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Authors: Kubokawa, Kaoru, Azuma, Nobuyuki, and Tomiyama, Minoru

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A New Population of the Amphioxus (*Branchiostoma belcheri*) in the Enshu-Nada Sea in Japan

Kaoru Kubokawa[1]*, Nobuyuki Azuma[2] and Minoru Tomiyama[3]

[1]*Ocean Research Institute, University of Tokyo, Minamidai, Nakano, Tokyo 164-8639, Japan*
[2]*Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori 036-8561, Japan*
[3]*Marine Resources Research Center, Aichi Fisheries Research Institute, Toyohama, Minamichita, Aichi 470-3412, Japan*

ABSTRACT—The amphioxus, *Branchiostoma belcheri*, is currently listed in the registry of "Endangered Animals of Japanese Marine and Fresh Water Organisms" issued by the Japan Fisheries Resource Conservation Association. We surveyed a new population of this species in an area near the Irago Channel and the Enshu-Nada Sea in Aichi Prefecture, Japan. This population was originally discovered by a research group of the Aichi Fisheries Research Institute when they were collecting Japanese sand lances (*Ammodytes personatus*) in this area. Every month from June to November of 1995 with the exception of October, we collected amphioxi and sand lances in an area of about 1 km^2 to study the population profile of the amphioxus and the relation between these two animals. The newly discovered population of the amphioxus was suspected to be larger than any other Japanese populations of this species. Animals of this population could be divided into two groups according to body size distribution: those of approximately 20 mm long on the average (termed small) and those of approximately 50 mm long on the average (termed large). No small amphioxus was found in June; 10% or fewer of the animals were of small size in July and August; and about 40% of the animals were of small size in September and November. The breeding season was estimated to last from June (or earlier) to August based on observation of the gonadal size. Amphioxi share a habitat with sand lances, which stay in the sand during the summer dormant period. The newly discovered amphioxus population may provide an important source of material for modern biological researches on Cephalochordata in Japan, although special care for protection of this population should be taken.

INTRODUCTION

The subphylum Cephalochordata of the phylum Chordata contains about 29 species that represent valid taxa (Poss and Boschung, 1996). As is well known, the amphioxus is the closest living invertebrate relative of vertebrates (Wada and Satoh, 1994). Because of this position in the phylogenic tree, they have attracted the attention of comparative and evolutionary biologists. In Japan, the amphioxus is represented primarily by a major species, *Branchiostoma belcheri*, whose distribution has been reported in a total of more than 40 habitats. The habitats are widely spread along the coasts of Kyushu, the Seto Inland Sea, the southwest part of Honshu (Nishikawa, 1981). The natural monument in Japan has designated on the habitats of amphioxi in shores of Oshima Island in Aichi Prefecture and of Nouji in Hiroshima Prefecture. Not only these populations but a number of others as well are drastically disappearing or already extinct presumably due to water and sediment pollution. These conditions have made it difficult or almost impossible to conduct studies on Japanese amphioxus by means of modern biological techniques, in spite of the important phylogenic position of this species. In fact, research into Japanese amphioxus has been very limited. The ecology of this species, for example, has been the subject of only a single paper (Kikuchi, 1977: on the Tsuyazaki population).

Almost monthly since 1989, researchers of the Aichi Fisheries Research Institute (AFRI) have conducted a survey of sand lances (*Ammodytes personatus*) during their summer dormancy in the Deyama Shallow of the Irago Channel. Using a large hook for collection, these researchers unintentionally collected a small number of amphioxi along with the sand lances, thereby discovering a new population of habitat for this species. Motivated by this occurrence, in the summer and autumn of 1995 we surveyed the same area in order to determine the precise location of the new amphioxus population and to obtain information on the abundance and body size of the animals in the population.

* Corresponding author: Tel. +81-3-5351-6487;
FAX. +81-3-5351-6488.

METHODS

The surveys were performed once each on 16 June, 10 July, 9 August, 21 September, and 14 November of 1995 in a square-shaped area of about 1 km^2 within two diagonal points of 34.40N, 137.03E and 34.30N, 137.04E in the Deyama Shallow (Deyama hereafter). Deyama is located in the Irago Cannel at the junction of Mikawa Bay, Ise Bay and the Enshu-Nada Sea, and about 10 km south from the western edge of the Atsumi Peninsula (Fig. 1). An AFRI research boat (4 tons) was used for the survey. For the collection of amphioxus, a cylinder dredge (20 cm in diameter and 40 cm in length) was used, and the dredging was performed at five points (the center and each of four corners of the square) for each survey date. Collected sediment was moved to a container and carefully searched for amphioxus. When found, amphioxus was picked up individually by hand and moved to a tank. The search was repeated more than three times for each sediment sample. If amphioxus was not found, dredging was repeated up to 4 times.

At the same time, sand lance was collected by means of a specialized fishing apparatus known as a "Karazuri" to which about 20 hooks are attached with a steel bar of about 3 m in length. This device was lowered to the ocean floor and trailed along the floor surface behind the boat for 5 min per passage. The collected amphioxus was carried back to the laboratory, where their body lengths were measured and the developmental condition of their gonads was observed externally.

RESULTS

A few of the amphioxi collected in Deyama were sent to Dr. Teruaki Nishikawa, Nagoya University, for taxonomic iden-

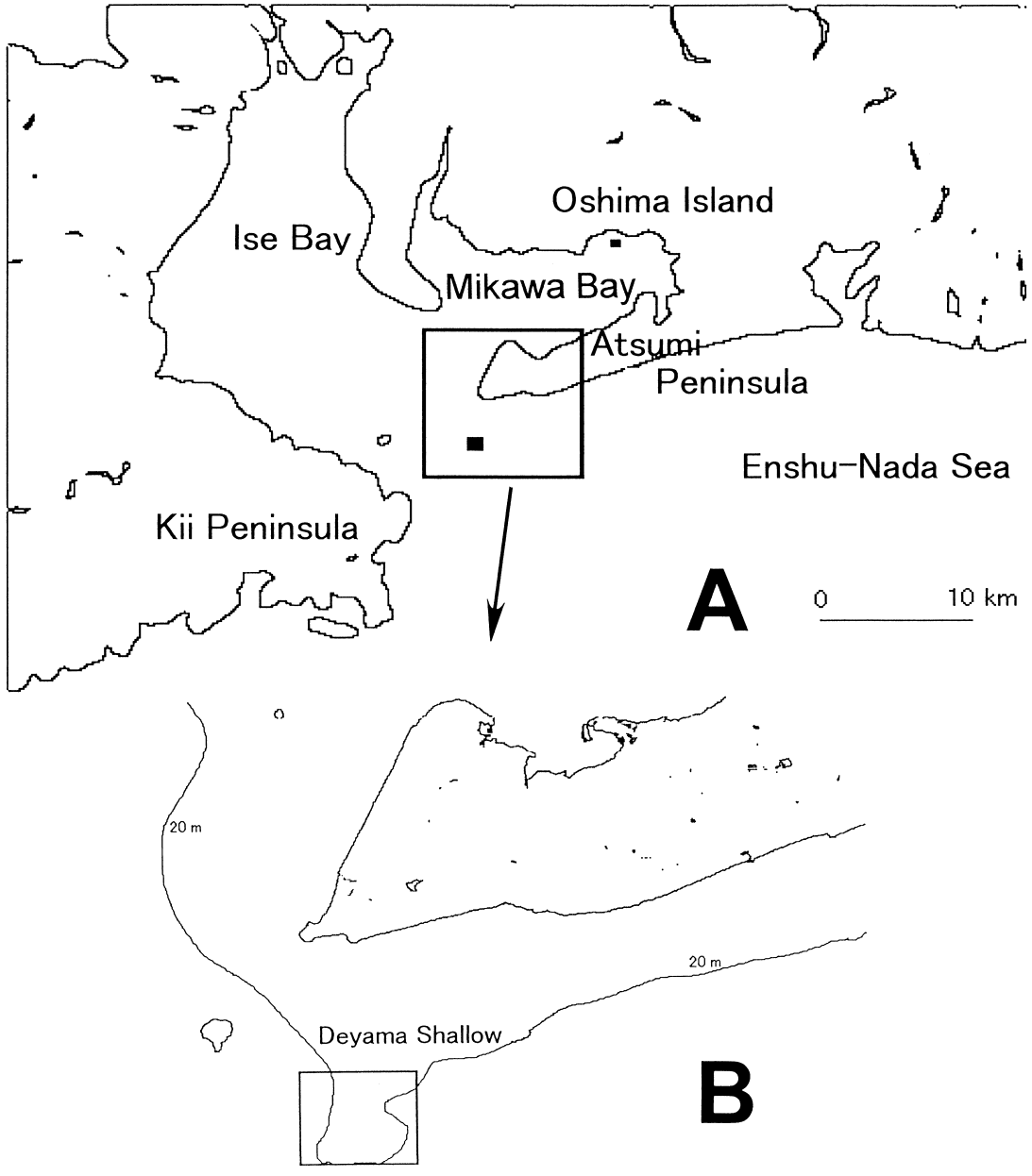

Fig. 1. (**A**) Map of the study area (surrounding by a square) in the Irago Channel at the junction of Mikawa Bay, Ise Bay and the Enshu-Nada Sea. (**B**) Location of the study area in the Deyama Shallow. The thin lines indicate depth in 20 m intervals.

Table 1. Profiles of body length in small and large amphioxus in various months

	Small individuals				Large individuals				
	July	August	Sept	Nov	June	July	August	Sept	Nov
Minimum length (mm)	17	15	14	20	33	28	35	35	40
Maximum length (mm)	20	25	24	24	63	54	68	58	64
Average (mm)	19.00	18.75	19.13	22.00	50.59	42.90	52.18	43.92	51.73
Standard deviation (mm)	1.73	4.79	2.58	1.58	6.21	7.61	5.92	7.22	6.64
Median (mm)	20	17.5	19	22	50.5	44.5	53	44	51
Number	3	4	38	9	46	20	150	53	15

tification. They were all identified as *Branchiostoma belcheri* Gray.

The water depth of the collection sites of these amphioxi varied from 18 to 23 m. Bottom sediments were mainly composed of fine sands in most cases, and in some cases terrigenous silt, gravel, or shell fragments dominated. No amphioxus was found in areas where silt or gravel where the dominant components of the sediment. The surface water temperature was 18.6 and 22.4°C in August and September respectively. The total number of amphioxus collected was 46 in June, 23 in July, 154 in August, 91 in September and 24 in November (Table 1). The maximum population density observed was about 8 individuals/liter of sediment.

The distribution of the amphioxus body length varied from month to month (Fig. 2A). When paired comparisons of the body length distribution were made between neighboring months, all the pairs showed statistically significant differences: $P \leqq 0.01$ between June and July, between July and August, and between August and September; $0.01 \leqq P \leqq 0.05$ between September and November by the two-sided Kolmogorov-Smirnov two sample test. The body length started to show a tendency of bimodal distribution beginning in July and a clear bimodality was observed in September and November. Cumulative frequency curves (ogives) of the body length distributions also indicated the presence of two groups of amphioxi with different body length distributions in all the months except June (Fig. 2B). One group had amphioxi of body length smaller than 26 mm (small individuals described in Table 1), and one group those larger than 26 mm (large individuals described in Table 1). Therefore, we analyzed a biomodality of the body length distribution by dividing into the two groups at 26 mm in the body length. Amphioxi of small body length composed about 40% of the total sample in September and November, 10% in July, 3% in August and 0% in June. The body size parameters of these two groups are given in Table 1. In the small individuals, the body length varied within a small range from July to September, but increased suddenly in November as judged from the mean and median values. The difference in body length distribution between September and November was highly significant ($P \leqq 0.01$; Mann-Whitney U-test). Furthermore, the individual variation of the body length tended to decrease with month, and the difference in the body length variance between September and November was found to be highly significant ($P \leqq 0.01$; ANOVA).

In the large-size group, the body length differed significantly ($P \leqq 0.01$; one way layout ANOVA) with month, and the mean body lengths in July and September were significantly smaller ($P \leqq 0.01$; Duncan's multiple range test) than those in the other months. On the other hand, the individual variation (represented by the standard deviation) of the body length was almost constant through the period from June to November in the large individuals.

By external visual examination of the animals, amphioxi of the small individuals were judged to be sexually immature animals. In the large-size group, well-developed gonads were discernible in a number of individuals in June, July and August. In September, all individuals of the large-size group possessed the regressed gonads, and at the November collection, the gonads were hardly recognizable in any individuals collected.

In order to confirm that amphioxi share their habitat with sand lances during the summer dormant period of the sand lance, we tried to collect sand lances at the same sites and times. The number of sand lances collected showed a significant correlation with that of amphioxus collected on the same day at the same site (Table 2).

DISCUSSION

The benthic population of *Branchiostoma belcheri* Gray in the Deyama Shallow in Aichi Prefecture reported here is of larger density than any population reported for *B. belcheri* (Nishikawa, 1981) based on the limited number of individuals collected in Japan. The maximum body length (68 mm) observed in this population is also larger than any other values ever reported for Japanese *B. belcheri*, *B. belcheri* in Xiamen (57 mm) (Chin, 1941), *B. floridae* in Florida (58 mm) (Stokes, 1996) or for other *Branchiostoma*. The habitat in Deyama might thus be suitable for encouraging amphioxus growth. The location of the current Deyama Population is close to the famous population in Oshima Island in Mikawa Bay (see Fig. 1). However, it is believed that the Oshima population has already disappeared as a result of terrigenous sludge. The last recorded collection from the Oshima population was made in 1957 (Nishikawa and Mizuoka, 1990). The relation between the current Deyama and previous Oshima populations is not known.

The breeding season of the Japanese amphioxus is known to be short, though its actual occurrence is variously reported within a range from May to July (Nishikawa, 1981).

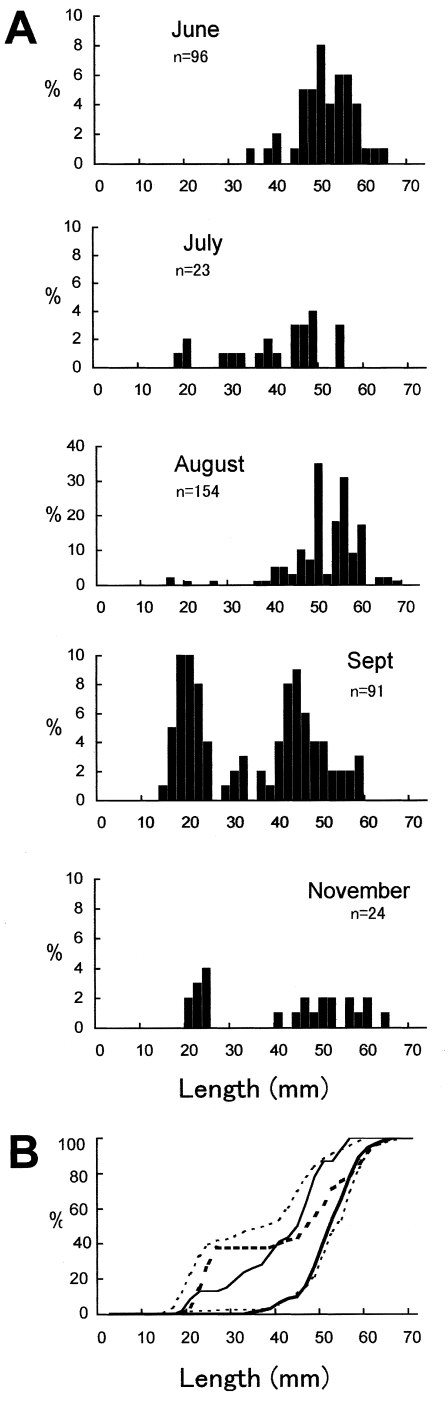

Fig. 2. (**A**) Length-frequency histograms taken in June, July, August, September and November. The frequency scale is described as a percentage. The maximum scale is 40% in August and 10% in over the rest of the year. (**B**) Cumulative frequency curves of the body length distributions in June (thick solid line), July (thin solid line), August (thin dashed line, right), September (thin dashed line, left) and November (thick dashed line).

However, none of these previous data have been based on continuous study over a period of months. In China, Chin (1941) reported that the breeding season of *B. belcheri* in Xiamen is from May to July. In Tampa bay, Florida, *B. floridae* has been reported as breeding through early May to early September (Stokes and Holland, 1996). Our results clearly show that breeding of most individuals lasts from June (or earlier) to August. Because individuals collected in June in the present study had fully developed gonads, the start of the breeding period is likely to be May, although further study will be needed to confirm this.

The sudden appearance of a large number of small animals in September, as well as the appearance of the smallest individual (14 mm) in the same month, suggests that these were individuals hatched in the same year and who had just settled in this habitat. Stokes (1996) and Stokes and Holland (1995) reported in a study on *B. floridae* that juveniles settled when they were approximately 4 mm long. It is estimated from data reported by Chin (1941) on *B. belcheri* that juveniles reach 8 mm long for a month after fertilization. Furthermore, the growth rate of *B. belcheri* was estimated as 2.5 mm/month in early juveniles in the summer in Qingdao (Wu *et al.*, 1994) and as 1.5 mm/month in post-settlement lancelets in Xiamen (Chin, 1941). In other species, the summer growth rate was estimated to be 0.5 mm/day for *B. floridae* in Florida (Stokes, 1996) and the post-settlement growth as being 10 to 12 mm/month for *B. nigeriense* in Nigeria (Webb, 1958). Judging from the above information on growth rate, it would seem that the small individuals collected in September and November were hatched between May and August, although there is a discrepancy between the size of this settlement and that of Florida amphioxus reported by Stokes (1996). A possible explanation for the difference of body length between settlements might be that the juveniles of the present population had a larger growth rate than any individuals previously reported. In addition, it may be that the some juveniles were plankton in June, and were therefore not collected by the dredging at that time. On the other hand, it is difficult to explain the presence of individuals of 17 mm in length in July, even considering the rapid growth rate. It is suspected that these amphioxi were hatched in the previous year, and relocated to and settled in the present habitat in July or later. In this case, there would have been a widely spread habitation of amphioxus in June, and the failure to collect any juveniles by dredging would have to be ascribed to coincidence. Clearly, additional samplings of this population will be needed to clarify settlement of juveniles and the reproduction.

Among the five months studied, the number of amphioxi was the largest in August. A possible explanation for this result is that amphioxus aggregates in patches for reproduction and we happened to hit such a patch during our dredging. The precise density of amphioxus in Deyama is difficult to estimate because of the method of collection, i.e., dredging. Nonetheless, we calculated a maximum population of 8 individuals/liter. The density of amphioxus in Tampa Bay has been variously reported as 100 to 1200 individuals/m^2 (Stokes,

Table 2. Percentages of the number of amphioxus and sand lance at various stations in Deyama in summer

		Stations					
		1	2	3	4	5	total
June	Amphioxus	0	12.7 (7)	54.5 (30)	5.5 (3)	27.3 (7)	100 (47)
	Sand lance	33.7 (46)	3.6 (5)	54.7 (75)	4.4 (6)	3.6 (5)	100 (137)
July	Amphioxus	–	3.6 (3)	96.4 (81)	–	–	100 (84)
	Sand lance	8.3 (71)	17.5 (149)	57.8 (491)	0.4 (3)	16 (136)	100 (850)

Values in parentheses; the number of animal. –; no data.

1996), 183 individuals/liter (Pierce, 1965) and approximately 340 individuals/m^2 (Bloom *et al*., 1972). The density was 156 individuals/liter in Xiamen (Chin, 1941) and 4000/m^2 in Shantung in China (Tchang and Koo, 1936). The density in the Deyama population is smaller than that of any of these populations, though it is currently the largest density in Japan. It is important to protect this population and at the same time to use animals of this population efficiently for scientific studies without affecting the population size.

The sand lance has a slender body shape and a body length of 7 to 10 cm during its aggregation in the sand over the summer dormant period, which is followed by the breeding season. Both sand lance and amphioxus may prefer the same habitat type, i.e., sand sediment. Since sand lances are widely distributed along the shores of Japan northward from the Seto Inland Sea, there is a possibility of finding new amphioxus populations by surveying sites where sand lances have been collected during the summer dormant season.

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