Context-dependent group size preferences in large shoals of three-spined sticklebacks

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Grouping behaviour is widespread in animals. One important reason for grouping is the reduction of individual predation risk; the larger a group, the greater the protection for the individual. Fishes, in particular, have become a model taxon in experimental research to study proximate and ultimate causes of grouping. Accordingly, numerous studies have so far demonstrated that fishes prefer to shoal with larger groups. Thus far these studies have usually examined small groups, with up to 20 individuals. However, in nature groups are often much bigger (up to several hundreds of individuals), and theory predicts that benefits, for example due to dilution effects, decline exponentially with increasing group size. Furthermore, discrimination might be absent because of limited cognitive ability. Thus, it is essential to test whether the findings from small groups also apply to large groups. Here, we examined group size preferences in the three-spined stickleback, Gasterosteus aculeatus, a small fish that forms large shoals in nature. In five experiments, subadult sticklebacks were given the choice between two shoals differing in group size (numerical contrasts: 15 versus 60, 20 versus 60, 30 versus 60, 40 versus 60 and 50 versus 60). Test fish on average preferred the larger group; this preference was stronger in the beginning of the respective trial and decreased over time. Moreover, preferences for the larger shoal decreased with decreasing group size differences, implying context-dependent preferences. We found significant discrimination up to numerical contrasts of 40:60. Our results are in accordance with the findings of group size discrimination in small groups and with optimality hypotheses, but might also reflect the impact of cognitive constraints.

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might be different when all available shoals are relatively large. Thus, under natural conditions (Stone & Beran, 2012).

...larger than 20 individuals. The knowledge gained from small group experiments cannot necessarily be transferred to larger groups, because the benefits of choosing a larger group might be different when all available shoals are relatively large. For example, benefits might decline exponentially (Cresswell & Quinn, 2011 and references therein). Furthermore, owing to cognitive constraints quantification discrimination might be affected when the total number is large (Agrillo, Piffer, & Bisazza, 2010). However, little is known about group size preferences in groups exceeding 20 individuals. A recent study on trained western mosquitofish, Gambusia affinis, showed that these fish can discriminate between large sets at a ratio of 1:2, in this case between 100 and 200 artificial objects (Agrillo et al., 2010), whereas a comparative study reported that none of five investigated fish species discriminated numerical contrasts of 25 versus 50 (Agrillo et al., 2012).

In the present study, we investigated spontaneous group size discrimination in the three-spined stickleback, Gasterosteus aculeatus. The three-spined stickleback is a small fish widely distributed across the northern hemisphere. Outside the breeding season, it forms shoals with group sizes ranging from a few individuals to several hundred fish (Poulin, 1999; Wootton, 1984). Sticklebacks have evolved several morphological features, such as spines for protection against predators (e.g. Frommen et al., 2011). Living in shoals is assumed to reduce individual predation risk further (Magurran, 1990). Shoaling in sticklebacks has been intensively studied (e.g. reviewed in Frommen, Mehlis, Brendler, & Bakker, 2007). When given the choice, sticklebacks prefer to shoal with the larger of two groups at different numerical contrasts: for example, 3 versus 20, 3 versus 5 (Krause, 1993), 5 versus 10, 5 versus 9 (Krause, Godin, et al., 1998), 8 versus 12 (Frommen, Hiermes, & Bakker, 2009) or 3 versus 6 (Fischer & Frommen, 2013). In the present study the first aim was to test whether sticklebacks discriminate between two shoals differing in group size when both are relatively large, using numerical ratios similar to those used in previous ‘small group’ studies. Therefore, we conducted five experiments with numerical contrasts ranging from 15:60 to 50:60. The second aim was to test whether the strength of the preference for the large group changes according to the benefits that are assumed to decrease with decreasing group size differences. Finally, we tested whether preferences change over time, because habituation effects might influence shoaling preferences.

METHODS

Experimental Subjects

Sticklebacks used in the experiments were subadult (aged between 3 and 4 months), F1 offspring originating from wild-caught fish. Parental fish were purchased in April 2010 from a commercial fisherman who had the permission to catch sticklebacks during their spring migration on the island of Texel, the Netherlands. Here, fish can pass the dyke from the sea to freshwater habitats via an artificial passage, where they can be easily caught by netting (Kemper, 1995).

The fish were transported in three large plastic tanks (75 × 50 cm and 40 cm high, with approximately 150 fish per tank, half filled with sea water and tap water and aerated by battery-run membrane pumps) in an air-conditioned vehicle (at approximately 17 °C air temperature) within 5 h to the Institute for Evolutionary Biology and Ecology in Bonn, Germany. There they were kept together for 1 week in a large outdoor tank (750 litres), with air ventilation and a constant supply of tap water at a flow rate of 3 litres/min. They were fed with defrosted red mosquito larvae (Chironomus spp.). Then they were transferred under similar conditions to those already described within 12 h to the Konrad Lorenz Institute for Ethology, Vienna, Austria, where they were kept in a large outdoor tank (750 litres, air ventilation, regular water exchange).

No permits were required for the export and import of the fish. During both periods of transport the condition of the fish was frequently monitored. No fish died during the transport, and afterwards they showed normal shoaling behaviour. Fifty pairs of these fish were bred under standardized laboratory conditions (see Frommen et al., 2013 for details). Resulting offspring were kept in four large outdoor tanks (750 litres, approximately 250 fish per tank) with air ventilation and regular water exchange. All fish were fed daily in excess with defrosted mosquito larvae.

Before the experiments, 120 F1 fish were haphazardly caught using a hand net and were transferred to the laboratory using a water-filled bucket (10 litres). Here, they were equally distributed among three tanks (130 × 65 cm and 50 cm high) located in an air-conditioned room under standardized winter light regime (8:16 h light:dark cycle, temperature 18 ± 1 °C). Light was provided by a fluorescent lamp (36 W) placed above the tanks. Each tank was equipped with an internal filter to clean and aerate the water and with five clay pots for shelter. The tank water was partially replaced by fresh water once a week; water nitrite concentration was regularly checked. The fish were checked daily for health (e.g. the constitution of the fish was estimated during feeding). During the experiments these fish functioned as stock for test fish, while the fish in the outdoor tanks provided the stimulus shoals.

Experimental Design and Procedure

The test aquarium measured 130 × 65 cm and 50 cm high and was filled with 1-day-old tap water up to a water level of 30 cm. It was divided into two stimulus compartments (32.5 × 65 cm and 50 cm high) on the right- and left-hand sides with a test compartment in the middle (65 × 65 cm and 50 cm high). The compartments were separated by glass plates, allowing visual contact only (cf. Frommen et al., 2008; Fischer & Frommen, 2013). The set-up was illuminated by a fluorescent tube (36 W), which was centred lengthwise above the aquarium. Experiments were videotaped from the side using a webcam (LifeCam Cinema, Microsoft) mounted on a tripod in front of the experimental tank.
To avoid disturbance from outside, a green curtain was stretched around the set-up.

For each trial, stimulus fish were randomly caught from a stimulus fish tank. All of the fish were fed shortly before the experiments to avoid confounding effects caused by different hunger levels (e.g., Frommen, Luz, & Bakker, 2007; Krause, 1993). In five experiments the following numerical contrasts were examined: 15 versus 60 ($N = 15$), 20 versus 60 ($N = 15$), 30 versus 60 ($N = 25$), 40 versus 60 ($N = 25$) and 50 versus 60 ($N = 25$). These equal ratios of 0.25, 0.33, 0.5, 0.67 and 0.83.

After stimulus fish were transferred into the test tank they were allowed to acclimatize for 5 min. Stimulus shoals swarmed evenly in their respective stimulus compartments. Meanwhile, the test fish was randomly caught out of the test fish tank. After the acclimatization period, the test fish was placed into a transparent Plexiglas cylinder (diameter 12 cm; height 35 cm) in the centre of the test fish compartment. The size of the cylinder was big enough to allow sufficient free moving space for the small test fish (body size 2–3 cm). The cylinder could be lifted by a string from outside the curtain. As soon as the test fish had tried to approach both stimulus shoals (indicated by nudging the plastic cylinder on both sides), but after no longer than 2 min, the cylinder was lifted up and the test fish could swim freely within its compartment. Shoaling preferences were estimated by measuring the time the test fish spent in association zones measuring 15 cm in front of the two stimulus shoals. The webcam recorded the test fish compartment for 30 min. Because little is known about shoaling preferences for large shoals, we chose this extended experimental time (usually an experimental duration of up to 15 min is standard, e.g., Gómez-Laplaza & Gerlai, 2013; Piffer et al., 2012). To control for potential side preferences, the position of the larger shoal alternated between the trials.

Experiments were conducted in August and September 2010. After each test day, the test tank was rinsed and the water was renewed. After the trial, fish were released into a further stock tank similar to the one described above. Each test fish was used only once, whereas stimulus fish were used several times. However, stimulus fish were never used twice a day or in the same combination. After the experiment, experimental fish were added to the breeding stock of the institute.

**Data Analysis and Statistics**

Videos were analysed with Observer XT (version 7.0; Noldus Information Technology, Wageningen, The Netherlands) and the time the test fish spent in the association zones near the stimulus shoals was measured. We calculated the proportion of time the test fish spent with the large shoal, relative to the total time in both choice zones. Eight out of 105 test fish spent 100% of the time with one of the shoals. As these fish did not show any abnormal behaviours, these data points were kept in the analyses. Excluding these fish did not change the results qualitatively. Preferences for the large groups were analysed using a one-sample t test when data did not differ significantly from a normal distribution; otherwise Wilcoxon signed-ranks tests were conducted. We conducted a linear mixed-effect model (LME) with percentage of time with the large shoal as the dependent variable, group size ratio (range 0.25–0.83) as an explanatory variable and experiment (experiments 1–5) as a random factor (to control for repeated testing of the same numerical ratio) in order to test whether the preference for the large shoal decreases with decreasing group size differences. To examine changes over time, we split the experimental time into two blocks of 15 min (an often-used timeframe; see Piffer et al., 2012 and references therein). Changes over time were analysed with an LME as described above, with time period as an additional factor. We assessed whether the removal of a variable caused a significant decrease in the model fit. Reported P values of the LMEs refer to the increase in deviance when the respective variable was removed. Hence, degrees of freedom differ by 1. Tests of statistical significance were based on likelihood ratio tests (LRT), which follow a chi-square distribution. These routines use maximum likelihood parameter estimation. Probabilities are two tailed throughout.

**Ethical Note**

The experiments comply with the current laws of Austria and Switzerland. The experiments were discussed and approved by the institutional ethics committee in accordance with Good Scientific Practice guidelines and national legislation.

**RESULTS**

**Total Experimental Time (30 min)**

Across all experiments, fish preferred to shoal with the larger group (one-sample t test: $t_{104} = 2.704$, $P = 0.008$; Fig. 1).
Preferences for the larger group decreased with increasing numerical ratio (LRT: $\chi^2 = 9.228, P = 0.002$; Fig. 2). Considering the single experiments, we found significant discrimination at ratios of 0.25 (15 versus 60) and 0.33 (20 versus 60; see Fig. 1 and Table 1 for statistics).

Changes Over Time

Across all experiments fish spent significantly more time with the large shoal in the first 15 min than in the second 15 min (LRT: $\chi^2 = 9.909, P = 0.002$; Fig. 3). In the first 15 min, fish on average preferred to shoal with the large shoal (one-sample t test: $t_{\text{choose}} = 3.964, P < 0.001$; Fig. 3a), whereas in the second 15 min this preference was no longer significant (one-sample t test: $t_{\text{choose}} = 0.806, P = 0.422$; Fig. 3b). There was no significant interaction between 'numerical ratio' and 'time period' (LRT: $\chi^2 = 0.124, P = 0.724$). The preference for the larger group decreased with decreasing differences in group sizes in both time periods (first 15 min: LRT: $\chi^2 = 8.542, P = 0.004$; second 15 min: LRT: $\chi^2 = 7.948, P = 0.005$; Fig. 3). The separate analysis revealed that in the first 15 min fish discriminated at ratios 0.25 (15 versus 60), 0.33 (20 versus 60) and 0.67 (40 versus 60), whereas in the second 15 min significant discrimination occurred only at the smallest ratio: 0.25 (15 versus 60; see Fig. 3 and Table 1 for statistics).

**DISCUSSION**

The results of our study indicate that sticklebacks are spontaneously (i.e. without prior learning) able to discriminate between two large groups and that they prefer to shoal with the group containing more individuals. They also show that group size choice is context dependent. Preference for the large group decreased with decreasing group size differences. This finding could be interpreted as an adaptive behavioural response, because the benefits may change accordingly. The relative benefits of associating with the larger group (owing to dilution, confusion or vigilance effects, for example) are assumed to increase as the difference in number of individuals increases. For instance, the dilution effect, and thus survival probability, is much higher in a group of 60 individuals compared with a group of 15 individuals. However, when the groups are similar in size (e.g. 50 versus 60) the effects are probably negligible. Furthermore, in this case the cost of an accurate assessment might be too high as it could require repeated switches between the shoals, which may lead to increased conspicuousness of the fish and thus higher predation risk.

Alternatively or additionally, reduced large group discrimination might be caused by cognitive constraints resulting in higher error rates when group sizes become more similar. This means that fish might simply not be able to differentiate between two large shoals when they are rather similar in size. Several studies in fishes examining size discrimination showed similar patterns in small groups (e.g. Gómez-Laplaza & Gerlai, 2011; Krause, Godin, et al., 1998). Using pure behavioural experiments cognitive abilities can only be measured indirectly, by examining whether and to what extent animals do or do not discriminate. However, possessing the cognitive abilities to solve a task does not necessarily lead to differential responses, as for example motivation to show a given behaviour might be absent. Thus it is generally difficult to distinguish between adaptive response and cognitive constraints, in particular when behaviour is predicted to change in the same direction as in our case. Testing trained animals in nonfunctional contexts might be a potential solution to this problem (e.g. Agrillo et al., 2010), but with the cost that the result may not relevant under natural conditions.

**Table 1**

Performance of three-spined sticklebacks in the five experiments differing in numerical contrasts

<table>
<thead>
<tr>
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<th>15 vs 60</th>
<th>20 vs 60</th>
<th>30 vs 60</th>
<th>40 vs 60</th>
<th>50 vs 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 30 min</td>
<td>$t = 4.949, P &lt; 0.001$</td>
<td>$t = 3.018, P &lt; 0.001$</td>
<td>$t = 0.974, P = 0.339$</td>
<td>$t = 1.711, P = 0.100$</td>
<td>$t = 1.152, P = 0.261$</td>
</tr>
<tr>
<td>First 15 min</td>
<td>$t = 5.258, P &lt; 0.001$</td>
<td>$V = 103, P = 0.015$</td>
<td>$t = 1.707, P = 0.101$</td>
<td>$t = 2.676, P = 0.013$</td>
<td>$V = 181, P = 0.627$</td>
</tr>
<tr>
<td>Second 15 min</td>
<td>$t = 3.090, P = 0.008$</td>
<td>$V = 86, P = 0.147$</td>
<td>$t = 0.022, P = 0.983$</td>
<td>$t = 2.666, P = 0.793$</td>
<td>$V = 217, P = 0.141$</td>
</tr>
</tbody>
</table>

Results across the total experimental period (30 min) and of the first and the second 15 min intervals are shown. Statistically significant discrimination ($P < 0.05$) is shown in bold.

![Figure 3](image-url) Preferences for the large group across the five experiments examining different numerical contrasts in (a) the first and (b) the second 15 min of the experiments. The black continuous line indicates the 50% threshold. The boxes show the median, 1st and 3rd quartiles and interquartile range (IQR). The whiskers indicate the highest/lowest value within the range between the 1st quartile and 1.5 × IQR. Circles indicate outliers.
Using our data set we are not able to elucidate definitely the underlying mechanism of quantity discrimination. Large quantity discrimination (≥4) in animals is thought to rely on an analogue magnitude system (Feigenson, Dehaene, & Spelke, 2004; see also Jones & Brannon, 2012; Rugani, Cavazzana, Vallortigara, & Regolin, 2013; Stancher et al., 2013), which is not based on precise counting but on approximation. According to Weber’s law, approximation is based on relative differences, that is ratios between sets rather than on absolute differences (e.g. Gómez-Laplaza & Gerlai, 2011). In fishes, numerical ratios up to 0.5 (e.g. 3 versus 6 or 5 versus 10) are frequently differentiated, for instance, by angelfish, Pterophyllum scalare (Gómez-Laplaza, 2012), guppies, Poecilia reticulata (Piffer et al., 2012) and zebrafish, Danio rerio (Ruhl & McRobert, 2005). Mosquitofish can even discriminate smaller ratios (i.e. 0.67, e.g. 8 versus 12; Agrillo et al., 2010). In our study, numerical ratio was correlated with absolute size difference. Thus, it is not possible to infer whether discrimination was based on absolute or relative differences. In the first 15 min, fish discriminated up to size differences of 40 versus 60, which correspond to a ratio of 0.67. This ratio was often found as the upper limit in small group discrimination. In the present study, preference for the larger group decreased with increasing numerical ratio, which is in agreement with Weber’s law; however, experiments with similar ratios at different absolute group size differences are required to draw definitive conclusions.

The question of whether quantity discrimination is based on numerical abilities or continuous attributes such as density, cumulative surface area or overall space occupied by a group is currently disputed (e.g. Frommen et al., 2009; Gómez-Laplaza, 2012; Piffer, Miletto Pettazzini, & Agrillo, 2013). In our case the larger group might occupy more space or might show higher density. Thus, instead of using any numerical system, preferences for the larger shoal might in fact be explained by preferences for the denser shoal. Several studies using sophisticated experimental approaches have dealt with that topic in small sets (e.g. Agrillo, Dadda, Serena, & Bisaza, 2009; Frommen et al., 2009; Gómez-Laplaza & Gerlai, 2013; Piffer et al., 2013; Rugani et al., 2013). However, ultimately both mechanisms will lead to a preference for the larger of two groups in our study. The exact mechanisms underlying quantity discrimination in larger groups should be the aim of future studies.

The preference for the large group was particular pronounced in the first 15 min and decreased over experimental time, which might be expected because after being placed into a novel environment fish may seek the greatest safety, that is the large group. With increasing time, the fish become more familiar with the new tank and thus bolder. This result is in accordance with other studies showing that anxious fish show increased shoaling tendencies (e.g. Kelley, Morrell, Inskip, Krause, & Croft, 2011).

In summary, our study revealed that three-spined sticklebacks prefer the larger of two groups containing high numbers of individuals. Furthermore, test fish were able to distinguish between numerical ratios up to 0.67. These results correspond to those reported in small groups, indicating that large group sizes do not significantly hamper sticklebacks’ discriminatory abilities. Furthermore, we found that the choice for larger groups is flexible and might depend on perceived danger and thus change according to the expected benefits.

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