The Effects of Different Types of Intruders on the Level and Characteristics of Territorial Defence Response of Domesticated Male Siamese Fighting Fish

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ABSTRACT

This study investigated the effects of types of intruders on the level and types of male aggressive acts at different stages of the egg guarding period in domesticated fighting fish (*Betta splendens*). The degree of aggressiveness was measured at two reproductive phases in response to three types of intruders: male, female and female that had laid eggs. The nest-holding males displayed the same level of aggressiveness towards intruders before and after the hatching of eggs. Male intruders elicited the highest number of aggressive acts followed by female, and mated female intruders. The highest incidence of gill cover erection, biting, and tail beating by nest-holding males was directed towards male intruders whilst female and mated female intruders were subject to lower and lowest incidence of these aggressive acts respectively. However, female intruders were attacked and chased most frequently and mated female intruders comparatively less frequently whereas male intruders were least subject to these types of aggression.

Key words: Aggressive behaviour - *Betta splendens* - Egg guarding – Siamese fighting fish - Territorial defence

INTRODUCTION

Territorial defence is costly for both territorial males and their intruders (1,2). Strategic decisions, either defending or giving up its territory, are made in relation to the costs that territorial holders and their intruders inflict on each other (3,4). The degree of parental effort displayed in any activity, determined on the basis of this risk to future reproductive potential, will depend on the value of the present offspring compared to the parent's expected value of future offspring (5).

A few studies have been undertaken on the changes in parental care over the breeding cycle of fish in response to different intruders including those of three-spined stickleback (*Gasterosteus aculeatus*; 6), smallmouth bass (*Micropterus dolomieui*; 7,8), bluegill sunfish (*Lepomis macrochirus*; 9), cichlid (*Cichlasoma nigrofasciatum*; 10), freshwater goby (*Padogobius martensii*; 11), common goby (*Pomatoschistus microps*; 12), sand goby (*Pomatoschistus minutus*; 13) and wild fighting fish (*Betta splendens*; 14). 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 for the parents (15,16,17). Parents spend substantial amounts of time and energy on egg guarding, egg ventilating to provide a flow of oxygenated water, and removing dead eggs from the nest (18,16). The domesticated Siamese fighting fish (*Betta splendens* Regan) conforms to this pattern in its parental care behaviour. Males construct their bubble nests, entice females to spawn in them, and care for fertilised eggs and fry (19). Therefore, the ability to guard eggs and fry is critical to the reproductive success of male Siamese fighting fish.

We studied the level of male aggressiveness in domesticated Siamese fighting fish towards potential intruders during egg guarding. The degree of aggressiveness was measured at two reproductive phases in response to the territorial intrusion by a male, a female and a female that had laid eggs. By quantifying territorial behaviour during encounters, the following hypotheses were investigated:

1. Males should become more aggressive after the eggs have hatched.
2. Males should be most aggressive towards male intruders, less aggressive towards female intruders, and least aggressive towards females that have laid eggs. This is because nest-holding males may perceive in male intruders an additional threat of territorial take-over, and perceive female intruders as potential mates. Therefore, the nest-holding males should display different levels of aggression towards different types of intruders.

Fish Biology

The Siamese fighting fish is an Anabantid native to Southeast Asia. Males of 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. They defend a territory in the water column near the surface. Each territory is centred on the bubble nest built by the male (20). 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 (19,21,22,14). It takes fertilised eggs approximately 36 hours to hatch. Males exhibit parental care behaviour both in the field and in the laboratory for 5-7 days after hatching (21,22,14). They do not cannibalise their eggs and larval fish during the period of parental care. Highly aggressive social displays by the males include gill cover erection, fin spreading, biting, and tail beating (23,24). Fighting usually results in physical damage and even death. Females are duller in colour. After the females finish laying eggs, the males chase the females out of the bubble nest areas, and provide parental care for the developing eggs and larval fish themselves.

MATERIALS AND METHODS

The test subjects were 180 domesticated males and 120 females bought from a fighting fish farm in Bangkok. They were kept in the laboratory with natural light (i.e. approximately 12:12 light: dark cycle) and fed daily with mosquito larvae. Males and females were housed in separated 1-litre bottles that were wrapped around with a piece of paper to prevent visual contact. Prior to the test, the nest-holding male was placed in its 1-litre bottle next to a female in its 1-litre bottle until she became gravid.

To control the effect of size differences, the males were measured prior to the experiments. The following procedure was followed in measuring the fish body length

(21,22,14). First, each fish was placed in an aquarium (20.0x12.0x16.0 cm high) filled with water to the depth of 3 cm. The fish was not anaesthetised because most types of anaesthetic alter fish appearance (25). 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 was 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 then placed in a 37-litre aquarium, measuring 0.50x0.25x0.30 m high, densely planted with aquatic vegetation. Males built their bubble nests within 24 hours after being placed in the aquarium. A gravid female was placed in the aquarium with the nest-holding male in the evening at approximately 1530 hours of the second day after the nest-holding male had been 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 0700-1000 hours the morning after she had been placed with the nest-holding male. As soon as fertilisation was completed, the mated female was immediately removed from the breeding tank.

During the two reproductive phases (i.e. 1 hour after the eggs were laid and within 1 hour of the eggs hatching), three types of intruders were introduced to the nest-holding males (i.e. males, gravid females and mated females which had laid eggs). Each 15-min trial was followed by a 10-min interval, and conducted in random order to minimise an order effect. Observation began after an intruder had been placed in the breeding tank for 5 minutes. The aggressive responses of the nest-holding male were observed, including gill cover erection, biting, tail beating, attacking and chasing. At the end of the observation period, the intruder was removed from the experimental aquarium and returned to its home tank. Sixty replicates were conducted with new sets of fish in order to avoid pseudo-replication. No male or female was 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 (24). Gill cover erection occurred when the nest-holding male raised upright its gill cover while moving towards or parallel to the intruder. The act ended when the defender lowered the gill cover or swam away from the intruder. Biting was recorded when the nest-holding male used its mouthpart to bite or tear at the intruder. Tail beating was defined as each separate beat of the tail towards the intruder. Attacking was recorded when the nest-holding male swam rapidly towards its intruder. Chasing was defined as rapid and continuous following by the defender in order to put the intruder to fight. Total aggressive acts were the sum of all different five aggressive acts that the nest-holding male performed during the 15-min observation period.

Statistical Analyses

All variables were tested for normality using Lilliefors' test. The equality of variances was evaluated using Levene's test. The one-way ANOVA was used to test for body size differences among groups of intruders. The two-way repeated-measures ANOVA was used to test for types of intruders (i.e. male, female and mated female that had laid eggs), stages of reproductive phase (i.e. before and after egg hatching), and the interaction of these factors prior to pairwise comparisons. The two-way repeated-measures ANOVA was also used to test for types of intruders, types of

aggressive acts (i.e. gill cover erection, biting, tail beating, attacking, and chasing) and the interaction of various factors prior to Dunnett T3 tests for pairwise comparisons.

RESULTS

The nest-holding males, male intruders before egg hatching, and male intruders after egg hatching were not different in size (nest-holding males ($\bar{x} \pm SD$ = 4.51 ± 0.71), male intruders before egg hatching($\bar{x} \pm SD$ = 4.49 ± 0.67), and male intruders after egg hatching($\bar{x} \pm SD$ = 4.45 ± 0.62) (one-way ANOVA: $F_{2, 177}$ = 0.13, ns). Females who laid the eggs were the same size ($\bar{x} \pm SD$ = 4.95 ± 0.64) as female intruders before egg hatching ($\bar{x} \pm SD$ = 4.88 ± 0.56) and female intruders after egg hatching ($\bar{x} \pm SD$ = 4.82 ± 0.53) (one-way ANOVA: $F_{2, 177}$ = 0.75, ns).

The numbers of aggressive acts displayed by the nest-holding male per each 15-min observation period towards the three different types of intruders varied **(Table 1, Figure 1)**. The highest number of total aggressive acts was recorded when male intruders were present. Less aggression was directed towards female intruders, and least towards mated females that had laid eggs **(Figure 1).** The nest-holding male did not become more aggressive towards the intruders after the eggs had hatched than before egg hatching **(Table 1, Figure 1).** There was an interaction between types of intruders and reproductive phases **(Table 1, Figure 1)**.

Table 1. Two-way repeated-measures analysis of variance (Wilks' Λ) of types of intruders at two reproductive phases. ***P*<0.001, **P*<0.05

Effect	Hypothesis *d.f.*	Error *d.f.*	Value	*F*	*P*
Intruder	2	293	0.537	126.19	**
Reproductive phase	1	294	0.997	0.81	NS
Intruder x Reproductive phase	2	293	0.978	3.27	*

The frequency of each of the five types of aggressive acts significantly varied in relation to the types of intruders **(Table 2, Figure 2a-e).** The highest rate of gill cover erection, biting, and tail beating occurred in the presence of male intruders. These aggressive acts were less frequent towards female intruders, and least of all towards mated females **(Table 2, Figure 2a-c).** However, the nest-holding males attacked and chased away female intruders most frequently, mated female intruders less so, and male intruders least of all **(Table 2, Figure 2d-e)**. There was an interaction between types of intruders and types of aggressive acts per each 15-min observation period **(Table 2).**

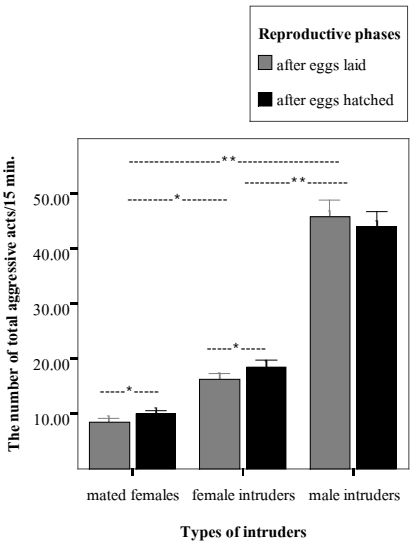

Figure 1. Mean (±SE) of the number of aggressive acts per 15-min observation period among three types of intruders at two reproductive phases. Dash bars link those groups that were found to be significantly different. Two-tailed paired *t* test, ***P*<0.001, **P*<0.05

Table 2. Two-way analysis of variance (Wilks' Λ) of types of intruders at five aggressive acts. **P*<0.001

Effect	*SS*	*d.f.*	*MS*	*F*	*P*
Intruder	415101.65	2	207550.83	1096.96	*
Aggressive acts	837792.27	4	209448.07	1106.99	*
Intruder x aggressive acts	438872.09	8	54859.01	289.94	*
Error	334894.34	1770	189.21		
Total	3044574.00	1785			

DISCUSSION

The result does not support the findings of other studies that the nest-holding males become more aggressive as the reproductive value of the young increases as they age. This may be because the domesticated males have been subject to intensive artificial selection for stage fighting for hundreds of generations. Consequently, there is no powerful selective force acting on the probability of survival of domesticated offspring. This finding is different from the results of the study on wild fighting fish (14). Two factors may account for this divergence. First, wild fighting fish have a restricted reproductive season. The probability of being able to breed again decreases with the time spent caring for a particular brood. Therefore, investments in territorial defence should increase in direct proportion to the age of offspring (14). Second, wild

fighting fish offspring would have a lower survival rate than domesticated offspring due to higher predation risks and lower food availability in their natural environment.

Yamamoto et al (26) found that an Amazonian cichlid, *Pterophyllum scalare* Lichtenstein, was more aggressive before than after the eggs hatched. The contrary findings of Yamamoto's study and our study may be explained by the fact that domesticated fighting fish does not cannibalise its offspring during the period of parental care whereas the Amazonian cichlid male has some tendency towards eating the eggs under its care. Amazonian cichlid eggs are at greater risk than newly hatched fry because they are immobile compared to the newly hatched fry (26).

Our results showed that the domesticated nest-holding males performed varying numbers of aggressive acts towards different types of intruders. Nest-holding males were most aggressive towards male intruders as male intruders could pose an additional threat of territorial take-over. Male fighting fish needs to own and hold a specific territory in order to obtain mates. This also applies to Tanganyikan cichlid (*Lamprologus ocellatus*; 27), Amazonian cichlid (*Pterophyllum scalare*; 26), and wild fighting fish (14). On the other hand, male fighting fish was less aggressive towards female intruders as it might perceive female intruders as potential mates. The nest-holding males were least aggressive towards mated females that had laid eggs. This suggests that they may be able to distinguish between mated females and other gravid female intruders. Females that have laid eggs may pose less treat of eating their own eggs than other females.

However, there is an alternative explanation for the different responses to the two types of female intruders. Courtship and aggressive behaviour of male fighting fish share the same components of display (i.e. attacking and chasing; 28,29,14). Courtship and aggressive behaviours are differentiated by the intensity of display and the amount of time the male spends at the nest. During courtship display the male mostly attacks and chases the female, and spends more time at the nest. Biting acts are few. However, during an aggressive interaction the male bites more, increases the frequency of tail beats and gill cover erection, and spends less time under the nest (28,29,14). When confronted with the gravid female intruder, the nest-holding male displayed more attacking and chasing than raising gill cover, biting and tail beating. This behaviour could represent the male's motivation and intention to court rather than repel the gravid female intruder.

ACKNOWLEDGMENTS

We thank J Endler and T na Nagara for comments on previous versions of the manuscript. Invaluable assistance in the laboratory was provided by N Kongthong, P Suebchana, P Sirisuk, N Klanniwat, and U Chavalit. This work was supported by the TRF/BIOTEC Special Programme for Biodiversity Research and Training grant BRT R-144003 and Complex System Key University Research Unit, the Institute of Research and Development, Walailak University.

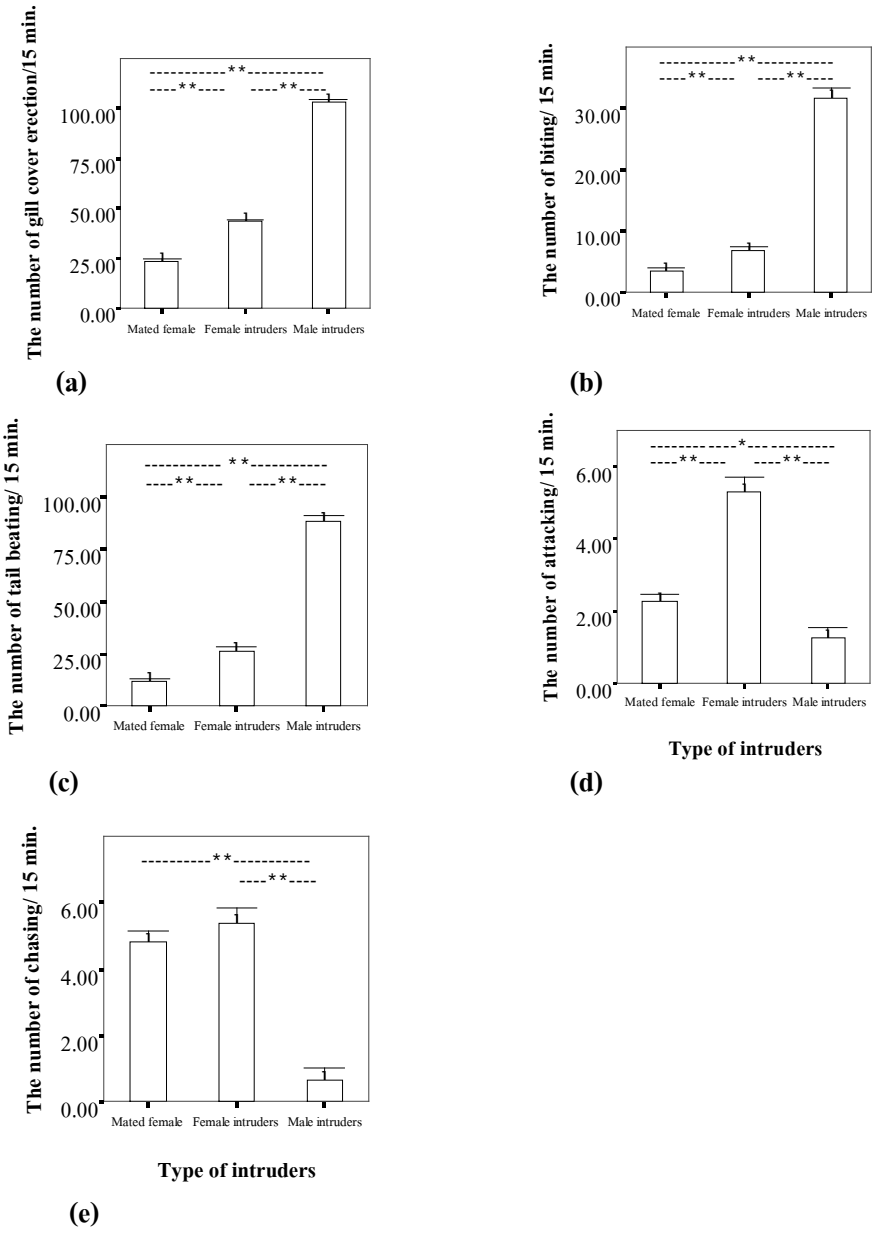

Figure 2. Mean (±SE) of the number of aggressive acts per 15-min observation period among three types of intruders. Dash bars link those groups that were found to be significantly different. (a) Gill cover erection, (b) biting, (c) tail beating, (d) attacking, and (e) chasing. Two-tailed paired *t* test, ***P*<0.001, **P*<0.01

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บทคัดย่อ

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ผลของชนิดผู้บุกรุกต่อระดับและลักษณะของการป้องกันอาณาเขตของปลากัดเพศผู้

การศึกษานี้ได้ทดสอบผลของชนิดผู้บุกรุก และช่วงเวลาในการฟักไข่ต่อระดับความก้าวร้าวของปลากัด (*Betta splendens*) เพศผู้ ระดับความก้าวร้าวของปลากัดเพศผู้ได้ทำการวัดที่สองช่วงเวลาคือ หลังจากที่ปลากัดเพศเมียเพิ่งวางไข่และหลังจากที่ไข่เพิ่งฟักเป็นตัว โดยทดสอบกับชนิดของปลาผู้บุกรุกสามชนิดคือ ปลากัดเพศผู้ ปลากัดเพศเมีย และปลากัดเพศเมียที่เพิ่งวางไข่ พบว่า ปลากัดเพศผู้เจ้าของหวอดแสดงพฤติกรรมก้าวร้าวไม่แตกต่างกันระหว่างสองช่วงเวลาต่อปลาบุกรุกทั้งสามชนิด แต่ปลากัดเพศผู้เจ้าของหวอดแสดงพฤติกรรมก้าวร้าวสูงสุดกับปลาบุกรุกเพศผู้ แสดงพฤติกรรมก้าวร้าวรองลงมากับปลากัดเพศเมีย และน้อยที่สุดกับปลากัดเพศเมียที่เพิ่งวางไข่ โดยที่ปลากัดเพศผู้เจ้าของหวอดจะแสดงพฤติกรรมการแผ่เหงือก กัดและตีหางใส่ปลาบุกรุกเพศผู้มากที่สุด อย่างไรก็ตาม ปลากัดเพศผู้เจ้าของหวอดไล่และจู่โจมเข้าใส่ปลาบุกรุกเพศเมียมากกว่าปลากัดเพศเมียที่เพิ่งวางไข่ และปลากัดเพศผู้เจ้าของหวอดไล่และจู่โจมเข้าใส่ปลาบุกรุกเพศผู้น้อยที่สุด

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