Decoy effect in shoaling decision making in zebrafish *(Danio rerio)*

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ABSTRACT

Several organisms, from slime molds to humans, are known to violate normative principles of economic rationality in decision making. In animals, the neural circuitry underlying behaviors that violate or conform to normative rationality is relatively poorly understood. We investigated whether zebrafish, a model organism with a strong suite of functional neuroimaging and genetic manipulation tools, showed a decoy effect with respect to the principle of the Independence of Irrelevant Alternatives (IIA). We examined IIA in social decision-making by measuring revealed preferences from spatial trajectories of freely swimming individual zebrafish in an arena where they could view and perform shoaling behavior near conspecific zebrafish in adjacent display tanks. IIA was tested in terms of the invariance of shoaling choices between binary and ternary sets of various display fish group sizes. We provide the first report of evidence for the decoy effect in male zebrafish, but not in females. This opens up a range of possibilities to study the neural basis of context-dependent decision making.

Introduction

The normative principles of economic rationality define individuals as behaving rationally when they make consistent choices that maximize their utility. Such consistent choices can be represented through a stable ordinal utility function that maximizes 'expected utility', from which axiomatic principles of transitivity and independence of irrelevant alternatives (IIA) follow[1]. Transitivity is complied with if in a choice set of A, B and C, an individual who has a stable preference of A over B, and B over C, always prefers A over C. IIA refers to the consistency of preference of A over B regardless of the presence of other items in the choice set[2]. Specifically, Luce's choice axiom of constant ratios IIA(L) is upheld when the ratio of the probabilities of choosing A and B in the choice set A, B remains constant even with a larger choice set A, B, C[3]. Another specific derivative principle of IIA, the principle of regularity, is met when the addition of an alternative to the choice set does not increase the absolute preference for any existing option[4,5].

Choices made by behavioral agents can be tested for whether they meet the conditions of these axiomatic principles. If so, these agents would be considered rational relative to each operationalized definition of rationality. Tests suggest transitivity and IIA are violated by humans[6–8] and several other animals[9–12]. These violations of rationality largely stem from the context dependence of decision making and suggest that the brain may calculate value relative to context, or through a process of comparison between choice attributes, across available options[13]. On the other hand, models of value-first decision making, posit an absolute context independent process of valuation underlying rational choices, where choice attributes have stable values represented in a cognitive utility scale[13]. Dynamic models of decision making, where option valuation, comparison and decision formation are dynamic and intertwined, have accounted for several context effects[14–16].

IIA refers to one specific aspect of context that can change preferences between two options: the availability of further options- known as distractors or decoys- in the choice set. These context effects are largely reported in studies where the options differ in two or more attribute dimensions with the decoy asymmetrically placed such that its effect dominates relative to one of the attributes, which might result in context specific interactions between the psychophysical value functions of the attributes[4,5,9,10,17–23]. Decoy effects of various kinds: for example, the commonly reported attraction effect, the similarity effect, and the compromise effect, have been reported in multi-attribute human[8] and animal[5] studies where the two main choice options are on an iso-preference line relative to at least two attributes that trade-off. The attraction effect describes the phenomenon where a decoy that is slightly inferior to one of the option values enhances the relative preference for that option[24]; while the similarity effect describes the opposite phenomenon when a decoy reduces the relative preference for the option to which it is most similar[25]. The compromise effect is seen when the decoy has an extreme value and therefore enhances the

preference for the option which represents a compromise between the decoy and the competitor[26].

More comprehensive work on integrating these decoy effects into a single model[8,27] suggests that the effect of a decoy on changing the relative accuracy of choice of the more highly valued of two options depends both on the value of the decoy relative to those two options, as well as the task difficulty i.e. the gap between the values of these two options[27]. Studies suggest that the relative accuracy of choosing the more highly valued of two options decreases with increasing decoy value, consistent with a divisive normalization model where the neural activity encoding a stimulus is modulated based on the values of other stimuli encoded by the pooled activity of neurons contributing to the spatiotemporal receptive field[12,28–31]. Other studies suggest that increasing the value of the decoy improves choice accuracy[28], consistent with a biophysical cortical attractor model based on the competing representation of the values of the two options in a neural network, with larger decoys strongly activating a common pool of inhibitory interneurons which in turn have a larger inhibitory effect on neurons representing the smaller valued option[28]. Some of these results have not been replicated and a third model of value-based attentional capture has been posited instead, where the decoy distracts from the two options and reduces absolute, if not relative choice accuracy[32]. Another explanation for these different effects is a model incorporating neural imaging data that suggests that different models might operate in different parts of the brain - divisive normalization in the intraparietal sulcus but not in the ventromedial prefrontal cortex, with both decision making processes running in parallel to produce different decoy effects in different regions of decision space[28].

The neural computations underlying behaviors that conform to and violate rationality are best studied in systems that enable large scale, high-resolution neural recordings, and genetic manipulations. So far, IIA has been only investigated in two model organisms: mice[18] and *C. elegans*[33]. We investigate the decoy effect in social decision making in zebrafish- a larger, more complex vertebrate model system than *C. elegans* that is likewise equipped with neural imaging and electrophysiological tools, and amenable to genetic and pharmacological manipulation. This is crucial in order to understand the neural basis of context-dependent decision making.

The behavior in which we investigate IIA is shoal size choice. Very few studies have investigated decoy effects with the two options varying with respect to a single attribute, and these have revealed that different decoy effects may be seen with different attributes[10,18,34]. A number of fish species show preferences for larger shoal sizes[35–40]. Zebrafish also has been reported to show sex based differences in shoaling decisions[41,42]. Shoal size decisions are usually attributed to numerical competence and have been reported to follow Weber's law i.e. the perceived difference between shoal size sets, or numerical sets in general, is represented on a log scale rather than linearly[39,43,44]. Reding & Cummings (2019) investigated rationality in shoal size decision making relative to the use of a decoy in mosquitofish and reported no IIA violations. In the present study, we investigate decoy effects in adult zebrafish (*Danio rerio*) using a shoal size choice behavioral task design adapted from Reding & Cummings (2019)[45].

Results

Change in relative preference and absolute preference at the population level

We presented two shoal size choice sets, to the focal fish in the central arena - one set with a display group of 4 fish versus another of 2 fish, and a second choice set comparing 6 fish to 3; and for each set, we used a single fish as the decoy for trichotomous choices (Figure 1. A, B, and D). The focal fish was tracked using the DeepLabCut pose estimation software[46] for a period of 5 minutes, and the time spent in each sector was quantified using the fish's trajectory data (Figure 1. C). Based on the time spent in each zone in initial trials with no display fish, we concluded that there was no zone bias of the focal fish in the setup (Supplementary Figure S1).

At first, we checked for the population-level shift in relative preference with respect to chance (relative preference of 0.5). The relative preference for the larger shoal did not significantly depart from random chance (0.5) for the dichotomous presentation, but shifted to being statistically significantly above chance for the trichotomous presentation with a decoy shoal set size of 1 fish, for both the shoal choice size sets used: 4 versus 2 and 6 versus 3 (Figure 2. A.i. and B.i. ; 4 versus 2: dichotomous:Mean = 0.54, SD = 0.19; one sample t-test: t = 1.2927, p-value = 0.2075, Cohen's d = 0.24, 95% CI [0.47,0.62] , n = 27; trichotomous: Mean = 0.59, SD = 0.17; one sample t-test: t = 2.6366, p-value =0.013,Cohen's d = 0.50, 95% CI [0.52,0.66], n= 27. 6 versus 3: dichotomous: Mean = 0.56, SD = 0.16; one sample t-test: t = 1.9766, p-value = 0.059, Cohen's d = 0.39, 95% CI [0.49,0.63], n = 25; trichotomous: Mean = 0.60, SD = 0.15; one sample t-test: t = 3.5775, Cohen's d = 0.71, 95% CI[0.54,0.67], p-value =0.001,n = 25). In all cases there was a considerable spread of the preferences of individual fish in the dichotomous presentations, rendering a non-significant departure away from the chance.

The population level shift in preference index was largely due to a shift in the male subpopulation towards a significant preference for the larger shoal (against chance) in the trichotomous trials - both in 4 versus 2 (Figure 2. A.ii. ; Males, dichotomous: Mean = 0.50, SD = 0.23; one sample t-test: t = 1.11774, bootstrapped p-value = 0.86, Cohen's d = 0.03, 95% bootstrap CI [0.38,0.63], n = 13; Males, trichotomous: Mean = 0.60, SD = 0.18; one sample t-test: t = 1.98, bootstrapped p-value = **0.048**, Cohen's d = 0.55, 95% bootstrap CI [0.51,0.69], n = 13) and 6 versus 3 choice sets (Figure 2. B.ii. ; Males,

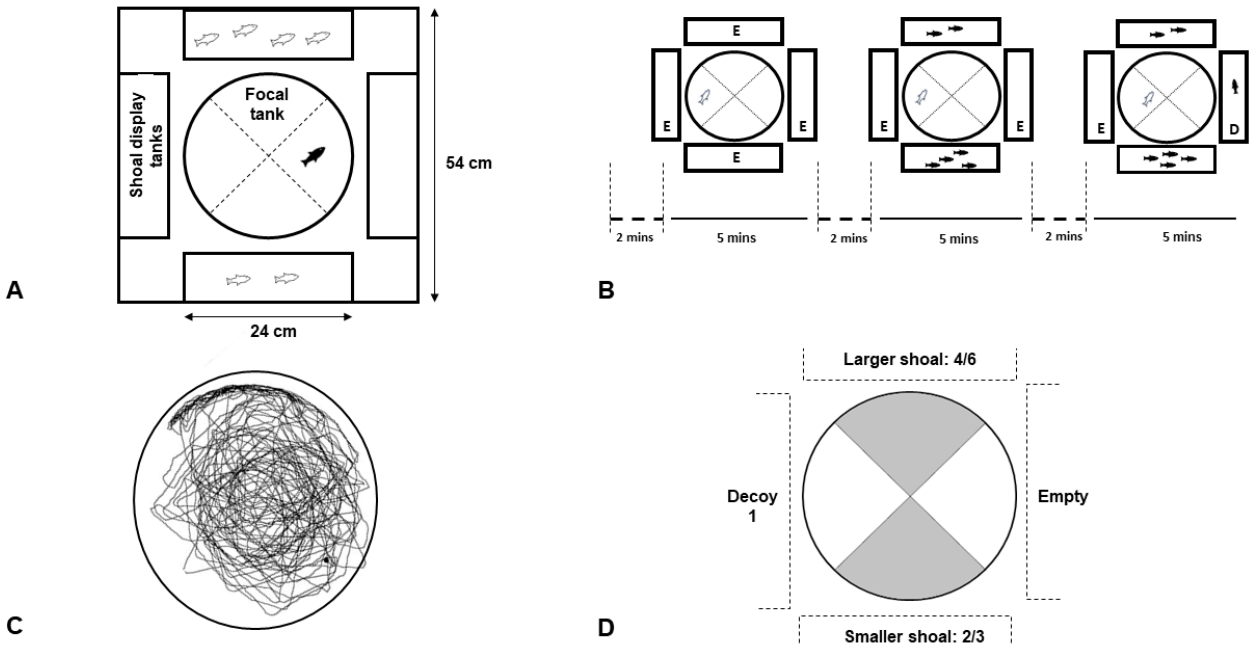

Figure 1. A. Shoal choice set up. **B.**The order of trials: After two minutes of acclimatization to the setup after the introduction of the focal fish, we conducted an initial baseline trial lasting five minutes to ensure no spatial bias across sectors in the absence of display fish. Following another 2-minute break, a sequence of counterbalanced dichotomous and trichotomous display shoal choice conditions was administered (interspersed by a 2-minute break) and for each condition, responses were recorded for five minutes.**C.** Example of focal fish trajectory data **D.** Diagrammatic representation of shoal arrangement in a trichotomous choice trial (main choice sectors in grey and decoy and empty sectors in white).

dichotomous : Mean = 0.51, SD = 0.23; one sample t-test: t = 0.33147, bootstrapped p-value = 0.63, Cohen's d = 0.09, 95% bootstrap CI [0.42,0.61] , n = 13; Males, trichotomous : Mean = 0.61, SD = 0.18; one sample t-test: t = 2.5874, bootstrapped p-value = **0.02**, Cohen's d = 0.71, 95% bootstrap CI [0.53,0.68] , n = 13). The female subpopulation showed a preference for the larger shoal in both dichotomous and trichotomous conditions, for both the 4 versus 2 (Figure 2. A.ii. ; Females, dichotomous: Mean = 0.58, SD = 0.16; one sample t-test: t = 2.0256, bootstrapped p-value = **0.04**, Cohen's d = 0.54, 95% bootstrap CI [0.50,0.66], n = 14; Females, trichotomous: Mean = 0.58, SD = 0.18; one sample t-test: t = 1.6814, bootstrapped p-value = **0.08**, Cohen's d = 0.44, 95% bootstrap CI [0.49,0.67], n = 14) and 6 versus 3 choice sets (Figure 2. B.ii. ; Females, dichotomous : Mean = 0.61, SD = 0.12; one sample t-test: t = 3.2353, bootstrapped p-value = **0.05**, Cohen's d = 0.93, 95% bootstrap CI [0.54,0.67], n = 14; Females, trichotomous : Mean = 0.60, SD = 0.15; one sample t-test: t = 2.3636, bootstrapped p-value = **0.01**, Cohen's d = 0.68, 95% bootstrap CI [0.52,0.69] , n = 14). As with the overall population, the male subpopulation did not show any significant difference in a direct pairwise comparison between dichotomous and trichotomous relative preferences, in both the 4 versus 2 (paired t-test: t = -1.0432, bootstrapped p-value = 0.3174, Cohen's d = 0.44, 95% bootstrap CI [-0.26,0.07], n = 27) and 6 versus 3 (paired t-test: t = -1.5038, bootstrapped p-value = 0.134, Cohen's d = 0.55, 95% bootstrap CI [-0.20,0.02], n = 25) choice sets.

In order to test for interaction and order effects we ran a linear mixed effect model, incorporating fixed effects of condition (dichotomous or trichotomous), order of presentation (dichotomous first or trichotomous first), and sex along with random effects, such as that of fish identity (ID), incorporating both intercept and intercept-plus-slope models for ID. We used the AIC score to select the most parsimonious model that also represented all the necessary variables (Supplementary Tables 1 and 2). We did not find any significant effect of the condition [CI = (-0.15)-(0.14), p = 0.93] or of the interaction of sex and condition [CI = (-0.11)-(0.30),p =0.35] on relative preference in the 4 vs 2 decoy choice set (Supplementary Table 3). Similarly, we did not find any significant effect of the condition [CI = (-0.13)-(0.12), p = 0.89] or of the interaction of sex and condition [CI = (-0.07)-(0.27), p = 0.26] on relative preference in the 6 vs 3 decoy choice set (Supplementary Table 4). The effect size (Cohen's d) for the order of presentation (Order) was within the range of 0 to 1 for both 4 versus 2 decoy 1 (d= 0.06) and 6 versus 3

decoy 1 (d = -0.07) choice set, thus ruling out order effects (Supplementary table 5 and 6).

However, with the lack of statistical significance using traditional hypothesis testing methods, we cannot rule out the presence of a worthwhile effect size. Therefore, we used a 'Two One-Sided Test' (TOST) approach[47] where we set a theoretical value of upper (ΔU) and lower ($-\Delta L$) equivalence bounds based on the smallest effect size of interest (SESOI). Two one-sided hypotheses are tested: H 01: $\Delta \leq -\Delta L$, H 02: $\Delta \geq \Delta U$, and upon rejection of both i.e. if the 90 % confidence interval (CI) is fully bound by the SESOI, we conclude $-\Delta L \leq \Delta \leq \Delta U$ i.e., the observed effect size is statistically smaller than the smallest effect size of interest. Equivalence bounds for the paired t-test were calculated using the powerTOSTpaired.raw function of the R package TOSTER[48] with statistical power of 80% and alpha 0.05, separately for 4 versus 2 - decoy 1 choice set (with a sample size of 27 for whole-population analysis and n=13 for the male subpopulation); and for the 6 versus 3- decoy 1 choice set (with a sample size of 25 for the whole population and 13 for the male subpopulation). For the total population the equivalence bounds for 4 versus 2 and 6 versus 3 choice sets were ±0.14 and ±0.12 around the means respectively, and for male sub-populations, the equivalence bounds were ±0.24 and ±0.17 around the means respectively. The TOST null hypothesis i.e that the effect size is worthwhile, could be rejected only at the whole population level (Supplementary Figure S2 and S4, 4 versus 2: TOST 90% CI[-0.134,0.034]; 6 versus 3: TOST 90% CI[-0.115,0.0035]) but not for the male sub-populations in either choice set (Supplementary Figure S3 and S5, 4 versus 2: TOST 90% CI[-0.244,0.044]; 6 versus 3: TOST 90% CI[-0.216,0.016]).

In literature the violation of rationality has been strictly inferred only when the relative preference differs significantly by direct comparisons[22,45]. Here we show evidence for a decoy effect in the male sub-population only when comparing performance in each condition against chance (0.5), which contributed to a significant difference from a chance at the whole population level aggregated across the sexes. Through an equivalence analysis (TOST) we also show that even though pairwise comparisons in male sub-populations are statistically non-significant, the effect sizes are not too small to be neglected.

We analyzed the proportion of time spent in each sector to investigate if there was a violation of the principle of regularity. At the population level, fish spent significantly more time in the decoy+empty sectors in the trichotomous trials in both 4 versus 2- decoy 1 (paired t-test: t = -2.5135, p-value = **0.018**, Cohen's d = 0.5, 95% CI [-0.12,-0.01], n=27) and 6 versus 3- decoy 1 (paired t-test: t = -3.2352, p-value = **0.003**, Cohen's d = 0.5, 95% CI [-0.08,-0.01], n=25) choice sets. The introduction of the decoy significantly reduced the absolute time spent shoaling in the dichotomous shoal choice sectors taken together, but this consisted of a nonsignificant but large decrease in the time spent in the sector with the smaller display fish shoal size (2 or 3) (Figure 2. C.i.; paired t-test: 4 versus 2-1: t = 1.8703, p-value = 0.07275, Cohen's d = 0.5, 95% CI [-0.007,0.151], n = 27; Figure 2. D.i.; paired t-test: 6 versus 3-1: t = 1.51, p-value = 0.14, Cohen's d = 0.45, 95% CI [-0.015,0.1], n = 25). The fish spent a similar amount of time investigating the smaller shoal and the decoy during trichotomous choices (4 versus 2- decoy 1: Proportion of time with 2 = 0.23 ±0.09, Proportion of time with 1: 0.24 ±0.10; 6 versus 3- decoy 1: Proportion of time with 3 0.22 ±0.07, Proportion of time with 1: 0.22 ±0.08).

The reduction of the time spent with the smaller shoal in the trichotomous condition was due to the behavior of the male subpopulation. Males showed a significant decrease in the proportion of time spent in the sector facing the smaller-sized shoal (2 or 3, respectively) (Figure 2. C. iii. ; paired t-test; 4 versus 2-1: t = 2.2366, bootstrapped p-value = **0.038**, Cohen's d = 0.8, 95% bootstrap CI [0.02,0.24], n = 13; Figure 2. D. iii. ; 6 versus 3-1: t = 1.9745, bootstrapped p-value = **0.05**, Cohen's d = 0.75, 95% bootstrap CI [0.004,0.136], n = 13). The female subpopulation showed no significant difference in the proportion of time spent in the sector facing the smaller-sized shoal (2 or 3) in the trichotomous condition (Figure 2. C. ii. ; paired t-test : 4 versus 2-1: t = 0.30592, bootstrapped p-value = 0.78, Cohen's d = 0.1, 95% bootstrap CI [-0.08,0.09], n = 14; Figure 2. D. ii.; 6 versus 3-1 : t = 0.2665, bootstrapped p-value = 0.78, Cohen's d = 0.1, 95% bootstrap CI [-0.07,0.09], n = 12).

The principle of regularity assumes IIA: that the introduction of a decoy would distract the focal fish equivalently from all previous options, and therefore symmetrically reduce the share of time spent with those options while maintaining the relative proportion of time spent with each option from dichotomous trials[14]. We report that in the case of the male sub-population, while the introduction of the decoy reduced the time spent shoaling with the smaller shoal, the absolute time spent with the larger shoal was unchanged. There was no increase in the absolute share of the proportion of time spent with any of the dichotomous choice shoals upon introduction of the decoy, which is a more stringent criterion in a strict interpretation of the principle of regularity.

Relative preference and absolute preference at the individual level

At an individual level, some fish display rational behavior while others display choice reversal - the addition of the decoy reversed the relative preference for a larger shoal. The individual fish whose data fall along the inclined line of a plot of the preference index for the larger shoal in the trichotomous choice set against the same preference index in the dichotomous choice set, display rational choice consistency (Figure 3. A and B). The individual fish in the top left quadrant showed a choice reversal from preferring the smaller shoal in dichotomous choice to preferring the larger shoal in the presence of the decoy, outnumbering those in the bottom right quadrant which showed a choice reversal towards preferring the smaller shoal in trichotomous choices.

In the 6 versus 3- decoy 1 choice set, the choice reversals were mostly in the direction of preferring the larger shoal in the presence of the decoy (top left quadrant; 7 out of 10 choice reversals i.e., 70%). In the 4 versus 2- decoy 1 choice set, the choice reversals were in both directions (6 out of 11 choice reversals i.e., 54%). These choice reversals were mainly contributed by the males both in 4 versus 2 decoy 1 (7 out of 11 choice reversals i.e., 63%) and 6 versus 3 decoy 1 (6 out of 10 choice reversals i.e., 60%) choice sets. The opposite choice reversals i.e., switching from choosing the larger shoal to the smaller shoal, were also observed, more in the 4 versus 2- decoy 1 than the 6 versus 3- decoy 1 choice sets.

In the ternary overlay of individual changes in the proportion of time allocation to each sector, this same phenomenon is seen as a shift in the density of points towards the larger shoal–decoy axis among the males in both the 4 versus 2- decoy 1 and 6 versus 3- decoy 1 choice sets (Figure 3. C.i. and D.i.), with no noticeable shift among the females (Figure 3. C.ii. and D.ii.). The shift for the males is also visible at the whole population level (Supplementary Figure S6 and S7)). Among the males (Figure 3. C.i. and D.i.), the fish that spent more time shoaling with the smaller shoal in the dichotomous choice trials (in red) in both the choice sets, showed a shift towards the decoy in the trichotomous choice set (in green). The point density cloud in the trichotomous choice trials among males, was most dense near the smaller shoal-decoy indifference region with a few points near the larger shoal (6 and 3) time allocation region (Figure 3. C.i. and D.i.).

The fact that the male fish with a weaker preference index for the larger shoal spent more time with the decoy also manifests as a significant negative correlation between the relative preference index for the larger shoal (4 and 6 respectively) in the trichotomous condition and the proportion of time spent in decoy+empty zone (Figure 3. E., 4 versus 2 Males; R = -0.67, p-value = **0.0012**; 3. F., 6 versus 3 Males; R = -0.62, p-value = **0.025**). This indicates that male fish that show a strong relative preference for the larger shoal in trichotomous choices also spend relatively less time investigating the decoy. This also contributes to a whole population level significant negative correlation between the relative preference index for the larger shoal size of 4 in the trichotomous condition and the proportion of time spent in decoy+empty zone (Supplementary Figure S8; R = -0.51, p-value = **0.0067**). There was only a weak negative relationship between the preference index and the proportion of time allocated in decoy+empty zones for the larger shoal size of 6 at the population level (Supplementary Figure S9; R = -0.23, p-value = 0.25).

Trajectory density analysis

We rotated and overlapped the individual trajectories of fish during the shoaling period along the 4 versus 2 and 6 versus 3 choice axis to produce Kernel density estimation (KDE) based plots to infer the probability density underlying the position trajectory data of fish.

We found support for the existence of a decoy effect only in the males in both the 4 versus 2 (Figure 4 A.i. and ii.) and 6 versus 3 (Figure 4 B.i. and ii.) trichotomous choice sets with a decoy of 1 fish. With the introduction of the decoy fish, we observed a dampening of density around the smaller shoal size (2 and 3) without any effect on the density near the larger shoals (4 and 6). However among female fish, the effect was not as sharp in either the 4 versus 2 (Figure 4 A.iii. and iv.) or 6 versus 3 choice sets (Figure 4 B.iii. and iv.). The density shift among the males was also reflected in the whole population level trajectory density plots (Supplementary Figure S10; 4 versus 2 decoy 1 : A and B , 6 versus 3 decoy 1 : C and D). We performed a Kernel density-based location two-sample test to find locally significant differences in regions of density space using the *kde.loct.test* of the R package *ks:kernel smoothing*. The trajectory region facing the smaller shoals (2 and 3, respectively, in the two choice sets) was the major region that was significantly different in the two distributions only in the case of male fish sub-population (Figure 4. Red region in C.i. and D.i.) and not in female fish sub-population (Figure 4. Red region in C.ii. and D.ii.). The density regions facing smaller shoals were significantly dampened even at the population level (Supplementary Figure S10; E and F).

This supported our previous results of finding a dampening of the preference of male fish for the smaller shoal in the trichotomous choice set. Individual trajectory probability density plots for these fish using the kernel density estimation approach revealed several instances of fish demonstrating choice reversal in the preference between two shoal sizes upon introducing the decoy fish, in both the 4 versus 2- decoy 1 and 6 versus 3- decoy 1 choice sets (Supplementary Figure S11 and S12).

Repeatability analysis of change in relative preference at the individual level

We tested whether the similarity in the decoy effect observed in males and at the population level in both the 4 versus 2 and 6 versus 3 choice sets is reflected in repeatability at the level of individual performance. We compared the difference of the Preference Index for the larger shoal between dichotomous and trichotomous trials (D-T) for the same set of fish across the two choice sets (4 versus 2- decoy 1 and 6 versus 3- decoy 1) using a Pearson correlation test. We failed to find any significant correlation when comparing 4 versus 2-1 and 6 versus 3-1 choice trials for the same set of fish in both males and females (Figure 5A; females: R= -0.072, p = 0.82, males: R= 0.15, p = 0.62). We also estimated repeatability using a linear mixed effect-based approach where repeatability R is calculated as the fractional group-level variance relative to the sum of variance from the group-level and population-level and found no significant or sizeable estimate of R (adjusted for Sex) using parametric bootstrapping (Figure 5B).

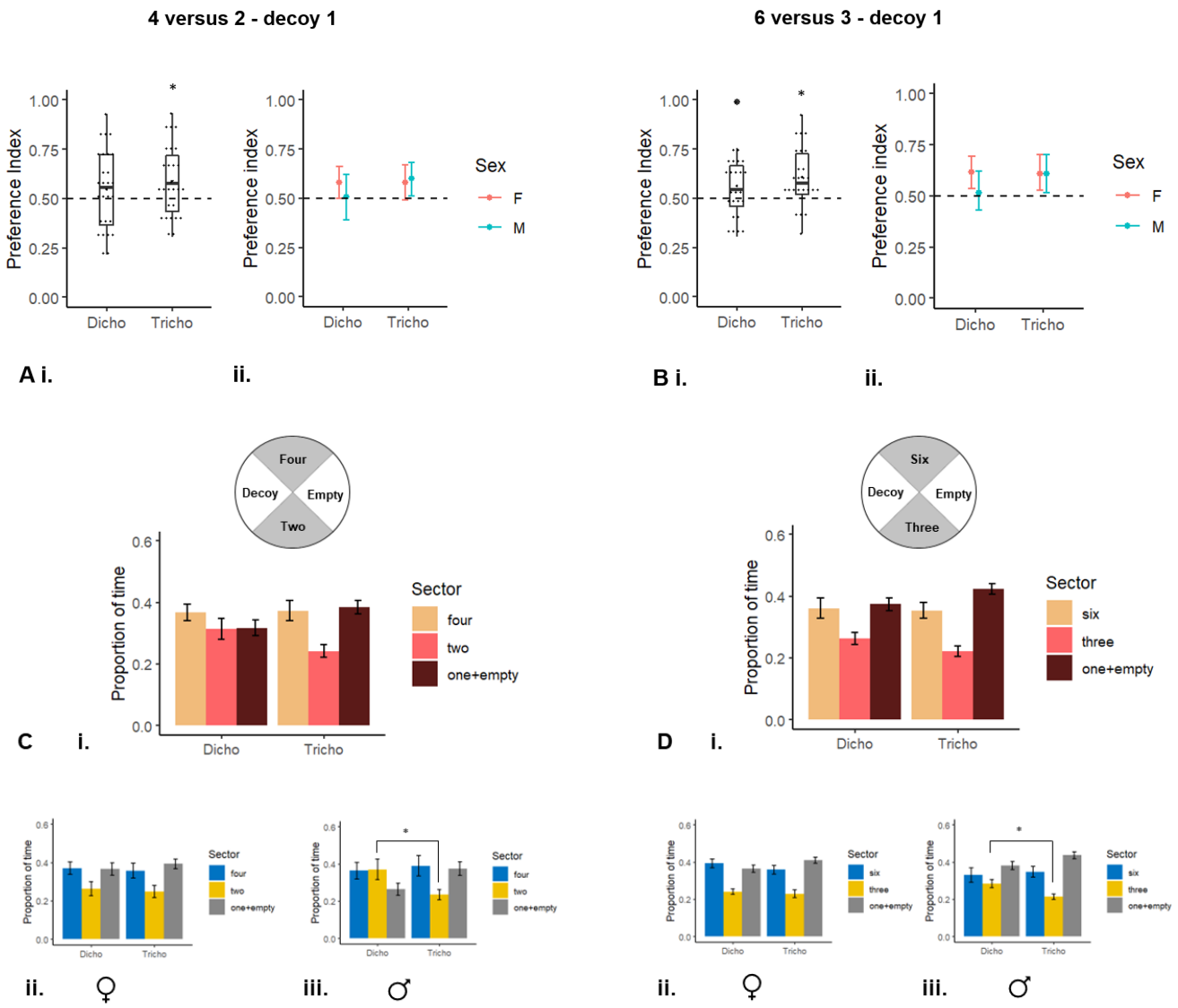

Figure 2. Relative preference for the larger shoal against chance (0.5) in dichotomous versus trichotomous trials at **A.i.** the population level (significant against chance in trichotomous trials) and **ii.** disaggregated for male and female subpopulations with 95% bootstrap CI against chance for the 4 versus 2 choice set (significant against chance for males only in trichotomous trials, but significant against chance for females in both dichotomous and trichotomous trials) and for the 6 versus 3 choice set **B.i**. at the population level (significant against chance in trichotomous trials) and **ii**. for male and female subpopulations with 95% bootstrap CI against chance (significant against chance for males only in trichotomous trials, but significant against chance for females in both conditions). **C** The proportion of time allocated to each sector (absolute preference; Mean ±SE) at **i.** the population level, **ii.** for females and **iii.** for males for the 4 versus 2 choice set, and for the 6 versus 3 choice set at **D.i.** the population level, **ii.** for females and **iii.** for males.

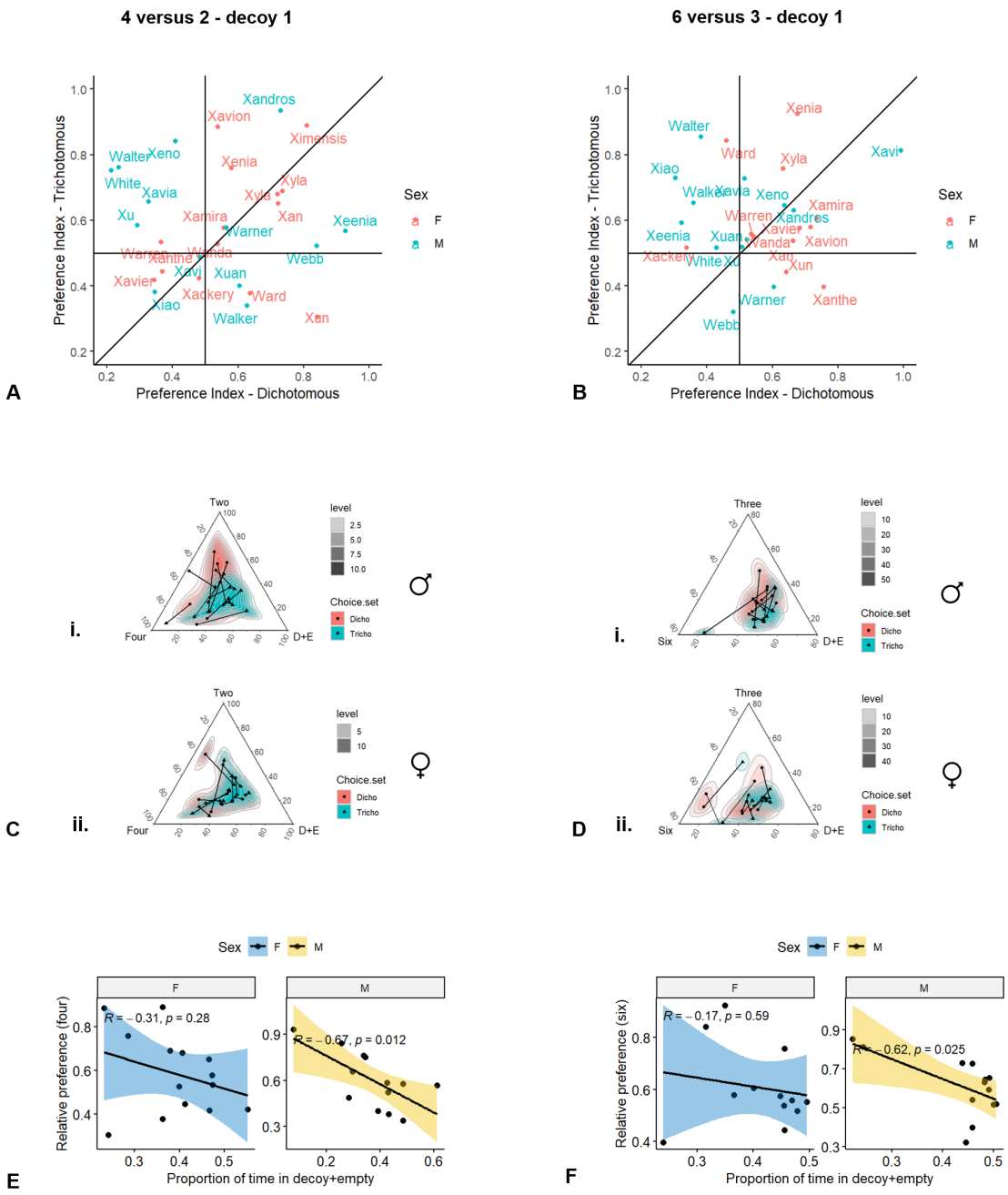

Figure 3. Relationship between the relative preference for the larger shoal in dichotomous and trichotomous choice trials. Note that the top right quadrant displays the preference for the larger shoal in both conditions for the **A.** 4 versus 2 choice set and **B.** the 6 versus 3 choice set. Individual fish are indicated by name and sex (red: females, blue: males). The top left quadrant indicates a reversal of preference for the smaller shoal in dichotomous trials, towards preferring the larger shoal, in trichotomous conditions. The points in the bottom right quadrant represent a reversal of preference for the larger shoal in dichotomous trials to the preference for the smaller shoal in trichotomous trials. **C.** and **D.** Ternary plots displaying the shift in the proportion of time allocated to shoaling in the two main choice sectors (top and bottom left corner), as well as the decoy+empty sectors together (bottom right corner) in dichotomous (in red) versus trichotomous conditions (in blue) for **C.** the 4 versus 2 choice set - for **i.** Males and **ii.** Females and **D.** the 6 versus 3 choice set - for **i.** Males and **ii.** Females. **E.** and **F.** The correlation between the relative preference for the larger shoal and the proportion of time allocated to the decoy+empty sectors for the **E.** 4 versus 2- decoy 1 choice set - Females (left) and Males (right) and **F.** the 6 versus 3- decoy 1 choice set - Females (left) and Males (right).

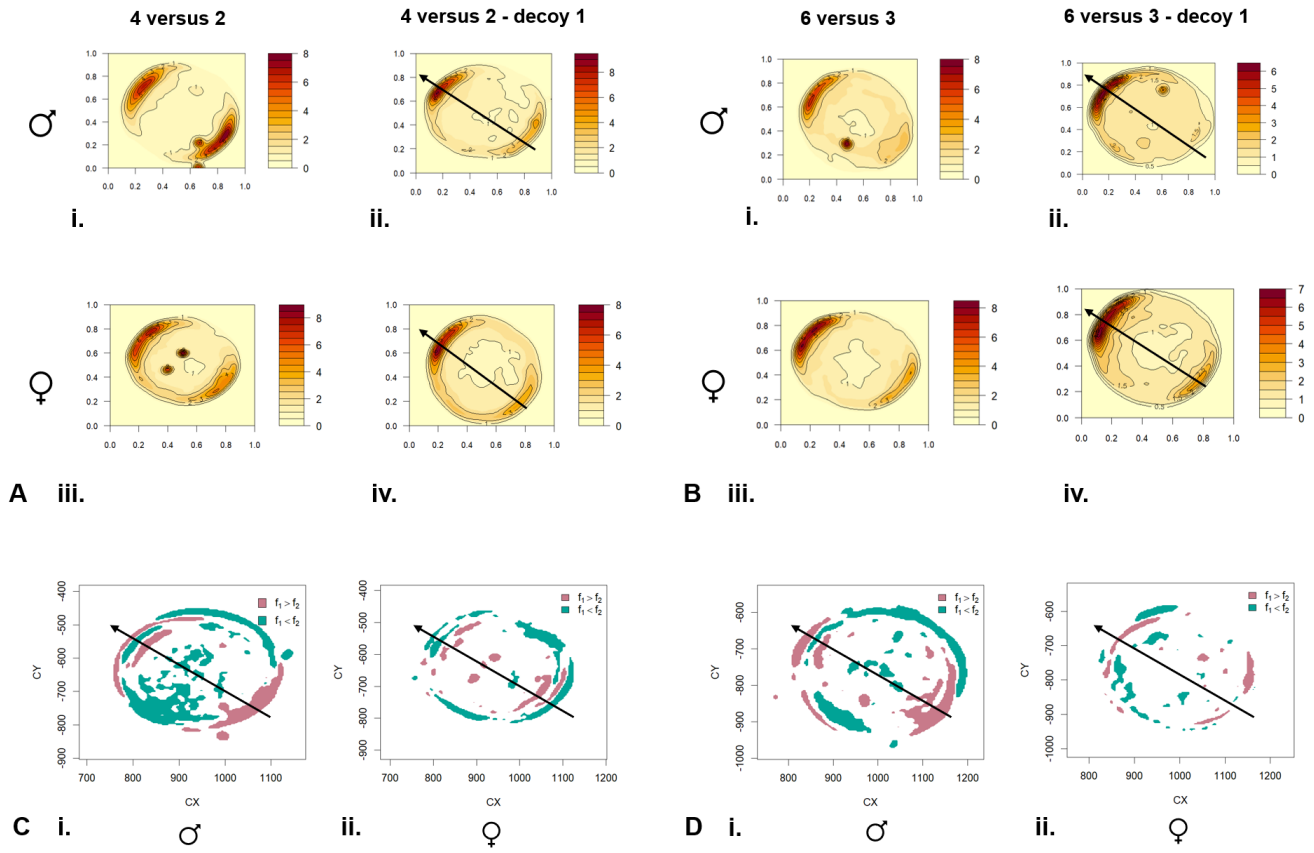

Figure 4. Population Trajectory kernel density estimate (KDE) plots of the **A.i.** and **iii.** dichotomous and **A.ii.** and **iv.** trichotomous trials of the 4 versus 2 decoy 1 choice set (male subpopulation above and female subpopulation below) showing a shift in the direction of the arrow towards the larger shoal in trichotomous trials. Population trajectory kernel density estimate (KDE) plots of the **B.i.** and **iii.** dichotomous (males and females) and **B. ii.** and **B.iv.** trichotomous trials (males and females) of the 6 versus 3 choice set, (males above and females below). Regions of significant difference between dichotomous and trichotomous choice trials are shown for **C.** The 4 versus 2 choice set for **i.**. males and **ii.** females, and for **D.** the 6 versus 3 choice set for **i.** males and **ii.** females.

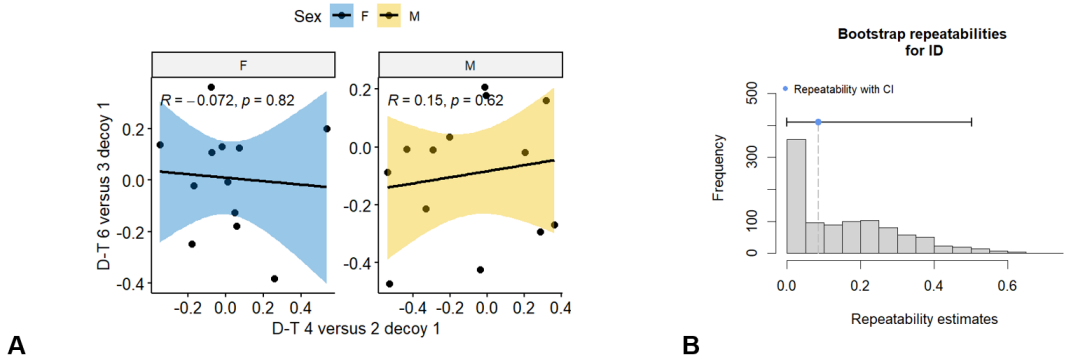

Figure 5. **A.** The change in relative preference between dichotomous and trichotomous choice sets (D-T value) for the 4 versus 2- decoy 1, relative to the 6 versus 3- decoy 1 choice set - females in red and males in blue **B.** A bootstrap estimate of repeatability between individual performance in the two choice sets using the linear mixed-effect based approach.

Discussion

Our data provide the first evidence for the decoy effect in decision making along a single attribute dimension of shoal size choice in male zebrafish (*Danio rerio*), a neurobiologically and genetically tractable model organism.

The decoy effect and the independence of irrelevant alternatives are well-documented human cognitive biases that have been measured only in a few animal model systems. Previous attempts in mice[18] reported no violation of IIA, while work on *C. elegans* showed both rationality in some decisions and the violation of rationality with respect to specific combinations of odorants. Context effects in decision making have been reported in the fish literature, largely with respect to multi-attribute mate choice by females - for example in the peacock blenny, *Salaria pavo*[49] and the green swordtail, *Xiphophorus helleri*[35] . The investigation of IIA in shoal size choice decisions has only been conducted in mosquitofish, *Gambusia affinis*, where no violation of the constant ratio rule was found[45]. We used a similar experimental design to theirs in our shoal size choice task in order to avoid the replication crisis seen in human decoy effect studies[18,27,28,32] where small variations in the task and stimulus modality may have been responsible for the differing decoy effects found. Our finding of a decoy effect in the same task in a different model system is unlikely to be based on differences in the setup or stimulus modality and could suggest that decision making and IIA for the same task varies between species, and between sexes within a species.

We report a shift in relative preference in the presence of the decoy option since the male zebrafish in our study showed a population-level sharpened relative preference for the larger shoal in the presence of the decoy which did not exist in dichotomous condition. We also see a significant dampening of the absolute preference of male zebrafish for the smaller shoal in the presence of decoy fish, as revealed by the trajectory kernel density-based analysis, with these fish allocating a similar proportion of time to the smaller shoal and the decoy in the trichotomous trials, in line with the similarity effect, rather than the attraction or compromise effect.

Rationality is violated to the extent of observing individual choice reversals from preferring smaller shoals to preferring larger shoals in the presence of a decoy, largely among male zebrafish. In its most minimal form, the principles of IIA propose choice order stability. Choice reversals also rule out the possibility that the change in relative preference that we see here is due to the "random dilution effect" proposed by Bateson et al. (2002)[4], where a proportion of choices are randomly allocated between options. A third option may absorb these even with no change of bias between the two main options, thereby diluting the masking effect of the random noise on the existing choice bias and appearing to change the relative preference[34]. Apart from choice reversals at the individual level, we observe that the time allocation by the population of male zebrafish to the decoy in the trichotomous choices does not uniformly portion out from the time allocated to the existing two choices, with a significantly large portion of the time allocated to the smaller shoal being redirected towards the decoy, and with no significant change in the time allocated to the larger shoal. Female zebrafish in our study upheld rationality both in terms of the constant ratio rule and the principle of regularity.

We show sex based differences in the preference for the larger shoal - males did not prefer the larger shoal in both the dichotomous shoal choice sets of 4 versus 2 and 6 versus 3, while females consistently preferred the larger shoal for both choice sets. This is consistent with previous reports of such sex based differences in shoal discrimination performance[42,50–52]. However, the inability of male zebrafish in our study to prefer the larger shoal in a dichotomous choice of 4 versus 2 is different from the finding of Potrich et al (2015) that male zebrafish show a significant preference at the population level for a shoal size of 4 over 2[53]. This difference could be because Potrich et al (2015) used only females in the display shoals, whereas we randomized the sex of the fish in the display shoal. Many other shoal discrimination studies have not tested for sex based differences in shoaling behaviour[39,54]. Most shoal choice studies also use a different apparatus from us - a rectangular three-tank set up, and one study[39] used a perforated divider through which shoaling decisions were based on olfactory and not just visual cues from the display fish. Our results indicate that there is sex based difference in the shoal choice strategies upon the introduction of a third inferior option. Female zebrafish in our study maintained their relative preference between the dichotomous options when spending time with the decoy option in trichotomous trials. Male zebrafish in our study, however, asymmetrically portioned out time spent shoaling with the smaller shoal size while shoaling with the decoy fish. This suggests the use of an absolute valuation mechanism for shoaling decisions in females and a comparative mechanism in the case of males. These differences could arise due to different evolutionary shoal choice pressures for males and females[41,55].

Unidimensional violation of IIA has been rarely reported[10]. Such work is essential because of the difficulty with interpreting multi-attribute context effects, with multiple plausible underlying models mapping to the same outcome. Morgan et al. (2012) also demonstrate different decoy effects at play during single attribute manipulations for different attributes[10]. Different mechanisms might also be expected to mediate context effects in various single attribute choices, and these must be investigated to arrive at a comprehensive understanding of decision making relative to various attributes considered together. Our use of visual stimuli, where context effects and the underlying neural circuitry are very well understood in zebrafish, rather than gustatory stimuli, will also help establish this system as a useful model to explore context effects.

We did not find any significant correlation between the context-dependent change in preference for individuals across choice contexts of 4 versus 2 and 6 versus 3 in either males or females, suggesting that fish may not use the same general rule

or mechanism for these two sets of comparison pairs at the individual level. We would also need to investigate repeatability within the context of a given shoal size set to further examine the possibility that context-dependent effects may not be stable or repeatable over time[56].

In our study, we establish decoy effects that violate normative economic principles of behavioral rationality in the male, but not the female zebrafish model system relative to a single attribute: shoal size decision making. A wide range of pharmacological, genetic, neurobiological, and behavioral tools available in this system will enable future work on comprehensively understanding context effects in this model organism, and examining the underlying mechanisms behind sex-specific rationality in contextual decision making.

Methods

Subjects

Two batches of adult zebrafish (*Danio rerio*) (age <1 year) were bought from a local pet store in Daryaganj, New Delhi, India, and were used for two sets of experiments. Local pet stores capture and breed zebrafish from local rivers and ponds where wild zebrafish are found. The fish were procured in March 2021 and experiments were carried out in August-October 2021. All fish were maintained in the ZebTec Active Blue - Stand Alone system (Tecniplast, PA, USA) and maintained on a 12:12 light: dark (10 a.m. - 10 p.m.) circadian cycle at 7.50-8.50 pH, 28-30°C and 650-700 µS conductivity at Ashoka University, Sonipat, Haryana, India. Fish were fed ad libitum twice a day with powdered Tetra-Tetramin flakes.

For the experiments, 30 fish were randomly picked from the population. Two fish died while the experiments were underway, and time stamps for some recordings could not be verified. A complete data set for analysis was obtained for only 27 fish for the 4 versus 2 choice task, out of which 14 were females. For the 3 versus 6 choice task, data for 25 fish were completely processed through the analysis pipeline, out of which 12 were females. We computed the sample size (N) required to attain significance level at 0.05 with statistical power of 80% for one sample t-test against chance (0.5) using the pwr.t.test() function in R - this N was 26, and thus we approximated the minimum sample size for the study. Fish were kept isolated in separate tanks for 2-4 weeks before the shoaling experiments began in the ZebTec fish system, such that they could see and smell each other through a shared water circulation system with olfactory cues. The remaining fish from the same population were randomly chosen as display fish for the shoal choice assay.

Shoal choice apparatus

The shoaling apparatus consisted of a cylindrical shoaling transparent acrylic tank (referred to henceforth as the focal tank) of diameter 24cm (4mm thickness), centered within a larger 54cm x 54cm x 31 cm square plexiglass tank (henceforth referred to as the main tank), similar to the setup used by Reding and Cummings, (2019)[45]. Transparent rectangular plexiglass 27 cm x 10 cm x 30cm display tanks were centrally placed and glued to the sides of the main square tank, such that their occupants could be viewed equidistant from the central focal tank. The distance between the boundary of the central tank and display tanks was 4cm. All edges were sealed using a water-resistant silicone sealant (DOWSILTM GP silicone sealant). The bottom surface of the main tank was placed on a raised platform. All the outer faces of the main tank were covered with opaque paper to block visual cues from the outside, and each of the perpendicular faces of each of the display tanks was also covered with laminated black paper to bar the display fish from seeing each other.

An LED light strip (Mufasa Copper Non-Waterproof LED Strip 5050) of uniform length was attached to each of the display tanks to provide a light source from within the setup and to avoid any shadow formation of the tripod. We placed a dummy tripod (without a camera), rising to the same height and approaching the existing camera tripod from the diagonally opposite end. The entire set-up was covered by a 10ft X 10ft black photography cloth curtain of the dimensions to prevent any influx of external visual cues, such that the sole light source came from the display tanks. The space between the display tanks and the focal tank was filled with water to reduce any distortion caused by refraction. The background face of the display tanks, behind the display fish, was covered with a green sheet of laminated paper, as per Lucon-Xiccato et. al (2017)[57]. The camera used for these recordings was a GoPro Hero 8 (1080p|30fps|linear) and all recordings were made in .MP4 format.

Experimental design

A cylindrical sheet of white paper laminated with transparent plastic was used to cover the central focal tank to keep the focal fish and display fish mutually visually isolated before the trial began. Before every treatment trial, 5 minutes of no-fish trials were performed where the subject fish were exposed to empty display tanks after 2 minutes of visual isolation from display tanks. During the 2-minute visual isolation period, fish were introduced to the display tanks in group sizes corresponding to the choice set. Each trial ended after 5 minutes of video recording the shoaling responses of the focal fish, followed by a 3-minute resting period during which the display fish was changed. For each trial, we used the *Sample()* function in R to first randomly decide the axis of presentation of the dichotomous choice (axis with respect to the camera view), followed by randomizing the side of the presentation of the larger shoal, and finally the side of the presentation of the third choice (for trichotomous

trials). The order in which fish were tested on each choice set was kept constant across the two choice sets, such that each fish was tested first on the choice set of 2 versus 4, then after a period of two weeks, on the choice set of 3 versus 6 in that order. Within each choice set, the order of dichotomous and trichotomous choice set trials was counterbalanced i.e., in half of the trials dichotomous choices were presented first and in the other half the order was reversed (Figure 1. B).

DeepLabCut based fish tracking

All videos were converted to .MP4 format and cropped for any extra recording time to a fixed duration of 5 minutes, starting from the moment of raising of the sheet covering the focal tank, using the Python-based video editing tool Moviepy. The videos were then processed using DeepLabcut, a Python-based pose estimation package[46] to track the head, body, and tail of the fish.

A few videos were randomly chosen to generate the training frames where the pre-defined body parts were manually marked. Default Artificial Neural Network ResNet 50 was trained for at least 1,50,000 iterations for any set of analyses. The videos were then processed, and labeled videos were generated to check the efficiency of tracking. The tracking data for each of the body parts were converted and stored in .csv format. We chose the body part which was most efficiently tracked by inspecting the labeled tracked videos, which in most cases was the 'body' point, rather than the head or tail.

Analysis

1 Relative preference and absolute preference

The trajectory data obtained from the DLC tracking was processed using a custom R script DLC-Analyzer[58] for the time spent in each sector data. The preference index for the larger shoal was calculated using the time spent in each sector data as :

$$\text{Preference Index (PI)} = \frac{\text{Time spent in larger shoal sector}}{\text{Time spent in larger shoal sector} + \text{Time spent in smaller shoal sector}} \tag{1}$$

As a test for the Independence of Irrelevant Alternatives (IIA), we tested for the change in the relative preference for the larger shoal with and without the decoy option of a single display fish. The change in the relative preference upon adding a decoy was tested both in a direct comparison between performance with the dichotomous and trichotomous choice sets using linear mixed effect model-based analysis and for each set preference shift was also tested against random chance (0.5). The proportion of time the fish spent shoaling in each sector in a choice set was calculated as the time spent in that sector divided by the total time in each trial (300 seconds, or 5 minutes). The proportion of time allocated to each of the options in the dichotomous and corresponding trichotomous choice set was calculated to test the principle of regularity. Both the distributions of relative preference and the proportion of time allocation did not deviate from normality when tested using the Shapiro-Wilk's test for all choice sets.

We used the boot.t.test() function in R in order to calculate 95% t-CI values against chance (0.5) for males and females subpopulations due to small sample size. The bootstrap replication value was always kept at 1000. We also performed a test of equivalence called TOST (two one sided tests) in order to test if insignificant comparisons had worthwhile effect sizes using the package TOSTER in R. We calculated the equivalence bounds based on the smallest effect size of interest (SESOI) calculated for paired t-tests for each of the choice sets (4 versus 2 and 6 verses 3) based on the statistical power of 80%, the given sample size, and an alpha of 0.05. When the 90% Confidence Interval was not completely bound by SESOI based equivalence bounds, the effect size was considered worthwhile.

2 Individual-level relative preference and absolute preference

Choice reversals at the individual level were visualized by plotting the relative preference for the larger shoal in trichotomous versus dichotomous trials for both the 4 display fish versus 2 and the 6 versus 3 fish choice sets. The individuals along the inclined line of the plot showed invariance in performance with or without decoy i.e., following the principle of the independence of irrelevant alternatives. The top left and bottom right sector on such a plot represent individual fish that exhibit choice reversals. The proportion of time allocation to each of the choice options for individual fish was visualized using a ternary plot using the ggtern package in R. The proportion of time spent in the decoy and empty sectors in the case of the trichotomous trials were pooled to reduce the variables to three for a ternary plot visualization of the time spent with the decoy+empty sectors, relative to the two sectors with the same number of display fish as the corresponding dichotomous trial. The *stat_density_tern()* function of the package ggtern was used to represent the kernel density estimates to visualize the shift in the density of points between the dichotomous and trichotomous conditions.

3 Trajectory analysis

The trajectories of individual fish in all trials were rotated to fit the same dichotomous choice axis, directed toward the larger shoal in the top left sector and toward the smaller shoal in the bottom right sector. All the rotated trajectories were then superimposed by concatenating each of the X and Y coordinate vectors for all fish, separately for dichotomous and trichotomous

trials for the 4 versus 2- decoy 1 choice set and 6 versus 3- decoy 1 choice set. Bivariate kernel density plots were generated for each of the choice conditions and choice sets using the *k2de()* function from the library *MASS* in R. We used default parameters, except that the grid numbers on each axis were set to 100. KDE plots for individual trajectories were also produced in the same way. Trajectory KDE-based global two-sample comparisons between the dichotomous and trichotomous conditions for each of the choice sets were performed using the *kde.test()* function of the package *ks:kernel* smoothing in R on the population trajectory data. The locally significant region plot was produced using the *kde.local.test()* function of the package *ks:kernel smoothing* in R.

4 Repeatability analysis

The repeatability of the difference in individual relative preference for the larger shoal, between dichotomous and trichotomous choice conditions (D-T) was estimated across the 4 verses 2 and 6 versus 3 choice set, using a Pearson correlation test and a linear mixed effect model-based repeatability estimation. The Pearson correlation test was performed along with the *ggscatter()* plot function of the package *ggpubr* in R software. The linear mixed-effect model approach partitions the variance into within-group and between-group sources[59]. The repeatability estimate R is calculated as :

$$\text{Repeatability R} = \frac{\text{Group-level variance VG}}{\text{Group-level variance VG} + \text{Residual(data-level) variance VR}} \tag{2}$$

We calculated adjusted repeatability using the package *rptR* in R by including the fixed effect of the choice set (4 versus 2-decoy 1, and 6 versus 3- decoy 1), and Sex along with the random effect of fish ID. We set the number of bootstraps to 1000 and the permutation number to 10 to account for uncertainty around the estimation of R.

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Author contributions statement

The study was designed by AS, KK, and BKR; the apparatus was made by AS and KK; the data was collected by AS and KK; data analysis was performed by AS and BKR; and the manuscript was written by AS and BKR.

Competing Interest

The authors declare no competing interest.

Additional Information

The ARRIVE international guidelines were followed in the reporting in this manuscript and CSPCA national and institutional guidelines were followed for the care and use of animals. Ethical approval was obtained from Ashoka University's Institutional Animal Ethics Committee (approval no. 01/Bio/BKR/2020).

Data Availability

Supplementary information and all raw data files that support the findings of this study are openly available in figshare at http://doi.org/10.6084/m9.figshare.20745865.

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