LAY SUMMARY

Gammarids showed no predator-specific grouping response, and only formed groups in response to conspecific injury cues. This contrasted with our prediction that different predators that vary in their tendency to attack grouped prey ought to elicit specific grouping responses. There are a number of reasons that may account for this finding.

Gammarus pulex show a grouping response to conspecific injury cues but not to predator kairomones

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ABSTRACT

Many species gain protection from predators by forming groups, but there is also evidence that some predators are better able to detect or more likely to attack grouped prey. Given this, it might pay prey to be flexible in their group behaviour, forming groups upon detecting certain predators, but dispersing when detecting others. In the first of two experiments, we found that flounders (*Platichthys flesus*) were more likely to attack larger groups of gammarids (*Gammarus pulex*) than smaller ones, while sticklebacks (*Gasterosteus aculeatus*) showed no such bias. This gave us the opportunity to test the idea that prey might show predator-specific grouping responses. Accordingly, our second experiment compared the grouping behaviour of gammarids exposed to kairomones from either of the two predators, to conspecific injury cues (a non-specific predation cue), to combinations of predator kairomone plus conspecific injury cues and finally to two control treatments. We predicted, based on our first experiment, that the gammarids would disperse in response to flounder kairomones, and group more cohesively in response to stickleback kairomones and conspecific injury cues. In fact only the treatments including conspecific injury cues elicited a grouping response in the gammarids, while predator kairomones alone had no effect whatsoever on group cohesion or dispersal. We discuss possible explanations for these findings and briefly consider other systems that might be better suited to exploring predator-specific anti-predatory grouping behaviour.

KEY WORDS: Alarm substance; Anti-predator; Collective response; Predator-prey; Selfish herd; Schreckstoff

INTRODUCTION

Group formation is a common response to predation (Krause and Ruxton, 2002). Aggregating prey potentially gain a range of benefits, including diluted per capita risk of capture (Foster & Treherne 1981; Godin 1986; Morgan & Colgan 1987), confusion of predators (Tosh et al. 2006) and shared vigilance costs (Roberts 1996). Probably for these reasons, grouping behaviour has evolved as an anti-predator response in many different taxa. Grouping behaviour is not inherently advantageous, however: aggregating brings various costs, including increased competition for resources (Krause and Ruxton 2002), and exposure to horizontally-transmitted parasites and pathogens (Rifkin, et al. 2012). Given these costs and benefits, observed group sizes often vary depending upon the ecological context, with smaller, more dispersed groups occurring when animals are foraging for dispersed food, and larger, denser groups forming when predators are detected (Hoare et al. 2004).

The dynamic, flexible group-size responses to the costs and benefits of grouping are further complicated by the fact in certain predator-prey interactions, grouping may sometimes actually be maladaptive. While some predatory species may avoid attacking larger groups in favour of smaller ones, where their likelihood of capturing prey is greater, in other cases, larger groups may be more likely to be attacked by predators (e.g. Botham et al. 2005; Botham & Krause 2005). This may occur if these predators are better able to detect larger groups, or if they preferentially target larger over smaller groups. If the likelihood of being attack by a predator is directly proportional to the number of individuals in the group then the prey may do no better by grouping than they would if they were alone, and if the risk of a successful attack increases

disproportionately with increasing group size then prey might actually be expected to form smaller groups, or not to group at all (Krause & Ruxton 2002). In nature, many prey species are hunted by a range of different predators. Given this, grouping by prey individuals may be an appropriate response to some predators, but a maladaptive one to others, leading to the prediction that prey species with multiple predators should flexibly and adaptively vary their grouping response, according to the type of predator that they are faced with.

To test this prediction, that prey animals should modify their grouping response depending upon predators that they are faced with, we used groups of gammarids (*Gammarus pulex*) as a model prey system. *G. pulex* are small (<15 mm length) detrivorous amphipod crustaceans that occur in large aggregations in freshwater streams and rivers throughout northern Europe (Williams & Moore 1986). They are an appropriate model species for addressing this question, since they are an important prey species for many invertebrate, bird and fish predators (MacNeil et al. 1999), and exhibit readily quantifiable anti-predator behaviours (Andersson et al. 1986; Wudkevich et al. 1997; Wisenden et al. 1999; Åbjörnsson et al. 2000; Kullmann et al. 2008; Ahlgren et al. 2011). Of particular relevance to our study, *Gammarus* are known to exhibit grouping behaviour in response to both predator kairomones (Kullmann et al. 2008) and conspecific injury cues (Wisenden, et al., 2001). Conspecific injury cues, *schreckstoff* (von Frisch 1938), are a non-specific indicator of predation threat that consist of chemicals released in bodily fluids from individuals that have suffered mechanical damage, such as through mastication by a predator. Detection of conspecific injury cues is known to bring about anti-predatory responses such as fleeing, hiding or aggregating in a range of aquatic species (Chivers & Smith 1998; Brown 2003; Ferrari et al. 2010). As predators we used juvenile flounders (*Platichthys flesus*) and threespine

sticklebacks (*Gasterosteus aculeatus*). Both species co-occur with and prey upon *Gammarus pulex* (Radforth 1940; Hynes 1950).

We performed two experiments. The first was designed to determine whether different predators differed in their tendency to attack larger groups of prey. This experiment revealed that when given a binary choice of attacking differently sized groups of gammarids, flounders were more likely to first attack the larger group, while the sticklebacks showed no such preference, being equally likely to attack larger or smaller groups first. This suggests a cost to grouping when attacked by flounders, and given that the flounders are capable of consuming many gammarids in one feeding bout, it further suggests that dispersing might be a more adaptive response than grouping upon detecting a flounder. In contrast, grouping may be the most appropriate response to predation from sticklebacks, in order to minimise per capita predation risk.

This gives rise to a prediction of a bidirectional grouping response -grouping or dispersing- in response to stickleback or flounder predator cues, which forms the basis of our second experiment. Here, groups of gammarids were exposed to direct predator cues, in the form of kairomones contained in water that had held equal biomasses of either flounders or sticklebacks; to indirect predator cues, in the form of the filtrate of crushed conspecifics, simulating the release of bodily fluids from preyed upon individuals; to combined treatments that included both conspecific injury cues plus either flounder or stickleback kairomones; and to each of two controls, consisting of the addition of clean tank water or no addition of any water to the arena containing the gammarid groups. We compared two measures of grouping behaviour, mean nearest neighbour distance, a measure of local interactions, and mean distance from group

centroid, a measure of group-wide cohesion. We predicted that the gammarids would form more cohesive groups in response to indirect, conspecific injury cues and direct predator cues from sticklebacks, but that they would disperse in response to cues from the flounders. We further predicted that this bidirectional, predator-specific grouping effect would be compounded by the combined presentation of predator cues and conspecific injury cues, since this could indicate both that predators were present and actively foraging.

METHODS

Experiment 1: Do flounders and sticklebacks attack larger groups of gammarids?

Experimental Animals

Gammarids, threespine sticklebacks and flounders were collected from the Kinnessburn, a small stream in St Andrews, UK (56.3349° N, 2.7885° W) in September 2012. Several hundred Gammarids were collected using aquarium nets. Individuals infected with the parasite *Pomphorhynchus laevis*, as identified by a conspicuous orange patch on the cuticle, were rejected, as these parasites are known to affect the anti-predator behaviour of their hosts (Bakker et al. 1997; Perrot-Minnot et al. 2007). In the laboratory the gammarids were divided between three 90L aquaria containing de-chlorinated fresh water and a shallow covering of coarse gravel. They were fed daily with Aquarian brand goldfish flakes, as well as being provided with dead leaves collected from the Kinnesburn to supply additional food and a source of shelter. They were left to acclimatise for a period of approximately six weeks before experimental trials began.

Approximately 100 juvenile flounders (35-55mm long) and 100 adult sticklebacks (45-55 mm long) were captured using mesh cage traps. These were held in single-species groups of 25 in 90L aquaria containing de-chlorinated fresh water and a shallow covering of sand. The aquaria with the sticklebacks also contained artificial plants, for cover. They were fed frozen bloodworms once per day. The aquaria for all three species were equipped with independent external filters, ensuring no water mixing between aquaria. Laboratory temperature was maintained at 8°C and the light: dark regime was held at 12: 12 hours. At the end of the experiment all animals were released at the point of capture.

Experimental Arena and Procedure

We set up an experimental arena in a black plastic vat measuring 100x80x40cm (LxWxH). This contained a 1 cm deep sand substrate and a water depth of 15cm. Two holding cylinders were placed in the arena, 25 cm from either end, along the longest axis. These had a diameter of 12 cm and were 25cm tall. They were constructed from colourless, perforated plastic (Penn Plax brand tank dividers), with 5 1mm diameter holes per cm^2. These were used to house the groups of gammarids. Water was pumped into each of the cylinders at a rate of 0.1L / minute via a 5mm silicone hose from an external reservoir. This water was drawn from the same source as the water in the test arena and was intended to carry gammarid chemical cues out of the cylinders and into the main arena. This caused the water level in the experimental arena to rise by approximately 0.025 cm per minute, giving an increase in depth of approximately 0.9 cm over the duration of the longest trial. A test fish holding unit measuring 10x10x30cm and constructed from the same material as the holding cylinders was placed against the centre of one of the long

walls. This was attached to a pulley allowing it to be raised, to release the test fish. The whole apparatus was surrounded by white plastic screening to minimise external disturbance. A Logitech C600 webcam mounted above the arena was used to film the trials.

Trials took place in October and November 2012. The experiment had three group size treatments, 6 vs 0, 5 vs 1 and 4 vs 2, and 20 fish of each species were tested within each gammarid group size treatment. The species and group size trials were run in a randomly predetermined order. No fish or gammarid was used more than once. In between trials the arena and holding units were thoroughly cleaned and the water, sand and gammarids were replaced.

At the start of the trial we added 6 male gammarids measuring 8-10mm in length to the cylinders. The gammarids were distributed between the two cylinders according to the group size distribution to be tested. The cylinder containing the larger number of gammarids was selected at random. As soon as the gammaraids were added, the water pumps feeding each cylinder were switched on, and a single test fish (flounder or stickleback) was added to the test fish holding unit. The test fish and gammarids were allowed to settle for 15 minutes. Following this, the fish holding unit was raised 10 cm, releasing the fish and beginning the trial. The trial was allowed to continue until the test fish attacked one of the cylinders containing the gammarids. Attacks took the form of a series of sharp bursts or lunges directed against the wall of the cylinder. Typically these appeared to be directed at a gammarid on the other side of the cylinder wall, though there were several trials in the 6 vs 0 gammarid trial where fish of both species attacked the empty cylinder. None of the gammarids were actually consumed or damaged during these trials.

Statistical Analyses

Here the response variable was the first group of gammarids attacked, larger or smaller, generating a binary variable. We used a binary logistic regression to analyse these data, with predator species (flounder or stickleback), the gammarid group size treatment (6 vs 0, 5 vs 1 or 4 vs 2) and the location of the cylinder containing the larger group of gammarids (left or right) included as fixed factors, and latency to attack included as a covariate. Additionally, a Cox regression was used to compare the latency to first attack between the two species and across the three group size conditions.

Experiment 2: Do gammarids display different grouping responses to different predator cues?

Experimental Animals

Threespine sticklebacks (40-45 mm in length) and flounders (40-60mm in length) were collected from the Kinnessburn, St Andrews, UK in August 2013. Gammarids were collected in November 2013. All were captured from the same location and using the same methods, and housed in the same laboratory under the same conditions as described for Experiment 1. In order to avoid sexual or related behaviour which may have interfered with the measurement of grouping behaviour, only male gammarids measuring between 8-12mm were used in the experimental trials described below. No individual was tested more than once. At the end of the experiment all animals were released at the point of capture.

Experimental Procedure

Experimental Arena

Trials were performed in rectangular arenas (60 x 35 x 5 cm). These were lined with white adhesive plastic to maximise the contrast between the animals and the base of the arena. Two such arenas were set up side by side, allowing two trials to be run simultaneously. Each was illuminated by a bank of LED lights placed around the sides of the arenas and directed upwards onto a white plastic reflector sheet, providing low, even illumination across the arenas. A Logitech C600 webcam was fixed centrally above each arena, and whole set up was screened off with rigid white plastic sheets to minimise external disturbance. Trial videos were filmed at 15 frames per second.

General Procedure

At the start of each trial, each arena was filled with 2.9l of de-chlorinated fresh water. The experimental cue (described below) was then added to the arenas. We applied the same treatment cues to each arena in the pair. In all treatments except the no water control treatment, the experimental cue, in the form of de-chlorinated tank water containing either a predation cue, or in the case of the second control treatment, no additive, was sprayed onto the surface of the water in the arena, using a misting spray. Five sprays were delivered to each arena, one to each corner and one to the centre. Following Hoare, et al. (2004) pilot trials were first run in which we

sprayed blue food colouring onto the water to demonstrate that the liquid spread rapidly within the arenas. Where stickleback, flounder or conspecific injury cues were presented alone, a total of 0.5ml of the cue was delivered. Where both conspecific injury cues and predator cues were presented in the same trial, each cue was sprayed five times (0.5ml each), such that 1 ml total of cues were presented. Immediately following this, two groups of six gammarids were collected from their tank and each group was placed into a plastic cup containing 0.1 l of de-chlorinated fresh water. Five minutes after the treatment cues had been added to each arena, the cups were gently tipped into the centre of each arena, releasing the six gammarids and bringing the total volume of water in each arena up to just over 3l. The gammarids were left to acclimatise to the arenas for five minutes before the webcams were turned on to record their movements from above for a period of ten minutes. At the end of each trial the gammarids were removed to new housing tanks and the arenas were emptied and rinsed thoroughly to avoid interference with subsequent trials. For each of the seven treatments outlined below we performed ten replicates, carried out as five sets of paired trials each.

Preparation of Experimental Cues

i. Direct predator cue treatments

Here we looked at the effects of direct predator cues, in the form of kairomones from sticklebacks and flounders upon gammarid grouping behaviour. To prepare the predator cues, six stickleback (45-55mm length) and five flounder (40-60mm length, approximately equal biomasses of fish) were placed into single-species aquaria (30 x 30 x 30 cm) containing sand, 15l de-chlorinated fresh water and artificial plants. The sides of each tank were wrapped in black

plastic to minimise stress and the fish were left for 24 hours. After this 24-hour period had passed, the fish were returned to new housing tanks and two 1l spray bottles were filled with the water, one from each of the flounder and stickleback tanks.

ii. Indirect predation cue treatment: conspecific injury cues

Eight male (8-10mm) and eight female (6-8mm) gammarids were crushed into a fine paste using a mortar and pestle (Wudkevich et al. 1997; Wisenden et al. 1999; Wisenden et al. 2001). De-chlorinated fresh water was added to the paste to bring the final volume up to 50ml. This was filtered through a muslin cloth to remove any larger pieces of remaining cuticle. The filtrate was then immediately transferred to a spray bottle.

iii. Combined direct and indirect cue treatments

The predator kairomone and conspecific injury cues were prepared as described above. Each was decanted into a separate spray bottle, and applied to the arenas as described above in the *General Procedure* section.

iv. Control treatments

We ran two control treatments. The first was a comparison control, in which nothing was added to the arena prior to the release of the gammarid groups. The second was a delivery control. Here, 0.5ml of water containing no additional cues was applied to the surface of the arena. The water was drawn from the same reservoir as was used to fill the arenas.

Response variables

From the trial videos we collected data on the distance of each individual from the group centroid, and to its nearest neighbour. We also calculated the mean swimming speed of each individual gammarid for every replicate group. Because the water in the experimental areas was very shallow we treated the trials as two dimensional systems. This allowed us to use basic trigonometry to calculate grouping metrics and individual swimming speeds. Individual measures of distance from group centroid and nearest neighbour distance are by definition not independent within groups, while individual swimming speeds are likely to be influenced to a lesser or greater extent by the behaviour of others within the replicate group. For these reasons, we use the group means of these measures averaged across the ten minute trial duration as our units of analysis. We saw no trends of increase or decrease in any of these measures over the trial. To confirm this, for each replicate group we determined distance from centroid, nearest neighbour distance and mean swimming speed (mean for the preceding 60 seconds) at 60 second intervals (procedure described below), yielding ten values covering the duration of the trial. These were compared within treatments using Friedman non-parametric repeated measures tests, which revealed no differences in any of the three response variables across sampling intervals in any of the treatment groups. Test statistics are provided in Table 1. For illustration, correlation coefficients (median and 20th and 80th percentiles) are also included in Table 1, and also provide no evidence for a change in any of these measures over the duration of the trial. Because neither distance from centroid, nearest neighbour distance nor mean swimming speed were seen to increase or decrease consistently in any of our treatment groups, we used whole-trial mean measures of each of these response variables in our analyses.

Videos were processed using Logger Pro (Vernier Software & Technology). This was used to obtain coordinates for each animal at 30 frame intervals (corresponding to two second sampling intervals). The mean coordinate of all individuals within the trial group was calculated at each sampling interval, giving the location of the group centroid. From these measures we calculated the mean distance of each individual from the group centroid per sampling interval, and from these in turn, the mean for the trial as a whole. Inter-individual distances were used to identify nearest neighbour distances for each individual. Again, we used these measures to first calculate mean nearest neighbour values per sampling interval, and from these, the mean value per trial. Finally, we collected data on individual swimming speed. We calculated the distance travelled by each individual between sampling intervals and from these measurements determined the mean swimming speed per second for each individual for the whole trial duration.

Statistical Analyses

We compared mean distance from group centroid, mean nearest neighbour distance and mean swimming speed between each of the seven treatments using one-way ANOVAs with Tukey post-hoc comparisons.

RESULTS

Experiment 1: Do flounders and sticklebacks attack larger groups of gammarids?

A binomial logistic regression revealed effects of species and treatment upon direction of first attack towards larger or smaller gammarid groups / empty holding cylinder (X^2=5.78, df=1, P=0.016 and X^2=9.34, df=2, P=0.009), as well as an interaction between these variables (X^2=3.87, df=2, P=0.040). Specifically, both species were more likely to attack the largest gammarid groups first in the 6 versus 0 treatment, while only the flounders showed a bias towards attacking the larger gammarid groups in the 5 versus 1 and four versus 2 treatments (Figure 1a). We saw no effects of latency to attack (X^2=0.36, df=1, P=0.59) or the location of the larger group (X^2=0.50, df=1, P=0.48).

A Cox regression revealed that the flounders took longer to attack the gammarids than did the sticklebacks (X^2=13.31, df=1, P<0.001, Figure 1b). We saw no effect upon attack latency of gammarid group size treatment (X^2=0.74, df=2, P=0.68) and no interaction between predator species and group size treatment (X^2=0.02, df=2, P=0.98). We also saw no effect upon attack latency of which group, larger or smaller, was attacked first (X^2=0.51, df=1, P=0.48), and finally, no effect of the location of the larger group (X^2=0.21, df=1, P=0.65).

Experiment 2: Do gammarids display different grouping responses to different predator cues?

Both mean distance to group centroid and nearest neighbour distances were lower in the three treatments that included conspecific injury cues (injury cues alone, injury cues plus stickleback kairomones and injury cues plus flounder kairomones) than they were in the treatments that presented only predator kairomones, and two controls (one-way ANOVA: $F_{(6, 63)}$= 64.28,

366 $P<0.001$ and $F_{(6, 63)}= 80.36$, $P<0.001$, Figure 2a and b). Mean swimming speeds were lower in
367 the five treatments that included injury cues, predator kairomones or combinations of both than
368 they were in the two control treatments (one-way ANOVA: $F_{(6, 63)}= 73.36$, $P<0.001$, Figure 2c).
369
370 DISCUSSION
371
372 Our first experiment revealed that flounders were more likely to attack larger groups of
373 gammarids, while sticklebacks showed no such bias. The flounders presumably attacked the
374 larger groups of gammarids either because they detected them first or because the larger group
375 acted as a stronger or more attractive stimulus. Both visual and chemical cues were available to
376 both predators. Flounders certainly rely on olfactory cues (Nevitt 1991), and are often found in
377 highly turbid estuaries where visual cues are limited or unavailable, though less is known about
378 the extent to which they rely on visual information when foraging in clear water. Sticklebacks
379 are able to forage effectively using both visual and prey chemical cues (Webster at al. 2007). The
380 difference between the two predators in their tendency to attack the larger group of prey may be
381 due to either differences in their sensitivity to prey visual or chemical cues, in their ability to
382 perceive different group sizes or differences in their motivation to attack larger groups.
383 Irrespective of the mechanism, based upon this finding we predicted that gammarids ought to
384 disperse when exposed to flounder kairomones, and aggregate when exposed to kairomones from
385 sticklebacks. In fact, we found that only the treatments including conspecific injury cues elicited
386 a grouping response in the gammarids, seen both in reduced nearest neighbour distance and
387 lower mean distances to group centroid, while predator kairomones alone had no effect on group
388 dispersal. We saw no differences in either of these measures of cohesion between treatments

where conspecific injury cues were presented alone or in combination with predator kairomones, suggesting no interaction effect between these.

Why did we not see the predicted effects of predator kairomones upon grouping behaviour? We are confident that the gammarids were able to detect the predator cues, since they were less active when exposed to predator kairomones and / or conspecific alarm cues than they were in the two control treatments. Reduction or cessation of activity in response to predator cues has previously been recorded in gammarids (Andersson et al. 1986; Andersen et al. 1993; Wudkevich et al. 1997) and in other aquatic species too (e.g. Stein & Magnuson 1976; Lawler 1989: Spivey et al. 2015), and is thought to be an adaptive response to predation risk, since less active animals may be less likely to encounter or be detected by predators. One explanation for the lack of effect of predator kairomones on grouping behaviour in our study may relate to opportunity costs. It may be that predator kairomones are sufficiently common under natural conditions that always and unconditionally responding to them incurs significant disadvantages. It may pay to respond to direct cues of predation, such as conspecific injury cues, since these indicate that predators are actively hunting, rather than to indirect cues, since doing so could limit opportunities for foraging, feeding or searching for mates. We have no data on how frequently gammarids are exposed to predator cues at the location from which they were collected, but this idea seems at least plausible, since sticklebacks, flounders and brown trout (*Salmo trutta*) occur at high densities there. In a study of the antipredator behaviour of the freshwater isopod *Caecidotea intermedius*, Spivey et al. (2015) found that they also did not respond to predator kairomones, but did respond to conspecific injury cues, by reducing their

411 activity. These authors similarly suggest that the isopods may only respond to cues that indicate
412 that predators are actively consuming prey nearby.
413
414 A second explanation may be that gammarids rely on hiding rather than grouping as the primary
415 means of avoiding predation. This was not possible to determine in our study, since no refuge
416 was provided, and is worthy of further investigation. Another possibility is that they cannot
417 discriminate between fish predator species using chemical cues. In a study by von Elert &
418 Pohnert (2000), bioassays of the chemical compounds found within kairomones obtained from
419 threespine sticklebacks, northern pike (*Esox lucius*) and crucian carp (*Carassius carassius*) were
420 found to be very similar in terms of their active compounds, supporting the possibility that
421 kairomones may not vary sufficiently between species to facilitate recognition at the species
422 level. The compounds comprising the kairomones to which the gammarids respond are unknown,
423 but it is plausible that the compounds used in predator recognition by the gammarids may be
424 same for both predator species. This seems to be the case for recognition of fish predators in of
425 mayfly larvae (*Baetis* sp.); in a series of experiments Alvarez et al. (2014) studied the
426 behavioural responses of mayfly larvae to a variety of co-occurring and unfamiliar predatory
427 fish. They showed that the cue by which the mayfly larvae likely detect their predators is
428 contained in the fish's cutaneous mucous. When exposed to cues from different natural predators
429 that differed in the risk that they posed to the mayfly larvae, the number of individuals that were
430 observed out of cover was lower than under control conditions irrespective of the predator
431 species. This suggests that they either cannot distinguish between them or that if they can, they
432 may not modify their behaviour according to predator type. A further experiment showed that
433 they also responded in this way to cues from both a natural fish predator and also to an

unfamiliar freshwater fish species, but not to cues from a novel marine fish or to a frog. This could suggest that while they do not or cannot distinguish between species using these cues, some component of the cue may allow for a more general or class-level of recognition of predators. A similar effect could explain the lack of a bidirectional response of the gammarids in our study to the flounders and sticklebacks, as we had predicted based upon our first experiment, though it does not account for the overall lack of a grouping response.

A final potential explanation for the lack of any group cohesion effect may be related to the methods that we employed. The groups of gammarids presented to the predators in experiment 1 were held in units measuring 12 cm in diameter. The free-moving gammarids in experiment 2 formed groups that were less dense than this, and this was true even for the most cohesive groups seen in the conspecific injury cue treatments. It may therefore be that the bias for flounders to attack larger groups was an artefact of unnaturally high density of these groups. We think that this is unlikely however, both because the gammarids naturally occur in far higher densities than that used in experiment 1 at the site from which they were collected, and also because while this might explain the lack of a bi-directional effect of predator kairomones on grouping, it does not explain the lack of an effect overall.

It should be noted that our findings contrast with those of another study, by Kullmann, et al. (2008), who found that gammarids exposed to stickleback-conditioned water, a predator-type cue, were more likely to spend more time close to a confined group of conspecifics compared to those exposed to tap water. The difference between the findings of the present study and Kullmann et al.'s (2008) study may be due to differences in experimental design, or differences

between the gammarid populations, perhaps reflecting adaptive or phenotypically plastic responses to different predation regimes (e.g. Ahlgren et al. 2011).

Grouping aside, we know from a large body of existing research, that many species exhibit other predator-specific behavioural responses. For example, vervet monkeys (*Cercopithecus aethiops*) produce predator-specific alarm calls, with receivers fleeing up trees when leopards (*Panthera pardus*) approach and scanning the sky or the ground respectively when raptors or snakes have been detected by others (Seyfarth et al. 1980). Tadpoles of the frog *Rana temporaria* hide from predatory waterboatmen (*Notonecta* sp.), but not from aeshnid dragonfly larvae (van Buskirk 2001). In chickadees (*Poecile atricapilla*), alarm calls encode information about predator size, which in turn mediates the intensity of the mobbing behaviour directed towards the predator by the receivers (Templeton et al. 2005). The aquatic snail *Physa acuta* responds to different predators by taking appropriate evasive action, spending more time close the surface of the water when exposed to chemical cues from predatory crayfish, and more time beneath cover when they have detected predatory fish (Turner et al. 2006). Botham et al. (2008) compared anti-predatory behaviour of guppies (*Poecilia reticulata*) from a range of high- and low-predation intensity populations when faced by several different predator species that differed in the severity of the risk that they posed. They found an interaction effect between the level of predation pressure that a population had been exposed to the type of predator presented, suggesting that guppies from higher predation sites tended to react most strongly only to the most dangerous predators. While these and other examples demonstrate that prey species can respond differently and seemingly adaptively to different kinds of predators, there remains little evidence that prey species might adaptively modulate their grouping responses to specific predators that impose different costs on

grouped versus ungrouped prey. Our study would seem to suggest that while gammarids exhibit grouping responses to conspecific alarm cues, they are not the most appropriate study species for addressing this question. We suggest instead that guppies (*Poecilia retulata*) might instead be a very useful model system. Their evolutionary ecology, particularly as regards predation pressure is already well studied (Magurran 2005). Guppies from populations that are intensely predated upon by nocturnal crustaceans that primarily hunt by olfaction shoal to a lesser degree than do those from populations where predation from crustaceans is less intense. Moreover, guppies, like many other fish species often disband their shoals at night, and it is possible that this represents an adaptive grouping strategy associated with the transition from risk of predation by diurnal and primarily visual pisciverous fishes to crepuscular and nocturnal chemosensitive predators (Helfman 1986; Magurran 2005). The dynamics of grouping behaviour is an interesting subject, with implications for our understanding predator-prey interactions and further research in this area would be valuable.

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640 **Table 1.** Stability of response variables over trial time within treatments

Metric (Treatment)	*r*			Friedman test (df=9)	
	Median	**20th percentile**	**80th percentile**	X^2	*p*
Distance from centroid					
Control 1. Fresh water	0.01	-0.17	0.09	4.70	0.81
Control 2. No Spray	0.01	-0.17	0.17	7.20	0.60
Flounder	0.07	-0.12	0.15	2.75	0.95
Stickleback	-0.02	-0.17	0.22	3.60	0.93
Gammarid injury	0.09	-0.06	0.25	9.84	0.40
Gammarid injury & flounder	0.06	-0.06	0.23	5.61	0.75
Gammarid injury & stickleback	0.10	-0.07	0.25	7.89	0.58
N**earest neighbour distance**					
Control 1. Fresh water	-0.12	-0.23	0.03	5.59	0.77
Control 2. No Spray	-0.07	-0.18	0.19	7.11	0.62
Flounder	-0.01	-0.29	0.12	3.90	0.92
Stickleback	-0.09	-0.14	0.06	6.67	0.66
Gammarid injury	0.06	-0.02	0.18	11.06	0.27
Gammarid injury & flounder	0.10	-0.21	0.33	6.64	0.66
Gammarid injury & stickleback	-0.12	-0.26	0.12	10.12	0.34
Swimming speed					
Control 1. Fresh water	0.04	-0.07	0.19	7.45	0.59
Control 2. No Spray	0.08	-0.09	0.22	9.90	0.45
Flounder	-0.08	-0.19	0.02	7.30	0.52
Stickleback	-0.02	-0.30	0.22	4.11	0.86
Gammarid injury	0.10	-0.07	0.28	10.02	0.31
Gammarid injury & flounder	-0.05	-0.26	0.27	7.80	0.54
Gammarid injury & stickleback	0.05	-0.24	0.22	9.52	0.45

641

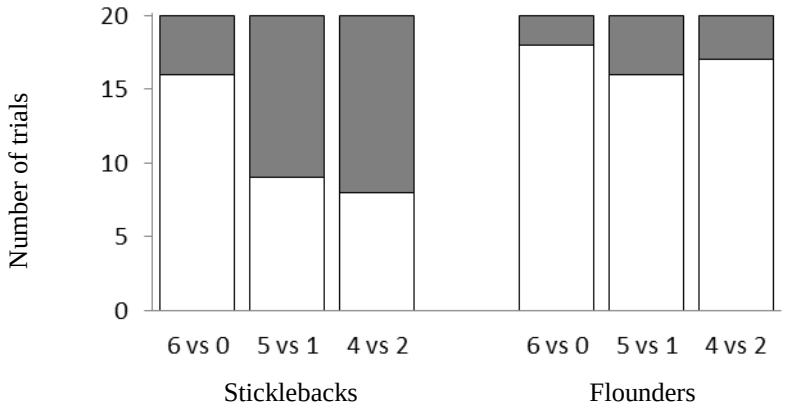
FIGURE LEGENDS

Figure 1. (a) The number of trials in which the stickleback and flounder predators first attacked the larger (white) or smaller (grey) group of gammarids. (b) Survival curves showing the latency to attack either gammarid group by the sticklebacks (*i*) and flounders (*ii*). The flounders took longer to attack than did sticklebacks, though within species there were no differences between the group size treatments.

Figure 2. (a) Mean distance from group centroid, (b) mean nearest neighbour distance and (c) mean swimming speed for each of the seven treatment groups. There were 10 replicates per treatment. Differences between treatments, inferred from Tukey post-hoc tests, are indicated by the letters above the bars- bars with the same letter above them indicate that mean values did not differ between treatments. Error bars indicate 95% confidence intervals.

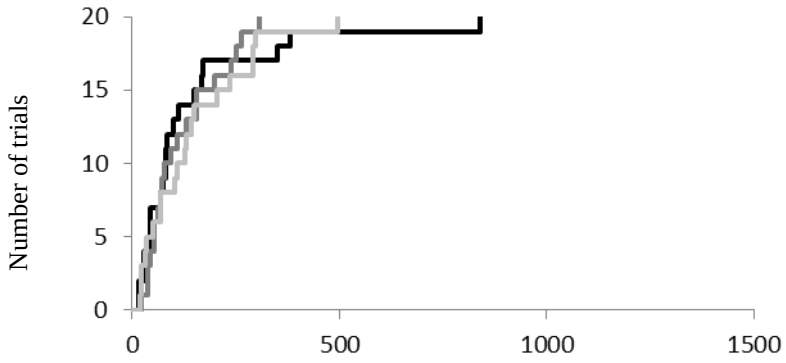
Figure 1.

(a)

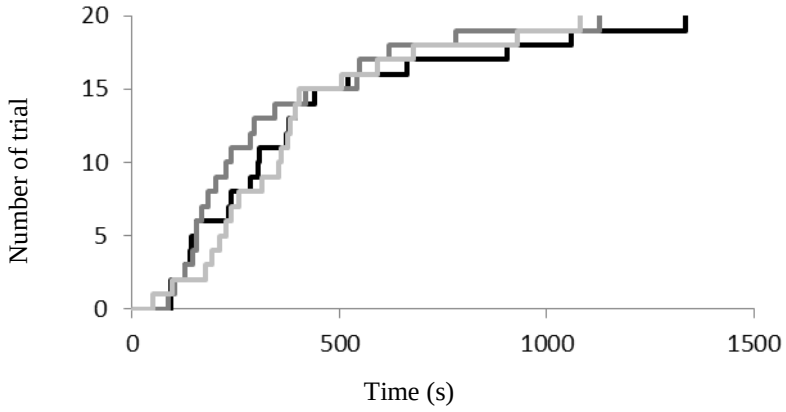


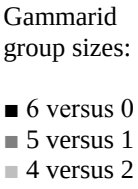
(b)

(*i*) Sticklebacks



(*ii*) Flounders





671 **Figure 2.**
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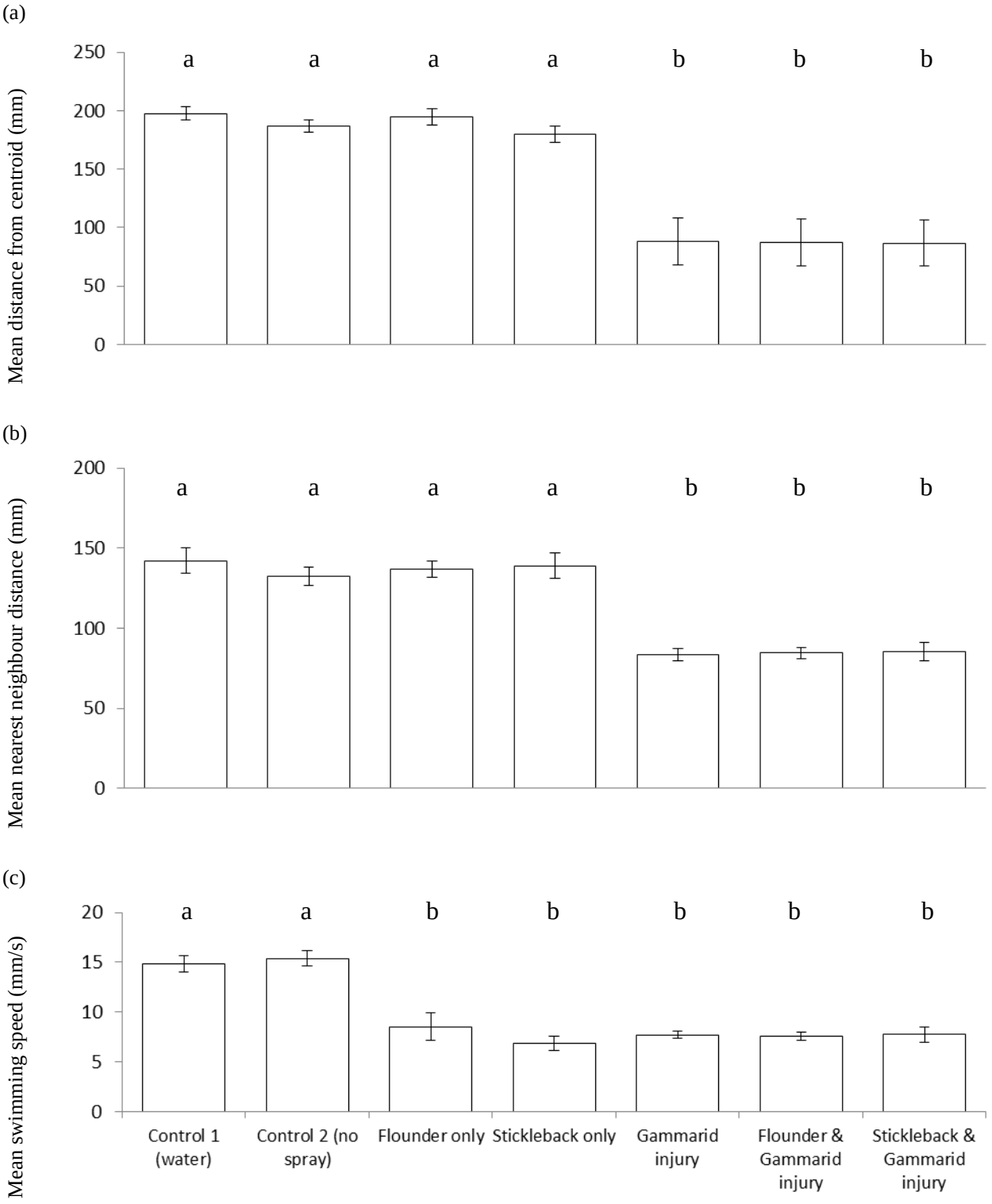

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