

Growth and reproduction of the glassperch *Parambassis siamensis* (Teleostei: Ambassidae) in Central Laos

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Age, growth and maturation of the glassperch *Parambassis siamensis*, collected in the Mekong basin, Vientiane Province, Central Laos were investigated during the low temperature season (LTS) in February and March 2010 and the high temperature season (HTS) in May and June 2010. Histological observations of gonads and otolith daily increment analyses were undertaken. Gonadal sexual dimorphism is histologically identifiable in fish larger than ca. 15 mm SL, but not easily so in smaller fish. The reproductive size of females was estimated as larger than ca. 30 mm SL for both HTS and LTS, compared with ca. 20 mm SL for males. Ages estimated from otolith daily increment counts were 37–123 days in fish collected during the HTS (14.4–38.2 mm SL), and 32–148 days during the LTS (10.0–41.3 mm SL). On the basis of growth patterns (logistic models) in the HTS and LTS [$L = 36.97 / (1 + \exp(-0.036 \times (T - 47.09)))$] for the former period, $L = 40.98 / (1 + \exp(-0.026 \times (T - 76.37)))$ for the latter], fish reached 30 mm SL (reproductive size) faster (ca. 90 days) during the HTS than in the LTS (ca. 115 days). Considering the daily ages at reproductive size and their maximum ages (123 in the HTS and 148 in the LTS) observed in the present study, *P. siamensis* is considered to spawn throughout the year with plural generation alternations per year. Feeding incidence rates (%) were high, 86.1 % in the HTS and 92.9 % in the LTS, stomach contents comprising mainly zooplankton and insect larvae. This indicated that *P. siamensis* is carnivorous, the habitats from which fish were sampled being rich in dietary organisms regardless of seasons. Hence, the faster growth and earlier maturation in fish during the HTS are considered to be attributable to the higher temperature of that season.

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Introduction

The glassperch *Parambassis siamensis* (Fowler, 1937) is a common, small-sized freshwater fish (maximum size less than 50 mm SL; Kottelat, 2001) of the Indochinese Peninsula, locally named Pa khap khong in Laos. This species was originally distributed in tropical and subtropical freshwaters in Laos, Thailand, Cambodia, Vietnam and Peninsular Malaysia (Roberts, 1995; Kottelat, 2001). The breeding populations in Singapore and Indonesia result from introductions (Ng et al., 1993; M. Kottelat, pers. comm.). This species is well known to occur in various habitat types, including large rivers and flooded fields (Taki, 1978), swamps (Vidthayanon, 2002), irrigation canals and reservoirs, almost year-round (Morioka et al., 2008). It is an important food fish for the local inland communities of the region (utilized in dried and fermented forms, occasionally as feed for cultured carnivorous fishes, e.g., *Channa* spp.), although its commercial value is limited (Hung et al., 2011). However, the establishment of nearly 20 invasive exotic fish species (e.g. *Oreochromis niloticus*, *Clarias gariepinus*, *Hypophthalmichthys molitrix*) in the Mekong River basin (Phillips, 2002; Welcomme & Vidthayanon, 2003; Hung et al., 2011) and the recent environmental changes (e.g., urbanization and land exploitation for agriculture) are a potential concern for the decline in the region's native and endemic fish diversity and their stock level. For fish diversity conservation as well as stock assessment, information on the life history of the concerned species, such as growth, sexual development and generation time, is a basic requirement. However, none of these aspects have been investigated so far in *P. siamensis*.

External gonad features are widely used as indicators of sexual maturity stages (Ricker, 1971), detailed analyses of germ cell differentiation and development by means of histological observations being possible on gonads (van Aerle et al., 2004). Moreover, reproductive cycles and spawning patterns may also be predictable from histological observations of oocyte development and their distribution in the ovaries (Lowerre-Barbieri et al., 1996). As well as sexual development analyses, growth analysis based on daily age determination is an important investigation factor in fish biology. Daily age determination in larval and juvenile teleost fishes using otolith increments has progressed since Pannella (1971), the method having since been broadly applied to age and

growth estimations (Campana & Neilson, 1985). In aspects of stock management as well, both age and growth information and gonad histology make important contributions to an understanding of population structures (sex ratio and reproductive size).

This study of growth and sexual development in *Parambassis siamensis* was based on an analyses of otolith daily increments and histological analyses of gonads. In addition, observations were made on stomach contents in order to determine food supply at the collection sites.

Materials and methods

Characteristics of fish collection sites. The pond and irrigation canal from which the fish samples were collected contained water year-round. The shore and part of the bottom of the pond comprised leaf mold and fine mud, and the river bottom various-sized pebbles and sand.

Sampling in the wild. A total of 298 *P. siamensis* were used in the present study. They have been collected from a reservoir (ca. 300 × 500 m) of ca. 30 cm depth (18°14'40.32" N 102°29'42.66" E) and from an irrigation canal of <30 cm depth (18°14'56.29" N 102°28'00.34" E), both located in the Namxuang area (44 km north of Vientiane City) and connected by agricultural irrigation canals to the Nam Gum River (Fig. 1), a tributary of the Mekong River. Fish collections were made on 4 February (10.0–41.3 mm SL, n=65 in 90 % ethanol; 11.6–39.8 mm SL, n=63 in 10 % formalin), 3 March (12.0–37.4 mm SL, n=33 in 90 % ethanol), 12 May (17.0–38.2 mm SL, n=84 in 90 % ethanol), 17.8–37.8 mm SL, n=23 in 10 % formalin), 3 June (14.4–38.2 mm SL, n=10 in 90 % ethanol) and 20 June 2010 (n=20) using a seine net (5 mm mesh, 4 m width, 1 m height). Based on the general pattern of monthly average temperature fluctuations [lower during November to February (ca. 20–25 °C) and higher during March to May (ca. 25–30 °C)] (Morioka et al., 2009), fish samples were divided into two seasonal groups, viz. fish grown under the lower temperature season (collected in February and March, referred to as LTS) and those under the higher temperature season (collected in May and June, HTS).

Fish caught on 20 June 2010 were kept alive and used for validating the periodicity of otolith increment deposition. Specimens preserved in

90 % ethanol were used for otolith daily increment analysis and observations on stomach contents, and those in 10 % formalin for histological analyses of gonads.

Histological observations of gonads. Only fish preserved in 10 % formalin were used, their abdominal portions [11.6–39.8 mm SL, n=63 (LTS), 17.8–37.8 mm SL, n=23 (HTS)] was dehydrated in an ethanol progressive series, embedded in paraffin wax and cut into 5 μm sections using a microtome. The paraffin sections were mounted on glassslides, dewaxed and dehydrated by immersion in a xylene-ethanol series. The sections were then stained with hematoxylin-eosin for histological observations on the gonads, and sex identification and gonad developmental analyses were made.

Validation of periodicity of otolith increment deposition. In order to validate the periodicity of otolith increment deposition, 20 specimens were collected on 20 June 2010 and immediately transferred to the Living Aquatic Resources Research Center, Vientiane, Laos. Fish were kept in a 60 l aquarium for two days so as to become acclimatized to the laboratory environment. During rearing, fish were fed *Moina* sp. and *Artemia* nauplii 3 times a day. They were then live-stained by alizarin complexone (ALC, 100 ppm) on 22–23 June for 15 hours (17:00 to 08:00) in a 5 l aquarium. After ALC staining, fish were returned to the 60 l aquarium and rearing continued as above. Water temperature during rearing was 27.8–29.7 °C. Fish were sampled on 2 July 2010 (9 days after ALC staining) and preserved in 90 % ethanol, their standard length (SL) at sampling was 16.4–26.1 mm SL. The otoliths (sagittae) were removed after measurement of SL, mounted on glass slides with epoxy resin, and ground by sandpaper (# 1500) and lapping films (6 and 9 μm mesh). The ground otolith surfaces were occasionally etched by 0.1 N hydrochloric acid to emphasize increment contrast. The otoliths were initially observed under a fluorescent microscope with a G-filter in order to identify the ALC-stained increment. Subsequently, the number of increments deposited outside the ALC mark was counted under normal transmitted light ($\times 200$ –400).

Stomach content and age determination in wild-caught fish. After measurement of SL (mm) of fish preserved in 90 % ethanol, 94 (HTS) and 98

(LTS) specimens were dissected under a binocular dissecting microscope to observe stomach contents and feeding incidence rates [%; = (number of fish with food material in stomach)/(total number of fish) $\times 100$] were determined in each season. After the stomach content observations, the otoliths (sagittae) were removed under a binocular dissecting microscope, and prepared for increment analysis as described.

Results

Gonadal development. Histological observations were made on the gonads of 86 specimens. In the smallest size class 10.0–14.9 mm SL (n=9), oocytes were observed in the ovaries of three specimens (Fig. 2b), therefore, identified as females. However, in the remainder (n=6), only gonial germ cells were observable. Because oogonia and spermatogonia were not readily identifiable on the basis of morphology (Fig. 2a), the germ cells were judged being at a stage prior to sexual dimorphism in the gonads. In specimens 15.1–39.8 mm SL (n=77), sex was readily identifiable in all specimens (Fig. 2), 35 females and 42 males (maximum sizes 39.8 mm SL and 38.1 mm SL, respectively). Including the three specimens (in the smallest size class) for which sex could be determined, the sex ratio (male number/total number) was determined as 0.53. All of the females in the 15.0–19.9 mm SL size class (n=4) had ovaries occupied predominantly by primary growth (peri-nucleolus) oocytes of ca. 40 μm diameter (Figs. 2d, 3). The 20.0–24.9 mm SL size class (n=10) had 80 % (8 of 10) of females with primary growth oocytes of ca. 40–60 μm diameter (slightly enlarged) (Figs. 2f, 3), the rest having previtellogenic (oil droplet) oocytes (ca. 70–90 μm of diameter, Fig. 3). Subsequently, the 25.0–29.9 mm SL size class (n=4) had two specimens with dominant primary growth oocytes (ca. 50 μm diameter), one with previtellogenic oocytes (ca. 100 μm diameter, Fig. 2h) and the rest with vitellogenic oocytes (ca. 350 μm diameter) (Fig. 3). The 30.0–34.9 mm SL size class (n=9) had 33 % (3 of 9) with dominant primary growth oocytes (ca. 60–70 μm diameter) and the remainder with dominant vitellogenic oocytes (ca. 150–370 μm diameter, Figs. 2j, 3). In the size class >35.0 mm SL (n=7), dominant vitellogenic oocytes (>350 μm diameter) were observed in all specimens (Figs. 2l, 3). Additionally, in all females ≥ 30 mm SL



Fig. 1. Laos; arrows indicate collection sites of *Parambassis siamensis*.

($n = 16$), partially spent and post-ovulated ovaries with preovulatory follicles were absent. This trend in oocyte development and fish standard length was similar between fish collected during the both HTS and LTS.

In the testes of 8 males in the 15.0–19.9 mm SL size class, one had testis containing a few cysts with spermatozoa, although the most advanced germ cells in the remaining specimens were spermatids, including immaturity stage (Figs. 2c, 4). However, the 20.0–29.9 mm SL size class ($n = 21$) had 85.7 % (18 of 21) of males with mature testes dominantly occupied by spermatozoa (Fig. 2e,g, 4). Mature testes occurred in 100 % of specimens > 30 mm SL ($n = 13$) (Figs. 2i,k, 4). This trend in testis development and SL was similar between fish collected during HTS and LTS.

Fig. 2. Gonadal development of *Parambassis siamensis*. **a**, sex not identifiable (SL < 15 mm); **b**, ovary (SL < 15 mm); **c**, testis (15–20 mm SL); **d**, ovary (15–20 mm); **e**, testis (20–25 mm SL); **f**, ovary (20–25 mm SL); **g**, testis (25–30 mm SL); **h**, ovary (25–30 mm SL); **i**, testis (30–35 mm SL); **j**, ovary (30–35 mm SL); **k**, testis (SL ≥ 35 mm); **l**, ovary (SL ≥ 35 mm). SL: standard length of examined fish. Arrows in **a** indicate gonial germ cells (sex not identifiable), arrowheads in **b** primary growth oocytes, arrows in **c** spermatogonia, arrow in **h** oil-droplet oocyte, solid and open arrowheads in **j** and **l** primary growth oocytes and vitellogenic oocytes, respectively. Broken, solid and red line circles in **c** and **e** indicate spermatocytes, spermatids and spermatozoa, respectively. Scale bars: 20 μm in a–f, 50 μm in g–i and k, 100 μm in j and l.

Validation of periodicity of the sagittae increment deposition. The number of increments formed outside the ALC (alizarin complexone) mark in the sagittae mostly coincided with the actual number of days after ALC staining (mean \pm SD: 8.95 ± 0.23 increments, $n = 20$) (Fig. 5), not differing significantly from 1 (t-test, $p > 0.05$). This indicated that the increments of the sagittae were formed daily and that increment counts would be useful as indicators of age in days in the specimens examined.

Daily age of fish and growth based on otolith increment analyses. The sagitta increments were clearly observable (Fig. 6), the number of increments varying from 32 to 148 in the LTS (10.0 to 41.3 mm SL, $n = 92$) and from 37 to 123 in the HTS (14.4 to 38.2 mm SL, $n = 85$). Relationships between the number of increments in the sagittae (T) and fish standard length (L) in each period were regressed by the following logistic formulae (Fig. 7a):

$$L = 36.97 / (1 + \exp(-0.036 \times (T - 47.09)))$$

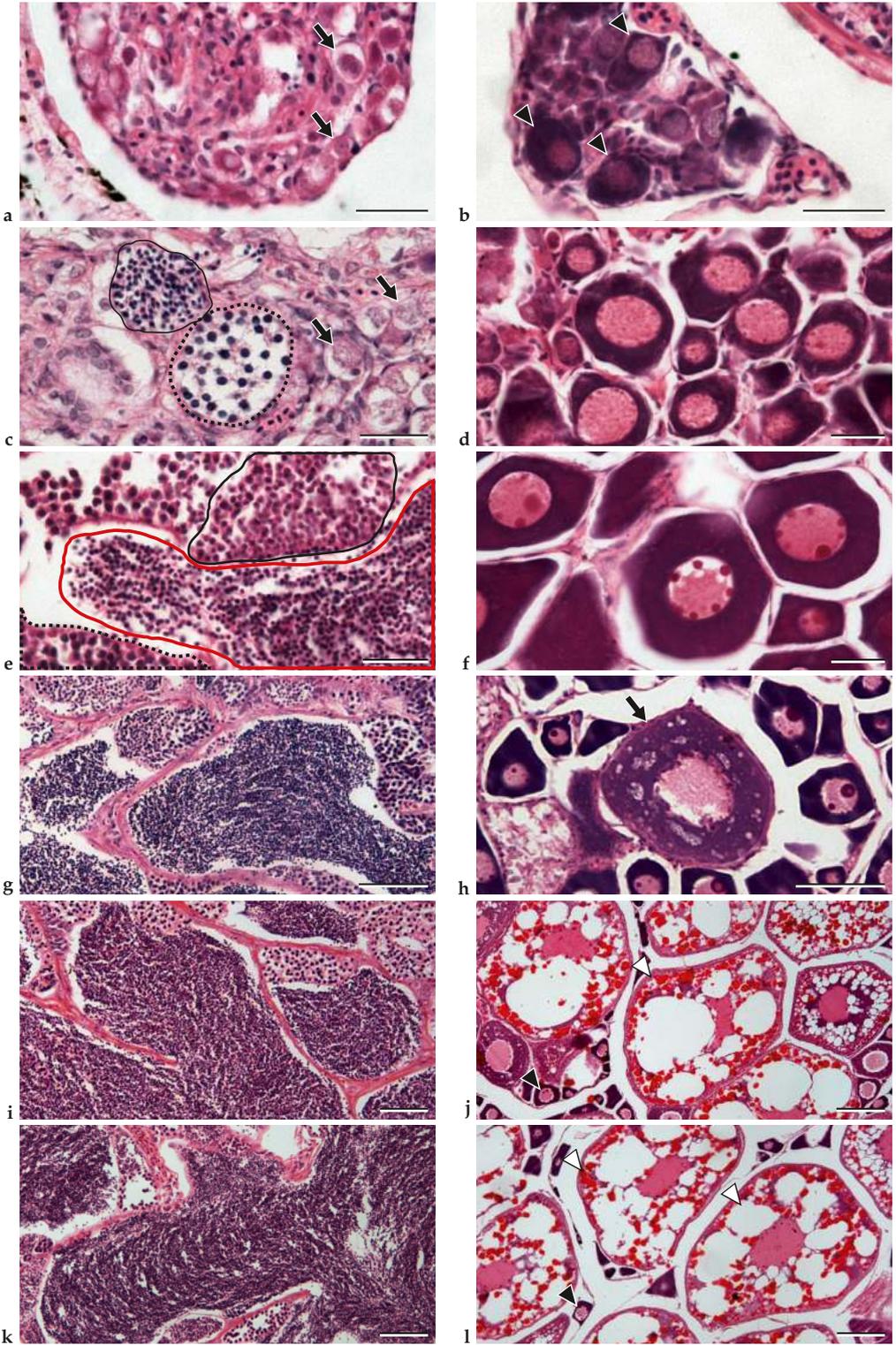
($R^2 = 0.86$, $n = 85$) in the HTS;

$$L = 40.98 / (1 + \exp(-0.026 \times (T - 76.37)))$$

($R^2 = 0.91$, $n = 92$) in the LTS.

These formulae indicated that the fish in the HTS grew significantly faster than those in the LTS (F test, $p < 0.01$). In addition, relative growth ($RG = L_{T+1} - L_T$, mm) obtained on the basis of the logistic formulae for each period, illustrated that growth was accelerated over ca. 50 days in the HTS and 90 days in the LTS, subsequently slowing down (Fig. 7b). RG in the HTS, thereafter, became less than that in the LTS prior to ca. 70 days (Fig. 7b).

Stomach contents and feeding habit. Feeding incidence rates were 86.2 % in the HTS and 92.9 % in the LTS. Fish stomach contents most often included aquatic insects (mostly insect larvae and occasionally terrestrial insects) (83 of 94 specimens in the HTS and 74 of 98 in the LTS), followed by



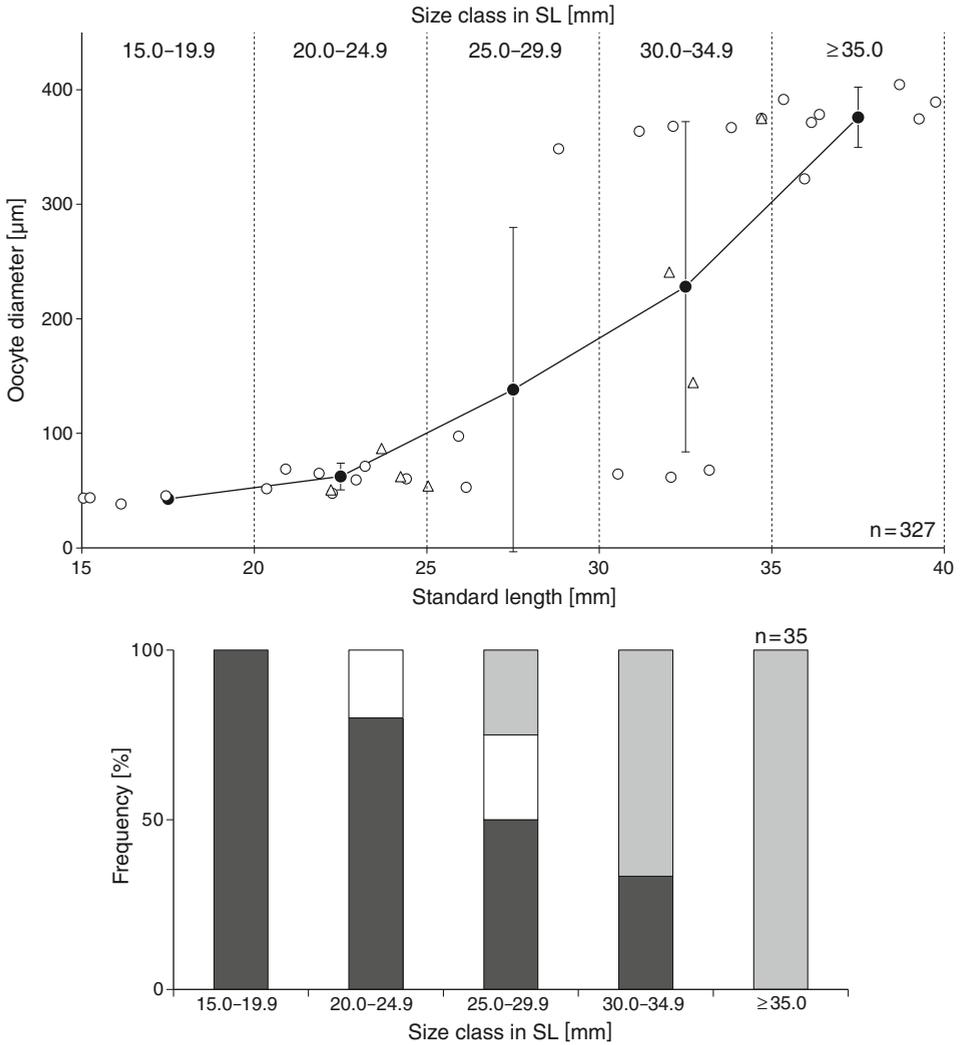


Fig. 3. a, Relationship between standard length (mm) and oocyte diameter (µm) of female *Parambassis siamensis*. Oocytes of 36 fish ranging from 15.0–38.2 mm SL were measured. Δ, \circ : average oocyte diameter (µm) per fish in the HTS and LTS, respectively; \bullet : average oocyte diameter (µm) of each fish size class. Vertical bars: standard deviations of oocyte diameter in each size class. b, Frequency distribution (%) of females with primary growth (■), previtellogenic (oil droplet or yolk vesicle; □) and vitellogenic (▒) oocytes observed in their ovaries in each size class (mm SL).

Copepoda (including nauplii, copepodites and adults, 4 of 94 in the HTS and 27 of 98 in the LTS) and Branchiopoda (Daphniidae and Chydoridae, 8 of 94 in the HTS and 5 of 98 in the LTS). No plant materials were observed, indicating clearly that *P. siamensis* is carnivorous (insectivorous/planktivorous).

Discussion

Based on the oocyte diameter and the high frequency of vitellogenic oocytes (13 females out of a total of 16) in females larger than 30 mm SL (Figs. 2, 3), females of *P. siamensis* were considered to be partially reproductive in the size class of 30.0–35.0 mm SL, and entirely reproductive in the size class of ≥ 35.0 mm SL. In addition, Roberts

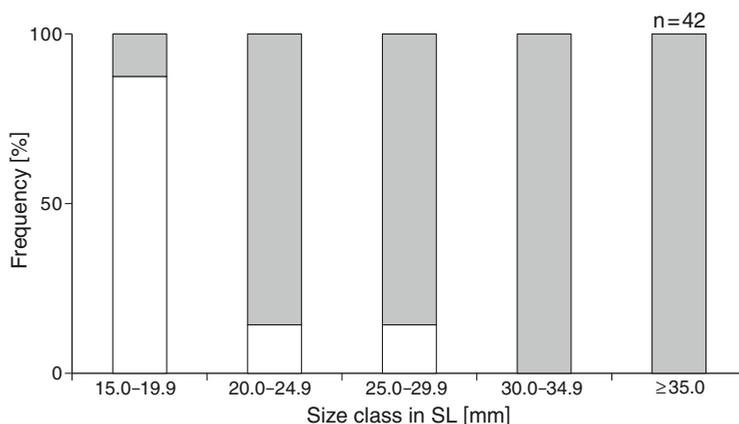


Fig. 4. Frequency distribution (%) of male *Parambassis siamensis* with spermatid predominant testes (□) and spermatozoon predominant testes (■) by size class (mm SL).

(1995) reported the maturation size of female *P. siamensis* as ca. 30 mm SL. These observations suggest that the size of reproductive females in this species is ca. 30 mm SL, regardless of temperature. Moreover, partially-spent ovaries having post-ovulatory follicles that are often observed in multiple spawners (Lowerre-Barbieri et al., 1996), were not found in any females examined in the present study (maximum size 41.3 mm SL). These observations likely suggest that this species spawns only once in its lifetime. However, considering the known maximum size of the species (ca. 50 mm SL; Kottelat, 2001), *P. siamensis* may in effect spawn multiple times after reaching a larger size, although further confirmation is required. In contrast, spermatozoa were observed in the testes in more than 80 % of all males of 20.0–29.9 mm SL and 100 % of all males ≥ 30 mm SL (Fig. 4), indicating the earlier occurrence of gametogenesis in males than in females. A similar phenomenon has often been observed in other fish species (Hard et al., 1985; Rijnsdorp, 1989; Rodriguez et al., 2001; Doiuchi & Yoshimoto, 2009).

The similar reproductive size for both females and males observed in the HTS and LTS, despite faster growth in the former period (Fig. 7), indicated that sexual development is more size-dependent than temperature-dependent in this species, since higher temperatures lead to faster growth within a population (Morioka, 2002; Morioka & Kaunda, 2005), as well as earlier maturation (Dotsu, 1982; Kon & Yoshino, 2003). Based on the reproductive sizes estimated here (ca.

30 mm SL in females and 20 mm SL in males) (Figs. 3–4), the reproductive ages were estimated to be ca. 90 days in the HTS and ca. 115 days in the LTS (females), and ca. 50 days in the HTS and 80 days in the LTS (males) (Fig. 7a). Relative growth in the HTS and LTS increased over ca. 50 and 90 days, respectively, and declined, thereafter (Fig. 7b). The sizes at the point from which relative growth declined were ca. 20–21 mm SL (estimated from the logistic growth formulae; Fig. 7a), generally corresponding to the timing of a rapid increase in the number of males having spermatogenic testes (Fig. 2e), followed by an increase in the number of females larger than 25 mm SL having vitellogenic oocytes (Fig. 3). This suggests that the growth rate of the species declines with gonadal development, or simply decreases when approaching the maximum size of the species (41.3 mm SL observed in the present study, ca. 50 mm SL in Kottelat, 2001). However, considering the aforementioned difference in maturation sizes in males and females that may suggest the difference in growth between sexes even in the same season, more detailed investigation is necessary to identify the growth pattern for each sex.

The occurrence of various sized specimens regardless of season (10.0–41.3 mm SL in LTS, 14.4–38.2 mm SL in HTS) and the distribution of reproductive size/age estimated in the present study (Figs. 2–3, 7), indicate that *P. siamensis* spawns throughout the year, and the multiple generations may occur within a single year. Further, the observation of high feeding incidence

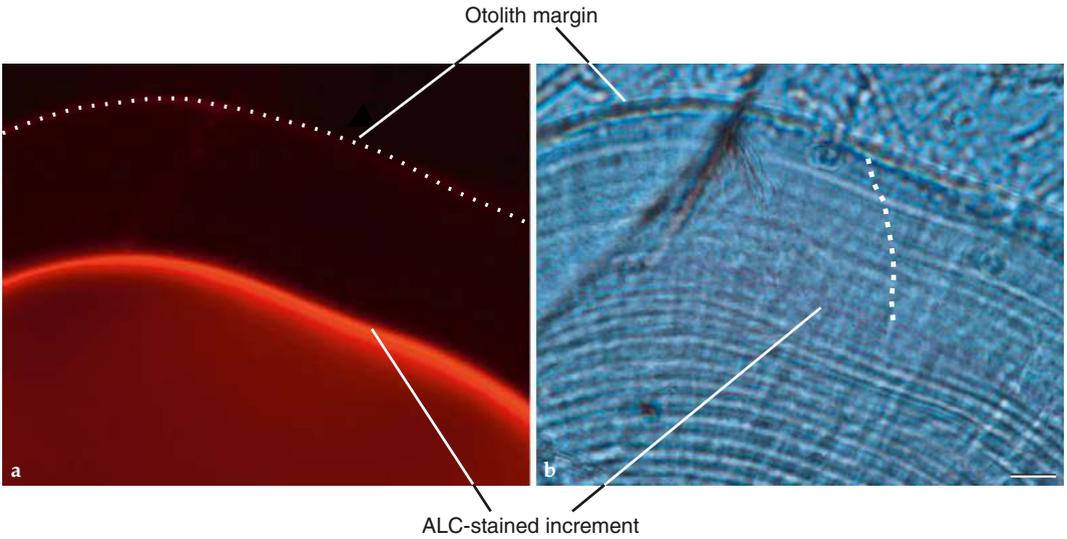


Fig. 5. Sagittae of *Parambassis siamensis* (13.2 mm SL), stained by ALC. **a**, sagitta observed under fluorescent light (G-filter); **b**, normal transmitted light. Dots on the right image indicate daily increments. Scale bar: 20 μm .

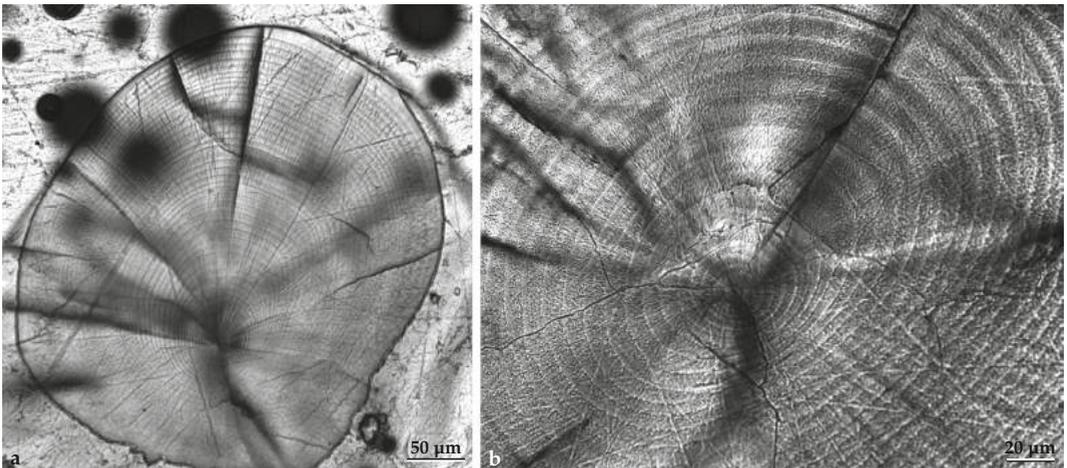


Fig. 6. The sagitta of *Parambassis siamensis* (12.0 mm SL). **a**, entire sagitta; **b**, core portion of sagitta.

rates during both seasons (86.2 % in the HTS and 92.9 % in the LTS) and the presence of a considerable quantity of stomach content regardless of season and therefore differing water temperature, demonstrated that the habitat of *P. siamensis* is sufficiently stable to ensure a consistent supply of food, and suitable water temperature for growth and maturity. Year-round reproduction, reported in other tropical/sub-tropical species (Shimizu, 1999) and observed here, is considered to be dependent upon environmental suitability,

i. e. acceptable temperature and consistent food supply throughout the year (Cushing, 1990; Kon & Yoshino, 2002).

The genus *Parambassis* comprises more than 10 species that are widely distributed throughout the subtropical and tropical areas of India, Indochina, Malaysia, Indonesia and Oceania (see Allen, 1991; Allen et al., 2002; Roberts, 1995; Kottelat, 2003), and maximum size varies from ca. 30 mm SL (*P. lala*, see Talwar & Jhingran, 1991) to ca. 240 mm SL (*P. gulliveri*, Allen et al., 2002). Fur-

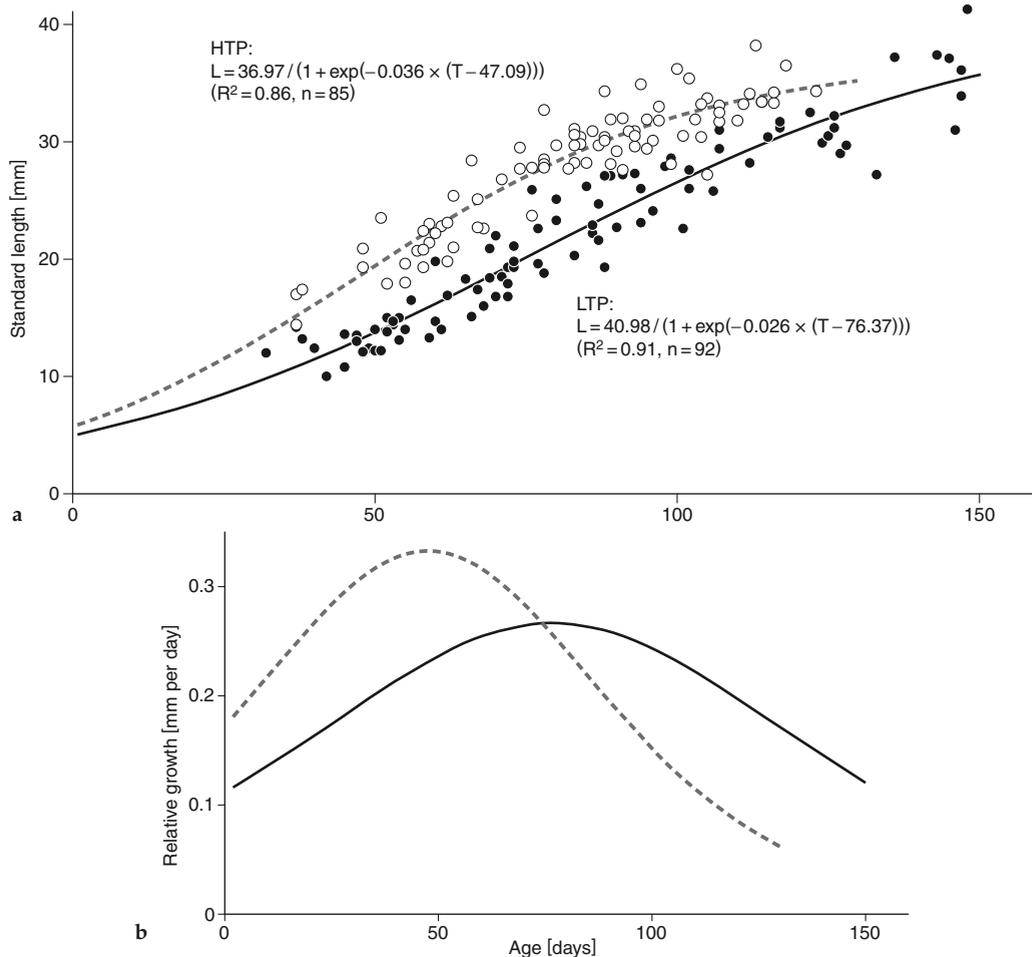


Fig. 7. **a**, Growth patterns of *Parambassis siamensis* collected in the HTP (○) and LTS (●); **b**, relative growth (mm per day) of fish in the HTP (broken line) and LTS (solid line) estimated from the logistic growth formulae.

thermore, habitats also vary from marine/estuary (e.g. *P. lala* and *P. thomassi*) (Kapoor et al., 2002) to freshwater areas. In addition, unique reproductive behavior, i.e., oral brooding (*P. apogonoides*, Roberts, 1995), has been reported. Such diverse ecological characteristics among congeners have probably resulted from speciation due to environmental adaptation, and possibly the development of polyphyletic array within the taxon (Roberts, 1995). Hence, comparisons in growth/reproductive patterns (including early-life stages) of congeners would be important in terms of evolutionary ecology. However, such information has not been reported to a great extent as of yet, and further confirmation is required.

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