented in Fig. 7. The GLI distribution was not significantly different between sexes in all samples (each $P>0.05$, Kolmogorov-Smirnov two-sample test). Individuals with gonads that were not filled with gametes but contained spaces (S-individuals), first appeared on 18 June and constituted ca. 29% of the sample; this value increased to around 43% on 20 June. However, further increases were not observed in the following two samples. On 1 July, S-individuals increased to 83.2% (males) and 88.7% (females), suggesting that the data of first spawning in 2002 was between 13 and 18 June, while concentrated spawning occurred between 28 June and 1 July. On 8 July, a very slight increase in average GLI was recorded in both sexes, and the number of individuals whose gonads were filled with gametes increased. However, the percentage of these individuals was not as high as on 9 July 2001. On 12 July 2002, we observed further decreases in average GLI, but the percentage of individuals whose gonads were filled with gametes increased.

The sudden increase of S-individuals on 1 July 2002 suggests the occurrence of mass spawning. The second spawning was confirmed by changes in the GLI observed between 1 to 12 July. However, the percentage of individuals whose gonads were filled with gametes was not as high as 2001. This difference suggests that the occurrence of the second spawning may be affected by the environmental conditions in a given year and not always occur in every individual.

Size at maturity

Fig. 8 shows the size frequency distribution of male and female amphioxus on 10 July 2000. This sample consisted of two large groups: one peaked at ca. 37 mm BL and mainly comprised three-year-old individuals, while the other group peaked at ca. 20 mm BL and mainly comprised one-year-olds. Among the one-year-olds, very few individuals had gonads (9/368 individuals <25 mm BL). The smallest mature male was 20.5 mm BL, whereas the smallest mature female was 21.5 mm BL, indicating that almost no one-year-olds develop gonads.

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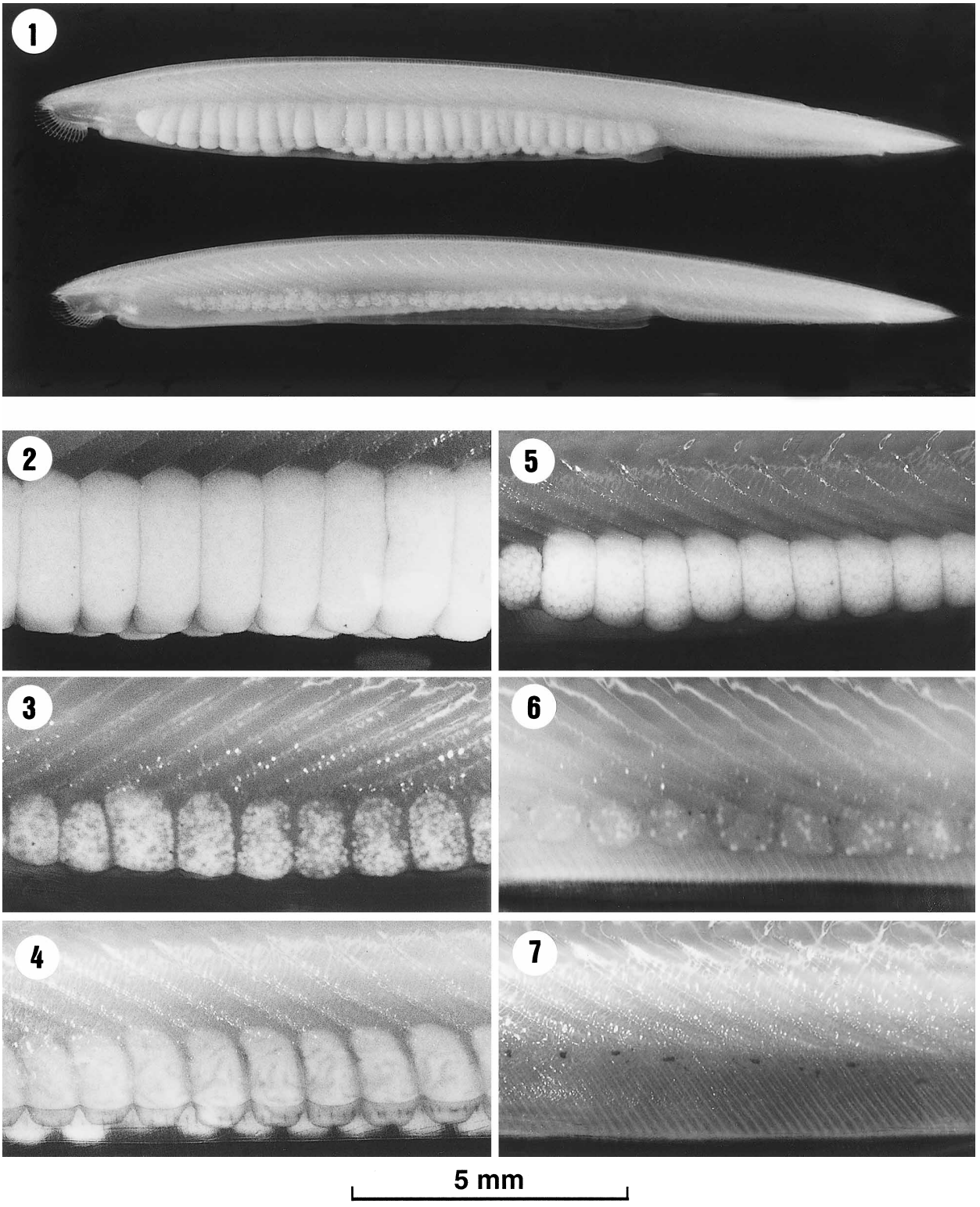


Fig. 5. Photographs illustrating morphological changes before and after spawning in *B. belcheri*. (1) 21 June 2002: two females (55 mm BL) with well-developed gonads (above) and after the first spawning (below). After the first spawning, the ovaries shrunk, but several oocytes remained. (2) 19 June 2002: fully developed ovaries (54-mm BL female). The GLI of this specimen was 5.46. (3) 19 June 2002: shrunken ovaries after the first spawning. Ovaries contain oocytes, but in less dense conditions. This specimen measured 54.5 mm BL and had a GLI of 3.12. (4) 19 June 2002: after the first spawning, testes are shrunken and contain groove-like spaces. The GLI of this specimen was 3.4. (5) 9 July 2002: ovaries before the second spawning. Although the ovaries are filled with oocytes, they have not increased in size. This specimen measured 45 mm BL and had a GLI of 3.6. (6) 9 July 2002: ovaries after the second spawning in a 53.5-mm BL female. Very few oocytes can be observed. (7) 9 October 2002: gonads of an individual (53 mm BL) during the non-reproductive period. Gonads have disappeared almost completely, and thin blackish dots can be seen where they used to be. At this stage, it is impossible to determine sex.

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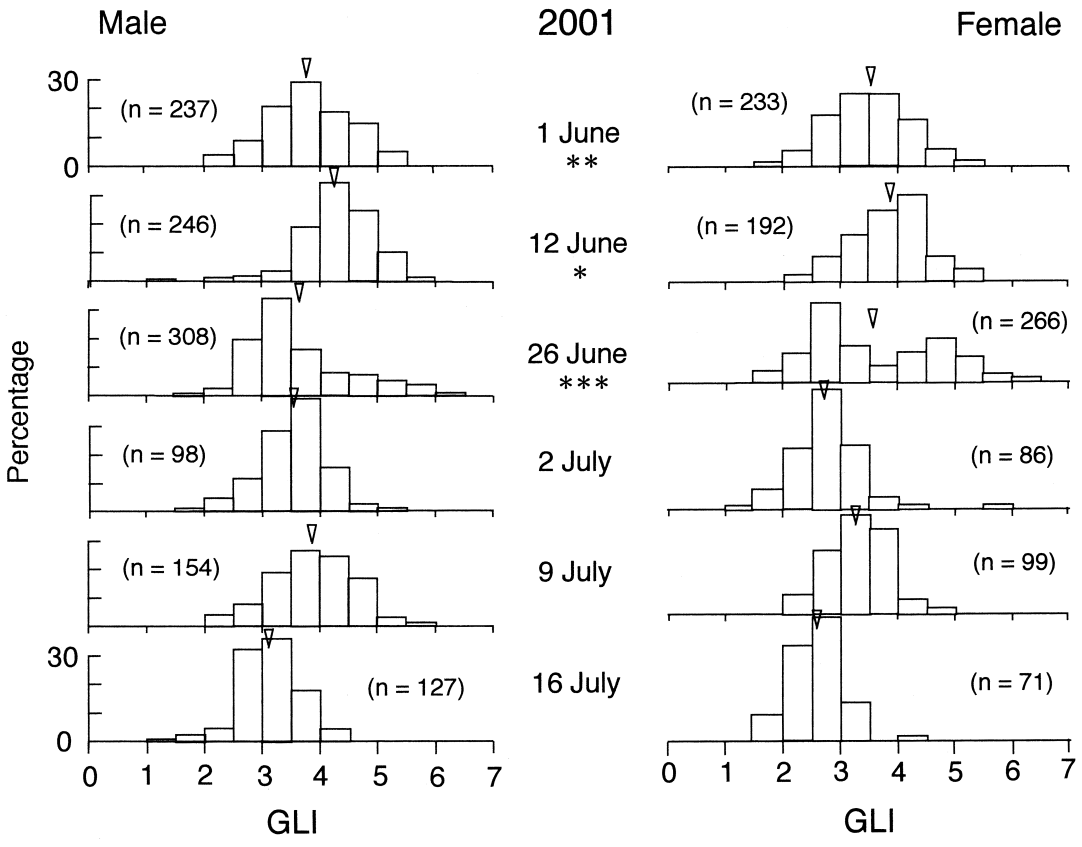


Fig. 6. Change in the percentage distribution of the GLI in 2001. Sexes are shown separately. The white triangle indicates the average value. Differences of the GLI distribution between sexes $*P<0.05$, $**P<0.01$, $***P<0.001$, Kolmogorov-Smirnov two-sample test.

Gonad weight

To assess reproductive burden in a amphioxus, specimens in the following four conditions were weighed:

A: Fully developed gonads. Specimens were obtained on 25 June 2002 (male n = 47, female n = 48; Fig. 5 [1 and 2]).

B: Shrunken gonads after the first spawning. Specimens were obtained on 25 and 28 June 2002 (male n = 47, female n = 58; Fig. 5 [1, 3 and 4]).

C: Gonads present, before the second spawning. Gonads were small but filled with gametes. Specimens were obtained on 12 July (n = 22; Fig. 5 [5]).

D: During the non-reproductive season. Specimens were obtained on 25 September 2002 (n = 29; Fig. 5 [7]).

The results are summarized in Fig. 9. Males and females from categories A and B are shown separately (A(M), A(F) and B(M), B(F)). The regression equations of A(M) and A(F) were very similar, as were the regression lines of B(M) and B(F). These results indicate that there were no sexual differences in body weight based on gonad condition. Males and females of the same body length and in the same category weighed the same. Males and females in category C were not separated. The regression equation indicates that C-individuals were slightly heavier than B-individuals. Specimens in category D had the lowest values; their gonads were very small and transparent, and it was impossible to determine their sex.

Based on the equations, the weights of individuals of six different body sizes and in four gonad conditions were calculated and are presented in Table 1. The percentage of the weight of animals in categories A, B, and C compared to that of animals in category D is presented below. Specimens with fully developed gonads were average of 63.4% (males) and 65.6% (females) heavier than animals in non-reproductive condition. By the first spawning, these animals lost a considerable amount of weight; although they were still heavier than animals in category D, the difference was average of only 12.5% (males) and 12.7% (females). The increase in body weight before the second spawning (C/D-B/D) was very slight: average of only 5.6% (males) and 5.4% (females). Although the animals spawned a second time, the number of gonads involved in the second spawning was very small.

A very large reduction in body weight occurred with the first and the second spawning. It is reasonable to assume that the weight difference between category A and B is the weight of gametes extruded during the first spawning. The weight of gametes lost during the second spawning is similar estimated by the weight difference between categories C and D. Total weight loss over the two spawning event was 113.8+41.7=155.5 mg (male) and 117.9+41.7=159.6 mg (female) in 40-mm BL individuals. These values indicate that a 40-mm BL male weighing 223.4 mg during the non-repro-

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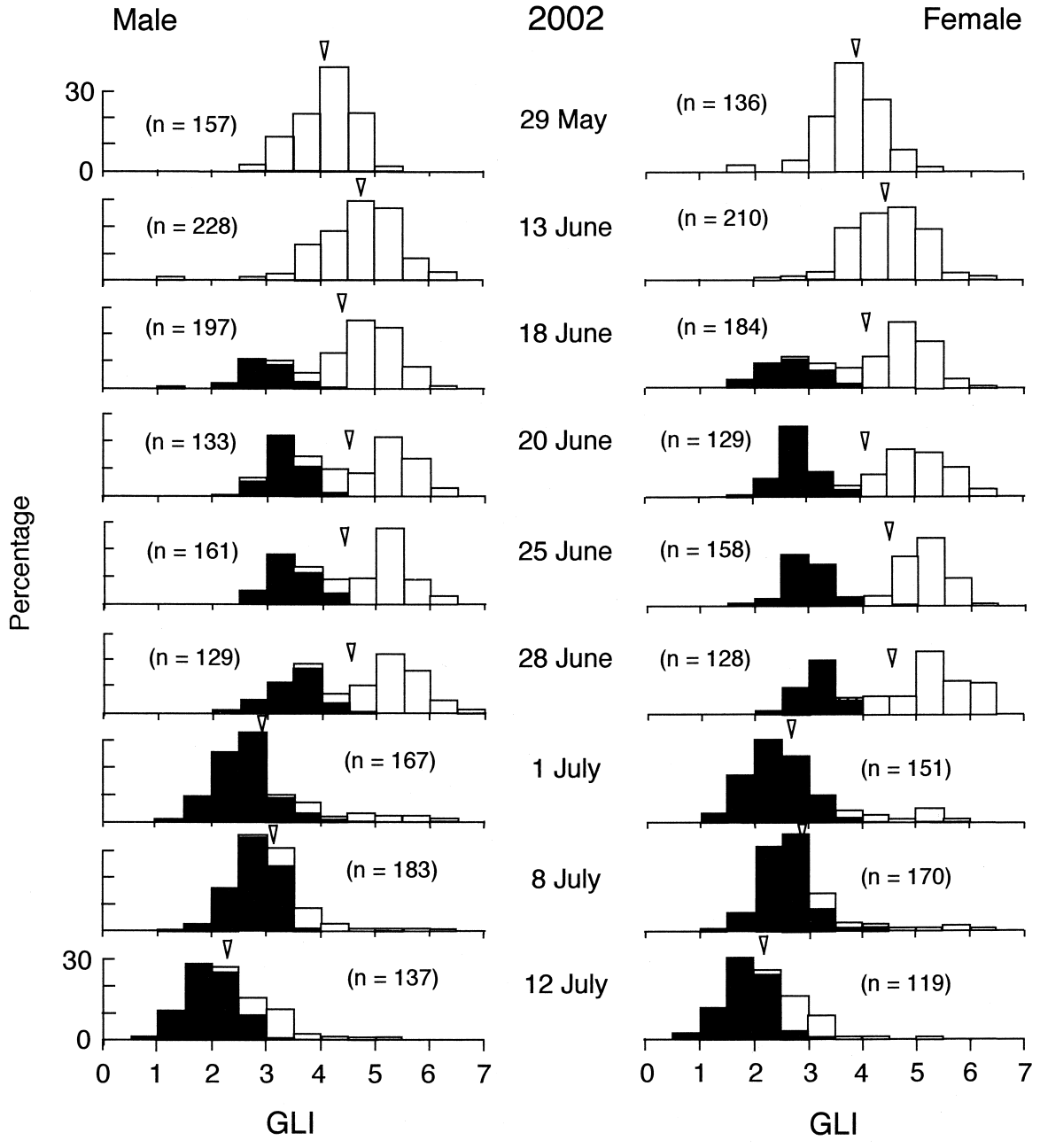


Fig. 7. Change in the percentage distribution of GLI in 2002. Black bars represent individuals whose gonads were not filled with gametes but instead had spaces inside them. Further details as in legend to Fig. 6.

ductive period accumulates and extrudes a total of 155.5 mg of nutrient-rich substances as gametes, while a 40-mm BL females weighing 223.4 mg must consume 159.6 mg to spawn twice. Thus, nutrient expenditure during reproduction is a large burden to amphioxus, particularly because the animals require nutrients not only for reproductive activities but also for somatic growth.

Hermaphrodites

We found two hermaphrodites among 11,184 specimens examined. The first individual was found among 125 specimens obtained on 13 May 2002. Its BL was 53.5 mm, and the maximum length of the ovaries and testes was 0.9 mm and 0.6 mm, respectively. The total number of gonads was 20 (one ovary and 19 testes) on the left side and 25 (3 ovaries and 22 testes) on the right. A photograph of the specimen is presented in Fig. 10 [1 and 2]. The second hermaphrodite was found on 25 June 2002 among 319 collected specimens. Its BL was 54 mm and the number of gonads on the left and right sides was 26 and 28, respectively. Only three gonads on the left side were ovaries; one was large and two were small (maximum length of 1.2 mm; Fig. 10 [3]).

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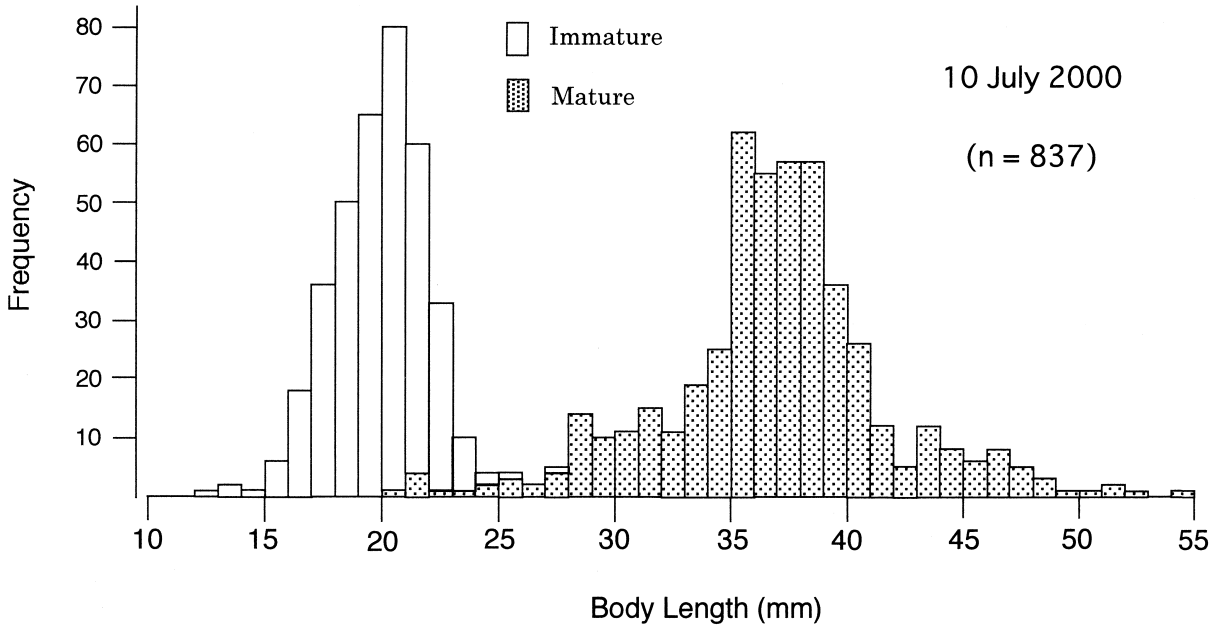


Fig. 8. Size frequency distribution of male and female *B. belcheri* obtained on 10 July 2000. Shaded bars represent individuals with developed gonads.

DISCUSSION

Number of gonads

A most unusual and puzzling feature of amphioxus is its left-right asymmetry (Whittaker, 1997). Not only the number of gonads, but also the positions of muscle segments and nerves are asymmetrical. Branchiostomids typically have one or two more gonads on the right side than on the left, with a typical count of ca. 27 on the left side and ca. 28 on the right (Wickstead, 1975). In our study, we found this difference to be larger in *B. belcheri*. Chin (1941) also demonstrated an average number (range) on the left side of 25 (22–28) compared to 27 (23–29) on the right in *B. belcheri*. Each gonad is a separate sac and has no common duct (Wickstead, 1975). It is interesting in some individuals that one to several gonads are missing. Some of the gonads did not develop and a space was found where they should be. Such individuals were found from January to August, the period from the beginning of development of gonads to the disappearance of them. Therefore, the missing or absence of gonads was not the results of partial extrusion of gamates. Chin (1941) reported a very exceptional male with only 5 and 6 testes on the left and right sides; however, we did not encounter such an individual. In *B. nigeriense*, Webb (1955) reported a sexual difference in the number of gonads; the number of gonads ranged from 28 to 34 with a peak at 30 in males, whereas 24 to 30 with a peak at 28 in females. However, we found no such sexual differences in our study species.

Reproductive period and multiple spawning

Our results on the reproductive period of *B. belcheri* generally agree with the results or suggestion by Nakagawa (1897), Oyama and Yoshii (1940) and Kubokawa *et al*. (1998). We found that from September to December, gonads were very small and it was impossible to determine sex. Kubokawa *et al*. (1998) also reported that in September, all individuals of the large-sized group possessed the regressed gonads, and at the November collection, the gonads were hardly recognizable in any individuals collected. Fang *et al*. (1991) studied annual changes in the gonads of amphioxus at Xiamen (Amoy), and reported that the reproductive period was restricted in summer, from June to July. Although the authors did not provide the scientific name of the species, it may reasonably be assumed to have been *B. belcheri*.

Chin (1941) reported that *B. belcheri* bred twice a year, from May to July and again in December. Azariah (1965a, b) reported that Indian lancelets (*B. lanceolatum*) collected in Madras, from July to August and from December to February, had fully developed gonads, suggesting that this species bred twice a year. Wickstead (1975) reported that release of mature gametes occurs through a rupture in the atria wall of the gonads, which releases the gametes into the atrium. The rupture subsequently closes, and the gonads redevelop; this presupposes that amphioxus is capable of breeding more than once.

Stokes and Holland (1996) studied the spawning patterns of Florida amphioxus *B. floridae* in detail. The gonad index was first defined as the ratio of the wet weight of the whole gonads to the whole body (SGI). Because it was extremely time consuming to measure the SGI, they adopted a simpler index (PGI) calculated from the single gonad size, and both the SGI and PGI were indiscriminately

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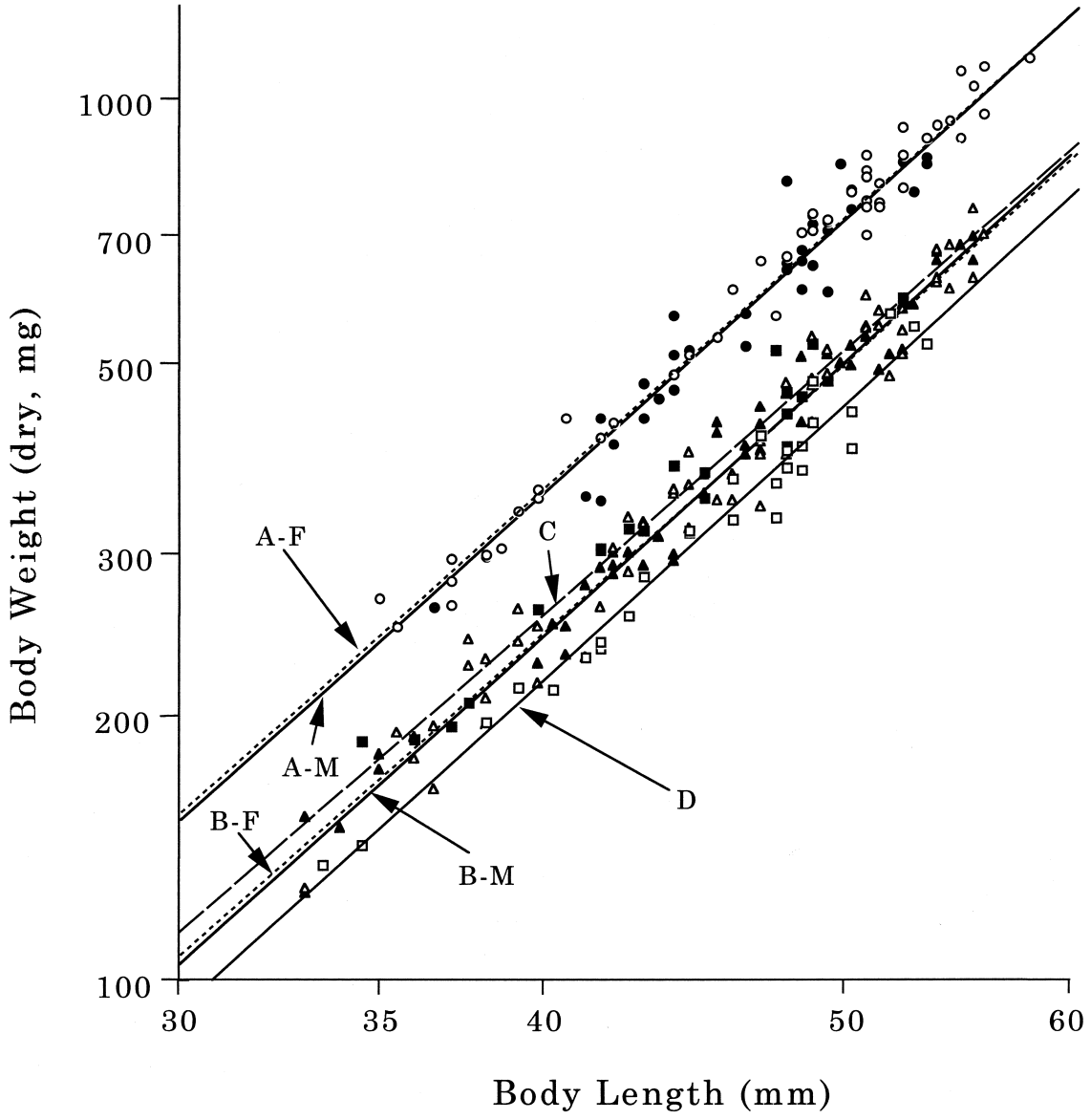


Fig. 9. Relationships between the body length (mm) and body weight (dry, mg) of *B. belcheri* before and after spawning. A: males (M) and females (F) with fully developed gonads. B: males (M) and females (F) after the first spawning. C: individuals before the second spawning. D: individuals during the non-reproductive period.

A:	● — male	LogY = 3.059LogX – 2.338 (r=0.981, n=48)	○ — female	LogY = 3.055LogX – 2.326 (r=0.991, n=48)
B:	▲ — male	LogY = 3.061LogX – 2.503 (r=0.989, n=47)	△ — female	LogY = 3.007LogX – 2.416 (r=0.981, n=58)
C:	■ —	LogY = 2.946LogX – 2.296 (r=0.979, n=22)		
D:	□ —	LogY = 3.065LogX – 2.560 (r=0.985, n=27)		

used as gonad indices. Specimens were collected daily for 24–60 days during the reproductive period from1991 to 1995. From daily changes in the gonad indices, the author assumed that amphioxus repeatedly spawned at intervals of one to15 days, with an average interval of roughly one week. The spawning pattern observed in our study species differed from that of the Florida species. The animals extruded almost all gametes during the first spawning. The second spawning occurred roughly two weeks later; however, the number of available gametes was very small compared to the first spawning. The occurrence of a third spawning has not been confirmed. Because Stokes and Holland (1996) treated their specimens as populations, they were unable to determine how many times a single individuals spawned; however, they suggested that the animals spawned several times at interval of one to two weeks.

To spawn this frequency, the Florida lancelet must accumulate a very large amount of nutrients. The maximum gonad index reported by Stokes and Holland (1996) was 35, indicating that some animals developed gonads that constituted 35% of their total body weight. Azariah (1965b) suggested that the fin rays provide nutrient deposits that can be used by lancelets during the breeding season, and that the fin-ray chambers act as storage compartments. Stokes and

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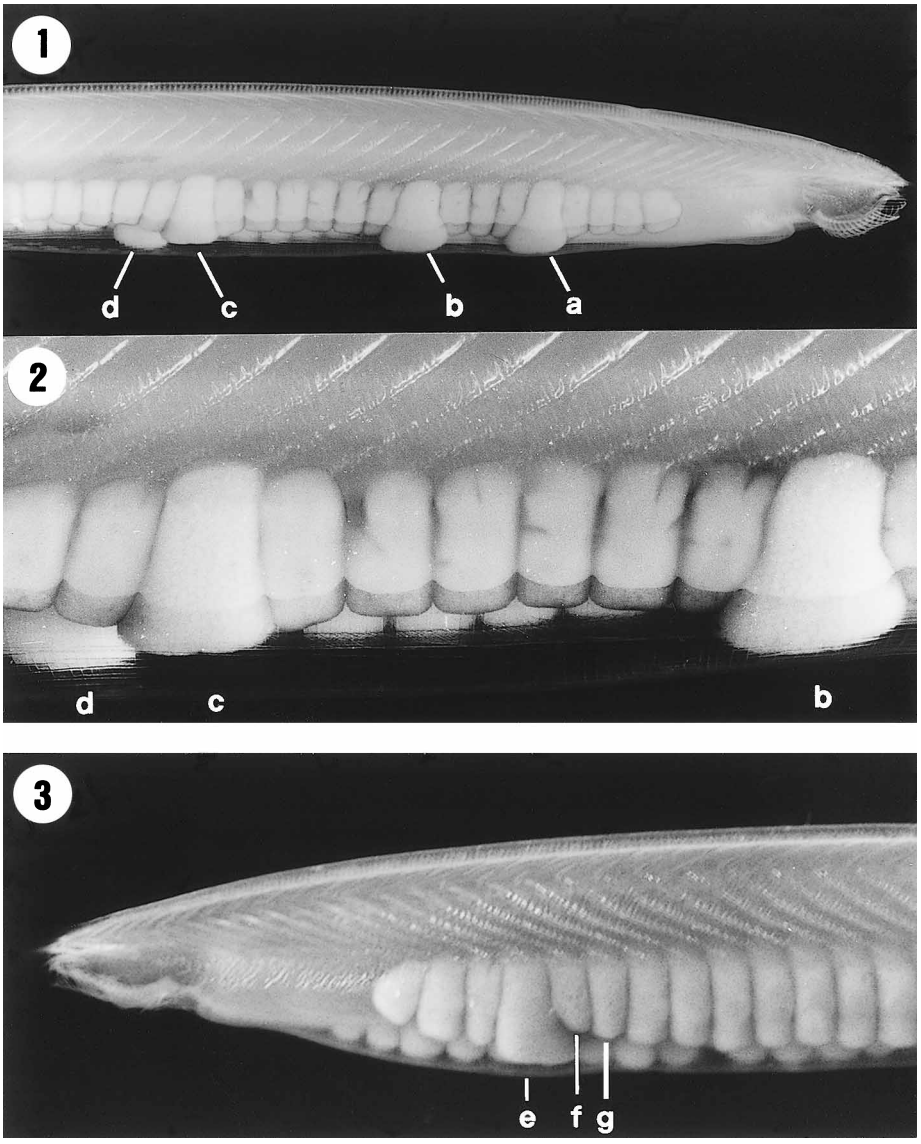


Fig. 10. Two hermaphroditic specimens of *B. belcheri*. (1): The individual obtained on 13 May 2002 possessed four ovaries and 41 testes. The ovaries were larger than the testes. Three ovaries were located on the right side (a-c) and the other on the left side (d); all of them can be seen in this photograph. (2): Enlargement: Three axe-shaped ovaries are apparent among the smaller testes. (3): A second hermaphrodite obtained on 25 June 2002. The anterior fourth (e) to sixth (g) gonads located on the left side of the body are ovaries. Only the fourth gonad is very large and axe-shaped.

Holland (1996) also suggested that fin-rays are nutritional reserves. They examined fin-rays and measured a fin-ray index, which was high in winter when little somatic growth and no gametogenesis took place. Fin-ray indices did not rise between spawning events. The authors assumed that most nutrition derived from food during the breeding season was not stored, but was continuously drained to support gametogenesis in addition to somatic growth. We also examined fin-rays of amphioxus; however, we observed no clear seasonal changes in the morphology of fin-rays (Yamaguchi and Henmi, unpub. data). Azariah (1965b) reported that the color of fin-rays changed from pale yellowish to golden yellow with progressive development. We could not confirm this color change. Therefore, the fin-rays in our study species are not likely to be nutritional reserves.

The difference in patterns of reproduction between the Florida amphioxus and our study species may be partly explained by the absence of adequate nutritional reserves. In our study species, body weight increased only slightly prior to the second spawning. Although the gonads were filled with gametes, they were very small compared to the size before the first spawning. *B. belcheri* has no efficient nutritional reserve; the nutrients required for gonad development to allow the second spawning may therefore be derived from food consumed shortly after the first spawning. A shortage of nutrients may lower the number of gametes available for the second spawning.

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Table 1. Body weights of *B. belcheri* with gonads in various developmental stages (upper). A(M), males with fully developed testes; A(F), females with fully developed ovaries; B(M), males with shrunken testes after the first spawning; B(F), females with shrunken ovaries after the first apawning; C, individuals of both sexes before the second spawning; D, individuals of both sexes during the non-reproductive period, with very small transparent gonads. The percentage of the weight of animals in categories A, B and C campared to that of animals in category D (below).

Body length	Body weight (dry, mg)					
(mm)	A(M)	A(F)	B(M)	B(F)	C	D
30	151.5	153.7	104.2	106.2	113.6	92.5
35	242.7	246.2	167.0	168.9	178.9	148.4
40	365.1	370.2	251.3	252.3	265.1	223.4
45	523.5	530.5	360.5	359.6	375.1	320.5
50	722.6	731.9	497.7	493.6	511.6	442.7
55	967.2	979.3	665.3	657.4	677.4	592.9

Body length	Percentage				
(mm)	A(M)/D	A(F)/D	B(M)/D	B(F)/D	C/D
30	163.8	166.2	112.6	114.8	122.8
35	163.5	165.9	112.5	113.8	120.6
40	163.4	165.7	112.5	112.9	118.7
45	163.3	165.5	112.5	112.2	117.0
50	163.2	165.3	112.4	111.5	115.6
55	163.1	165.2	112.2	110.9	114.3
Average	163.4	165.6	112.5	112.7	118.1

Size at maturity

Chin (1941) reported that the smallest individual of *B. belcheri* with fully developed gonads in Amoy, China was 29 mm BL. Nakagawa (1897) reported that the smallest individuals of *B. belcheri* attained sexual maturity at 26 mm BL (male) and 25 mm BL (female) in Amakusa, Japan. In our study, the minimum mature males and females was 20.5 mm BL and 21.5 mm BL, respectively. These were included in the group of one-year-old; however, most of one-year-olds did not develop gonads. Therefore, these small individuals with developed gonads were exceptions; most individuals developed gonads after their second year.

Hermaphrodites

Chin (1941) observed one hermaphroditic *B. belcheri* with ten ovaries and ten testes on both sides. This differed from two specimens that we obtained, which had only four or three ovaries among a total of 45 or 54 gonads. Although the testes predominated in number, the ovaries were more developed. According to Wickstead (1975), several authors have observed hermaphroditic individuals of *B. lanceolatum*. In contrast, Stokes and Holland (1996) encountered no hermaphroditic *B. floridae* during an examination of 21,500 specimens.

ACKNOWLEDGEMENTS

We thank two anonymous referees for their helpful comments on this paper and H. Shimasaki, K. Mizoguchi and T. Kai for their help in the field.

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(Received November 6, 2002 / Accepted April 24, 2003)

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