Picky predators and odd prey: colour and size matter in predator choice and zebrafish’s vulnerability – a refinement of the oddity effect

Angela N. Aivaz, Andrea Manica, Peter Neuhaus & Kathreen E. Ruckstuhl

To cite this article: Angela N. Aivaz, Andrea Manica, Peter Neuhaus & Kathreen E. Ruckstuhl (2019): Picky predators and odd prey: colour and size matter in predator choice and zebrafish’s vulnerability – a refinement of the oddity effect, Ethology Ecology & Evolution, DOI: 10.1080/03949370.2019.1680445

To link to this article: https://doi.org/10.1080/03949370.2019.1680445

Published online: 30 Oct 2019.
Picky predators and odd prey: colour and size matter in predator choice and zebrafish’s vulnerability – a refinement of the oddity effect

ANGELA N. AVAZ¹, ANDREA MANICA ², PETER NEUHAUS¹,* and KATHREEN E. RUCKSTUHL¹

¹Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta T2N 1N4, Canada
²Zoology Department, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

Received 28 February 2019, accepted 17 September 2019

Many fish species typically assort into homogeneous groups and thus reduce the risk of predators targeting individuals that are phenotypically odd compared to the majority of the group. The confusion and oddity effect hypotheses predict that phenotypically odd individuals in a group are preferentially targeted by predators. Therefore, heterogeneous groups of prey should be more vulnerable to predator attention and attacks than homogeneous (all prey is the same colour, size, etc.) groups. Predators might use different cues to select a target, depending on their sensory abilities (sound, smell, vision, etc.). Visual-hunting predators that can see colour, for example, may be more likely to select prey based on colour than size oddity of prey. Previous studies on the oddity effect have found support for the colour-oddity and predator response, and to a lesser extent on size oddity of prey. We thus predicted that, given a choice, a group with an odd-coloured individual would be targeted more often by a predator than a group with an odd-size fish but not odd in colour. Heterogeneous groups of six zebrafish (Danio rerio) containing either one odd-coloured (wildtype or red) or one odd-sized (large or small) individual were used as stimulus prey in dichotomous choice experiments examining prey preference in needlefish (Xenentodon cancila). Needlefish preferred heterogeneous groups regardless of the colour of the odd individual and when the odd individual was smaller than the other group members. However, when the odd individual was larger than the rest of the group, the predators preferred homogeneous groups of small fish. These new findings are inconsistent with the predictions of the oddity effect and warrant investigation of alternative hypotheses for size-assorted grouping in fish.

KEY WORDS: predation, oddity effect, size-assorted, colour-assorted, Danio rerio, Xenentodon cancila.

*Corresponding author: Peter Neuhaus, Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta T2N 1N4, Canada (E-mail: pneuhaus@ucalgary.ca).
Animals that live in groups are known to assort phenotypically by species, body size, age, and sex (Krause & Ruxton 2002). Shoaling fish are useful organisms for examining phenotypic assortment, as they are easy to observe and maintain in the laboratory for experiments on behavioural studies (McCann et al. 1971; Croft et al. 2005). Size-assorted grouping has been documented in both the lab and in the wild for fish species such as, zebrafish (Danio rerio; McCann et al. 1971), three-spined sticklebacks (Gasterosteus aculeatus; Ranta et al. 1992b; Croft et al. 2005) and guppies (Poecilia reticulata; Croft et al. 2005). The preference to form schools is not restricted to body size similarities: fish have also exhibit shoaling preferences with other but has been shown also preferring conspecifics, unparasitised fish and fish with similar body colouration (Krause et al. 2000). These preferences may lead to assorted grouping, especially for individuals that are preyed upon by primarily visual predators (Foster 1973).

The oddity effect hypothesis states that individuals that differ phenotypically from the rest of the group are visually odd and are preferentially targeted by predators (Ohguchi 1978; Landeau & Terborgh 1986; Theodorakis 1989; Perry-Williams et al. 2018). Movements of organisms in a group distract/confuse a predator trying to focus on one prey item (Tosh & Ruxton 2006). A phenotypically odd individual in a group, however, can reduce these effects of predator confusion as the predator is better able to focus on this individual for target, attack and capture (Tosh et al. 2006; Ruxton et al. 2007).

An extension of the oddity effect hypothesis is that heterogeneous groups are more vulnerable to predation than homogeneous groups. Predation risk may increase for majority group members if odd individuals remain part of their group because these odd individuals likely attract predator attention to the entire group (Ohguchi 1978; Landeau & Terborgh 1986). Specifically, they showed that predator attack (Ohguchi 1978) and capture success (Landeau & Terborgh 1986) was significantly greater on heterogeneous than on homogeneous prey schools. Theodorakis (1989), who also tested the oddity effect, focused on size-oddity and not colour-oddity as with the previous two studies. Although the predator in this study ate odd-sized minnows in greater proportion than predicted by their frequency in the group, this proportion was not significantly different from random choice.

Since the oddity effect directly links to predation it makes it adaptive for prey to perceive their own oddity and behave in such a way as to reduce their oddity. Therefore, individuals should choose to group with other phenotypically similar individuals to prevent looking odd compared to the rest of the associate. Mollies (Poecilia latipinna; McRobert & Bradner 1998; Bradner & McRobert 2001), female fighting fish (Betta splendens; Blakeslee et al. 2009) and Western rainbowfish (Melanotaenia australis; Rodgers et al. 2010) have all demonstrated significant preferences for grouping with conspecifics similar in colour.

Similar grouping patterns have been observed with size-oddity, however, with differing results for differing sizes. Consequently, social decisions and thus the strength of the oddity effect may depend upon body size (Rodgers et al. 2011). Theodorakis (1989) and Ranta et al. (1992a) found that small but not large fish significantly increased the magnitude of preference for similar-sized individuals in the presence of a predator, suggesting that odd small fish but not odd large fish suffer from the effects of oddity. However, Pitcher et al. (1986) and Svensson et al. (2000) found opposite results, suggesting that large fish but not small fish are concerned with their oddity.
Peuhkuri (1999) found that large- and small-sized majority members exhibited random choice between homogeneous and heterogeneous groups with respect to grouping preference. Majority members should have preferred the homogeneous groups if the oddity effect was indeed a mechanism acting in the decision-making process of prey.

These inconsistencies between sizes and forms of oddity need to be addressed from the perspective of the predator. The oddity effect is considered to be a driving force in shaping the composition of prey groups and is based upon predation. It is important to distinguish between colour- and size-oddity – depending on the visual system of the predator; colour differences may be more visually intense or more conspicuous than size differences. Tosh et al. (2006) showed that increased relative visual intensity of the odd individual (defined as a single tadpole, highlighted by a small arrow, among a group of non-highlighted tadpoles on a computer screen) increased targeting success of human predators (who had to select/click on the highlighted moving target). We further hypothesized that colour-oddity may not elicit the same response from visual predators as size-oddity. We predicted that predators would react more strongly to and prefer groups with colour-odd rather than size-odd prey.

If the oddity effect does apply to both colour and size, and heterogeneous groups are more vulnerable than homogeneous groups, then predators should prefer heterogeneous groups containing odd-coloured or odd-sized individuals. We are only referring to the group choice stage of attack by the predator and not which individual to attack from that group. Oddity in size versus oddity in colour has not been compared in a single study before and thus we are the first to explore this hypothesis using a needlefish-zebrafish predator-prey system.

**MATERIAL AND METHODS**

**Study organisms**

Red transgenic and wildtype zebrafish were bred in the lab to obtain the small size class. A total of 37 zebrafish per phenotype (small wildtype, small red and large wildtype), 3 to 9 months old, were used, each fish was re-used approximately 6 to 7 times in total in the 60 trials. Groups composed of six zebrafish were chosen for the experiments 2 days in advance; we used groups of six fish because the oddity effect may only apply to relatively small groups (Landeau & Terborgh 1986; Ruxton et al. 2007). All zebrafish were randomly selected for each trial, and each individual was never used in consecutive trials. Habituation may have played a role in grouping behaviour; however, the zebrafish did not show any discernable visible changes in their behaviour and appeared to behave normally throughout each trial. No large red fish were used, as there were too few to spare for purposes other than breeding. The small zebrafish had a mean total length (TL ± SE) of 24.12 ± 0.97 mm (wildtype) and 23.46 ± 0.65 mm (red) and a mean mass (± SE) of 0.14 ± 0.02 g (wildtype) and 0.11 ± 0.01 g (red). The large wildtype zebrafish had a mean TL (± SE) of 36.06 ± 0.49 mm and a mean mass (± SE) of 0.40 ± 0.02 g. There was a 33-35% difference in total length between the two size classes, a difference used during observations of size-assorted grouping in other fish species (European minnows, Phoxinus phoxinus: 33% difference between small and large sizes (Pitcher et al. 1986); three-spined sticklebacks: 34% difference between small and large sizes (Peuhkuri 1999). The zebrafish were maintained on a 12:12 LD (light/dark) cycle in a flow-through system composed of several 10 L holding tanks (Aquatic Imports™) filled with dosed reverse osmosis (RO) water (1 gram Instant Ocean/gallon water), which had a conductivity of 430 mS, a pH of 7.0–7.5 and a constant temperature of 26 °C. The zebrafish were fed a maintenance diet of live brine shrimp nauplii (Artemia salina) once daily. These fish
were anaesthetized with clove oil to determine their mass and length and were allowed to recover behaviourally (swimming upright) before being put back into their holding tanks. The fish were kept in their experimental groups until trials began 2 days later.

Zebrafish are well fitted organisms for studying the dynamics of phenotypic assortment as they are available in different colours (transgenic-genetically modified), are readily abundant and are easily bred and maintained in a lab setting. In addition, they do not assort by sex (Ruhl & McRobert 2005) and there are no sex differences in colour as observed with other fish species, such as guppies (Seghers 1974; Croft et al. 2004). Red transgenic and wildtype zebrafish were used so that we could observe two colours of zebrafish while controlling for size. Similar to Gong et al. (2003) we did not note any differences in swimming behaviour or morphology between red transgenic and wildtype (Aivaz & Ruckstuhl 2011), indicating that transgenic fish are not behaviourally odd when shoaling with the wild type fish. Zebrafish can see and distinguish the colour red (Snekser et al. 2006), indicates. In Cortemeglia and Beitinger (2006) study transgenic zebrafish were not eaten more than the wildtype, while Hill et al. (2011) found that the transgenic fish were twice as likely to get eaten than the wildtype. These differences could partially be due to study design (Hill et al. 2011). Both varieties of fish show no preference for either wild or transgenic fish (Snekser et al. 2006), suggesting that colour might not be important in shoal choice.

Little is known about the aquatic predators that prey on zebrafish in their natural habitat (Spence et al. 2006); however, they noted that needlefish (Xenentodon cancila) were found among zebrafish in shallow clear waters of Bangladesh (DeWitt 1960; Spence et al. 2006; Engeszer et al. 2007), and as, since they are predatory, they may feed on zebrafish, we found this predator-prey system appropriate for our study. As we were using red fish, it was important to consider the visual pigment sensitivity of the predator. Needlefish belong to the Order Beloniformes, which includes medaka (Oryzias latipes, Nelson 2006). Although needlefish visual pigment sensitivity has not been studied before, it is known that medaka can see red (Matsumoto et al. 2006) and since both species belong to the same Order, they are likely to have similar visual pigments. Needlefish are also known to prey on a variety of prey and colour vision may be an important asset (Ward-Campbell et al. 2005).

The 12 needlefish used for these experiments had a mean TL ± SD of 153.75 ± 12.05 mm and a mean mass ± SD of 6.19 ± 1.35 g. They were kept in similar water conditions as the zebrafish but occupied larger aquaria (113.6 L; length × width × height: 91.4 × 30.5 × 40.6 cm). During the experimental period, the needlefish were only transferred twice daily – once to the experimental tank (113.6 L) and once to the “used fish” tank (113.6 L). Before conducting experiments, it was necessary to test if the needlefish would pursue and consume both colours and sizes of zebrafish. Therefore, in a pre-experiment trial session we offered red, wildtype, small and large zebrafish to the needlefish, which they consumed in equal proportions.

**Experimental setup and procedure**

Dichotomous choice experiments, where a predator chooses between a homogeneous and a heterogeneous group of zebrafish, allowed us to address our prediction that the latter is preferred over the former. All procedures were performed in strict accordance with the policies formulated by the Canadian Council on Animal Care (CCAC) and the University Animal Welfare Committee (UAWC). The protocol was approved by the Animal Care Committee (ACC Protocol: BI 2008–24) and complied with all the relevant laws of Canada. Experiments were conducted between 08:00 and 16:00 hr. Initially, two choice experiments were run to see if the needlefish showed a preference for a particular zebrafish size (treatment A: large wildtype versus small wildtype zebrafish) or colour (treatment B: small red versus small wildtype zebrafish; Table 1). Following analyses of these two experiments, four choice experiments were conducted in which eight groups of zebrafish were used, two groups for each experiment. The two experiments dealing with colour oddity kept the size of the fish constant using following groups: treatment C (1 small wildtype, 5 small red versus 6 small red) and D (1 small red, 5 small wildtype versus 6 small wildtype; Table 1). The two experiments concerned with size oddity used only wild type fish comparing the following groups: treatment E (1 large wildtype, 5 small wildtype versus 6 small wildtype...
wildtype) and F (1 small wildtype, 5 large wildtype versus 6 large wildtype; Table 1). The zebrafish were removed from the stimulus chambers after every trial and a different single predator was used for each trial per treatment. Ten trials were run for each treatment and each needlefish was randomly selected from a pool of 12 needlefish. Pseudoreplication was not a factor as we ran tests on each treatment in isolation and in random order (see Statistical analyses in Material and Methods).

Experiments were conducted in a 113.6 L aquarium (91.4 × 30.5 × 40.6 cm), which was filled with water to a height of 16.5 cm to yield a volume of approximately 37.9 L. The water used in this tank was in the same condition as the water in the holding tanks. In order to score the movement of the needlefish, the aquarium was divided into three equal sections by drawing two lines on the outer surface, one at 30.5 cm and one at 61 cm. The central third of the tank was considered a “no choice” or neutral zone and the left and right thirds of the tank were considered the “choice” zones. The outside of the aquarium was covered with blue paper with the exception of the front glass, which was left uncovered for video-taping. The tops were cut off of two cylindrical-shaped plastic bottles (chamber height: 20.5 cm; diameter: 10.6 cm) that were filled half full with rocks to weigh down the chambers when inside the experimental tank. These stimulus chambers were then filled with water (to the same height as the tank water, 16.5 cm) and one group of zebrafish was put into each chamber; a lid was placed on top to prevent any zebrafish from escaping. The height of the stimulus chambers exceeded that of the aquarium water, and thus, mixing of water between the chambers and the tank was prevented; predators could not use olfaction to detect their prey. Each stimulus chamber was placed in the centre of the end thirds of the aquarium. This setup allowed the needlefish to swim between and around the zebrafish stimuli chambers (Fig. 1). The length of the experimental tank equated to approximately six predator body lengths, which was ample room for the predators to move freely. A lid was positioned on top of the aquarium during each trial as it provided the lighting necessary to illuminate the tank. A Panasonic PV-GS200 digital video camcorder was used to tape record all trials.

Table 1.

Means in seconds ± SDs for within-treatment comparisons of dichotomous choice experiments examining preference of predatory needlefish for different group compositions of zebrafish stimuli. Effect sizes calculated as unstandardized mean differences (Mean₁ – Mean₂); ☞ = large brown zebrafish; ◊ = small brown zebrafish; ◣ = small red zebrafish. Stimulus group figures represent the composition of each treatment group. Oddity describes the type of odd fish (size-odd, colour-odd, or no oddity).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oddity</th>
<th>Stimulus group₁ (n = 10)</th>
<th>Stimulus group₂ (n = 10)</th>
<th>Mean ± SD₁</th>
<th>Mean ± SD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No oddity</td>
<td>☞</td>
<td>☣</td>
<td>285 ± 133.6</td>
<td>222 ± 76.4</td>
</tr>
<tr>
<td>B</td>
<td>No oddity</td>
<td>☣</td>
<td>☞</td>
<td>237 ± 147.3</td>
<td>294 ± 143.4</td>
</tr>
<tr>
<td>C</td>
<td>Colour oddity</td>
<td>☣</td>
<td>☞</td>
<td>327 ± 103.4</td>
<td>159 ± 114.9</td>
</tr>
<tr>
<td>D</td>
<td>Colour oddity</td>
<td>☣</td>
<td>☞</td>
<td>336 ± 120.7</td>
<td>225 ± 86.3</td>
</tr>
<tr>
<td>E</td>
<td>Size oddity</td>
<td>☣</td>
<td>☞</td>
<td>168 ± 68.1</td>
<td>252 ± 119.3</td>
</tr>
<tr>
<td>F</td>
<td>Size oddity</td>
<td>☞</td>
<td>☣</td>
<td>291 ± 146.3</td>
<td>147 ± 84.2</td>
</tr>
</tbody>
</table>
The needlefish were not observed to strike at the stimulus chambers as the three-spined sticklebacks did in Ohguchi’s (1978) study. However, needlefish are very curious predators and were observed to swim between and around the stimulus chambers while fixedly observing the zebrafish inside. This fixative behaviour is a common characteristic of these predators (Foster 1973), therefore, the time spent near each stimulus chamber was inferred as behavioural choice by the needlefish preferring a specific chamber.

At the start of the experiment, one needlefish was introduced into the tank and allowed to acclimate to its surroundings. Acclimatisation was determined by the time when a needlefish settled down, starting to explore its surroundings. Mean (± SD) acclimation time for all predators was 93.5 ± 71.61 sec. Once the needlefish began swimming, scoring was initiated. Every 30 sec, we recorded in which third of the tank the needlefish was swimming for a total of 15 min and a total of 30 scores. The central “no choice” zone was included in the scoring but not included in the analyses as only the mean times spent in the left third and right third of the tank were examined for differences. The stimulus chambers containing the zebrafish were randomized every trial to reduce the risk of side biases. Any fish predator that did not move within 5 min was removed from the tank and the trial terminated; these fish were not used again in the same treatment – these trials were not considered in any analyses. Each needlefish was removed from the experimental tank at the end of each trial and any debris remaining was siphoned out. Needlefish were fed fathead minnows (Pimephales promelas) at the end of each day. Half the aquarium water was changed after every experiment and during the time when no experiments were running, the water was kept aerated and filtered. Habituation of needlefish to the experimental setup is very unlikely as it can take up to a month before some fish species habituate to an experimental setup as determined for sticklebacks (Nakayama et al. 2013).

Statistical analyses

All statistics were performed in R version 2.10.1 (R Development Core Team 2009). Predator choice of zebrafish stimulus group was dichotomous and the data were recorded binomially. There were repeated measures as several scores (observations) were recorded for each predator during its 15-min trial. In order to account for any autocorrelation between observations (sometimes the predators remained on a given side of the test tank for a duration of several observational scores) we used a generalized estimating equation (GEE) with first-order autoregressive correlation (ar1) and with a binomial distribution for each experiment; the models

Fig. 1. — Experimental tank setup: demarcation line visually divided the tank (113.6 L, length x depth x height: 91.4 x 30.5 x 40.6 cm) equally into thirds. Stimulus chambers contained six zebrafish and were weighted down with rocks. The predator was placed in the centre of the tank to begin trials. The water level of the stimulus chamber and tank were at 16.5 cm.
had no intercept. Since we only ran 10 trials per experiment, we calculated the effect sizes as odds ratios along with their associated 95% CIs. Odds ratios range from 0 to infinity and were calculated by multiplying the observed scores for the heterogeneous groups \( W_i \) by the expected scores for the homogeneous groups \( Z_i \) and then dividing the resulting number by the product of the expected scores for heterogeneous groups \( X_i \) and the observed scores for homogeneous groups \( Y_i \) (odds ratio = \( \frac{W_i Z_i}{X_i Y_i} \); refer to Table 2 in Results). The null hypothesis would be that there is no preference exhibited by predators, which would yield an odds ratio of 1 (see Simon 2001 for understanding odds ratios). If the CIs associated with the odds ratios do not include 1 (the null hypothesis), then there truly is an effect. See Whitlock and Schluter (2009) for the equation used for the calculation of CIs for odds ratios. Not correcting for multiple testing [decreasing alpha, \( \alpha = 0.05 \) (critical \( P \)-value)] inflates the type I error rate; however, correcting for multiple testing inflates the type II error rate (Whitlock & Schluter 2009). As a result, we used mainly odds ratios in interpreting the results. Interpreting solely \( P \)-values limits statistical inference as it does not provide an estimate of the magnitude of an effect (as do odds ratios) and it does not give the precision of that estimate (as do CIs); it only addresses the probability of the data given that the null hypothesis (no preference is shown by predators) is true (Nakagawa & Cuthill 2007). Examining odds ratios provides a more meaningful interpretation of the results as it not only addresses the probability of our hypothesis given the data but provides an assessment of degrees of likelihood and addresses the biological importance of the effects (Nakagawa & Cuthill 2007). We thus used both odds ratios and CIs when reporting our results.

We compared the magnitudes of effect for colour-oddity in our study to those of Landeau and Terborgh (1986), a study in which the oddity effect is said to have empirical support (Ruxton et al. 2007). Only two other studies [Ohguchi (1978) — colour-oddity; and Theodorakis (1989) — size-oddity] are considered to have empirical data for the oddity effect (Ruxton et al. 2007); however, not enough information was provided in these studies for us to directly compare our results to theirs. Ohguchi (1978) and Landeau and Terborgh (1986) showed that different colours of odd individuals suffer from the oddity effect and both studies used different predator-prey systems and different colours of odd prey. Their results suggest that odd individuals should be preferentially targeted regardless of the pigment of their odd colour and that the oddity effect is a general phenomenon which may apply across taxonomic groups. We also used a different predator-prey system and different colours of prey than the previous studies. Our needlefish predators are primarily visual hunters (McMahon & Holanov 1995) that can only consume one prey item at a time (an assumption of the oddity effect), similar to the largemouth bass predators used in Landeau and Terborgh (1986) study. Therefore, our comparison with Landeau and Terborgh (1986) study should lend greater insight into the adaptive value of assorted grouping.

RESULTS

When the predators were given a choice between two different homogeneous groups of zebrafish no significant preferences were found (treatment A: large wildtype versus small wildtype; and treatment B: small red versus small wildtype \( P > 0.05 \); Table 2). When the predators were given a choice between a homogeneous group and a heterogeneous group, predators generally preferred heterogeneous groups, both odd in size and colour experiments C-F (results from experiments D, E and F were strong trends, \( P > 0.05 \); Table 2). Homogeneous groups were preferred over heterogeneous groups when the latter contained a single odd large fish (treatment E: 1 large wildtype, 5 small wildtype versus 6 small wildtype; Table 2).

The magnitudes of predator preference were higher for colour oddity treatments (C and D) compared to those of size oddity treatments (E and F). Odds ratios were similar for the former two treatments (\( \sim 2 \)) and comparable for the latter two treatments. The magnitudes of preference were the same for treatments D and E but for treatment E, preference was in the opposite direction from what was predicted, hence
Table 2.

Observed and expected predator preference scores for zebrafish prey groups in dichotomous choice experiments. Expected outcome was determined by frequency of no choice (e.g., equal amounts of time spent on each side of the choice chamber = 50%). Wald test statistics and P-values were generated from the generalized estimating equations (GEEs), odds ratios (effect sizes) and associated 95% confidence intervals (CIs) are shown. Number of test per treatment = 10. Significant terms are highlighted in bold. P-values that are close to the $P < 0.05$ threshold are in italics. Red zebra fish = empty fish symbol, wt = wild type zebrafish = black fish symbol. L = large body size fish, S = small body size fish. Test = Group phenotype refers to treatment A and B and group oddity refers to treatment C-F. Odds ratio = $W_iZ_i/X_iY_i$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group symbols</th>
<th>Type</th>
<th>N for each group</th>
<th>Observed N (%</th>
<th>Expected N (%)</th>
<th>Wald value</th>
<th>P-value</th>
<th>CI &lt; Odds Ratio &lt; CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>6 L wt</td>
<td>$W_1 = 95$ (56%)</td>
<td>$X_1 = 84$ (50%)</td>
<td>0.75</td>
<td>0.39</td>
<td>0.84 &lt; 1.28 &lt; 1.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>6 S wt</td>
<td>$Y_1 = 74$ (44%)</td>
<td>$Z_1 = 84$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S red</td>
<td>6 small red</td>
<td>$W_1 = 79$ (45%)</td>
<td>$X_1 = 88$ (50%)</td>
<td>0.229</td>
<td>0.63</td>
<td>0.53 &lt; 0.81 &lt; 1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S wt</td>
<td>6 small wt</td>
<td>$Y_1 = 98$ (55%)</td>
<td>$Z_1 = 88$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colour-oddity</td>
<td>1 small wt, 5 small red</td>
<td>$W_1 = 109$ (67%)</td>
<td>$X_1 = 81$ (50%)</td>
<td>4.57</td>
<td>0.03</td>
<td>1.31 &lt; 2.06 &lt; 3.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No oddity</td>
<td>6 small red</td>
<td>$Y_1 = 53$ (33%)</td>
<td>$Z_1 = 81$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colour-oddity</td>
<td>1 small red, 5 small wt</td>
<td>$W_2 = 112$ (60%)</td>
<td>$X_2 = 93$ (50%)</td>
<td>3.803</td>
<td>0.05</td>
<td>0.99 &lt; 1.49 &lt; 2.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No oddity</td>
<td>6 small wt</td>
<td>$Y_2 = 75$ (40%)</td>
<td>$Z_2 = 93$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Size-oddity</td>
<td>1 large wt, 5 small wt</td>
<td>$W_3 = 56$ (40%)</td>
<td>$X_3 = 70$ (50%)</td>
<td>3.72</td>
<td>0.05</td>
<td>0.42 &lt; 0.67 &lt; 1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No oddity</td>
<td>6 small wt</td>
<td>$Y_3 = 84$ (60%)</td>
<td>$Z_3 = 70$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Size-oddity</td>
<td>1 small wt, 5 large wt</td>
<td>$W_4 = 97$ (66%)</td>
<td>$X_4 = 73$ (50%)</td>
<td>3.246</td>
<td>0.07</td>
<td>1.23 &lt; 1.98 &lt; 3.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No oddity</td>
<td>6 large wt</td>
<td>$Y_4 = 49$ (34%)</td>
<td>$Z_4 = 73$ (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the lower odds ratio value (Table 2). If the numerator and denominator are switched in the odds ratio equation for treatment E, the odds ratio is 1.5, a value similar to that of treatment D — see Statistical analyses for calculation of odds ratios.

**DISCUSSION**

The needlefish predators did not prefer a particular size or colour of prey when presented with a dichotomous choice of homogenous groups varying in size or colour (large versus small, red versus wildtype). Therefore, any preferences exhibited for heterogeneous over homogeneous groups of zebrafish were likely due to the presence of the odd individual in the group (i.e., the odd individual attracted predator attention to the group). The confidence intervals (CIs) of the odds ratios were large for all experiments (likely a result of small sample size), which indicated that larger sample sizes would be needed to achieve greater precision in the range of probable effect size estimates.

The results from both colour-oddity experiments were consistent with the oddity effect hypothesis — predators preferred heterogeneous over homogeneous groups. However, when the odd fish was red, the lower CI for the odds ratio just barely included 1 (the null hypothesis) (– CI = 0.99), suggesting a weaker effect when the odd fish was a novel red colour. On the other hand, the results for the size-oddity experiments were inconsistent with the predictions of the oddity effect hypothesis — predators preferred heterogeneous over homogeneous groups only when the odd fish was smaller and not when it was larger than the rest of its group members [the latter effect was weaker than that of the former – the upper CI for the odds ratio just barely included 1 (+ CI = 1.07)]. It may have been possible that the differences between large and small fish were not large enough or that the scale was not appropriate. However, a 33% difference between size classes should have been adequate for a visual predator to detect size-oddity among prey group members ≤ 6 predator body lengths away. Alternatively, there could have been a large size-oddity effect when the majority of fish in the group were small, but not when the majority were large. In this case, predators would have preferred heterogeneous groups when the odd fish was larger than the rest of the group members but they did not. An alternate explanation could be that the probability of capture success is based on the swimming speed of fish in relation to their body size. If size-oddity affects the capture success of the predator then perhaps a small fish in a group with larger fish is more vulnerable as it may have a slower swimming speed than its larger companions (Krause et al. 2005), a theory that could explain predator preference for size-heterogeneous groups containing one small odd fish in our study. However, it still does not explain why predators tended to prefer homogenous groups of small fish over a heterogeneous group containing one odd large fish. Perhaps other constraints imposed by size-oddity were influencing decision-making by predators.

To examine colour-oddity more closely, we can compare our results to those of Landeau and Terborgh (1986). For both studies, we compared groups with oddity (1 odd colour:7 similar colour, Landeau & Terborgh 1986; 1 odd colour:5 similar colour, our study) to groups with no oddity (4 blue:4 brown, Landeau & Terborgh 1986; 6 similar colour, our study). Both studies had similar odds ratios when the odd fish was its natural colour [wildtype zebrafish in our study and brown silvery minnows in Landeau & Terborgh (1986) study]. However, the odds ratios were not similar when the odd fish was a novel colour [i.e., blue (Landeau & Terborgh 1986) or red (our
study) – the odds ratio of the former was twice that of the latter]. The larger effect size observed in Landeau and Terborgh (1986) study could be due to olfaction or other sensory communication because in this study predators were in the same water as their prey while they were separated in our experiments. Our fish were born with their red colour and therefore, these factors would not have played a role in prey group selection; they also didn’t share the same water with the predator (they were in plastic water bottles). However, there are other factors that need to be considered which could influence prey selection when examining the effects of oddity, such as the visual environment (background complexity, Bradner & McRobert 2001; reactive distance, Krause et al. 1998; turbidity, Shoup & Wahl 2009; light intensity, Meager et al. 2010), the frequency of colour morphs in the population (Jönsson et al. 2011), the size distribution of fish (Croft et al. 2009), group size (Krause et al. 1998) and the previous experience of the predator (Webster & Hart 2006).

The results from our study are relevant because throughout the literature the oddity effect has been assumed more often than it has been tested (Blakeslee et al. 2009). To our knowledge, only one study exists that has tested size-oddity from the perspective of the predator (Theodorakis 1989). However, while the predator was allowed to consume the prey in that study, and the number of fish remaining at the end of the trial was known, there is no detailed information on the order of attacks.

Until now, it has been assumed that predators should prefer both large- and small-sized odd prey, yet there is evidence that both sizes of prey make different decisions about their own oddity in terms of magnitude of preference for similar-sized individuals (Theodorakis 1989; Ranta et al. 1992a). Interestingly, the different results we obtained for size-oddity correspond more to experiments examining the oddity effect from the perspective of the prey: large fish do not appear to be concerned with their oddity but small fish might be (Theodorakis 1989; Ranta et al. 1992a; Peuhkuri 1999). Depending on the system and the preferences of the predator this may in special cases even be turned around where small prey may be safer in groups of big conspecifics than in groups of many other small individuals when the nutritional factor plays a role in prey choice by the predator (Rodgers et al. 2015; Raveh et al. 2019).

If the effects of oddity were the same for differing sizes as for differing colours, any size- or colour-odd individual should elicit the same response from predators, and by the same token, any size- or colour-odd prey should behave similarly when making decisions about which group to join, stay or leave. Since the former (predator response) was not observed and the latter (prey response) is disputable, we caution that size-oddity may differ from colour-oddity, although to what extent they differ has yet to be determined. Without further demonstrations of size-oddity from the predator’s perspective, we propose that predictions for size-oddity cannot be based on evidence of colour-oddity alone. While more research is needed to clarify, a likely explanation for size-assorted grouping in prey fish may not only be visual oddity but also on size, as constraints imposed by size-oddity such as competitive abilities (Krause 1994; including hunger levels, Reebs & Saulnier 1997), differences in activity budgets (Ruckstuhl 2007; Aivaz & Ruckstuhl 2011), levels of predation risk (Ashley et al. 1993), different swimming speeds (Krause et al. 2005) or choice of optimal group sizes (Hager & Helfman 1991), may be influencing decision-making by both predators and prey. It is important to test which factors are contributing the most to prey selection and size-assortedness in order to gain a better understanding of the adaptive value of size-assorted grouping.

Our results highlight the need for more empirical evidence of size-oddity from the perspective of the predator. Of primary importance would be to determine if the
specific predictions of the oddity effect are supported for differing sizes, in terms of strike efficiency and capture success. Our results suggest that size-oddity elicits a similar response from predators as colour-oddity and both can play a role in shaping predator and prey interactions.

ACKNOWLEDGEMENTS

We would like to thank Jack Hogg and Warren Fitch for assistance in obtaining fish, Aly Carter and Sarah Childs for access to their brine shrimp decapsulation protocol and Petra Hermann, Wic Wildering, and Jeff Goldberg for sharing their animal holding facilities and treated water.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

FUNDING

This study was supported by the National Science and Engineering Research Council discovery grant [RT732497 to K.E. Ruckstuhl] and the Department of Biological Sciences of the University of Calgary Research Starter Grant (K.E. Ruckstuhl).

ORCID

Andrea Manica @ http://orcid.org/0000-0003-1895-450X

REFERENCES