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รายงานฉบับสมบูรณ์

โครงการลักษณะของแหล่งที่อยู่อาศัยของปลากริม
และการเลือกคู่ของปลากริมเพศเมีย

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บทคัดย่อ

จากการศึกษาแหล่งที่อยู่อาศัยของปลากริม (croaking gouramis *Trichopsis vittata*) ในนาข้าวที่มีน้ำท่วมขังและหนองน้ำขนาดเล็กในประเทศไทย พบว่าปลากริมจะอาศัยอยู่ในแหล่งน้ำนิ่งที่ตื้นและมีพืชน้ำขึ้นอยู่อย่างหนาแน่น น้ำมีค่าต่างๆ เหล่านี้ต่ำคือ ความเป็นกรดเบส ปริมาณออกซิเจนละลายน้ำ ความขุ่น ความต้องการออกซิเจนทางชีวเคมี ไนไตรท์ ไนเตรท และฟอสเฟต แต่จะมีค่าเหล่านี้สูงคือการนำไฟฟ้า ปริมาณการละลายของของแข็ง และอุณหภูมิ ปลากริมเพศผู้มีขนาดตัวยาวกว่าปลากริมเพศเมีย ในการผสมพันธุ์ ปลากริมเพศผู้จะรัดปลากริมเพศเมียหลายครั้ง จากการศึกษาผลของความยาวลำตัวของปลากริมเพศผู้ที่มีต่อความพอใจของปลากริมเพศเมียและการต่อสู้ของปลากริมเพศผู้ โดยแบ่งคู่ของปลากริมเพศผู้เป็น 2 กลุ่ม คือ กลุ่มของคู่ที่มีขนาดตัวเท่ากันกับกลุ่มของคู่ที่มีขนาดตัวต่างกัน พบว่าปลากริมเพศเมียจะเลือกคู่โดยไม่ได้พิจารณาจากความยาวลำตัวของปลากริมเพศผู้ และในการต่อสู้พบว่า ปลากริมเพศผู้ตัวใหญ่มักจะชนะปลากริมเพศผู้ตัวเล็ก สรุปได้ว่าขนาดของลำตัวของปลากริมเพศผู้ที่ใหญ่กว่าปลากริมเพศเมียนั้นอาจมีผลดีต่อการต่อสู้ของปลากริมเพศผู้เท่านั้น นอกจากนี้เมื่อศึกษาผลของขนาดหวอดต่อความชอบของปลากริมเพศเมียและการต่อสู้ของปลากริมเพศผู้ โดยแบ่งคู่ของปลากริมเพศผู้ออกเป็น 3 กลุ่มคือ (1) กลุ่มของคู่ที่ปลาสร้างหวอดทั้งสองตัว (2) กลุ่มของคู่ที่ปลาสร้างหวอดเพียงตัวเดียว และ(3) กลุ่มของคู่ที่ปลาทั้งสองตัวไม่สร้างหวอด พบว่าปลากริมเพศเมียชอบปลากริมเพศผู้ที่สร้างหวอดขนาดเล็กมากกว่าปลากริมเพศผู้ที่สร้างหวอดขนาดใหญ่ และชอบปลากริมเพศผู้ที่ไม่สร้างหวอดมากกว่าปลากริมเพศผู้ที่สร้างหวอด แสดงว่าการที่ปลากริมเพศผู้ได้สร้างหวอดหรือสร้างหวอดขนาดใหญ่อาจมีผลทำให้ประสิทธิภาพของปลากริมเพศผู้ลดลง ส่วนในการต่อสู้พบว่าปลากริมเพศผู้ที่สร้างหวอดขนาดใหญ่ไม่ได้ชนะมากกว่าปลากริมเพศผู้ที่สร้างหวอดขนาดเล็ก ทั้งนี้อาจเป็นไปได้ว่าการที่ปลากริมเพศผู้สร้างหวอดขนาดใหญ่หรือการที่ได้สร้างหวอดแล้วไม่ได้แสดงถึงการมีความสามารถสูงในการเลี้ยงดูลูกอ่อนหรือในการต่อสู้

ABSTRACT

Croaking gouramis *Trichopsis vittata* were investigated in rice paddy fields and small ponds in Thailand. They inhabited shallow standing waters with dense vegetation which were low values in pH, DO, transparency, BOD, nitrite, nitrate and phosphate but high in specific conductance, TDS and temperature. Males were bigger than females. Male and female embraced many times during mating. To examine the role of male body size on the female sexual preference and on the male-male competition in croaking gouramis, two treatments were set up. In one treatment, males of the same size were matched, while in the other males of different sizes were placed together. The results showed that the male body size did not affect the sexual preference among female croaking gouramis. Regarding the male-male competition study, large males were found to win more fights than small ones. The results indicate that the sexual size dimorphism in croaking gouramis, in which species the male is larger than the female, may be associated with the male-male competition, not with the female preference. To examine the role of the bubble nest size on the female sexual preference and on the male-male competition in croaking gouramis, three treatments were set up: (1) two-bubble-nest treatment, (2) one-bubble-nest treatment, and (3) no-bubble-nest treatment. Females preferred small bubble nest males over large ones and preferred males with no bubble nest over males with bubble nests. In the male-male competition, large bubble nest males did not win more fights. This study suggests that males with large bubble nests or males with bubble nest might not always reflect the male's contribution to paternal care and the outcome of contests.

บทสรุปสำหรับผู้บริหาร

ปลากริม (*Trichopsis vittata* (Cuvier, 1831)) เป็นปลา anabantid ที่มีแหล่งที่อยู่อาศัยในประเทศไทย เวียดนาม มาเลเซียและอินโดนีเซีย ความเข้าใจเกี่ยวกับระบบการสืบพันธุ์ในธรรมชาติอาจจะช่วยในการพัฒนาระบบการเพาะเลี้ยง เพื่อพัฒนาเพื่อการส่งออกต่อไป เราเข้าใจเกี่ยวกับระบบนิเวศของปลากริม *Trichopsis vittata* (Cuvier, 1831) ในธรรมชาติน้อยมาก การศึกษาส่วนใหญ่จะเกี่ยวกับพฤติกรรมก้าวร้าว (Bischof, 1996) และเสียงของปลากริม (Ladich et al., 1992a, b; Ladich, 1997a, b; Ladich & Yan, 1998; Ladich & Popper, 2001; Wysocki & Ladich, 2002; Wysocki & Ladich, 2003) นอกจากนี้การศึกษาทั้งหมดนี้ใช้ปลากริมจากร้านขายปลา ซึ่งปลาผ่านการผสมพันธุ์และคัดเลือกพันธุ์มาหลายชั่วรุ่น ทำให้ปลากริมเหล่านี้ไม่เหมือนปลากริมในธรรมชาติ นับจนถึงปัจจุบันงานวิจัยทางด้านวิวัฒนาการและนิเวศวิทยาในสิ่งแวดล้อมตามธรรมชาติยังมีน้อยมาก การทดลองครั้งนี้จะทำเพื่อเพิ่มความเข้าใจเกี่ยวกับปลากริมในธรรมชาติ จะทำการเปรียบเทียบปลากริมที่พบในนาข้าวกับหนองน้ำขนาดเล็กในฤดูกาลผสมพันธุ์ จะทำการศึกษาถึงความหนาแน่นของประชากร อัตราส่วนทางเพศ ลักษณะของหวอดได้แก่ ความยาว ความกว้าง ความลึกและ พื้นที่ของหวอด จะทดสอบสมมุติฐาน 3 ข้อดังนี้ (1) ขนาดและรูปร่างของปลากริมมีความแตกต่างกันระหว่างปลากริมที่พบในนาข้าวกับหนองน้ำขนาดเล็ก (2) ปลากริมเพศผู้จะสร้างหวอดที่มีขนาดเป็นสัดส่วนกับขนาดความยาว และน้ำหนักตัว และ (3) จำนวนไข่ต่อหวอดสัมพันธ์กับขนาดพื้นที่ของหวอดและความยาวของปลากริมเพศผู้ที่สร้างหวอด

ขนาดของปลาเพศผู้อาจจะมีผลต่อความสำเร็จในการสืบพันธุ์ โดยอาจจะมีอิทธิพลต่อจำนวนเพศเมียที่ยอมผสมพันธุ์ด้วย และคุณภาพของการดูแลลูกๆให้อยู่รอด ปลาเพศผู้ขนาดใหญ่หลายชนิดมักจะชนะการต่อสู้ ครอบครองอาณาเขตที่ดีและมีเพศเมียมาผสมพันธุ์ด้วยจำนวนมาก เช่น ปลากัด (e.g. Jaroensutasinee & Jaroensutasinee 2001) การศึกษาครั้งนี้จะหาความสัมพันธ์ระหว่างขนาดปลากริมเพศผู้ การต่อสู้ระหว่างเพศผู้ และ ความชอบของปลากริมเพศเมีย โดยได้ทำนายว่า (1) ถ้าการต่อสู้ระหว่างปลากริมเพศผู้มีความสำคัญในการประสบความสำเร็จในการผสมพันธ์แล้ว ปลาเพศผู้ขนาดใหญ่จะชนะการต่อสู้ (2) ระยะเวลาในการต่อสู้จะลดลงเมื่อความแตกต่างระหว่างขนาดปลาเพศผู้ที่จะต่อสู้เพิ่มขึ้น และ (3) ถ้าปลากริมเพศเมียชอบปลาเพศผู้ที่มีขนาดใหญ่แล้ว ปลากริมเพศเมียจะต้องใช้เวลาอยู่กับปลากริมเพศผู้ที่มีขนาดใหญ่นานกว่าปลากริมเพศผู้ที่มีขนาดเล็ก

ความสำเร็จในการสืบพันธุ์ของสิ่งมีชีวิตเพศผู้มีความสัมพันธ์แบบบวกกับขนาดของหวอดในปลาหลายสปีชีส์ (e.g. Natsumeda, 1998) หวอดขนาดใหญ่จะมีพื้นที่ผิวใหญ่ให้ไข่ไปเกาะ แต่อย่างไรก็ตามหวอดขนาดใหญ่ก็อาจจะเป็นการเพิ่มโอกาสให้ตัวผู้ตัวอื่นเข้ามาแย่งได้มากขึ้น อาจจะมีการแอบเข้ามาโดยตัวผู้ตัวเล็กได้ง่ายขึ้น หรือถูกผู้ล่าเข้ามาล่าได้ง่ายขึ้น (Magnhagen, 1992) การศึกษาครั้งนี้ทำนายว่า (1) ถ้าปลากริมเพศเมียชอบปลาเพศผู้ที่มีหวอดขนาดใหญ่แล้ว ปลากริมเพศเมียจะต้องใช้เวลาอยู่กับปลากริมเพศผู้ที่สร้างหวอดขนาดใหญ่นานกว่าปลากริมเพศผู้ที่สร้างหวอดขนาดเล็ก และ (2) ถ้าหวอดขนาดใหญ่เป็นดัชนีที่ดีที่จะบ่งชี้ถึงสภาพของปลากริมเพศผู้แล้ว ปลากริมเพศผู้ที่สร้างหวอดขนาดใหญ่น่าจะชนะการต่อสู้มากกว่าปลากริมเพศผู้ที่สร้างหวอดขนาดเล็ก

Executive Summary

Wild croaking gourami (*Trichopsis vittata* (Cuvier, 1831)) is an anabantid fish native to Thailand, Vietnam, Malaysia and Indonesia. An understanding of their natural breeding system may help the development of captive rearing systems to provide fish for petshop. Understanding of the basic ecology and natural history of wild croaking gourami in its natural habitat is limited. Most studies of croaking gourami have concerned agonistic behaviour (Bischof, 1996) and significance of sounds in vocalizing fish (Ladich et al., 1992a, b; Ladich, 1997a, b; Ladich & Yan, 1998; Ladich & Popper, 2001; Wysocki & Ladich, 2002; Wysocki & Ladich, 2003). In addition, all those studies have used heavily inbred pet-shop lines of fish. These fish bear little resemblance to the wild croaking gourami. Up until now, use of croaking gourami in evolutionary, ecology and life history research in the context of natural environments has little been done. This present study will conduct to further understanding of wild croaking gourami in its environment and physiological ecology of habitat use. The study will be made in rice paddy fields and small ponds during breeding season. Population density, adult sex ratio and bubble nest characteristics including length, width, depth and area will be examined. Three hypotheses will be tested (1) croaking gourami sizes and shapes differ between two habitats: rice paddy and small ponds (2) the male croaking gourami builds a bubble nest in proportion to its length and weight and (3) the number of eggs per nest is correlated with the bubble nest area and male length.

Male body size may affect reproductive success by influencing the number of mates obtained, and the quality of care provided to the broods. Larger males tend to win in male contests, hold higher quality territories and gain greater access to females in several fish species (e.g. Jaroensutasinee & Jaroensutasinee 2001). In this study, the relationship between male body size, male contest, and female preference in croaking gourami will be investigated. This study predicts that (1) if males contest plays an important role in mating success, then larger males should win fights, (2) fighting duration should decrease as the size difference between male increases and (3) if females prefer larger males as mates, then they should spend more time with larger males than smaller males.

Male reproductive success correlates positively with nest size in many nest-holding fish species (e.g. Natsumeda, 1998). Larger nest ensures a greater surface area for egg deposition; however, larger nests are more susceptible to take-over by other males, sneak by smaller males or be eaten by predator (Magnhagen, 1992). In this study, we will investigate the relationship between bubble nest area, female preference and male contest in croaking gourami. This study predicts that (1) if females prefer males with large bubble nest, then they should spend more time with larger bubble nest males than smaller bubble nest males, and (2) if larger bubble nest area is a good indicator of male condition, then males who built larger bubble nests should win more fights against smaller bubble nest males.

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CHAPTER 1
INTRODUCTION

Croaking gourami (*Trichopsis vittata* Cuvier, 1831) is a small freshwater fish native to many tropical areas in Asia such as Java, Borneo, Sumatra, Malaya, Indo-China and Thailand (Smith, 1945). It is uniquely adapted to the stagnant waters with dense aquatic or floating plants. At present, this fish species becomes a popular home aquarium fish in occidental countries because of its slim body, long fins and peaceful behaviour. Therefore, croaking gourami has been selected artificially for many generations in the pet trade with the decline of it in natural habitats. The studies of this fish were done in the lab using these heavily inbred fish. Due to its sound production during fighting, croaking gourami has been used in the study of agonistic behaviour (Bischof, 1998; Henglmüller and Ladich, 1999), vocalisation (Ladich et al., 1992; Ladich, 1997; Ladich and Yan, 1998; Wysocki and Ladich, 2001) and hearing (McKibben and Bass, 1998; Yan, 1998; Ladich and Popper, 2001; Wysocki and Ladich, 2002).

Although croaking gourami is not in the IUCN Red List and is available in Thailand, little is known about it. Therefore, it is necessary to study its ecological habitat requirements and sexual behaviour of this wild croaking gourami. Field data on the water quality parameters of its nesting site would be very valuable for behavioural ecology knowledge. This study aims to investigate characteristics of wild reproducing croaking gourami, its nesting habitats and reproductive biology. Furthermore this study is the first to investigate sexual selection and how larger male body size and the larger bubble nest in this species maintain through female preference, male-male competition or both.

This study was divided into three parts. The first part is about mating habits and nesting *habitats of croaking gouramis. The second part is about the role of male body size on female* preference and male-male competition. The third part is about their bubble nest size on both mechanisms.

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CHAPTER 2
MATING HABITS AND NESTING HABITATS IN CROAKING GOURAMIS *Trichopsis vittata*

PUBLICATION

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ABSTRACT

Croaking gouramis *Trichopsis vittata* were investigated in the two types of habitats in which they occur in Thailand: rice paddy fields and small ponds. Croaking gouramis inhabit shallow standing water with dense vegetation near the margin of both habitats. Nest sites in rice paddies were higher in pH, dissolved oxygen, and temperature but lower in specific conductance, total dissolved solids and water depth than in small ponds. Population densities of croaking gouramis were lower in rice paddies than in small ponds. Males were heavier and bigger than females. There were positive correlations between male weight and body length and width, whereas female weight was positively correlated only with body length. There was no correlation between the bubble nest area and the male body length and weight. Male and female embraced many times during mating. There were (Mean ± SD) 3.74 ± 1.02 eggs per embracement, 84 ± 12.97 embracement/spawning, and 314 ± 109.77 eggs/spawning with a spawning duration of 178 ± 51.17 min. Fish larvae hatched within 30.75 ± 0.55 hr and 2-day old fry's sizes were 3.21 ± 0.29 mm.

Keywords: Mating habits, Nest habitats, Croaking gouramis, *Trichopsis vittata*

INTRODUCTION

Many fish species commonly select particular habitat types in an apparent effort to increase their reproductive success (Jenkins and Wheatley, 1998; Bremset and Berg, 1999; Chaves and Bouchereau, 2000; Morrow and Fischenich, 2000; Olivotto et al., 2003; Wildhaber and Lamberson, 2004). These habitat choices could influence the costs and benefits of time and energy devoted to different activities (Hart and Reynolds, 2002). Habitat requirements can influence many aspects of fish biology-they can affect distribution (Darling, 2000; Clay et al., 2004) and colonisation (Roberts and Poore, 2006), interact with genetic parameters to influence the timing of sexual maturity (Wild et al., 1994), interact with environmental cues in regulating the reproductive cycle (Omori, 1995), influence mating and breeding systems (Sato, 1994; Reynolds, 1996; Dickerson et al., 2004), impede and/or enhance the evolution of reproductive isolation (Magurran and Phillip, 2001) and reflect the distinctive functional morphology of fishes (Motta et al., 1995).

The croaking gourami (*Trichopsis vittata*, Cuvier, 1831) is a small dull coloured freshwater fish native to Indonesia, Vietnam and Thailand (Smith, 1945; Sandford, 2000). This fish inhabits shallow standing freshwater habitats such as rice paddy fields and small ponds. They are most notable for their audible rattling or croaking noise, which can be heard during breeding or fighting. This fish has been selected artificially for many generations in the pet trade, and it has mostly been studied in the lab with heavily inbred pet shop lines. Previous studies have focused on its agonistic behaviour (Bischof, 1998; Henglmüller and Ladich, 1999), vocalisations (Ladich et al., 1992; Ladich, 1997; Ladich and Yan, 1998; Wysocki and Ladich, 2001) and hearing (Yan, 1998; Ladich and Popper, 2001; Wysocki and Ladich, 2002). Little is known about the ecology, habitat preferences and reproductive behaviour of wild populations.

Although the croaking gourami is not in the IUCN Red List, the artificial selection in many regions of the world and their introduction of pet shop breeds into natural populations, together with the decline of this native species in its natural habitats, makes it important to study the natural ecology of the species. Therefore, field data on the water quality parameters of its nesting site would be valuable for understanding habitat associations and behavioural ecology.

This study aims to investigate the characteristics of two kinds of croaking gourami habitats: rice paddy fields and small ponds, sexual size dimorphism of wild croaking gouramis, relationship between bubble nest size and male body size, and reproductive biology. Two hypotheses were tested. First, if the characteristics of the rice paddy fields and the small ponds were significantly different, then the sizes of the fish and their bubble nest areas from both habitats should be significantly different. This is because this fish may have some morphological adaptation or natural selection to particular habitats. Second, if bubble nest areas depended on the sizes of the builders, then the bubble nest areas should be positively correlated with the male body length and/or weight. This is because the larger males should be capable of building larger bubble nests.

MATERIALS AND METHODS

Species Description

The croaking gourami is a tropical freshwater fish of the suborder Anabantoidea, family Belontiidae (Smith, 1945; Sandford, 2000). This fish is capable of utilising the oxygen present at the water surface via its labyrinth organ (i.e., a bony structure formed by a segment of gill arch and covered with a respiratory epithelium) (Graham, 1997). Males and females usually attain sexual maturity after 4 months of age with a standard body length of 3.0-4.0 cm (Henglmüller and Ladich, 1999). Both sexes are brown-bodied with three horizontal stripes. Males have more reddish and elongated dorsal fins than females. Females are smaller, and duller than males. Males build bubble nests that consist of mucus-covered bubbles that stick together in a mass and bind with emergent plants on the water surface (Helfman et al., 1997).

Fish Sampling

All field work was carried out during 07:00-10:00 hr in the middle of the breeding season (i.e., April and May 2004) in Nakhonsithammarat Province, southern Thailand (08° 29.381′ - 08° 29.384′ N, 99° 55.138′ - 99° 55.437′ E). After 10:00 hr, nest-holding males tend to leave their bubble nest to find food (M. Jaroensutasinee Pers. Obs.). We divided our study into two parts: plot study and outside plot study. For plot study, we set out 10 plots in rice paddies and 10 plots in small ponds. For outside plot study, we collected additional data from 112 bubble nests found outside plots.

For plot study, ten 4 m2 (2×2 m) sampling plots in rice paddy fields and ten 4 m2 (2×2 m) plots in small ponds were used. We selected rice paddy fields and small ponds where there were bubble nests present. Then at each rice paddy field or small pond, we set one plot per rice paddy field or small pond in order to avoid a pseudo-replication and randomly sampled between these two habitats with respect to days. The water depth of bubble nests was measured to its nearest 1.0 mm. The bubble nests were photographed with a ruler for scale and width (a) and length (b) were measured using a Vernier Calliper. Bubble nest area (A) was estimated using the elliptic equation {$A = \pi(ab/4)$}. If there were other bubble nests nearby within 1 m, the distance from each bubble nest to the nearest neighbouring bubble nest was measured by tape before the male nest builders were captured.

We collected three water samples at three points of the plot along diagonally straight line from corner to corner: two samples from the corners and one from the centre of the plot. Water characteristics measured in each plot included temperature, transparency, pH using a pH metre, dissolved oxygen (DO) using Orion-Model 835, specific conductivity and total dissolved solids (TDS) using Mettler Toledo-Model MX300. The water samples were taken to the laboratory for measurement of biochemical oxygen demand (BOD) by using a standard method for the examination of water and wastewater (APHA, 1998), nitrite, nitrate and phosphate by using ion chromatography based on U.S. EPA method 300 (EPA, 1993). After the water samples were collected, all of the fish in a plot were captured and counted in order to calculate population density and adult sex ratio. The adult sex ratio was calculated as the number of sexually active males divided by the total number of sexually active adults of both sexes in the plot (Jirotkul, 1999).

For outside plot study, 112 nest-holding males were collected outside the sample plots and their bubble nests were measured in order to improve estimates of average male body size, water depth, and bubble nest sizes. We recorded the number of bubble nests with eggs. If there were eggs in bubble nests, all eggs were collected from the nests and counted in the laboratory. Ten randomly selected eggs from each nest were measured for diameter to the nearest 0.01 mm using a Vernier Calliper.

Body Weight and Body Size Measurements

All fish from both groups were taken to the laboratory and their body weight measured to the nearest 0.01 g by using a Mettler digital balance. The following procedure was followed by measuring fish body length and width. First, each fish was placed in an aquarium, measuring $25.0 \times 12.5 \times 16.0$ cm high filled with water to depth of 3.0 cm. Second, a piece of Plexiglas with a ruler was placed in the aquarium to provide scale. Finally, after 1-min acclimatisation period, the fish was photographed with a digital camera (Sony Mavica, model-FD91). The digital pictures were then used to estimate the fish body length from the tip of the upper jaw to the caudal peduncle, and body width. This procedure decreased the probability of killing or damaging fish from using anaesthetic drugs.

Reproductive Biology

The test subjects were wild caught croaking gouramis collected in April and May 2004 from Nakhonsithammarat. The fish were maintained in the laboratory with natural light approximately 12:12 light:dark cycle and fed daily with ant eggs. Twenty males and twenty females were housed in separated 1-L bottles to prevent fighting. A female was placed in her 1-L bottle next to a male in his 1-L bottle until she became gravid. Gravid females had yellowish ovaries apparent through their scales (Liengpornpan Per. Obs.). The male was then placed in a 37-L tank, measuring $50.0 \times 25.0 \times 30.0$ cm high, densely planted with aquatic vegetation. Males built their bubble nests within 24 hr after being placed in the tank. A gravid female was placed in the tank with the nest-holding male in the evening at approximately 15:30 hr of the second day after the nest-holding male had been placed in the tank. We recorded male and female courtship behaviours, the number of eggs released per embracement, the number of embracement per spawning, the total number of fertilised eggs per spawning, spawning duration, and the total time spent from fertilised eggs to fish larvae. Ten randomly selected fish larvae from each nest were measured for total body length to the nearest 0.01 mm using a Vernier Calliper.

Data Analysis

All variables were tested for normality using Lilliefors' test and log transformed when necessary to achieve normality, as in fish body weight and bubble nest area. Independent-sample t-tests were used for comparisons between rice paddy field and small pond habitats. Pearson correlations were used to test the association among body measurements, and body size and bubble nest area. All significant tests were two tailed.

RESULTS

Habitat Characteristics

There were 11 bubble nests found in ten plots within rice paddy fields and 12 bubble nests found in ten plots within small ponds. Croaking gouramis built their bubble nests in clear shallow standing water with dense vegetation, floating plants or other structurally complex features along the edges of their habitats. Habitats used were characterised by low values in pH, DO, transparency, BOD, nitrite, nitrate, and phosphate but high values in specific conductance, TDS and temperature (Table 1), relative to water qualities measured from natural streams and rivers. Differences were found between rice paddy fields and small ponds in pH, specific conductance, DO, TDS, water depth, temperature and population density (Table 1 and Table 2). There were other fish species found in plots within both rice paddy fields and small ponds, including wild Siamese fighting fish, other gourami species, needle fish, serpent-head fish, eel and catfish.

Fish Size and Bubble Nest Size

Males were heavier and bigger than females (Table 3). There were positively correlated between male weight and body length and width (Pearson correlation coefficient, length: $r_{175} = 0.797$, $P < 0.001$; width: $r_{175} = 0.823$, $P < 0.001$), whereas female weight was positively correlated only with its body length (Pearson correlation coefficient, length: $r_{10} = 0.735$, $P < 0.05$; width: $r_{10} = 0.430$, NS).

We collected all males found both in plots and outside plots during field surveys. We found 175 males but only 112 of these males built bubble nests. All 112-bubble nests were located in the shallow water near the edges of rice paddy fields and small ponds. There were only two out of 112 nests that had eggs (Table 4). Most nests were built in newly flooded areas. There was no correlation between the bubble nest area and the male body length and weight (Pearson correlation coefficient, length: $r_{112} = 0.049$, NS; weight: $r_{112} = 0.024$, NS).

Table 1 The Mean ± SD of water characteristics of croaking gourami nest sites in rice paddy fields and small ponds* $P < 0.001$

Characteristics	Mean ± SD		*t*-test
	Rice paddy field	Small pond	
pH	6.83 ± 0.57	5.00 ± 0.36	$t_{18} = 3.89$*
Specific conductance (μS/cm)	35.92 ± 6.38	51.38 ± 12.71	$t_{18} = -3.44$*
Dissolved oxygen (mg/L)	5.44 ± 1.13	1.67 ± 0.70	$t_{18} = 8.95$*
Total Dissolved Solids (mg/L)	18.20 ± 3.17	25.77 ± 6.33	$t_{18} = -3.38$*
Transparency (cm)	12.17 ± 6.07	11.55 ± 4.74	$t_{18} = 0.26$
BOD (mg/L)	2.61 ± 0.83	2.64 ± 0.74	$t_{18} = -0.085$
Nitrite (mg/L)	ND	ND	-
Nitrate (mg/L)	ND	ND	-
Phosphate (mg/L)	ND	ND	-
Water depth (cm)	11.0 ± 3.6	50.4 ± 23.0	$t_{18} = -5.366$*
Temperature (°C)	31.6 ± 1.79	29.8 ± 1.1	$t_{18} = 2.65$*

ND = not detected ($NO_2 < 0.17$ mg/L, $NO_3 < 0.21$ mg/L, $PO_4^{2-} < 0.08$ mg/L)

Table 2 The Mean ± SD of population parameters, body size and bubble nest dimensions of croaking gouramis in rice paddy fields and small ponds *$P < 0.05$

Characteristics	Mean ± SD		*t*-test
	Rice paddy	Small pond	
Population density (individuals/m^2)	2.23 ± 2.21	7.83 ± 7.41	$t_{21} = -2.29$*
Adult sex ratio	0.81 ± 0.29	0.80 ± 0.32	$t_{21} = 0.10$
Bubble nest width (cm)	1.83 ± 0.79	2.45 ± 0.88	$t_{21} = -1.79$
Bubble nest length (cm)	1.97 ± 0.89	2.60 ± 0.96	$t_{21} = -1.63$
Bubble nest area (cm2)	3.09 ± 2.02	5.18 ± 3.38	$t_{21} = -1.78$
Water depth at bubble nests (cm)	10.6 ± 4.8	15.4 ± 16.2	$t_{21} = -0.94$
Male length (cm)	3.58 ± 0.38	3.39 ± 0.17	$t_{21} = 1.60$
Male width (cm)	1.09 ± 0.14	1.03 ± 0.09	$t_{21} = 1.21$
Male weight (g)	0.89 ± 0.15	0.79 ± 0.14	$t_{21} = 1.61$

Table 3 The Mean ± SD of croaking gourami weight, length and width *$P < 0.05$, **$P < 0.001$

Body measurement	Males (N = 175)	Females (N = 10)	*t*-test
Weight (g)	0.86 ± 0.23	0.59 ± 0.10	$t_{183} = 370$**
Length (cm)	3.51 ± 0.35	3.17 ± 0.21	$t_{183} = 3.07$*
Width (cm)	1.03 ± 0.12	0.91 ± 0.04	$t_{183} = 7.30$**

Table 4 The Mean ± SD and range of bubble nest size, water depth, distance to the nearest nest, and egg diameter.

Measurement	Sample size	Mean ± SD	Range
Bubble nest width (cm)	112	1.74 ± 0.79	0.32 - 4.15
Bubble nest length (cm)	112	1.87 ± 0.95	0.40 - 5.57
Bubble nest area (cm2)	112	2.79 ± 2.02	0.10 - 8.55
Water depth (cm)	112	13.80 ± 16.36	3.00 - 98.00
Distance to the nearest nest (cm)	7	60.4 ± 42.6	9.0 - 120.0
Number of bubble nest with fertilised eggs	2	373.5 ± 38.9	346 - 401
Egg diameter (mm)	20	0.78 ± 0.08	0.64 - 0.92

Reproductive Biology

Fifteen out of 20 pairs were spawning. During 16:00-18:00 hr, a nest-holding male enticed a gravid female to visit his bubble nest. The nest-holding male chased this female vigorously until she swam towards him and followed him to his bubble nest. Then he wrapped his body around the female juxtaposing their genital pores. These embraces were accompanied by the release of eggs with the Mean ± SD of 3.74 ± 1.02 eggs per embracement. Following each embrace, the male chased the female from the immediate vicinity of the nest. A male released his sperm to fertilise the eggs, picked the fertilised eggs up in his mouth and spewed them into the bubble nest. A mating sequence consisted of an average of 5-6 pseudo-spawning bouts (i.e. embraces without the release of eggs) and 84 ± 12.97 spawning bouts (i.e. embrace with egg release). The couple repeated this embracement process over the Mean ± SD of spawning duration of 178 ± 51.17 min. This resulted in the Mean ± SD of 314 ± 109.77 eggs released per spawning. When this external fertilisation was over, the nest-building male chased the mated female out of his bubble nest, placed the fine layers of bubble beneath the eggs to ensure that the eggs remained in the bubble nest and solely defended the nest and surrounding territory. The fry hatched within the Mean ± SD of 30.75 ± 0.55 hr and continued developing under the bubble nest. Two days after hatching, fish larvae were free swimming with the Mean ± SD of 3.21 ± 0.29 mm.

DISCUSSION

Habitat Characteristics

Croaking gouramis live in shallow lentic water, which is rather turbid (i.e., low in transparency and high in TDS) from organic substances and clay particles. This murky condition is common to rice paddy fields and small ponds throughout Thailand. Our results showed that population density was higher in small ponds than rice paddies. This may be due to two possible reasons. First, the environment of rice paddies is more extreme than small ponds. During dry season, there will be almost no water available in the rice paddies, but lots of water in small ponds. During the early wet season, rice paddies will be used for rice cultivation, lots of water present in the paddies, less vegetation, lots of animal disturbance (e.g., water buffalos, and ducks), and a high amount of fertilisation and pesticide used from rice cultivation. Second, the higher fish density in small ponds may be related to the highly dense vegetation in small ponds (Liengpornpan Per. Obs.). However, we did not measure vegetation density in our study. For future study, the effect of vegetation complexity on fish density should be examined.

Croaking gouramis may prefer to live in shallow water with dense vegetation due to three possible reasons. First, dense aquatic vegetation in shallow water could minimise predation risks from piscivorous fishes, crabs, prawns and snakes (Kramer et al., 1997) and reduce intraspecific interactions (Corkum and Cronin, 2003). Second, males could use these emergent plants to hold the bubbles together or prevent them from drifting into unsuitable habitat for the fry. Third, males could easily pick up the fertilised eggs and fish larvae, and spew them back to their bubble nests in shallow water. In deeper cloudy water, males might not be able to see fertilised eggs or fish larvae at the bottom very well or males might spend too much energy on picking up eggs and larvae up from the bottom. Many studies have demonstrated that prey organisms select microhabitats with high structural complexity as a way of reducing predation risks (Pamala and Kenneth, 1994; Prenda and Granado-Lorencio, 1996; Elkin and Baker, 2000). Many fishes are most abundant in shallow areas because these habitats enhance growth and survival of young, e.g., pinfish (Jordan et al., 1997), Atlantic salmon (Bremset and Berg, 1999), cod (Linehan et al., 2001), and winter flounder (Manderson et al., 2004).

Rice paddy fields and small ponds differed in water characteristics (i.e., pH, conductivity, DO, TDS, water depth, and temperature). Therefore, croaking gouramis can occupy a wide range of habitat types, especially in poorly oxygenated water (1.67 mg/L), which is lethal to most fishes within 24 hr (i.e., < 3.5 mg/L) (Moore, 1942) and slightly acidic water (pH = 5) which could cause slow growth in fish species (i.e., pH < 6.5) (Boyd, 1996). These limiting factors can influence fish distribution (Matthews, 1998), increase mortality rate and depress hatching success in many fish species (Keckeis et al., 1996; McNatt and Rice, 2004). However, this physical constraint does not apply to croaking gouramis because this species has a labyrinth organ. In addition, this fish builds a bubble nest to hang its eggs and young to expose them to oxygen directly. Therefore, building bubble nests may be an adaptive behaviour, which enables gouramis to live in a hypoxic environment.

Fish Size and Bubble Nest Size

Large body size in males of many species may have evolved because of the advantages of large size in promoting success in male-male competition (Bisazza et al., 1989; Poncin, 1996; Bisazza et al., 2000). Sexual size dimorphism (i.e., males are larger than females) has been shown in many fish species including Pacific salmon (Fleming and Gross, 1994), sockeye salmon (Quinn and Foote, 1994), tilapia (Toguyeni et al., 1997), neotropical armoured catfish (Hostache and Mol, 1998), dark chub (Katano, 1998), rainbow trout (Bonnet et al.,1999), and wild Siamese fighting fish (Jaroensutasinee and Jaroensutasinee, 2001). Our results showed that male croaking gouramis were generally heavier and bigger than females. In croaking gouramis, only males exhibit parental care including building bubble nests, caring for the eggs and fish larvae and defending the territory. Therefore, larger males in this species would have a selective advantage over small males. However, there is no data available on the relationship between male size and reproductive success in croaking gouramis. It has been demonstrated that male body size is positively correlated with reproductive success in some fish species with male parental care (e.g., mottled sculpins (Downhower and Brown, 1980) and lumpfish (Goulet and Green, 1988).

From our field surveys, we found that croaking gourami's bubble nests were difficult to find. Once we found bubble nests, there were only two out of 112 nests that had eggs in them. The low number of bubble nests with eggs found during our field surveys could be because we might have collected our field surveys early in the breeding season. However, this would be very unlikely because the field surveys were conducted covering the two months period in April and May.

This study offered clear evidence that male croaking gouramis built their bubble nests similar in size. This means that larger males did not build larger bubble nests. There could be two possible reasons. First, a small bubble nest would be energetically less costly to build and guard. Therefore, by spending less time and energy building a small bubble nest, a nest-holding male will have more time and energy to spend on searching for mates, courting them and defending his territory from other potential rivals. Second, large gourami males should not spend too much energy in increasing bubble nest size because females have a limited number of eggs per brood. Therefore, males may not increase their reproductive success by building larger bubble nests. Males should spend their energy on other activities that would directly increase their reproductive successes such as enticing females into their nests, defending territory from other males, and egg fanning to increase the amount of oxygen flow to fertilised eggs and fish larvae.

Our results for croaking gourami were not consistent with previous observations of an ecologically similar species, the Siamese fighting fish *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001). Male fighting fish built their bubble nests according to their size (Jaroensutasinee and Jaroensutasinee, 2001). The difference between our study and the fighting fish's study could be due to several reasons. First, fighting fish might be under different selective pressure than gourami. Second, female fighting fish might vary in size and the number of eggs per brood. Therefore, larger fighting males might only choose larger females as mates. Third, there might be less nest-holding fighting fish males in the wild than females. This would cause an operational sex ratio biased towards females. As a result, larger nest-holding males are very rare and should become the choosier sex. Therefore, these larger nest-holding males may prefer larger gravid females that can provide larger broods as mates. In addition, fighting fish females did not show any preference towards males with larger bubble nest sizes (Jaroensutasinee and Jaroensutasinee, 2003). This implies that females in this species are not the choosier sex.

Reproductive Biology

Many studies have been published on belontiid mating behaviours and emphasised on the behaviour of single pairs with the confines of small aquaria (e.g., Miller and Robinson, 1974; Pollak et al., 1981). Our results confirmed previous findings about gourami reproductive behaviours. Males establish territories that they defend against conspecifics and in which they construct bubble nests at the water's surface. Our results showed that croaking gourami tended to spawn in the evening. Similar finding has been reported in wild Siamese fighting fish *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001).

Our results confirm that wild-caught croaking gourami can breed in the laboratory condition successfully as shown in many fish species including catfish *Heteropneustes fossilis* (Bloch) (Thankur and Nasar, 1977), blue gourami *Trichogaster trichopterus* (Pollak et al., 1981), mollies *Poecilia* spp. (Woodhead and Armstrong, 1985), common goby *Pomatoschistus microps* (Svensson and Forsgren, 2003). However, our experimental design was set up as a single pair within the confine of small aquarium. The observations were terminated after a single spawning episode in order to save the female from serious injury or death (Miller, 1964). Therefore, we cannot conclude whether the reproductive biology of croaking gourami is a single mating. Previous study on blue gourami *Trichogaster trichopterus* has demonstrated that the reproductive biology of this fish is a multiple mating (i.e., the mating occurrence of two or more days with a single partner or with two or more partners on one or more days) (Pollak et al., 1981).

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CHAPTER 3
MALE BODY SIZE, FEMALE PREFERENCE AND MALE-MALE COMPETITION IN CROAKING GOURAMIS *Trichopsis vittata*

PUBLICATION

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ABSTRACT

This study examined the rôle of the male body size on the female sexual preference and on the male-male competition among croaking gouramis (*Trichopsis vittata* Cuvier, 1831). Two treatments were set up for the study of the female sexual preference and the male-male competition. In one treatment, males of the same size were matched, while in the other males of different sizes were placed together. The results showed that the male body size did not affect the sexual preference among female croaking gouramis. Regarding the male-male competition study, large males were found to win more fights than small ones. The fighting duration under the treatment of the size matched fish were longer than that in the treatment of the size mismatched fish. A comparison of different agonistic behaviours of the fish under both treatments showed that sound producing, biting and total agonistic behaviours were found more frequently among the size matched fish than among the size mismatched group. A further observation of the large and small fish in the treatment of the size mismatched group also revealed that large males performed more chasing and less escaping than small ones. The results indicate that the sexual size dimorphism in croaking gouramis, in which species the male is larger than the female, may be associated with the male-male competition, not with the female preference.

Keywords: Male body size, Female preference, Male-male competition, Croaking gourami, *Trichopsis vittata*

INTRODUCTION

The evolution of sexually selected traits is driven by the outcomes of two main mechanisms of sexual selection: mate preference and mate competition. They are traditionally considered to act in concert (Jennions and Petrie, 1997; Qvarnstrom and Forsgren, 1998). In most animals, females choose males as their mates because females are generally more limited in their reproductive possibilities than males (Dawkin, 1995; Maier, 1998), and males generally compete for mating opportunities (e.g. Forsgren, 1997; Jaroensutasinee and Jaroensutasinee, 2001a; Reichard et al., 2005). However, despite females being choosy, they have limited control over their reproductive success and/or paternity for their offspring (e.g. Mays and Hill, 2004; Reichard et al., 2005). Male-male competitive interactions can both have beneficial or detrimental effects on the female preference process (reviewed in Wong and Candolin, 2005). For example, dominant males are often able to exclude inferior subordinates so that females end up mating with high quality males, clearly beneficial (Berglund et al., 1996). On the other hand, dominant males may increase their opportunities by excluding subordinate males, thereby eliminating female choice and even reducing female fitness (e.g. Holland and Rice, 1999; Moore and Moore, 1999; Byrne and Roberts, 2000; Moore et al., 2001; Sih et al., 2002; reviewed in Wong and Candolin, 2005).

In sexual size dimorphic fish species, which males defend their territories, males are often larger than females. Such dimorphism has been found, for example, in tilapia, *Oreochromis niloticus* (Toguyeni et al., 1997), armoured catfish, *Hoplosternum littorale* (Hostache and Mol, 1998), dark chub *Zacco temmincki* (Katano, 1998), rainbow trout, *Oncorhynchus mykiss* (Bonnet et al., 1999), and Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001b). Male body size can act as a reliable indicator of health and vigour (Nicoletto, 1993) and may have significant father-son heritability (Reynolds and Gross, 1992). Therefore, male body size may be an important trait for female preference and/or male-male competition (Reynolds and Gross, 1992; Reichard et al., 2005). Moreover, the degree of size difference between a pair of males may strongly affect female preference (Basolo, 2004). Variation in male body sizes has also been positively correlated with reproductive fitness and brooding success (Hsu and Wolf, 1999; Basolo, 2004; Fujii et al., 2005).

Some female fish can discriminate between large and small males and often prefer larger ones e.g., Atlantic molly, *Poecilia mexicana* (Plath et al., 2004) and sailfin molly, *Poecilia latipinna* (MacLaren et al., 2004). These large males typically possess characters that can provide many benefits to increase their reproductive successes (Aspbury and Basolo, 2002; Fujii et al., 2005). Many studies reveal that there are positive correlations between male body size, age and their benefits (Andersson and Iwasa, 1996) i.e. increasing fecundity with larger testes (Quinn and Foote, 1994; Katano and Maekawa, 1997), maturing at a larger size, producing bigger clutches (Paine, 1990), building larger and more complex nests (Bisazza et al., 1989; Sato, 1994), courting more vigorously (Reichard et al., 2005), increasing sperm competition (Parker, 1992), giving better paternal care (Beacham and Murray, 1988; Bisazza et al., 1989; Reynolds and Gross, 1992; Östlund and Ahnesjö, 1998; Kolm, 2002), defending larger territories (Candolin and Voigt, 2001), having greater success in territorial defence against potential egg predators (Wiegmann and Baylis, 1995; Poncin, 1996; Kitevski and Pyro, 2003), having a longer reproductive and life span (Paine, 1990), and developing more aggressive behaviours to win contests (Bisazza et al., 1989; Poncin, 1996; Bisazza et al., 2000; Candolin and Voigt, 2001).

The most obvious form of male-male competition is the obvious physical fighting. However, male contests often do not escalate into fighting. Although in many fish species, for example, sand goby, *Pomatoschistus minutus* (Forsgren, 1997), Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001a), and European bitterling, *Rhodeus sericeus* (Reichard et al., 2005), large males have high fighting ability and may win fights. In other species, male body size is not a good predictor of success in male competition as in guppies, *Poecilia reticulata* (Kodric-Brown, 1993). Large males may ultimately gain preferential access to resources such as food, territories and females. They tend to feed better, grow faster, and become more aggressive, while smaller ones may grow at a slower rate and be less aggressive (Godin, 1997; Quinn, 1999; Rincón and Grossman, 2001). Male-male competition may take place before mating and the winner is indeed involved in more successful mating by the losing rival (Andersson et al., 2002; Dickerson et al., 2004). In particular female eavesdroppers spend significantly more time with the winners of the aggressive interactions than with the losers (Doutrelant and McGregor, 2000).

This study is the first to investigate the role of male body size on sexual selection among croaking gouramis (*Trichopsis vittata* Cuvier, 1831), among which the male is larger than the female. We experimentally separated female preference from male-male competition. Since males solely provide parental care for eggs and fry, females are under pressure to select their mates carefully. We examined how sexual size dimorphism in this species is maintained through both female preference and male-male competition. We predict that: (1) if sexual size dimorphism functions through female preference, then females should prefer large males and spend more time with large males than small ones; (2) if sexual size dimorphism functions through male-male competition, then large males should win more fights than small ones and (3) if sexual size dimorphism functions in both female preference and male-male competition, then both of these should occur in this species.

MATERIALS AND METHODS

Fish Biology

The croaking gourami (*Trichopsis vittata* Cuvier, 1831) is an anabantid fish that is sexually size dimorphic, and is widely distributed from eastern India through Malaysia and Thailand (Sandford, 2000). This species is a small (i.e. 3.0-4.0 cm) brown-bodied fish with three horizontal stripes. Males are more reddish and have elongated anal and dorsal fins. Females are smaller and duller than males. During the breeding season, gravid females have yellowish ovaries, which can be seen by holding them up against a light (Liengpornpan Pers. Obs.). Male builds a bubble nest on the water surface and tries to entice female to visit the nest and mate. After completing external fertilisation, the nest-holding male picks up the fertilised eggs, puts them into his bubble nest, and spends time guarding the nest and surrounding its territory until the fish larvae can swim. Therefore, a territorial male should be attractive to females and be strong enough to win contests with rivals for offspring survivorship.

Measuring Fish Body Length

All croaking gouramis used in this study were field caught using a one-person seine net during June-September 2005 from Nakhonsithammarat, Thailand (08° 29.381′ - 08° 29.384′ N, 99° 55.138′ - 99° 55.437′ E). Croaking gouramis are very abundant here and the number removed and transferred to the laboratory represents only a small proportion of the local population. Therefore, the number of the fish removed for this study would be expected to have little impact on the genetic variation of the croaking gourami population. Body size has been measured for all fish before the experiments were run. The following procedure was followed in measuring the fish body length. First, each fish was placed in an aquarium, measuring 25.0 x 12.5 x 16.0 cm high and filled with water to a depth of 3.0 cm. Second, a piece of Plexiglas with a ruler was placed in the aquarium as a measuring scale. Finally, after a 1-min acclimatisation period, the fish was photographed with a digital camera (Sony

Mavica, model-FD91). The digital pictures were then used to estimate the fish body length from the anterior-most point of the upper jaw to the end of the caudal peduncle. This procedure decreased the probability of killing or damaging the fish by using anesthetic drugs.

Female Preference Test

The Mean ± SD of croaking gourami male and female body length was 3.51 ± 0.35 cm (N = 175) and 3.34 ± 0.20 cm (N = 175), respectively. Males were larger than females (Independent sample t-test: $t_{348} = 5.672$, $P < 0.05$). The study was conducted through two treatments in which males of a body length difference of ± 0 SD (i.e. size matched treatment with 0.00 cm difference between males) and ± 1 SD (i.e. size mismatched treatment with 0.35 cm difference between males) were used. Fifty-eight trials were conducted in both treatments. In the size matched treatment, there were three fish: one gravid female and two males of the same body length. In the size mismatched treatment, there were also three fish: one gravid female and two males of the different body sizes.

The female preference experiments were carried out in an aquarium (37-L measuring 50.0 x 25.0 x 30.0 cm high) divided into three compartments. The two equally small compartments were separated by a removable opaque Plexiglas partition and each compartment housed a male. The large compartment comprised 67% of the total tested arena and contained a gravid female. The removable clear Plexiglas divider between the large and two small compartments allowed the tested female to see both males but did not allow the two males to see each other. The preference zone was demarcated in the female compartment by drawing a line 7.0 cm away from the clear Plexiglas partition on the outside of the aquarium. The region next to the partition of each male was the preference zone for that male and the rest of the female compartment was a no preference zone. Trials were conducted from 07:00 to 18:00 hr and all tested fish were fed before testing.

Each trial was comprised of two 10-min observation sessions with a 5-min interval. At the beginning of each trial, a gravid female was randomly selected, introduced into the centre of the no preference zone and given 5-min to acclimatise to the experimental situation. The first observation period was then initiated. During this period, the female could choose which of the males she preferred; her preference was indicated by the side of the partition where she spent her time. The behaviours of the fish trio were noted and the number of seconds the females spent in each position of the three compartments was recorded on a computer. At the completion of this first observation period, the following activities were included: switching the location of the two males to control for any female side bias, carrying out the second 10-min observation period for mate preference trials with the same female for a 5-min acclimation period, and adding the total times females spent near the two males for statistical analyses. To ensure that both males were viewed by the female and all of the three participants were actively involved in the target courtship, the tested female had to be seen, for each individual trial, approaching each male at least once. We excluded trials where females spent more time outside the preference zone than inside the preference zone. Each trial was conducted with new females and new pairs of males. For the size mismatched treatment, we defined the female preference for a male as the difference between the total time spent near the large male and the total time spent near the small male divided by the total time spent near both males. For the size matched treatment, we defined the female preference for a male as the difference between the total time spent near a male on the right hand side of the aquarium and the total time spent near a male on the left hand side of the aquarium divided by the total time spent near both males.

Male-Male Competition Test

For the male-male competition test, the same pairs of tested males from female preference tests were placed in a 1.5-L square tank with dimensions 10.0 x 10.0 x 15.0 cm high. These same pairs of tested males had never seen each other prior to the male-male competition test. Croaking gouramis displayed substantial differences in agonistic behaviours which could be classified into the following eleven gestures: approaching, lateral displaying, sound producing, biting, mouth opening, going round in a circle, adhering to each other, surfacing, mouth defying, chasing and escaping. These agonistic behaviours displayed by both males were being observed and recorded until one fish retreated. Approaching (A) was defined as one fish moving rapidly towards another by movements of the caudal peduncle and all fins closed. Lateral displaying (D) was recorded when both fish oriented in a head-to-tail position and spread the median fins in a lateral position. Sound producing (P) was defined as the males shaking their bodies and beating pectoral fins rapidly for sound emission. Biting (B) was recorded when males used their mouthparts to bite or tear one another. Mouth opening (O) was observed when both fish moved rapidly toward the opponent and apparently opened their mouths at the same time. Going round in a circle (G) was recorded when both males followed in a circle and beat their pectoral fins. Adhering to each other (Ad) was recorded when both males locked jaws and nipped each other. Surfacing (S) was recorded when the males float to the water surface to breathe. Mouth defying (De) was recorded when both males rested and only one male opened and pointed his mouth at his opponent. Chasing (C) was observed when one individual swam rapidly to displace another. Escaping (E) was defined as one fish moving rapidly away from another. At the beginning of the male-male competition tests, both fish displayed high visual stereotypic movements, composed of going round in a circle, lateral displaying, mouth opening and biting. Four types of agonistic behaviour, which both males in all pairs showed simultaneously, were going round in a circle, lateral displaying, mouth opening, and adhering to each other. The records of the male-male competition cover the period from the observation of the first agonistic behaviour until one party's retreat.

Data Analysis

Parametric statistics were used when underlying assumptions were met; otherwise, non-parametric tests were used. Chi-square tests were used to test the number of competitions won by the large males. Independent sample t-tests were used to test the fighting duration and the female preference observed during both treatments. Paired t-tests were used to test the time females spent between large and small males and the level of agonistic behaviour differences between large and small males. Two-way ANOVA tests were used to test the level of agonistic behaviour differences between the two treatments, the winning status as well as the interaction between the two treatments and the winning status.

Ethical Note

All croaking gouramis were housed at the freshwater laboratory, Walailak University, Thailand. Extreme care was taken during the animal handling to minimise stress to the fish used in this study. The male-male competition took the form of ritualised agonistic displays, chases and escapes. The fish were monitored closely at all times to ensure that no physical injuries were being inflicted. Since competitive interactions may be stressful for the test subjects, the length of time when males were placed in a competitive environment was kept to a minimum and the fish were separated immediately after the completion of the study, at which point the fish were then retained in the laboratory as breeding stock. Other fish captured but not used in this study were also retained for use in future studies in order to minimise the number of collection trips subsequently required. No licences were required for the study.

RESULTS

Female Preference Test

Female preference was not found to be influenced by the greater body length, {(Mean ± SE) the size matched treatment: 0.039 ± 0.034, N = 58; the size mismatched treatment: 0.037 ± 0.029, N = 58; Independent sample t-test, t_{114} = 0.039, NS}. Females did not spend time differently either with large or with small males (Paired t-tests: t_{57} = 1.368, NS).

Male-Male Competition Test

Of all the 58 pairs in both treatments, 40 pairs of males fought and displayed the other nine types of agonistic behaviour except chasing and escaping, but the other 18 pairs of males displayed only the chasing and escaping behaviours. The fighting duration in the size matched treatment was longer than that in the size mismatched treatment {(mean ± SE) the size matched treatment: 460.88 ± 69.17 s, N = 40; the size mismatched treatment: 275.63 ± 47.52 s, N = 40; Independent sample *t*-test: t_{69} = -2.208, $P < 0.05$}. Large males won more fights in the size mismatched treatment (44 out of 58 fighting pairs, Chi-square test: χ^2_1 = 15.52, $P < 0.05$).

Behavioural Differences between the Size Matched and the Size Mismatched Treatment

Males from the size matched treatment were found to perform a higher number of sound producing, biting, and total agonistic behaviours than those in the size mismatched treatment (Two-way ANOVA tests: sound production: $F_{1,156}$ = 5.833, $P < 0.05$; biting: $F_{1,156}$ = 5.345, $P < 0.05$; total agonistic behaviours: $F_{1,156}$ = 8.765, $P < 0.05$, Table 1).

Behavioural Differences between Winners and Losers

The study showed that there were no differences in the following six agonistic behaviours: approaching, sound producing, biting, surfacing, mouth defying, and total agonistic behaviours (Two-way ANOVA tests: approaching: $F_{1,156}$ = 3.291, NS; sound producing: $F_{1,156}$ = 0.305, NS; biting: $F_{1,156}$ = 0.033, NS; surfacing: $F_{1,156}$ = 0.054, NS; mouth defying: $F_{1,156}$ = 3.356, NS; total agonistic behaviours: $F_{1,156}$ = 0.134, NS, Table 1).

Behavioural Differences between Large and Small Males

Large males performed more chasing than small ones (Paired *t*-tests for chasing: t_{39} = 3.147, $P < 0.05$, Figure 1). Small males displayed more escaping than large males (Paired t-tests for escape: t_{39} =-3.147, $P < 0.05$, Figure 1). Large and small males displayed the same amount of total agonistic behaviours {(Mean ± SE): large males: 38.95 ± 7.07 times; small males: 38.15 ± 6.94 times, Paired *t*-tests: t_{39} = 0.672, NS}.

Table 5 The Mean ± SE of the amount of agonistic behaviours between the winners and the losers in the size matched and the size mismatched treatment. N = 40

Agonistic behaviour	Amount of agonistic behaviour (Mean ± SE)			
	Size matched treatment		Size mismatched treatment	
	Winner	Loser	Winner	Loser
Approaching	1.35 ± 0.80	0.43 ± 0.22	0.88 ± 0.18	0.25 ± 0.12
Sound production	7.28 ± 1.50	6.60 ± 1.27	4.45 ± 0.91	3.85 ± 0.78
Biting	12.65 ± 2.19	12.48 ± 2.05	8.05 ± 2.03	7.48 ± 2.03
Surfacing	5.45 ± 1.17	5.35 ± 1.55	3.50 ± 0.95	4.18 ± 1.23
Mouth defying	2.65 ± 1.54	0.75 ± 0.50	1.38 ± 0.32	0.25 ± 0.12
Chasing	1	0	1	0
Escaping	0	1	0	1
Total behaviours	65.13 ± 9.85	61.33 ± 9.03	39.68 ± 6.95	37.43 ± 7.05

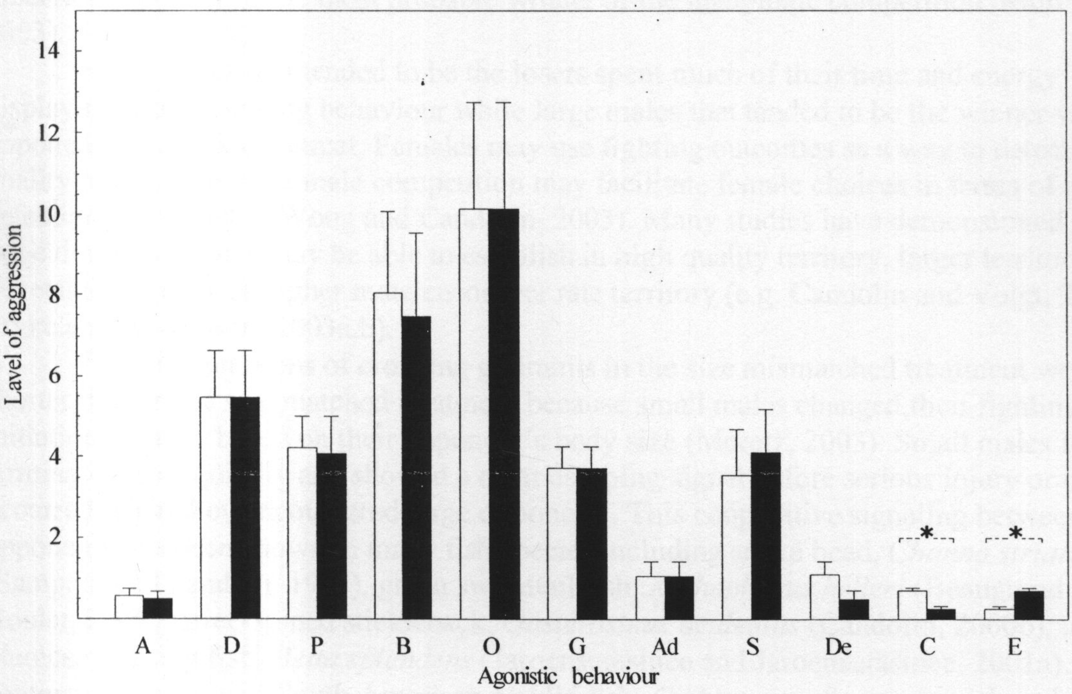

Figure 1 The Mean ± SE of agonistic behaviours between large (□), and small (■) males in the size mismatched treatment, N = 40 fighting pairs. Male agonistic behaviours include approaching (A), lateral displaying (D), sound producing (P), biting (B), mouth opening (O), going round in a circle (G), adhering to each other (Ad), surfacing (S), mouth defying (De), chasing (C), and escaping (E).* $P < 0.05$

DISCUSSION

The study showed that sexual size dimorphism among croaking gouramis was not associated with the female preference because females did not choose their mates based on the male body size. In some fish species, females use other male traits as a cue for choosing their mates, such as coloration in three-spined sticklebacks, *Gasterosteus aculeatus* (Candolin, 2000a), courtship behaviour in fifteen-spined sticklebacks, *Spinachia spinachia* (Östlund and Ahnesjö, 1998), and nest characters in blenny, *Istiblennius enosimae* (Sunobe et al., 1995), common goby, *Pomatoschistus microps* (Jones and Reynolds, 1999) and three-spined sticklebacks, *Gasterosteus aculeatus*, (Barber et al., 2001). Additionally, among the fighting fish, *Betta splendens*, females choose the winner males from male-male contests as their mates (Doutrelant and McGregor, 2000). However, our experiments did not test this possibility because we did not allow females to choose mates after male-male competition tests.

This study confirmed that there was an advantage of the large size fish in winning fights as seen in several fish species, including European grayling, *Thymallus thymallus* (Poncin, 1996), one-sided livebearer, *Jenynsia multidentata* (Bisazza et al., 2000) and wild Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001a). Fighting ability or aggressiveness has been found, in several species of fish, to correlate positively with standard metabolic rate (Metcalfe et al., 1995), growth rate (Jönsson et al., 1998; Lahti et al., 2001) and cortisol level (Sakakura et al., 1998). Standard length proved to be a moderate predictor of the most probable winner of the male-male competition (Moretz, 2003).

Small males that tended to be the losers spent much of their time and energy displaying more escaping behaviour while large males that tended to be the winner waited for opponents to attack or retreat. Females may use fighting outcomes as a way to detect the best quality mates. The male-male competition may facilitate female choices in terms of mate detection (reviewed in Wong and Candolin, 2005). Many studies have demonstrated that large dominant males may be able to establish in high quality territory, larger territory, more vegetated territory or higher mate encounter rate territory (e.g. Candolin and Voigt, 2001; Pryke and Andersson, 2003a,b).

Fighting durations of croaking gouramis in the size mismatched treatment were shorter than in the size matched treatment because small males changed their fighting initiation strategy based on their opponent's body size (Moretz, 2003). Small males tended to terminate fights quickly and showed a clear escaping signal before serious injury or death occurred when they encountered large opponents. This cooperative signaling between opponents has been shown in many fish species including snake head, *Channa striatus* (Sampath and Pandian, 1985), green swordtail fish, *Xiphophorus helleri* (Beaugrand and Goulet, 2000), three-spined stickleback, *Gasterosteus aculeatus* (Candolin, 2000b), and Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001a). In contrast, opponents of South American cichlid fish, *Cichlasoma dimerus*, continued their fighting with highly damaging escalation even when they seemed to know which individual will be the winner (Maan et al., 2001).

The amount of sound producing, biting, and total agonistic behaviours in the size matched treatment was higher than that observed in the size mismatched treatment. Similar findings have been reported for other fish species including Arctic charr, *Sahelinus alpinus* (Brännäs et al., 2002), zebrafish, *Danio rerio* (Kitevski and Pyron, 2003), and Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001a). Croaking gouramis may use both morphological and sound characteristics to assess their opponents' fighting ability. This is because croaking gouramis may change from using visual signals to assess their rivals to acoustic signal in highly dense freshwater vegetation (Ladich, 1997; 1998).

In conclusion, this study suggests that the sexual size dimorphism in croaking gourami (*Trichopsis vittata* Cuvier, 1831) functions through the male-male competition, not through the female preference. Since males solely provide parental care for eggs and fry, males in this species may be under a selective pressure such that larger males are better able to defend territories against potential egg predators (Wiegmann and Baylis, 1995; Poncin, 1996; Kitevski and Pyron, 2003).

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CHAPTER 4
BUBBLE NEST SIZE, FEMALE PREFERENCE AND MALE-MALE COMPETITION IN CROAKING GOURAMIS *Trichopsis vittata*

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ABSTRACT

This study examined the role of the bubble nest size on the female sexual preference and on the male-male competition among croaking gouramis (*Trichopsis vittata* Cuvier, 1831). Three treatments were set up for the study of the female sexual preference and the male-male competition: (1) two-bubble-nest treatment, (2) one-bubble-nest treatment and (3) no-bubble-nest treatment. The results showed that females preferred small bubble nest males over large ones. There are four possible reasons: small bubble nests are available, will be enlarged during mating, contain more oxygen for the eggs and hatching young, and more camouflaged than large bubble nests. Surprisingly, females did not prefer males with bubble nest over males without bubble nests. In addition, when we compared female preference among three treatments, females spent more time with males in no-bubble-nest treatment than in two-bubble-nest treatment. The results indicate that females may perceive bubble nest presence and large bubble nest size as a negative clue of male quality because this may suggest that these males already spend their energy reserve in nest building and territorial defence. In the male-male competition, large bubble nest males did not win more fights. There were no differences in the numbers of agonistic behaviour, the total agonistic behaviour and the winning status between males who built bubble nests and males who did not build bubble nest. There was no significant difference in fighting duration between the three treatments. This study suggests that males with large bubble nests or males with bubble nest might not always reflect the male's contribution to paternal care and the outcome of contests because they do not win in most fights. Therefore, both natural selection and sexual selection may act on male nest-building in croaking gouramis.

Keywords: Bubble nest size, Female preference, Male-male competition, Croaking gouramis, *Trichopsis vittata*

INTRODUCTION

Nest of fishes should provide females with important information on builder health status and other valuable qualities such as potential parental care that can have strong effects on reproductive success (Sunobe et al., 1995; Barber et al., 2001). Peacock wrasse, Symphodus tinca, females prefer males with nests early in the nesting cycle, and they spawn less with non-nesting males in late-cycle nests (Warner et al., 1995). In some fish species, therefore, females prefer to mate with large nest males including sand goby, *Pomatoschistus minutes* (Lindström, 1992), cichlid, *Lamprologus callipterus* (Schütz and Taborsky, 2000), and large spawning pit males including African cichlid, *Oreochromis mossambicus* (Nelson, 1995). Competitive interactions between rival males could affect female detection of mates by influencing the size of the territories (Wong and Candolin, 2005).

Large nest males or the territory owners may increase female reproductive success by being stronger (Bisazza et al., 1989; Magnhagen and Kvarnemo, 1989; Takahashi et al., 2001), performing a high amount of courting (Pampoulie et al., 2004), providing better care of both the eggs and young (Östlund and Ahnesjö, 1998; Kolm 2002), and winning more fights (Johnsson et al., 1999). In common goby, *Pomatoschistus microps*, females base their mate choice on nest appearance (Jones and Reynolds, 1999). However, in a few fish species apparent male quality does not appear to directly affect female nest-choice behaviour, for example three-spine stickleback, *Gasterosteus aculeatus* (Sargent, 1982) and Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2003).

A few fish species construct floating nests by using mucous-covered bubbles such as armoured catfish, *Liposarcus pardalis* (Andrade and Abe, 1997), neotropical armoured catfish, *Hoplosternum littorale* (Hostache and Mol, 1998), Siamese fighting fish, *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001) and croaking gourami, *Trichopsis vittata* (Liengpornpan et al., 2006). We used the croaking gourami as a suited model for investigating the effect of nest size on female preference and male-male competition for several reasons. First, males build bubble nests on the water surface that are easy to reduce in size. Second, croaking gouramis are very abundant and easily captured in natural habitats in Thailand (Smith, 1945). Third, this fish can be collected and maintained in a large number because of their small body size (approximately 3.0-4.0 cm, Henglmüller and Ladich, 1999). Finally, croaking gouramis adapt well to captive conditions and seem to perform fundamentally identical courtship behaviour in the laboratory and in the field (Liengpornpan Per. Obs.).

Several experimental studies have shown that females seem to base their mate preference mainly on the quality of territory or nest site rather than on male traits (Jones, 1981; Warner, 1987). This study aimed to investigate the effect of bubble nest presence/absence and bubble nest size on female preference and male-male competition in croaking gouramis. Four hypotheses were tested: (1) if there is a female preference for a large bubble nest, then females should spend more time with large bubble nest males than small ones while controlling for male body size; (2) if there is a female preference for males with bubble nests, then females should spend more time with bubble nest males than no bubble nest males while controlling for male body size; (3) if bubble nest size is a good indicator of male quality, then (3a) large bubble nest males should display more agonistic behaviours and win more fights than small bubble nest males while controlling for male body size, and (3b) fighting duration between large and small bubble nest males should decrease as the size difference of bubble nest areas between a fighting pair increases; and (4) if holding a bubble nest is a good indicator of male quality, then bubble nest males should win more fights and behave more aggressively than non-bubble-nest males.

MATERIALS AND METHODS

Fish Biology

The Croaking gourami (*Trichopsis vittata* Cuvier, 1831) is an anabantid fish that is sexually size dimorphic, and is widely distributed from eastern India through Malaysia and Thailand (Sandford, 2000). This species is a small brown-bodied fish with three horizontal stripes. Males are more reddish and have elongated anal and dorsal fins. Females are duller and smaller than males (the Mean ± SD of female and male body sizes are 3.16 ± 0.21 cm and 3.51 ± 0.35 cm, respectively (Liengpornpan et al., 2006). During breeding seasons (i.e. March-May), pheromones released by fish males induce female ovarian maturation (Degani and Schreilbman, 1993). Gravid female croaking gouramis have yellowish ovaries, which can be seen by holding them up againt light (Liengpornpan Pers. Obs.). The defining characteristics of the croaking gourami mating system are that male builds a bubble nest on the water surface and tries to entice female to visit the nest and mate. After completing external fertilisation, the nest-holding male picks up the fertilised eggs, puts them into his bubble nest, and spends time guarding the nest and surrounding its territory until the fish larvae can swim.

Measuring Fish Body Length and their Bubble Nest Size

All croaking gouramis used in this study were field caught using a one-person seine net during April - June 2005 from Nakhonsithammarat, Thailand (08° 29.381′ -08° 29.384′ N, 99° 55.138′ - 99° 55.437′ E). Body size (SL) has been measured for all fish before the experiments were run. The following procedure was followed in measuring the fish body length. First, each fish was placed in an aquarium, measuring 25.0 x 12.5 x 16.0 cm high and filled with water to a depth of 3.0 cm. Second, a piece of Plexiglas with a ruler was placed in the aquarium as a measuring scale. Finally, after a 1-min acclimatisation period, the fish was photographed with a digital camera (Sony Mavica, model-FD91). The digital pictures were then used to estimate the fish body length from the anterior-most point of the upper jaw to the end of the caudal peduncle. This procedure decreased the probability of killing or damaging fish from using anaesthetic drugs. The bubble nests were photographed with a ruler for scale and width (a) and length (b) were measured using a Vernier Calliper. Bubble nest area (A) was estimated using the elliptic equation {$A = \pi (ab/4)$, where a and b were the semimajor and semiminor axes}.

Female Preference Test

To investigate the role of the bubble nest size on the female sexual preference, three treatments were set up: (1) two-bubble-nest treatment, (2) one-bubble-nest treatment and (3) no-bubble-nest treatment. Each trial of these three treatments composed of three fish: one gravid female and two males with the same standard body length. Female preference experiments were carried out in a 37-L aquarium (measuring 50.0 x 25.0 x 30.0 cm high) divided into three compartments. The two equally small compartments were separated by a removable opaque Plexiglas partition and each compartment housed a male. The large compartment comprised 67% of the total tested arena and contained a gravid female. The removable clear Plexiglas divider between the large and two small compartments allowed the tested female to see both males but did not allow the two males to see each other. Focal females in each trial could not directly interact with both males because this study examined only visual conditions for bubble nest size. The preference zone was demarcated in the female compartment by drawing a line 7.0 cm away from the clear Plexiglas partition on the outside of the aquarium. The region next to the partition of each male was the preference zone for that male and the rest of the female compartment was a no preference zone. Trials were conducted from 07:00 to 18:00 hr and all tested fish were fed before testing.

(1) Two-Bubble-Nest Treatment

Thirty-two trials were conducted. Each trial consisted of two 5-min acclimation periods and two 10-min observation periods. At the beginning of each trial, a pair of matched males was placed in two similar adjacent compartments. Both males were allowed to build their bubble nests in each equal compartment. After that, a focal gravid female was placed in the centre of the no preference zone. Each successful trial was that in which all three subjects actively courted and the focal female approached each male at least once in the first 5-min acclimation period. The first observation period was initiated after a 5-min acclimation period. During the observation period, the number of seconds which the female spent in each of the three zones was recorded on a computer. At the completion of the first observation period, another opaque Plexiglas was placed into the aquarium, barring female sight and the large bubble nest was cut approximately in half. This opaque Plexiglas was taken back so the female could see both males again for a second 5-min acclimation period. This was followed by a second observation period.

(2) One-Bubble-Nest Treatment

Thirty-six trials were conducted. Each trial consisted of a 5-min acclimation period and followed by a 10-min observation period. At the beginning of each trial, a pair of matched males was placed in two similar adjacent compartments. Only one male was allowed to build his bubble nest in one equal compartment. After that a focal gravid female was placed in the centre of the no preference zone. Each successful trial was that in which all three subjects actively courted and the focal female approached each male at least once in the 5-min acclimation period. The observation period was initiated after a 5-min acclimation period. During the observation period, the number of seconds which the female spent in each of three zones was recorded on a computer.

(3) No-Bubble-Nest Treatment

Fifty-eight trials were conducted. Each trial consisted of a 5-min acclimation period and followed by a 10-min observation period. At the beginning of each trial, a focal gravid female and a pair of matched males were placed in the centre of the no preference zone and in two similar adjacent compartments respectively. Both matched males were not allowed to build bubble nests. Each successful trial was that in which all three subjects actively courted and the focal female approached each male at least once in 5-min acclimation period. The observation period was initiated after a 5-min acclimation period. During the observation period, the number of seconds which the female spent in each of three zones was recorded on a computer.

Male-Male Competition Test

For the male-male competition test, the same pairs of tested males from female preference tests were placed in a 1.5-L square tank with dimensions 10.0 x 10.0 x 15.0 cm high. These same pairs of tested males had never seen each other prior to the male-male competition test. Croaking gouramis displayed substantial differences in agonistic behaviours which could be classified into the following 11 gestures: approaching, lateral displaying, sound producing, biting, mouth opening, going round in a circle, adhering to each other, surfacing, mouth defying, chasing and escaping. These agonistic behaviours displayed by both males were being observed and recorded until one fish retreated. Approaching (A) was defined as one fish moving rapidly towards another by movements of the caudal peduncle and all fins closed. Lateral displaying (D) was recorded when both fish oriented in a head-to-tail position and spread the median fins in a lateral position. Sound producing (P) was defined as the males shaking their bodies and beating pectoral fins rapidly for sound emission. Biting

(B) was recorded when males used their mouthparts to bite or tear one another. Mouth opening (O) was observed when both fish moved rapidly toward the opponent and apparently opened their mouths at the same time. Going round in a circle (G) was recorded when both males followed in a circle and beat their pectoral fins. Adhering to each other (Ad) was recorded when both males locked jaws and nipped each other. Surfacing (S) was recorded when the males float to the water surface to breathe. Mouth defying (De) was recorded when both males rested and only one male opened and pointed his mouth at his opponent. Chasing (C) was observed when one individual swam rapidly to displace another. Escaping (E) was defined as one fish moving rapidly away from another. At the beginning of the male-male competition tests, both fish displayed high visual stereotypic movements, composed of going round in a circle, lateral displaying, mouth opening and biting. Four types of agonistic behaviour, which both males in all pairs showed simultaneously, were going round in a circle, lateral displaying, mouth opening, and adhering to each other. The records of the male-male competition cover the period from the observation of the first agonistic behaviour until one party's retreat.

Data Analysis

Parametric statistics tests were used when underlying assumptions were met; otherwise, non-parametric tests were used. Linear regressions were used to test (1) female preference for large bubble nest males, (2) the constancy of female preference for the bubble nest size, and (3) the relationship between the fighting duration and the bubble nest area difference. Paired t-tests were used to test (1) the time females spent between nest-holding males and non nest-holding males and, (2) the number of total agonistic behaviour between large and small bubble nest males, and (3) the level of agonistic behavioural differences between males in each treatment. One-way ANOVA tests were used to test for the total time which females of each treatment spent with their males and the fighting duration among the three treatments. Two-way ANOVA tests were used to test the level of agonistic behaviour differences between three treatments, winning status as well as the interaction between treatments and winning status. Pearson correlations were used to test the association between male body size and bubble nest area, and between male body size and bubble nest length. Chi-square tests were used to test the number of fights won by large bubble nest males in the two-bubble-nest treatment and by males with bubble nests in the one-bubble-nest treatment.

Ethical Note

All croaking gouramis were housed at the freshwater laboratory, Walailak University, Thailand. Extreme care was taken during the animal handling to minimise stress to the fish used in this study, and the water conditions approximated natural conditions. The male-male competition took the form of ritualised agonistic displays, chases and escapes. The fish were monitored closely at all times to ensure that no physical injuries were being inflicted. Since competitive interactions may be stressful for the test subjects, the length of time when males were placed in a competitive environment was kept to a minimum and fish were separated immediately after the completion of the study, at which point the fish were then retained in the laboratory as breeding stock. Other fish captured but not used in this study were also retained for use in future studies in order to minimise the number of collection trips subsequently required. No licences were required for the study.

RESULTS

Female Preference Test

From the two-bubble-nest treatment, there was no female preference for large bubble nest males (Linear regression: time difference: $r^2 = 0.007$, $F_{1,30} = 0.205$, NS; time ratio difference: r^2= 0.009, $F_{1,30}$ = 0.267, NS). After reducing the size of large bubble nest by half, there was no female preference from the same female for the new large bubble nest male (Linear regression: time difference: r^2 = 0.001, $F_{1,30}$ = 0.348, NS; time ratio difference: r^2 = 0.001, $F_{1,30}$ = 0.032, NS).

From the one-bubble-nest treatment, females did not spend time differently between either with males with bubble nests or with males without bubble nest (Paired *t*-test: t_{35} = 0.015, NS). From the no-bubble nest treatment, females did not spend time differently between both males (Paired *t*-test: t_{57} = 0.670, NS). When we compared the time females spent with males among three treatments, females in no-bubble-nest treatment spent longer time with males than in two-bubble-nest treatment (One-way ANOVA: $F_{2,123}$ = 4.895, P < 0.05, Post Hoc Tests with Bonferroni adjustment: P < 0.05, Figure 2).

Male-Male Competition Test

Male body size did not correlate with bubble nest area and bubble nest length (Pearson correlation, bubble nest area: r_{100} = -0.061, NS; bubble nest length: r_{100} = -0.020, NS). Males from 25 out of 32 pairs in the two-bubble-nest treatment, 31 of 36 pairs in the one-bubble-nest treatment and 40 of 58 pairs in the no-bubble-nest treatment fought and displayed other nine types of agonistic behaviours except chasing and escaping behaviours. For the rest, males displayed only chasing and escaping behaviours. The fighting duration among the three treatments was not significantly different (One-way ANOVA, $F_{2,93}$ = 0.362, NS). From the two-bubble-nest treatment, large bubble nest males did not win the fights more than small ones (Table 6). Large and small bubble nest males displayed the same amount of total agonistic behaviours {(Mean ± SE): large males: 54.04 ± 15.54 times; small males: 55.64 ± 16.66 times, Paired *t* tests: t_{24} = -0.737, NS}.

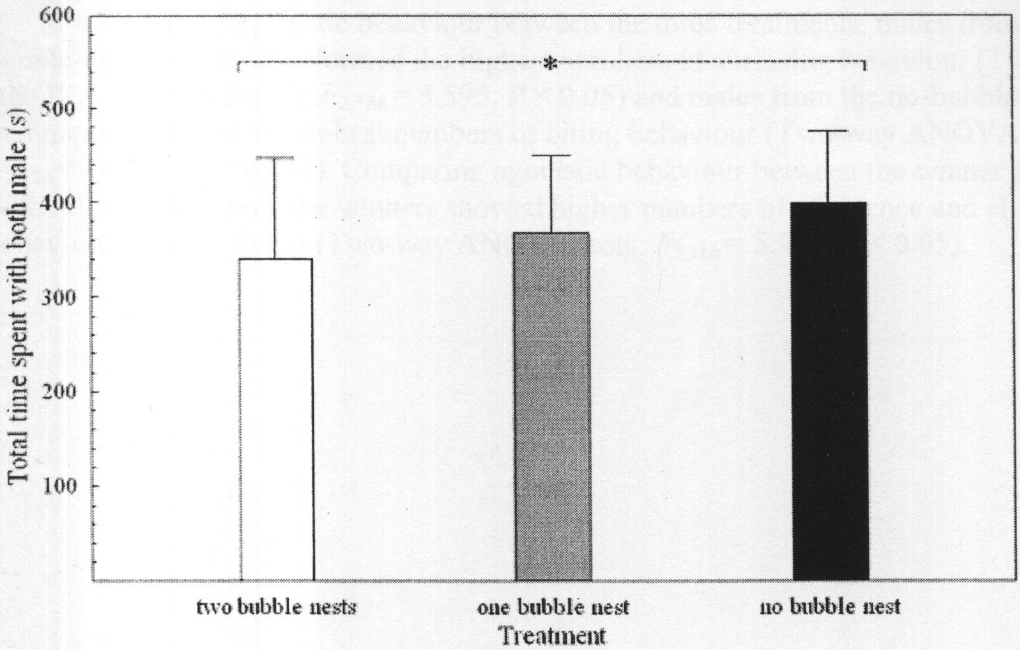

Figure 2 The Mean ± SD of total nearness time among two-bubble-nest treatment (□), one-bubble-nest treatment (■), and no-bubble-nest treatment (■). *P < 0.05

Table 6 Winning status in two-bubble-nest treatment and one-bubble nest treatment.

Treatment		Winner (N)	Statistical test
Two-bubble-nest treatment	large bubble nest males	20	$\chi^2_1 = 2.00$, NS
	small bubble nest males	12	
One-bubble-nest treatment	males with bubble nests	18	$\chi^2_1 = 0.00$, NS
	males with no bubble nest	18	

Considering each agonistic behaviour separately, large bubble nest males performed more chasing and less escaping behaviour than small bubble nest males (Paired t-test for chasing: $t_{24} = 2.40$, $P < 0.05$; escaping: $t_{24} = -2.40$, $P < 0.05$, Figure 3a). There was no relationship between the fighting duration and the bubble nest area difference in the two-bubble-nest treatment (Linear regressions: $r^2 = 0.026$, $F_{1,30} = 0.787$, NS).

From the one-bubble-nest treatment, males with bubble nests did not win more fights than males without bubble nest (Table 6). Males with and without bubble nests displayed the same amount of total agonistic behaviours during fighting {(Mean ± SE): males with bubble nests: 53.45 ± 13.53 times; males without bubble nests: 52.48 ± 13.35 times, Paired t-tests: approach: $t_{30} = -0.183$, NS; sound producing: $t_{30} = 0.334$, NS; biting: $t_{30} = -0.362$, NS; surfacing: $t_{30} = 0.859$, NS; mouth-defying: $t_{30} = -0.049$, NS; chasing: $t_{30} = -0.177$, NS; escaping $t_{30} = 0.177$, NS, total agonistic behaviour: $t_{30} = 0.381$, NS, Figure 3b}.

From the no-bubble-nest treatment, both males displayed the same amount of total agonistic behaviour during fighting (Paired t-tests: approach: $t_{39} = -1.221$, NS; sound producing: $t_{39} = 0.418$, NS; biting: $t_{39} = -0.299$, NS; surfacing: $t_{39} = -0.610$, NS; mouth-defying: $t_{39} = -1.203$, NS; chasing: $t_{39} = 0.158$, NS; escaping $t_{39} = 0.000$, NS; total agonistic behaviours: $t_{39} = -1.129$, NS).

Comparing agonistic behaviour between the three treatments, males from the two-bubble-nest treatment performed the highest numbers of surfacing behaviour (Two-way ANOVA tests: surfacing: $F_{2,186} = 3.595$, $P < 0.05$) and males from the no-bubble-nest treatment displayed the highest numbers of biting behaviour (Two-way ANOVA tests: biting: $F_{2,186} = 10.101$, $P < 0.001$). Comparing agonistic behaviour between the winner and the loser of the three treatments, the winners showed higher numbers of adherence and chasing behaviour than the losers (Two-way ANOVA tests: $F_{1,186} = 5.915$, $P < 0.05$).

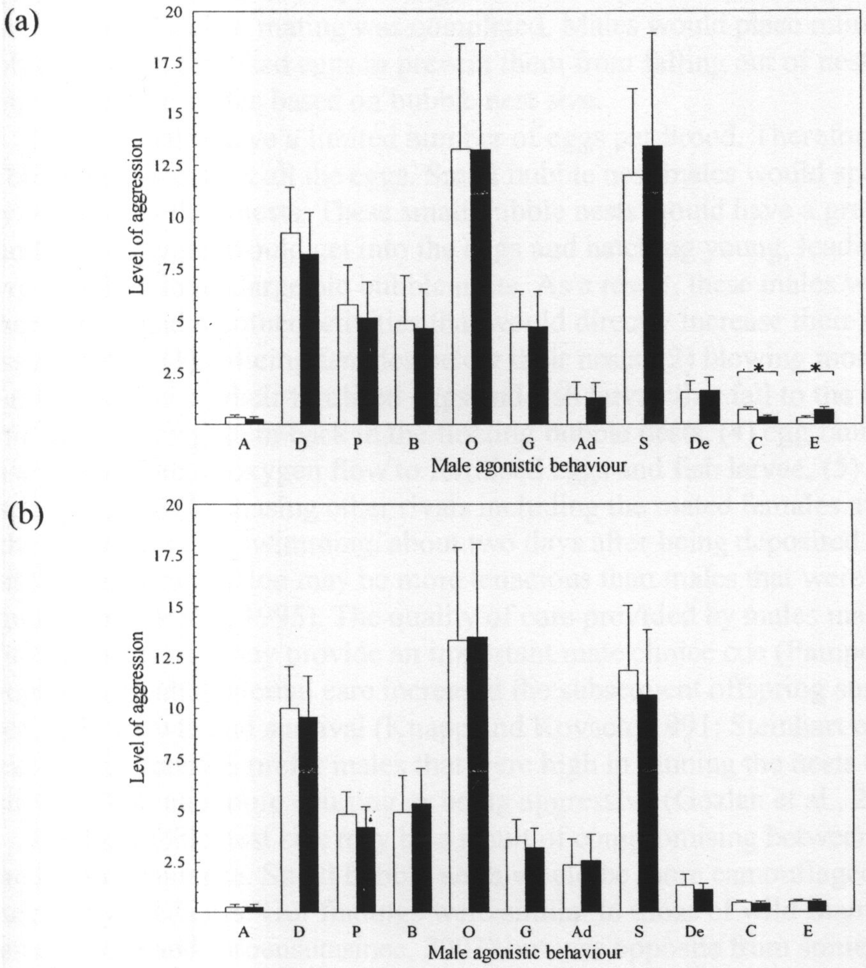

Figure 3 The Mean ± SD of male agonistic behaviours. (a) Two-bubble-nest treatment. (□) and (■) represent large bubble nest males and small bubble nest males, N = 25 fighting pairs. (b) One-bubble- nest treatment (□), and (■) represent males with bubble nests and males without bubble nest, N = 31 fighting pairs. Male agonistic behaviour includes approaching (A), lateral displaying (D), sound producing (P), biting (B), mouth opening (O), going round in a circle (G), adhering to each other (Ad), surfacing (S), mouth defying (De), chasing (C), and escaping (E). * $P < 0.05$

DISCUSSION

Our results showed that female croaking gouramis preferred small bubble nest males over large bubble nest males. This could be presumably due to four possible reasons. First, in natural habitats, males with small bubble nest may simply be available and there was no correlation between the bubble nest area and the male body length (Liengpornpan et al., 2006). This pattern may be reliable to indirect selection models (e.g. runaway selection), which predict that female mating preference and small bubble nest male evolve in concert due to a genetic correlation between preference and the male trait (Morris et al., 1996).

Second, we observed that the newly built bubble nests are bright white and have small dome shape. If no mating occurs in a few days after bubble nests are constructed, the bubble nests become pale brown, and getting larger and flatter. Small bubble nests may be a good indicator of newly built bubble nests. Males would increase the number of bubbles in order to increase the size of their own bubble nests during three periods: (1) a few minutes before mating started, (2) during each spawning, while mating male spewed fertilised eggs into the bubble nest and (3) when mating was completed. Males would place more several fine layers of bubbles beneath fertilised eggs to prevent them from falling out of nest. Therefore, females did not choose their mates based on bubble nest size.

Third, females have a limited number of eggs per brood. Therefore, males may build small bubble nests that fit all the eggs. Small bubble nest males would spend less time and energy in building their nests. These small bubble nests would have a greater surface/volume ratio and more oxygen would get into the eggs and hatching young, leading to higher survivorship than in the large old bubble nests. As a result, these males would have more time and energy to spend on other activities that would directly increase their reproductive successes, that is, (1) enticing females below their nests, (2) blowing more bubbles during mating, (3) picking up their fertilised eggs and fish larvae that fall to the bottom after each spawning and placing them back in the floating bubble nests, (4) egg fanning with water to increase the amount of oxygen flow to fertilised eggs and fish larvae, (5) removing dead or diseased eggs, and (6) chasing other rivals including the mated females away from the nests until the spawn are free swimming, about two days after being deposited. Males guarding eggs at the time of intrusion may be more tenacious than males that were guarding larvae (Wiegmann and Baylis, 1995). The quality of care provided by males may be of a direct benefit to females and may provide an important mate choice cue (Pampoulie et al., 2004). Although this greater paternal care increased the subsequent offspring survival, it also reduced male growth and survival (Knapp and Kovach, 1991; Steinhart et al., 2000). However, some females prefer males that were high in fanning the nests (St Mary and Lindström, 2003) and more courting or being aggressive (Gozlan et al., 2003).

Lastly, bubble nest size may be a result of compromising between sexual selection and predation avoidance. Small bubble nests would be more camouflaged and less likely to be detected by predators. Our findings were similar to those of wild Siamese fighting fish (Jaroensutasinee and Jaroensutasinee, 2003) but was opposite from some fish species in which male mating success was related markedly with increasing nest size such as sand goby (Lindström 1992; Kvarnemo, 1995), blenny, *Istiblennius enosimae* (Sunobe et al., 1995), and Japanese stream goby (Takahashi et al., 2001). Some fish build small opening nests, reducing predation risks including sand goby, *Pomatoschistus minitus* (Svensson and Kvarnemo, 2003) and allowing successful egg-ventilation including common goby, *Pomatoschistus microps* (Jones and Reynolds, 1999).

Our results showed that female croaking gouramis did not prefer males with bubble nests over males without bubble nests. This indicates that the visual aspects could be irrelevant or only minor in criteria for female preference. Sound emission might be a very effective way of assessing the physical strength of the producer (Ladich et al., 1992). Therefore, female croaking gouramis may assess male quality through sound characteristics.

Generally, bubble nest size can be an indicator of vigorous and healthy of builders (Barber et al., 2001). For example, in Azorean rock-pool blenny, *Parablennius sanguinolentus*, nest-holder males were more aggressive towards other males (Oliveira et al., 2002). Our results did not show that bubble nest presence/absence or bubble nest size was associated with wining status, fighting duration or male body size. Similar findings were showed in Siamese fighting fish. Large bubble nest males did not win more fights than small bubble nest males (Jaroensutasinee and Jaroensutasinee, 2003).

This study shows the optimal response from females when holding a bubble nest and paternal care were in conflict. In croaking gouramis, large nest size and holding bubble nest did not affect females for sexually accepting males. Males with large bubble nests might not always reflect the male's contribution to paternal care and the outcome of contests because they do not win in most fights. Further studies are needed to clarify other cues that females should use for predicting male characters or paternal quality. Fish nests may be used as only a courtship strategy but bubble nest size may provide honest information about male quality. Males who invested less in nest building may reserve their energy for parental effort. Thus females had a marked preference and seemed to mate with small bubble nest males, although these characteristics may decrease mating success. However, female croaking gouramis may use other nest-cues to increase their reproductive success including territory quality (nest site concealment) (Sargent and Gebler, 1980; Sargent, 1982), nest structure (Jones and Reynolds, 1999) or structurally complex nest site such as densely vegetated site (Candolin and Voigt, 1998). The structural complexity of habitats is known to affect predator foraging success (Candolin and Voigt, 1998). Furthermore, there were some male characteristics that females did not prefer such as well-fed males in common goby, *Pomatoschistus microps* (Svensson and Forsgren, 2003) and foreign males in Pacific blue-eye, *Pseudomugil signifer* (Wong et al., 2004). However, nest structure or time span before a male starts to build his nest may be of a direct benefit to females for providing an important mate preference cue (Barber, 2001; Svensson and Kvarnemo, 2003). This suggests that females may gain valuable information regarding male health status from nest inspection. Otherwise, both natural selection and sexual selection may act on male nest-building in croaking gouramis.

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CHAPTER 5
ภาคผนวก

International Publication

1. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2006. Mating habits and nesting habitats of the croaking gourami *Trichopsis vittata.* Acta Zool. Sinica 52: 846-853.
2. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2007. Male body size, female preference and male-male competition in croaking gourami. Acta Zool. Sinica 53: in press. (ดังในบทที่ 3)

Conference presentation

1. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2004. Habitat characteristics of croaking gouramis (*Trichopsis vittata*). 8 th BRT Annual Conference. Surat Thani, Thailand. 14-17 October.
2. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2004. Habitat characteristics of croaking gouramis (*Trichopsis vittata*). 30 th Congress on Science and Technology of Thailand. Impact Exhibition and Convention Center, Muang Thong Thani, Thailand. 19-21 October 2004.
3. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2005. Male body size, female preference and male-male competition in croaking gouramis. 9 th BRT Annual Conference. Khonkaen, Thailand. 10-13 October.
4. Liengpornpan S, Jaroensutasinee M, Jaroensutasinee K, 2006. Male body size, female preference and male-male competition in croaking gouramis. 10 th BRT Annual Conference. Krabi, Thailand. 8-10 October.

บทความเผยแพร่แก่สาธารณะชน

1. ความหมายที่ซ่อนเร้นในเสียงปลากริม
2. ลีลารักปลากริม

คู่มือสำหรับเยาวชน

1. คู่มือการเลี้ยงปลากริม

动物学报 52(5):846-853，2006
Acta Zoologica Sinica

Mating habits and nesting habitats of the croaking gourami *Trichopsis vittata* *

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Abstract Croaking gouramis *Trichopsis vittata* were investigated in the two types of habitats in which they occur in Thailand: rice paddy fields and small ponds. Croaking gouramis inhabit shallow standing water with dense vegetation near the margin of both habitats. Nest sites in rice paddies were higher in pH, dissolved oxygen, and temperature but lower in specific conductance, total dissolved solids and water depth than in small ponds. Population densities of croaking gouramis were lower in rice paddies than in small ponds. Males were heavier and bigger than females. There were positive correlations between male weight and body length and width, whereas female weight was positively correlated only with body length. There was no correlation between the bubble nest area and the male body length and weight. Male and female embraced many times during mating. There were (Mean ± *SD*) 3.74 ± 1.02 eggs per embracement, 84 ± 12.97 embracement/spawning, and 314 ± 109.77 eggs/spawning with a spawning duration of 178 ± 51.17 min. Fish larvae hatched within 30.75 ± 0.55 h and 2-day old fry's sizes were 3.21 ± 0.29 mm [*Acta Zoologica Sinica* 52 (5): 846-853, 2006].

Key words Croaking gouramis, *Trichopsis vittata*, Mating habits, Nest habitats

条纹短攀鲈的交配习性和筑巢生境 *

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摘 要 条纹短攀鲈栖息于静止、植被茂密、靠近稻田和池塘边缘的浅水中。本文研究了它在泰国稻田和小池塘两种生境中的繁殖特征。与池塘相比，稻田筑巢地的 pH 值、溶解氧的含量和温度较高，而电导、溶解的固体物浓度较低，水也较浅。稻田中的种群密度低于池塘。雄性个体的体重和体长均大于雌性个体，且其体重与体长和体宽正相关，但雌性个体的体重仅与体长正相关；泡巢的面积与雄性个体的体长和重量均不相关。繁殖期间，条纹短攀鲈雌、雄个体交尾多次。每次交尾产卵 3.74 ± 1.02（Mean ± *SD*）粒，每个繁殖季节交尾 84 ± 12.97 次；在一个持续 178 ± 51.17 min 的产卵时段里，条纹短攀鲈平均产卵 314 ± 109.77 粒。鱼苗孵化需 30.75 ± 0.55 h，2 日龄个体的大小平均为 3.21 ± 0.29 mm [动物学报 52 (5): 846-853, 2006]。

关键词 条纹短攀鲈 交配习性 筑巢生境

Many fish species commonly select particular habitat types in an apparent effort to increase their reproductive success (Jenkins and Wheatley, 1998; Bremset and Berg, 1999; Chaves and Bouchereau, 2000; Morrow and Fischenich, 2000; Olivotto et al., 2003; Wildhaber and Lamberson, 2004). These habitat choices could influence the costs and benefits of time and energy devoted to different activities (Hart and Reynolds, 2002). Habitat requirements can influence many aspects of fish biology—they can affect distribution (Darling, 2000; Clay et al., 2004) and colonisation (Roberts and Poore, 2006),

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interact with genetic parameters to influence the timing of sexual maturity (Wild et al., 1994), interact with environmental cues in regulating the reproductive cycle (Omori, 1995), influence mating and breeding systems (Sato, 1994; Reynolds, 1996; Dickerson et al., 2004), impede and/or enhance the evolution of reproductive isolation (Magurran and Phillip, 2001) and reflect the distinctive functional morphology of fishes (Motta et al., 1995).

The croaking gourami (*Trichopsis vittata*, Cuvier, 1831) is a small dull coloured freshwater fish native to Indonesia, Vietnam and Thailand (Smith, 1945; Sandford, 2000). This fish inhabits shallow standing freshwater habitats such as rice paddy fields and small ponds. They are most notable for their audible rattling or croaking noise, which can be heard during breeding or fighting. This fish has been selected artificially for many generations in the pet trade, and it has mostly been studied in the lab with heavily inbred pet shop lines. Previous studies have focused on its agonistic behaviour (Bischof, 1998; Henglmüller and Ladich, 1999), vocalisations (Ladich et al., 1992; Ladich, 1997; Ladich and Yan, 1998; Wysocki and Ladich, 2001) and hearing (Yan, 1998; Ladich and Popper, 2001; Wysocki and Ladich, 2002). Little is known about the ecology, habitat preferences and reproductive behaviour of wild populations.

Although the croaking gourami is not in the IUCN Red List, the artificial selection in many regions of the world and their introduction of pet shop breeds into natural populations, together with the decline of this native species in its natural habitats, makes it important to study the natural ecology of the species. Therefore, field data on the water quality parameters of its nesting site would be valuable for understanding habitat associations and behavioural ecology.

This study aims to investigate the characteristics of two kinds of croaking gourami habitats: rice paddy fields and small ponds, sexual size dimorphism of wild croaking gouramis, relationship between bubble nest size and male body size, and reproductive biology. Two hypotheses were tested. First, if the characteristics of the rice paddy fields and the small ponds were significantly different, then the sizes of the fish and their bubble nest areas from both habitats should be significantly different. This is because this fish may have some morphological adaptation or natural selection to particular habitats. Second, if bubble nest areas depended on the sizes of the builders, then the bubble nest areas should be positively correlated with the male body length and/or weight. This is because the larger males should be capable of building larger bubble nests.

1 Materials and methods

1.1 Species description

The croaking gourami is a tropical freshwater fish of the suborder Anabantoidea, family Belontiidae (Smith, 1945; Sandford, 2000). This fish is capable of utilising the oxygen present at the water surface via its labyrinth organ (i.e., a bony structure formed by a segment of gill arch and covered with a respiratory epithelium) (Graham, 1997). Males and females usually attain sexual maturity after 4 months of age with a standard body length of 3 – 4 cm (Henglmüller and Ladich, 1999). Both sexes are brown-bodied with three horizontal stripes. Males have more reddish and elongated dorsal fins than females. Females are smaller, and duller than males. Males build bubble nests that consist of mucus-covered bubbles that stick together in a mass and bind with emergent plants on the water surface (Helfman et al., 1997).

1.2 Fish sampling

All field work was carried out during 07:00 – 10:00 h in the middle of the breeding season (i.e., April and May 2004) in Nakhonsithammarat Province, southern Thailand (08° 29.381′ – 08° 29.384′ N, 99° 55.138′ – 99° 55.437′ E). After 10:00 h, nest-holding males tend to leave their bubble nest to find food (M. Jaroensutasinee pers. obs.). We divided our study into two parts: plot study and outside plot study. For plot study, we set out 10 plots in rice paddies and 10 plots in small ponds. For outside plot study, we collected additional data from 112 bubble nests found outside plots.

For plot study, ten 4 m^2 (2 m × 2 m) sampling plots in rice paddy fields and ten 4 m^2 (2 m × 2 m) plots in small ponds were used. We selected rice paddy fields and small ponds where there were bubble nests present. Then at each rice paddy field or small pond, we set one plot per rice paddy field or small pond in order to avoid a pseudo-replication and randomly sampled between these two habitats with respect to days. The water depth of bubble nests was measured to its nearest 1.0 mm. The bubble nests were photographed with a ruler for scale and width (a) and length (b) were measured using a Vernier Calliper. Bubble nest area (A) was estimated using the elliptic equation $\{A = \pi(ab/4)\}$. If there were other bubble nests nearby within 1 m, the distance from each bubble nest to the nearest neighbouring bubble nest was measured by tape before the male nest builders were captured.

We collected three water samples at three points of the plot along diagonally straight line from corner to corner: two samples from the corners and one from the centre of the plot. Water characteristics measured in each plot included temperature, transparency, pH using a pH metre, dissolved oxygen (DO) using Orion-Model 835, specific conductivity and total dissolved solids (TDS) using Mettler Toledo-Model MX300. The water samples were taken to the laboratory for measurement of biochemical oxygen demand (BOD) by using a standard method for the examination of water and wastewater (APHA, 1998), nitrite, nitrate and phosphate by using ion chromatography based on U.S. EPA method 300 (EPA, 1993). After the water samples were collected, all of the fish in a plot were captured and counted in order to calculate population density and adult sex ratio. The adult sex ratio was calculated as the number of sexually active males divided by the total number of sexually active adults of both sexes in the plot (Jirotkul, 1999).

For outside plot study, 112 nest-holding males were collected outside the sample plots and their bubble nests were measured in order to improve estimates of average male body size, water depth, and bubble nest sizes. We recorded the number of bubble nests with eggs. If there were eggs in bubble nests, all eggs were collected from the nests and counted in the laboratory. Ten randomly selected eggs from each nest were measured for diameter to the nearest 0.01 mm using a Vernier Calliper.

1.3 Body weight and body size measurements

All fish from both groups were taken to the laboratory and their body weight measured to the nearest 0.01 g by using a Mettler digital balance. The following procedure was followed by measuring fish body length and width. First, each fish was placed in an aquarium, measuring 25.0 cm × 12.5 cm × 16.0 cm high filled with water to depth of 3 cm. Second, a piece of Plexiglas with a ruler was placed in the aquarium to provide scale. Finally, after 1-min acclimatisation period, the fish was photographed with a digital camera (Sony Mavica, model-FD91). The digital pictures were then used to estimate the fish body length from the tip of the upper jaw to the caudal peduncle, and body width. This procedure decreased the probability of killing or damaging fish from using anaesthetic drugs.

1.4 Reproductive biology

The test subjects were wild caught croaking gouramis collected in April and May 2004 from Nakhonsithammarat. The fish were maintained in the laboratory with natural light approximately 12 : 12 light:dark cycle and fed daily with ant eggs. Twenty males and twenty females were housed in separated 1-L bottles to prevent fighting. A female was placed in her 1-L bottle next to a male in his 1-L bottle until she became gravid. Gravid females had yellowish ovaries apparent through their scales (Liengpornpan, per. obs.). The male was then placed in a 37-L tank, measuring 50.0 cm × 25.0 cm × 30.0 cm high, densely planted with aquatic vegetation. Males built their bubble nests within 24 h after being placed in the tank. A gravid female was placed in the tank with the nest-holding male in the evening at approximately 15:30 h of the second day after the nest-holding male had been placed in the tank. We recorded male and female courtship behaviours, the number of eggs released per embracement, the number of embracement per spawning, the total number of fertilised eggs per spawning, spawning duration, and the total time spent from fertilised eggs to fish larvae. Ten randomly selected fish larvae from each nest were measured for total body length to the nearest 0.01 mm using a Vernier Calliper.

1.5 Data analysis

All variables were tested for normality using Lilliefors' test and log transformed when necessary to achieve normality, as in fish body weight and bubble nest area. Independent-sample *t*-tests were used for comparisons between rice paddy field and small pond habitats. Pearson correlations were used to test the association among body measurements, and body size and bubble nest area. All significant tests were two tailed.

2 Results

2.1 Habitat characteristics

There were 11 bubble nests found in ten plots within rice paddy fields and 12 bubble nests found in ten plots within small ponds. Croaking gouramis built their bubble nests in clear shallow standing water with dense vegetation, floating plants or other structurally complex features along the edges of their habitats. Habitats used were characterised by low values in pH, DO, transparency, BOD, nitrite, nitrate, and phosphate but high values in specific conductance, TDS and temperature (Table 1), relative to water qualities measured from natural streams and rivers. Differences were found between rice paddy fields and small ponds in pH, specific conductance, DO, TDS, water depth, temperature and population density (Table 1 and 2). There were other fish species found in plots within both rice paddy fields and small ponds, including wild Siamese fighting fish, other gourami species, needle fish, serpent-head fish, eel and catfish.

2.2 Fish size and bubble nest size

Males were heavier and bigger than females (Table 3). There were positively correlated between male weight and body length and width (Pearson correlation coefficient, length: $r_{175} = 0.797$, $P < 0.001$; width: $r_{175} = 0.823$, $P < 0.001$), whereas female weight was positively correlated only with its body length (Pearson correlation coefficient, length:

Table 1 The Mean ± *SD* of water characteristics of croaking gourami nest sites in rice paddies and small ponds

Characteristics	Mean ± *SD*		*t*-test
	Rice paddy field	Small pond	
pH	6.83 ± 0.57	5.00 ± 0.36	$t_{18}=3.89^*$
Specific conductance (μS/cm)	35.92 ± 6.38	51.38 ± 12.71	$t_{18}=-3.44^*$
Dissolved oxygen (mg/L)	5.44 ± 1.13	1.67 ± 0.70	$t_{18}=8.95^*$
Total Dissolved Solids (mg/L)	18.20 ± 3.17	25.77 ± 6.33	$t_{18}=-3.38^*$
Transparency (cm)	12.17 ± 6.07	11.55 ± 4.74	$t_{18}=0.26$
BOD (mg/L)	2.61 ± 0.83	2.64 ± 0.74	$t_{18}=-0.085$
Nitrite (mg/L)	ND	ND	-
Nitrate (mg/L)	ND	ND	-
Phosphate (mg/L)	ND	ND	-
Water depth (cm)	11.0 ± 3.6	50.4 ± 23.0	$t_{18}=-5.366^*$
Temperature (℃)	31.6 ± 1.79	29.8 ± 1.1	$t_{18}=2.65^*$

ND = not detected ($NO_2<0.17$ mg/L, $NO_3<0.21$ mg/L, $PO_4^{3-}<0.08$ mg/L), $P<0.001$.

Table 2 The Mean ± *SD* of population parameters, body size and bubble nest dimensions of croaking gouramis in rice paddies and small ponds

Characteristics	Mean ± *SD*		*t*-test
	Rice paddy field	Small pond	
Population density (individuals/m^2)	2.23 ± 2.21	7.83 ± 7.41	$t_{11}=-2.29^*$
Adult sex ratio	0.81 ± 0.29	0.80 ± 0.32	$t_{18}=0.10$
Bubble nest width (cm)	1.83 ± 0.79	2.45 ± 0.88	$t_{21}=-1.79$
Bubble nest length (cm)	1.97 ± 0.89	2.60 ± 0.96	$t_{21}=-1.63$
Bubble nest area (cm^2)	3.09 ± 2.02	5.18 ± 3.38	$t_{21}=-1.78$
Water depth at bubble nests (cm)	10.6 ± 4.8	15.4 ± 16.2	$t_{21}=-0.94$
Male length (cm)	3.58 ± 0.38	3.39 ± 0.17	$t_{21}=1.60$
Male width (cm)	1.09 ± 0.14	1.03 ± 0.09	$t_{21}=1.21$
Male weight (g)	0.89 ± 0.15	0.79 ± 0.14	$t_{21}=1.61$

$^*P<0.05$.

Table 3 The Mean ± *SD* of croaking gourami weight, length and width

Body measurement	Males ($n=175$)	Females ($n=10$)	*t*-test
Weight (g)	0.86 ± 0.23	0.59 ± 0.10	$t_{183}=3.70^{**}$
Length (cm)	3.51 ± 0.35	3.17 ± 0.21	$t_{183}=3.07^*$
Width (cm)	1.03 ± 0.12	0.91 ± 0.04	$t_{17}=7.30^{**}$

$^*P<0.005$, $^{**}P<0.001$.

$r_{10}=0.735$, $P<0.05$; width: $r_{10}=0.430$, NS).

We collected all males found both in plots and outside plots during field surveys. We found 175 males but only 112 of these males built bubble nests. All 112-bubble nests were located in the shallow water near the edges of rice paddy fields and small ponds. There were only two out of 112 nests that had eggs (Table 4). Most nests were built in newly flooded areas. There was no correlation between the bubble nest area and the male body length and weight (Pearson correlation coefficient, length: $r_{112}=0.049$, NS; weight: $r_{112}=0.024$, NS).

2.3 Reproductive biology

Fifteen out of 20 pairs were spawning. During 16:00 - 18:00 h, a nest-holding male enticed a gravid female to visit his bubble nest. The nest-holding male chased this female vigorously until she swam towards him and followed him to his bubble nest. Then he wrapped his body around the female juxtaposing their genital pores. These embraces were accompanied by the release of eggs with a Mean ± *SD* of 3.74 ± 1.02 eggs per embracement. Following each embrace, the male chased the female from the immediate vicinity of the nest. A male released his sperm to fertilise the eggs, picked the fertilised eggs up in his mouth and spewed them into the bubble nest. A mating sequence consisted of an average of 5 – 6 pseudo-spawning bouts (i.e. embraces without the release of eggs) and 84 ± 12.97 spawning bouts (i.e. embrace with egg release). The couple repeated this embracement process over a Mean ± *SD* of spawning duration of 178 ± 51.17 min. This resulted in a Mean ± *SD* of 314 ± 109.77 eggs released per spawning. When this external fertilisation was over, the nest-building male chased the mated female out of his bubble nest, placed the fine layers of bubble beneath the eggs to ensure that the eggs remained in the bubble nest and solely defended the nest and surrounding territory. The fry hatched within a Mean ± *SD* of 30.75 ± 0.55 h and continued developing under the bubble nest. Two days after hatching, fish larvae were free swimming with a Mean size ± *SD* of 3.21 ± 0.29 mm.

Table 4 The Mean ± *SD* and range of bubble nest size, water depth, distance to nearest nest, and egg diameter

Measurement	Sample size	Mean ± *SD*	Range
Bubble nest width (cm)	112	1.74 ± 0.79	0.32 – 4.15
Bubble nest length (cm)	112	1.87 ± 0.95	0.40 – 5.57
Bubble nest area (cm^2)	112	2.79 ± 2.02	0.10 – 8.55
Water depth (cm)	112	13.80 ± 16.36	3.00 – 98.00
Distance to the nearest nest (cm)	7	60.4 ± 42.6	9.0 – 120.0
The number of bubble nest with fertilised eggs	2	373.5 ± 38.9	346 – 401
Egg diameter (mm)	20	0.78 ± 0.08	0.64 – 0.92

3 Discussion

3.1 Habitat characteristics

Croaking gouramis live in shallow lentic water, which is rather turbid (i.e., low in transparency and high in TDS) from organic substances and clay particles. This murky condition is common to rice paddy fields and small ponds throughout Thailand. Our results showed that population density was higher in small ponds than rice paddies. This may be due to two possible reasons. First, the environment of rice paddies is more extreme than small ponds. During dry season, there will be almost no water available in the rice paddies, but lots of water in small ponds. During the early wet season, rice paddies will be used for rice cultivation, lots of water present in the paddies, less vegetation, lots of animal disturbance (e.g., water buffalos, and ducks), and a high amount of fertilisation and pesticide used from rice cultivation. Second, the higher fish density in small ponds may be related to the highly dense vegetation in small ponds (Liengpornpan, per. obs.). However, we did not measure vegetation density in our study. For future study, the effect of vegetation complexity on fish density should be examined. Croaking gouramis may prefer to live in shallow water with dense vegetation due to three possible reasons. First, dense aquatic vegetation in shallow water could minimise predation risks from piscivorous fishes, crabs, prawns and snakes (Kramer et al., 1997) and reduce intraspecific interactions (Corkum and Cronin, 2003). Second, males could use these emergent plants to hold the bubbles together or prevent them from drifting into unsuitable habitat for the fry. Third, males could easily pick up the fertilised eggs and fish larvae, and spew them back to their bubble nests in shallow water. In deeper cloudy water, males might not be able to see fertilised eggs or fish larvae at the bottom very well or males might spend too much energy on picking up eggs and larvae up from the bottom. Many studies have demonstrated that prey organisms select microhabitats with high structural complexity as a way of reducing predation risks (Pamala and Kenneth, 1994; Prenda and Granado-Lorencio, 1996; Elkin and Baker, 2000). Many fishes are most abundant in shallow areas because these habitats enhance growth and survival of young, e.g., pinfish (Jordan et al., 1997), Atlantic salmon (Bremset and Berg, 1999), cod (Linehan et al., 2001), and winter flounder (Manderson et al., 2004).

Rice paddy fields and small ponds differed in water characteristics (i.e., pH, conductivity, DO, TDS, water depth, and temperature). Therefore, croaking gouramis can occupy a wide range of habitat types, especially in poorly oxygenated water (1.67 mg/L), which is lethal to most fishes within 24 h (i.e., <3.5 mg/L) (Moore, 1942) and slightly acidic water (pH 5) which could cause slow growth in fish species (i.e., pH<6.5) (Boyd, 1996). These limiting factors can influence fish distribution (Matthews, 1998), increase mortality rate and depress hatching success in many fish species (Keckeis et al., 1996; McNatt and Rice, 2004). However, this physical constraint does not apply to croaking gouramis because this species has a labyrinth organ. In addition, this fish builds a bubble nest to hang its eggs and young to expose them to oxygen directly. Therefore, building bubble nests may be an adaptative behaviour, which enables gouramis to live in a hypoxic environment.

3.2 Fish size and bubble nest size

Large body size in males of many species may have evolved because of the advantages of large size in promoting success in male-male competition (Bisazza et al., 1989; Poncin 1996; Bisazza et al., 2000). Sexual size dimorphism (i.e., males are larger than females) has been shown in many fish species including Pacific salmon (Fleming and Gross, 1994), sockeye salmon (Quinn and Foote, 1994), tilapia (Toguyeni et al., 1997), neotropical armoured catfish (Hostache and Mol, 1998), dark chub (Katano, 1998), rainbow trout (Bonnet et al., 1999), and wild Siamese fighting fish (Jaroensutasinee and Jaroensutasinee, 2001). Our results showed that male croaking gouramis were generally heavier and bigger than females. In croaking gourami, only males exhibit parental care including building bubble nests, caring for the eggs and fish larvae and defending the territory. Therefore, larger males in this species would have a selective advantage over small males. However, there is no data available on the relationship between male size and reproductive success in croaking gouramis. It has been demonstrated that male body size is positively correlated with reproductive success in some fish species with male parental care (e.g., mottled sculpins (Downhower and Brown, 1980) and lumpfish (Goulet and Green, 1988).

From our field surveys, we found that croaking gourami's bubble nests were difficult to find. Once we found bubble nests, there were only two out of 112 nests that had eggs in them. The low number of bubble nests with eggs found during our field surveys could be because we might have collected our field surveys early in the breeding season. However, this would be very unlikely because the field surveys were conducted covering the two months period in April and May.

This study offered clear evidence that male croaking gouramis built their bubble nests similar in size. This means that larger males did not build larger bubble nests. There could be two possible reasons. First, a small bubble nest would be energetically less costly to build and guard. Therefore, by spending less time and energy building a small bubble nest, a nest-holding male will have more time and energy to spend on searching for mates, courting them and defending his territory from other potential rivals. Second, large gourami males should not spend too much energy in increasing bubble nest size because females have a limited number of eggs per brood. Therefore, males may not increase their reproductive success by building larger bubble nests. Males should spend their energy on other activities that would directly increase their reproductive successes such as enticing females into their nests, defending territory from other males, and egg fanning to increase the amount of oxygen flow to fertilised eggs and fish larvae.

Our results for croaking gourami were not consistent with previous observations of an ecologically similar species, the Siamese fighting fish *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001). Male fighting fish built their bubble nests according to their size (Jaroensutasinee and Jaroensutasinee, 2001). The difference between our study and the fighting fish's study could be due to several reasons. First, fighting fish might be under different selective pressure than gourami. Second, female fighting fish might vary in size and the number of eggs per brood. Therefore, larger fighting males might only choose larger females as mates. Third, there might be less nest-holding fighting fish males in the wild than females. This would cause an operational sex ratio biased towards females. As a result, larger nest-holding males are very rare and should become the choosier sex. Therefore, these larger nest-holding males may prefer larger gravid females that can provide larger broods as mates. In addition, fighting fish females did not show any preference towards males with larger bubble nest sizes (Jaroensutasinee and Jaroensutasinee, 2003). This implies that females in this species are not the choosier sex.

3.3 Reproductive biology

Many studies have been published on belontiid mating behaviours and emphasised on the behaviour of single pairs with the confines of small aquaria (e.g., Forselius, 1957; Miller and Robinson, 1974; Pollak et al., 1981). Our results confirmed previous findings about gourami reproductive behaviours. Males establish territories that they defend against conspecifics and in which they construct bubble nests at the water's surface. Our results showed that croaking gourami tended to spawn in the evening. Similar finding has been reported in wild Siamese fighting fish *Betta splendens* (Jaroensutasinee and Jaroensutasinee, 2001).

Our results confirm that wild-caught croaking gourami can breed in the laboratory condition successfully as shown in many fish species including catfish *Heteropneustes fossilis* (Bloch) (Thakur and Nasar, 1977), blue gourami *Trichogaster trichopterus* (Pollak et al., 1981), mollies *Poecilia* spp. (Woodhead and Armstrong, 1985), common goby *Pomatoschistus microps* (Svensson and Forsgren, 2003). However, our experimental design was set up as a single pair within the confine of small aquarium. The observations were terminated after a single spawning episode in order to save the female from serious injury or death (Miller, 1964). Therefore, we cannot conclude whether the reproductive biology of croaking gourami is a single mating. Previous study on blue gourami, *Trichogaster trichopterus*, has demonstrated that the reproductive biology of this fish is a multiple matings (i.e., the mating occurrence of two or more days with a single partner or with two or more partners on one or more days) (Pollak et al., 1981).

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ลักษณะของแหล่งที่อยู่อาศัยของปลากริม (*Trichopsis vittata*)

HABITAT CHARACTERISTICS OF CROAKING GOURAMIS (*Trichopsis vittata*)

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บทคัดย่อ: จากการศึกษาพบว่าปลากริม (*Trichopsis vittata*, Cuvier, 1831) ในประเทศไทยจะอาศัยอยู่ในน้ำนิ่งและตื้นที่มีพืชน้ำขึ้นเป็นจำนวนมากตามขอบของนาข้าวและหนองน้ำขนาดเล็ก คุณสมบัติของน้ำที่มีค่าต่ำคือ ความเป็นกรดเบส ปริมาณออกซิเจนละลายน้ำ ความเค็ม ความขุ่นใส ความต้องการออกซิเจนทางชีวเคมี ไนไตรท์ ไนเตรท ฟอสเฟต และความลึกของน้ำแต่คุณสมบัติที่มีค่าสูงคือ การนำไฟฟ้า ปริมาณของแข็งละลายได้ทั้งหมด และอุณหภูมิ ความแตกต่างของคุณสมบัติของน้ำและลักษณะของปลากริมที่อาศัยอยู่ในนาข้าวและในหนองน้ำคือ ความเป็นกรดเบส การนำไฟฟ้า ปริมาณออกซิเจนละลายน้ำ ปริมาณของแข็งละลายได้ทั้งหมด ฟอสเฟต ความลึกของน้ำ อุณหภูมิและความหนาแน่นของประชากร โดยทั่วไปแล้วปลาตัวผู้มีน้ำหนักมากกว่าและมีขนาดใหญ่กว่าปลาตัวเมีย น้ำหนักของปลาตัวผู้มีความสัมพันธ์เชิงบวกกับความยาวและความกว้างของลำตัว แต่น้ำหนักของปลาตัวเมียจะมีความสัมพันธ์เชิงบวกเฉพาะกับความยาวของลำตัวเท่านั้น พื้นที่ของหวอดไม่มีความสัมพันธ์กับความยาวลำตัวและน้ำหนักของปลาเพศผู้

Abstract: In Thailand Croaking gouramis (*Trichopsis vittata*, Cuvier, 1831) were found to inhabit shallow water with dense vegetation around the edges of rice paddy fields and in small ponds. The shallow nesting water was low in pH, dissolved oxygen, salinity, turbidity, biochemical oxygen demand, nitrite, nitrate, and phosphate, but had high levels of conductivity, temperature, and total dissolved solids. Water and fish characteristics in the rice paddy fields differed from those found in the ponds in terms of pH, conductivity, dissolved oxygen, total dissolved solids, phosphate, water depth, temperature, and population density. Males were generally heavier and bigger than females. There was positive correlation between the weight of male fish and its body length and width. However, female weight correlated positively only with body length. There was no correlation between the bubble nest area and the body length and weight of males.

Introduction: During breeding seasons, many fish seek out potential mating partners and suitable spawning habitats [1]. This study was conducted to gain further understanding of the croaking gouramis, which are native to Thailand, in their natural environment and the physiological ecology of their habitats.

Methodology: All fieldwork was carried out in Nakhonsithammarat Province during a breeding season from April to May 2004. Ten individual 4 m^2 (2×2 m) plots were marked on the ground surface of rice paddy fields and small ponds. The water depth of each bubble nest was measured.

The bubble nests were then photographed so that the width (*a*) and length (*b*) could be measured in the laboratory. The nest area was estimated using the ellipsoid equation (A = ¶*ab*). The shortest distance between bubble nests was measured before the male nest builders were captured. The water characteristics were measured before all the fish in the plot were captured, and population density and adult sex ratio calculated. In order to achieve great accuracy in estimating average male and female body sizes, water depth, and bubble nest size, additional fish outside the twenty plots were collected and their bubble nests measured. All eggs were collected from the nests, counted, and ten randomly selected eggs from each nest were measured.

Table I. Mean ± S.D. of water characteristics in habitats of croaking gouramis, their bubble nest size and body size. *$P < 0.05$, ** $P < 0.001$.

Characteristics	Mean ± S.D.		*t* - test
	Rice Paddy Field	Small Pond	
pH	6.82 ± 0.66	6.00 ± 0.53	t_{58} = 5.28**
Conductivity (mS•cm $^{-1}$)	35.43± 7.49	51.38 ± 13.33	t_{58} = -5.71**
Dissolved oxygen (mg•l $^{-1}$)	5.51 ± 1.34	1.47 ± 0.76	t_{46} =14.33**
Total dissolved solids (mg•l $^{-1}$)	17.95 ± 3.73	25.77 ± 6.63	t_{58} = -5.63**
Salinity (ppm)	0.0 ± 0.0	0.0 ± 0.0	-
Turbidity (cm)	11.8 ± 7.6	11.6 ± 6.6	t_{58} = 0.12
Biochemical oxygen demand (mg•l $^{-1}$)	2.6 ± 1.0	2.6 ± 0.8	t_{58} = -0.04
Nitrite (mg•l $^{-1}$)	0.00 ± 0.00	0.02 ± 0.08	t_{29} = -1.44
Nitrate (mg•l $^{-1}$)	0.01 ± 0.06	0.00 ±0.00	t_{29} = 1.00
Phosphate (mg•l $^{-1}$)	0.07 ± 0.17	0.00 ± 0.00	t_{29} = 2.28*
Depth (cm)	11.1 ± 4.0	50.4 ± 27.4	t_{30} = -7.79**
Temperature (°C)	31.65 ± 1.71	29.80 ± 1.09	t_{49} = 5.00**
Population density (individuals•m $^{-2}$)	2.23 ± 2.21	7.83 ± 7.41	t_{11} = -2.29*
Adult sex ratio	0.81 ± 0.29	0.80 ± 0.32	t_{18} = 0.10
Bubble nest width (cm)	1.83 ± 0.79	2.45 ± 0.88	t_{21} = -1.79
Bubble nest length (cm)	3.58 ± 0.38	3.39 ± 0.17	t_{21} = -1.60
Bubble nest area (cm)^2	12.36 ± 8.07	20.71 ± 13.52	t_{21} = -1.78
Water depth (cm)	10.6 ± 4.8	15.4 ± 16.2	t_{21} = -0.94
Male length (cm)	3.58 ± 0.38	3.39 ± 0.17	t_{14} = 1.55
Male width (cm)	1.09 ± 0.14	1.03 ± 0.09	t_{21} = 1.21
Male weight (g)	0.89 ± 0.15	0.79 ± 0.14	t_{21} = 1.61

Results, Discussion, and Conclusion: We investigated the physical and biological features required by the croaking gouramis to complete their life cycle. They inhabited shallow bodies of water (Table I) with dense vegetation that were similar to the natural habitat of wild Siamese fighting fish [2]. They produced bubble nests on the water surface consisting of mucus-covered bubbles that stuck together. The water in which the fish bred was characterized by low values in pH, dissolved oxygen, salinity, turbidity, biochemical oxygen demand, nitrite, nitrate, and phosphate but high values in conductivity, temperature, and total dissolved solids (Table I). Thus this species is a labyrinth fish that is well adapted to warm water with very low oxygen levels. Water and fish characteristics in the rice paddy fields differed from those found in the ponds in terms of pH, conductivity, dissolved oxygen, total dissolved solids, phosphate, water depth, temperature, and population density (Table I). Significant sexual dimorphism was noted as males were heavier and bigger than females (Table II). There was a high correlation between male weight and body length and width (Pearson correlation coefficient: length: r_{175}=0.797, P<0.001; width: r_{175}=0.823, P<0.001) whereas female weight correlated only with body length (Pearson correlation coefficient: length: r_{10}=0.735, P<0.05; width: r_{10}=0.430, NS). All 114 bubble nests (Table III) were located near the edges of rice paddy fields and in small ponds. There was no correlation between the bubble nest area and the male body length and weight(Pearson correlation coefficient: length: r_{114}=0.056, NS; width: r_{114}=0.041, NS).

Table II. Mean ± S.D. of croaking gouramis weight, length and width. *n*, Sample sizes; *t* – tests were two-sample tests with separated variance and Bonferroni adjustment, $*P < 0.005$, $**P < 0.001$

Body measurement	Males (n = 175)	Females (n = 10)	t - test
Weight (g)	0.85 ± 0.23	0.59 ± 0.10	t_{183} = 3.70**
Length (cm)	3.51 ± 0.35	3.16 ± 0.21	t_{183} = 3.07*
Width (cm)	1.03 ± 0.12	0.91 ± 0.05	t_{17} = 7.30**

Table III. Mean ± S.D. and range of bubble nest width, length, depth, area, the shortest distance between nests, and egg diameter. *n*, Sample sizes

Measurement	n	Mean ± S.D.	Range
Bubble nest width (cm)	114	1.78 ± 0.82	0.37 – 4.15
Bubble nest length (cm)	114	1.94 ± 1.08	0.18 – 6.00
Bubble nest area $(cm)^2$	114	12.12 ± 10.80	3.00 – 98.0
Water depth (cm)	114	14.08 ± 16.53	0.21 – 75.4
Distance to the nearest nest (cm)	7	60.4 ± 42.6	9.0 – 120.0
The number of eggs per nest	2	373.5 ± 38.9	346 - 401
Egg diameter (mm)	20	0.78 ± 0.08	0.64 – 0.92

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[2]. Jaroensutasinee, M., Jaroensutasinee, K. *J. Fish Biol.* 2001, 58, 1311-1319.

Keywords: habitat characteristics; bubble nest size; croaking gouramis; *Trichopsis vittata*

Male body size, female mate choice and male contest in croaking gouramis (*Trichopsis vittata*)

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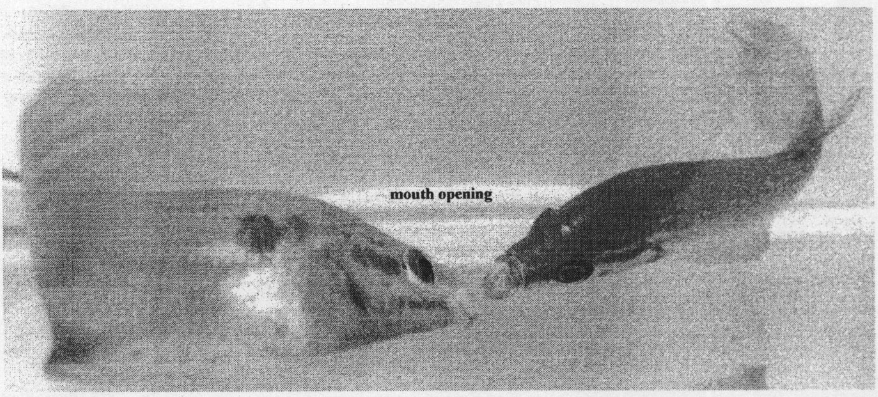
Abstract: Female croaking gourami (*Trichopsis vittata* Cuvier, 1831) did not choose their mates based on male size, although the larger males won more fights. There were differences in fighting duration between ±0 SD treatment (the same body size treatment) and ±1 SD treatment (the different body size treatment). Comparing agonistic behaviours between both treatments, the fighters of ±0 SD treatment performed more vocal display, biting, and total agonistic behaviour than in the ±1SD treatment. Considering each agonistic behaviour separately between large and small males in ±1SD treatments, the larger males performed more chasing than the smaller ones and the smaller males displayed more escaping than the larger males. The results suggest that sexual size dimorphism in this species which males are larger than female may derive only from the male-male competition.

Introduction:

This study is first to investigate whether a larger male gourami has an influence on female choice and/or male-male competition.



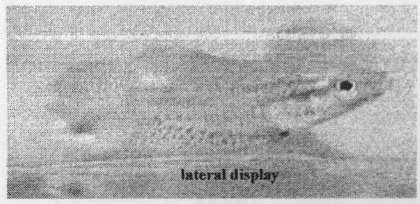
Hypothesis:

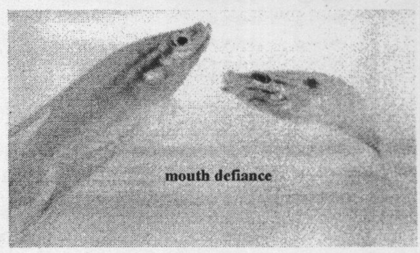
* If female choice is influenced by male body size, then female should spend more time with the larger male than the smaller one.
* If male contest provides an additional cue in mating success, then the larger male should win the fight.
* Fighting duration should decrease as the body size difference between the males increases.

Methodology:

◆ The female preference experiments were being carried out in an aquarium divided into three compartments. The two equal smaller compartments were housed males and the larger one contained the gravid female. Each trial comprised of two 10-min observation sessions with a 5-min interval. During observation periods, the behaviours of the trio of fish were noted and the number of seconds the females spent in each position of the three compartments was recorded in the computer. Two treatments were conducted using males in which there was a body length difference of ± 0 SD and ± 1 SD of male body length.

◆ For the male-male competition tests, the same pairs of tested males from the female preference tests were placed in a 1 litre bottle. Agonistic behaviours were recorded including approach, lateral display, vocal display, biting, mouth opening, going round in a circle, adherence, surfacing, mouth defiance, chasing and escaping. Fighting duration were recorded until one of the males retreated.





Results and Discussion:

There was no female preference with greater male body size (independent sample *t*-test, t_{114} = 0.39, $P<0.001$; Fig.1). Larger males won more fights (χ^2 test for ±1SD treatment: 15.52, $P<0.05$). The larger males performed more chasing than the smaller ones (paired *t*-tests for chasing: t_{39} =3.147, $P<0.05$) and the smaller males displayed more escaping than the larger males (paired *t*-tests for escape: t_{39} =-3.147, $P<0.05$; Fig. 2). Thus sexual size dimorphism in this species may evolutionarily derive, easily maintain and be more obvious by only male contest. The male-male competition and female mate choice can sometimes work contemporaneously in the opposite directions (Qvarnstrom & Forsgren *1998*). This balancing sexual selection on the male body size of gouramis may act to maintain the genetic variation in sexually selected traits (Moore & Moore *1999*).

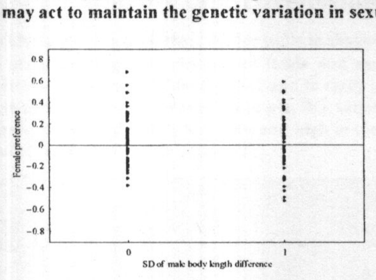


Fig. 1 Female preference and male size differences of ± 0 SD and ±1 SD in male standard body length.

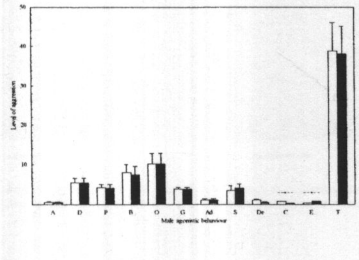


Fig. 2 Mean (±SE) of agonistic behaviour between large (□), and small (■) males in ± 1SD treatments, n = 40 fighting pairs. Male agonistic behaviour includes approach (A), lateral display (D), sound producing (P), biting (B), mouth opening (O), Going round in a circle (G), adherence (Ad), surfacing (S), mouth defiance (De), chasing (C), escaping (E) and total agonistic behaviour (Total). * $P<0.05$.

Reference:

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Keywords:

male body size, female mate choice, male contest, croaking gouramis, *Trichopsis vittata*

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HABITAT CHARACTERISTICS OF CROAKING GOURAMIS (*Trichopsis vittata*)

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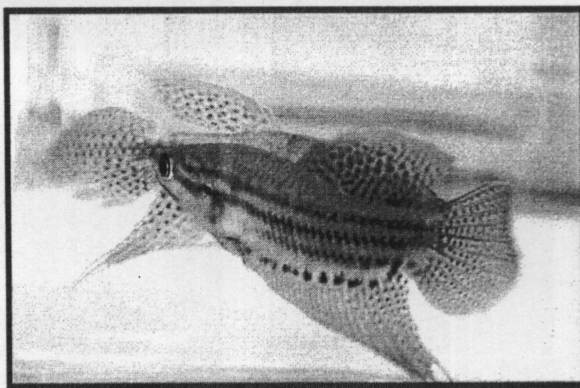
Abstract

We investigated two types of croaking gourami habitat in Thailand: rice paddy fields and small ponds. We found that gouramis inhabit shallow water with dense vegetation near the margin of rice paddy fields and small ponds. Nesting water was low in pH, dissolved oxygen, salinity, transparency, biochemical oxygen demand, nitrite, nitrate, phosphate and water depth but high in specific conductance, total dissolved solids and temperature. We compared the water characteristics and croaking gourami body sizes between two natural habitats. We found that the different characteristics were pH, specific conductance, dissolved oxygen, total dissolved solids, phosphate, water depth, temperature and population density. Generally, males were heavier and bigger than females. Male weight was positively correlated with its length and width but female weight was only positively correlated with length. Bubble nest area was not correlated with male length and weight.



Introduction

During breeding seasons, many fish seek out potential mating partners and suitable spawning habitats [1]. This study was conducted to gain further understanding of the croaking gouramis, which are native to Thailand, in their natural environment and the physiological ecology of their habitats. Understanding of their basic ecology is limited.

Hypotheses

1. **If the characteristics of the rice paddy fields and the small ponds are significantly different, then the sizes of the fish and their bubble nest areas from both habitats are significantly different.**
2. **If bubble nest areas depend on the sizes of the builders, then the bubble nest areas should be positively correlated with the male body length and/or weight.**

Methodology

All fieldwork was carried during the middle of a breeding season (April and May 2004) in Nakhonsithammarat Province, Thailand. Ten individual 4 m^2 plots were marked on the ground surface of the rice paddy fields and small ponds. The water depth of bubble nests was measured. The bubble nests were photographed for measuring width (*a*) and length (*b*) in the laboratory. Bubble nest area was estimated using the ellipsoid equation ($A = \pi ab$). The shortest distance between bubble nests was measured by tape measure before capturing the male nest builders. The water characteristics were measured before capturing all the fish in the plot and calculating the population density and adult sex ratio. The adult sex ratio was calculated as the number of sexually active males divided by the total number of sexually active adult of both sexes in the plot [2]. To improve estimate of average male and female body sizes, water depth and bubble nest sizes, additional fish outside the 20 plots were collected and their bubble nests were measured. All eggs were collected from the nests and counted. Ten randomly selected eggs from each nest were measured to the nearest 0.01 mm using a Vernier Caliper.

Results and Discussion

Most croaking gouramis built their bubble nests in shallow water with dense vegetation along the edges of rice paddy fields and small ponds to minimise predation risks, to hold the bubbles together and to easily pick up the eggs back to their bubble nests. Nesting water was low in pH, DO, salinity, transparency, BOD, nitrite, nitrate, phosphate and water depth and high in specific conductance, total dissolved solids and temperature (Table I).

Table I. The mean ± S.D. of water characteristics in habitat use by croaking gouramis, their bubble nest sizes and their sizes. *$P < 0.05$, ** $P < 0.001$.

Characteristics	Mean ± S.D.		*t* - test
	Rice Paddy Field	Small Pond	
pH	6.82 ± 0.66	6.00 ± 0.53	t_{58} = 5.28**
Specific conductance ($\mu S \cdot cm^{-1}$)	35.43 ± 7.49	51.38 ± 13.33	t_{58} = -5.71**
Dissolved oxygen ($mg \cdot l^{-1}$)	5.51 ± 1.34	1.47 ± 0.76	t_{46} = 14.33**
Total dissolved solids ($mg \cdot l^{-1}$)	17.95 ± 3.73	25.77 ± 6.63	t_{58} = -5.63**
Salinity (ppm)	0.0 ± 0.0	0.0 ± 0.0	-
Transparency (cm)	11.8 ± 7.6	11.6 ± 6.6	t_{58} = 0.12
Biochemical oxygen demand ($mg \cdot l^{-1}$)	2.6 ± 1.0	2.6 ± 0.8	t_{58} = -0.04
Nitrite ($mg \cdot l^{-1}$)	0.00 ± 0.00	0.02 ± 0.08	t_{29} = -1.44
Nitrate ($mg \cdot l^{-1}$)	0.01 ± 0.06	0.00 ± 0.00	t_{29} = 1.00
Phosphate ($mg \cdot l^{-1}$)	0.07 ± 0.17	0.00 ± 0.00	t_{29} = 2.28*
Water depth (cm)	11.1 ± 4.0	50.4 ± 27.4	t_{30} = -7.79**
Temperature (°C)	31.65 ± 1.71	29.80 ± 1.09	t_{49} = 5.00**
Population density ($individuals \cdot m^{-2}$)	2.23 ± 2.21	7.83 ± 7.41	t_{11} = -2.29*
Adult sex ratio	0.81 ± 0.29	0.80 ± 0.32	t_{18} = 0.10
Bubble nest width (cm)	1.83 ± 0.79	2.45 ± 0.88	t_{21} = -1.79
Bubble nest length (cm)	3.58 ± 0.38	3.39 ± 0.17	t_{21} = -1.60
Bubble nest area (cm^2)	12.36 ± 8.07	20.71 ± 13.52	t_{21} = -1.78
Water depth at bubble nests (cm)	10.6 ± 4.8	15.4 ± 16.2	t_{21} = -0.94
Male length (cm)	3.58 ± 0.38	3.39 ± 0.17	t_{14} = 1.55
Male width (cm)	1.09 ± 0.14	1.03 ± 0.09	t_{21} = 1.21
Male weight (g)	0.89 ± 0.15	0.79 ± 0.14	t_{21} = 1.61

Between two natural habitats, some water characteristics were significantly different but fish body sizes and their bubble nest sizes were not significantly different. This reject the first hypothesis. **This result implies that croaking gouramis could occupy a wide range of habitat types.**

No correlation between the bubble nest area and the male body length and weight (Pearson correlation coefficient: length: r_{114} = 0.056, NS; width: r_{114} = 0.041, NS). This reject the second hypothesis. **Croaking gouramis bubble nests are small and similar in size because they would be less costly to build and guard.**

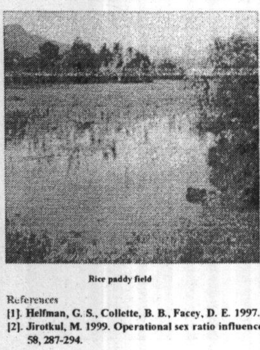
Significant sexual dimorphism was noted as males were heavier and bigger than females

Male weight was positively correlated with body length and width.

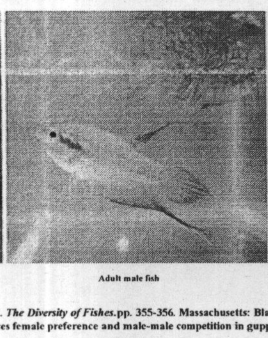
(Pearson correlation coefficient: length: r_{175} = 0.797, $P < 0.001$; width: r_{175} = 0.823, $P < 0.001$)

Female weight was correlated only with body length.

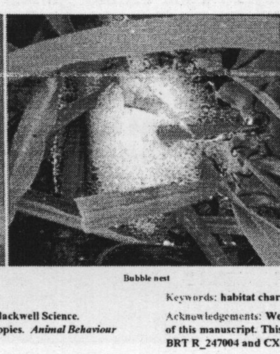
(Pearson correlation coefficient: length: r_{10} = 0.735, $P < 0.05$; width: r_{10} = 0.430, NS)



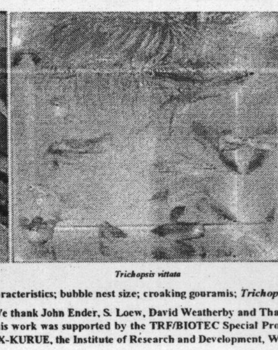
Rice paddy field



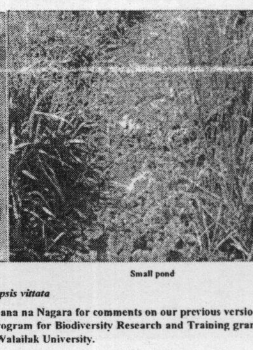
Adult male fish



Bubble nest



Trichopsis vittata



Small pond

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[2]. Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour* 58, 287-294.

Keywords: habitat characteristics; bubble nest size; croaking gouramis; *Trichopsis vittata*

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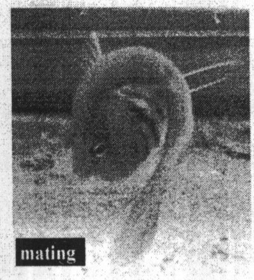
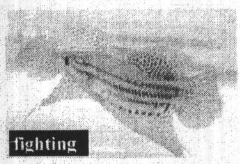
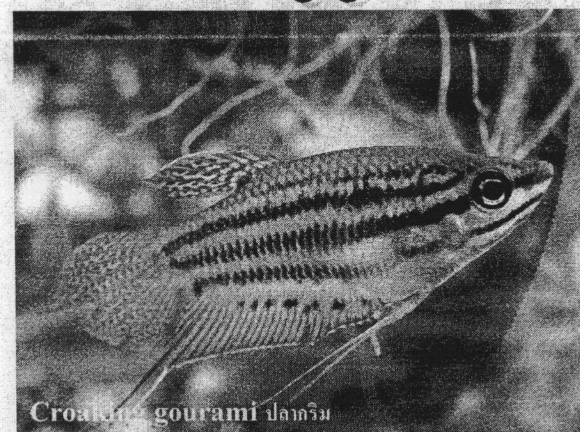
Habitat characteristics and sexual selection of croaking gouramis



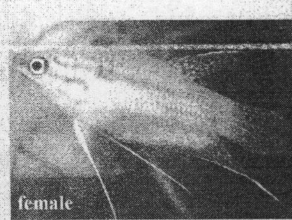
mating



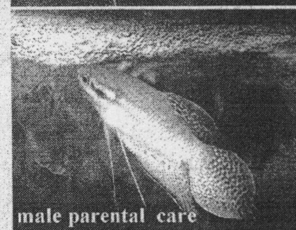
fighting



Croaking gourami ปลากริม



female

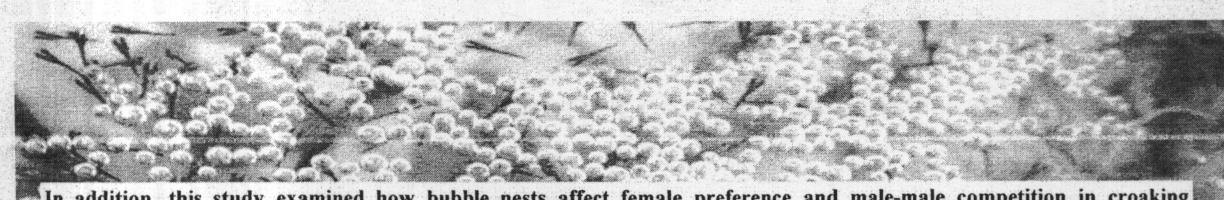


male parental care

Croaking gouramis *Trichopsis vittata* Cuvier, 1831) were investigated in rice paddy fields and small ponds. The fish inhabit shallow standing water with dense vegetation near the margin of both habitats. Nest sites were characterised by water that was relatively low in pH, DO, transparency, BOD, nitrite, nitrate, phosphate and water depth but high in specific conductance, total dissolved solids and temperature. Compared to those in ponds, nest sites in rice paddies were higher in pH, DO, and temperature but lower in specific conductance, total dissolved solids and water depth. Population densities of croaking gouramis were lower in rice paddies than in small ponds. Males were heavier and bigger than females. There was no correlation between the bubble nest area and the male body length and weight. Male and female embraced many times during mating. There were (mean $\pm$ SD) 3.74$\pm$1.02 eggs/embracement, 84$\pm$12.97 embracement/spawning, and 314 $\pm$ 109.77 eggs/spawning with a spawning duration of 178 $\pm$ 51.17 min. Fish larvae hatched within 30.75 $\pm$ 0.55 hr and 2-day old fry's sizes were 3.21 $\pm$ 0.29 mm

Female croaking gouramis did not choose their mates based on male size, although large males won more fights than small males. Fighting durations in $\pm$ 0 SD treatment (i.e., same male body size treatment) were longer than in $\pm$ 1 SD treatment (i.e., different male body size treatment). Comparing antagonistic behaviour between these two treatments, males in $\pm$ 0 SD treatment performed more sound production, biting, and total antagonistic behaviour than males in $\pm$ 1 SD treatment. Comparing between large and small males in $\pm$ 1 SD treatment, large males performed more chasing and less escaping than small ones. The results suggest that sexual size dimorphism in croaking gourami, in which males are larger than females, may function for male-male competition rather than female preference.

In addition, this study examined how bubble nests affect female preference and male-male competition in croaking gouramis. We divided our study into three treatments: (1) two-bubble-nests, (2) one-bubble-nest and (3) no-bubble-nests. We found that females preferred small bubble nest males over large ones. There are four possible reasons: small bubble nests are available, will be enlarged during mating, contain more oxygen for the eggs and hatching young, and more camouflaged than larger bubble nests. Surprisingly, females preferred males with no bubble nest over males with bubble nests. In addition, when we compared female preference among three treatments, females spent more time with males in no-bubble-nest treatment than in two-bubble-nest treatment. These results suggest that females may perceive bubble nest presence and large bubble nest size as a negative clue of male quality because this may suggest that these males already spend their energy reserve in nest building and territorial defence. In male-male competition, large bubble nest males did not win more fights. There were no differences in the numbers of agonistic behaviour, the total agonistic behaviour and the winning status between males who built bubble nests and males who did not build bubble nests. Winners in all treatments performed a higher number of adherence and chasing behaviour than losers. There was no significant difference in fighting duration between the three treatments.



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ความหมายที่ซ่อนเร้นในเสียงปลากริม

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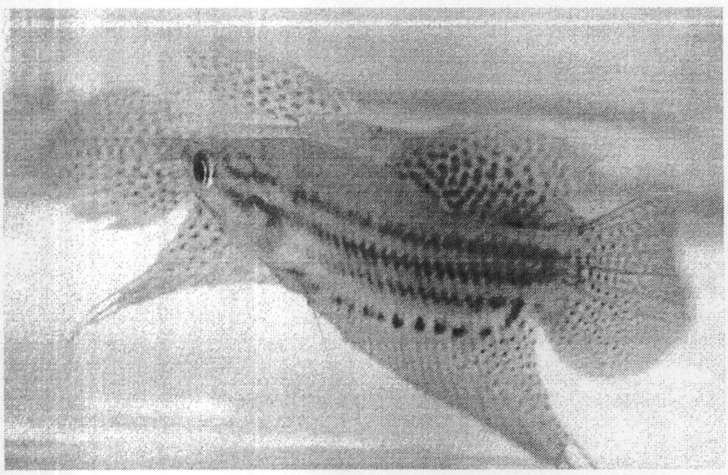
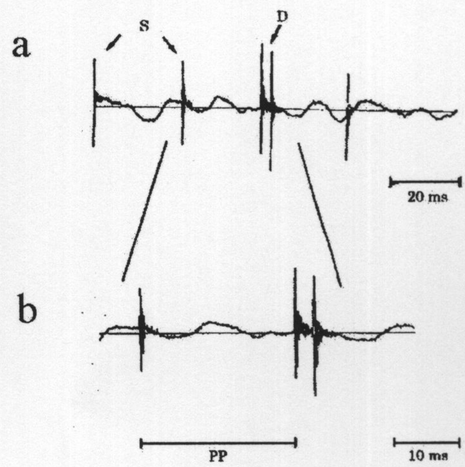
"กร่อดๆๆๆๆๆๆๆๆๆๆๆ กร่อดๆๆๆๆๆๆๆๆๆๆ" เสียงอะไรดัง หันซ้ายแลขวาก็ไม่เห็นมีอะไรที่จะทำให้เกิดเสียงดังได้ "กร่อดๆๆๆๆๆๆๆๆ กร่อดๆๆๆๆๆๆๆๆ" ดังอีกแล้ว ใครเอาไม้มาเคาะขวดเล่นแถวนี้นะ เสียงนี้จะเป็นอย่างอื่นไปไม่ได้นอกจากเสียงไม้เคาะขวด ถ้าไม่บังเอิญหันไปเห็นปลากริมสองตัวที่เพิ่งจับได้จากร่องสวนหลังบ้านเมื่อเช้า และนำมาใส่ขวดโหลไว้ กำลังเข้าเทียบคู่กันแบบสลับหัวสลับหางพร้อมทั้งสั่นลำตัวและครีบอกอย่างรวดเร็วพร้อมๆ กันทั้งสองตัว (lateral display) และจังหวะการสั่นนี้ก็เข้าได้ดีกับจังหวะของเสียงที่ดังขึ้นพอดี ทำให้ต้องวางหนังสือที่กำลังอ่านค้างอยู่และเข้าไปดูใกล้ๆ ใช่จริงๆ ด้วย ปลากริมสร้างเสียงได้ !!! ไม่น่าเชื่อเลย แต่เมื่อได้ไปค้นคว้าเพิ่มเติมก็ทำให้ทราบว่าชื่อสามัญของปลากริม คือ croaking gourami ก็มีที่มาจากการที่ปลากริมสามารถสร้างเสียงได้นั่นเอง





ปลากริมสร้างเสียงได้อย่างไร

ปลากริมมีอวัยวะสร้างเสียงอยู่ที่บริเวณครีบอก (pectoral fin) ซึ่งจะประกอบด้วยเอ็นขนาดใหญ่ 2 เส้นที่ยึดติดกับแถบของครีบอกเส้นที่ 4 และเส้นที่ 5 คล้ายกับสายของกีตาร์ ดังนั้นเมื่อสั่นครีบอกอย่างเร็วและแรงก็จะให้เอ็นนี้สั่นด้วยและทำให้เกิดเสียงดังขึ้นได้

ปลากริมทั้งตัวผู้และตัวเมียจะเริ่มสร้างเสียงได้เมื่ออายุประมาณ 2 เดือน โดยเสียงในช่วงแรกนี้จะเป็นเสียงจังหวะเดี่ยว (single pulse) เนื่องจากครีบอกแต่ละข้างจะมีเอ็นเพียงเส้นเดียวที่เจริญได้เต็มที่ ทำให้เสียงที่ได้มีลักษณะแหลมสูงแต่เบา ต่อมาเมื่อปลากริมโตเต็มที่ คือมีอายุได้ประมาณ 4 เดือน อวัยวะสร้างเสียงคือเอ็นทั้งสองเส้นจะเจริญเต็มที่ ทำให้เสียงที่สร้างในช่วงหลังนี้เป็นเสียงจังหวะคู่ (double pulse) โดยเสียงนี้จะทุ้มต่ำและดังกว่าเดิม นอกจากนี้ยังพบว่าถ้าตัดเอ็นเส้นใดเส้นหนึ่งออกในระยะนี้ เสียงที่เคยเป็นจังหวะคู่ก็จะกลายเป็นเสียงจังหวะเดี่ยวได้

ทำไมปลากริมต้องสร้างเสียง

ปลากริมมีวิวัฒนาการให้สามารถสร้างเสียงได้ก็เพราะว่าปลากริมชอบที่จะอาศัยอยู่ในแหล่งน้ำตื้นที่นิ่งมีพืชน้ำขึ้นอยู่อย่างหนาแน่น มีตะกอนตกทับถมมากมายทำให้น้ำขุ่น ดังนั้นการสื่อสารด้วยการมองเห็นจึงทำได้ยาก ดังนั้นจึงมีวิวัฒนาการให้สามารถสื่อสารด้วยเสียงได้ และเสียงนี้สำคัญต่อปลากริมอย่างมาก เชื่อไหมว่าเสียงสามารถบอกอายุและขนาดตัวของเจ้าของเสียงได้ เนื่องจากเสียงยิ่งทุ้มต่ำและดังมากเท่าไรขนาดตัวของเจ้าของเสียงก็ยิ่งใหญ่เท่านั้น ดังนั้นถ้าเอาปลากริมสองตัวมากัดกัน ตัวที่ชนะก็มักจะเป็นตัวที่สร้างเสียงทุ้มต่ำและดังกว่า ดังนั้นการประเมินอายุ ขนาดตัว และความสามารถของคู่ต่อสู้ได้จึงช่วยลดการได้รับบาดเจ็บหรือการตายจากการต่อสู้ได้ด้วย และคาดว่าปลากริมตัวเมียอาจจะประเมินคุณภาพของปลากริมตัวผู้จากเสียงที่สร้างขึ้นในระหว่างที่มีการเกี้ยวพาราสีก็ได้ แหมไม่น่าเชื่อเลยว่าเสียงจะมีความหมายได้มากขนาดนี้ เข้าทำนองคารมเป็นต่อรูปหล่อเป็นรอง

ลีลารักปลากริม

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ใครจะรู้บ้างว่า "ปลาเอ๋ยปลากริม" คำที่พวกเราร้องเล่นกันตั้งแต่เด็กๆ นั้น ความจริงแล้วปลาชนิดนี้มีชื่อเรียกได้หลายชื่อ เช่น ปลากริมควาย ปลากริมข้างลาย ปลาหมัด หรือปลากัดป่า เนื่องจากมีลักษณะหลายอย่างคล้ายปลากัดมาก เช่น รูปร่างลักษณะ การสร้างหวอด รวมทั้งแหล่งที่อยู่อาศัย แต่ชื่อสามัญนิยมเรียกเหมือนกันว่า croaking gourami ตามความสามารถในการสร้างเสียง มีชื่อวิทยาศาสตร์ว่า *Trichopsis vittata* ตามแถบสีดำที่ปรากฏอยู่ทางด้านข้างของลำตัว

ปลากริมตัวผู้และปลากริมตัวเมียมีลักษณะคล้ายกันมาก ปลากริมตัวผู้มักจะมีสีสันสดใสกว่า มีครีบและหางที่ยาวกว่าปลากริมตัวเมียเล็กน้อย แต่ลักษณะที่ใช้แยกเพศปลาได้อย่างถูกต้อง คือ การยกภาชนะใสที่แสงสามารถส่องผ่านได้ดี เช่นขวดแก้วที่ใส่ปลาขึ้นส่องกับแสงไฟ หรือแสงแดด ถ้าเห็นรังไข่ในท้องก็แสดงว่าเป็นปลากริมตัวเมียอย่างแน่นอน

เมื่อปลากริมอายุได้ประมาณ 4 เดือน ปลากริมตัวผู้จะสร้างรังหรือที่เรียกกันทั่วๆ ไปว่า "หวอด (bubble nest)" ซึ่งเป็นฟองอากาศขนาดเล็กจำนวนมากยึดติดกันเป็นแพลอยอยู่ที่ผิวน้ำ ส่วนรังไข่ของตัวเมียจะขยายขนาดใหญ่ขึ้นมากและมีสีเหลืองนวล เนื่องจากภายในมีไข่บรรจุอยู่เต็ม เมื่อสภาพร่างกายพร้อมและสภาพแวดล้อมเป็นใจ คือ ช่วงเวลาบ่ายที่เงียบสงบปลากริมหนุ่มก็จะเริ่มเกี้ยวพาราสีปลากริมสาว โดยปลากริมหนุ่มจะว่ายน้ำวนเข้าไปหาปลากริมสาว พร้อมทั้งสร้างเสียงดังกร่อดๆๆๆๆๆๆ ทำอย่างนี้ไปเรื่อยๆ ถ้าปลากริมสาวตกลงปลงใจด้วยก็จะใช้ปากชนไปที่ลำตัวของปลากริมหนุ่ม เมื่อได้รับการสนองตอบดังนี้แล้วปลากริมหนุ่มก็จะเริ่มบรรเลงลีลารักทันทีโดยการงอลำตัวของตนเข้าโอบรัดท้องของปลากริมสาว เมื่อปลากริมสาวปล่อยไข่ออกมาปลากริมหนุ่มก็จะฉีดน้ำเชื้อเข้าผสมกับไข่ทันที แล้วรีบกลับตัวเพื่อว่ายน้ำไปเก็บก้อนสีขาวขุ่นที่ประกอบด้วยไข่ประมาณ 3-5 ฟองนี้ไปเก็บไว้ภายในหวอดที่สร้างรอไว้ พร้อมทั้งพ่นฟองอากาศเพิ่มปิดทับไข่ไว้อีกชั้นเพื่อไม่ให้ไข่ตกลงมาจากหวอด จากนั้นปลากริมหนุ่มก็จะว่ายน้ำกลับไปหาปลากริมสาวอีกและแสดงลีลารักเช่นนี้ซ้ำๆ อีกประมาณ 80-100 ครั้ง ซึ่งกว่าจะเสร็จสิ้นภารกิจรักนี้ก็ใช้เวลาไปประมาณ 2-3 ชั่วโมง แต่ก็ได้ผลงานที่น่าพอใจคือสามารถอมไข่ไปเก็บไว้ภายในหวอดได้มากถึงประมาณ 300-400 ฟองต่อการผสมพันธุ์หนึ่งครั้ง

เมื่อรัดจนไข่ถูกปล่อยออกจากตัวปลากริมสาวหมดแล้ว ปลากริมหนุ่มก็จะหมดความสนใจในตัวปลากริมสาวแถมยังเบื่อเอามากๆ เพราะมันจะว่ายน้ำไล่ให้ปลากริมสาวออกไปให้ห่างไกลจากหวอดด้วยท่าทางที่แสนจะก้าวร้าว ถ้าเราสงสารกลัวปลากริมหนุ่มจะเหนื่อยไปมากกว่านี้ หรือกลัวปลากริมสาวจะถูกทำร้ายก็ควรแยกปลากริมสาวออกไปจากตู้ปลาทันทีหลังการผสมพันธุ์สิ้นสุดลง

ในระยะนี้ปลากริมหนุ่มจะไม่สนใจอาหาร แต่จะคอยเฝ้าอยู่ใกล้ๆ หวอดตลอดเวลา พร้อมทั้งคอยพ่นฟองอากาศเพิ่มจนหวอดมีขนาดใหญ่มาก และภายใน 30-33 ชั่วโมงไข่ก็จะฟักออกเป็นตัว แต่ยังคงเกาะติดอยู่กับหวอดต่อไป หากมีตัวอ่อนบางตัวหลุดจากหวอด พ่อปลาก็จะรีบเข้าไปอมแล้วนำไปปล่อยไว้ที่หวอดอย่างเดิม พ่อปลาจะเฝ้าดูแลลูกๆ ของมันอย่างใกล้ชิดและทะนุถนอม แต่อย่าเพิ่งซาบซึ้งกับวีรกรรมของพ่อปลามากเกินไป เพราะหลังจากนั้นอีกประมาณ 2 วัน เมื่อลูกปลาสามารถว่ายน้ำเองได้อย่างอิสระ และมีความยาวลำตัวประมาณ 3-4 มิลลิเมตร พ่อปลาซึ่งอดอาหารมานานตั้งแต่เริ่มผสมพันธุ์ก็จะหิวมากแล้วว่ายน้ำไล่กินลูกของมันเอง ดังนั้นจึงควรแยกพ่อปลาออกไปในระยะนี้ หรืออาจใส่พืชน้ำให้หนาแน่นขึ้นเพื่อให้ลูกปลาได้ใช้หลบซ่อนจากพ่อปลาผู้หิวโหย

อยากรู้จังว่าลูกๆ ของมันจะรอดพ้นจากปากพ่อของมันได้สักกี่ตัว และปลากริมสาวใช้เกณฑ์อะไรในการเลือกปลากริมหนุ่มมาเป็นพ่อดูแลลูกของมัน

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ปลากริม หรือในบางท้องที่อาจเรียกว่าปลากริมควาย ปลากริมข้างลาย ปลาหมัด หรือปลากัดป่า มีชื่อสามัญว่า croaking gourami ตามความสามารถในการสร้างเสียง มีชื่อวิทยาศาสตร์ว่า *Trichopsis vittata* ตามแถบสีดำ 3 แถบที่พาดไปตามยาวของลำตัว ปลากริมเป็นปลาเขตร้อนที่พบแพร่กระจายอยู่ทั่วไปตามแหล่งน้ำจืดที่นิ่งและตื้นมีพืชน้ำขึ้นอยู่อย่างหนาแน่น เช่น หนองน้ำเล็กๆ ท้องร่องสวน หรือแม้แต่ในนาข้าวที่มีน้ำท่วมขัง

ปลากริมเป็นปลาขนาดเล็กมีความยาวลำตัวประมาณ 3-5 เซนติเมตร รูปร่างคล้ายปลากัด ปากค่อนข้างแหลม ที่ขากรรไกรทั้งสองข้างมีฟันรูปสามเหลี่ยมซี่เล็กๆ เรียงอยู่หนึ่งแถว ครีบหลังยาวเรียวมีขนาดสั้นกว่าครีบท้อง หางมีลักษณะคล้ายใบโพธิ์ ปลากริมแต่ละตัวมีสีสันแตกต่างกันไปแม้จะเป็นปลาจากแหล่งเดียวกัน โดยทั่วไปแล้วจะมีสีน้ำตาล สีน้ำเงิน สีเขียว และสีแดงผสมกัน ปลากริมตัวผู้และปลากริมตัวเมียมีลักษณะคล้ายกันมาก แต่ปลาตัวผู้มักจะมีสีสันสดใสกว่า มีครีบและหางยาวกว่าปลาตัวเมียเล็กน้อย ซึ่งลักษณะที่ใช้แยกตัวปลาได้อย่างถูกต้อง คือ การยกภาชนะที่ใส่ปลาขึ้นส่องกับแสงไฟ หรือแสงแดด ถ้าเห็นรังไข่ในท้องก็แสดงว่าเป็นปลากริมตัวเมียอย่างแน่นอน

โดยทั่วไปไม่นิยมใช้ปลากริมเป็นอาหาร แต่มักเลี้ยงไว้ดูเล่นในขวดแก้ว เนื่องจากเป็นปลาท้องถิ่นที่หาได้ง่าย มีสถานภาพที่ไม่เสี่ยงต่อการสูญพันธุ์ในธรรมชาติ แข็งแรง กินง่าย และที่สำคัญเลี้ยงง่าย จึงเป็นปลาเริ่มต้นที่ดีเหมาะสำหรับนักเลี้ยงปลามือใหม่ นอกจากนี้ในบ้านเราอาจมีการนำปลากริมตัวผู้มากัดกันเช่นเดียวกับปลากัด แต่กัดได้ไม่ทนเหมือนปลากัด เนื่องจากปลากริมมีนิสัยไม่ก้าวร้าว เวลากัดกันปลาทั้งสองตัวจะสั่นลำตัวอย่างแรงและตีครีบอกอย่างเร็วทำให้เกิดเสียงดังสามารถได้ยินอย่างชัดเจน ในประเทศแถบยุโรปจะนำปลากริมมาคัดเลือกพันธุ์และผสมพันธุ์เพื่อให้มีลักษณะที่สวยงามมากขึ้น นิยมขายเป็นปลาตู้ เนื่องจากสามารถเลี้ยงร่วมกับปลาชนิดอื่นได้ มีนิสัยรักสงบ

การเลี้ยงปลากริม สามารถทำได้ 2 วิธี คือ การเลี้ยงเดี่ยวไว้ดูเล่นในขวดแก้ว และการเลี้ยงคู่เพื่อผสมพันธุ์ในอ่างเลี้ยงปลา ซึ่งการจับปลาจากสภาพธรรมชาตินั้นควรนำน้ำในแหล่งนั้นมาด้วย เพื่อใช้เลี้ยงปลาในระยะแรก จากนั้นจึงค่อยเปลี่ยนมาใช้น้ำบ่อหรือน้ำประปาที่ได้เปิดพักไว้แล้วประมาณ 1 สัปดาห์ และอาหารที่ใช้มีได้อย่าง เช่น ลูกน้ำ ไรแดง หรือแม้แต่ไข่ขนาดเล็กและตัวอ่อนของมดแดง

การเลี้ยงปลากริมในขวดแก้วเพื่อดูเล่น

การเลี้ยงปลากริมแบบนี้ไม่ยุ่งยากมากนัก เนื่องจากเป็นการเลี้ยงปลาเพียงตัวเดียวในขวดขนาด 1 ลิตร ให้นำปลากริมและน้ำจากแหล่งน้ำมาเทใส่ในขวดแก้วให้มีความสูงของน้ำประมาณ 12 เซนติเมตร ถ้าจำเป็นต้องวางขวดใกล้กันก็ควรคั่นด้วยกระดาษ เพื่อบังไม่ให้ปลามองเห็นกันได้ เพราะถ้าปลามองเห็นกันได้ ปลาจะแสดงพฤติกรรมก้าวร้าวต่อกันตลอดเวลา ทำให้ปลาอ่อนเพลียและเกิดความเครียด ควรให้อาหารวันละ 2 ครั้งเช้าและเย็น และเปลี่ยนน้ำทุกๆ 3-4 วัน โดยใส่เกลือเม็ดลงไปในขวดด้วย 1-2 เม็ด และฉีกใบหูกวางแห้งใส่ลงไปในขวดด้วยเล็กน้อย เพื่อไม่ให้ปลาเป็นโรค นอกจากนี้อาจปักก้านของพืชน้ำลงไปในขวดด้วยก็ได้ แต่จะทำให้น้ำเสียเร็ว ต้องเปลี่ยนน้ำบ่อยขึ้น ถ้าโชคดีเราอาจเห็นปลากริมตัวผู้สร้างหวอดในขวดก็ได้

การเลี้ยงปลากริมในอ่างเลี้ยงปลาเพื่อเพาะพันธุ์

การเลี้ยงปลากริมเพื่อเพาะพันธุ์นี้ต้องใส่พืชน้ำลงในอ่างเลี้ยงปลาด้วย และต้องวางอ่างนี้ไว้ในที่ที่เงียบสงบ โดยในระยะแรกใส่เฉพาะปลากริมตัวผู้ลงในอ่างเลี้ยงปลา ส่วนปลากริมตัวเมียที่มีไข่สุกเต็มที่จะแยกใส่ไว้ในขวดแก้วขนาด 1 ลิตร แล้ววางขวดนี้ไว้ในอ่างเลี้ยงปลาเพื่อใช้กระตุ้นให้ปลาตัวผู้ในอ่างสร้างหวอด เมื่อปลาตัวผู้สร้างหวอดแล้ว จึงเทปลาตัวเมียลงในอ่าง ในช่วงบ่ายถึงเย็นปลากริมตัวผู้จะเริ่มเกี้ยวพาราสีปลากริมตัวเมียโดยการว่ายน้ำไปรอบๆ ปลาตัวเมีย แล้วสร้างเสียง ถ้าปลากริมตัวเมียตกลงที่จะผสมพันธุ์ด้วยก็จะใช้ปากชนไปที่ลำตัวของปลากริมตัวผู้ เมื่อได้รับการสนองตอบดังนี้แล้วปลากริมตัวผู้ก็จะงอลำตัวของตนเข้าโอบรัดท้องของปลากริมตัวเมียเพื่อให้ปลากริมตัวเมียปล่อยไข่ออกมา จากนั้นปลากริมตัวผู้ก็จะฉีดน้ำเชื้อเข้าผสมกับไข่ทันที แล้วรีบกลับตัวเพื่อว่ายน้ำไปเก็บก้อนสีขาวขุ่นที่ประกอบด้วยไข่ประมาณ 3-5 ฟองนี้ไปเก็บไว้ภายในหวอดที่สร้างรอไว้ พร้อมทั้งพ่นฟองอากาศเพิ่มปิดทับไข่ไว้อีกชั้นเพื่อไม่ให้ไข่ตกลงมาจากหวอด จากนั้นปลากริมตัวผู้ก็จะว่ายน้ำกลับไปหาปลากริมตัวเมียอีกและผสมพันธุ์เช่นนี้ซ้ำๆ อีกประมาณ 80-100 ครั้ง ซึ่งกว่าจะเสร็จสิ้นก็ใช้เวลาไปประมาณ 2-3 ชั่วโมง โดยสามารถอมไข่ไปเก็บไว้ภายในหวอดได้มากถึงประมาณ 300-400 ฟองต่อการผสมพันธุ์หนึ่งครั้ง เมื่อรัดจนไข่ถูกปล่อยออกจากตัวปลากริมตัวเมียหมดแล้ว ปลากริมตัวผู้ก็จะหมดความสนใจในตัวปลากริมตัวเมีย เพราะมันจะว่ายน้ำไล่ให้ปลากริมตัวเมียออกไปให้ห่างไกลจากหวอด ในระยะนี้ควรแยกปลากริมตัวเมียออกไปจากตู้ปลาทันทีหลังการผสมพันธุ์สิ้นสุดลง

ในระยะนี้ปลากริมตัวผู้จะไม่สนใจอาหาร แต่จะคอยเฝ้าอยู่ใกล้ๆ หวอดตลอดเวลา พร้อมทั้งคอยพ่นฟองอากาศเพิ่มจนหวอดมีขนาดใหญ่มาก และภายใน 30-33 ชั่วโมงไข่ก็จะฟักออกเป็นตัว แต่ยังคงเกาะติดอยู่กับหวอดต่อไป หากมีตัวอ่อนบางตัวหลุดจากหวอด ปลากริมตัวผู้ก็จะรีบเข้าไปอมแล้วนำไปปล่อยไว้ที่หวอดอย่างเดิม แต่หลังจากนั้นอีกประมาณ 2 วัน เมื่อลูกปลาสามารถว่ายน้ำเองได้อย่างอิสระ และมีความยาวลำตัวประมาณ 3-4 มิลลิเมตร ปลากริมตัวผู้ที่อดอาหารมานานตั้งแต่เริ่มผสมพันธุ์ก็จะหิวและว่ายน้ำไล่กินลูกของมันเอง ดังนั้นจึงควรแยกปลากริมตัวผู้ออกไปในระยะนี้ หรืออาจใส่พืชน้ำให้หนาแน่นขึ้นเพื่อให้ลูกปลาได้ใช้หลบซ่อน และให้อาหารลูกปลาด้วยไรแดง