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Shoaling and aggressive behaviour in juvenile fighting fish (*Betta splendens*)

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Abstract

The fighting fish (*Betta splendens*) is known for its aggressive behaviour. In the wild, the adult fighting fish are territorial and show aggressive behaviour. Previous studies showed that adult fighting fish associated with conspecifics showed shoaling behaviour in the laboratory. However, no previous studies have investigated shoaling behaviour in juvenile fighting fish and it could have implication for commercial breeding. Therefore, we examined shoaling preference and aggressive behaviour of the juvenile male fighting fish by using binary choice experiments in the laboratory. When testing these two kinds of behaviour, the test fish were given a choice between a female juvenile fish shoal and a male juvenile fish shoal in order to evaluate whether juvenile male fighting fish preferred shoaling with the same sex or different sex before being reared individually. It was found that males showed no significant difference either in shoaling preference or aggressive behaviour. However, the size of male test fish was positively correlated with aggressive behaviour. Therefore, large juvenile male fighting fish should be reared separately from shoals in the fish culture.

Keywords: fighting fish, shoaling preference, aggressive behaviour

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1. Introduction

Shoaling in fish is a behaviour that allows them to remain together through social attraction [1]. This behaviour is widespread among fish species and it has been estimated that over 50% of approximately 25,000 species of fish engage in shoaling in at least one stage of development during their ontogeny and about 25% of fish species shoal throughout all of their lives [2]. Such groups range from small aggregations of Cyprinid fishes and Perciform fishes in freshwater habitats to huge pelagic groups of marine fishes such as cod, herring or tuna. Due to the existence of enormous shoals of marine fishes, shoaling contributes to the commercial importance of fisheries [2, 3]. Also, fish shoals have attracted biologists including behavioural ecologists, because they are model systems to investigate several aspects of social behaviour and organisation [4]. Shoaling in freshwater fishes has been studied more often than shoaling in marine species, probably because pelagic fish move fast and usually form enormous groups. This makes direct observation difficult and the capture of complete shoals impossible in the wild for marine species. In contrast, these constraints are reduced by studying freshwater fishes [5].

Betta splendens, the Siamese fighting fish, is a small freshwater fish known primarily for the aggressive behaviour demonstrated by males [6]. In nature adult fighting fish disperse to a density of 1.7 fish/m^2 [7] and are not typically seen forming shoals [8]. However, in the laboratory, adult fighting fish have been shown to associate with conspecifics, showing shoaling behaviour [9]. In binary choice tests, female and male fighting fish were shown to associate with other fighting fish over empty chambers, to prefer larger groups of females over individual females, and to prefer groups of females over individual males [9]. These results show that *B. splendens* demonstrates social behaviour similar to shoaling under specific conditions. However, no previous studies have investigated shoaling behaviour in juvenile fighting fish and this is relevant to fish breeders as they may prefer to maintain them in shoals rather than individually [10].

For local sport games and other commercial reasons in Thailand, the ornamental fighting fish is one of the most popular fishes (it is one of the two most popular ornamental fishes) for local sale and exporting globally. This requires raising the fish for mass production for professional breeders. There are many issues that arise when rearing fish for large scale breeding and propagation. One of these is the excessive aggressive behaviour of the juvenile male fighting fish raised in a litter [11]. This behaviour leads to loss of favourable

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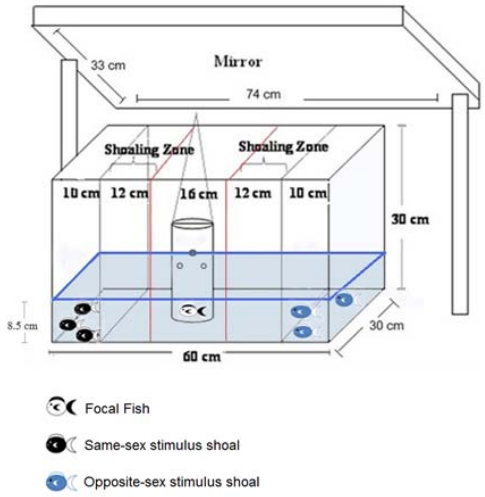

Figure 1. The test tank.

characteristics (eg. complete fin or complete scales) and this damage can reduce the monetary value of the fish for commercial breeders [10]. Therefore, efficient techniques for culturing are required in order to reduce the budget costs of breeding and propagation and to avoid damage to the physical appearance of the fish due to aggressive behaviour. Here, we investigated shoaling and aggressive behaviour in juvenile male fighting fish with a view to improve the commercial production of this fish species.

2. Materials and methods

Fish maintenance

Two hundred juvenile fighting fish (1.5 months old, with total length between 25 mm and 29 mm) were obtained from a local fish farm in Nakhon Pathom province (WGS84: 13° 86' 44.55" N, 100°7' 30.88" E), Thailand, in August 2013. They were born in the same brood (full sibs) in the farm. When they were brought into the laboratory, they were transferred to three aquaria (60×30 with 30 cm water depth) at the Department of Biology, Faculty of Science, Burapha University for raising in the laboratory. They were reared together in the same stock aquarium. The experiments were conducted in October 2013 when they were nearly 3 months old and still considered to be juveniles [10]. The focal and stimulus fish were directly chosen from the same stock aquarium in order that we could control for any effects of familiarity on shoaling preference [12]. Temperature (26º-28º C) and lighting conditions were natural and from the sun light. All fish were fed twice daily ad libitum with dried commercial food pellets.

Experimental apparatus

The experimental tank consisted of one tank (60 cm ×30 cm with 16 l. water) covered with opaque paper to minimise disturbing stimuli from outside the test tank. To observe experiments, a full length mirror was held with clamp stands at 45° at the back and above the tank. It was subdivided into three sections, with one central compartment (16 cm in length) and two side compartments (10 cm in length for each side) separated by porous plexiglass to allow both chemical and visual cues. The stimulus zone was marked out with a thin rope as 12 cm from either side compartment to the central compartment. According to a protocol used to study shoaling preference by [13], the size of this stimulus zone was defined as within approximately 3 body lengths of test fish in this study. Also, a transparent release tube was used by the observer and remotely raised with a pulley and cord. (Figure 1)

Experiment on shoaling behaviour and aggressive behaviour

All trials were conducted in October 2013, during daylight hours, between 9.00 and 17.00 and all the fish came from the same stock tank from the same litter. Using a binary choice for each trial, there was a test fish (a focal fish) and 2 stimulus shoals with 3 fish for each shoal (a same-sex stimulus shoal VS an opposite-sex stimulus shoal). For each experiment, each stimulus shoal was randomly chosen and placed in each of the end compartment of the test tank to acclimate for 12 hours. After the acclimatisation period, a focal male fish was then placed in a clear porous plastic cylinder in the centre of the middle compartment and allowed to acclimatise for 10 minutes. Following this period, an

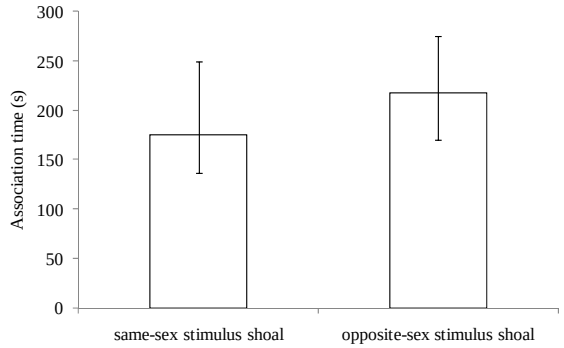

Figure 2. The histogram shows association time (medians ± quartiles) displayed by male focal fish.

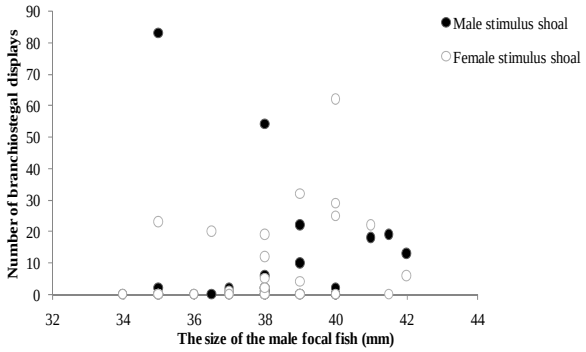

Figure 3. The correlation between level of aggression (number of branchiostegal displays) of the male focal fish interacting with a female stimulus shoal (open circles) and a male stimulus shoal (close circles) and the size of the male focal fish (mm).

observer sat motionless 2 metres in front of the test tank and the pulley over the test tank was raised to release the focal male fish from the release tube. As soon as the focal male fish entered the stimulus zone for the first time, the trial was commenced. Each trial lasted for 10 minutes (600 seconds), with the observer measuring how long the test fish spent in the shoaling zone next to a same-sex stimulus shoal VS an opposite-sex stimulus shoal by using two stop watches to measure time spent in each shoaling zone. The test fish was considered to have either entered or left the zone as soon as its snout either entered or left the shoaling zone.

To quantify aggressive behaviour, the level of aggression was recorded by tallying the number of branchiostegal displays or opercular erections [14]. All fish were naïve to the experiment. Thirty replicates for the juvenile male fighting fish were carried out. For each trial, a focal fish was in the sized range of stimulus fish. All juvenile fish were used in this experiment had a total length (TL) between 29-42 mm.

Statistical analysis

Nonparametric tests, Wilcoxon Signed Rank Test and Spearman rank correlation, were used throughout these experiments as none of the data conformed to a normal distribution and could not be satisfactorily transformed. Exact tests were used throughout. The significance level at $P < 0.05$ was used to indicate 'significant statistical difference'. All statistical tests were performed using SPSS version 14.

3. Results

For shoaling behaviour (Figure 2), there was no difference in the time spent in the shoaling zone between a same-sex stimulus shoal and an opposite-sex stimulus shoal (Wilcoxon Signed Rank Test: $z = -0.956$, $P = 0.339$, $n= 30$). For aggressive behaviour, there was no difference in the number of branchiostegal displays in these two zones (Wilcoxon Signed Rank Test: $z = -1.416$, $P = 0.157$, $n= 30$).

However, the level of aggression against the male stimulus shoal in the shoaling zone was positively and significantly correlated to the size of the juvenile male test fish (Spearman correlation: $r2 = 0.369$, $P= 0.045$) whereas there was no such correlation between this behavioural trait and the size of these male focal fish when interacting with the female stimulus shoal (Spearman correlation: $r2 = 0.304$, $P= 0.103$), as seen in Figure 3.

4. Discussion

In theory, fish should assort in shoals on the basis of similar phenotypic traits and shoal members should have phenotypic assortment on the basis of: species, sex, phenotype, parasitism, familiarity and kinship [4]. By using binary choice test in this study, sex difference was not a factor for the juvenile male fighting fish to make a decision to choose a partner shoal. As all the fish in this study were less than 3 months old, it was likely that they were still juvenile and did not have motivation to reproduce [10]. Therefore, the male focal fish did not significantly show female preference when making the shoaling decision.

Our juvenile male fighting fish did not show sex difference in aggressive behaviour even though they are territorial and show aggressive behaviour [10], they are capable of living in groups and with a pecking order [9]. In this study, all the fish were full sibs and reared together after fertilization. It might be useful to investigate whether there is a difference in the level of aggression between kin and non-kin in the future to test hypotheses about inclusive fitness [15]. We found that there was a significant positive relationship between the size of focal male juveniles and the level of male aggression against the male stimulus shoal. It is possible that larger male juveniles might have higher androgen

levels than smaller ones and this hormone might have made the larger ones show more aggression [16].

Excessive aggression in territorial fishes might be bad for commercial rearing operations. It may lead to stress for the fishes, which could inhibit growth and make fish more susceptible to disease, injury and even death. It is likely that this aggressive behaviour of the male fighting fish raised in a brood could lead to the above damage [10], and this is significant because they need a good appearance with complete fins and scales to be sellable within large scale commercial aquaculture [11]. This study indicates that larger male juvenile fighting fish should be reared in isolation from the brood to reduce production costs and damage of physical appearance for smaller males. This finding may also be applicable to territorial fish cultures for reducing production costs.

5. Conclusion

In conclusion, this study provides basic knowledge on the behaviour of Siamese fighting fish that will be beneficial to commercial aquaculture of this species [17]. Large juvenile male fighting fish should be raised separately from their own brood to avoid excessive aggressive behaviour and consequently reduce damage to the ornamental appearance the fish.

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