

Original Article

Shaping the antipredator strategy: flexibility, consistency, and behavioral correlations under varying predation threat

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Recent ecological and evolutionary research emphasizes the importance of adaptive trait integration. For instance, antipredator defenses are built up of several morphological and behavioral components in many species, yet their functional relationships are still poorly documented. Using field-collected freshwater crustaceans *Gammarus fossarum* in a within-subject design, we investigated the flexibility and consistency of refuge use, photophobia, and exploration behavior as well as their associations, quantified both when predation risk was absent or artificially simulated. In agreement with the “threat-sensitivity” hypothesis, both refuge use and photophobia increased under predation risk, whereas exploration behavior did not vary. For all behaviors, individuals exhibited low but significant consistency between risk treatments. However, intraindividual variability did not vary with weight or between sexes. Finally, a positive relationship between photophobia and refuge use was observed only under predation risk in females, whereas it was found in both risk treatments in males. Our findings demonstrate that predation risk may promote behavioral flexibility and behavioral correlations, or syndromes, in wild populations, and call for an integrative study of antipredator mechanisms where the evolution of each dimension has to be examined in combination with all others. Additional phenotypic traits should be included in future studies to better characterize the multidimensionality of antipredator defense and the functional relationships among traits. The survival consequence of behavioral correlations between risk-sensitive traits also remains to be estimated in mortality selection experiments.

Key words: behavioral syndrome, boldness, crustacean, *Gammarus fossarum*, intraindividual variability, multiple trait, personality.

INTRODUCTION

Several morphological and behavioral traits, such as microhabitat use, temporal activity, response to predator cues, or conspicuousness, contribute to the evasive response to predation risk and defensive response to predator attacks (Brodie et al. 1991). Besides, it is widely documented that individuals of a given population exhibit diverse behavioral, morphological, and life-history traits with antipredator functions (DeWitt et al. 1999; Hatle et al. 2001; Boeing et al. 2006; Hedrick and Kortet 2006; Miyatake et al. 2008; Mikolajewski et al. 2010; Pruitt et al. 2012). From an adaptive perspective, multiple antipredator traits are thought to merge into a common ultimate function: increasing survival through optimized predation avoidance (Lind and Cresswell 2005; Mikolajewski et al. 2010). Yet, the functional role of these multiple traits in mounting an efficient antipredator strategy remains underinvestigated (Pearson 1988; Brilot et al. 2012). Indeed, it remains rarely tested

whether different antipredator behaviors act as complementary or compensatory defenses, are expressed sequentially or simultaneously, in an additive or a synergistic way, or are intended towards different predators (Pearson 1989; Olmstead and Denno 1993; DeWitt et al. 1999; Hatle et al. 2001; Van Son and Thiel 2006; Brilot et al. 2012).

To what extent these multiple behaviors interact to merge into a common adaptive antipredator function is unknown. For instance, a recent study on the crustacean amphipod *Gammarus pulex* found that experimentally reduced photophobia does not impinge on predation by a benthopelagic fish (Perrot-Minnot et al. 2012), a result contrasting with the common interpretation of photophobia as an antipredator behavior in aquatic invertebrates (Wisenden et al. 1999; Perrot-Minnot et al. 2012 and references therein). This suggests that photophobia taken alone is not responsible for any significant protection from predation. Instead, photophobia is likely to be one component of general antipredator mechanisms that involve several behavioral and perhaps other phenotypic traits (Perrot-Minnot et al. 2012). Adopting an integrative framework is

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therefore crucial to understand the evolution of multidimensional antipredator defenses (DeWitt et al. 1999) and to determine how individuals can optimally and flexibly combine several dimensions of their antipredator behavioral repertoire.

Investigating how individuals shape their antipredator strategy through adjusting multiple behaviors involves several steps. First, it requires estimating the level of flexibility of each trait across risky and safe situations between which behavioral patterns are known to vary. For instance, under the “threat-sensitivity” hypothesis, prey species should adjust their antipredator strategy depending on the current threat imposed by potential predators (Sih 1986; Ferrari et al. 2010; Higginson et al. 2012; Brown et al. 2013). Thus, most evasive and defensive behaviors are expected to exhibit phenotypic flexibility in response to the level of predation risk (Kats and Dill 1998; Lass and Spaak 2003; Ferrari et al. 2010; Higginson et al. 2012; Brown et al. 2013). Second, individual differences in behavioral adjustment across risky and safe situations can be estimated to assess the potential for adaptive behavioral flexibility within a population. Third, determining how individuals combine multiple antipredator behaviors is a necessary step toward an integrative approach to the evolution of antipredator mechanisms. Recent studies suggest that predation risk may promote different patterns of association between risk-sensitive behaviors (Bell and Sih 2007; Dingemans et al. 2007; Adriaenssens and Johnsson 2013). Phenotypic correlations between antipredator behaviors should then be assessed in both risky and safe environments to improve our understanding of the role of behavioral flexibility in causing association patterns.

In this study, we investigate the effect of predation risk simulated by fish chemical cues on the expression, flexibility, and consistency of 3 antipredator behaviors and their association patterns in the freshwater amphipod *Gammarus fossarum*. In many freshwater organisms, evasive behaviors against predators extensively rely on abiotic and biotic cues such as light, shelter, and chemical cues and on selecting microhabitat and time of activity safe from predation (Kats and Dill 1998; Ferrari et al. 2010). In gammarid species in particular, antipredator mechanisms generally consist of several risk-sensitive behaviors such as activity, reaction to light or to gravity, refuge use, and aggregation (Wisenden et al. 1999; Åbjörnsson et al. 2000; Lind and Cresswell 2005; Perrot-Minnot et al. 2007; Kullmann et al. 2008; Miyatake et al. 2008; Durieux et al. 2012; Perrot-Minnot et al. 2012). The freshwater amphipod *G. fossarum* thus represents an appropriate model to investigate the multidimensionality of antipredator behavior across situations varying in risk levels. We used a within-subject design to address 3 questions: 1) Are photophobia, refuge use, and exploration tendencies risk-sensitive behaviors as predicted by the “threat-sensitivity” hypothesis? 2) Are between-individual behavioral differences consistent between safe and risky situations? 3) Do the patterns of association between these 3 behaviors vary between safe and risky situations?

MATERIALS AND METHODS

Amphipod collection and maintenance

We collected *G. fossarum* individuals (44 females and 30 males), a species closely related to *G. pulex*, from a population located in river Norges, Burgundy, eastern France (47.36°N, 05.158°E). Gammarids were acclimated to the laboratory for a week prior to experiments. They were maintained at 15 °C under a 12:12h light:dark cycle in large tanks filled with oxygenated dechlorinated UV-treated tap water and fed ad libitum with elm leaves. Two days

prior to behavioral assays, individuals were removed from the holding tank and isolated in 50-mL glass vials with food and shelter. All the manipulations presented here meet the ABS guidelines for ethical treatment of animals.

Behavioral assays

In studies of behavioral syndromes, the overall procedure requires the selection of a time lapse between repeated behavioral measurements and a specific order (fixed or randomized) (David et al. 2011; Bell 2013). Following Wilson et al. (2012) and Bell (2013) recommendations to take potential carryover effects into account, we decided to conduct behavioral trials with predator scent after trials without scent to avoid any prolonged aversive reaction from individuals after experiencing a risky situation (Wisenden et al. 1999). Two behavioral trials within the same treatment were run in a day (one in the morning and one in the afternoon). Trials in scented water were run the day after the last trial in water with no predator scent. We measured photophobia, refuge use, and exploration behavior in a fixed order first in the absence of predation threat and then with simulated predation risk. Each individual was tested sequentially for the 3 behaviors in oxygenated dechlorinated UV-treated water during the first 2 days and then in oxygenated dechlorinated UV-treated water with fish chemical cues during the next 2 days. After each behavioral trial, individual gammarids were returned to their glass vial provided with food and shelter.

Refuge use was quantified as the tendency to seek refuge in an open lit arena. To quantify sheltering tendency while controlling for potential interindividual differences in photophobia, we used a pile of translucent glass pebbles sealed with silicone adhesive as a refuge, placed close to the edge of an 11-cm diameter plastic circular box filled with 100 mL of water. Boxes were illuminated from above. After a 10-min acclimation period, the position of the focal gammarid was recorded by scan sampling every 20 s during 10 min. A score of 1 was given when the individual was found under or against the refuge and 0 when found out of it. Scores were then summed up and ranged from 0 (always outside) to 30 (always under refuge).

We assessed the exploratory behavior of individual gammarids in a 24 × 15 × 6 cm arena comprising 6 virtual areas of equal sizes, each offering the focal gammarid a vertical piece of wire mesh to cling onto. A focal gammarid was introduced in the middle of the arena, with a 5-min acclimation period. Then its behavior was observed for 10 min and its position was recorded every 20 s. Individuals were given a score of 1 if they had moved to a different area between 2 consecutive observations or 0 if they had remained in the same one, with a maximal score of 30. Scores were summed up to give a measure of exploration behavior, ranging from 0 to 30. Due to a technical problem, female sample size was reduced to 43 for exploration behavior.

Reaction to light was measured following Perrot-Minnot et al. (2012). The apparatus consisted in a 23 × 3 cm glass tube containing 100 mL of aerated dechlorinated UV-treated water at 15 °C, placed below 2 solar spectra fluorescent tubes (OSRAM Lumilux-865, Molsheim, France), providing a light intensity of 600 Lux. Half of the tube was covered with black elastomer (dark section of the apparatus). The focal gammarid was gently introduced inside through a hole placed at the middle of the apparatus and was given a choice between 1 lit and 1 dark section. Its position was recorded by scan sampling every 20 s during 5 min. Photophobia

was thereafter considered as a binary response variable (with individuals either emerging at least once from or staying in the opaque section of the apparatus throughout the whole trial).

Simulating predation threat using fish chemical cues

Simulating predation threat through the use of predator chemical cues is commonly used in ecological studies with aquatic prey (Kats and Dill 1998; Sih et al. 2003; Wilson et al. 2012) and more specifically with *G. pulex* (Wellnitz et al. 2003; Perrot-Minnot et al. 2007; Kullmann et al. 2008; Dianne et al. 2011; Durieux et al. 2012). We used the European chub *Leuciscus cephalus* as a predator. This cyprinid fish is commonly found in western-central European rivers and consumes small freshwater invertebrates such as gammarids. Ten European chubs ranging from 15 to 30 cm long were maintained in a large tank filled with oxygenated dechlorinated water for several weeks prior to the experiments and fed with live gammarids. Behavioral trials under predation threat were performed in scented water diluted from the chub tanks, at a final concentration of fish cues corresponding to 3 g of fish biomass per liter.

Statistical analyses

In our sample, males and females differed in their median weight (median [first quartile;third quartile]: males: 19.5 mg [14.0;23.7]; females: 10.3 mg [9.1;12.3]; $\chi^2 = 66.6$, degrees of freedom [df] = 1, $P < 0.0001$). In order to disentangle the effects of sex versus weight in further analyses, we computed for each individual an adjusted weight, hereafter referred to as “sex-adjusted weight,” calculated following this formula: “sex-adjusted weight” = (“individual weight” – “averaged weight for the corresponding sex”)/“standard error of weight for the corresponding sex.”

In all models, refuge use and exploration scores were transformed as a proportion of the highest value possible, allowing us to fit a binomial error structure to the models.

The influence of the risk treatment (predator scent present vs. absent) on individual behavior was assessed using generalized linear mixed models with a binomial error structure. Models were built with the behavioral variable (photophobia, refuge use, or exploration behavior) as the response variable and risk treatment (predator scent vs. no predator scent), sex, sex-adjusted weight, and all 2-level interactions as predictors. Individual identity was set as a random intercept variable. Risk treatment was added as a random slope variable to test for individual differences in behavioral responses across predation risk treatments (Schielzeth and Forstmeier 2009), aka behavioral reaction norms. This approach allows estimation of both the group-level mean effect of adding predator scent (fixed effect) and the individual variation in this response (random effect). The random slope variable was removed from the analyses involving photophobia to enable the model to converge. We provided Cohen's d as the effect size of behavioral change across risk treatment (Nakagawa and Cuthill 2007).

Behavioral consistency across the 2 risk treatments was estimated using generalized linear models (GLMs) with individual behavior in the predator scent treatment as the response variable and individual behavior in the no-predator scent treatment as a predictor. Error structure was set to binomial. The influence of sex and sex-adjusted weight on individual consistency in refuge use and exploration behavior across treatments was assessed using the following procedure: we extracted for each individual

the absolute value of the residuals of the above regressions to assess intraindividual variability (see David et al. 2012; Stamps et al. 2012). Then we tested the influence of sex and sex-adjusted weight on these residuals using GLMs, which error structures were set to Gaussian. To assess the consistency of photophobia, we compared, in males and females separately, the number of individuals that consistently stayed in or emerged from the opaque section of the apparatus in both risk treatments to the number of individuals that changed their behavior between risk treatments using a Fisher's Exact test. If individuals are overall consistent in their behavior, we expect the number of gammarids changing their behavior between treatments to be significantly lower than those that do not.

Correlations between behavioral traits were estimated within each sex and each risk treatment using Spearman's correlation coefficients and their 95% confidence interval, estimated using bootstrapping methods. To relate exploration behavior and refuge use to photophobia (with individuals staying in or emerging from the opaque section of the apparatus), we ran GLMs with exploration behavior or refuge use as a response variable and photophobia as a predictor. Error structure was set to binomial.

All statistical analyses were performed using the statistical free-ware R version 2.13.0 (R Development Core Team 2011) and the R package “lme4” (Pinheiro and Bates 2000). In all analyses, the minimum adequate model was obtained by backward stepwise term deletion of nonsignificant variables ($P > 0.05$).

RESULTS

Behavioral flexibility across risk treatments

Refuge use was higher when predator scent was present (average refuge use score = 16.2 vs. 11.7 when predator scent was absent; $b = 1.25$, standard error [SE] = 0.42, Cohen's $d = 0.430$) (Figure 1a and Table 1) and in heavier individuals ($b = 0.46$, SE = 0.21, $r_s = 0.09$; Table 1); but it was not affected by sex ($b = 0.70$, SE = 0.40; Table 1). Intraindividual variation in refuge use across treatments significantly differed between individuals (significant random effect in Table 1).

Exploration behavior did not vary between the predator scent (average exploration score = 20.2) and the no-predator scent treatment (average exploration score = 21.0; $b = -0.17$, SE = 0.15; Cohen's $d = -0.106$) (Figure 1b and Table 1). Sex ($b = -0.09$, SE = 0.23) and sex-adjusted weight ($b = -0.06$, SE = 0.11) had no influence on exploration behavior (Table 1). Intraindividual variation in exploration behavior across risk treatments significantly differed between individuals (significant random effect in Table 1).

In photophobia trials, fewer individuals emerged at least once from the opaque section of the apparatus when predator scent was present (15.6% of them) than when they were absent (37.2% of them) ($b = -1.63$, SE = 0.49; Table 1). Sex-adjusted weight ($b = 0.54$, SE = 0.31; Table 1) and sex ($b = -0.22$, SE = 0.63; Table 1) had no effect on photophobia.

Behavioral consistency across risk treatments

Refuge use when predator scent was absent significantly but only slightly predicted refuge use when predator scent was present ($b = 0.032$, SE = 0.005, $\zeta = 6.50$, $P < 0.0001$, $R^2 = 0.04$). Intraindividual variability in refuge use was not influenced by sex ($b = -0.33$, SE = 0.53, $t = -0.62$, df = 72, $P = 0.54$,

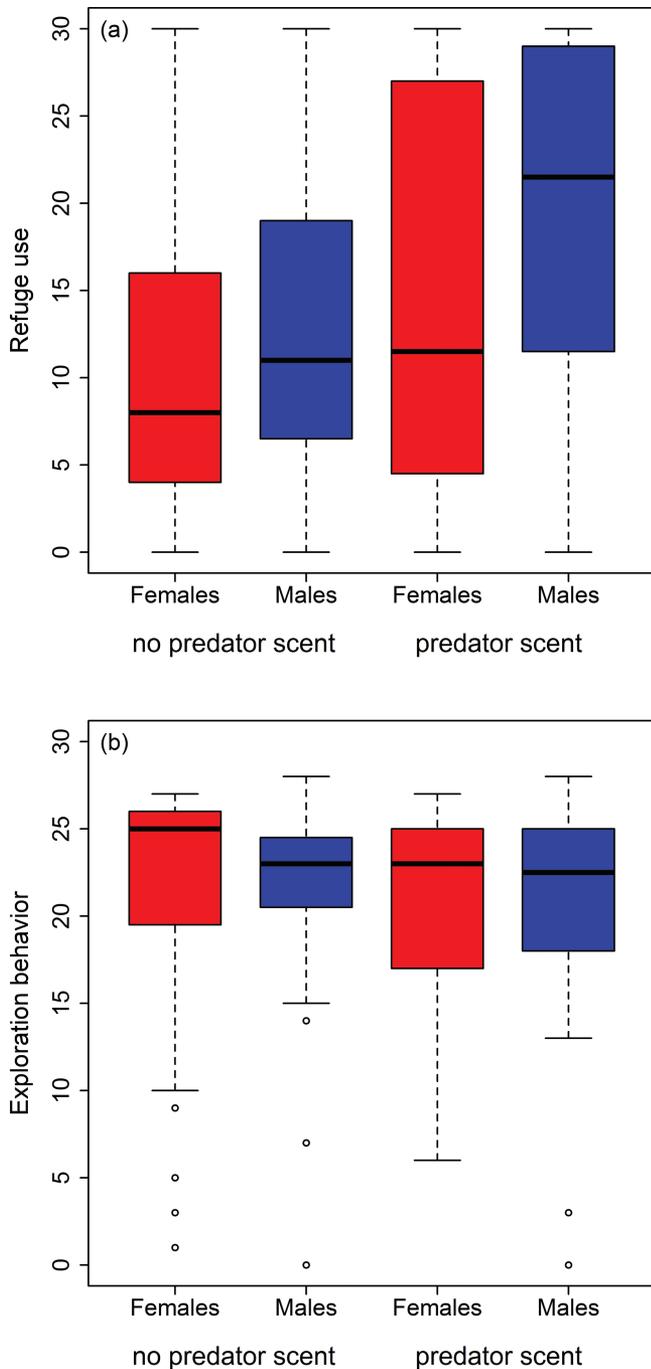


Figure 1

Refuge use by *Gammarus fossarum* females and males in the 2 risk treatments (a) (boxplots show median, interquartile range, and minima and maxima extreme values; $N = 45$ females and 33 males). Refuge use was quantified as the relative position of the gammarid during a 10-min trial, ranging from 0 (always outside the refuge) to 30 (always under the refuge). Exploration tendencies of *G. fossarum* females and males in the 2 risk treatments (b) (boxplots show median, interquartile range, ± 1.5 interquartile range and outliers; $N = 45$ females and 33 males). Exploration tendencies were assessed by scan sampling the focal gammarid and quantifying its movements during a 10-min trial and range from 0 to 30.

$R^2 < 0.01$) nor by sex-adjusted weight ($b = 0.28$, $SE = 0.26$, $t = 1.08$, $df = 73$, $P = 0.29$, $R^2 = 0.02$). Exploration behavior when predator scent was absent significantly predicted exploration behavior

when predator scent was present ($b = 0.056$, $SE = 0.007$, $z = 8.15$, $P < 0.0001$, $R^2 = 0.14$). Intraindividual variability in exploration behavior was not influenced by sex ($b = 0.09$, $SE = 0.13$, $t = 0.75$, $df = 72$, $P = 0.46$, $R^2 < 0.01$) nor by sex-adjusted weight ($b = 0.01$, $SE = 0.13$, $t = 0.22$, $df = 71$, $P = 0.83$, $R^2 < 0.01$). Overall, individuals tended to be significantly, though weakly, consistent in their exploration behavior and their use of the refuge across the 2 risk treatments.

In photophobia trials, the number of females that were consistent in their reaction to light, scored as the tendency to stay in or emerge from the opaque section of the apparatus (Fisher's Exact test: $P = 0.017$; Table 2), was higher than the number of female changing their behavior across treatments. No significant difference was found in males (Fisher's Exact test: $P = 0.13$; Table 2).

Correlations among behavioral traits within risk treatments

In females, exploration behavior was negatively related to photophobia only when predator scent was absent (Table 3a and Figure 2a). Photophobia and refuge use were positively linked when predator scent was present (Table 3a and Figures 2b and 3b), but not when predator scent was absent (Table 3a and Figures 2a and 3a).

Males that were more photophobic were more likely to use the refuge both when predator scent was absent (Table 3b and Figures 2c and 3c) and when predator scent was present (Table 3 and Figures 2d and 3d). Exploration behavior was negatively related to both refuge use and photophobia in males when predator scent was absent (Table 3b and Figure 2c), whereas this relationship was not significant when predator scent was present (Table 3b and Figure 2d).

DISCUSSION

Behavioral responses to predation risk

In this experiment, we assessed refuge use, exploration behavior, and photophobia in field-collected *G. fossarum*. Our assumption was that these 3 behaviors had risk-sensitive properties and that individuals should thus be flexible across risk treatments. In accordance with the "threat-sensitivity" hypothesis (Sih 1986), our results indeed provide evidence that *G. fossarum* are sensitive to change in fish predation threat: both males and females increased photophobia and refuge use in the presence of fish chemical cues. These findings are in agreement with previous studies reporting an increase in refuge use under predation risk in the closely related species *G. pulex* (Perrot-Minnot et al. 2007; Dianne et al. 2011) and in many other aquatic animals (Kats and Dill 1998; Wisenden et al. 1999; Ferrari et al. 2010). They also confirm the importance of chemical cues as reliable information about potential predator threat in aquatic organisms (see Kats and Dill 1998 and Ferrari et al. 2010 for reviews). This suggests that altering photophobia and refuge use may be a behavioral adaptation aimed at deterring predation threat and that behavioral flexibility is being selected in this respect. More studies involving differential predation experiments are yet needed to test this assertion.

However, contrary to expectations, individuals did not decrease their exploration behavior in the presence of fish chemical cues. Between-individual variation in our exploration measure was low, with a lot of individuals reaching the highest scores possible.

Table 1

Effect of risk treatment—predator scent present versus absent—on 3 behaviors (refuge use, exploration, and photophobia) in the crustacean *Gammarus fossarum*

	Refuge use		Exploration		Photophobia	
	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value
Fixed effects						
Sex	2.85	0.09	0.16	0.69	0.11	0.74
Risk treatment	8.14	0.004	1.15	0.28	11.03	<0.001
Adjusted weight	4.53	0.033	0.30	0.59	2.80	0.09
Sex × risk treatment	0.72	0.40	0.06	0.80	0.20	0.65
Sex × adjusted weight	<0.01	0.93	1.06	0.30	3.24	0.07
Risk treatment × adjusted weight	3.33	0.07	0.03	0.88	<0.01	0.93
Risk treatment × adjusted weight × sex	2.24	0.14	0.46	0.50	0.69	0.41
Random effect						
Risk treatment	839.49	<0.0001	149.46	<0.0001	—	—

P values were obtained through a backward stepwise method by successively removing the least significant term of the model. The “risk treatment” random slope effect corresponds to the among-individual differences in behavioral changes between the 2 risk treatments. It was not added to the analyses involving photophobia to enable the model to converge. Values in bold indicate significant terms at the 5% threshold.

Table 2

Consistency of photophobia of *Gammarus fossarum* between the 2 risk treatments—predator scent present versus absent

	Predator scents	
	=0	>0
No predator scents		
Females		
=0	6	10
>0	2	27
Males		
=0	3	8
>0	1	18

Photophobia is estimated from the tendency to stay in (=0) or emerge at least once (>0) of the opaque section of the apparatus during a 5-min trial. The number of individuals that consistently stayed in or emerged in both risk treatments was compared with the number of individuals that changed their behavior between the 2 treatments, using a Fisher's Exact test: females, $P = 0.017$ and males, $P = 0.13$. Numbers in bold indicate the number of individuals that were consistent in their behavior between treatments.

Our experimental design might thus have been inappropriate to assess interindividual variation and intraindividual consistency in exploration behavior. To date, few protocols have been designed to measure exploration tendencies in aquatic crustaceans (Mowles et al. 2012; Chapman et al. 2013). Given these technical limitations, our findings tend to suggest that exploration behavior may not be functionally related to antipredator defense in *G. fossarum*, possibly because individuals do not benefit from reducing their exploration behavior in the presence of fish chemical cues. Behavioral adjustments in the face of predation risks may then be more likely to have evolved in a domain-specific than in a domain-general manner (Brilot et al. 2012). In other words, *G. fossarum* behavioral responses to predation risk are less likely to manifest themselves as a decrease in general activity but rather as changes in functionally selected behavioral tendencies. Yet, previous findings show that *Gammarus minus* and mosquitofish *Gambusia holbrooki*, respectively, decrease their general activity and their exploration behavior in the presence of injury-released cues from conspecifics (Wisenden et al. 1999; Ward 2012). Decreased activity was also found to confer

amphipods a higher protection from predation (Wisenden et al. 1999). Further studies linking exploration to survival in controlled settings are thus needed to identify the viability selection pressures applying on exploration behavior.

Sex-adjusted weight was found to be related to refuge use although the effect of weight cannot be distinguished from that of age in a species with continuous growth over lifetime. Heavy individuals were more likely to use the refuge than lighter individuals. These findings could be interpreted as heavier individuals acting more cautiously because they are more likely to be predated than lighter individuals. The reason for this could lie in a higher probability of being detected by predators or in a lower number of safe microhabitats to hide in. Such size-related predation susceptibility has been reported in other aquatic crustaceans (Straile and Hälbig 2000). Thus, heavier individuals may behaviorally compensate for their phenotypically driven higher susceptibility to predation. Further ecological and longitudinal studies are needed to determine whether individuals of different sizes are more or less likely to get predated and to what extent general boldness covaries with size in this species.

Evidence for behavioral consistency across risk treatments

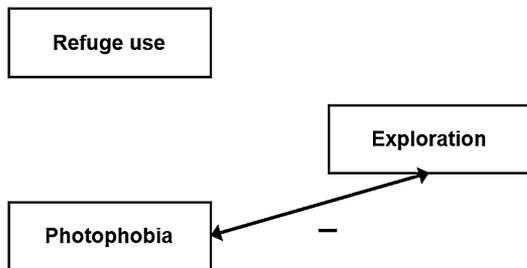
Similar to what has been observed in other species (Sih et al. 2003), we found evidence for low but significant behavioral consistency (sometimes called “personalities” or “behavioral carryover”) in the antipredator behavior of *G. fossarum*, with individuals consistently differing in refuge use, in exploration, and in photophobia across the 2 risk treatments. Behavioral consistency across risk treatments indicates that some individuals are found to be bolder or shyer than others both in the absence and in the presence of predation threat. Given that individuals are thought to optimally adjust their behavior between both safe and risky treatments, we expect the optimal behavior to differ between these 2 risk treatments. However, differences among individuals are observed, which suggest that all individuals do not adopt the optimal behavior in both risk treatments, or alternatively, that optimal strategies depend on other traits or state-dependent variables that also differ between individuals. Further studies should investigate the forms of antipredator behaviors' fitness functions to identify the selection pressures shaping

Table 3
Among-behavior correlations in females (a) and males (b), and in the 2 risk treatments

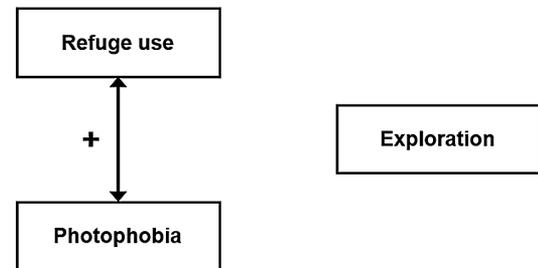
	No predator scents				Predator scents			
	r_s/χ^2	95% CI	P value	n	r_s/χ^2	95% CI	P value	n
a) Females								
Exploration–refuge use	−0.15	−0.46, 0.18	0.34	43	−0.13	−0.45, 0.20	0.39	43
Photophobia–refuge use	0.91	—	0.34	44	69.18	—	<0.0001	44
Exploration–photophobia	10.01	—	0.002	43	1.60	—	0.21	43
b) Males								
Exploration–refuge use	−0.46	−0.71, −0.11	0.012	30	−0.22	−0.59, 0.18	0.25	30
Photophobia–refuge use	29.81	—	<0.0001	30	9.52	—	0.002	30
Exploration–photophobia	22.18	—	<0.0001	30	1.53	—	0.22	30

We used Spearman's correlation analyses (r_s and 95% confidence interval [95%CI]) or GLMs. Photophobia was expressed as a binary variable (with individuals either staying in or emerging from the opaque section of the apparatus). It was related to exploration behavior and refuge use by using GLMs fitted with a binomial error structure. Values in bold indicate significant terms at the 5% threshold.

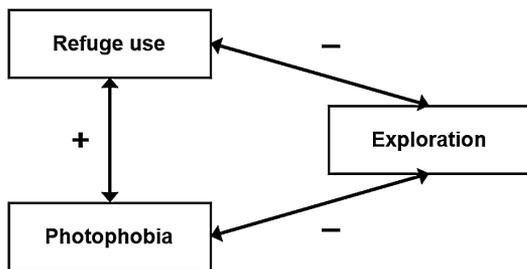
a) Females – no predator scents



b) Females – predator scents



c) Males – no predator scents



d) Males – predator scents

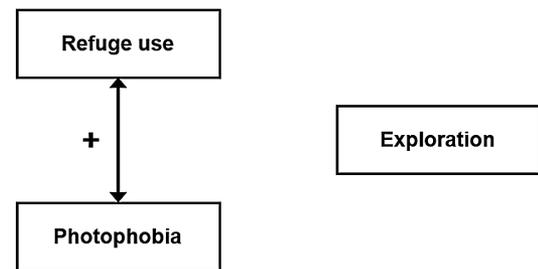


Figure 2

Diagram showing the dynamic relationships between the 3 behaviors measured in *Gammarus fossarum*, according to sex—females (a, b) and males (c, d)—and risk treatment—without (a, c) or under (b, d) predation risk simulated by chub scent. Arrows and signs indicate the direction of significant correlations between behaviors. Despite exploration behavior being related to other behaviors, it has been shown not to be sensitive to predation threat and thus is unlikely to have a significant role in individuals' general antipredator strategy (see Discussion for more details).

these behaviors and the potential for the maintenance of intraspecific variation (Kight et al. 2013).

The emergence of behavioral syndromes

Our within-subject experimental design enabled us to investigate the association patterns between behaviors and, in particular, to determine whether a risky environment can promote

correlations between them (Bell and Sih 2007; Dingemanse et al. 2007). Exploration behavior was not related to any other behavior when fish cues were present. Thus, predation risk does not seem to promote any correlations involving exploration. This result may not be surprising as exploration behavior was found not to be risk sensitive (i.e., to vary between safe and risky treatments). Behavioral correlations involving exploration might then be negligible in shaping effective antipredator defenses.

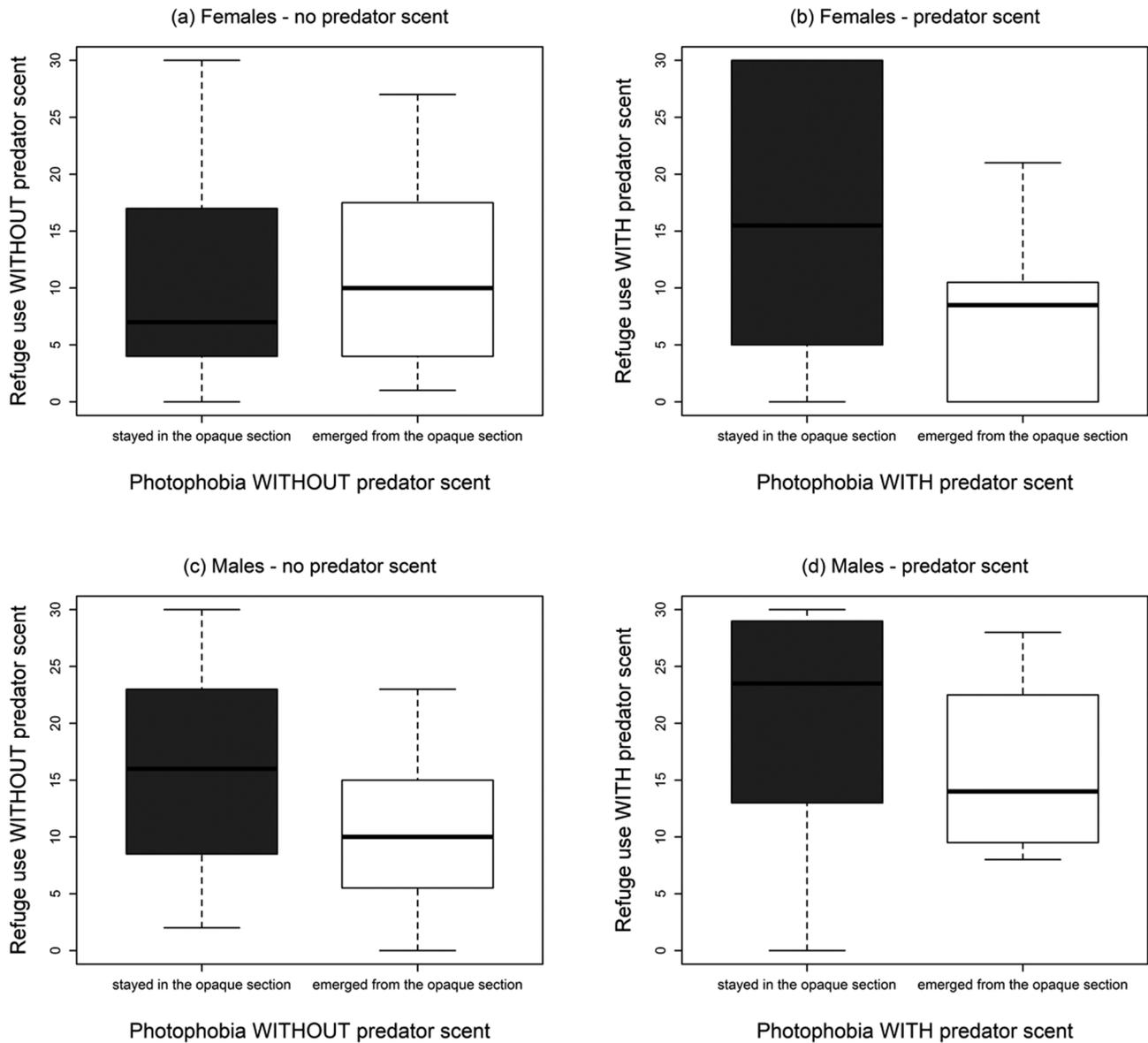


Figure 3

Relationships between refuge use and photophobia (scored as a binary variable) in *Gammarus fossarum* females (a, b) and males (c, d), according to risk treatment—without (a, c) or under (b, d) predation risk simulated by chub scent. More photophobic individuals (gray boxplots) significantly spent more time under a translucent refuge than less photophobic ones (white boxplots), except in (a).

Focusing on the 2 risk-sensitive behaviors, we provide evidence that predation risk may promote associations between them in one sex. In females, no relationship between photophobia and refuge use was found in the absence of fish cues, whereas photophobia was positively linked to refuge use when predator scent was present. In males, refuge use was positively linked to photophobia both in the absence and in the presence of fish chemical cues. Males that were more photophobic were also more willing to use the refuge. Such relationship was not due to individuals responding to the same stimulus (light) because the shelters offered to gammarids were translucent. It then seems that simulated predation threat can promote correlations between 2 consistent antipredator behaviors in *G. fossarum*, at least in females, which is in accordance with findings in other freshwater species (Bell and Sih 2007; Adriaenssens and Johnsson 2013). The fact that the correlation appeared in the

risky treatment suggests that some combinations of photophobia and refuge use may be particularly efficient in the face of predation. The reasons why behavioral correlations between photophobia and refuge use are present in both treatments in males remain unknown. It may be that males overall incur greater predation risks than females, forcing them to adopt appropriate behavioral combinations all the time. The particular reproductive ecology of *G. fossarum*, where costly precopulatory mate-guarding lead to a high sexual competition, may explain why sex-based differential predation could occur and maybe why the multiple dimensions of antipredator behaviors follow different patterns between sexes in *G. fossarum*.

Individuals may adjust their behavior to match optimal behavioral combinations when predation risk becomes salient. An important point here is that not all individuals appeared

to maximize safety through maximal refuge use and maximal photophobia. In other words, the benefits of sheltering and photophobia may not be additive. Rather, individuals seem to flexibly converge to a particular combination supposed to provide increased protection from predation (Bell and Sih 2007; Lee and Berejikian 2008). Behavioral traits taken in isolation might not contribute to increased avoidance or defense, but rather, multiple traits may complement each other (DeWitt et al. 1999). In support of this interpretation, decreased photophobia taken alone does not seem to increase the susceptibility to predation of the closely related species *G. pulex* (Perrot-Minnot et al. 2012). Our study is however restricted to 3 behavioral traits, and a more comprehensive view of multidimensionality of antipredator defenses should be reached by including additional behavioral, morphological, and life-history traits. Further experimental studies are also needed to investigate the fitness consequences of alternative combinations of antipredator behaviors. Overall, our results call for an integrative study of antipredator mechanisms where the evolution of each dimension has to be examined in combination with all others (DeWitt et al. 1999; Lailvaux and Kasumovic 2011).

CONCLUSIONS

Our study provides original findings on the multidimensionality of antipredator strategy and an experimental demonstration that predation risk may affect behavioral correlations, at least in females. First, we have demonstrated that *G. fossarum* adjust their photophobia and refuge use between a safe and a risky treatment following the “threat-sensitivity” hypothesis. Exploration behavior was not affected by fish chemical cues, which suggests that exploration may not be functionally related to survival in this species. We also found weak but significant evidence for the existence of consistent individual differences in *G. fossarum*. Additional work is needed to understand why individuals are not completely flexible, what the forms of behavioral traits’ fitness functions are, and how intraspecific variation in behavioral traits is maintained (Kight et al. 2013).

Finally, we showed that predation threat can promote the association between 2 different behavioral dimensions of antipredator defenses, at least in females, providing a potential explanation for the emergence of behavioral correlations or syndromes. Also, such correlation indicates that the benefits of combined antipredator behavioral dimensions may be synergistic (or complementary, sensu DeWitt et al. 1999) rather than additive in a freshwater crustacean. It still remains to understand why, from a functional point of view, some behavioral combinations may be particularly efficient in the face of predation. This can be achieved through controlled predation experiments (Perrot-Minnot et al. 2007, 2012; Dianne et al. 2011).

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