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Research Article

Reproductive Behavior and Parental Role of Giant Gourami (*Osphronemus goramy* Lacepède, 1801)

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ABSTRACT
The giant gourami (*Osphronemus goramy* Lacepede, 1801), a popular aquaculture species in Southeast Asia, exhibits unique cooperative biparental care behaviour. To support captive breeding efforts, this study aimed to visually document the reproductive activity of giant gourami, elucidate each stage in detail, and provide insights into the distinct parenting roles of males and females. Underwater cameras were used to observe a breeding pair of gourami in a pond for five days, conducted three times with different pairs during different spawning periods. The male and female contributions to nest building were quantitatively analysed using the T-test, while their parental care involvement was qualitatively assessed and statistically analysed using the Mann-Whitney U test. The results revealed three main phases of giant gourami reproduction: pre-spawning (including adaptation, nest building, and courtship), spawning and fertilisation, and post-spawning with parental care. Our observation confirmed the biparental tendency, with males being more involved in pre-spawning activities and females taking on a prominent role in post-spawning care. In conclusion, males focused on mating preparations and courtship, while females invested more in parental care.

INTRODUCTION

Anabantoids or labyrinth fish (order: Anabantiformes, suborder: Anabantoidei) are a group of ray-finned fish classified by the presence of an air-breathing organ. Members of this order consist of facultative and obligate air-breathing fish, whose vascularized additional breathing organs help them obtain oxygen directly from the air (Mendez-Sanchez & Burggren 2019). Due to their physiology and air-breathing nature, anabantoid fish can thrive in seemingly unfavourable aquatic environments, particularly in shallow and oxygen-deprived waters. Their typical habitat choices have led to the evolution of unique behaviours related to the utilization of their labyrinth organ (Huang & Lin 2016). These behaviours are particularly evident in their reproductive processes, where mating behaviours vary across different genera (Rüber et al. 2006).

Most anabantoids exhibit intricate mating processes, incorporating specific courtship behaviours, nest building, and parental care in most species. There are two main known modes of parental care in anabantoid fish: mouthbrooders and nest builders, with a few members showing free-spawning tendencies, such as the genus *Anabas* (Zworykin 2012). The predominant behaviour in the group is nest building, which can be categorised into three main types: bubble-nest builders, as observed in most small gourami fish; substrate nest spawning, seen in the genus Sandelia; and nest construction using plant materials, as observed in the genus Osphronemus (Rüber et al. 2004; Rüber et al. 2006; Tate et al. 2017).

Giant gourami is a native species found in the Southeast Asia region. The fish is highly valued as a commercial species in Indonesia, Malaysia, Thailand, and Philippines (Ling 1977; Yap 1999; Rimmer et al. 2013). Its habitat is similar to that of other anabantoid relatives, as it inhabits slow-moving waters such as rivers, lakes, swamps, and other isolated bodies of water (Ismail et al. 2018). Giant gourami is also equipped with a labyrinth organ, allowing it to tolerate low-oxygen environments and even stay out of water for short periods (Cole et al. 1999). Their ability to adapt to harsh environments is reflected in their reproductive behaviour, as extra care is necessary for their offspring to survive in low-oxygen and confined waters filled with potential predators.

By nature, the fish is a nest builder and exhibits a parental care system that involves contributions from both parents. This trait persists even when the fish is bred in captivity (Slembrouck et al. 2020). Farmers have recognised the nest-building and parental care nature of giant gourami and traditionally provide nest supports, such as adding baskets or holes in the breeding pond, for the fish to construct their dwellings (Kristanto et al. 2019). Previous studies have addressed practical methods and analyses regarding breeding techniques (Arifin et al. 2013; Nafiqoh & Nugroho 2013; Amornsakun et al. 2014; Slembrouck et al. 2019). However, the intricacies of such breeding behaviour have been seldomly studied, including the extent of parental role assignment for each parent.

Our study aims to provide a more comprehensive understanding of giant gourami's reproductive behaviour, focusing on the pre-spawning phase, through the mating process, and up to the parental egg care in the post-spawning phase. Several studies have previously used underwater video to capture the behaviours of fish in the wild, including *Lepomis auritus* (Martin & Irwin 2011), *Moapa coriacea* (Ruggirello et al. 2020), and *Thymallus arcticus* (Kupferschmidt et al. 2019). Additionally, previous studies have employed video recording as a tool to investigate the mating behaviors of zebrafish *Danio rerio* (Zempo et al. 2021) and American poeciliid *Brachyhaphis olomina* (Garita-Alvarado et al. 2018) in greater detail. Our methods similarly involve video recordings using underwater cameras to visually capture and provide evidence of the mating behavior of giant gourami. The resulting output would offer visual evidence of the intricate phases of mating behavior that have been discussed by local farmers and other practitioners for years.

We also identify the extent of reproductive roles performed by each brood parent by collecting quantitative data based on their behaviour. Quantitative description of fish behaviour has been employed for anabantoid fish studies previously by Miller & Hall (1968). The analyses were also recently employed for aquaculture studies (Settle et al. 2018; An et al. 2021). By observing the mating process, we seek to elucidate the behaviour of both sexes in each phase, from courting to caregiving periods. Furthermore, we emphasize the analysis of the distinctive roles played by each sex throughout the entire reproductive period. Behavioural studies such as steps of mating behaviour could be a stepping stone to further investigate their neurobiological functions regarding to their trigger and response to environmental and social cues (Mes et al. 2018; Tripp et al. 2020). Understanding fish behaviour is essential for advancing intelligent aquaculture system and enhancing breeding efficiency (Du et al. 2022). Basic knowledge about the behavioural intricacies of mating process in fish is ultimately a prerequisite to develop an intensive and commercial level aquaculture system (Araujo et al. 2022).

We hope that our results will establish a solid foundation of knowledge on giant gourami breeding. The development and improvement of new fish breeding techniques require a thorough understanding of the complete reproductive process of the fish. Therefore, it is crucial to build a knowledge base from the ground up, particularly regarding the natural behaviours of endemic fish such as the giant gourami.

MATERIALS AND METHODS

Broodstock selection and preparation

The experiment was conducted in fish farm owned by a local gourami breeder and farmer in Duku village, Minggir district, Sleman Regency, in The Special Region of Yogyakarta. The giant gourami broodstock was also obtained from said farm. Three pairs of giant gourami were selected and prepared for mating. The parent fish's qualities were evaluated using Indonesian standards for giant gourami broodstock (SNI 2000) which stipulates that the fish must be in good health, have a complete organ structure, is lack physical abnormalities in the external morphology, including operculum and genitalia and vibrant external coloration. Male and female broodstock weighed 2 - 3 kg and 1.5 - 2 kg respectively and were both regarded as sexually mature. The male body length ranged between 40 - 50 cm, while females had 35 - 40 cm length. The body colour of mature male giant gourami was vivid red and black, with a pointed stomach section, a regular scale pattern, and active mobility. Mature female broodstock was characterised by having a rounded stomach, the edges of the scales were slightly opened, and slower movement compared to the males.

Breeding pond preparation

The giant gourami broodstocks were reared in a rectangular concrete pond with dimensions of 2 m length, 1.5 m width, and 1.5 m height filled with 1 m water level for observation, amounting to about 3 m^3 water volume in the pond (Figure 1). The experimental pond was prepared with nest-building materials and a pair of underwater cameras (Brica B-PRO5 α Edition mark II with 16 MP CMOS sensor, fixed focus angle lens, and aperture of F/2.8, f=2.9 mm). Nest materials were prepared with a braided bamboo basket and dry palm fibre to facilitate the fish in constructing their nest. The materials were put on top of a woven bamboo table inside the pond. The pond was filled with water after the basket, bamboo table, and observation cameras were installed, before adding the fish.

The broodstocks were initially reared in separate rearing pond before being transferred to the experimental pond. We measured the temperature in the initial rearing pond, with temperature at 27 – 28 °C and pH at 7.2. The experimental pond had 28 - 30 °C water temperature and pH at 7.4. The pond was filled with water from a nearby irrigation canal, leading to reduced visibility in the video recording due to turbidity.

The underwater camera was prepared as depicted in Figure 2. The camera was submersible and held inside a structure of PVC pipe and supported with two bamboo stakes, mounted on a concrete block. Two sets of cameras were prepared in the pond, one for capturing activities in the nest and one for capturing activities around the bamboo table. Both cameras were set to capture a video with a 1920 x 720 pixels ratio in 30 frames per second. The lens covered around 30 - 40 cm field of vision.

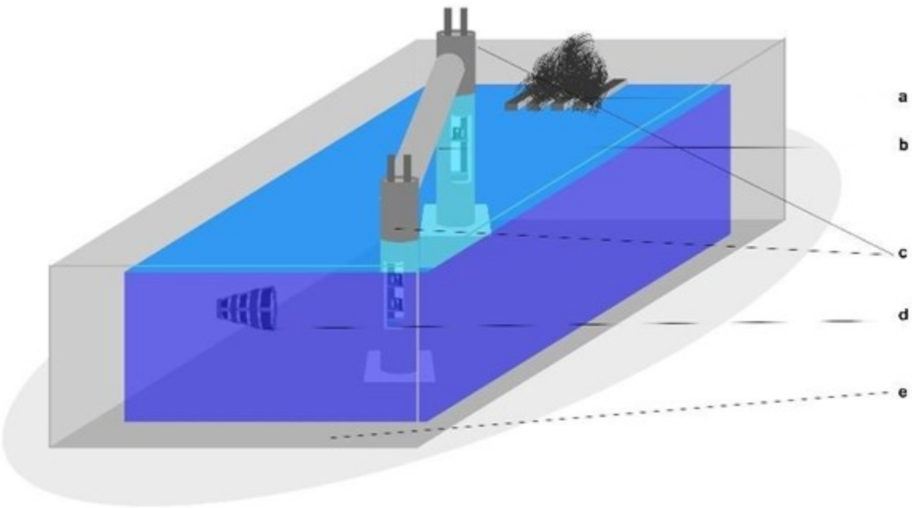

Figure 1. Observation pond design: a. Woven bamboo table, b. PVC pipe to mount the camera, c. Underwater camera, d. Bamboo basket for nesting, e. Breeding pond area.

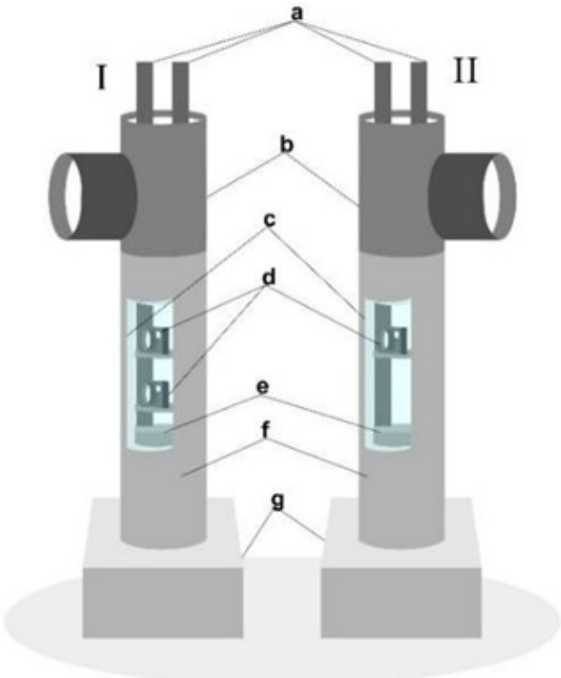

Figure 2. Underwater camera design: a. Bamboo stakes for supporting the structure, b. T-shaped PVC pipe fitting, c. 3 mm Acrylic, d. Underwater camera, e. Styrofoam, f. Four-inch PVC pipe, g. Concrete block for support. Camera I is facing toward the nest, and camera II is facing toward the bamboo table.

Observation of reproductive behaviour

All observations were conducted in three replications, using a separate pond for each breeding process. Each pair of broodstock was reared in separate ponds, with one breeding cycle in one pond considered as one replication. In total, three broodstock pair was observed in three separate ponds. Data were collected in the form of video recordings from two cameras in each pond, facing both the nest and bamboo table (Figure 2). All pairs reproduced naturally without hormonal induction. Our observation is limited to the adaptation, courtship phase, and until the early stage of parental behavior (egg guarding).

The observation period started when the fish were reared inside the pond and lasted until the post-spawning period when the parents took care of the eggs. To study the significance of gender roles in nest construction, we observed the fish twice: in the morning between 08.00 - 11.00 AM and in the afternoon between 02.00 - 05.00 PM. Data were collected every 15 minutes, four times in the span of one hour, both in the morning and afternoon period.

For parental care analysis, we observed the behaviour of both male and female fish while tending to their egg in the nest. Observation was conducted four times in the span of one hour only at the last day. In captive breeding, the nest containing the eggs is typically removed from the breeding pond to be hatched in separate container. Hence, the parental care observation was only conducted at day + 1 post fertilisation. The data were collected based on qualitative scoring: 1 – Seldom (The parent never swam directly in front of the nest or being next to the egg at least once in the observation period); 2 – Occasional (The parent visited the nest more than once or spent less than half of the observation period next to the nest); 3 – Frequent (The parent frequently visited the nest or spent more than half of the observation period next to the nest); 4 – Always (The parent never left the nest).

Data analysis

We conducted both qualitative and quantitative analyses in this study. Qualitative data were collected based on reproductive behaviour such as courtship, mating, and parental care observed within the pre-spawning, spawning, and post-spawning period. Data were collected from three ponds as three replications. The descriptive analysis was performed for every behaviour during the pre-spawning and spawning periods. Quantitative analysis was conducted on the frequency of exhibited nest building behaviour between parents. The results of the quantitative data were statistically analysed using T-test. Parental care behaviour of each parent was scored and statistically analysed using non-parametric test.

RESULTS

The overview of the mating process

In this study we observed three pairs of giant gourami each in different observation pond. Generally, we classified the pattern of mating behaviour into five phases as depicted in Figure 3, which are adaptation, courtship, nest-building, spawning, and parental care.

The initial adaptation phase started as soon as the pair was transferred into the new pond from the previous broodstock pond. At this point the fish scours the area of the pond, finding the potential nest location, and the building materials (ijuk on top of the bamboo table). The phase continued with courtship interactions between the pair, which lasted around three hours. Following the successful courtship, the pair constructed the nest with each defined role. The fish behaviour throughout the mating process are recorded in the ethogram (Table 1). The behaviour pattern of the pair of broodstock is further elaborated in the following sections.

Adaptation and Courtship

The initial phase of the pre-spawning process is to adapt the fish to the new pond habitat. The most noticeable difference from the previous rearing habitat was the reduced light penetration to the water column due to turbidity. This, however, does not seem to hamper the fish's locomotion and interaction, as they recognized the nest (bamboo basket) and the area

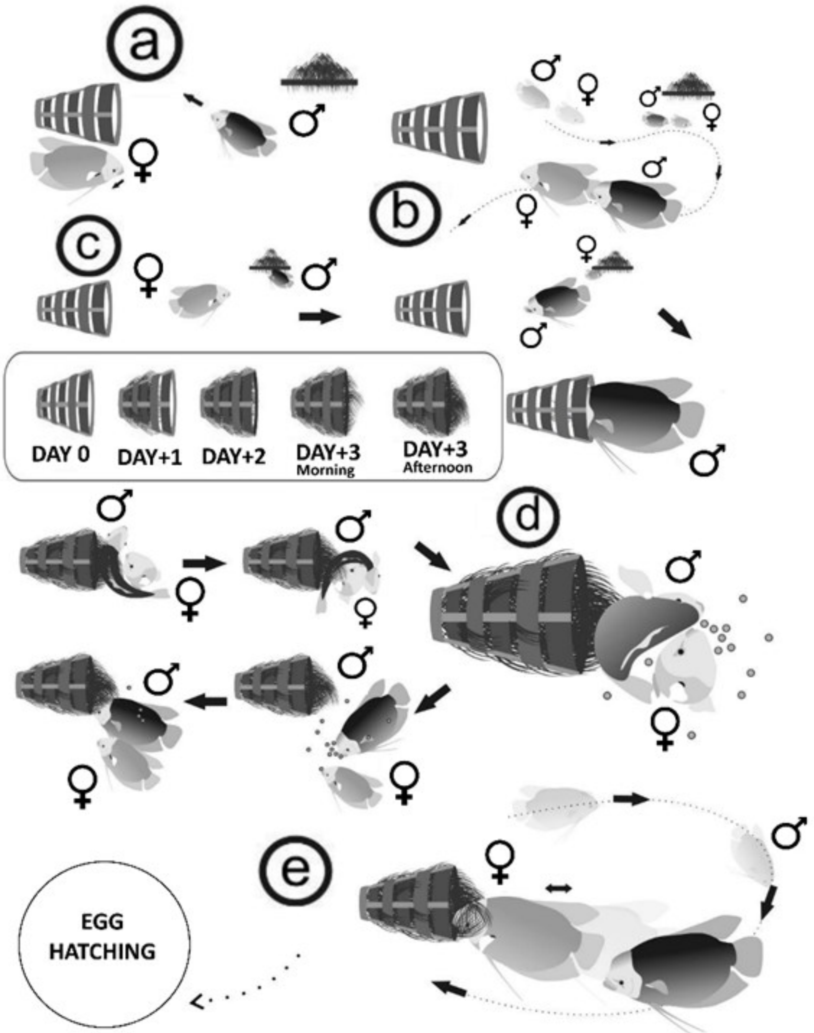

Figure 3. The mating and parental care process in *Osphronemus gorami* pair. (a) Adaptation phase; (b) Courtship phase; (c) Nest-building phase; (d) Fertilization/Spawning phase; (e) Parental care phase.

Table 1. Ethogram of giant gourami mating behaviour. The information is split between the male and female broodstock.

Phase	Male	Female
Adaptation	• Swimming around the basket • Scouting the place where the ijuk fibre is located	• Swimming around the basket • Swimming in and out of the basket
Courtship	• Showing aggressive swimming pattern near the female • Aggressively chase the female while occasionally flaring operculum • Actively syncing swimming movement with the female	• Slowly swims away from the male, occasionally stops • Actively swimming away from the male, circling the pond • Female shows appeasement by slowing down
Post-Courtship	• Swimming in pair with the female, following their movement • Following the female from behind • Swimming outside of the basket, circling the perimeter	• Swimming in pair with the male around the pond • Female actively swimming in search for the basket, swimming around it • Female enters and prepares the basket by cleaning the inside with pectoral and tail fin
Nest Building	• Actively swim in the direction of the bamboo table • Transported the ijuk fibres with its mouth to the inside of the basket while arranging it	• Female got out from the basket, swimming behind the male • Occasionally picked the ijuk fibres, just swims behind the male, or stayed inside the basket to arrange the fibres

Table 1. Contd.

Phase	Male	Female
Spawning / Fertilisation	• Male swims circling the female just outside the basket after it was fully filled with fibres • Performed anabantoid embrace by skewing his body into U-shape, wrapped around the female • While bending, the male expelled the sperm to fertilize the eggs • Gathered the eggs and put them inside the nest with the female	• Female stayed just outside the basket, leaning its body towards the direction of the male • Performed a receptive pose by leaning the body towards the male, while it embraces • Eggs were expelled while being embraced by the male • Helped gathering the eggs with the male
Parental Care	• Mainly circled outside the perimeter of the nest. • Seldomly swim near the nest	• Swim at the entrance of the nest • Constantly fanning the nest with its pectoral fin.

around it. Male fish were seen swimming around the entire pond during this phase, whereas female fish were seen swimming in circles around the bamboo basket that had been prepared as a potential nesting area and entering and exiting the basket (Figure 4). It was observed that in the adaptation phase, the fish surveyed the area of their habitat to identify and establish their territorial area before the mating phase.

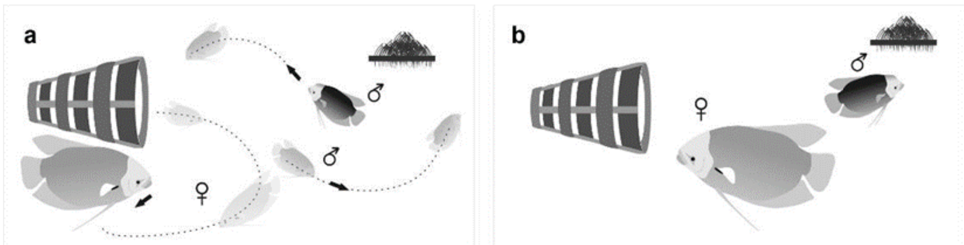

Figure 4. Adaptation phase: a. Male broodstock scours the habitat while female broodstock swims in and out of the nest, and checks the perimeter of the nest. b. Both broodstock identify the present objects in the vicinity of the pond.

The second phase of the pre-spawning behaviour was courtship, illustrated in Figure 4. Three steps of courtship behaviour were observed in this study: Identification, courting by the male fish and courting response from the female fish. In the first step, the male fish exhibited aggressive swimming behaviour while the female fish were swimming around and re-entering the bamboo basket in sequential order. Male courting starts when the male fish swim aggressively towards the female fish, effectively chasing the female around the habitat area. The aggressive swimming behaviour was also accompanied by the fish frequently opening its mouth and operculum. Subsequently, the female fish gave a response to the male chase and swims more slowly. In this step, the movement of the male slowed and gradually synchronized with the female's swimming pace.

The two fish then swim in pair around the habitat, around the nest territory, and in and out of the bamboo basket, with the male fish supposedly guarding the female (Figure 5). This behaviour continued until the male fish started searching for materials to build their nest.

Nest building

After the female was courted and the fish formed a pair, the fish started to swim around the habitat to search for nest-building materials. Palm fibres or "Ijuk" were provided on the woven table for the fish to build their nest (Figure 6). The fibres were picked by the fish by swallowing and saving them in their mouth, subsequently transported into their bamboo basket nest.

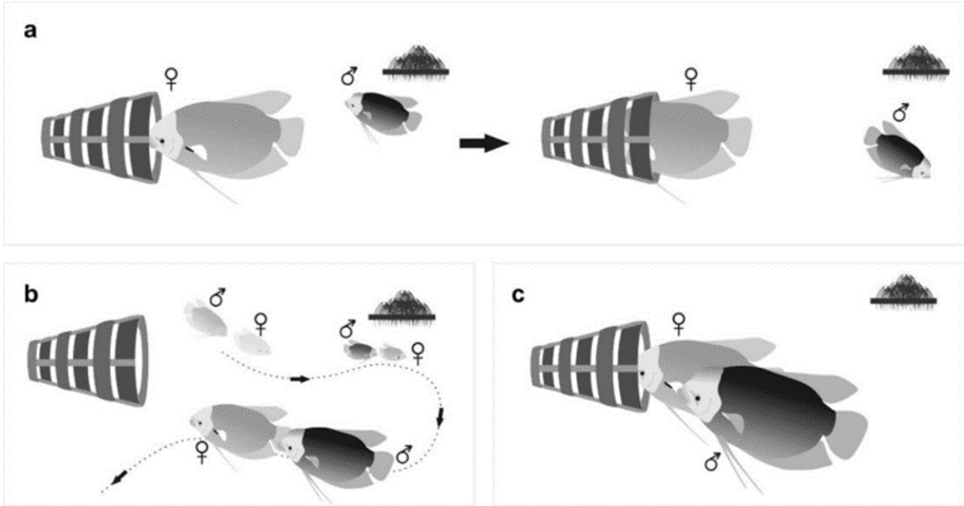

Figure 5. Courtship phase: a. Both broodstock identifying their potential mate. b. Female fish swims around while male fish court the female by chasing it throughout the pond area. c. Male fish synchronize their movement with the female, following it swimming around the nest.

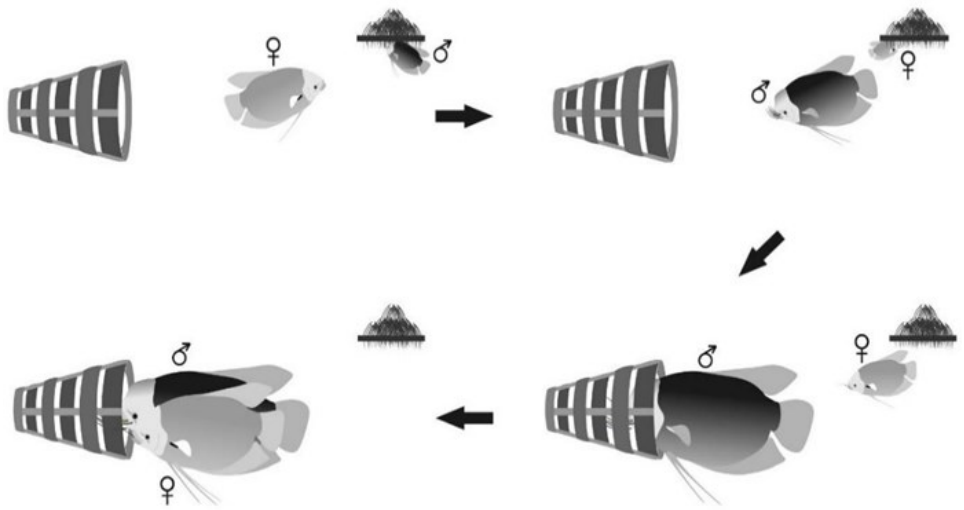

Figure 6. Nest construction. The basket acted as a hole for the nest. Male fish first initiate the nest building by picking up palm fiber from the woven table. Female fish followed the male and occasionally assisted in gathering the materials.

The fibres were expelled from the fish's mouth, noticeable by the presence of bubbles from their mouth, and arranged inside the basket. The fibres were arranged in a circular pattern akin to a bird nest and started from the innermost of the basket to the outer parts.

The nest-building behaviours were exhibited by both male and female fish (Figure 7). The male fish however clearly showed a more dominant role in the process. The role of picking nest materials and building was predominantly observed in the male fish, while the female fish mainly followed the male while only occasionally picking up the nest materials and help arranging the nest.

The process of nest building was continuous for three observation days (Figure 8). Observation on the first day of the nest building showed that the bamboo basket started to be scarcely filled with palm fibres in the afternoon. Afternoon observations on the second day revealed that the fibres had already filled the interior of the basket but had not yet covered the exterior or the entrance part of the nest. The basket was filled to the entrance in the morning of the third day, with materials transporting activities significantly reduced compared to the days before. In the afternoon, female fish was occasionally seen neatly arranging fibres by pulling loose fibres and pushing them into the inner part of the basket.

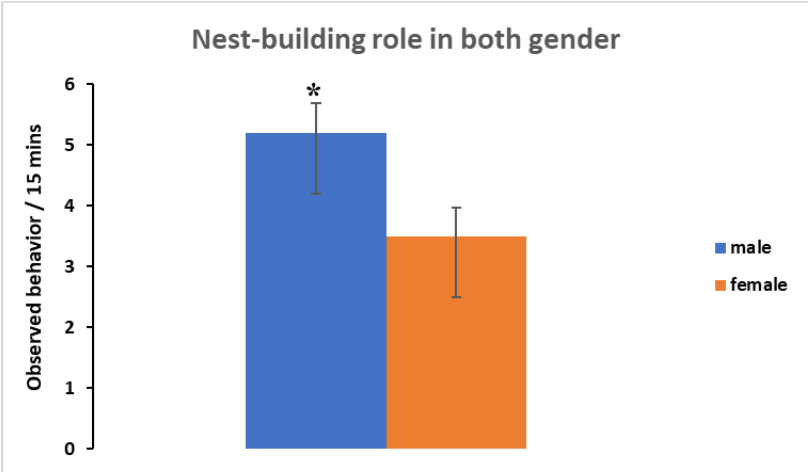

Figure 7. Comparison of the average frequency of nest building between male and female parents (3 replications) observed every fifteen minutes. The difference between both data is statistically significant *) T-test p value = 0.008, $p < 0.05$).

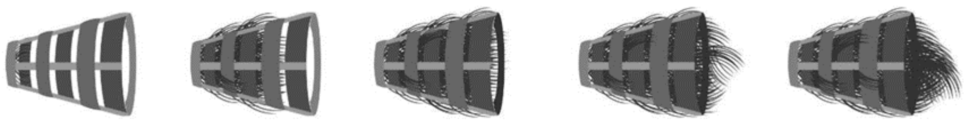

Figure 8. Nest construction process: a. The bamboo basket for nesting at the start of the mating period (D0), b. The bamboo basket started to be filled with palm fibers (D+1 afternoon), c. Partially filled nest (D+2 afternoon), d. Filled nest (D+3 morning), e. Completed nest filled with fibers (D+3 afternoon).

Spawning

The spawning process of giant gourami was observed after the nest was established. The whole spawning ritual is illustrated in Figure 9. The ritual started with the male fish swimming encircling the female fish, which was followed by the female who skewed its body angle until it leaned on the body of the male fish. The male in return arched its body, forming an upside-down "U" shape, and wrapped it around the female's body. At this point, the female expelled the eggs and externally fertilised by the male fish.

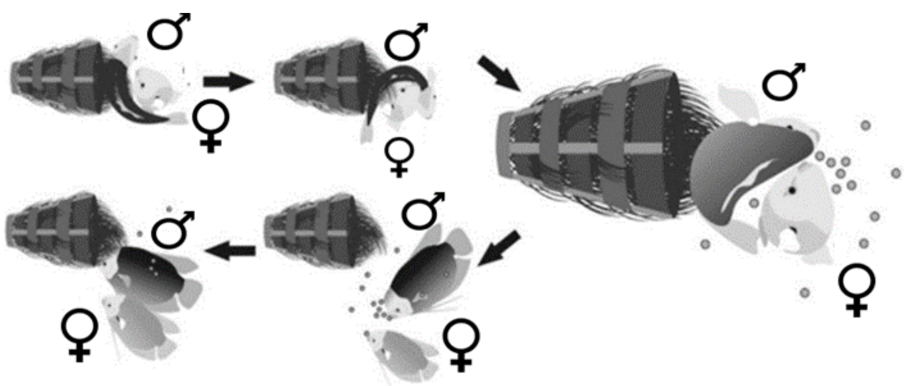

Figure 9. Giant gourami spawning process: Right after the completion of the nest building, both fish entwined their body with the female fish released the eggs, and subsequently fertilized by the male. The fertilized eggs were then moved to the nest by both parents.

The fertilization process occurred twice in one spawning cycle. The floating eggs were then subsequently gathered by both parents and carried to the nest. In our observation, the spawning and fertilization process occurred between 4.00 - 5.00 PM.

Post-spawning

The reproductive behaviour observed after the spawning period is parental care. The giant gourami exhibited parental care behaviour in the form of nest guarding and egg care (Figure 10). The male fish predominantly guard the territory by swimming around their nest area. The female fish mostly swim near the entrance of the nest while fanning the nest with its pectoral fin.

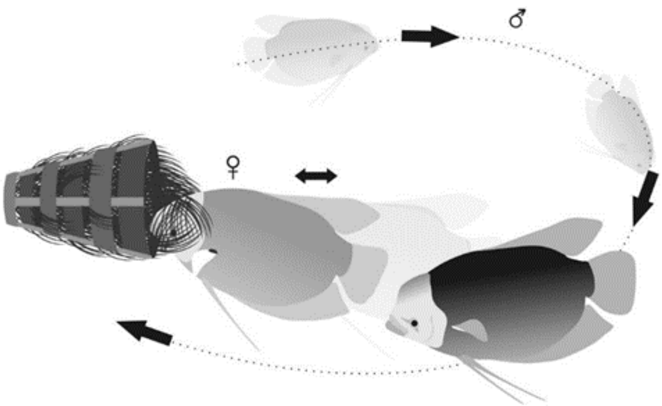

Figure 10. Egg care and parental behaviour: Female fish predominated the role of egg care by fanning the egg from the entrance of the nest using its pectoral fin, while male fish mostly swims around in the vicinity of the nest in a protective manner.

Based on the observation, both parents had different roles in parental care. Figure 11 showed the overall presence of each parent near the nest. The difference between male (median = 2) and female (median = 4) is statistically significant (Mann-Whitney U test p value < 0.05, U = 0). The female fish primarily hovers close to the nest opening and stays in the vicinity. The male fish only occasionally swims up to the nest to examine the eggs before swimming away. It is assumed that the male fish also supposedly guards the nest but covers a wider area around their territory. The female fish largely served as the caregiver because she was closest to the eggs and occasionally fanned the nest.

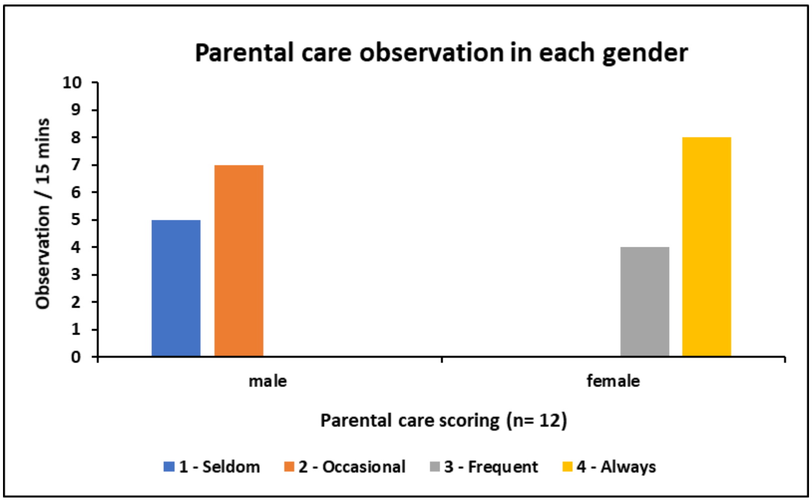

Figure 11. Comparison of the average frequency of parental care behaviour between male and female parent (3 replications) observed every fifteen minutes. The behaviour which was interpreted as parental care are swimming and caretaking activities in the vicinity of the nest. The scoring criterias are: (1) Seldom,

which the parent never swam directly in front of the nest or being next to the egg at least once in the observation period; (2) Occasional, which the parent visited the nest more than once or spent less than half of the observation period next to the nest; (3) Frequent, which the parent frequently visited the nest or spent more than half of the observation period next to the nest; (4) Always, which the parent never left the nest. The statistical difference between both gender is significant (n = 12, Mann-Whitney U test p value = 0.00; $p < 0.05$)

DISCUSSION

Timeline of reproductive behaviour

The findings of this study shed light on the reproductive behaviour of *Osphronemus goramy* in a captive environment. The observed behaviours can be categorized into different phases, including pre-spawning phases of adaptation, courtship, and nest building, spawning, and post-spawning parental care. By examining each phase, we can gain insights into the reproductive strategies and roles of male and female fish in this species.

The overall mating process takes about five days (Figure 12). The pre-spawning phase started on day 1 after the fish were acclimatized. The courtship process was observed in the first afternoon and nest building was observed starting the next day when the fish started gathering fibre material. The nest-building process comprised most of the pre-spawning phase, in which the fish took three days to finish. The spawning process, which includes embracing rituals and fertilization, happened right after the nest was finished in the afternoon of the fourth day. On the fifth day, the eggs were already placed and settled inside the nest and both parents already assumed their respective parental roles, guarding and fanning the nest.

Our result is consistent with previous reference regarding to steps of giant gourami mating, where the whole process could take about a week and nest building process took about 3 days to finish (Tanjung & Jhonly 2015). Each stage of the reproductive process saw distinct behaviours from both parents. In the pre-spawning stage, we saw male-centric behaviour, but in the post-spawning stage, caring behaviour was predominately the responsibility of the female.

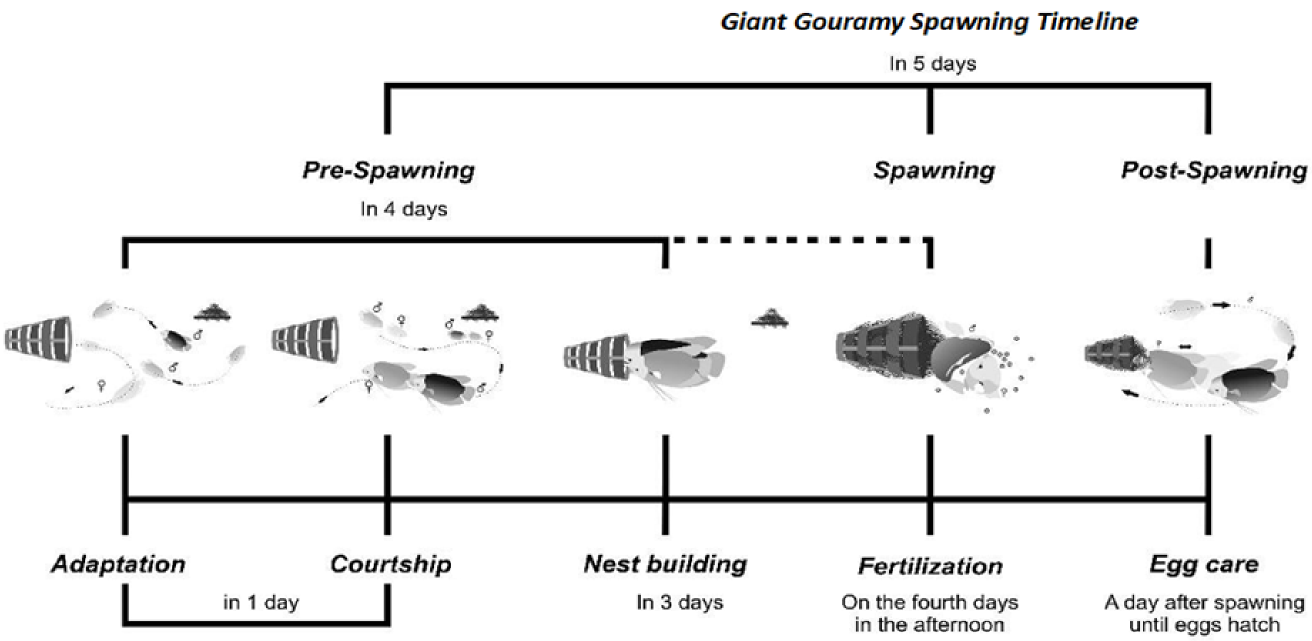

Figure 12. Timeline of the entire reproductive process of giant gourami pair in five days period. The adaptation and courtship rituals were observed on the first day, followed by nest building which took three days to complete. On the fourth day, spawning occurred and was subsequently followed by parental care (egg) on the fifth day.

Rüber et al. (2006) documented four main modes of reproductive behaviour in anabantoid fish: Free-spawning without parental care (genus Anabas, Helostoma, Ctenopoma), mouthbrooding (genus Ctenops, Spheerichtys, and several Betta species including *Betta macrostoma*), substrate spawning with male parental care (*Sandelia capensis*), and bubble nesting with parental care. Among the members of the anabantoids, bubble nesters are the most common, exhibited in at least five genus (Betta, Trichopsis, Trichogaster, Colisa, Macropodus). In fish, there are generally three types of nest construction: excavating burrows and other substrates for egg-placement, piling up walls with materials such as sands, pebbles, animal materials, and plant matter, and constructing cemented-nest with materials glued together by kidney secretions (Navarrete-Fernández et al. 2014). In this case, giant gourami exhibited piling up nest construction, filling the walls of a hole with plant materials for depositing eggs. This behaviour is also unique in the Osphronemid family (Rüber et al. 2006; Arifin et al. 2020; Slembrouck et al. 2020).

Generally, the giant gourami displayed similar stages and behaviour as other anabantoid fish. Males tend to show aggressive display to entice the female, accompanied by chasing the female (Bronstein 1982; Miller & Jearld 1983). The initial response of the female is avoidance and fleeing for several hours until it showed response of appeasement / submission by slowing down and swims with the male (Bronstein 1982). However, compared to other nest-building anabantoids, particularly in the family of Osphronemidae, the giant gourami performed courtship before building nest. Other nest builder members from the gourami family such as Trichogaster, Trichopsis, and several species of Betta including Betta splendens had the males build a nest first before performing courtship (Liengpornpan et al. 2006; Saha et al. 2017; Lichak et al. 2022). The activity and behaviour of both parents and how it varies between the sexes will be further discussed in the section that follows.

Pre-spawning phase

The early pre-spawning rituals we observed in this study were adaptation and courtship behaviour. Preeminent behaviours were observed in both male and female fish. The male directly shows aggressive behaviour towards the female, including opercular flares accompanied by frequent mouth-openings, circling and chasing the female throughout the area, synchronised swimming, and leading-to-nest movement.

The first prominent display of courtship by the male is the opercular flare, in which the fish puffed up the gills and opened the operculum wide. This behavioural pattern is apparent in the early courtship where the male showed aggressive swimming in front of the female and later while chasing the female around. The opercular flare signifies the intensity of the male to attract the female (Goddard & Mathis 1997). This type of aggressive behaviour is also displayed by other anabantoid fish to compete between males for female attraction and deter intruders from their territory (Forsatkar et al. 2016). The act of flaring is taxing to their physiology, as it prevents water from passing through gills making the fish experience a hypoxic state (Abrahams et al. 2005; Castro et al. 2006). To the female fish, the display of intensity could signal the health and virility of the prospective male, and a show of fierceness and aggressivity would give an impression of a strong potential mate although eventually, the preferences differ between species (Dzieweczynski et al. 2014). Male fitness and vitality would be defined by the display of aggressiveness and opercular flare, indicating a preferable mate with ideal genetic resources.

The female fish occasionally displayed fleeing behaviour in courtship, in which the males chase it throughout the pond area while flaring its operculum and opening its mouth. The males increased their intensity of aggressive behaviour when the females are shown to be unreceptive (Rainwater & Miller 1968), hence the male may chase the female relentlessly until it showed a positive signal or gesture of appeasement by swimming slower. By that time, the movement of the male also slowed and eventually synchronized with the female's swimming movement. The paired swimming would lead to the male enticing the female to swim in the direction of the nest. In giant gourami, the receptive female swim beside the male as if it was escorted by the male towards the nest and then circles and swims in and out of the nest. Similar escorting behaviour was also observed in other anabantoid fish in the family Belontiidae, where the female returns to the nest with the male by swimming in a zig-zag pattern (Hall & Miller 1968; Miller & Jearld 1983).

After successfully courting the female and leading it into the nest area, the giant gourami male started the nest-building phase, or in this case adding plant materials into the prepared braided conical bamboo basket, which acts as its nest. The palm fibres or "ijuk" were used as a structure for the eggs to latch inside the nest. The bamboo basket, is typically prepared by giant gourami farmers, particularly in Indonesia, as a nesting frame, while palm fibres are provided inside the pond area as nest -building material (FAO 2023). The courted female fish were led into the nest area where the female familiarize herself with it by circling and swimming in and out from the bamboo basket. Both fish were then searched for plant materials to fill the basket. This behaviour was predominantly observed in males, actively seeking palm fibres and bringing them to the nest. The fish accomplishes this by swallowing the plant matter, exhaling it with bubbles, and depositing it inside the nest. Females also participate in nest-building albeit seldom.

In this study, bamboo basket was provided as a safe nest location for the fish and also serves as the supporting structure of the nest. Palm fibres are supplied as a substitute for fibrous vegetation materials that the fish actively seek in their natural habitat. The provision of palm fibres or ijuk also speeds up the nest-building process, since the fish does not have to spend the extra effort of biting stems and leaves of vegetation to obtain the fibrous materials. The well-covered nest cushioned with plant fibres is the haven for storing the eggs. Both sexes performed spawning after the process of building the nest was complete.

Spawning and egg-care

Spawning started when the male performed a circling movement and curved its body, clasping around the female's body. The clasping behaviour itself is not exclusive to anabantoids, as it is also observed in other families of fish such as cyprinids (Family: Cyprinidae) which the male clasped to the female body with inclination to one side, keeping the vent area close, and subsequently eggs and milt were released (Maurakis et al. 2001; Kottelat et. al. 2006; Jacob 2013; Chacko & Sekharan 2022). In anabantoids, it had a different pattern of embracing and clasping, as the male bends into a reverse U-shape and wraps around the female. At that moment, eggs were expelled by the female straight into the vicinity of the male's urogenital opening, where it emits sperm and subsequently fertilized the eggs. The bent portion of the male body exerts pressure on the lateral region of its testes like a lever to release sperm (Hayakawa & Kobayashi 2009). The behaviour, known as anabantoid embrace, is regarded as a distinctive spawning method of anabantoid fish (Rainwater & Miller 1968; Chandran et al. 2013; Tate et al. 2017).

The female parent's egg-guarding and egg-fanning action is the most prevalent parental care behaviour that we have seen in this study. This behaviour is the most common parental care in nest-building teleosts (Ishimatsu et al. 2018), and is generally exhibited by anabantoid fish (Jaroensutasinee & Jaroensutasinee 2001; Mitra et al. 2006). The female fish fans its pectoral fin in the direction of the nest to create water movement, removing impurities from the nest and ventilating the eggs (van Lieshout et al. 2013). The level of dissolved oxygen in the water is crucial to the survivability and growth of the embryo inside the egg. Oxygen gas is diffused into the egg through the egg's outer layer (Kranenbarg et al. 2000). The rate of oxygenation is dependent on the level of ambient DO and the velocity of the water current (O'Brien et al. 1978). The fish egg had a capsule-like layer called chorion that protects the embryo and the egg from external perturbation, however, it also hinders oxygen diffusion into the egg (White & Seymour 2011). The fanning process by the parent fish ensures the water circulation inside the nest, removing stagnated water and oxygenizing the water around the eggs, and the resulting current helps with oxygen diffusion into the eggs (Green & McCormick 2004).

Being a fish with additional air-breathing apparatus in the form of a labyrinth organ, anabantoids are physiologically well-equipped to thrive in a low-oxygen environment. The hypoxic environment, in the form of stagnant and confined waters, is the kind of habitat that anabantoid fish calls home, including giant gourami. Thus, the general breeding and reproductive strategies are also adapted to the hypoxic condition. This trait is exhibited by the members of anabantoids with parental care behaviour by creating bubble-nest, mouth-brooding, and constructing nests on substrates such as vegetations; although several species free-spawns (Tate et al. 2017). Parental care is a plesiomorphic condition in anabantoids, and the evolutionary transition from one form of parental care to others is a common occurrence among the genera (Rüber et al. 2004). The form of bubble-nest building behaviour was indicated to have independently evolved into distinct types, most prominently occurring in the genus *Osphronemus* and *Microtenopoma* (Rüber et al. 2006). In the case of giant gourami (*Osphronemus goramy*), it is interesting to note that the fish is a nest builder by nature; but it deviates from the common method of creating bubble-nest, as observed within their relatives, in favour of constructing a submerged nest with vegetation materials. Divergence in evolutionary behaviour may also alter the role of parental care between male and female fish.

Parental role

Giant gourami generally had a biparental role, and parental care is mainly observed to be the female's role. In our observation, the propensity of a biparental role was already apparent in the pre-spawning period, where female fish also contributed to nest building aside from the male albeit not as significant. The role is, however, reversed in the post-spawning parental care period. The female fish took a more prominent role in tending the eggs while the male assumed the role of a nest guard, swimming in the outer vicinity of its territory. The type of parental role that we encounter is different compared to other anabantoids, especially bubble-nest builders. In paradise fish (*Macropodus opercularis*), the egg care behavior is mostly paternal, with the male fanning and creating bubbles to keep the eggs stay afloat (Cole et al. 1999; Huang & Chang 2011; Rácz et al. 2021). A similar male-oriented role was also observed in *Trichogaster tricopterus*, where it also chased out the female from the vicinity of the nest before retrieving and tending the eggs (Kramer & Liley 1971; Kramer 1973; Pollak et al. 1981). The female is usually chased out by the male because of their tendency of eating the eggs (Kramer 1973).

Cooperation between two parents is the defining characteristic of biparental care behaviour. A different parent is inherently assigned to a specific role in providing care. In the case of giant gourami, females tended to eggs and fry while the males typically assumed the guarding role and had less direct interaction with their offspring. The sex-specific role between female and male giant gourami is also typically present in other biparental caregivers, for instance in biparental convict cichlids, male fish had more tendency to leave their eggs to chase out intruders (Itzkowitz et al. 2001). The pattern is similar as the female took a more active role in the egg/fry care compared to the male, which is also reflected in our results where post-spawning egg care is typically displayed by females.

The assignment of parental roles in both parents could be rooted in the difference in investment in their reproductive process. There is an asymmetry in gamete size and reproductive energy requirement in both sexes, as males typically produced smaller, less energy-demanding gametes (sperm) and females generate bigger and high-energy gametes (egg) but more metabolically costly (Sutton & Wilson 2019). The result is that individuals with larger reproductive stakes will invest more in parental role, while sex with lower parental investment will likely spend more effort in competing for reproductive chances (Trivers 1972). The notion that gamete size would directly affect reproductive decisions has been criticised in recent studies (Jennions & Kokko 2010; Liker et al. 2015) for reproductive decision also depends on other factors such as the current environmental situation; however, the theory applies regarding parental investment in giant gourami, relating to how much investment each parent had spent for their reproductive needs. For instance, females spent much metabolic energy on producing and laying eggs, while males spend their energy on courtship and competition.

The assumption that female fish spend more energy to create eggs suggests that they had bigger stakes in spawning than male fish. This might prompt the female fish to expend more effort to protect their significant investment through more frequent, hands-on care for the eggs. This condition leads to the assumption that females choose mates who are perceived as vigorous and fit in terms of reproduction, which is reflected in the courtship ritual, in which the males must compete for mating chances with females (Trivers 1972). Females are choosier because the courtship and spawning ritual could be costly for them, as a male's display of intensity could mean aggravated assault on the female, which could reduce their reproductive capability (Krasnec et al. 2012).

Our findings show that males made significant pre-spawning investments, particularly in courtship rituals and nest building. In theory, a part of their investment is already paid off in the form of successful reproductive chance, thus their stake in post-spawning care is arguably lower. Furthermore, because gamete production requires less energy, men recover faster than females, who need time to produce eggs (Kokko & Jennions 2008). As males consume less energy for reproductive needs, their post-spawning activity may be directed toward actions that might facilitate future mating. Previous findings have found that males with territorial dominance are more reproductively active (Limberger 1983; Maruska & Fernald 2010; Kustan et al. 2012) and they are more likely to obtain more reproductive success (Paull et al. 2010; Yokoi et al. 2016). Protecting territorial dominance may be the favoured mode of behaviour because it offers a better trade-off than investing energy in providing direct care for the eggs, such as constant fanning.

The behavioural pattern of the male also supports polygamous mating. This breeding strategy involves a male mate with multiple females, and it may favour increased breeding success in males. Our present work does not represent the polygamous nature of male giant gourami because reproductive activity was observed using a 1:1 sex ratio. Commercial breeders, on the other hand, have been known to use many females in a breeding pond, with a 1:3 male-to-female ratio (Slembrouck et al. 2019; Arifin et al. 2020; Slembrouck et al. 2020). The increased male ratio has been known to result in competition between males for courting females, and could result in aggravated aggression between fish that could potentially reduce the breeding efficiency (Arifin et al. 2020). Due to the nature of our methodology, we are unable to provide information on fish behaviour in situations when there are several females and several male competitors. Future studies should explore the various parental roles and behaviour that may arise in those conditions.

CONCLUSION

Overall, our findings revealed that giant gourami exhibits complex mating behaviours, including specific courtship rituals and nest building. Their biparental care trait is reflected in both male and female parents actively participating in the reproductive process. The parental role was assigned differently, as male-dominated behaviours occurred in the pre-spawning phase while the female-centred role was exhibited in direct egg care after spawning. The difference in the parental role might be influenced by each gender's reproductive stake, as the male is more involved in the courting process, fertilization, and territorial behaviour; while the female invested more energy in producing and laying eggs, thus had a higher stake in the parental care phase. The egg care behaviour of constantly fanning the eggs by the female may be an evolutionary response to low-oxygen environments. In the end, our research highlights the importance of understanding the natural behaviours of giant gourami's effective breeding. By unravelling the intricacies of their reproductive processes, we provide a foundation for the development of improved breeding techniques.

Further studies could focus on investigating the genetic factors influencing reproductive behaviour in giant gourami, as well as the impact of environmental factors on their breeding success. The physical and chemical factors such as water quality parameters could also affect the mating process of the fish. In addition, specific social behaviour regarding competition and reproductive fitness in multiple-pair situations should be investigated. This would provide insight into the degree to which parental role in the face of rivals, the propensity for polygamy, and the precise length of time each parent needs to recover from reproduction before being ready for the next spawning cycle. Basic behavioural knowledge should be built from the ground up as it would serve as a reference to develop and improve breeding strategies for commercial giant gourami production in the future.

AUTHORS CONTRIBUTION

T.I.J. designed the process, performed data collection and analysis, and wrote initial manuscript draft. I.H. conceptualized and supervised the study. H.B. performed data analysis, wrote and edited the manuscript. D.W.K.S. designed and supervised the study, wrote and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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