

REPRODUCTIVE BIOLOGY OF THE MUDSKIPPER *Boleophthalmus boddarti* IN SOC TRANG

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ABSTRACT: The mudskipper *Boleophthalmus boddarti* is an amphibious fish widely distributed in the mudflat regions in Asia, but its reproductive biology was poorly studied. This study was conducted in the Tran De, Soc Trang, Vietnam, from March 2013 to February 2014, to investigate reproductive parameters of *B. boddarti*. Data analysis based on a total of 360 collected fish (188 females and 172 males) suggest that the sex ratio of this mudskipper was not significantly different between dry and wet seasons. This fish was a multiple reproducer and spawned in the wet season with a peak spawning in three main months of this season (August to October) as difference stages of oocytes were observed in the ovaries. The male *B. boddarti* released sperms during the reproductive season since different type of spermatogonia were found in stage V testis sections. This fish reached 11.52 cm total length at first sex maturation and was high fecundity (9,800-33,800 eggs), indicating a possible adaptation of this species to the monsoonal climate. The understanding of the reproductive biology of *B. boddarti* provides useful knowledge of its reproductive adaptation to the mudflat toward sustainable management in the study region.

Keywords: *Boleophthalmus boddarti*, fecundity, length at first maturity, mudskipper, spawning.

INTRODUCTION

Knowing of fish reproductive biology is useful for fishery management [15, 22]. Fecundity is related to egg size and used to estimate fish recruitment and abundance [16], and information on the fish length at first maturity is helpful for fish stock management [11, 36]. However, information on the reproductive biology of fishes, especially gobies, in the Mekong Delta, where fish stocks have been subjected to overexploitation [24], is limited.

The sympatric mudskipper *Boleophthalmus boddarti* (Pallas, 1770) is an elongated fish [23] and widely distributed in the Indo-Pacific regions including India, Thailand, China, Indonesia, Malacca, Myanmar, Guam and Vietnam [10, 29]. This gobiid species mainly occurs in the inter-tidal mudflat areas during ebb tide [25, 35]. The *B. boddarti* is a distinct residential fish who builds the burrows for refuging from predator and feeding in the soft bottom patterns [7, 8, 28].

Although the Boddart's goggle-eyed goby *Boleophthalmus boddarti* (Pallas, 1770) is a commercial fish in some Asian countries [13,

26] and has a wide range of salinity from marine to freshwater [32], its information has been limited to external morphology [10] and living habitat [8, 28]. This gobiid species shows isometric growth [26] and feeds mainly on diatom [30]. The fecundity of this goby vary with geographic location and is affected by environmental factors (e.g., pollution), and its ovary development is reported in Bhayandar, Kalyan and Vasai creeks around Mumbai regions [6]. However, the testis development, spawning period, and size at first mature of this fish has been limited. Although this gobiid species is a commercially important fish in Vietnam [26], the understanding on its reproductive biology has been unknown. Therefore, this study aims to understand reproductive biological characteristics including spawning pattern and season, size at first mature and fecundity of this fish that provides useful information for future sustainable exploitation and conservation this gobiid species in the present study area.

MATERIALS AND METHODS

Study sites and sampling

This study was conducted for a year, from March 2013 to February 2014, in Tran De district, Soc Trang province, Vietnam. Deep gill nets with 1.5 cm mesh aperture in the cod end were set at the highest tide and retrieved after 2-3 hours during ebb tide along the margin of mangrove forest areas to collect monthly fish specimens in the study site (9°28'47.41"N, 106°12'25.96"E). After being sexed using external morphology of genital papilla, which was oval for female and triangle for male [27], fish specimens were measured total length (TL, nearest 0.1 cm) and body weight (W, nearest 0.01 g). Fish specimens were identified based on their external description and classification [39] before being stored in 5% formalin and transported to the laboratory.

Gonadal maturity stage classification

Ovaries and testes, in the laboratory, were removed from fish specimens and visually classified into six maturation stages according to the criteria on the black goby *Gobius niger* described by Vesey & Langford (1985) [37]. The ovaries and testes were examined histologically as a biological indicator for oocyte development patterns using the staining procedure of Carleton et al. (1980) [5].

Length at first maturity and fecundity determination

The length at first maturity (L_m) is the length at which 50% of the population is sexually mature [14]. This threshold was determined using the simple logistic curve equation $P = 1/(1+\exp[-r \times (TL-L_m)])$, where, P is the proportion of mature individuals in a length class; L_m is the length with 50% of fish reaching sexual maturity, and r is a model parameter [40]. Thirty mature (stage IV) and ripe (stage V) ovaries were placed in the Gilson fluid for seven days to release oocytes [2], that were then counted in a petri dish to determine the batch fecundity of this mudskipper.

Data analyses

The positive relationships between fecundity and fish length and weight were estimated using logarithmic regression [21]. The difference in sex ratio between wet and dry seasons was examined using the χ^2 test. The SPSS software v.21 was

used for data analysis, and the alpha value in all tests was set at 0.05 or less.

RESULTS AND DISCUSSION

Sex ratio

A total of 360 individuals (188 females and 172 males) were collected at the study site during the period of study. Data analysis showed that the female to male ratio of *B. boddarti* was 1.0:0.9 in total, 1.0:0.8 in wet season and 1.0:1.1 in dry season, respectively, but the sex ratio of this fish was not significantly different from the ratio of 1:1 (χ^2 , $P > 0.05$ in all cases). The sex ratio of *B. boddarti* was similar to that of *Pseudapocryptes elongatus* collected from the same region reported by Dinh (2008) [9]. In other gobies, sex ratios are deviated from 1:1. For example, more females than males *Gobius vittatus* are caught in the spawning season, as males *G. vittatus* guard their nests [17]. Silva & Gordo (1997) [33] indicate that the cryptobenthic behavior of male *Gobius niger* results in more female being caught during the spawning season. Moreover, the 1:1 sex ratio of *B. boddarti* and *P. elongatus* may indicate that these gobies shared the same reproductive behavior.

Ovary and testis development

Ovaries: Stage I ovaries were too small, elongated and paired with a smooth surface. The ovarian sections composed of germ cell (GC) and oogonia (PO, fig. 1a). The diameter of ovaries increased to roughly 1 mm in stage II that were connected by connective tissues and covered by the rough surface with prominent blood vessels. The ovarian sections contained germ cells (SC), oogonia (PO) and some primary and secondary vitellogenic oocytes (PVO, SVO, fig. 1b). Stage III ovaries were larger compared to stage II, separated and became transparent with visible orange-yellow translucent eggs. Ovaries were covered with prominent blood vessels. Mature oocytes showed migratory nucleus toward the center and contained more yolk accumulation (e.g., secondary vitellogenic oocytes, SVO) and fewer oogonia (PO) in the ovarian sections (fig. 1c).

At stage IV, ovaries contained small whitish eggs at the posterior end, and blood vessels were still prominent and eggs were extrudable with slight pressure on the abdomen. The ovarian sections mainly consisted of post vitellogenic oocytes (PsVO) with the nucleoli in the center of the nucleus and a few oogonia (PO), primary and secondary vitellogenic oocytes (fig. 1d). The ovaries reached the

largest diameter at stage V, became opaque and were covered by a firm membrane, containing mainly hydrated oocytes. The post vitellogenic oocytes were absent, but oogonia (PO) were scattered between primary and secondary vitellogenic oocytes (PVO, SVO) and hydrated mature oocytes (e.g., eggs, HMO, fig. 1e). No fish with stage VI of ovaries were found in this study (figs. 1, 2a).

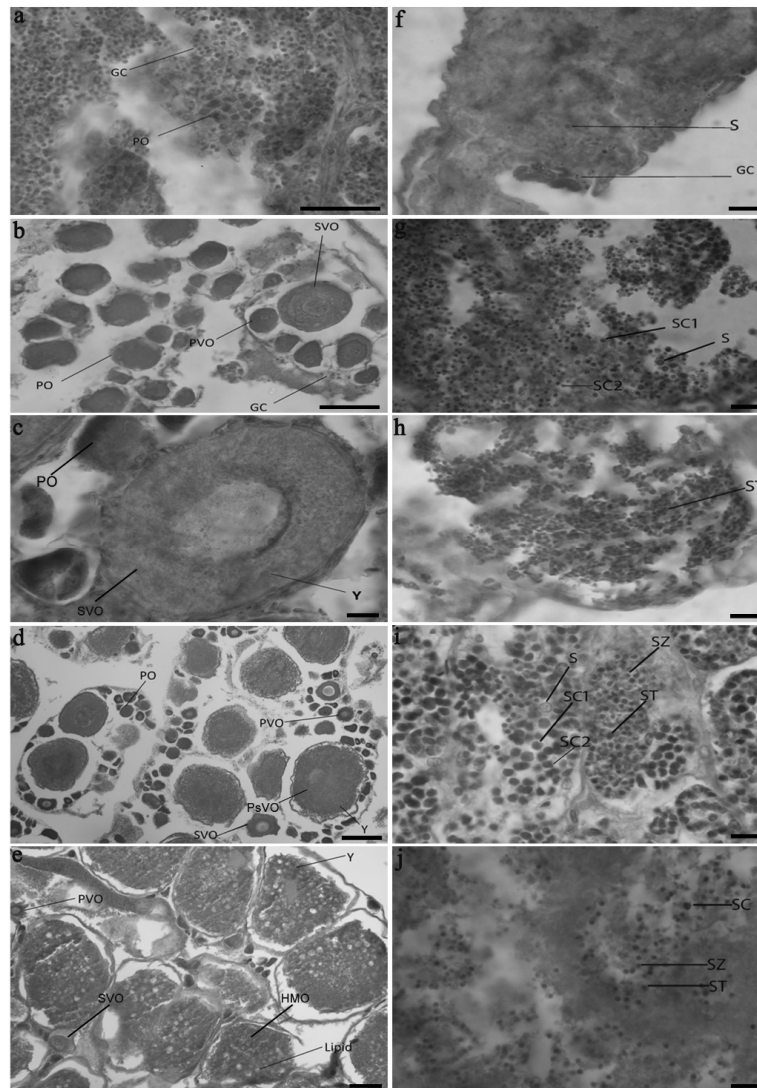


Figure 1. Gonad cross-section of *B. boddarti*

a-e: stage I-V of ovary; f-j: stage I-V of testis; GC: germ cell, PO: oogonia, PVO: primary vitellogenic oocytes, SVO: secondary vitellogenic oocytes, PsVO: post vitellogenic oocytes, Y: yolk, HMO: hydrated mature oocyte, S: Spermatogonia, SC1: primary spermatocytes, SC2: secondary spermatocytes, ST: spermatid and SZ: spermatozoa; scale bar: 200 μ m.

Testes: Stage I testes were too small, elongated and connected by connective tissue with a smooth surface, and were not easy to differentiate from the ovaries. There were many spermatogonia (S) and germ cells (GC) appeared in the testis sections (fig. 1f). Stage II testes were pale with a rough surface and about 1 mm in width. Many primary and secondary spermatocytes (SC1 and SC2) and a few spermatogonia (S) appeared in testis sections (fig. 1g). Testes became white, separate and translucent in stage III. The testis sections consisted of mainly primary and secondary spermatocytes (SC1 and SC2), a few spermatids

(ST) and spermatozoa (SZ) in the sperm ducts (Fig. 1h). Stage IV testes were milky in color and swollen with a few blood vessels on the rough surface. The testis sections comprised many spermatozoa (SZ) and a few spermatids, primary and secondary spermatocytes in the sperm ducts (fig. 1i). Stage V testes reached the largest size with milky color. The testis sections contained mainly spermatozoa (SZ), a few primary spermatocytes (SC1), secondary spermatocytes (SC2) and spermatid (ST, fig. 1j). Similar to ovaries, fish with stage VI testes were not found in the present study (figs. 1, 2b).

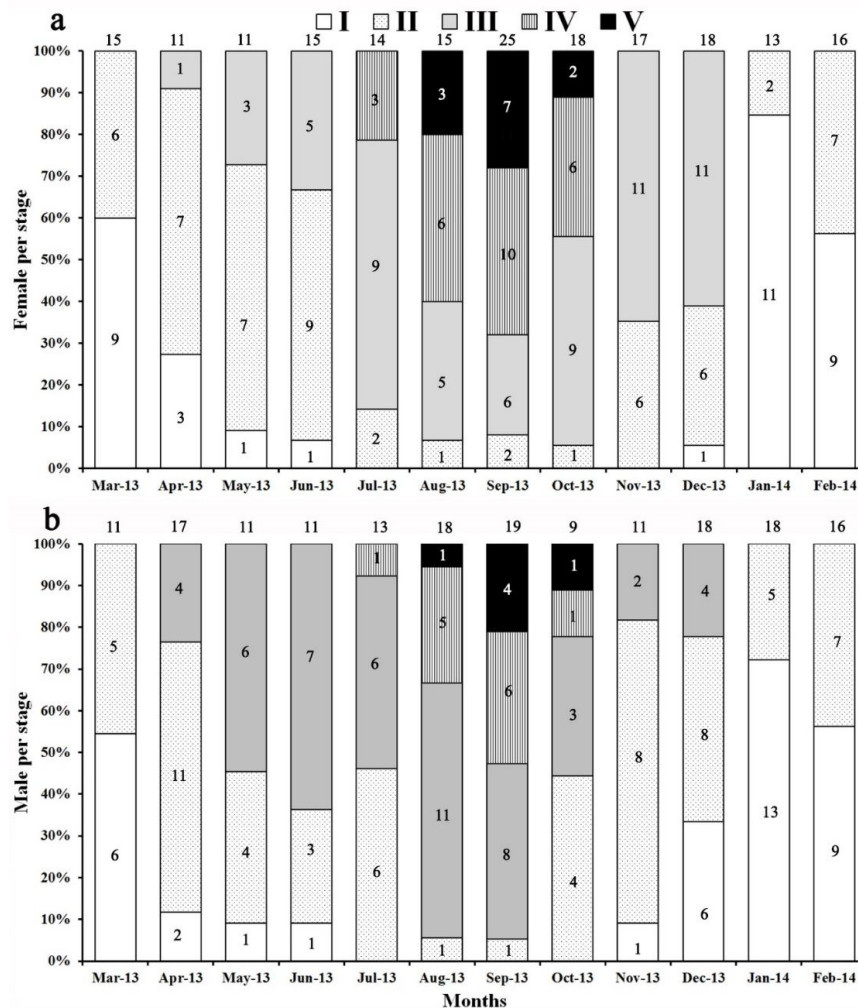


Figure 2. Gonadal stage compositions of female (a) and male (b) of *B. boddarti* (number on the top: total fish collected; number within a column: fish at each gonad development stage)

In four months (July to October), the ovary developed from stage I and II to stage V. Most females with stage V ovaries were found in late September (fig. 2a). Testis development shared a similar pattern with ovaries (fig. 2b). The oogonia and primary oocyte were found in all stages of ovaries. The sympatric mudskipper *B. boddarti* in the present study was a multiple batch spawner, supported by the coexistence of four oocyte types (oogonia, primary and secondary vitellogenic and hydrated mature oocytes) in the stage V ovaries. Moreover, different types of spermatogonia (primary spermatocytes, secondary spermatocytes, spermatid, and spermatocyte) were found in ripe testes (stage V), suggesting that male *B. boddarti* can release sperm several times during the reproductive season. This assumption was similar to most gobies that are serial spawners shedding eggs more than once through a spawning season rather than giving a single spurt of egg release [18].

Immature (stage I) and maturing ovaries (stage II) were found throughout the study, while early matured ovaries (stage III) appeared from the late dry to the end of wet season (April to December, fig. 2a). The mature and ripe ovaries appeared mostly in the wet season, whereas the ripe ovaries were mainly found in the mid-wet season (August to October). Similarly, the testis development showed the same as ovaries (fig. 2b). This pattern suggests that *B. boddarti* was a multi-spawner releasing eggs during a period of three months (August to October) with high precipitation, being similar to some fishes in the Mekong Delta as *Pseudapocryptes elongatus* [9].

Length at first maturation and fecundity

The length at sexual maturity (L_m) was estimated from the maturity curve, and 50% *B. boddarti* reached sexual maturity at 11.52 cm (fig. 3). The size at first sexual maturation is influenced by the biotic factors such as parental care [1], e.g., male *Pomatoschistus marmoratus* reaches first maturation at larger size compared to females due to parental care [20]. Additionally, length at first maturity of fish depends on environmental factors (location)

[38], e.g., *P. marmoratus* reaches 2.4 cm in TL at Suez Canal [12] but 2.7 cm in Mauguio Lagoonare [3]. The size at first mature of *B. boddarti* was strongly shorter than that of co-occurring goby *P. elongatus* (15.4 cm for females and 16.3 for males) [9].

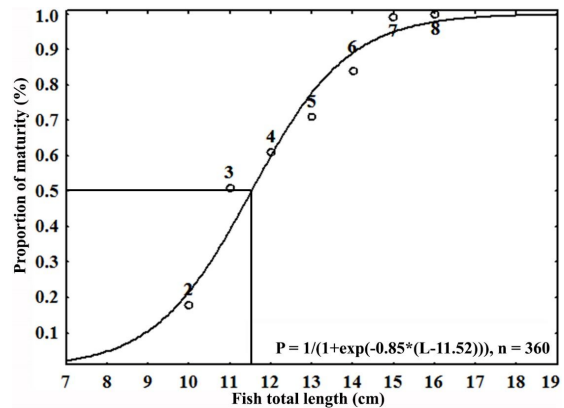


Figure 3. Length at first mature of *B. boddarti*

The female goby was a multiple spawner and a large fish released more eggs at each spawning event as the fecundity had a positive relationship with total length (Fig. 4a) and body weight (fig. 4b). Most gobies are repetitive spawners, and the fecundity and egg size depend on fish size [17, 22]. Similarly, the present study revealed that *B. boddarti* was a serial spawner, and its fecundity positively related to fish size. Abundance and geographic location also cause the variation of fish fecundity [38], e.g. the difference in fecundity of *B. boddarti* among three creeks in Mumbai, India [6]. The *B. boddarti* fecundity in the previous study is 2,100-12,300 eggs and lower than that in the present study (9,800-33,800 eggs) as a consequence of the polluted environment in these creeks in Mumbai, India [6]. The batch fecundity of *B. boddarti* in this study is higher than that of *Neogobius melanostomus* (84 to 606 eggs) the upper Detroit River in North America [19] and *Crystallogobius linearis* (200-700 eggs) in the coastal central area of the Adriatic Sea [4], but lower than *Valenciennea strigata* (60,000-160,000 eggs) in the north shore of Moorea, Society Islands, French Polynesia [31] and *Amblygobius phalaena* (37,000-38,000 eggs) in

the coral reef at Sesoko Island, Okinawa, Japan [34], and similar to *P. elongatus* (2,600-29,400 eggs) in the Mekong Delta, Vietnam [9]. This

suggested that the different geographic regions lead to the different fecundity of *B. boddarti* in comparison with other gobioid species.

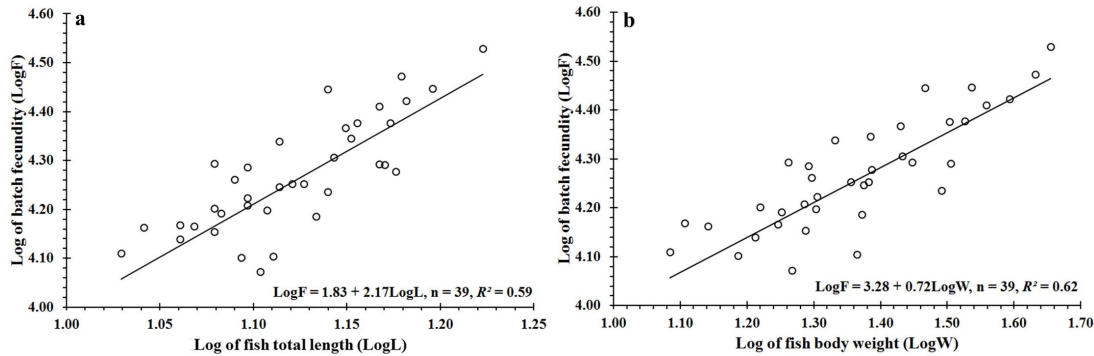


Figure 4. Relationship between fecundity (logF) and (a) fish total length (logTL) and (b) body weight (logW) of *B. boddarti*

CONCLUSION

Boleophthalmus boddarti was a serial spawner shedding eggs over three months in the wet season. This fish reached 11.52 cm in total length and was high fecundity. The present study can provide a basis for further study on the culture and breeding of this mudskipper toward sustainable management. Knowing the size at maturity could allow fisherman and local authorities to set the appropriate size limit for fish catches and the fishing period in the dry season or late wet season to avoid catching a spawning fish population.

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SINH HỌC SINH SẢN CÁ BÔNG SAO *Boleophthalmus boddarti* Ở KHU VỰC BÃI BÒI TỈNH SÓC TRĂNG

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TÓM TẮT

Cá bông sao *Boleophthalmus boddarti* là một loài cá bụn phân bố rộng ở vùng bãi bồi của nhiều khu vực ở châu Á, nhưng đặc điểm sinh học của chúng còn ít được biết đến. Nghiên cứu này được thực hiện ở vùng Trần Đề, Sóc Trăng, Việt Nam từ tháng 3 năm 2013 đến tháng 2 năm 2014 để tìm hiểu chỉ số sinh học sinh sản của cá bông sao. Kết quả phân tích 360 mẫu cá (188 cá cái và 172 cá đực) cho thấy tỷ lệ giới tính của cá bông sao không khác biệt giữa mùa khô và mùa mưa. Loài này sinh sản vào mùa mưa, đẻ trứng nhiều lần trong suốt mùa sinh sản và tập trung chủ yếu vào 3 tháng chính của mùa này (tháng 8 đến tháng 10) bởi vì nhiều giai đoạn khác nhau của tế bào trứng được tìm thấy trong tiêu bản lát cắt ngang tuyến trứng chín (giai đoạn V). Cá đực có thể phóng tinh trùng suốt mùa sinh sản bởi nhiều giai đoạn phát triển khác nhau của tinh trùng được phát hiện trong tiêu bản lát cắt ngang tuyến tinh chín (giai đoạn V) của loài này. Loài cá này đạt chiều dài thành thực đầu tiên là 11,52 cm và có sức sinh sản cao (9.800-33.800 trứng), điều này cho phép chúng có thể thích nghi tốt ở vùng nhiệt đới gió mùa. Kết quả nghiên cứu về đặc điểm sinh học sinh sản của cá bông sao như sự thích sinh sản của loài này ở khu vực nghiên cứu làm cơ sở khoa học trong việc quản lý bền vững ở khu vực bãi bồi ở tỉnh Sóc Trăng.

Từ khóa: *Boleophthalmus boddarti*, cá bụn, chiều dài thành thực đầu, sinh sản, sức sinh sản tiên.

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