Type of intruder and reproductive phase influence male territorial defence in wild-caught Siamese fighting fish

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Abstract
This study investigated how parental care increases with offspring age by examining the level of male aggressiveness toward potential intruders during egg guarding in a natural population of Siamese fighting fish (Betta splendens Regan). The degree of aggressiveness was measured at two reproductive phases in response to three types of intruders: male, female and females that have laid eggs. The nest-holding males became more aggressive after their eggs hatched than after the eggs were laid. Nest-holding males displayed gill cover erection, biting, tail beating and attacking at the highest rate towards male intruders, intermediate towards female intruders and the least aggressive towards females, which have laid eggs.

Keywords: Territorial defence; Egg guarding; Aggressive behaviour; Siamese fighting fish; Betta splendens

1. Introduction

To understand the evolution of mating systems, it is necessary to determine how parental care increases offspring survival. Many theoretical studies have examined the parental effort and the optimisation of fecundity with respect to adult mortality (Williams, 1966; Pianka, 1976; Pressly, 1976, 1981; Ricklefs, 1977). These models predict that during the period of parental care, increases in the cost of parental effort will be favoured when (1) the average reproductive value of the young increases, (2) the number of young under parental care increases, and (3) the parent’s reproductive value decreases.

In an animal with a restricted reproductive season, the probability of an animal to breed again decreases with the time spent caring for a particular brood. Therefore, investments should increase with offspring age (Weatherhead, 1982; Sargent and Gross, 1985; Corio, 1987). There are many studies of changes in parental care over the breeding cycle and in response to different intruders in birds (e.g. reviewed in Montgomerie and Weatherhead, 1988; Andersson et al., 1980; Brunton, 1990; Rytkönen et al., 1990; Pavel and Bureš, 2001). However, a few studies have been investigated in fishes including three-spined stickleback (Gasterosteus aculeatus; van Iersel, 1953), smallmouth bass (Micropterus dolomieui; Ridgway, 1988, 1989), bluegill sunfish (Lepomis macrochirus; Coleman et al., 1985), cichlid (Cichlasoma nigrofasciatum; Laverty and Keenleyside, 1990), freshwater goby (Pomatoschistus marinus; Tortonese et al., 1985), common goby (Pomatoschistus muelleri; Magnusson and Vesterhage, 1993) and sand goby (Pomatoschistus minutus; Lindström and Wennström, 1994). These studies showed that parents increase the
intensity of aggressive behaviours as their offspring get older.

Parental care is common among teleost fish and can be costly in terms of reduced survival, breeding rate and fecundity to the parents (Townshend and Wootton, 1985; Smith and Wootton, 1995, 1999). Parents spend substantial amounts of time and energy on care including egg guarding, egg ventilating to provide a flow of oxygenated water and dead egg removal from the nest (reviewed in Clutton-Brock, 1991; Smith and Wootton, 1995). The wild Siamese fighting fish (Betta splendens, Regan) conforms to this pattern in its care behaviour. Males in the breeding season establish and defend territories in rice paddies. They construct their bubble nests, entice females to spawn in them and care for fertilised eggs and fry (Gordon and Axelrod, 1968; Jaroensutasinee and Jaroensutasinee, 2001a). Therefore, the ability to compete for territories is critical to the reproductive success of male Siamese fighting fish. It could be due to male–male competition and possible direct benefits of female mate choice (Jaroensutasinee and Jaroensutasinee, 2001b).

We investigated the level of male aggressiveness towards potential intruder during egg guarding in wild-caught Siamese fighting fish. The degree of aggressiveness was measured at two reproductive phases in response to the territorial intrusion of a male, a female and a female that has laid eggs. By quantifying territorial behaviour during encounters, the following hypotheses were investigated.

1.1. Fish biology

The Siamese fighting fish is an Anabantid native to Southeast Asia. Typical fighting fish habitats in Thailand are quiet fresh water ponds with muddy bottoms or flooded rice paddies (Gordon and Axelrod, 1968). Unlike domesticated fighting fish, wild fighting fish are small, inconspicuous, and dull brown or green in colour (Jaroensutasinee and Jaroensutasinee, 2001a,b). They hide beneath water plants, presumably to minimise predation from fish-eating egrets, herons and kingfishers.

Males in this species build one bubble nest, court females, and care for a single brood of the developing eggs and newly hatched larval fish at a time. However, the successful males may have 2–3 successive broods per one breeding season. Each male defends a territory in the water column near the surface. His territory is centred on a bubble nest built by the male (Forsellius, 1957). Fertilised eggs need to be aerated by being attached to bubble nests. Males retrieve eggs or larval fish that fall out of the nest or stray and spew them back into the bubble nests (Gordon and Axelrod, 1968; Jaroensutasinee and Jaroensutasinee, 2001a,b). Fertilised eggs hatch approximately 36 h after eggs were laid. Males exhibit parental care behaviour both in the field and laboratory during when the young have hatched up to 5–7 days (personal observation). They do not cannibalise their eggs and larval fish during the period of parental care. They have very aggressive social displays including gill cover erection, fin spreading, biting, and tail beating (Clayton and Hinde, 1968; Simpson, 1968). Fighting usually involves physical damage and can result in death. Females are duller in colour and usually smaller than males. After the females finish laying eggs, the males chase the females out of the bubble nest areas and solely provide parental care for the developing eggs and larval fish. Wild fighting fish males are heavier and bigger than females. They build their nests in aggregations in dense emergent vegetation. Dense male nesting aggregations may help males to attract more mates. Their territory size would be less than half of the distance to the nearest nest.

2. Materials and methods

The test subjects were wild-caught fish collected from the same rice field from July–September 2001 in Nakhon Si Thammarat, Thailand (8°38'N 99°53'E). The fish were maintained in the laboratory with natural light (i.e. approximately 12 h-light/12 h-dark
cycle) and fed daily with mosquito larvae. Males and females were housed in separated 1-l bottles that were wrapped around with a piece of paper to prevent visual contact and fighting. Prior to the test, the nest-holding male was placed in his 1-l bottle next to a female in her 1-l bottle until the female became gravid.

To control for confounding effect of size differences between males, the fishes were measured prior to the experiments. The following procedure was followed in measuring the fish body length (Jaroensutasinee and Jaroenututypsum, 2001a,b). First, each fish was placed in an aquarium (20.0 cm × 12.0 cm × 16.0 cm high) filled with water to the depth of 3 cm. The fish were not anaesthetised because most types of anaesthetic alter fish appearance (Kodric-Brown, 1989). Secondly, a piece of Plexiglas with a ruler was placed in the aquarium to provide a standard calibration. Finally, after a 1-min acclimatisation period, the fish were photographed with a digital camera. The digital pictures were used to estimate the fish’s standard body length from the tip of the upper jaw to the caudal peduncle.

The nest-holding male was placed in a 37-l aquarium, measuring 0.50 m × 0.25 m × 0.30 m high, densely planted with aquatic vegetation. Males built their bubble nests within 24 h after being placed in the aquarium. A gravid female was placed in the aquarium 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 aquarium. After the female was placed in the aquarium, the nest-holding male chased the female and tried to entice her to come and spawn under his bubble nest. The female usually spawned between 07:00 and 10:00 h in the morning after she had been placed with the nest-holding male. After fertilisation was completed, the mated female was immediately removed from the breeding tank.

During two reproductive phases (i.e. 1 h after the eggs were laid and within 1 h of the eggs hatching), three types of intruders were introduced to the nest-holding males (i.e. males, gravid females and females which have laid eggs). Each 15 min trial was separated by a 10 min interval and conducted in random order to minimise an order effect. An intruder was placed in the breeding tank for 5 min before the observation began. The aggressive responses of the nest-holding male was observed, including gill cover erection, biting, tail beating, attacking and chasing. At the end of the observation period, the intruders were removed from the experimental aquarium and returned to their home tank. Sixty replicates were conducted with new sets of fish in order to avoid pseudo-replication. No males or females had been used in the experiment more than once to avoid an order effect.

The number of the five aggressive acts (i.e. gill cover erection, biting, tail beating, attacking and chasing) was recorded (Simpson, 1968). Gill cover erection was regarded as males erecting the gill cover while oriented towards or parallel to intruders. Lowering of the gill cover or swimming away from intruder ended gill cover erection. Biting was recorded when males used the mouthpart to bite or tear at intruder. Tail beating was defined as each separate beat of the tail towards intruder. Attacking was recorded when the focal male swam rapidly towards its intruder. Chasing was defined as rapid and continuous following. Male aggressiveness was the sum of each five aggressive act that the nest-holding male performed during the 15 min observation period.

2.1. Statistical analyses

All variables were tested for normality using Lilliefors’ test. The equality of variances was evaluated using Levene’s test. The three-way repeated-measures ANOVA was used to test for types of intruder (male, female and female that have laid eggs), times of reproductive phase (after eggs were laid and after eggs hatched), types of aggressive behaviour (gill cover erection, biting, tail beating, attacking and chasing) and the interaction among factors, and then followed by paired samples t-tests.

3. Results

The nest-holding males, male intruder after egg laid and male intruders after eggs hatched were not different in size (nest-holding males (¯ ± S.D. = 3.30 ± 0.26), male intruders after eggs laid (¯ ± S.D. = 3.23 ± 0.34) and male intruders after eggs hatched (¯ ± S.D. = 3.26 ± 0.31) one-way ANOVA: \( F_{2,177} = 0.81, \text{ns} \). Females who laid the eggs were the same size (¯ ± S.D. = 3.20 ± 0.22) as female intruder after egg laid (¯ ± S.D. = 3.14 ± 0.25) and female intruders after eggs hatched (¯ ± S.D. = 3.13 ± 0.21) (one-way ANOVA: \( F_{2,177} = 1.59, \text{ns} \).
Table 1
Three-way repeated-measures ANOVA (Wilks’ Λ) of types of intruders at two reproductive phases with five types of aggressive acts

<table>
<thead>
<tr>
<th>Effect</th>
<th>Hypothesis d.f.</th>
<th>Error d.f.</th>
<th>Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intruder</td>
<td>2</td>
<td>57</td>
<td>0.161</td>
<td>148.253 **</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>1</td>
<td>58</td>
<td>0.913</td>
<td>5.509  *</td>
</tr>
<tr>
<td>Aggressive behaviour</td>
<td>4</td>
<td>55</td>
<td>0.188</td>
<td>59.223 **</td>
</tr>
<tr>
<td>Intruder × reproductive phase</td>
<td>2</td>
<td>57</td>
<td>0.992</td>
<td>0.234  ns</td>
</tr>
<tr>
<td>Intruder × aggressive behaviour</td>
<td>8</td>
<td>51</td>
<td>0.214</td>
<td>23.383 **</td>
</tr>
<tr>
<td>Reproductive phase × aggressive behaviour</td>
<td>4</td>
<td>55</td>
<td>0.930</td>
<td>1.031 ns</td>
</tr>
<tr>
<td>Intruder × reproductive phase × aggressive behaviour</td>
<td>8</td>
<td>51</td>
<td>0.783</td>
<td>1.770 ns</td>
</tr>
</tbody>
</table>

* P < 0.05.
** P < 0.001.

The nest-holding males displayed differing amounts of aggressive acts per 15 min towards three types of intruders (Fig. 1a–e, Table 1). The nest-holding males were the most aggressive toward male intruders, intermediate towards female intruders and the least aggressive towards females that have laid eggs (Fig. 1a–e). For reproductive phase, the amount of aggressive acts significantly changed with the reproductive phases, being more frequent after egg hatching than egg laying (Table 1). For types of intruders and aggressive acts, these had no major effect since for intruder × aggressive behaviour, the interaction was significant (Table 1). The frequency of each of the five types of aggressive acts significantly varied in relation to the type of intruder.

The nest-holding males won all 120 fights with female intruder and 120 fights with females that have laid eggs (chi-square tests for both female intruders and females which have laid eggs: \( \chi^2 = 60, P < 0.001 \)) and lost 1 of 120 fights with male intruders (chi-square test: \( \chi^2 = 58.02, P < 0.001 \)).

4. Discussion
This study aimed to test only the first hypothesis of theoretical models that parents will increase their parental effort during the period of parental care when the average reproductive value of the young increases (Williams, 1966; Pianka, 1976; Pressley, 1976, 1981; Ricklefs, 1977). The result supports the hypothesis that the nest-holding males become more aggressive as the reproductive value of the young increases as they age. This is because the probability of the survival of offspring to reproduce increases. It has been shown in many fish species that parental males put more effort into egg guarding and caring as eggs become older, such as in three-spined sticklebacks (van Iersel, 1953), freshwater gobies (Torricelli et al., 1985), common gobies (Magnhagen and Vestergaard, 1993), and sand gobies (Lindström and Wennström, 1994). These males increase their fanning with increased egg age because larval fish need more oxygen, as they grow older (Reebs et al., 1984). However, Yamamoto et al. (1999) found the opposite result in that an Amazonian cichlid, Pterophyllum scalare Lichtenstein, was more aggressive after the eggs were laid than after the eggs hatched. The opposite finding between Yamamoto’s study and our study may be because wild-caught male fighting fish do not cannibalise their eggs during the period of parental care but the Amazonian cichlid has some tendency to eat its own eggs. Therefore, Amazonian cichlid eggs are at greater risk than newly hatched fry because they are immobile compared to newly hatched fry (Yamamoto et al., 1999).
Our results showed that the nest-holding males performed specific amounts of aggressive acts towards different types of intruders. Nest-holding males were the most aggressive towards male intruders. Male intruders may pose an additional threat of territorial takeover. Owning a territory is crucial for male fighting fish to obtain mates. This also has been shown in Tanganyikan cichlid (Lamprologus ocellatus; Walter and Trillmich, 1994) and Amazonian cichlid (P. scalare, Yamamoto et al., 1999). In addition, male fighting fish were less aggressive towards female intruders. These males may perceive opposite sex intruders as their potential mates. The nest-holding males were the least aggressive toward females that have laid eggs. This suggested that these males might be able to distinguish between females that have laid eggs and other female intruders. Females that have laid eggs might pose less threat of eating their own eggs than other females. However, there is an alternative explanation for the different response to the two types of female intruders. Courthouse and aggressive behaviour of male fighting fish share the same components of display (i.e. tail beating, gill cover erection and approach, Robertson and Sale, 1975; Doulrelant et al., 2001). The difference between these two types of display regards which behaviours are performed less or more commonly towards the intruders. During courtship behaviour, males perform very few bites and perform mostly tail beating, and gill cover erection, while males also spend more time at the nest. During an aggressive interaction male bites more, increases the amount of tail beats and gill cover erection, and decrease the time they spend under the nest (Robertson and Sale, 1975; Doulrelant et al., 2001). When we look at the gravid female treatment and compare between reproductive phases, one finds that males perform more tail beating and gill cover erections after hatching than after egg laying, but these males do not change their biting behaviour. This change in behaviour could represent and increase of the motivation of males to court gravid females as the eggs hatch and the males become more available to take care of new eggs.

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References


