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Density dependence and numerosity in fright stimulated aggregation behaviour of shoaling fish

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SUMMARY

Single three-spined sticklebacks, *Gasterosteus aculeatus*, were frightened with a light stimulus simulating an aerial predator while facing a choice between two conspecific display shoals of different membership sizes. We observed which shoal the test fish approached. Initially, both display shoals were equidistant from the test fish. The smaller shoal was then moved gradually closer whereas the larger shoal stayed at a constant distance. This experiment modelled an early stage of the aggregation behaviour of sticklebacks in response to perceived imminent predation risk. When the two display shoals were equidistant from the test fish, we found that the test animal preferred approaching the larger display shoal, and the magnitude of this preference increased with increasing display shoal size difference. This demonstrates that the aggregation behaviour of frightened sticklebacks is density dependent. Further, we found that sticklebacks made a trade-off between the distance to a display shoal and its membership size. In particular, for a given ratio of display shoal sizes, there was a critical distance at which half of all tested animals turned to one and the other half to the other display shoal. This demonstrates that the observed aggregation behaviour is also distance dependent. We introduce several elementary models which formalize individual predation risk and explore how distance and display shoal size contribute to total risk. In particular, we distinguish between total risk as a product or as a sum of the risk components associated with swimming distance and display shoal size, respectively. All models follow the 'partial preferences' paradigm of McNamara & Houston (*Anim. Behav.* **35**, 1084–1099 (1987)). We compare how closely these models match the observed data and how well they predict the empirical critical distances. We find a consistent discrepancy between theory and data, which we resolve by invoking a fundamental perceptual limit (numerosity) for shoal size discrimination.

1. INTRODUCTION

Many animal species aggregate in response to more-or-less simultaneously experienced fright stimuli caused by approaching predators. Often only a few seconds after an effective fright stimulus, a single large group of animals emerges from initially dispersed individuals (Krause & Tegeder 1994). Aggregation in response to fright stimuli is a very specific form of animal-grouping behaviour. Significant research effort has been directed at understanding the many functional aspects that underlie grouping behaviour in general (see, for example, Pulliam & Caraco 1984; Magurran 1990; Pitcher & Parrish 1993). In this respect, animal aggregation triggered by fright stimuli is a special case because of the clear functional context of imminent mortality risk. Further, this presents a paradigmatic system which displays social behaviour apparently lacking central control or coordination (e.g. Niwa 1994). The process of group formation from previously dispersed individuals seems literally self-organized, involving a sizeable number of individuals making concurrent and interdependent decisions.

The perceived imminence of a predator attack puts this process in a context where time counts. As a result, a behavioural mechanism for aggregation will presumably be subjected to a strong selection pressure aiming at the sharpest decrease in mortality risk in the shortest time possible. This led us to hypothesise earlier (Krause & Tegeder 1994) that individual animal behaviour during the early stages of aggregation can be characterized by a 'time minimisation principle'. To explain this further we introduce terminology borrowed appropriately from optimal control theory (see Whittle 1983). Consider a frightened animal that attempts to make a behavioural decision in a given initial context $C(0)$. Suppose that the animal can choose between several decisions which after execution lead to 'terminal' situations $C(1)$, $C(2), \ldots, C(n)$. Associated with every such situation is a mortality risk of the individual, respectively denoted by $K(1), \ldots, K(n)$. In addition, during behaviour leading from $C(0)$ to any one of the possible terminal $C(i)$ the animal is exposed to 'transit' risk. For simplicity we assume that the total transit risk $T(i)$ of the behaviour leading from $C(0)$ to $C(i)$ is proportional to the time it takes to attain $C(i)$. The time minimisation principle then says: whenever an animal faces choices $C(1), \ldots, C(n)$ for which the terminal risks $K(1), \ldots, K(n)$ are identical, then it should choose that behaviour for which $T(i)$ is smallest.

In Krause & Tegeder (1994) we tested this simple hypothesis on three-spined sticklebacks (*Gasterosteus aculeatus*), using an experimental design which presented a single 'test' fish with a choice of two single 'display' fish, each of which was encapsulated in a

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glass cylinder and positioned at different distances from the test fish. Thus the terminal risks $K(i)$ associated with joining one or the other display fish were the same, but respective transit risks $T(i)$ generally differed from one another. Our results gave evidence for the above hypothesis in that fish minimized the time needed to approach a conspecific by taking into account swimming times and body-turning speeds.

The experimental condition which allowed a test fish only choices between single (isolated) display animals seems adequate for modelling the initial stages of natural aggregation processes, during which single individuals approach one another. Once small subgroups have formed (Hamilton 1971) however, individuals face more complex behavioural choices $C(i)$ for which the risks $K(i)$ may not all be identical because group membership size is generally positively correlated with a reduction of mortality risk (Neill & Cullen 1974; Landeau & Terborgh 1986; Krause & Godin 1995). In this way the aggregation process becomes density dependent in the literal sense that the local density of conspecifics directly affects an animal's behaviour. The choice of target individuals now becomes one of groups. Okubo (1986) and Grünbaum & Okubo (1994) have given a more general definition of density dependence which requires that the behaviour of one animal depends on that of some or all of its conspecifics. Although Okubo (1986) has pointed out that density dependent dynamics should be widespread in general grouping behaviour, it has received comparatively little attention in the literature, particularly so in the case of group living vertebrates.

Previous investigations of group choice have measured the time a test animal spends on average with one group as opposed to another over periods of 10–30 min (Keenleyside 1955; Hager & Helfman 1991; Ranta *et al.* 1992; Krause 1993). For fright-stimulated aggregation such long observation periods are hardly relevant because a group forms within seconds of the fright stimulus. Instead it is the first group-choice of a frightened animal that strongly affects the strategic success of its behaviour during periods of imminent predation risk. Fast decision-making and short reaction-times are also obvious selective advantages. In particular, to the experimentalist this situation offers a possibility to investigate the nature and eventual limits of both perception and decision making in sticklebacks.

In the experiments reported here, a single test-fish is frightened with a light stimulus and must then choose to approach one of two display shoals which differ in their membership sizes and in the distances to the test fish. If the two distances (and therefore the associated transit risks) are equal, one predicts that the test fish approaches the larger shoal, just as in the above-cited long-duration studies of group choice the test fish have been observed to spend more time with a larger shoal. In both cases, fish presumably aim to benefit from the greater safety in numbers that larger shoals provide. If now the distance from the test fish to the smaller display shoal is decreased, it may happen that the reduction of transit risk outweighs the larger terminal risk and that the test fish therefore prefers to turn to the smaller display shoal. One can then find a critical distance, characterized by equal total risks associated with either group choice.

One aim of this study has been to predict the above critical distances and test the predictions against the empirical data. We therefore develop and compare several elementary models which differ in the way transit and terminal risks are combined to give total risk; for example, we distinguish an 'additive risk' model from a 'multiplicative' one. Our models follow the so-called 'partial preferences' paradigm of McNamara & Houston (1987), that is, a test fish is assumed to make total risk assessments subject to an additive noise term. This allows us to study the experimental data in more detail and we find evidence that sticklebacks systematically under-estimate the membership size of large shoals. This points to a fundamental perceptual limit for sticklebacks which in human psychophysics is known as 'numerosity' (see for example Atkinson *et al.* 1976).

2. MATERIALS AND METHODS

Several hundred three-spined sticklebacks (*Gasterosteus aculeatus*, $\bar{X} \pm$ s.d. standard length = 4 ± 0.3 cm) were collected from Morice Lake (near Sackville, Canada) and kept for about one month in flow-through holding tanks ($1.55 \times 0.38 \times 0.37$ m, L × W × H, 28 cm water depth) at 12.5 °C before they were used in experiments. The fish were fed on freeze-dried chironomids and euphausiids to satiation once a day. Sticklebacks were chosen for this study because they swim slowly enough that aggregation behaviour can be observed and analysed with video equipment.

The experimental tank ($1 \times 1 \times 0.3$ m, L × W × H, water depth 5 cm) was made of Plexiglas and placed inside a blind ($1.21.1 \times 1.2$ m, L × W × H), whose sides were covered with black plastic sheets to minimize external disturbance. The bottom of the test tank was made of white Plexiglas so that the fish bodies were clearly visible against the background. The test tank was lit by a 60 W bulb suspended about 1.5 m above its centre. A light regime of 12:12 h light:dark was used throughout the experiments. Video recordings were taken with a camera mounted 1.5 m overhead and centred on the tank. The behaviour of the fish was observed on a monitor.

Aggregation behaviour was elicited by switching off the light bulb above the tank for about 0.5 s and then switching it on again. This simulated the shadow of a predator but did not provide directional cues which could have biased the behaviour of the test fish. The shallow water depth of 5 cm kept the aggregation process two-dimensional. This did not impose artificial conditions as sticklebacks can often be found in shallow water in nature.

3. EXPERIMENTAL PROTOCOL

We employed the following method for controlling the positions of single fish or small fish-groups during experimental trials. One or more fish were put into glass cylinders which were made of clear plastic, contained no floor and measured 5.5 cm in diameter (for up to three fish) and 7 cm in diameter for shoals of five and eight display fish. Swift selective release was possible when lifting the cylinder by about 4 cm using a remote-pulling mechanism. The cylinder rim thus remained submerged in water, causing minimal disturbances of the water surface. This release process did not noticeably frighten the test fish.

We arranged three glass cylinders in the form of a triangle (see figure 1 *e*). The angle at the vertex of this triangle occupied by *T*, the test fish, measured 65°. The other two glass cylinders contained the 'display' shoals which stayed fixed and encapsulated throughout any one experimental trial. The test fish was released only when its body axis bisected the angle at the vertex of *T*. In this manner we strove to avoid any bias towards one of the shoals due to the initial body-orientation of the test fish. Concurrent with its release, the test fish was frightened with an off–on light stimulus.

Test fish were presented with a choice between the display shoals whose sizes in a given trial either equalled: (A) 1 conspecific versus 2 conspecifics; (B) 1 versus 3; (C) 1 versus 5; or (D) 1 versus 8. The larger shoal was always presented at a distance of 25 cm from the test fish whereas the smaller shoal was placed at variable distances ranging between 8 cm and 25 cm as follows: (A) 8 cm, 12, 17, 21, 25; (B) 8 cm, 10, 11, 12.5, 19, 25; (C) 10 cm, 11, 12, 15, 18, 25; (D) 8 cm, 10, 15, 20, 25 (see figure 1 *e*).

Ten replicates were carried out for each combination of shoal sizes and swimming distances (requiring 220 trials altogether). Each test fish was used only once, For each trial, display fish were chosen randomly from a separate tank of about 30 fish. About 10–15 min before the beginning of a trial, the display fish were introduced into the glass cylinders and habituated to the light stimuli. The test fish were given approximately 30–60 s between their introduction into the glass cylinder and the fright stimulus. This time period was chosen for three reasons: (i) the test fish needs a short time to get used to a new environment, for otherwise it is very likely to panic on release; (ii) the display fish have to move a few times to make sure that they are noticed by the test fish; and (iii) the test fish has to be oriented at release in the prescribed way, a condition that cannot be directly controlled. A trial was defined as successful and completed whenever the test fish came to within 4 cm (one body length) of either shoal. This is within typical inter-individual distances observed in fish shoals (Pitcher 1986). The experiment followed a formal 'forced choice' protocol in that trials were discarded whenever the test fish after release did not approach one of the display shoals (but darted to the tank wall, for instance).

4. RESULTS AND ANALYSIS

All data are displayed in figure 1 *a–d* (black dots), where figures have been labelled according to the various display-shoal combinations which we studied. Qualitatively, these data conform to what one expects intuitively. In particular, the choice behaviour of fish under fright stimulated aggregation is 'density dependent', that is dependent on display (or target) shoal sizes. For example, when the two display shoals were equidistant from the test fish so that transit risks were equal, then the test fish tended to prefer the larger shoal for approach. The magnitude of this preference increased with increasing size difference between display shoals. This is consistent with the general observation that a larger display-shoal offers greater protection from potential predators than a smaller one, see Pitcher & Parrish (1993) for a review.

In accordance with the notation set out in the introduction, let $C(s)$ and $C(l)$ respectively refer to the situations that the test fish either joins the smaller display shoal (s) or the larger one (l). Associated with these possible decisions are total risk of $R(\mathrm{s})$ and $R(\mathrm{l})$. Here $R(\mathrm{l})$ may depend on the distance d_l between the larger display shoal and the test fish as well as on the size n_l of that shoal. Similarly, $R(\mathrm{s})$ depends on d_s and n_s (which here always equals 1). With n_l, n_s and d_l given, the condition

$$R(d_{\mathrm{crit}}, n_\mathrm{s}) = R(d_\mathrm{l}, n_\mathrm{l}) \tag{1}$$

defines a critical distance d_{crit} between the smaller display shoal and the test fish at which the total risks associated with the two possible decisions are equal. The notation used in equation (1) makes it evident that the test animal is assumed to assess the total risks of $C(\mathrm{s})$ and $C(\mathrm{l})$ using the same routine (or function) R in both cases. As one can expect the total risk $R(\mathrm{s})$ to increase with increasing distance d_s, it follows that for all $d_\mathrm{s} > d_{\mathrm{crit}}$ the test fish will experience a smaller total risk turning to the larger group. Similarly, the condition $d < d_{\mathrm{crit}}$ should always induce the test fish to turn to the smaller shoal. At $d_\mathrm{s} = d_{\mathrm{crit}}$, the risks are equal so that on average one half of all test fish should decide for $C(\mathrm{s})$ and the other half for $C(\mathrm{l})$.

It is apparent from figure 1 that the recorded data are hardly compatible with an ideal, step-function behavioural response of the test fish, based on a critical ('switch point') distance d_{crit}. Instead, one observes a gradual decrease in the proportion p of test animals turning to the smaller display shoal as the distance d_s increases.

Two conditions must be met for an ideal step-function response: (i) all test animals use the same function R to evaluate risk; and (ii) all test animals determine the parameters d_s, d_l, n_s, n_l in the same precise way under identical circumstances. Because this lacks realism, one customarily relaxes either condition (i) or (ii) or both by allowing that perceptual measurements of parameters or the functional form of R are subject to noise ('partial preferences', see for example McNamara & Houston 1987). In particular, we assume that all noise can be summarized in one random variable $\in$ and that the total risk perceived subjectively by an animal equals $R+\in$. Here the random variable $\in$ is taken to follow a double exponential distribution with parameter β and is independent of the other noise variables which affect other test fish. The choice of this noise distribution can be motivated well by more general considerations (see, for example, Luce 1959; Yellot 1977; McNamara &

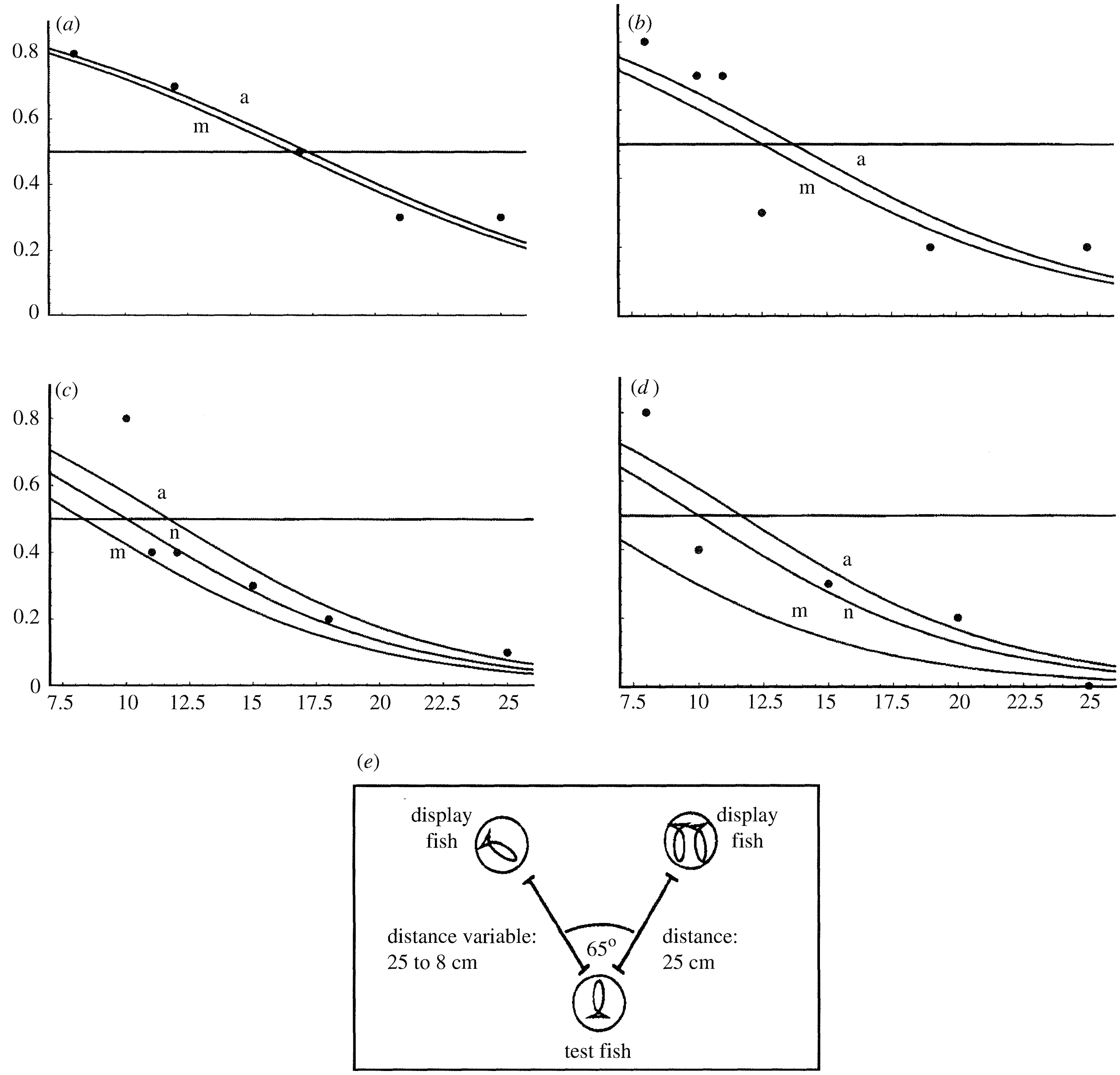

Figure 1. (*a*–*d*). The proportion of test fish that turned towards the smaller display shoal is plotted against the distance d_s between smaller shoal and test fish. The dots give the empirical data based on the response of ten different test fish. (*a*) $n_l = 2$, (*b*) $n_l = 3$, (*c*) $n_l = 5$, (*d*) $n_l = 8$. The multiplicative (m) and the additive (a) models of total risk were fitted to the empirical data points of all four experiments, using a generalised linear regression analysis within the framework of 'partial preferences' (McNamara & Houston (1987)). In experiments (*c*) and (*d*), a multiplicative model with 'numerosity' was also fitted (n). (*e*) Illustration of the experimental design.

Houston 1987). The probability that the perceived total risk for $C(\mathrm{l})$ exceeds that of $C(\mathrm{s})$, that is $R(\mathrm{s}) + \epsilon_s < R(\mathrm{l}) + \epsilon_l$, and that hence the test animal turns to (s) is then easily found to equal:

$$p := \mathbb{P}(R(\mathrm{s}) + \epsilon_s < R(\mathrm{l}) + \epsilon_l)$$

$$= \frac{\exp(\beta R \mathrm{l}))}{\exp[(\beta R(\mathrm{l})) + \exp(\beta R(\mathrm{s}))]}$$

$$= \frac{1}{1 + \exp[\beta(R(\mathrm{s}) - R(\mathrm{l}))]}. \qquad (2)$$

One may interpret the probability (2) as expressing a bias away from the case of an equiprobable (blind) choice between $C(\mathrm{s})$ and $C(\mathrm{l})$. The bias is due to the total risks $R(\mathrm{s})$ and $R(\mathrm{l})$ which enter the formula (2) multiplied by the common factor β. Indeed, the functional form of this bias is well known in elementary statistical theory as an 'induced exponential family' (see, for example, Lindgren (1976), see also the biomedical literature where expressions of the form (2) appear in bioassay, that is probability of response, studies of drug efficiency). Formally, one obtains this functional bias by multiplying the unbiased probabilities (here both equalling 1/2) by the exponential factors $\exp(\beta R(\mathrm{s}))$ and $\exp(\beta R(\mathrm{l}))$, respectively, and then dividing by the sum of these two resulting expressions. The unbiased probabilities of 1/2 cancel in the process, thus given the expression (2).

The parameter β can be interpreted as measuring the level of perceptual discrimination (here an antonym of 'noisiness') of the subjective total risks assessments. For example, if the discrimination β is zero, then (2) equals 1/2, yielding the case of blind choice. As the discrimination β increases to infinity, the probability (2) approaches either 1 or 0 depending on whether $R(s) < R(l)$ or the reverse inequality holds. In the limit $\beta = \infty$, one therefore recovers the ideal step function. The model (2) is thus a strict generalization of the simplest model (1). The value of β will in general depend on states internal to the test animal (alertness, degree of fright), on individual discrimination-ability or on external factors (e.g. visibility).

Formula (2) depends only on the difference of total risks and not on their absolute values. In particular, when the two risks $R(\mathrm{l})$ and $R(\mathrm{s})$ are equal, then (2) equals 1/2. Given d_1, n_1 and $n_s = 1$, we can then use (1) to determine a theoretical critical value of d_s at which on average one half of all tested animals turn to (s) and the other half to (l). It is also important to notice that the value of the theoretical critical distance d_{crit} is independent of the value assumed by the discrimination parameter β.

The model of expression (2) easily lends itself to statistical analysis. A log-odds transformation of expression (2) gives:

$$\xi := \log\left[p/(1-p)\right] = \beta(R(l) - R(s)),$$

thus allowing (weighted) linear regression analyses under the usual assumptions. As long as the probabilities p generally do not lie outside the interval [0.2, 0.8], weighting will not be necessary (see Cox 1970).

To complete the model (2) for data analysis, we have to specify the function $R(d, n)$. We shall distinguish two functional forms. According to a 'multiplicative risk' model, the total risk $R(i)$ associated with attaining the 'terminal' state $C(i)$ is of the form:

$$R(i) = T(i)\,K(i). \tag{3}$$

For comparison, we also consider an 'additive risk' model according to which the total risk $R(i)$ is taken to equal:

$$R(i) = (T(i) + \alpha K(i)). \tag{4}$$

Here the constant α is a parameter which measures the relative contributions of terminal risk $K(i)$ and transit risk $T(i)$ to the total risk. An additive risk hypothesis is classical in optimal control theory. It has a further conceptual advantage which in Tegeder & Krause (1995) we referred to as the 'sum rule' and which ties in well with the expression (2). Indeed, if we bias the blind-choice probabilities (here equalling both 1/2) first according to the transit risk contributions $R^{(1)}(i) = T(i)$ and then with the terminal risks $R^{(2)}(i) = \alpha K(i)$, we get the same result which is obtained by a direct bias of the blind-choice probabilities using the total risks (4). Thus every aspect contributing to total risk can be thought of as an independent bias factor whose contribution to total risk can easily be assessed by a generalized linear-regression analysis.

Both models formalize the intuitive property that total risk increases with increasing transit or terminal risks. We note, however, that an assessment of the relative contributions of transit and terminal risks cannot of course be made for the multiplicative risk model.

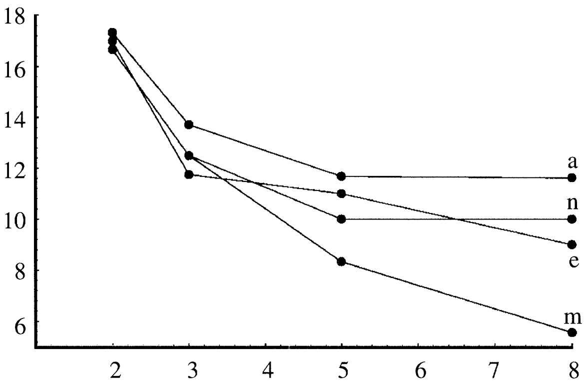

Figure 2. Critical (or switch-point) distances are plotted against n_1. (e): Empirically obtained critical distances (using piecewise linear interpolation of the data in figure 1), (a), (m), (n): predicted critical distances for the additive, multiplicative and the numerosity models. For clarity, points belonging to the same model are graphically linked.

Here we take the transit risk to be proportional to the distance between test fish and display shoal. (Effects of body turning times, which were found to be a significant factor in the context of time minimization (Krause & Tegeder 1994), were controlled experimentally by imposing a condition on the test animal's body orientation at release, see §3.) Without loss of generality we can take the constant of proportionality to be 1, that is $T(\mathrm{s}) = d_s$ etc. The terminal risk $K(\mathrm{s})$ we assume to be proportional to $1/(n_s+1)$, that is the inverse of the shoal size after it had been joined by the test animal. The constant of proportionality may again be taken to equal 1, for any other constant can be absorbed into α (for model (4)) or into β (for model (3)). Notice that with this choice of risk functions the multiplicative model becomes particularly intuitive; for R proportional to $d/(n+1)$ tallies well with the idea that total risk becomes twice as large when doubling the distance and about half as large when the target group size doubles. If total risk does not scale in this manner, then an additive risk model may be more appropriate. Because the additive model can handle varying relative contributions, it requires two parameters (α and β) whereas the multiplicative model involves only one meaningful parameter, that is β.

In the multiplicative model, the theoretical switch-point distances d_{crit} are given by

$$d_{\mathrm{crit}} = [d_1(n_s+1)]/(n_1+1). \tag{5}$$

As mentioned above, these predictions do not depend on β and are therefore independent of any estimated parameter. See figure 2, where critical distance is plotted against the size n_1 of the larger display shoal. All points belonging to the multiplicative model have been joined and labelled 'm'. We also obtained empirical switch-points from figure 1 by piecewise linear interpolation and labelled these switch-points by the letter 'e' in figure 2. While for (A) and (B) there is surprisingly good agreement between empirical and theoretical critical distances, which lie within less than 1 cm of one another, one observes a considerable discrepancy between data and predictions for the cases (C) and (D).

A similar observation is made when performing a regression analysis for the multiplicative model, that is when we regress ξ on:

$$c+\beta\{[25/(n_1+1)]-d_s/2\}.$$

The inclusion of a constant c in this procedure is more than a matter of formality in the regression routine. If this constant is found to be statistically significant, then one must conclude that the experimental data can be significantly described by the model:

$$p=\frac{\exp(c+\beta R(1))}{\exp(\beta R(s))+\exp(c+\beta R(1))},$$

as opposed to expression (2). In particular, whenever $R(1) = R(s)$, the probability p of turning to the smaller display-shoal must then equal $e^c/(1+e^c) \neq 1/2$ as opposed to 1/2. If we now recall the important contribution of body-turning times to total transit risk (Krause & Tegeder 1994), then a statistically significant, non-zero constant c would suggest that the release condition on the body axis of a test fish (see §3) was not well controlled.

For the four experimental trials, a regression analysis gives the following results (A) $\beta = 0.288$ ($T = 8.038$, $p = 0.004$); $c = 0.098$ ($T = 0.899$, n.s.); (B) $\beta = 0.336$ ($T = 3.427$, $p = 0.027$); $c = 0.204$ ($T = 0.6765$, n.s.); (C) $\beta = 0.372$ ($T = 3.5528$, $p = 0.024$); $c = 0.628$ ($T = 1.400$, n.s.); (D) $\beta = 0.388$ ($T = 2.447, p = 0.1341$); $c = 1.178$ ($T = 1.652$, n.s.). In all four cases, the parameter c is thus statistically insignificant (we have written n.s. whenever the p-value exceeded 0.2). We first ignore it and thus obtain the theoretical curves labelled 'm' in figure 1. These curves are very close to those which would have been obtained by a least squares fitting procedure as opposed to the generalised linear-regression. In case (A), the match is excellent. It is less good in case (B) and becomes progressively worse in cases (C) and (D), just as for the analysis of switch-points.

In case (D), the discrimination β is on the borderline of statistical significance. This is partly so because only four sampled distances could be used for the regression analysis. Sampling at the distance $d_s = 25$ gave a zero probability of turning to (s); and zero probabilities cannot be handled by regression (nor by maximum likelihood estimation). If only one of the ten test fish studied in (D) had turned to (s), the parameter β would become significant at a value very close to the one given above.

If we do not ignore the constant c despite its lack of statistical significance and thus allow for the possibility that the experimental release condition of the test fish was only imperfectly observed, we first note that the estimated value of c is positive in all four cases studied and has the smallest value in (A). Because in the risk neutral case p equals $e^c/(1+e^c)$, this means that release was systematically biased towards choosing the single display fish. The predicted curves for the multiplicative model with risk-neutral bias c included are labelled 'a' in figure 1. There is a corresponding curve for the predicted switch-points in figure 2. Inclusion of c gives much better agreement between data and theory in cases (C) and (D), at the price of assuming strongly increasing levels of systematic risk-neutral bias from $c = 0.098$ to $c = 1.178$. As the experimental protocol did not change between trials, such systematic bias seems very implausible to us. This and the statistical insignificance of the parameter c leads us to conclude that the multiplicative model is unsatisfactory.

Let us now examine the risk additive model. The predicted switch-points equal:

$$d_{\mathrm{crit}}=d_1+\alpha[1/(n_1+1)-1/(n_s+1)], \tag{6}$$

where the parameter α must be estimated from the data. This is unlike the multiplicative model where no estimation entered into the prediction of switch-points. We regress ξ on:

$$\beta\{(25-d_s)+\alpha[1/(n_1+1)-1/2]\}. \tag{7}$$

The following results are obtained: (A) $\beta = 0.144$ ($T = 8.03$, $p = 0.004$); $\alpha\beta = 6.63$ ($T = 5.93$, $p = 0.009$), (B) $\beta = 0.168$ ($T = 3.427$, $p = 0.027$); $\alpha\beta = 7.589$ ($T = 3.154$, $p = 0.0343$), (C) $\beta = 0.186$ ($T = 3.553$, $p = 0.024$); $\alpha\beta = 7.433$ ($T = 4.256$, $p = 0.01$), (D) $\beta = 0.194$ ($T = 2.447$, $p = 0.134$); $\alpha\beta = 6.673$ ($T = 2.589$, $p = 0.122$). The corresponding theoretical curves of probability p of turning to (s) and the plot of switch-points are shown in figure 1 and figure 2 respectively, both labelled 'a'. It is not surprising that the theoretical curves for the additive model coincide with those obtained for the multiplicative model with the risk-neutral bias c included. For in both cases, we regress ξ on a model of the form $c_1+c_2 d_s$ and obtain the same values for c_1 and c_2. We then plot a theoretical curve $p = 1/(1+\exp(-c_1-c_2 d_s))$ of p versus d_s. However, the coefficients c_1 ad c_2 depend variously on the parameters and functional form of the two models. The regression therefore may lead to statistical significance in one model and not in the other. The above figures show that all parameters of the additive model are statistically significant, except for the borderline case in (D), which is due to sparse sampling.

The observed fit of data and theory is good. It is excellent for (A) and fair for (D). We note, however, that the predicted switch-points systematically exceed the empirical points by a margin of about 2.5 cm, that is about half a body length of a typical test or display fish. Asymmetric sampling may be one reason for this systematic difference. Consider figure 1(*b*) for example. Although the theoretical curve is properly sigmoidal as expected, the empirical data seem to indicate a stronger fall-off around the switch-point than predicted. The theoretical curve does not render this well enough because of the two data points at $d_s = 18$ cm and $d_s = 25$ cm for which the measured probabilities p are surprisingly high (20 %). There are no data points at small values of d_s which could have balanced those obtained for the large values of d_s, for in practice distances below 8 cm made it difficult to observe unambiguously the group choice a test fish had made, bearing in mind that the average size of test and display animals was 4 cm. Analogous comments hold for cases (C) and (D).

The estimated levels of discrimination β, pooled over all test animals, are of the same order of magnitude in the four cases studied. The parameter α has the following estimated values: (A) 46.04, (B) 45.17, (C) 39.96 and (D) 34.40. So discrimination is slightly up (or 'noise' down) and the relative contribution α of terminal to total risk is substantially down in the cases (C) and (D) as compared to (A) and (B). Given that the experiments were performed over several weeks, the fairly small spread of parameter values found for the four studied cases may seem remarkable. In particular, the slight increase of β (that is stronger sigmoidality of the theoretical curves) from (A) to (D) may reflect that the animal's decisions become sharper (that is closer to a step function) with increasing difference of display shoal sizes. However, this effect seems very small, especially as the value of β does not affect the position of switch-points. There is therefore no pressing need for a more careful interpretation or analysis of the subtle β trend.

The trend of the parameter α is less intuitive and rather perplexing. Instead of a decrease of α from (A) to (D), one expects that the contribution of terminal to total risk remains roughly constant with increasing sizes n_1. Despite the fairly good agreement between prediction and data for the additive model, the inexplicable trend of α as well as the apparently systematic over-estimation of switch-points prevents us from accepting this model as satisfactory. However, the additive models seems to match the data better than the multiplicative one.

5. EVIDENCE FOR A 'NUMEROSITY' EFFECT IN FISH

The most remarkable feature of the empirical switch-points plotted in figure 2 is the strong kink in their trend at $n_1 = 3$. Instead of a decrease in the values of d_{crit} similar to that of the curve 'm', the decrease from (B) to (D) is much less than that from (A) to (B).

In the previous section, we proposed and discussed two ways of modelling this empirical phenomenon. In the multiplicative risk model, the observed kink could be explained only by invoking the statistically insignificant parameter c, that is by assuming that test fish showed a systematic and unexplained decision bias despite the experimental condition on an animal's body orientation at release. In the risk additive model, the kink is mirrored by the unexplained trend of the estimated contribution α of terminal to total risk.

For more convincing explanations of this phenomenon, we begin by recalling that the models of the previous section allowed for some variation or noise (within the 'partial preferences' paradigm of McNamara & Houston 1987) affecting the perception and brain processes of test animals; indeed, the subjective risk assessment $R+\epsilon$ of a test fish differed from the hypothesised objective risk function R by an additive 'noise' term ϵ. As is implicit in the terminology of 'noise', the dependence of R on the display shoal sizes and distances variables is hypothesised to be fundamentally correct, that is 'noise' only causes the scatter of subjective risk assessments around an hypothesised objective value. The scatter becomes less with increasing values of the discrimination parameter β. Important perceptual limits of test fish, however, are likely to give rise to empirical data which depart systematically from model predictions and which cannot be accounted for by fluctuation or scatter noise.

Let us therefore re-examine how distance or display shoal size enter the hypothesised risk formula R. Little is known about the mechanism and accuracy with which fish perceive distance. Li *et al.* (1985) suggested that fish use parallax while moving through the water. Stationary fish (Janssen 1982), however, would have to use binocular cues such as convergence or binocular disparity (Wetterer 1989). Walton *et al.* (1994) reported a binocular field of about 40 degrees for the sunfish (*Lepomis* spp). If we assume a similarly sized angle for sticklebacks, then a test fish in our experiment cannot have the two display shoals simultaneously in its binocular field before its release. Where distance assessments between conspecifics are concerned, fish might also rely on a mechanism which combines learnt allometric morphological relations (such as the ratio of eye/body size) and the visual angle that a conspecific subtends on the retina of a fish. However, we have been unable to find evidence or suggestions in the literature for a fundamental perceptual limit of distance assessments in teleost fish.

If we assume that during experiments (A)–(D) no perceptual limit constrains a test animal's shoal size assessments, then this means that the test fish must be able to judge the quantity n_1 for values up to 8 with sufficient accuracy and within comparatively short time spans (30–60 s). For comparison, we now recall that human observers are unable to report accurately a number of objects shown in a flash (or projected as after-images) whenever their number exceeds 4, a well-studied effect known as numerosity in the human psychophysical literature (Jevons 1871; Miller 1956; Atkinson *et al.* 1976). For example, the percentage of incorrect counting when eight objects are briefly presented to a human observer is known to exceed 50%. As the kink in the 'e' curve of figure 2 occurs between the data points for $n_1 = 3$ and $n_1 = 5$, let us examine the hypothesis that the tested fish also suffer a numerosity effect. The good agreement between data and theory in the cases (A) and (B), which holds for all models so far studied, suggests that the test fish can accurately assess or distinguish between shoal sizes n with values between 1 and 3. According to one very simple model of numerosity in sticklebacks, one then assumes that a test fish cannot make any accurate size distinction between shoals with $n > 3$ and that such sizes formally enter the function R through the default value $n_1 = 4$. So there are only four distinct size discriminations: 1, 2, 3 and 'large'.

We first study the impact of numerosity on the multiplicative risk model. In the regression analysis, numerosity leads to the modification

$$c+\beta[25/\min\{n_1, 4\}+1)-d_s/2)].$$

For (A) and (B), there are of course no differences. For (C) and (D) we naturally obtain the same estimate and statistical information on β as for the multiplicative model without numerosity. The constant c remains statistically insignificant with a changed estimated value. We ignore it and plot the resulting predicted curve with label '*n*' in figure 1 (*c*)–(*d*) and figure 2. The predictions of the multiplicative model with numerosity are remarkably close to the empirical data. Predicted and empirical switch-points differ by no more than 1 cm, and discrepancies are not one-sided as they were for the risk additive model.

More formally, we can appeal to the usual hypothesis testing philosophy to ask whether the data depart from the null hypothesis H_0 (unmodified multiplicative model) in the direction of the alternative hypothesis H_A (multiplicative model with numerosity) and whether this departure is of such magnitude that can reasonably be accounted as evidence against H_0. We sketch the details in the Appendix and report here the results. In the case of experiment (C), H_A is to be rejected at a level of 5%. At a level of 15%, H_0 is to be rejected. In the case of experiment (D), the hypothesis H_0 is to be rejected at levels well below 5%. These figures formally support the visual assessment that the distinction between modified and unmodified multiplicative models in (C) is a borderline case; see the closeness of the theoretical curves in figure 1 (*c*). For (D), however, the evidence is overwhelming in favour of rejecting H_0 and of accepting the multiplicative model with numerosity.

We next study the impact of numerosity on the risk-additive model of the previous section. It is not surprising that in this case the inclusion of a numerosity effect corrects the unexplained trend of the estimated contribution α of terminal to total risk so that the predictions of the modified additive model are those of the original model, but now with α roughly constant in the model with numerosity. For clarity, write α_0 and α_A for the values of parameter α in the original model and modified models, respectively. It is then clear from the regression routine (7) that we have the following relation between estimated parameter values:

$$\alpha_0[1/(n_1+1)-1/2] = \alpha_A[1/(\min\{n_1, 4\}+1)-1/2].$$

In cases (A) and (B), we have of course $\alpha_0 = \alpha_A$, whereas for (C) and (D), the two estimated values differ. For example, in the previous section we obtained $\alpha_0 = 39.96$ for (C). The above relation then shows that $\alpha_A = (1/6-1/2) \times 39.96 \times (1/5-1/2)^{-1} = 44.4$. Similarly, in (D) we obtain $\alpha_A = 44.6$. So the estimated values of the contributions α_A of terminal to total risk in the additive model with numerosity now vary between 46.04 and 44.4 as opposed to a range of 46.04 and 34.4 in the unmodified case. Inclusion of numerosity therefore rendered the parameter α roughly constant without affecting the model predictions.

The empirical data reported in this paper provide significant evidence for a numerosity effect in the aggregation behaviour of frightened sticklebacks. The inclusion of numerosity reinstates the multiplicative risk model as a good predictor. In the additive risk model, numerosity corrects an unexplained parameter trend without affecting the model predictions. Figure 2 indicates that the switch-point predictions of the modified multiplicative-model ('*n*') are better than those of the (modified) additive model ('*a*'). In particular, the former show no systematic over-estimation. We therefore single out the multiplicative-risk model with numerosity as the best of all models examined.

6. FURTHER DISCUSSION

One might argue that the restricted number of discriminated shoal sizes (1, 2, 3, 'large') in the preceding model is not caused by a fundamental perceptual limit but that it is instead the result of an adaptation; for it is conceivable that the individual predation risk in a group of homotypic conspecifics decreases sharply as the number of group mates increases from 1 to 4 and then decreases comparatively slowly, maybe even stays constant henceforth. Indeed, conceivably a large shoal might be more visible and so attract more attention and predator attacks, to the point that the effect of safety-in-numbers in a large shoal is reduced or even outweighed by an increased attack rate on the larger shoal. It then carries an adaptive value for a frightened stickleback not to base its strategic decisions on veridical shoal size assessments and thus to over-estimate the protection that group of conspecifics can provide. This argument is certainly consistent with the assumptions of our model. Yet we preferred the numerosity interpretation to this argument, because we do not understand why the visibility of a shoal and so presumably the rate of attacks on it should increase sharply as the shoal membership exceeds three. Instead, shoal visibility is likely to vary continuously with shoal size. A perceptual limit may, however, manifest itself rather abruptly, as for example in human numerosity. Further experimental work would be useful to probe the nature of the observed kink in curve 'e' of figure 2.

Although the empirical critical distance for experiment (C) exceeds that for (D) by about 2 cm, the two values predicted by the multiplicative model with numerosity are equal, see curves 'e' and 'n' in figure 2. This discrepancy between theory and data reflects the undue simplicity of the numerosity model which we used in the preceding section. Fish are unlikely to make a common default size judgement for shoals with more than three members. Other cues such as shoal diameter may help them distinguish large from small shoals. A more realistic model of numerosity in sticklebacks would therefore only assume consistent under-estimation of shoal sizes whenever the membership size exceeds three. Such a model would obviously give even better quantitative predictions of switch-points in experiments (C) and (D).

Our experimental results also show clearly that frightened sticklebacks do not always approach their nearest neighbours (Hamilton 1971) during an aggregation process. Turning times (Krause & Tegeder 1994), relative shoal sizes and swimming distances all systematically influence the behaviour of fish which aggregate in response to perceived imminent predation. The complexity of this behaviour is astonishing and evidence of the strong selection pressures which shaped it.

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APPENDIX

Here we sketch the elementary hypothesis testing argument employed in the section on numerosity in fish. Let H_0 and H_A refer to the hypotheses based on the original multiplicative model and the multiplicative model with numerosity, respectively. We assume that all experimental trials are independent of one another. In particular, the number k_i of turns to (s) at distance $d_s = d_i$ is binomially distributed with probabilities

$$p_0(i) = 1/1 + \exp\{\beta[d_i/2 - [25/(n_1+1)]\}$$

$$p_A(i) = 1/1 + \exp\{\beta[d_i/2 - [25/(\min\{n_1, 4\}+1)]\},$$

under H_0 and H_A, respectively. Here n_1 denotes the size of the larger display shoal, as usual. Write $q_0(i) = 1 - p_0(i)$ etc. The log-likelihood ratio for data $k := \{(k_i);\ i = 1, \ldots, l\}$ and N trials performed for every distance d_i then equals:

$$\log \frac{\mathbb{P}_A(k)}{\mathbb{P}_0(k)} = \sum_{i=1}^{l} k_i \log\left(\frac{p_A(i)\, q_0(i)}{p_0(i)\, q_A(i)}\right) + N \sum_{i=1}^{l} \log\left(\frac{q_0(i)}{q_A(i)}\right). \tag{8}$$

We can therefore consider Neyman–Pearson test regions of the form $\{X \geqslant x\}$ where

$$X(k) =: \sum_{i=1}^{l} k_i \phi_i$$

equals the first term on the right-hand side of (8). In fact, we obtain explicitly:

$$\phi_i = \log\left(\frac{p_A(i)\, q_0(i)}{p_0(i)\, q_A(i)}\right) = \beta\left(\frac{25}{\min\{n_1, 4\}+1} - \frac{25}{n_1+1}\right),$$

which is independent of $i = 1, \ldots, l$. We therefore write $\phi_i \equiv \phi$. Note that $n_1 \geqslant 4$ implies $\phi \geqslant 0$. To complete the test set-up, it suffices to compute probabilities:

$$\mathbb{P}_0\left(\sum_{i=1}^{l} k_i \geqslant x/\phi\right).$$

Using the fact that the k_i are independently binomially distributed, one can well approximate the above probability by:

$$\exp\left(\inf_{\theta}\left\{N \sum_{i=1}^{l} \log\left[(e^{\theta}-1)\, p_0(i) + 1\right] - x(\theta/\phi)\right\}\right).$$

The optimal value θ can be routinely computed. Going through the calculations, one obtains that for (C) $\mathbb{P}(X \geqslant X_{obs})$ approximately equals 0.14; for (D) it equals approximately 0.008. All conclusions now follow.

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