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Growth, secondary production and gonad development of two co-existing amphioxus species (*Branchiostoma belcheri* and *B. malayanum*) in subtropical Hong Kong

Yan Chen [a], Paul K.S. Shin [a,b], S.G. Cheung [a,*]

[a] *Department of Biology and Chemistry, City University of Hong Kong, Hong Kong*

[b] *Centre for Coastal Pollution and Conservation, City University of Hong Kong, Hong Kong*

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Abstract

The present study investigated the growth, secondary production and gonad development of two co-existing amphioxus species *Branchiostoma belcheri* and *B. malayanum* in subtropical Hong Kong from June 2005 to June 2006. Based on the modal progression analysis, amphioxus populations were decomposed into separate cohorts. The von Bertalanffy growth models were also estimated according to the size incremental data. From the growth models, the size ranges of one, two and three-year-old *B. belcheri* were estimated to be 5–28 mm, 28–38 mm and 38–45 mm BL, respectively; while the one and two-year-old *B. malayanum* were estimated to be 7–30 mm and 30–35 mm BL, respectively. The secondary production was calculated at 1.15 g m^{-2} yr^{-1} DW or 0.63 g m^{-2} yr^{-1} AFDW for *B. belcheri* with density 424 ind m^{-2}, and 0.51 g m^{-2} yr^{-1} DW or 0.40 g m^{-2} yr^{-1} AFDW for *B. malayanum* with density 121 ind m^{-2}. The production to biomass ratio (*P*/*B*) was 1.13 for *B. belcheri* and 0.98 for *B. malayanum*. Changes in the gonad length index indicated that *B. belcheri* spawned mainly in June and July, while *B. malayanum* mainly in April and August. As compared with *B. belcheri*, *B. malayanum* was characterized by rapid growth, shorter life span, early maturity and lower population density. Such differences in population dynamics may allow both species to share a similar habitat and co-exist in subtropical waters of Hong Kong.

Keywords: Amphioxus; Growth; Reproduction; Secondary production

1. Introduction

As the only existing animal group under Subphylum Cephalochordata and the closest living invertebrate to the vertebrates, the anatomy and embryology of amphioxus (=lancelet) have been studied extensively for over a century. Information on other aspects of their ecology and biology, however, is relatively limited. While there are 29 living amphioxus species with a wide geographic distribution in coastal waters all around the world (Poss and Boschung, 1996), reports on population studies are confined to a few species, including *B. belcheri* in China (Chin, 1984) and Japan (Henmi and Yamaguchi, 2003; Yamaguchi and Henmi, 2003), *B. lanceolatum* in North Sea and the Mediterranean (Courtney, 1975), *B. senegalense* in Africa (Gosselck and Spittler, 1979), *B. floridae* in USA (Stokes and Holland, 1996), and *B. nigeriense* in Nigeria (Webb, 1958a). These studies revealed that multiple cohorts exist in amphioxus populations. The animal also has various growth rates and reproduction seasons, depending on geographical locations and environmental conditions. In Asia-Pacific and around South China Sea, *B. belcheri* is commonly found in China and Japan, and listed as a second priority protection species and an endangered animal, respectively (Yang et al., 1993; Kubokawa et al., 1998).

In subtropical Hong Kong, large populations (>100 ind m^{-2}) of *B. belcheri* and *B. malayanum* have recently been discovered co-existing in similar sandy habitat (Chen, 2007). Of particular interest is *B. malayanum*, which has rarely been reported in the literature; only two specimens were recorded by Webb (1958a) and four individuals by Gibbs and Wickstead (1969) in Singapore

* Corresponding author. Tel.: +852 27887749; fax: +852 27887406.
E-mail address: bhsgche@cityu.edu.hk (S.G. Cheung).

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and Solomon Islands, and few specimens by Nohara et al. (2004) in Thailand. Thus, there is no detailed information on the biology and ecology of this species, which is deemed to be restricted to tropical or subtropical areas. The co-occurrence of *B. belcheri* and *B. malayanum* also raises an interesting question on how these two species co-exist in the same habitat. The present study aimed to investigate the population dynamics of *B. belcheri* and *B. malayanum* in terms of growth, secondary production and gonad development, and compare the findings with other amphioxus species.

2. Materials and methods

2.1. Study site and sample collection

Specimens of amphioxus were collected at roughly 1 month intervals from June 2005 to June 2006 in 10 samplings, from Pak Lap Wan, Hong Kong (22° 20′ N, 114° 21′ E), where high abundance (>100 ind m^{-2}) of *B. belcheri* and *B. malayanum* was found (Chen, 2007). Water depth at the sampling site was about 13 m and sediment comprised >90% fine sand. About 50 L sediments were obtained using a 0.1 m^2 van Veen grab. The sediment was gently washed on board with a 0.5 mm mesh screen and amphioxus retained were collected and placed in an aerated aquarium filled with seawater. Most amphioxus specimens survived the field treatment and were kept alive until measurements. Dead specimens were preserved in 5% neutralformalin, and included in individual count and size measurement.

2.2. Cohort separation and mortality calculation

All collected specimens were identified and the total body length (BL) (from the tip of the rostrum to the tip of the caudal fin) of each individual was measured to the nearest 0.1 mm using a vernier caliper. For each field collection, length–frequency data were grouped into size classes of 1 mm intervals, and the percentage frequency of the whole sample contributed by each size class was plotted as a length–frequency histogram. In order to follow the growth of separate cohorts over the sampling period, individual cohorts (size classes) were separated using the modal progression analysis of Fish Stock Assessment Tool II (FiSAT II, http://www.fao.org/fi/statist/fisoft/fisat). FiSAT II applies the maximum likelihood concept to separate the normally distributed components of size–frequency samples, allowing accurate demarcation of the component cohorts from the composite polymodal population size–frequency distribution. For each cohort identified, mean lengths with standard deviations, group sizes (in numbers), and separation index were estimated.

Mortality of amphioxus was deduced from the abundance data obtained from the frequency histogram for individual cohorts, as calculated by:

$$\text{Mortality} = 100 \times [1 - (N_t/N_{\max})] \quad (1)$$

where, $N_{\max}$ = maximum abundance; N_t = abundance at time t.

2.3. Growth analysis

Growth of amphioxus was evaluated by recording the size increment of each cohort collected sequentially in time throughout the sampling period, based on the data of cohort demarcation obtained in Section 2.2. The von Bertalanffy growth model (von Bertalanffy, 1938) was applied to the data, using the software Simply Growth (Pisces Conservation Ltd, www.irchouse.demon.co.uk). The growth model was based on the following equation:

$$L_t = L_{(\max)}\left(1 - b \cdot e^{-kt}\right) \quad (2)$$

where, L_t = body length at time t in days from initial settlement; $L_{(\max)}$ = maximum length or asymptotic length; k = instantaneous growth rate coefficient, and b = scaling factor:

$$b = e^{kt_0} \quad (3)$$

where, t_0 was included to adjust the equation for the initial size of the animal and was defined as age at which the animal would have had zero size.

2.4. Determination of length/mass relationships

In order to determine the length/mass relationships in amphioxus, 30 specimens each of *B. belcheri* or *B. malayanum* were blotted dry with paper towel, and the total body length of each individual was measured to the nearest 0.1 mm using a vernier caliper. Each individual was also weighed to the nearest 0.1 mg to provide the wet weight (WW), and further dried in an oven at 60 °C for 24 h to obtain the dry weight (DW). Ash-free dry weight (AFDW) of each individual was determined after combustion for 4 h at 500 °C in a muffle furnace.

2.5. Evaluation of secondary production

The secondary production of *B. belcheri* and *B. malayanum* was estimated utilizing the mass-specific growth rate (Crisp, 1984). This was based on an average length–frequency sample produced from the pooled samples, the parameters of von Bertalanffy growth model, and the calculated length/mass relationships. The secondary production (P) was calculated as the sum over i size classes:

$$P = \sum (N_i M_i S_i \Delta t) \quad (4)$$

where, N_i = number of animals (ind m^{-2}) in size class i; M_i = average individual body mass (DW or AFDW) in size class i; Δt = sample period; and S_i = mass-specific growth rate, as represented by:

$$S_i = b \cdot k\left[\left(L_{(\max)}/L_i\right) - 1\right] \quad (5)$$

where, L_i = mean length of size i; whereas other parameters, including b, k and $L_{(\max)}$, were depicted from Eqs. (2) and (3).

2.6. Sex determination

Both *B. belcheri* and *B. malayanum* belong to the genus *Branchiostoma*, which is characterized by bilateral series of gonads, with a row of testes or ovaries being on each side of the body (Poss and Boschung, 1996). The sex of amphioxus can be identified based on the conformation of the surface of the developed gonad observed under a microscope (×100). Ovaries are yellowish whereas oocytes are obvious inside ovaries and the surface of oocyte is granular. Testes are whitish, and the surface is smooth. In the present study, each specimen of the two amphioxus species obtained from the field was sexed and recorded as either unsexable, a male or female individual.

2.7. Seasonal changes of gonad conditions

The gonad length index (GLI) was adopted from Yamaguchi and Henmi (2003) to describe the gonad conditions of amphioxus. The body length of each individual was first measured to the nearest 0.1 mm using a vernier caliper and placed under a compound microscope (×100) equipped with an ocular micrometer to locate and measure the maximum gonad length. Post-spawning

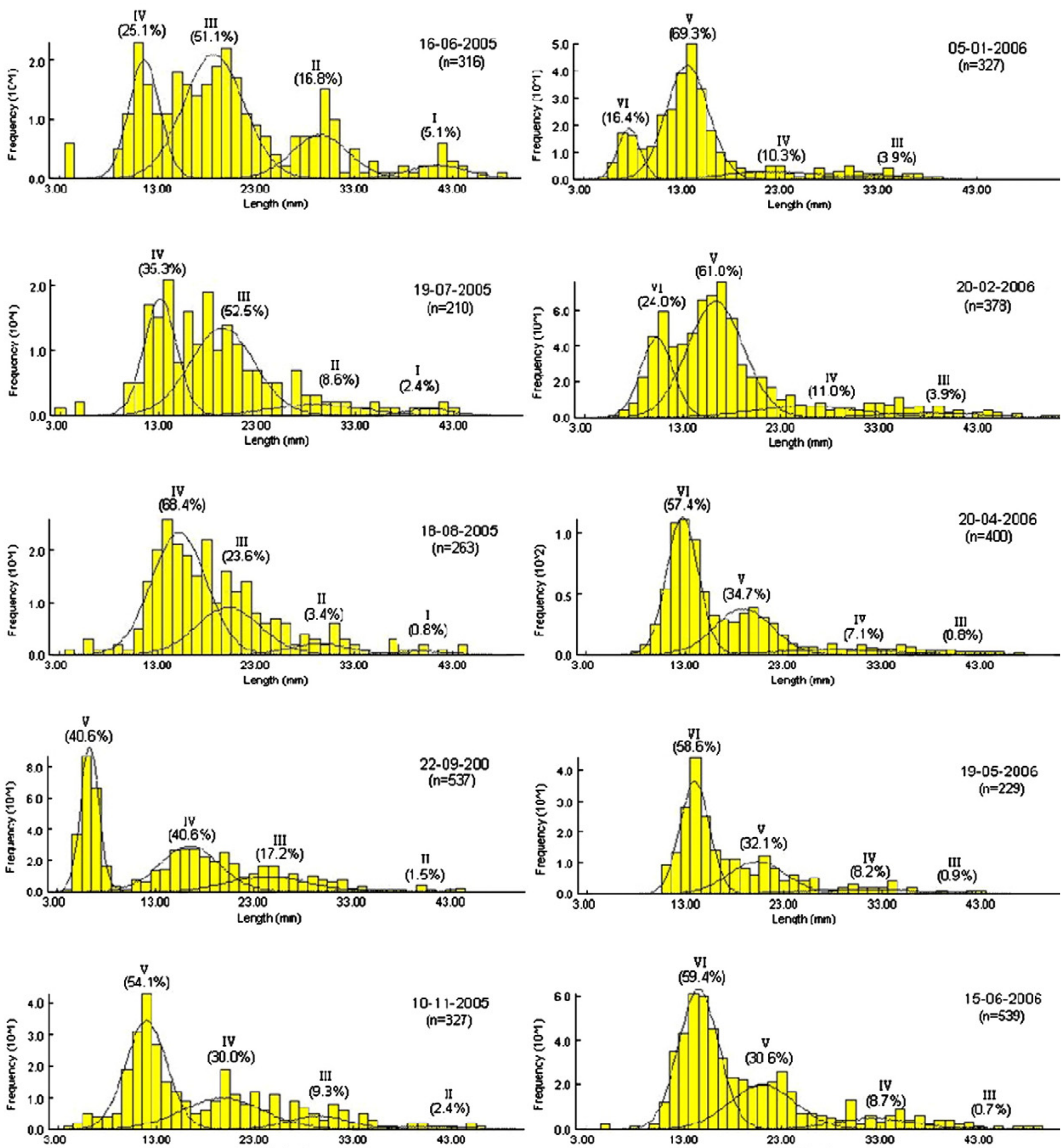

Fig. 1. Length–frequency histogram of *B. belcheri* collected from June 2005 to June 2006. Roman numbers indicate different cohorts demarcated by the modal progression analysis of FiSAT II. Proportion of each cohort was presented below by percentage.

individuals with regressed gonads were excluded from the GLI estimation. The value of GLI was then calculated as:

$$\mathrm{GLI} = (\text{maximum gonad length}/\text{body length}) \times 100 \quad (6)$$

Considering that gonad development may be asynchronous among amphioxus individuals, the maturity percentage was also included in the calculation:

$$\text{Maturity\%} = \text{number of maturity}/\text{number of adults} \quad (7)$$

where, "maturity" indicates individuals with detectable gonads, while "adult" indicates all individuals larger than the minimum size of sexual maturity no matter they have visible gonads or not.

3. Results

3.1. Amphioxus density

From June 2005 to June 2006, 4308 specimens of *B. belcheri* were collected ranging from 4 to 51 mm BL; and 1120 specimens of *B. malayanum* were collected ranging from 7 to 36 mm BL. The density of *B. belcheri* and *B. malayanum* at the study site was 423.8±111.1 m^{-2} and 121.2±59.9 m^{-2}, respectively.

3.2. Population structure and mortality of B. belcheri

Fig. 1 shows the polymodal population structure based on the length–frequency histograms of *B. belcheri* collected from June 2005 to June 2006. The number of survivors and the cumulative mortality calculated in each cohort are presented in Table 1.

As shown in Fig. 1, totally 6 separate cohorts were observed in the *B. belcheri* population. Cohorts I and II were large-sized groups (initial size 40.5 mm and 29.5 mm BL on average, respectively), which accounted for low proportions (5.1% and 16.8%, respectively) of the population in June 2005. Each of them kept diminishing in subsequent intervals. The last presence of cohorts I and II was recorded in August 2005 and November 2005, respectively. Cohorts III and IV were medium-sized groups (initial size 18.7 mm and 11.7 mm BL on average, respectively) and dominated the population from June to August 2005, with a combined proportion varying between 76.2% and 92.0%, and the average size varying between 11.7 and 22.3 mm BL. Small-sized individuals (<8 mm BL), representing newly-settled juveniles, were recorded at every intervals from June 2005 to January 2006, then in June 2006. Juveniles settled during this period could be split into two groups as cohorts V and VI. Cohort V emerged in September 2005 and consisted of all the juveniles settled in the period from June to September; while cohort VI emerged in January 2006, and consisted of all the juveniles settled in the period from September to January. Both cohorts V and VI appeared continuously at subsequent intervals. In September 2005, cohort V (average BL 6.3 mm) emerged in high abundance (proportion 40.6% of total population), and became the dominant group together with cohort IV, with a combined proportion of population at 81.2% in September and 84.1% in November, and the average size varying between 6.3 and 19.7 mm BL. In January 2006, cohort VI emerged and together with cohort V dominated the population in the following 6 months till the end of this investigation, with a combined proportion of population ranging between 85.1% and 92.1%, and the average size ranging between 7.7 and 21.0 mm BL. Over the study period, individuals varying between 6 and 23 mm BL constituted the dominant group in this *B. belcheri* population.

As shown in Table 1, during this one-year sampling period, cohort III had the highest abundance (201 ind m^{-2}) at the initial average size of 18.7 mm BL, followed by mortality of 31.3% occurring between June and July of 2005, further 37.8% between September and November of 2005 at the average size 24.4 mm BL; and 90.5% of the individuals died before February 2006 at the average size 38.1 mm BL. Cohort IV attained its highest abundance (271 ind m^{-2}) at the average size 16.5 mm BL, followed by mortality of 53.9% occurring between September and November of 2005; and 89.3% of the individuals died before June 2006 at the average size 32.3 mm BL. A similar case was observed in cohorts V and VI. Cohort V attained its highest abundance (288 ind m^{-2}) at the average size 16.4 mm BL, followed by mortality of 39.6% occurring between February and April of 2006. Cohort VI attained 281 ind m^{-2} at the average size 14.5 mm BL in May 2006. Mortality of individuals as high as 30.0% occurred between May and June. It appeared that death of *B. belcheri* generally started at the size around 16 mm BL (mortality 30%–50%), and the majority of individuals (around 90%) died before attaining 38 mm BL.

Table 1
Number of survivors and cumulative mortality (%) of each cohort of *B. belcheri* from June 2005 to June 2006

Time	Cohort I	Cohort II	Cohort III	Cohort IV	Cohort V	Cohort VI
16/06/05	20 (60.0%)	66 (65.2%)	201 (31.3%)	99		
19/07/05	6 (85.0%)	23 (83.3%)	138 (61.2%)	93		
18/08/05	3 (100%)	11 (84.8%)	78 (42.8%)	225		
22/09/05		10 (84.8%)	115 (80.6%)	271 (53.9%)	271	
10/11/05		10 (100%)	39 (92.0%)	125 (80.8%)	225	
05/01/06			16 (90.5%)	52 (84.5%)	283	67
20/02/06			19 (98.0%)	42 (86.7%)	288 (39.6%)	114
20/04/06			4 (98.0%)	36 (87.5%)	174 (54.2%)	287 (2.1%)
19/05/06			4 (98.5%)	34 (89.3%)	132 (64.2%)	281 (30.0%)
15/06/06			3	29	103	201

3.3. Population structure and mortality of B. malayanum

Fig. 2 shows the length–frequency histograms of *B. malayanum* collected in this annual investigation from June 2005 to June 2006. In the *B. malayanum* population, totally 5 separate cohorts were observed during the sampling period. Cohort I was the large-

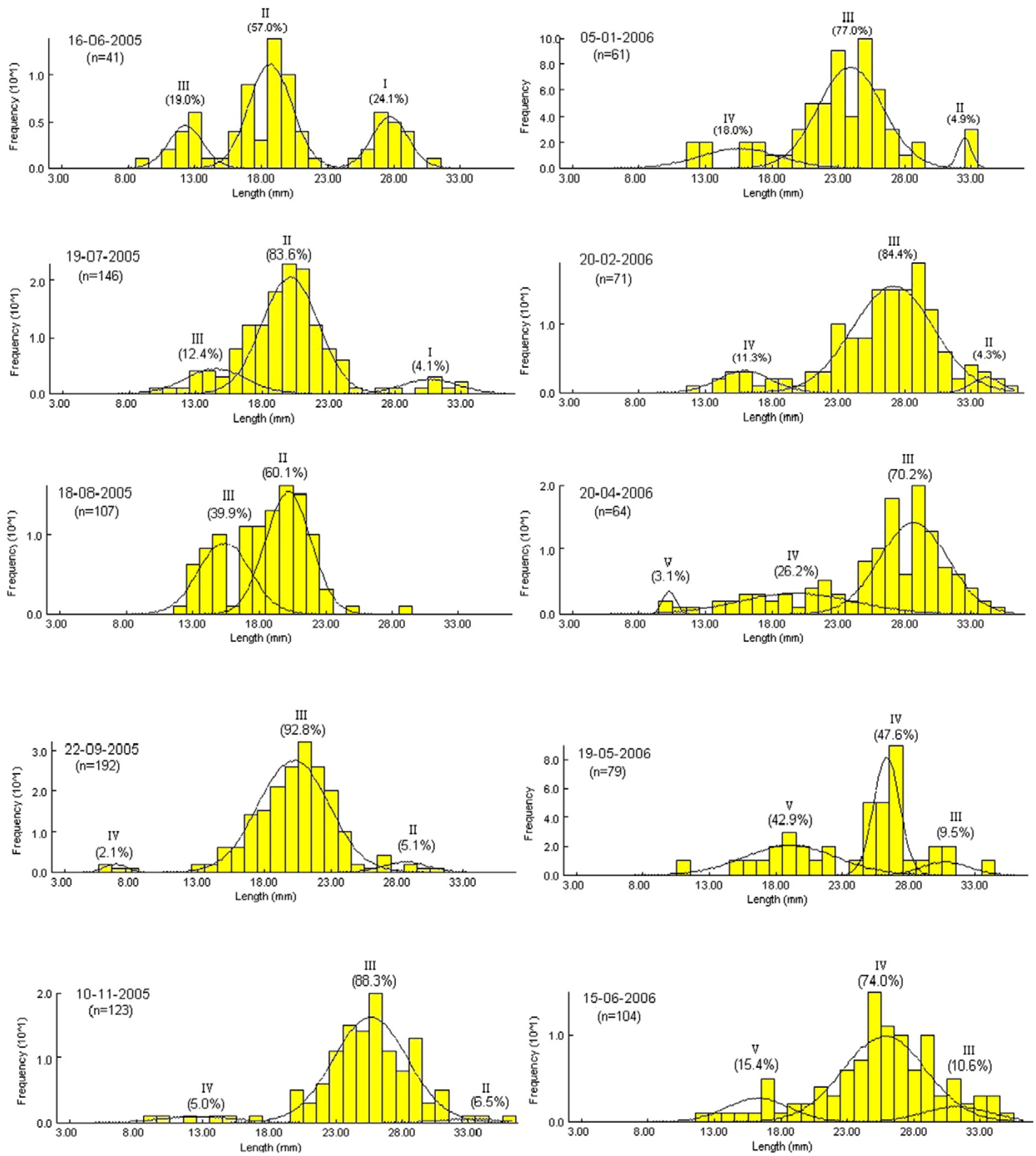

Fig. 2. Length–frequency distribution of *B. malayanum* collected from June 2005 to June 2006.

sized group (initial size 26.9 mm BL on average), whose proportion diminished from 24.1% to 4.1% at the first sampling interval between June and July of 2005. Cohort II dominated the population from June to August of 2005, with a proportion of population varying between 57.0% and 83.6%, and the average size ranging between 18.5 and 21.8 mm BL. Cohort III took over and dominated the population in the next 7 months from September 2005 till April 2006, with a significant proportion of population varying between 70.2.0% and 92.8%, and the average size ranging between 19.3 and 28.6 mm BL. Cohorts IV and V composed of newly-settled individuals were present during this sampling period, and both cohorts appeared continuously at subsequent sampling intervals. Cohort IV emerged in September 2005, increased in abundance, and dominated the population in May and June of 2006, with a proportion of population varying between 47.6% and 74.0%, and average size ranging between 20.7 and 24.3 mm BL. Cohort IV emerged in April 2006, which contributed significant proportion of 42.9% to the total population in May 2006 with the average size 12.3 mm BL. From the present data, individuals varying between 18 and 29 mm BL constituted the dominant group in this *B. malayanum* population.

Table 2 presents the number of survivors and the cumulative mortality calculated for each cohort of the *B. malayanum* population during this one-year sampling period. Cohort II attained its

Table 2
Number of survivors and cumulative mortality (%) of each cohort of *B. malayanum* from June 2005 to June 2006

Time	Cohort I	Cohort II	Cohort III	Cohort IV	Cohort V
16/06/05	24 (66.7%)	56	19		
19/07/05	8 (100%)	151 (47.0%)	23		
18/08/05		80 (91.4%)	54		
22/09/05		13 (93.4%)	226 (39.8%)	5	
10/11/05		10 (97.4 %)	136 (73.9%)	8	
05/01/06		4 (98.0%)	59 (68.1%)	14	
20/02/06		3 (100%)	72 (74.8%)	13	
20/04/06			57 (96.9%)	21	3
19/05/06			7 (96.9%)	36	32
15/06/06			7	48	10

highest abundance (151 ind m^{-2}) at the average size 19.1 mm BL in July 2005, followed by mortality of 47.0% between July and August, and another 44.4% between August and September. Therefore, totally 91.4% of the individuals died before September with the average size 28.1 mm BL. Cohort III attained its highest abundance (226 ind m^{-2}) at the average size 19.3 mm BL in September 2005, followed by mortality of 39.8% occurring between September and November, and 96.9% of the individuals died before May 2006 at the average size 29.7 mm BL. It appeared that death of *B. malayanum* generally started at the size around 19 mm BL (with mortality around 40%), and the majority of individuals (over 90%) died before attaining 30 mm BL.

3.4. Growth model

Fig. 3 (upper graph) shows the von Bertalanffy growth model of *B. belcheri*. $L_{(max)}$ was estimated as 45.56 mm (with 95% confidence between ±15.85 mm). The instantaneous growth rate coefficient k was estimated as 0.0025 (with 95% confidence between ±0.0017). t_0 was defined as 0.8874 day, and the scaling factor b was calculated as 1.0674. Based on this graph, the size and age of amphioxus could be correlated: the range of one, two and three-year-old *B. belcheri* was estimated to be 5–28 mm, 28–38 mm and 38–45 mm BL, respectively. As described before, 30%–50% individuals of *B. belcheri* died at the size around 16 mm BL, and 90% died at the size around 38 mm BL, which could be defined to be 2 years after settlement. The life span of *B. belcheri* thus could be estimated to be 2–3 years. The largest individual of *B. belcheri* collected in the present study, which was 51 mm BL, was presumed to be about 4 years old.

Fig. 3 (lower graph) shows the von Bertalanffy growth model of *B. malayanum*. $L_{(max)}$ was estimated as 35.17 mm (with 95% confidence between ±5.47 mm). The instantaneous growth rate coefficient k was estimated as 0.0049 (with 95% confidence

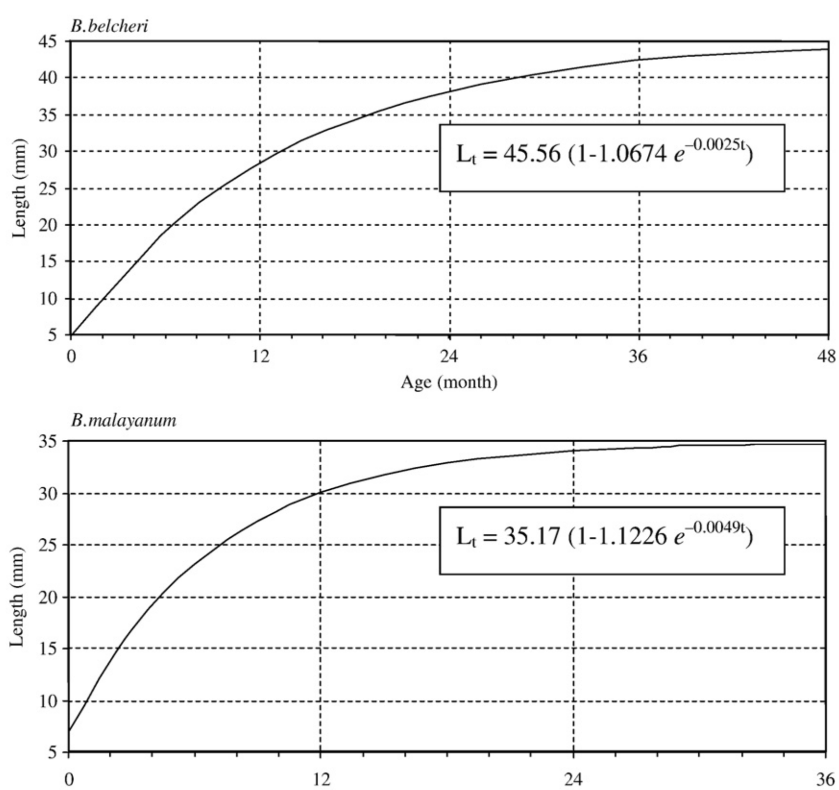

Fig. 3. The von Bertalanffy growth model of *B. belcheri* (upper graph) and *B. malayanum* (lower graph).

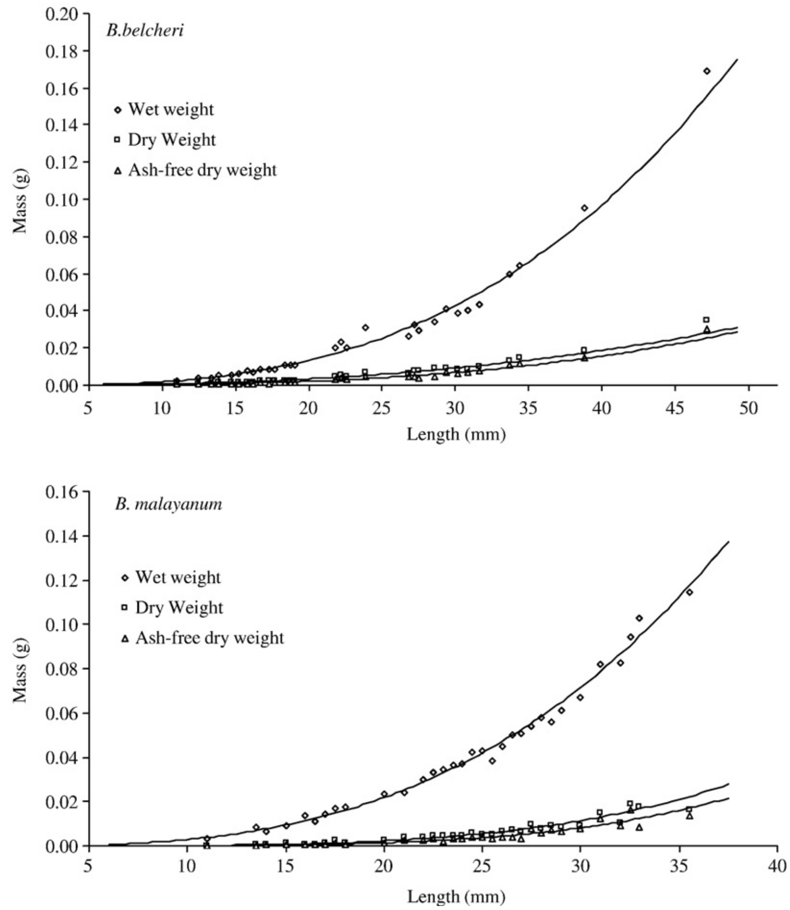

Fig. 4. Length/mass relations of *B. belcheri* (upper graph, $n=30$) and *B. malayanum* (lower graph, $n=30$).

between ±0.0019). t_0 was defined as 0.7948 day, and the scaling factor b was calculated as 1.1226. According to the results plotted, the size range of one and two-year-old *B. malayanum* was estimated to be 7–30 mm and 30–35 mm BL, respectively. As presented before, 40% individuals of *B. malayanum* died at the size around 19 mm BL, and 90% died at the size around 30 mm BL, which could be defined to be 1 year after settlement. The life span of *B. malayanum* thus could be estimated to be 1–2 years. The largest individual of *B. belcheri* collected in the present study, which was 35 mm BL, was presumed to be 2 years old.

The present findings thus showed that *B. malayanum* had a faster growth rate, and a shorter life span as compared with *B. belcheri*.

3.5. Length/mass relationships

In *B. belcheri*, the length/mass relationships, as shown in Fig. 4 (upper graph), were calculated as (with BL in mm, WW, DW and AFDW in g):

$$WW = 3 \times 10^{-6} \times BL^{2.8527}\left(R^2 = 0.9867,\ n = 30\right);$$

$$DW = 2 \times 10^{-6} \times BL^{2.441}\left(R^2 = 0.9535,\ n = 30\right);$$

$$AFDW = 3 \times 10^{-7} \times BL^{2.9057}\left(R^2 = 0.9701,\ n = 30\right).$$

In *B. malayanum*, the length/mass relationships, as shown in Fig. 4 (lower graph), were calculated as:

$$WW = 3 \times 10^{-6} \times BL^{2.9224}\left(R^2 = 0.9874,\ n = 30\right);$$

$$DW = 5 \times 10^{-7} \times BL^{2.8999}\left(R^2 = 0.9595,\ n = 30\right);$$

$$AFDW = 3 \times 10^{-7} \times BL^{2.9928}\left(R^2 = 0.9072,\ n = 30\right).$$

3.6. Secondary production

Based on the mass-specific growth rate method of calculating secondary production, the parameters of the above length/mass relationships, and the parameters of the von Bertalanffy growth model, the secondary production of amphioxus was estimated. In the *B. belcheri* population, the annual production was calculated to be 1.16 g m^{-2} yr^{-1} DW or 0.63 g m^{-2} yr^{-1} AFDW, the average biomass was 1.02 g m^{-2} DW, and the production to biomass ratio (P/B) was 1.13. In the *B. malayanum* population, the annual production was calculated to be 0.51 g m^{-2} yr^{-1} DW or 0.40 g m^{-2} yr^{-1} AFDW, the average biomass was 0.52 g m^{-2} DW, and the P/B was 0.98.

3.7. Minimal size at maturity and sex ratio

Among the 4308 specimens of *B. belcheri* examined throughout the investigation period, there were 324 sexable individuals, including 123 males and 121 females, with a sex ratio (male/female) of 1.02. The smallest specimens observed with gonads were 23 mm BL in male and 25 mm BL in female, while the largest specimens observed with gonads were 49 mm BL in male and 51 mm BL in female. Fig. 5 (upper graph) shows the size distribution of these mature individuals, indicating that the majority of them belonged to the two and three-year-old groups.

Amongst the 1120 specimens of *B. malayanum* examined in the present study, there were 476 sexable animals, including 275 males and 201 females, with a sex ratio (male/female) of 1.37. The smallest specimens observed with gonads were 15 mm BL in male and 18 mm BL in female, while the largest specimens observed with gonads were 35 mm BL in male and 36 mm BL in female. Fig. 5 (lower graph) shows the size distribution of these mature individuals, indicating that the majority of them belonged to the one-year-old group.

3.8. Seasonal changes of GLI in B. belcheri

Fig. 6 (upper graph) depicts the annual changes on the gonad development of *B. belcheri* as indicated by average GLI values (male and female) and maturity percentage. In July 2005, the maximum average gonad size was recorded (GLI value were 3.2 in male and 3.0 in female), so did the highest maturity percentage (39.8%). After July the average gonad size and maturity percentage all declined gradually. For the amphioxus collections in September and November, specimens were commonly observed with residues of gonads, indicating that they have extruded their gametes and completed spawning during this period. From January 2006, specimens were examined with developed gonads, which grew continuously in the following 6 months from January to June, and prepared for the next reproduction, with the average GLI increasing from 1.7 to 3.0 in male and from 1.7 to 2.9 in female, and the maturity percentage increasing from 14.6% to 36.2%. Hence, the spawning of *B. belcheri* mainly occurred around July according to the gonad development, and might continue for several months until December, since the larval settlement of *B. belcheri* was recorded from June 2005 to January 2006 (see Fig. 1).

Fig. 6 (lower graph) shows the annual change tendency of the gonad condition of *B. malayanum* as indicated by average GLI values and maturity percentage. There were two peaks of average gonad size, in August 2005 (GLI were 3.7 in male and 4.1 in female) and April 2006 (GLI were 3.4 in male and 3.5 in female); correspondingly two peaks of maturity percentage were recorded in July 2005 (65.0%) and April 2006 (69.1%). Therefore, the spawning of *B. malayanum* mainly occurred in April and August according to the gonad development, which

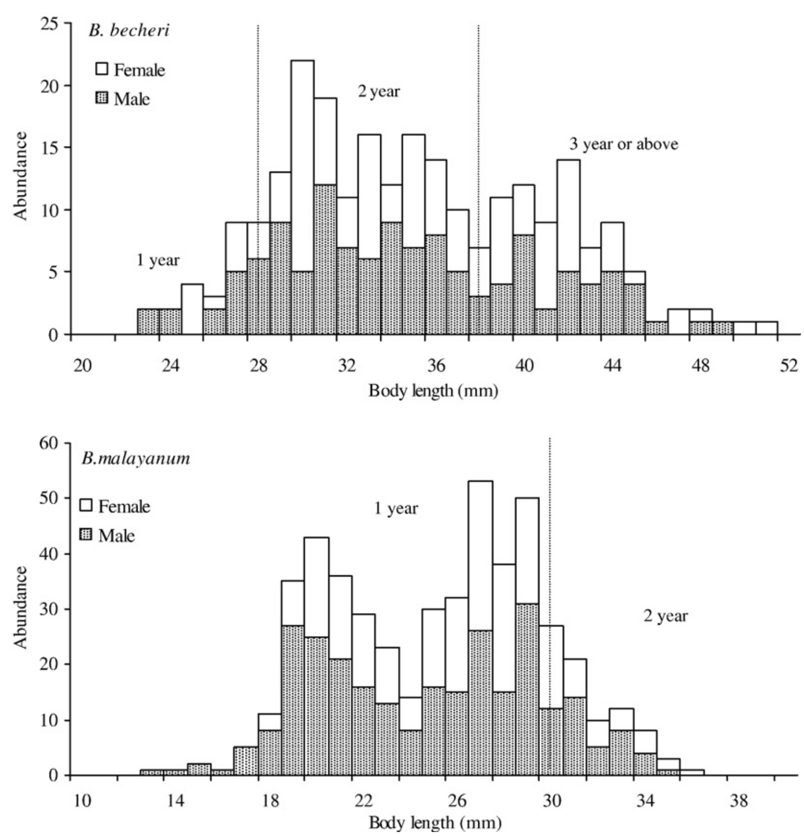

Fig. 5. Size–frequency histograms of mature *B. belcheri* (upper graph) and *B. malayanum* (lower graph) collected from June 2005 to June 2006.

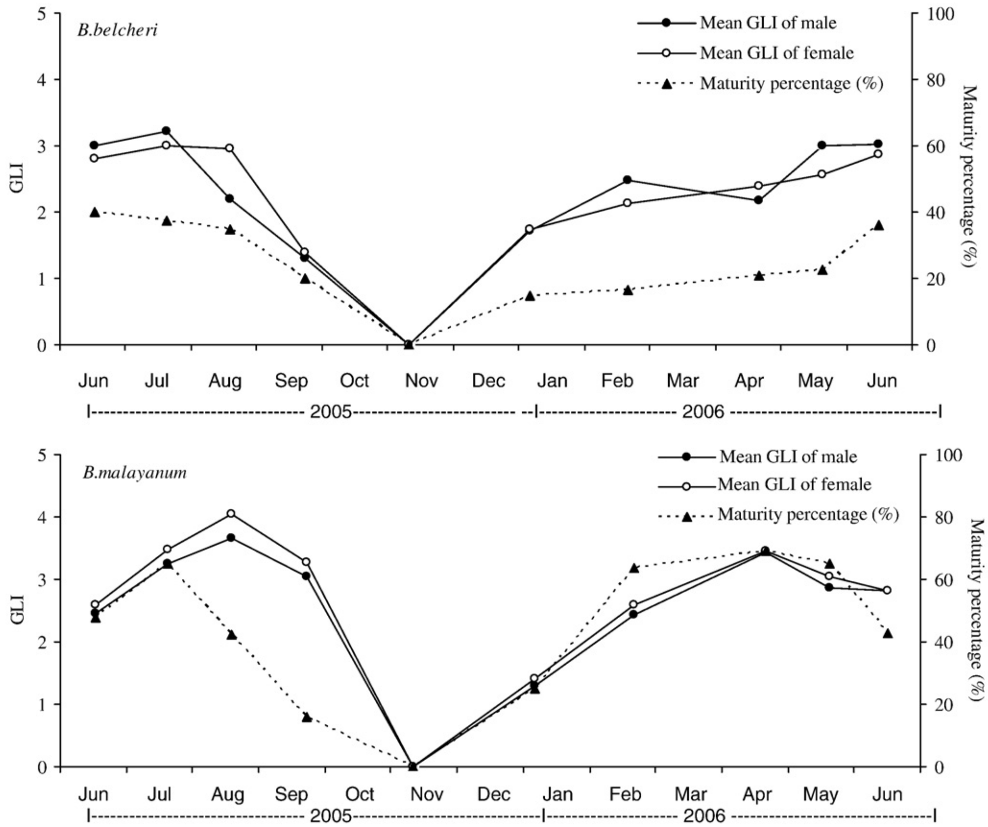

Fig. 6. Annual changes of average GLI values and maturity percentage of *B. belcheri* (upper graph) and *B. malayanum* (lower graph) during the period from June 2005 to June 2006.

was consistent with the data that the small-sized *B. malayanum* were recorded in September 2005 and April 2006 (see Fig. 2).

4. Discussion

4.1. Growth and secondary production

In the present study, using the modal progression analysis of FiSAT II, the amphioxus populations were decomposed into separate cohorts. Based on the size incremental data obtained, the von Bertalanffy growth models of *B. belcheri* and *B. malayanum* were established. The size ranges of one, two and three-year-old *B. belcheri* were estimated to be 5–28, 28–38 and 38–45 mm BL, respectively, which were consistent with the study by Chin (1984) on the growth of *B. belcheri* from Xiamen, China as depicted in Table 3. For *B. malayanum*, the size ranges of one and two-year-old groups were estimated to be 7–30 and 30–35 mm BL, respectively, in the present study. This was the first set of data on population growth for *B. malayanum* in the literature.

Previous studies showed that the growth of amphioxus varied in different species. *B. lanceolatum* from Helgoland in the German Bight was observed with a slow growth rate. The average size of one, two, three, four, five and six-year-old groups of *B. lanceolatum* was estimated at 13, 23, 33, 38, 44 and 47 mm BL, respectively, and the largest individual, which was 55 mm BL, was presumed to be 8 years old (Courtney, 1975). *B. senegalense* from North-West Africa were reported with a moderate growth rate. The one, two, three, four and five-year-old groups of *B. senegalense* was estimated at 8–30, 30–42, 42–51, 51–55 and 55–59 mm BL, respectively (Gosselck and Spittler, 1979). In contrast, *B. floridae* from Tampa Bay of Florida, USA was reported with a rapid growth rate. *B. floridae* grew from 5 to 40 mm BL within 1 year, and can live at least 2 years and reach a maximum length of 58 mm (Stokes and Holland, 1995).

Except for the inter-specific differences, environmental conditions, e.g., food supply, could also affect the growth rate of amphioxus. For example, the growth of *B. nigeriense* in Niger Delta varied significantly in different environments as reported by Webb (1958b). During the period from January to April 1953, newly-settled individuals of *B. nigeriense* (about 5 mm BL) grew to 35 mm BL in the brackish water of Lagos

Table 3
Growth of *B. belcheri* in average body length (mm) in Xiamen, China (Chin, 1984)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Year 1						4.5	7	10	13	15	16.5	18
Year 2	20	21.5	23.5	25.5	27	29	31.5	33.5	35	36	37	38
Year 3	39.5	40	40.5	41	41.5	42	43.5	44	45	46	47	48
Year 4	49	51	53.5	56								

Note: data based on 100,000 individuals of *B. belcheri* in 63 sampling collections in 2 years.

Lagoon, whereas they grew to only 10 mm BL in the seawater of Lagos Habour in open sea, and grew to 25 mm BL in Onikan in middle of these two locations, since the food supply in the open sea at Lagos was much inferior to that in the lagoons. In another case, variations in the body size of *B. belcheri* at different depths (10–80 m) of the Seto Inland Sea were examined to explore the effect of food supply on their growth rates. Results showed that the concentration of chlorophyll *a* in water had a significant correlation with the growth of one and two-year-old groups of *B. belcheri*, suggesting that the growth of amphioxus was mainly affected by phytoplankton supply (Saito et al., 2005).

The present study location, Pak Lap Wan, is a rather secluded beach in the coastal waters of Hong Kong and characterized by clear water, with near-bottom chlorophyll *a* concentration generally below 2 μg L^{-1} throughout the year (http://epic.epd.gov.hk/ca/uid/marinehistorical). In contrast, at the habitat of *B. floridae* in Tampa Bay of Florida, chlorophyll *a* concentration ranged 2–5 μg L^{-1} in winter and 12–16 μg L^{-1} in summer. The terrestrial runoff and stream input of this area led to a eutrophic environment and provided abundant food source for the suspension feeding amphioxus (Stokes and Holland, 1996). As a result of abundant phytoplankton supply, *B. floridae* in Tampa Bay thus showed a rapid growth rate as well as high density, as indicated by high values of secondary production and *P*/*B*. The secondary production was estimated to be 5.78 g m^{-2} yr^{-1} DW or 5.35 g m^{-2} yr^{-1} AFDW for *B. floridae* with a density of 100 ind m^{-2}, and 66.48 g m^{-2} yr^{-1} DW or 61.53 g m^{-2} yr^{-1} AFDW with a density of 1200 ind m^{-2}, the *P*/*B* was estimated at 11.64 (Stokes and Holland, 1996). In contrast, in the present study, the secondary production of *B. belcheri* was 1.15 g m^{-2} yr^{-1} DW or 0.63 g m^{-2} yr^{-1} AFDW with density 423 ind m^{-2}; and the secondary production of *B. malayanum* was 0.51 g m^{-2} yr^{-1} DW or 0.40 g m^{-2} yr^{-1} AFDW with density 121 ind m^{-2}. The *P*/*B* was estimated at 1.13 and 0.98 in *B. belcheri* and *B. malayanum*, respectively, which was significantly lower than that reported for *B. floridae*. Data on *P*/*B* for other species are lacking.

4.2. Reproductive period

Gonads of *B. belcheri* were measured with the maximum size in June and July, which was identified as the peak breeding season of *B. belcheri*. However, the larval settlement was recorded in the period from June to January of the next year, and mostly occurred in September. *B. belcheri* larvae generally spend 1 month of pelagic life prior to settlement (Chin, 1941). This could be thus deduced that the spawning of *B. belcheri* continued from May to December. Gonad development of amphioxus is generally related to the changes of water temperature, and suitable water temperature for the reproduction of *B. belcheri* in Xiamen, China was reported to be 23–25 °C (Fang et al., 1990). During the period from May to December, water temperature at the present study site was 23–26 °C at the near-bottom level (http://epic.epd.gov.hk/ca/uid/marinehistorical), which was suitable for the gonad development of *B. belcheri*.

The present observations on the annual gonad development of *B. belcheri* were in agreement with previous investigations. Chin (1941) reported two peak periods of the presence of newly-settled *B. belcheri* in Xiamen, China through a year, one from May to July, and another in December. However, Fang et al. (1990) reported that *B. belcheri* in Xiamen barely spawn after August, based only on observations on their gonad development. However, larval settlement can provide important clues for identifying the time range of breeding period, although it may not be reliable in identifying the peak period of reproduction due to difficulties in quantifying the number of juveniles in the field; for instance, small-sized juveniles can escape during the sieving process. Newly-settled juveniles are also active in vertical movements in the water column, depending on light intensity (Chin, 1941).

For *B. malayanum*, their gonads were measured with the maximum size in August and April, which thus can be identified as the spawning periods of *B. malayanum* in Hong Kong waters. This is the first report on gonad development in this species.

4.3. Size at sexual maturity

Chin (1941) reported that the smallest individual of *B. belcheri* with fully developed gonads in Xiamen was 29 mm BL, while Fang et al. (1990) reported that the smallest *B. belcheri* individual with detectable gonads was 27 mm BL. According to Yamaguchi and Henmi (2003), the minimum mature *B. belcheri* in Ariake Sea was 20.5 (male) and 21.5 mm BL (female). In the present investigation, the minimum mature *B. belcheri* was 23 (male) and 25 mm BL (female), the length of which was between those reported in the precious two studies. Results of the size distribution of mature amphioxus individuals showed that mature individuals of *B. belcheri* mainly belonged to two and three-year groups. This finding was in agreement with Chin (1941), which revealed that *B. belcheri* in Xiamen waters become mature until the second year of their life, again in the third year, but hardly in the fourth year. However, Fang et al. (1990) believed that many *B. belcheri* from the four-year group are also capable of reproduction.

In *B. malayanum*, the minimum mature specimen was observed to be 15 (male) and 18 mm BL (female), and the mature individuals mainly belonged to the one-year group. As compared with *B. belcheri*, the maturity of *B. malayanum* was about 1 year earlier.

4.4. Comparison between B. belcheri and B. malayanum

B. belcheri and *B. malayanum* were the two dominant amphioxus species co-occurring in Hong Kong waters. The findings revealed some interesting differences between *B. belcheri* and *B. malayanum*. As compared with *B. belcheri*, *B. malayanum* had (1) faster growth rate — the growth rate of *B. malayanum* (growth rate coefficient k=0.0025, as indicated in the von Bertalanffy growth model) was faster than *B. belcheri* (k=0.0049); (2) earlier maturity — the maturity of *B. malayanum* was 1 year earlier (at 1 year old) than *B. belcheri* (at 2 year old); (3) shorter life span — the life span of *B. malayanum* was 1 year shorter (2 years) than *B. belcheri* (3 years); and (4) lower population density — the density of *B. belcheri* was much higher (average 424 ind m^{-2}) than *B. malayanum* (average 121 ind m^{-2}). Such differences in population dynamics may allow both species to share a similar habitat and co-exist in subtropical waters of Hong Kong. McMahon (2002) studied the evolutionary and physiological adaptations of several invasive aquatic species in North America. Results showed that aquatic invasive animals are characterized by rapid growth, early maturity, short life span, and elevated fecundity, since these characteristics allow rapid population recovery of invasive animals after reductions by environmental extremes. The characteristics of invaders reported in the study of McMahon (2002) were consistent with those observed in *B. malayanum* in the present study, suggesting that *B. malayanum* might be a recent colonizer of Hong Kong waters as compared with *B. belcheri*. This may also explain the lower density of *B. malayanum*, possibly under the dominance of the well-established *B. belcheri*, despite the fact that both species share the same habitats. *B. malayanum* is restricted to tropical waters (Poss and Boschung, 1996) and was only reported upon in Singapore, Solomon Islands, and Thailand (Webb, 1958a; Gibbs and Wickstead, 1969; Nohara et al., 2004). Hong Kong is possibly at the northern limit for this species since it has not been reported upon at higher latitudes of Xiamen and Qingdao. If this is the case, *B. malayanum* in Hong Kong might have to live under relatively unfavourable conditions, such as a lower annual temperature range, and would be difficult to compete with the dominant *B. belcheri*, which has a wider northern limit and can adapt better to cooler water temperatures. Further studies on the temperature tolerance of *B. malayanum* as compared to *B. belcheri* will shed light on the above argument.

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