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Research Article

Sex determination, mating behavior and spawning frequency of captive wild blueband goby broodstocks, *Valenciennea strigata*

Siriwan Choosri[1,*], Wilaiwan Phuangsanthia[1] and Jarunan Pratoomyot[1]
The Institute of Marine Science, Burapha University, Chonburi, Thailand
* *Corresponding author: Siriwanc@go.buu.ac.th*

Abstract - This research was to examine the sex determination, mating behavior, and spawning frequency of captive wild blueband goby broodstocks, *Valenciennea strigata*, under laboratory conditions. The experiment was conducted over a 12 months period in a hatchery at Bangsaen Institute of Marine Science for a period of Chon Buri province, Thailand. Eight pairs of blueband goby broodstocks, derived from Chatuchak Market, Bangkok, were maintained in a biological recirculating system with tanks measuring 15 x 24 x 15 inches (70 liters of seawater). They were fed pelleted feed twice a day and supplemented with adults Artemia once a day for the duration of the study. Results revealed that the broodstocks had total weight ranging from 2.7 g-19.4 g (ave. 5.8 ± 3.8g) and total lengths between 7.1 and 13.8 cm (ave. 8.7 ± 1.5cm) (n = 16). Sex determination showed that sex can be differentiated by the genital papilla: males have small sex polyps, and the females have rounded sex lobes, along with a more open anus compared to males. These secondary sexual characteristics may be helpful in sexing blueband goby. Blueband gobies use external fertilization with breeding behaviour occurred in the evening after sunset and taking about 45 minutes to complete the laying process. The eggs were adhersive and oval-shaped, with sizes ranging from 1.0-1.1 mm in length and 0.3 mm in width. The spawning occurred every 10-12 days (10 ± 0.89 days) (n = 7), and the number of eggs ranged from 3,604 and 34,000 eggs (n = 5). The embryonic period lasted 56 hours and 30 minutes after fertilization. This study provided fundamental data that can serve as a guideline for raising blueband goby and other marine gobiidae.

Keywords: Blueband goby, mating behavior, sex determination, spawning frequency, *Valenciennea strigata*

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1. Introduction

Marine ornamental fish trading these days, mostly, is capturing from the nature which directly impacts on the resources in coral reefs. (Pouil et al., 2020). Due to overfishing, the blueband goby, *Valenciennea strigata*, has seen a decline in population and is now considered rare species (Robert, 1997). This species, a member of the Gobiidae family, is native to Thailand and is frequently sold in marine ornamental fish shops both domestically and internationally (Wabnitz et al., 2003). A survey of the trading marine ornamental fish trad at Chatuchak Market, Bangkok, found five species available for sale: *V. bella, V. puellaris, V. sexguttata, V. strigata, and V. wardii*. Among these five genera, *V. strigata* is particularly popular, with prices ranging from 250 to 350 baht per fish (Teskong et al., 2019). Therefore, it is imperactive to reduce the number of wild fish currently taken from the natural habitats, and one effective solution is aquaculture. A crucial step in successful cultivation is understanding the basic biological characteristics of the species (Molina & Segade, 2011; Gopakumar et al., 2011). In this study, we investigate the sex determination, mating behavior, and spawning frequency of *V. strigata* under laboratory conditions. The knowledgegained from this study provides essential foundational information regarding to the biological traits of broodstocks. This information will serveas a guideline for the productive breeding of *V. strigata* and indirectly supports to reduce the capture of wild species, thereby helping to prevent the *V. strigata* extincts in the future.

2. Materials and methods

2.1 Preparing the tank

Eight experimental glass tanks, each measuring 15 x 24 x 15 inches and containing 70 liters of seawater, were constructed for culturing *V. strigata* in a closed water recirculation system. The closed water recirculation system using algae, bio-balls, and artificial rocks as biological materials to maintain good water quality using algae. To simulate a natural environment, the tanks were added sands, artificial plants, gravels, artificial live rock, and PVC pipes (2.5 cm in diameter and 15 cm in length) to provide hiding places and areas for egg-laying (Figure 1). The tanks were aerated by airstones, connected to an ion unit, and covered blue plastic to prevent disturbances and reduce fish stress. The photoperiod during preconditioning was maintained under 12L:12D (L = light, D = dark) conditions. Water quality parameters were monitored with scientific instruments for laboratory analysis. Temperature and salinity were monitored daily, while remaining water quality parameters were assessed every 2 weeks. The water quality in each tank was maintained as follows: temperature ranged from 26.8-28.6 °C, salinity was 30-32 ppt, pH ranged from 7.8 to 8.3, ammonia was less than 0.02 ppm, nitrite and nitrate were less than 0.001 ppm, alkalinity was maintained at 100-120 mg/l and dissolved oxygen was maintained between 4.5-5 mg/l.

Figure 1. The experimental tanks unit for *V. strigata*

2.2 Collection of specimens

Valenciennea strigata were obtained from the fish market, Chatuchak, Bangkok, Thailand. (13.802879792336313, 100.54886486788841). Each fish was placed in a 5 x 12-inch areated plastic bag, and transported to the Institute of Marine Science, Bangsaen Chonburi province, Thailand (13.380891844272027, 100.97643551610513). The transportation period lasted approximately two hours.

Upon arrival, the plastic bags containing the *V. strigata* were floated in the tanks for 10-15 minutes to acclimate the fish before being slowly released into a 70-liter tank. The fish were acclimatized and maintained in the tanks for 20 days. During this time, they were fed pelleted feeds twice daily at a rate of approximately 2% of their body weight. The broodstock were received pelleted commercial feed (51% protein) supplemented with whole *Artemia* sp. Eight pairs were selected as broodstock, measured for weight and length, and their sex was determined based on differences in appearance, size, and color.

2.3 Sex determination

After approximately one month of experimenting, the sex of the *V. strigata* was determined using a 10% solution of tricaine methane sulfonate (MS-222) as an anesthetic agent. Each fish was placed in a plastic bowl 1,000 ml, and then add 3 drops of MS-222 were added. Once the fish stopped moving, each of them was removed from the bowl for sex assessment by examining its external appearance, size, colour and the shape of the genital papilla (Robert, 1997).

2.4 Mating behavior and spawning

The experiment was conducted for a period of 12 months. Each pair of *V. strigata* was maintained in a 70 liters tank equipped with a biological recirculation system. Prior to introducing the fish, a mesh net was placed in the center of the tank to divide it into two sections. A male and a female fish were introduced into the tank at a 1:1 ratio to allow them to acclimate to each other. The fish were observed to ensure that they accepted each other and did not exhibit aggressive behavior. Once they showed acceptance, Then, the mesh barrier was gradually removed from the middle of the tank, allowing them to being together. The acceptance behavior of the broodstock was closely monitored (Figure 2). Mating and spawning frequency were observed through direct observation and video recording. When egg clusters were found, fertilized eggs were randomly collected and examined under a stereo microscope. The total weight of each batch of eggs was measured to calculate egg production.

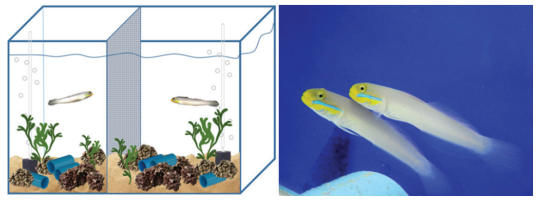

Figure 2. The exhibition of pairing *V. strigata*; The mess barrier in the middle of the tank was placed (left) prior to removing when ensuring they accepted each other (right).

2.5 Ethics statement

These experimental procedures were conducted under the approval of Burapha University's internal ethical review board (ethics project certificate ID# 15/2561).

3. Results

The *V. strigata* achieved reproductive maturity, with individuals ranging in total length from 7.1-13.8 cm (8.7 ± 1.5 cm, n = 16) standard length from 5.6 - 11.4 cm (7.1 ± 1.2 cm, n = 16) and total weight from 2.7-19.4 g (5.8 ± 3.8 g, n = 16).

3.1 Sex determination

External observations indicated females were generally larger than males. Female had a larger abdomen and were darker in color compared to males. Males had a more slender and elongated body, and their body size appeared more proportional than that of females. When examining the pattern on the body and the gill covers, both sexes displayed similar body patterns and gill cover markings, though males had more vivid coloration around the gill covers. Sexual characteristics, or secondary sexual characteristics, were used for gender identification. In this study, *V.*

strigata females had rounded sex organs and a larger anal opening, while males had smaller, pointed sex lobes (Figure 3).

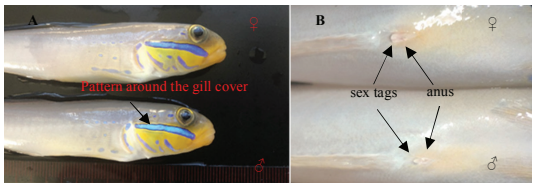

Figure 3. Sex determination between male and female *Valenciennea strigata*: (A) Males showed brighter in color pattern around the gill covers; (B) Females have rounded sex organs and while males have pointed polyps.

3.2 Mating behavior

Breeding in *V. strigata* involves external fertilization, where eggs are fertilized outside the female's body. The average breeding cycle involves laying eggs every 10 - 12 days (10 ± 0.89 days) (n = 7). Environmental conditions during this period were water temperature ranged from 26.8 - 28.6 °C and water salinity was maintained at 32 ppt. Spawning typically occurs in the evening starting approximately 12 minutes after sunset (start at 5:59 p.m. and terminated at 6:45 p.m.).

Prior to laying eggs 1-2 days, males cleaned the designated spawning sites, such as pipes or artificial piles of rocks, using their mouth. The female also participated in the cleaning process, beginning around 1:00 p.m. onwards or approximately 4 hours before egg-laying. During this time, the female exhibited increased aggression, herding the male to ensure the nest was thoroughly cleaned. It was noticed that the female's belly appeared enlarged and dark, with a protruding oviduct longer than usual. The female entered the PVC pipe, circled around it, and positioned themselves with their bellies facing up, touching the upper wall of the pipe to lay eggs. Meanwhile, male stood guarding, monitoring the surroundings for any potential danger. After laying eggs about 10 minutes, the female left the nest, allowing the male to fertilize the eggs. This spawning behaviour was repeated several times until the process ended. It was noted that the entire spawning events lasted approximately 46 minutes (n = 2), and consistently occurred in the evening, about 12 minutes after sunset (Figure 4).

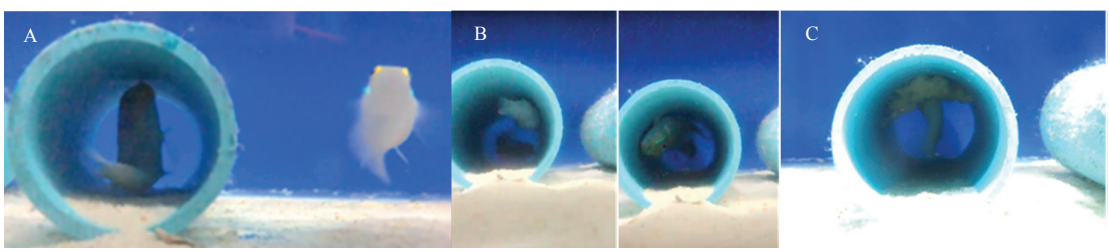

Figure 4. Mating behavior of *Valenciennea strigata*: (A) The male cleans the PVC pipe wall readily for laying eggs; (B) The female entered inside the pipe, circles around, then turn over with belly touching the PVC pipe wall and lays the eggs; (C) The male released sperms after the female has laid the eggs.

When spawning completed, the female immediately swam out of the spawning area, foraged for food by sifting through the sand, and covered the nest with sand to protect it from predators.

3.3 Spawning frequency

The spawning frequency of *V. strigata* was found to be different based on water temperature. At water temperatures of

27 - 29 °C (during February), eggs were laid every 8 days and at water temperatures of 27 - 30 °C (during March-April), eggs were also laid every 21 - 30 days. On average, eggs were laid every 10 - 12 days with an average interval of 10 ± 0.89 days (n = 7). The water temperature during these observations ranged from 26.8 - 28.6 °C, with an average of 27.2 ± 0.81 °C. The total number of eggs laid at each time was counted measured under a microscope. It was found that the total number of eggs was 3,604 with the eggs weight 0.15 g. This can be calculated to approximately 240 eggs per 0.01 grams. The number of eggs laid each time ranges from 3,604 to 34,000 eggs (n = 5). The eggs were found to stick together in bunch. They were oval in shape, rounded at the top, and had a sticky membrane at the bottom that allowed them to cling together and attached to a hiding spot, with a length was 1.0-1.1 mm and a width was 0.3 mm. Newly laid eggs were light yellow. By 4 - 5 days old, the eggs became clear white, the eyes of the embryos turned silver, and the eggs began to hatch (Figure 5).

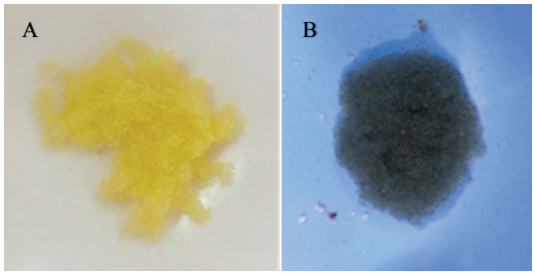

Figure 5. The eggs of *Valenciennea strigata*: (A) Newly laid eggs are light yellow; (B)

4. Discussion

Determining the sex of *V. strigata* based on the external morphology of female and male was quite challenging due to the similarity between males and females in sizes of head, body, fins, and body color. The only noticeable differences are the color patterns on the body and gill covers, and the shape and size of the female's abdomen when she is ready to spawn. This finding differs from Maciolek (1977), who reported that the external morphology of female and male hawaiian freshwater goby (*Lentipes concolor* (Gill, 1860), *L. seminudus* (Gunther, 1880), and *L. bustamantaei* (Boulenger, 1916) from the Gulf of Guinea is distinct. In those species, males and females have different sizes of head, mouth, dorsal fin and body color. Males appear to be more colorful than females.

In the present study, the sizes of the genital papilla were effective method to differentiate sexes of the *V. strigata* supporting Lau (1973), who reported that the genital papilla are commonly used for sex determination in fish. In this study, the maturation size of *V. strigata* ranged from 7.1 - 13.8 cm (TL) and 5.6 - 11.4 cm (SL). This range is similar to that reported by Kannan et al. (2013), who found that maturation size of the two-stripe goby, *Valenciennea helsdingenii*, was between 9.7 and 14.5 cm (TL). However, this differs from Suzuki (2016), who reported that maturation size of *Valenciennea yanoi* was between 4.62 to 5.42 cm (SL) and 6.5 cm (TL).

V. strigata exhibits external fertilization. After spawning, the eggs adhere to substrates, and the male release sperms over them. The fertilized eggs then develop within their shells before hatching into small larvae. The reproductive behaviour is consistent with that observed in the yellow prawn-goby, *Cryptocentrus cinctus* (Ruiz, 2012), and the Ice goby, *Leucopsarion petersii* (Shiro-uo) (Arakawa et al., 1999). The spawning frequency of *V. strigata* every 10-12 days is comparable to the 13-day cycle of *Valenciennea strigata* along the north shore of Moorea, Society Islands, French Polynesia, spawned every 13 days (Robert, 1997). However, This behaviour differs from the Caribbean cleaner goby (*Gobiosoma evelynae*), which spawns eggs every 6-8 days (Harding et al., 2003).

Blueband goby (*V. strigata*) eggs are adhesive and demersal. Newly released eggs are covered with a chemical layer

that helps them attach to substrates. This type of egg is also a characteristic of other goby fish eggs, including the yellow prawn goby, *Cryptocentrus cinctus* (Ruiz, 2012) and the ice goby, *Leucopsarion petersii* (Shiro-uo) (Arakawa et al., 1999) and Hawaiian *Lentipes* sp. (Maciolek, 1977). The eggs are oval-shaped and colour changes as they develop: 1-2 days old are yellow, they become clear by 4-5 days, with visible eyes. The incubation period from fertilization to hatching is about 56 h. 30 min at a temperature of 26.8-28.6 °C. Each cluster contains between 3,604 to 34,000 eggs. Ruiz (2012) reported that eggs of *C.cinctus* are 0.5 mm in size, with an incubation time of about 96 h, which is about 40h.30 min longer than that of *V. strigata.* Similarly, the development of eggs in *Gobiodon citrinus* takes about 100 h, which is longer than the duration observed for *V. stigmata* in the present study. In contrast, the eggs of Hawaiian *Lentipes* take about 24-72 hours (1-2 days) to develop from fertilization to hatching (Maciolek, 1977). The differences in egg development times among *V. strigata, C. cinctus* and *G. citrinus* may be due to the different genera, despite all being in the family Gobiidae.

5. Conclusion

The maturation size of *V. strigata* broodstock ranged from 7.1 to 13.8 cm in length and 2.7 and 19.4 g in total weight (n = 16). Sex determination by examining the genital papillae revealed that males have small sex polyps, while females have rounded sex lobes and a more open anus. The species exhibits external fertilization with spawning occurs every 10-12 days (n = 7). The eggs are adhesive, with clutch sizes ranging from 3,604 to 34,000 eggs (n = 5). and the embryonic development period lasts approximately 56 h 30 min after fertilization.

This study has provided fundamental data that, enhancein the understanding of the reproductive characteristics of the blueband goby. This information is valuable for aquaculture efforts, facilitating the breeding of offspring that can support further research.

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