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Balancing the response to predation—the effects of shoal size, predation risk and habituation on behaviour of juvenile perch

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Abstract Group size, predation risk and habituation are key drivers of behaviour and evolution in gregarious prey animals. However, the extent to which they interact in shaping behaviour is only partially understood. We analyzed their combined effects on boldness and vigilance behaviour in juvenile perch (*Perca fluviatilis*) by observing individuals in groups of one, two, three and five faced with four different levels of predation risk in a repeated measures design. The perch showed an asymptotic increase in boldness with increasing group size and the highest per capita vigilance in groups of two. With increasing predation risk, individuals reduced boldness and intensified vigilance. The interaction between group size and predation risk influenced vigilance but not boldness. In this context, individuals in groups of two elevated their vigilance compared to individuals in larger groups only when at higher risk of predation. Further, as only group size, they significantly reduced vigilance at the highest level of risk. With increasing habituation, solitary individuals became considerably bolder. Also, predation risk affected boldness only in the more habituated situation. Hence, repeated measures

may be essential to correctly interpret certain relationships in behaviour. Our results suggest that perch may adjust boldness behaviour to group size and predation risk independently. This is rather unexpected since in theory, natural selection would strongly favour an interactive adjustment. Finally, vigilance might be particularly effective in groups of two due to the intense monitoring and detailed response to changing levels of risk.

Keywords *Perca fluviatilis* · Group living · Boldness · Vigilance · Predator inspection · Novelty effect

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Introduction

Prey animals are constantly confronted with a starvation-predation conflict. They must gain resources to invest in growth and reproduction, but this often exposes them to a higher risk of being predated (Lima and Dill 1990; Ferrari et al. 2009). To cope with this challenge, group living has evolved in numerous species as one of various behavioural defense strategies. Joining together in groups has multiple benefits such as reducing predation risk (Krause and Ruxton 2002), but it also bears disadvantages such as increased competition (Hake and Ekman 1988; Grand and Dill 1999; Hoare et al. 2004).

In this trade-off, gregarious animals typically show a pronounced phenotypic plasticity in behaviour allowing an individual to adjust to changing conditions (Blumstein et al. 2001; Galef and Laland 2005; Magnhagen and Borcherding 2008). For instance, changing group sizes can considerably alter boldness-related behaviour in shoaling fish, especially when facing a predator (Seghers 1981; Allan and Pitcher 1986). Studies comparing the behaviour of different

fish species suggest this group-size effect to be species-specific (Magurran and Pitcher 1983; Saxby et al. 2010). Predation risk also affects behaviour in most cases by reducing boldness (Lehtiniemi 2005; Kim et al. 2009), although evidence exists for the reverse pattern (Brown et al. 2005; Martin et al. 2010; Saxby et al. 2010).

To react appropriately to predation risk, the prey animals must, however, first detect and assess the predator imposing the risk. Being vigilant facilitates this. Interrupting feeding is often used as indicator of vigilance since scanning for predators and feeding are assumed to be in some extent mutually exclusive (Treves 2000; Fernandez-Juricic et al. 2004; for fish feeding off the bottom, Krause and Godin 1996). The literature suggests the per capita vigilance to decrease with larger groups (Roberts 1996; Newey 2007) and to increase with predation risk (Walling et al. 2004; Benhaiem et al. 2008). After detection of a potential threat, the prey fish may demonstrate predator inspection. This phenomenon is suggested to help collect information about the predator (Häberli et al. 2005) and to serve as signal to the predator that it was detected (Godin and Davis 1995). Predator inspection is characterized by a hesitant approach of an individual towards a predator, a final pausing during which the predator is visually fixed, and a quick return to the shelter or group members (Pitcher 1992). Then, the gathered information about the predator can be shared with the group (Pitcher et al. 1986; Licht 1989). Hence, predator inspection may be seen as a function of vigilance in fish, with an expected group-size effect.

Animals can also strongly be affected by novel conditions or stimuli (Martin and Reale 2008; Magnhagen and Bunnefeld 2009). Previous studies proposed that an intermediate level of stress and habituation might be most appropriate to discover between-individual behavioural differences (e.g. Wilson et al. 1993). With increasing habituation, the heart rate stress response or behavioural responses might change non-proportionally between treatments or individuals (Magnhagen and Staffan 2005; Ellenberg et al. 2009; Rodriguez-Prieto et al. 2011). Thus, to avoid misleading results and to obtain a more complete picture of the investigated behavioural pattern, it may be essential to challenge individuals along a gradient of habituation.

As outlined, group size and predation risk are key drivers of behaviour and evolution in gregarious prey animals and are closely connected. However, previous studies mostly addressed only one of these factors (Frid 1997; Downes and Hoefer 2004). Also, since individuals were often only tested once, it cannot be ruled out that the described pattern was associated to a certain stage of habituation. Therefore, we analyzed how group size and predation risk shape behaviour in juvenile perch (Perca fluviatilis), how they interact and how their effects change with increasing habituation. We

observed boldness- and vigilance-related behaviour of individuals in groups of one, two, three and five faced with different levels of predation risk in a repeated measure design.

Methods

Study fish

Perch represent an ideal model organism for this study since they form large shoals during their juvenile stage (Probst et al. 2009) and show a pronounced phenotypic plasticity in behaviour (Snickars et al. 2004; Olsson et al. 2007; Hellström et al. 2011). In June 2010, 233 juvenile perch were caught in Lake Speldrop (Beeck et al. 2002; approximately 51° 46′ N, 6° 22′ E) using beach seines. They were immediately transported to the nearby zoological research station of the University of Cologne in Grietherbusch, Germany, where the study took place. Prior to the experiments, the perch were acclimated to indoor conditions in aquaria for 22 days (aquaria volume, 20 and 40 l; density, 0.75 fish per litre; food, pre-frozen chironomid larvae; filtering pump; 14:10 h day/night cycle; water temperature, 17.1–24.5 °C). For the last 6 days of acclimation, perch were daily relocated to a novel aquarium to habituate to being handled (Milinski 1997). The perch's body measurements during experiments were as follows: total length (TL)± SD=46±3 mm, weight=0.91±0.19 g.

Experimental design

Each individual participated in two experimental observations (referred to as run 1 and run 2), conducted on 2 consecutive days. On the evening prior to the first run, perch were randomly assigned to groups of one, two, three and five individuals. They were anesthetized with MS222, individually marked (tattoo) at the base of the caudal fin and stocked to the experimental aquaria. After overnight acclimation, the groups were tested in run 1 as described below in "Behavioural observations".

Then, the perch were regrouped shuffling the individuals to new groups of smaller, larger, or equal size and randomly restocked to the experimental aquaria. This protocol prevented non-random habituation to a specific aquarium, predator and social condition, e.g. to particular group members or group sizes, possibly biasing treatment effects. Online Supplementary Table S1 shows the replication for each of the possible combinations of group size change. After overnight acclimation, the newly created groups were tested again the following day in run 2 equivalently to the first run. On 12 consecutive days, the behaviour of 233 individuals was studied with replications for each factor level as shown in Table 1. The uneven distribution of group size replicates between the two

Table 1 Number of individuals included in the analysis for each possible combination of group size and predatory threat for run 1 and run 2

run 1		Group size				Sum		run 2		Group size				Sum	
		1	2	3	5	Ids	Groups			1	2	3	5	Ids	Groups
Predatory threat	0	13	10	9	30	62	27	Predatory threat	0	10	8	12	20	50	22
	1	4	4	9	10	27	11		1	6	10	6	10	32	15
	2	10	12	30	30	82	32		2	13	12	30	25	80	34
	3	3	14	15	30	62	21		3	2	10	15	35	62	19
Sum	Ids	30	40	63	100	233		Sum	Ids	31	40	63	90	224	
	Groups	30	20	21	20		91		Groups	31	20	21	18		90

The sums of individuals (Ids) and groups (≙truly independent replicates) across the respective other factor are given

runs resulted from the loss of data due to malfunctions of the computer program used for behavioural recording.

Test arena

The experimental aquaria (width × length × height, 44×82× 35 cm; filtering pump; water temperature, 21.7–24.3 °C) had three opaque sides and a transparent front side for the observations. The bottom was covered with gravel. A plastic net (mesh size 5 mm) partitioned the aquaria into two compartments of unequal size, and a removable opaque plate was placed next to it. The smaller compartment ($^1/_3$ of aquarium) held a predator (adult perch; TL=238±18 mm; n=18). The larger compartment ($^2/_3$ of aquarium) held the juvenile perch and comprised of two different areas of equal size. An open area was close to the predator's compartment and a shelter area containing green plastic strips to simulate vegetation furthest away from the predator's compartment (see also Magnhagen 2007).

Behavioural observations

After 16–21-h acclimation in the particular experimental aquaria, it was prepared for the behavioural observations. The opaque plate was carefully moved to the outer edge of the shelter area in which the group of tested juvenile perch was enclosed. Chironomid larvae (approximately 13 % of the individuals' body weight) were placed on the bottom in front of the net. After 15-min acclimation, the opaque plate was removed from the aquaria which made the predator visible to the perch for the first time and also made the food available. From this point on for 10 min, an observer recorded for every second one of four different activities for each juvenile perch individual using a computer program: (1) occurrence in the vegetated area, (2) occurrence in the open area, (3) feeding and (4) performing predator inspection. Thereby, feeding was defined as being oriented towards the bottom and attacking the food. Predator inspection was defined as being within 8-cm distance of the net (about two fish lengths) and being

orientated exactly towards the predator. To end observations, the opaque plate was relocated again next to the net.

Throughout each observation, the observer also noted changes in the predator's activity (not displacing or displacing itself) and distance (residing in the $^1/_3$ of its compartment distant, intermediate, or close to the net). This way, after observation, the predator's mean activity could be categorized as passive (not displaced once, level 0), partly active (displaced for less than 10 % of the time, level 1) or active (displaced for more than 10 % of the time, level 2) and its mean distance as distant, intermediate, or close (level 0, 1, or 2).

Data analysis—predatory threat and response variables

As indicator of predation risk, predatory threat was estimated by combining predator activity (level 0, 1, or 2) and distance (level 0, 1, or 2) of the respective observation: predatory threat = activity + distance. Since only five groups experienced the highest level of predatory threat, it was pooled with the second highest level. As a result, we obtained a factor with four levels ranging from 0 indicating the lowest to 3 indicating the highest predatory threat.

The data of the juvenile perch were used to calculate for each individual (1) the latency to move from the vegetated into the open area, (2) time spent in the open area, (3) duration of first feeding bout and (4) time inspecting predator (graphical overview in Online Supplementary Fig. S1). To combine these four non-independent behavioural measures, a principal component analysis (PCA) was performed including the data of all individuals of both runs (Bartlett's sphericity test X^2= 358, df=6, p<0.001; Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy=0.62; Budaev 2010; data of run 1 and run 2 have common principal components, Flury 1988).

The PCA generated two principal components (PC1 and PC2) with eigenvalues larger than 1 (Jackson 1993) explaining together 75 % of the variation (Table 2). Positive values on PC1 indicated a short latency until first leaving the vegetation, a long time spent in the open area, and a long duration of the first feeding bout, which corresponds to the general view of

Table 2 Factor loadings, eigenvalues and proportion of the total variance explained by the first three axes extracted from a PCA over the four different measures of behaviour

	PC1	PC2	PC3
Latency until first change	*−0.589*	−0.061	*0.516*
Time in open	*0.625*	−0.037	−0.198
Duration first feeding bout	*0.459*	*−0.470*	*0.724*
Time predator inspection	0.230	*0.880*	*0.414*
Proportion of total variance	0.497	0.253	0.164
Eigenvalue	1.991	1.010	0.654

All individuals of run 1 and run 2 are included. Interpretable factor loadings with absolute values >0.4 are in italic (Budaev 2010)

boldness (Magnhagen and Borcherding 2008). Therefore, PC1 was interpreted as indicator for boldness (PC1-boldness). Positive values on PC2 indicated a short duration of the first feeding bout and especially a long time spent with predator inspection, which corresponds to the general view of vigilance. Therefore, PC2 was interpreted as indicator for vigilance (PC2-vigilance). In PC1-boldness, the factor loading for the time spent with predator inspection was not sufficiently large for further interpretation (Budaev 2010). This suggests that predator inspection was not considerably correlated with the time individuals spent in the open area, which is important for the interpretation of PC2-vigilance in our study.

Data analysis—statistical models

Linear mixed effects (LME) models fitted by maximum likelihood were used to investigate the behaviour (R package nlme, Pinheiro et al. 2011). The extracted principal components PC1-boldness and PC2-vigilance were employed as response variables. All LME models included *groups* and *individuals nested within groups* as random effects to meet independency. Data analyses were performed with the software package R (R Development Core Team 2012, version 2.15.2).

First, we investigated the influence of group size and predatory threat on the behaviour of the individuals. For run 1 and run 2 separately, LME models were fitted incorporating the data of all individuals. We included group size and predatory threat and their interaction as fixed effects and PC1-boldness and PC2-vigilance as response variables, respectively.

Second, we analyzed changes of the effect of group size and predatory threat with repeated measure. Since each individual was tested in a different group in the two runs, *repeated measure within individuals* could not be included as random effect at the innermost level to avoid pseudoreplication. Instead, a combined dataset was created by randomly allocating individuals to either run 1 or run 2, which halved the statistical power but assured independency. LME models were fitted to this combined dataset including group size, predatory threat,

repeated measure and their two-way interactions as fixed effects and PC1-boldness and PC2-vigilance as response variables, respectively.

For each of the described global LME models, the most parsimonious sub-model regarding the fixed effects was derived using the second-order bias-corrected Akaike information criterion (AICc; Hurvich and Tsai 1989). Details on model selection are given in Online Supplementary Table S2. Additionally, the fixed effects of the most parsimonious sub-models were tested using Wald statistics. In case of a significant relationship ($\alpha = 0.05$), post hoc multiple comparisons adjusted by false discovery rate (FDR; Benjamini and Hochberg 1995) were employed.
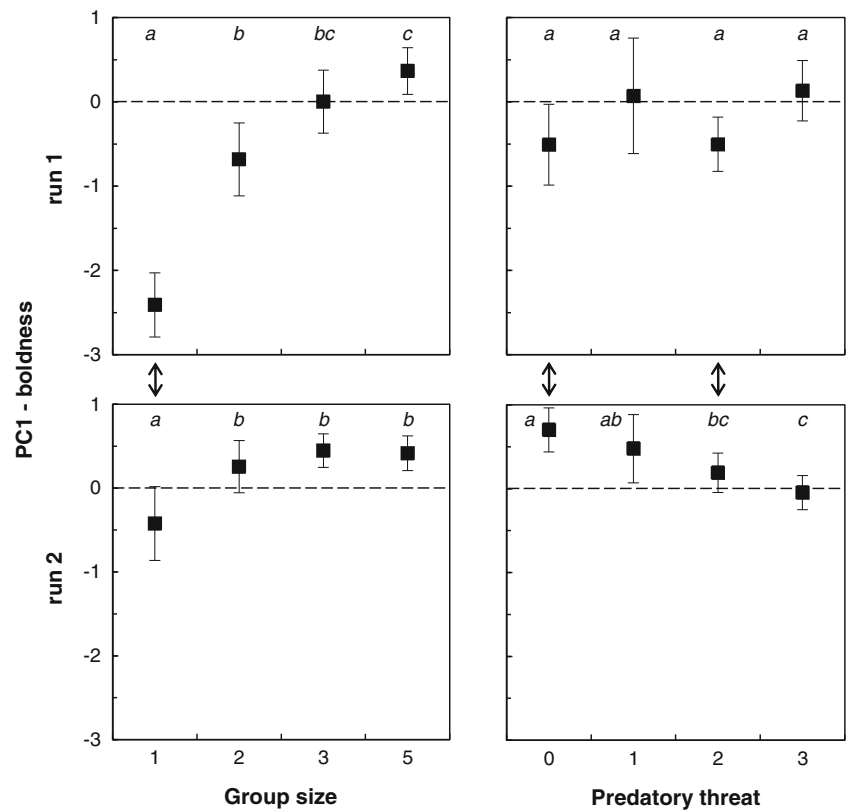
Results

Group size and predatory threat

Regarding boldness, there was practically no evidence for an interaction between group size and predatory threat in both runs (Akaike weights <0.015, Online Supplementary Table S2a). Thus, the models including their interaction were not used. Testing the fixed effects of the selected most parsimonious LME models, boldness was significantly influenced by group size in run 1 and by group size and predatory threat in run 2 (Table 3). Boldness increased significantly with larger groups, and this trend was of asymptotic character (Fig. 1). However, in run 2, significant differences could only be detected between solitary individuals and groups of two, three and five. Further, there was no obvious pattern in the response of the individuals towards the predator in run 1, but in run 2, a

Table 3 Wald statistics to test the effect of group size, predatory threat and their interaction (≙ global model) on the individuals' PC1-boldess and PC2-vigilance

Source of variation	df	F	p
PC1-boldness run 1			
Group size	3,87	25.8	<0.001
PC1-boldness run 2			
Group size	3,83	7.8	<0.001
Predatory threat	3,83	5.5	0.002
PC2-vigilance run 1			
Group size	3,84	8.2	<0.001
Predatory threat	3,84	8.5	<0.001
PC2-vigilance run 2			
Group size	3,74	1.9	0.132
Predatory threat	3,74	2.8	0.045
Group size×predatory threat	9,74	2.5	0.014

The most parsimonious LME models are given including all individuals separately for run 1 and run 2. Refer to the figures for FDR post hoc tests. *df* = numerator and denominator degrees of freedom; *F* = F-ratio

Fig. 1 PC1-boldness (mean with 95 % CI) for group size and predatory threat for all individuals shown separately for run 1 and run 2. Means within sub-graphs not including the same letter in their superscript are significantly different, as determined by FDR post hoc testing. Means changing significantly from run 1 to run 2 are marked with *up down arrows* (↕) between the sub-graphs

clear decrease in boldness with increasing predatory threat was observed.

Regarding vigilance, there was practically no evidence for an interaction between group size and predatory threat in run 1 (Akaike weight <0.02, Online Supplementary Table S2a). Thus, the model including their interaction was not used. In contrast, a significant interaction between group size and predatory threat was found in run 2 (Table 3). Analyzing the interaction, only solitary individuals showed a steady increase in vigilance with increasing predatory threat (Fig. 2). For the three larger group sizes, the vigilance saturated or dropped at higher levels of predatory threat. However, the pattern of maximum vigilance at an intermediate level of predatory threat was most pronounced and only significant in groups of two. Further, individuals in groups of two were only significantly more vigilant compared to individuals in the larger group sizes at the two highest levels of predatory threat (Fig. 2). Since the interaction mean of solitary individuals and predatory threat of three was based on too little replication (n=2, Table 1b), the three significant pairwise comparisons including it were not further interpreted.

Analyzing the main effects, the individuals' vigilance was significantly influenced by group size and predatory threat in run 1 (Table 3). Individuals in groups of two showed the highest vigilance during both runs (Fig. 3). In run 1, this higher vigilance of groups of two was significant towards solitary individuals and groups of five. A clear increase in vigilance with increasing predatory threat across all group sizes could be detected during both runs. However, in run 2, vigilance dropped, though non-significantly, with the highest level of predatory threat (Fig. 2).

Repeated measure and its interaction with group size and predatory threat

The individuals' boldness was significantly influenced by repeated measure and its interactions with group size and predatory threat (Table 4). Across all group sizes and levels of predatory threat, individuals increased in boldness with repeated measure. Solitary individuals were considerably bolder in run 2 compared to run 1 (Fig. 1). To note, this increase represents the largest effect size observed in our experiment. In contrast, boldness did not significantly increase in the larger group sizes. Further, through the repeated measure, the effect of predatory threat changed significantly to a linear decrease of boldness with higher predatory threat in run 2.

The individuals' vigilance was significantly influenced by the interaction between repeated measure and group size (Table 4). As the only significant change, individuals in groups of three decreased in vigilance from run 1 to run 2 (Fig. 3). There was no sufficient evidence for an interaction between repeated measure and predatory threat (Akaike weights=0.24, Online Supplementary Table S2b), and thus, the model including their interaction was not used.
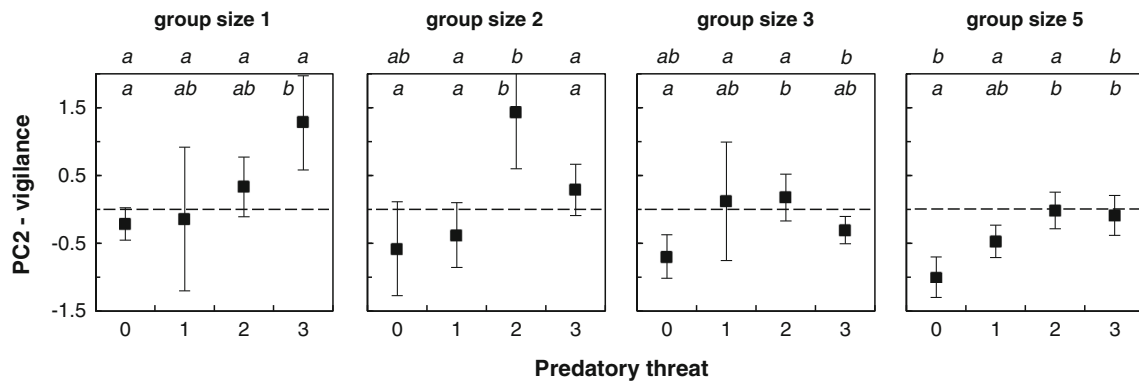
Fig. 2 PC2-vigilance (mean with 95 % CI) for the comparison among levels of predatory threat within group sizes in run 2. Means within sub-graphs not including the same letter in their superscript are significantly different, as determined by FDR post hoc testing. Superscripts above sub-graphs indicate results of post hoc tests for the comparison between group sizes within levels of predatory threat

Discussion

Our study combined the factors group size, predation risk and habituation to explore their effect on the boldness and vigilance of juvenile perch. In agreement with the literature on shoaling fish, we found a strong influence of group size on the behaviour of individuals resulting in bolder behaviour when group sizes became larger (Baird et al. 1991; Webster et al. 2007; Magnhagen and Bunnefeld 2009). Studying groups of 2, 4, 6, 12, and 20 individuals, Magurran and Pitcher (1983) observed a steady increase in the time individual minnows (*Phoxinus phoxinus*) spent on a food patch with larger groups.

In contrast, this time ceased to rise in goldfish (*Carassius auratus*) once a group size of 12 was reached. The authors suggested these differences to be a result of natural variation in shoaling tendency among species. In this context, our results would attribute a rather weak shoaling tendency to juvenile perch since the group-size effect was of asymptotic character with early saturation. Correspondingly, we observed the crucial behavioural difference between single perch and those having at least one companion. Signs of a strong unwillingness to be alone were previously shown in grouping animals including fish (Gomez-Laplaza and Morgan 2000; Apfelbeck and Raess 2008; Ward et al. 2008; Hellström et al. 2011).

Fig. 3 PC2-vigilance (mean with 95 % CI) for group size and predatory threat for all individuals shown separately for run 1 and run 2. Means within sub-graphs not including the same letter in their superscript are significantly different, as determined by FDR post hoc testing. Interpretations of superscripts for the second run need to be cautious due to the significant interaction group size × predatory threat (details in Fig. 2). Means changing significantly from run 1 to run 2 are marked with an *up down arrow* (↕) between the sub-graphs
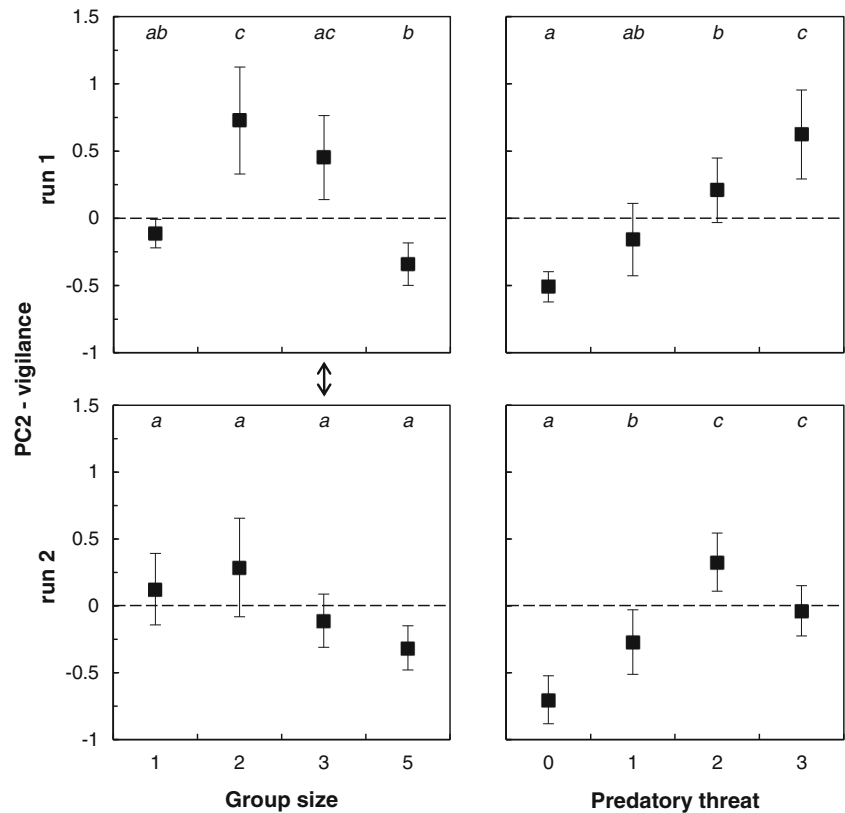
Table 4 Wald statistics to test for the effect of repeated measure, group size, predatory threat and their two-way interactions (≙ global model) on the individuals' PC1-boldess and PC2-vigilance

Source of variation	df	F	p
PC1-boldness			
Repetition	1,77	27.7	<0.001
Group size	3,77	21.4	<0.001
Predatory threat	3,77	2.7	0.054
Repetition × group size	3,77	4.3	0.007
Repetition × predatory threat	3,77	2.9	0.040
PC2-vigilance			
Repetition	1,80	0.0	0.924
Group size	3,80	10.2	<0.001
Predatory threat	3,80	5.9	0.001
Repetition × group size	3,80	3.1	0.033

The most parsimonious LME models fitted to the combined dataset are given, including individuals either for run 1 or run 2. Refer to Figs. 1 and 3 for FDR post hoc tests. Models shown in this table serve only to interpret the effect of repeated measure and its interaction with group size and predatory threat due to their reduced power compared to the models in Table 3. df = numerator and denominator degrees of freedom; F = F-ratio

Considering all this, the pattern of group-size effect in our study might indicate that social needs were already saturated in small groups. This is surprising since juvenile perch tend to form much larger shoals in nature than tested in our study (Probst et al. 2009).

Three possible explanations are conceivable for the bolder behaviour we observed in larger groups. First, stronger competitive interactions for food may enhance the individuals' foraging activity (Hake and Ekman 1988; Grand and Dill 1999; Hoare et al. 2004). Second, public information about the food location can favour foraging activity in the trade-off between risk avoidance and food intake (Valone and Templeton 2002; Dyer et al. 2009; Harcourt et al. 2009). Finally, being within a larger group promotes the individuals' sense of security by risk dilution, predator confusion and higher levels of overall vigilance (Roberts 1996; Krause and Ruxton 2002; Webster et al. 2007). As a result of the latter, the per capita vigilance within a social association of animals decreases asymptotically with increasing group size (Elgar 1989; Dehn 1990; Roberts 1996). In our study, vigilance was largely defined by predator inspection. To be beneficial to the group regarding per capita effort, the gathered information about the predator has to be transmitted from the inspecting individual to its group members. This was previously demonstrated but in other fish species (Pitcher et al. 1986; Licht 1989). Accordingly, we found higher levels of individual vigilance in smaller compared to larger groups in run 2, the situation less affected by novelty.

Still, our results partly contradict the theory of group-size-dependent vigilance as overall not solitary, but individuals in

groups of two were most vigilant. Previous studies observed that fish often perform predator inspections in pairs, thus, two individuals leave their shoal to investigate the predator (Magurran and Seghers 1990; Pitcher 1992). Also, two individuals can show coordinated cooperation over successive inspection events (Milinski et al. 1990; Dugatkin and Alfieri 1991; Croft et al. 2006). Short- or long-term cooperation between individuals is mainly explained in terms of tit-for-tat, which is described as a sequence of alternately performed, apparently selfless behavioural actions (e.g. risky predator inspection) of single individuals within a group (Pitcher 1992). This specific cooperation is assumed to work best in pairs (Packer 1986; Milinski et al. 1990) and to rapidly lose its strength as group size increases (Boyd and Richerson 1988). In this respect, the comparably high predator inspection activity in groups of two in our experiment would rather have been expected.

In addition to group size, our study fish were sensitive to predation risk, represented by the predator's activity and distance. We found a clear pattern of decreasing boldness with increasing risk in run 2. That perch react according to predators was seen in wild populations with high- or low-predation pressure, respectively (Magnhagen 2006), and in experiments with simulated predation risk (Christensen and Persson 1993). In both cases, perch reduce their risk-taking behaviour with increasing predation risk, which is also shown in other fish species (Bean and Winfield 1995; Chivers et al. 2001; Lehtiniemi 2005). Thereby, hiding within a structured habitat like the vegetated area in our setup is a common predator avoidance behaviour in perch (Snickars et al. 2004). Further, we observed higher vigilance with increasing predation risk. In agreement, Walling et al. (2004) induced an increase in predator inspection in three-spined sticklebacks (*Gasterosteus aculeatus*) using models with an increasing similarity to real predators, and Pitcher and Turner (1986) observed an increase in number of predator inspections by minnows with reduced distance of the predator.

As illustrated by our results, group size and predation risk are two main drivers of behaviour in juvenile perch. This leads to the question of whether and how they interact. Group living in prey animals has partly evolved as response to predation (Krause and Ruxton 2002), and thus, we might expect their effects to be strongly dependent on one another. For instance, the group-size effect in a given anti-predator behaviour may only be distinct in individuals under risk of predation (Frid 1997; Banks 2001; Downes and Hoefer 2004). This would allow individuals in smaller groups to invest as much into foraging or reproduction as conspecifics in larger groups when no predators are around. Since in nature, individuals are confronted with a continuous change in both of these factors, natural selection would strongly favor such a flexible behaviour. However, the extremely low weights of the interaction terms in our models suggest an independent adjustment of

boldness according to group size and predation risk in juvenile perch. In other words, our study does not provide evidence for an earlier saturation of boldness with increasing group size at low compared to high levels of risk or for a stronger influence of the predator on smaller compared to larger groups. This might indicate that no risk dilution, predator confusion, and the overall higher vigilance were mainly responsible for the observed group-size effect (Roberts 1996) but increased resource competition (Hake and Ekman 1988; Hoare et al. 2004). This is conceivable since food was limited and restricted to a small area in our experiments. Similarly, Grand and Dill (1999) found no difference in the form of the relationship between the behavioural measures 'amount of prey captured' and 'time under cover' and group size in the presence or absence of a predator in coho salmon (*Oncorhynchus kisutch*).

In contrast, our results for vigilance partially support an interactive adjustment of behaviour to group size and predation risk. In the second run, which was likely less disturbed by the novelty effect, individuals in groups of two showed only significantly higher vigilance than the larger group sizes at the two highest levels of risk. This strategy of individuals in smaller groups to not overemphasize safety at the expense of other activities under low risk of predation was previously demonstrated for lizards and sheep (Frid 1997; Downes and Hoefer 2004). Also, there is evidence that fish cease predator inspection activity and switch to predator avoidance behaviour above a certain threshold of risk (Magurran and Pitcher 1987). The significant drop in vigilance in groups of two at highest predation risk might indicate that we just passed this threshold. To conclude, the detailed response in vigilance of individuals in groups of two clearly stands out. They were overall most vigilant, elevated their vigilance compared to individuals in larger groups only when at higher risk and demonstrated a non-linear adjustment of vigilance in accordance with predator behaviour.

When comparing our first and second runs, noticeable differences became apparent for the effect of group size and predation risk. Especially, the individuals' boldness was influenced by the repeated measure characterized by a generally bolder behaviour as well as a more predictable reaction towards the predator in the second run. Based on the literature, we suggested this to be a result of progress in habituation to the novel environment (Gerlai and Hogan 1992; Wanzenböck et al. 2006; Martin and Reale 2008; Rodriguez-Prieto et al. 2011). The novelty effect in our study presumably comprised several factors which we cannot separate. Most likely, it included the unfamiliarity with the experimental procedure and test arena and the sudden exposure to olfactory cues of the predator during the short acclimation period (16–21 h).

Additionally, a social novelty effect might have been provoked by the sudden reduction in group size from 15 or more individuals during the holding period to only 1 to 5 individuals during the first day of observation. Hence, the smaller

group sizes experienced the greatest loss of social environment. Accordingly, over the two runs, we observed the most extreme increase in boldness in solitary perch. A particularly strong effect of novelty on solitary individuals is also suggested in flocking birds (Coleman and Mellgren 1994; Soma and Hasegawa 2004). Further, the three larger group sizes did not differ statistically in boldness in the second run. This indicates that in more stable circumstances, the influence of group size on the individual's boldness is only minor in juvenile perch. In contrast to our study in which perch seemed to have accepted the living in smaller groups after only 2 days' time, fear-induced behaviour in juvenile angelfish (*Pterophyllum scalare*) increased steadily over 4 days of solitariness (Gomez-Laplaza and Morgan 2000).

In summary, our study demonstrated the importance of group size and predation in shaping boldness and vigilance behaviour in juvenile perch. It suggests that perch may adjust boldness behaviour to group size and predation risk independently. This is rather unexpected since in theory, an interactive adjustment would strongly be favoured by natural selection. Our observations also indicate that only two individuals might be required in shoaling perch for a well-functioning social unit. At this group size, vigilance might be particularly effective in promoting fitness due to the combination of intense monitoring and detailed balancing of costs and benefits. Finally, our results suggest that habituation has a great potential to affect patterns in response to group size and predation risk and should ideally be considered through repeated measures when studying behaviour.

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