Plasticity and consistency of behavioural responses to predation risk in laboratory environments

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ABSTRACT

The individual animal is currently a major focus of behavioural research and an increasing number of studies raise the question of how between-individual behavioural consistency and behavioural plasticity interact. Applying the reaction norm concept on groups, our study addresses both of these aspects in one framework and within an animal's natural social environment. Risk-taking behaviour in one-year-old perch (*Perca fluviatilis*) was assayed in aquarium experiments before and after the fish were subjected to the presence or absence of a piscivorous predator for three weeks. To analyse the inter-individual behavioural variation across the repeated measurements, we dissected the behavioural change across the predator treatment into individual constant and plastic components using hierarchical mixed effects models. During the predator treatment juvenile perch increased in boldness and decreased in vigilance, the magnitude of these behavioural changes was influenced by group composition. However, the behavioural changes were not influenced by the presence of a predator, indicating the difficulties in generating realistic long-term predation pressure in the laboratory. Individuals differed in the relative increase in boldness across the predator treatment and, thus, varied in the shape of their reaction norms. In accordance, the best linear unbiased predictors (BLUP), extracted from the random effects of separate linear mixed effects models for the data before and after the predator treatment were only weakly correlated. Hence, between-individual variation seems to change under laboratory conditions and therewith not necessarily represents the initially present "natural" variation, giving important implications for the conduction and interpretation of behavioural experiments.

Keywords: reaction norm, *Perca fluviatilis*, phenotypic plasticity, boldness, personality, behavioural consistency

Running title: Behavioural plasticity and consistency in the laboratory

INTRODUCTION

Behaviour is considered to be one of the most plastic phenotypic traits (Price et al. 2003) and many studies have documented the potential plasticity of behavioural traits in a variety of animals [mammals (Hayes & Jenkins 1997), cephalopods (Sinn et al. 2007), insects (Agrawal 2001), birds (Cresswell & Quinn 2005; Miller et al. 2006), fish (Coleman & Wilson 1998)]. Since a shift in focus to the individual, an increasing amount of studies have reported consistent behavioural differences within animal populations (reviewed in Sih et al. 2004; Bell 2007), but also between individual plasticity *per se* (Dingemanse et al. 2010). The observed consistent behavioural differences across time or situations have been ascribed to an animal's underlying distinct personality, also termed coping style, temperament or behavioural syndrome (Koolhaas et al. 1999; Sih et al. 2004; Dingemanse & Reale 2005). These, initially contradictory, findings raise the question of how inter-individual variability and intra-individual stability interact (Nussey et al. 2007; Dingemanse et al. 2010).

Studies on fish have shown that behavioural stability along the shy-bold axis varies between species, but also within a species, when studied with regard to behavioural traits or situations. Wilson et al. (1993) found that pumpkinseed sunfish exhibited constant behavioural differences in the wild, and that these differences disappeared after the fish were held in the laboratory for some time. In sticklebacks, high predation risk altered the degree of aggression a stickleback displays towards conspecifics, and the observed overall decrease in boldness was due to selective predation, as the individual's degree of boldness did not change (Bell & Sih 2007).

Clearly, behavioural differences may have important fitness consequences (Dingemanse et al. 2004; Smith & Blumstein 2008). However, to assess the selective value of a trait, there is a need to understand how variable it is, both between and within individuals (Boake 1989; Hayes & Jenkins 1997; Dingemanse et al. 2010). One approach simultaneously addressing variability and consistency is the reaction norm concept (Via et al. 1995; Nussey et a. 2007), where an individual's behaviour is tested repeatedly along an environmental gradient. The behavioural differences along the environmental gradient represent the plastic ability at the population level. Deconstructed to the individual level, the presence of an interaction between individual reaction and environment (I x E) suggests that individuals differ in their plastic ability (Nussey et al. 2007; Briffa et al. 2008; Martin & Reale 2008; Dingemanse et al. 2010). In juvenile Eurasian perch, *Perca fluviatilis* L., relative differences in boldness towards a predator have been shown to be consistent between individuals across different social contexts (Magnhagen & Bunnefeld 2009). Perch also display a high degree of behavioural plasticity, changing their behaviour with status of nourishment (Borcherding & Magnhagen 2008), consumed prey (Heynen et al. 2010), social background (Magnhagen & Staffan 2005) or the experienced intensity of predation pressure (Magnhagen & Borcherding 2008; Hellström & Magnhagen 2011; Magnhagen et al. 2012).

The aim of the present study was to investigate whether the inter-individual behavioural variation within a group of perch changes with the adaptation to a novel situation. Furthermore, we wanted to test whether and how such changes, if any, are influenced by predation risk. Boldness towards a predator was measured in groups of juvenile perch, before and after participating in a three week tank treatment in absence or presence of an adult piscivorous perch. To analyse the consistency of the inter-individual variation across the repeated measurements, we compared the behavioural ranking within the same groups before and after the predator treatment and the variation in the groups that experienced a predator during the three weeks in the tank with those without predation risk. Using mixed effects models to analyse our data, we were able to dissect the obtained behavioural variation across the phenotypic response, at the group level, into individual constant and plastic components.

MATERIAL AND METHODS

In June 2009 one-year-old juvenile perch (body length, X ± SD; 90.3 ± 6.4 mm, weight 7.4 ± 1.7 g, N = 96) were caught with a beach seine in Lake Ängersjön, close to the city of Umeå, Sweden (63° 47´N; 20°17´E). The fish were transported to Umeå Marine Research Centre (UMF, 45 km south of Umeå). In the 100-litre transport vessel, a pump run by a car battery was constantly recirculating and oxygenating the water. The fish were stocked to a circular tank (60 cm high, 0.471 m^2) with continuously running water (13L:11D, 14-15 °C) to acclimate to indoor conditions (5 or 10 days). They were fed daily with pre-frozen red chironomid larvae (6% of total body mass). The predators, older perch (body length, X ± SD; 200.8 ± 13.5 mm, N = 15), were caught in a small stream near the laboratory and fed with earthworms daily. Fulton's condition factor was used as measure of the physical condition of the fish ($K=10^5M*TL^{-3}$, where M=weight in g and TL=total length in mm; Bagenal & Tesch 1978). After the experiments the tested fish were killed with an overdose of MS222. The predator perch were released into their natural habitat.

Experimental design

Before being handled all fish were sedated with MS222. The juvenile perch were marked with individual colour codes, generated with 4 different colours and carefully applied with a needle and tattoo colours (Tattoo-Flame©) on the upper and/or lower caudal fin. Subsequently, fish were weighed, measured and randomly assigned to groups of four (N = 24 groups). Each group participated in two sets of behavioural experiments, the first one directly before and the second one directly after being exposed to a three week predator treatment in tanks (Table 1). After the first set of behavioural experiments were conducted, 4 groups were added to each of the 6 tanks used for the predator treatment (N = 24 groups). Those six tanks (60 cm high, 0.471 m^2, 50% cover with artificial vegetation) were circular and had continuously running water. After three weeks all fish were collected from the tanks, sedated, weighed, measured and the behaviour of the same groups was re-assayed (second set of behavioural experiments), in the same way as during the first set of behavioural experiments. Due to the limited capacity of experimental aquaria, the study was conducted in two successive experimental blocks, starting five days apart. 12 groups (48 individuals) were tested in each block (see Table 1).

Predator treatment

In the experimental block 1 two tanks were stocked with a predator and one tank was predator free, while the opposite was done for block 2, resulting in 3 predator and 3 non-predator tank treatments. In each experimental block juvenile perch were allowed to acclimate to the tank for one day before the predators were added. To generate a real impression of danger, but to minimize the consumption of prey individuals the predator size ratio was chosen close to the maximal border of ingestability (literature data: 0.45, Claessen et al. 2000; our data: X ± SD; 0.39 ± 0.03 mm, range 0.34 – 0.45) and the predator was only allowed to swim free in the tank for 6 days out of the 21 days of the experiment. After 6 days the predator was transferred into a transparent perforated plastic box (41 x 26 x 29 cm) within the tank, so that visual and olfactory predator cues were still present. The tanks without predators were treated the same, a net was swept through the tank and the box was opened and closed. During the tank treatment juvenile fish were fed with pre-frozen chironomid larvae daily (6% of total body weight) and the predators with earthworms every second day.

Experimental aquaria

The experimental aquaria were 170 l (95 x 41 x 44 cm) and had continuously running water (17 °C; light regime 13L:11D). One-third of each aquarium was separated with a plastic net (mesh size 5 mm) and used for the predator, the rest for the group of juvenile perch. To prevent the fish habituating to the predator during the behavioural assays an opaque plastic screen was placed next to the net. The water inlet was placed in the section with the small perch and the outlet in the predator section to minimize olfactory cues between observations. The bottom of the aquaria was covered with gravel. Artificial vegetation was provided in the predator space and in the half of the space for the perch group that was furthest away from the predator.

Behavioural experiments

Both sets of behavioural experiments (before and after the predator tank treatment) consisted each of three repeated observations. Prior to each set of three behavioural observations the small perch were acclimatized to the aquarium for 3 days and fed daily with red chironomid larvae in the open area. The fish were then observed three times, twice on the first day with a break of three hours between experiments and once on the second day. Before each observation the juvenile perch were enclosed by the opaque screen in the half of their section that also contained the vegetation. Chironomid larvae (approx. 75 larvae, corresponding to 3% of the total fish weight) were poured into the open space produced between the net and the opaque screen and allowed to sink to the bottom. The observation started by lifting the opaque screen, making the large perch visible to the juvenile perch though the net. Each aquarium was observed for 10 min. The observer recorded (in real time) four different activities for each individual fish: occurrence in the vegetation, occurrence in the open, feeding and predator inspection. Thereby, feeding was defined as being oriented towards the bottom and attacking the food and predator inspection as being within two fish lengths distance of the net and being orientated exactly towards the predator. The activities were entered into a computer program, which record one behavioural unit every second. The recorded behavioural data were used to calculate 7 behavioural variables: (1) time spent in the open area, (2) total time spent feeding, (3) time to start feeding, (4) duration of the first feeding bout, (5) activity (number of changes between open area and vegetation), (6) time until first change of habitat, and (7) time spent with predator inspection. After each observation the opaque screen was put back next to the net. Each group was always tested in the same aquaria and in presence of the same predator. The predators used during the behavioural experiments were different from those used during the predator treatment in the tanks.

Statistical analyses

One group of four fish was excluded from the analyses, as two of its members were lost during the tank treatment (non-predator tank treatment), leaving a total of 23 groups and 92 individuals that were used for the analyses.

All 7 calculated behavioural variables were entered into a principal component analyses (PCA). By using a PCA we are able to use all data, and gain information about the relationship of the different variables to each other. The resulting scores illustrate an individual´s personality type rather than an isolated behaviour and retain the structure and variation present in the recorded data. We retained all extracted principal components with eigenvalues larger than 1 for further analyses.

To control for a possible ceiling effect due to the limit of the behavioural assays (10 min), we conducted a second PCA including an additional imaginary individual, which was given maximal values in each variable.

To analyse the effect of the predator tank treatment on the extracted principal components a linear mixed effect model approach was set-up. To avoid pseudoreplication in the analysis a nested design was created. Within individual repetition (behavioural observation 1-3) was added as random effect at the innermost level. Between-individuals within group was added as the next level, between-groups in one tank was added at the following level and between-tanks was added as random effect at the outer level. Treatment (before and after the predator tank treatment), experimental block (block 1 and 2) and their interaction were included as fixed effects with two factor levels each. Hence, the models for PC1 and PC2 were fit with the predictors of predator tank treatment, experimental block and their interaction and the random intercepts of within-individual repetition, individual, group and tank ID.

The effect of predator presence during the predator tank treatment was analysed incorporating only the data obtained after the predator tank treatment. Keeping the previously described structure of the random effects (within individual (repetition)/between individuals/between groups/between tanks), we included predator (predator or no predator during the treatment), experimental block (block 1 and 2) and their interaction as fixed effects.

The equivalent mixed-effect model was run using the behavioural data from before the tank treatment, including only experimental block (block 1 and 2) as fixed effect. The most parsimonious models for the separate data from before and after the tank treatment were derived by testing the fixed effects using Wald statistics (Pinheiro & Bates 2000).

To obtain an individual score for the relative ranking of an individual within its group we extracted the best linear unbiased predictors (BLUPs) of the random effect of `between individuals` from the two most parsimonious models for PC1 for before and after the tank treatment (Magnhagen & Bunnefeld 2009). Using a linear model, BLUPs from before were correlated with the BLUPs from after, to analyse the relative individual behavioural consistency across the predator tank treatment (before and after). Additionally, we analysed the relative individual behavioural consistency across the predator tank treatment (before and after) using the average (observation 1-3) of the seven calculated behavioral variables and Kendall correlations, as the data was not normally distributed.

To analyse the magnitude of the behavioural change, the difference between the behaviour before and the behaviour after the predator tank treatment was calculated (PC1 after – PC1 before). The difference between the behaviour before and the behaviour after the predator tank treatment was used as response variable to run a linear mixed effect model. In this model we used the same nested hierarchy for the random effects as stated above (within individual (repetition)/between individuals/between groups/between tanks). In addition to the categorical fixed effects experimental block and predator, the mean change of the accompanying group members (company change) and the change in condition factor during the predator tank treatment (condition factor after – condition factor before) as continuous fixed effects.

The free software R for statistical computing (R Development Core Team 2009) was used for all analyses. The PCA (*prcomp*) and the correlation (*lm*) were calculated with the standard *stats* library. The library *nlme* v.3.1.-90 was used to run the mixed effect models.

Ethical note

No prey individual was consumed or harmed (no bite marks or injuries) during the experiments. The experiments in this study comply with the guidelines of the Association for the Study of Animal Behaviour, and were approved by the Local Ethics Committee of the Swedish National Board for Laboratory Animals (CFN, license no A94-06).

RESULTS

The PCA on the 7 behavioural variables produced two principle components with eigenvalues > 1 (PC1 and PC2), explaining together 72.5% of the variation (Figure 1). Positive scores on PC1 indicated more time in the open, more time feeding, a longer duration of the first feeding bout, a lower latency to leave the vegetation and lower latency to start feeding, which would signify a fish with a high degree of boldness. Positive scores on PC2 indicated more time spent with predator inspection and a lower duration of the first feeding bout, specifying vigilance. Including an imaginary individual with maximal values in all variables in a second

PCA showed that the boldest observed individual in our study showed 71.9% of the possible maximum boldness, indicating that our results are not biased by any ceiling effect.

The effect of the predator treatment

The presence of a predator during the tank treatment did not have any effect on PC1 (boldness) and PC2 (vigilance) (Table 2), neither did experimental block, nor their interaction show a significant effect. However, after the predator treatment, perch were significantly bolder and less vigilant than before treatment (Table 3, Figure 2). For PC1 the predator treatment additionally showed a significant interaction with the experimental block (Table 3). Before the tank treatment PC1 scores were slightly lower for the first block than for the second, while these relations were absent after the predator treatment.

Magnitude of behavioural change

For PC1, the most parsimonious models using the separate data from before and after the predator treatment were the models without any fixed factors, which were used to extract the BLUPs. The BLUPs for individual perch before the predator treatment were correlated with the BLUPs from after the predator treatment ($t_{90} = 2.3$, $P = 0.02$, $r^2 = 0.05$), however, the data points are scattered (Figure 3), and the low r^2-value indicates that the correlation is weak and 95% of the variation remains unexplained. Similarly, out of the seven calculated behavioral variables, the behavior before and after the predator treatment was only significantly and weakly correlated for two behavioral variables (Total time spend in the Open: $p = 0.001$; Kendall's tau coefficient 0.235; Time until first change: $p < 0.001$; Kendall's tau coefficient 0.289).

During the predator treatment juvenile fish slightly decreased in condition factor ($t_{177.78} = 6.49$, $P = 0.001$; condition factor, $X \pm SD.$; before 0.99 ± 0.06, after 0.93 ± 0.08). This change in condition factor did not affect the magnitude of behavioural change (PC1 after the tank treatment – PC1 before the predator tank treatment), nor did predator presence or the experimental block (Table 4). An individual´s magnitude of change was significantly influenced by the mean difference between the scores before and after predator treatment of the accompanying group members (Table 4), in a way that the individual magnitude of change increased with increasing company change (Figure 4).

DISCUSSION

We could show that the behavioural reaction towards a predator in groups of juvenile perch can be expressed by two distinct behavioural axes. The behavioural measures that load on the first (PC1) axis (e.g. time spent in the open or time to start feeding), comply with those used in other studies on fish to investigate differences in boldness (Snickars et al. 2004; Magnhagen & Borcherding 2008). Further, the variation in PC2 scores mainly reflects whether the fish performed predator inspection or not, generally interpreted as vigilance (Pitcher 1992).

Comparing the scores of the same groups of juvenile perch, before and after they participated in the three week predator tank treatment, showed that juvenile perch increased in boldness (PC1) and decrease in vigilance (PC2) across the repeated measurements. Similar results were previously interpreted as habituation effects to a novel environment, represented by the laboratory conditions (Millot et al. 2009), but were also observed in response to decreasing predation risk (Goldenberg et al. 2014). Surprisingly, we found no behavioural difference between the fish that experienced a predator during the treatment and those without predator cues, although perch generally seem to adapt their behaviour to the experienced level of predation pressure (Magnhagen & Borcherding 2008; Magnhagen et al. 2012; Goldenberg et al. 2014). Laboratory studies have shown that juvenile fish rely on predator cues to optimize the trade-off between foraging and anti-predator reaction, where juveniles responded strongest to the connections of olfactory and visual cues (Mikheev et al. 2006; Martin et al. 2010). When predator and prey belong to the same species the diet of the predator (Mirza & Chivers 2001), but also the relative size of prey and predator may be important factors influencing the behavioural reaction (Lundvall et al. 1999). The predators in our study were fed earthworms, but the prey/predator size ratio in the tanks was below the maximal ratio for ingestability (Claessen et al. 2000) and juveniles responded to quick movements of the equally large predators in the aquaria. However, fish are capable of learning (Braithwaite & Salvanes 2008) and to habituate to initially threatening cues (Meliska & Meliska 1976), suggesting that, after a habituation period the predator, confined to a box, might not have been considered a real danger. Those results are indicative for a general problem and highlight the difficulty in generating naturalistic, but harmless scenarios of predation risk, to study the effects of long-term predation pressure in laboratory environments.

Analysing the behavioural consistency, we found a significant correlation of the BLUPs from before and after the predator tank treatment, this correlation explained 5% of the variation ($r^2 = 0.05$). This indicates that the consistency of inter-individual behavioural differences within a group of perch was rather low in this study. Individuals differed in the relative increase in boldness across the predator tank treatment and, thus, varied in the shape of their reaction norms. The analysis of the individual magnitude of behavioural change showed that the individuals in our study were not influenced by changes in condition, which is a known factor to alter boldness in juvenile fish (Vehanen 2003; Borcherding & Magnhagen 2008). Instead, there was a relationship between the individual and the accompanying group members, as individual magnitude of behavioural and the magnitude of behavioural change of the accompanying group members were positively correlated. In many social species the members of a group have been found to influence each other (Krause & Ruxton 2002). The behavioural conformity in a group facilitated by these social mechanisms has been suggested to further confuse an attacking predator (Zheng et al. 2005; Tosh et al. 2009). Thus, our result suggests that the individuals within a group influenced each other in the magnitude of their behavioural change, probably reflecting social constraints and increasing behavioural conformity (Laubu et al. 2016).

Comparing the influence of holding conditions on the outcome and comparability of behavioural experiments in birds, Miller et al. (2006) could show that the holding conditions (e.g. presented food or structure within the cage) may substantially alter the obtained results of behavioural experiments. Our results suggest that also the holding time might be a crucial factor affecting behaviour, as the between-individual variation seems to change along the temporal gradient and therewith not necessarily represents the initially present “natural” behavioural variation. These results emphasize the practical implications of the reaction norm concept and the benefits of measuring behavioural variation repeatedly across an influential gradient.

In conclusion, we found juvenile perch to increase in boldness across the repeated measurements, phenotypically adapting their behaviour to the predator tank treatment, but individuals differed in the shape of their reaction norms. The magnitude of behavioural change was influenced by group composition. However, there was no behavioural difference between the fish that experienced a predator during the tank treatment and those without predator cues, indicating the general difficulties in generating realistic long-term predatory threat in laboratory environments. Furthermore, the between-individual variation seems to change along the temporal gradient and therewith not necessarily represents the initially present “natural” variation, emphasizing the importance of repeated behavioural measurements and highlighting that initial holding/ laboratory acclimatisation time needs to be chosen carefully.

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Tables

Table 1: Experimental design

	Block 1	Block 2
Day 1-5	acclimatization in tank	acclimatization in tank
Day 6-8	acclimatization to aquaria	acclimatization in tank
Day 9-10	behavioural experiments “before”	acclimatization in tank
Day 11-14	predator treatment	acclimatization to aquaria
Day 15-16	predator treatment	behavioural experiments “before”
Day 16-31	predator treatment	predator treatment
Day 32-34	acclimatization to aquaria	predator treatment
Day 35-36	behavioural experiments “after”	predator treatment
Day 38-40		acclimatization to aquaria
Day 41-42		behavioural experiments “after”

Table 2: Wald statistic for the fixed effects for PC1 and PC2 before and after the predator tank treatment, tested separately with mixed effect models

		PC1 - boldness			PC2 - vigilance		
		F	df,df_{den}	P	F	df,df_{den}	P
Before							
	Exp block	1.42	1,4	0.29	0.74	1,4	0.43
After							
	Predator	1.18	1,2	0.39	0.24	1,2	0.67
	Exp block	1.89	1,2	0.30	4.47	1,2	0.16
	Predator x exp block	0.03	1,2	0.86	5.04	1,2	0.15

Table 3: Wald statistic for the fixed effects Treatment (before and after) experimental block and their interaction for PC1 and PC2 tested with a mixed effect model

	PC1 - boldness			PC2 - vigilance		
	F	df,df_{den}	P	F	df,df_{den}	P
Treatment	*1160*	*1,270*	*0.001*	*8.87*	*1,270*	*0.003*
Exp block	0.37	1,4	0.57	4.85	1,4	0.09
Treatment x exp block	*21.69*	*1,270*	*0.001*	2.22	1,270	0.13

Table 4: Wald statistic for the fixed effects company change (mean magnitude of behavioural change of the accompanying group members across the predator tank treatment), predator (predator presence or absence during the treatment), condition factor change and experimental block on the individual magnitude of change (before after difference in boldness score PC1 across the predator tank treatment)

	F	df, df_{den}	P
Company change	*43.28*	*1,179*	*0.001*
Predator	0.77	1,3	0.81
Condition factor change	0.14	1,68	0.52
Exp block	3.50	1,3	0.25

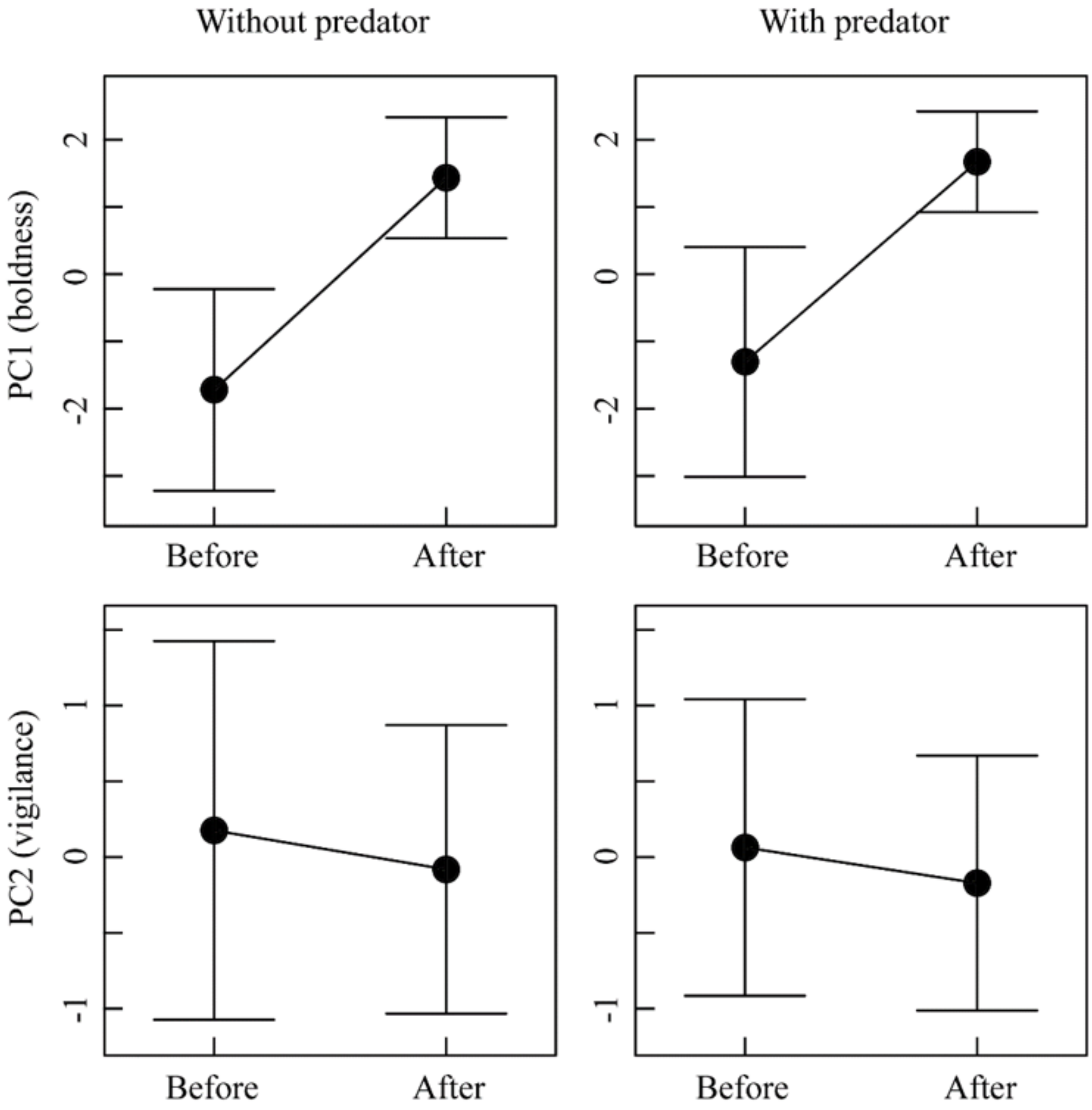
Figure legends

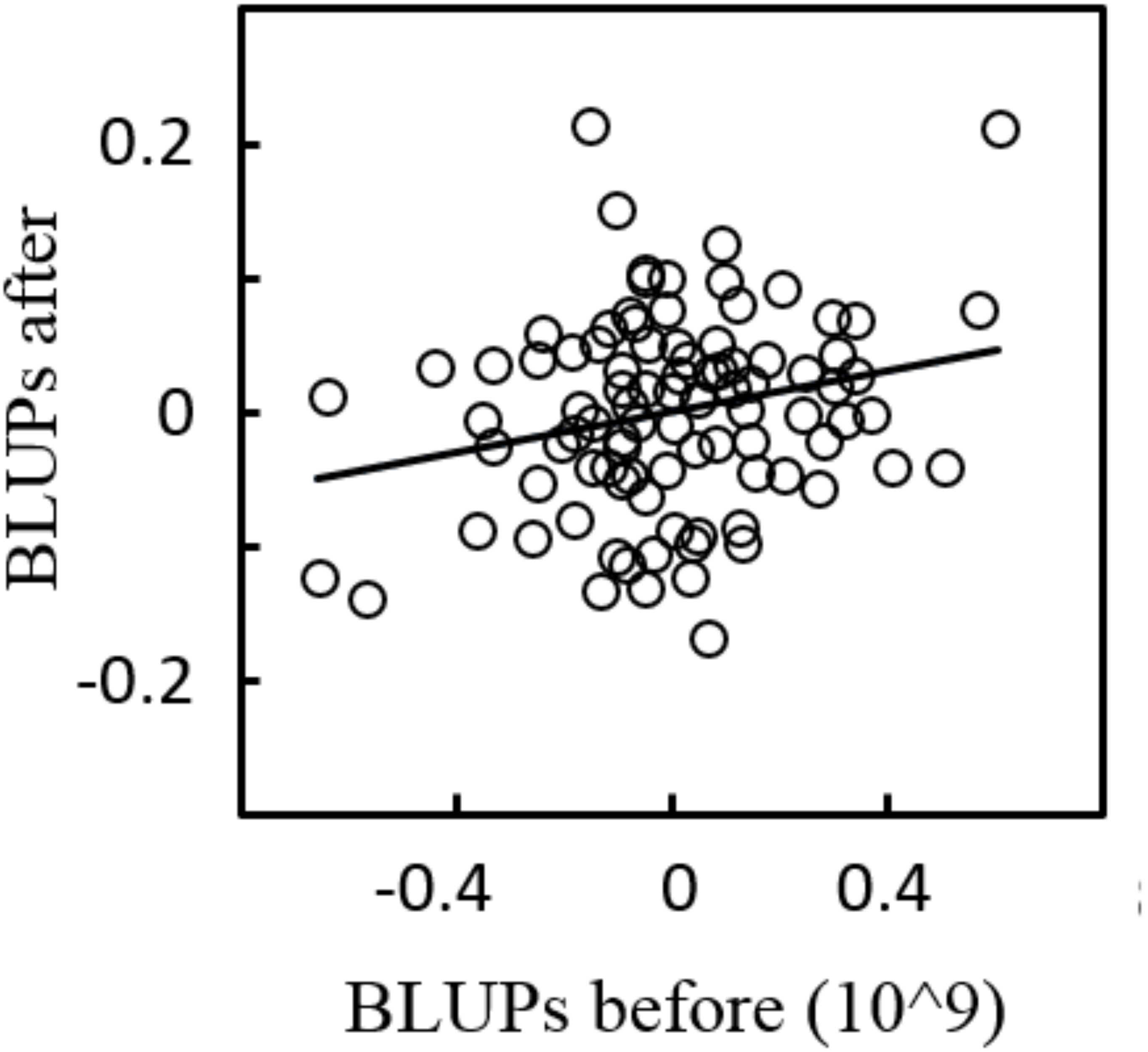
Figure 1: Biplot of the first two principal components, PC1 (Eigenvalue 4.02) and PC2 (Eigenvalue 1.06), extracted from a Principal Component Analysis (PCA) over the seven different measured behavioral variables [(1) time spent in the open area, (2) total time spent feeding, (3) time to start feeding, (4) duration of the first feeding bout, (5) activity (number of changes between open area and vegetation), (6) time to first change of habitat, and (7) time spent with predator inspection].

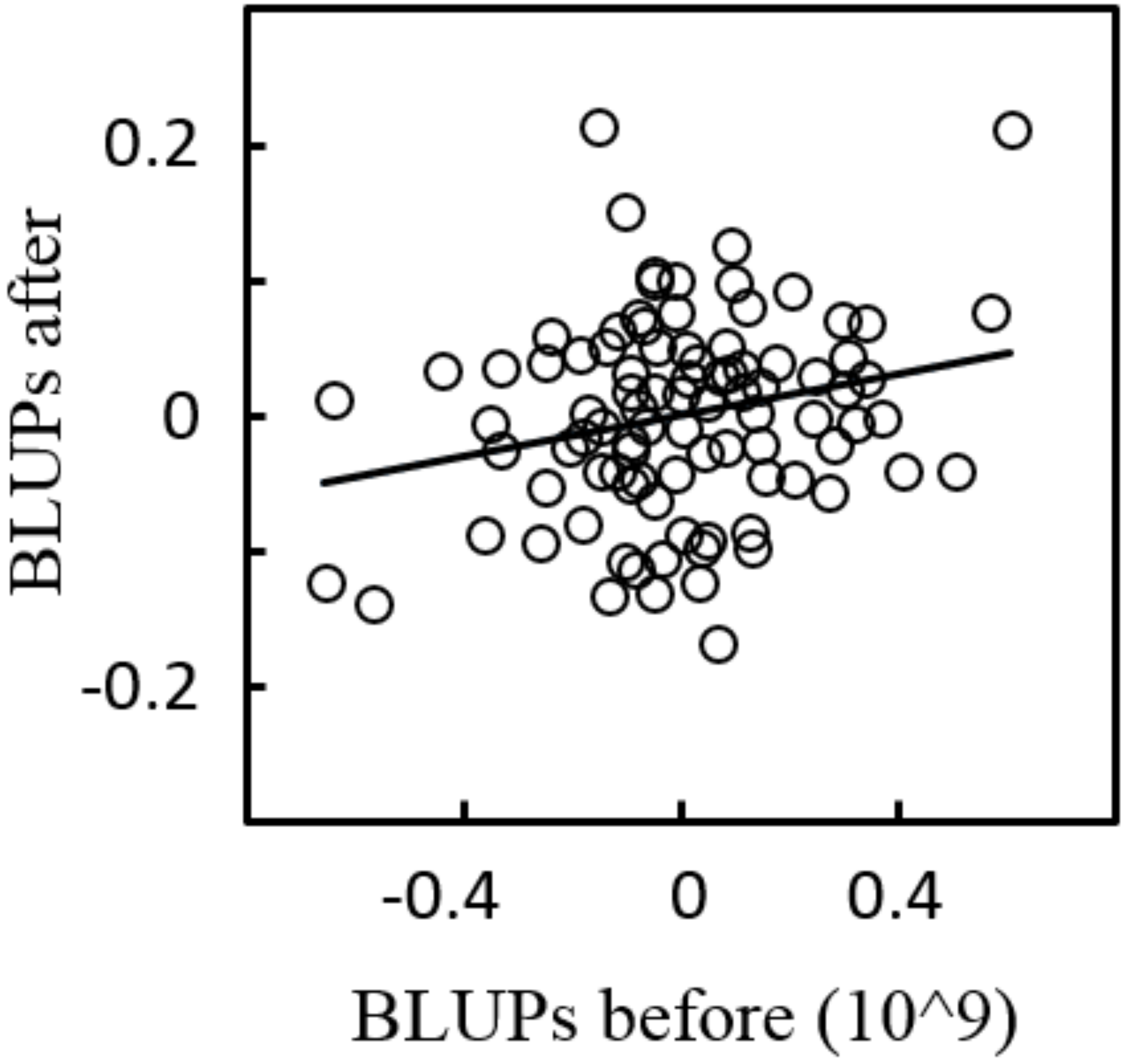
Figure 2: Mean boldness (PC1) and vigilance score (PC2) before and after the predator tank treatment, in absence or presence of a predator during the treatment

Figure 3: Correlation of the best linear unbiased predictors (BLUPs) from before the predator tank treatment with the BLUPs after the predator tank treatment, extracted on individual level from the most parsimonious mixed effect models for before and after the predator tank treatment, respectively

Figure 4: Mean individual increase in boldness (After – before difference PC1) plotted against the mean company difference (mean increase in boldness of the accompanying group members).


Without predator
With predator
PC1 (boldness)
PC2 (vigilance)
2
0
-2
1
0
-1
Before
After
Before
After
Before
After
Before
After


0.2
0
-0.2
-0.4
0
0.4
BLUPs after
BLUPs before (10^9)


0.2
0
-0.2
BLUPs after
-0.4
0
0.4
BLUPs before (10^9)