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RESEARCH ARTICLE

Dine or dash? Turbulence inhibits blue crab navigation in attractive–aversive odor plumes by altering signal structure encoded by the olfactory pathway

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SUMMARY

Blue crabs can distinguish and navigate to attractive (food) odors even when aversive odors (injured crab metabolites) are released nearby. Blue crabs in these conditions detect the aversive odor and avoid it, but find the attractive source with nearly the same success rate as when the attractive source is presented alone. Spatially and temporally distinct odor filaments appear to signal to foragers that the two odor sources are not co-located, and hence navigating to the attractive odor entails an acceptable risk of predation. However, environmentally produced turbulence suppresses tracking by homogenizing the two odors; blue crabs fail to track to the attractive source when the aversive source is present, even though turbulence does not substantially inhibit tracking to the attractive source alone. Removal of sensory input from aesthetascs on the antennules, but not chemosensors on the legs, rescues navigation to attractive–aversive dual plumes in turbulent conditions. These results suggest that mixing in the natural environment may amplify the effects of predators by suppressing tracking to food odors when aversive cues are present, and that the olfactory pathway mediates the response.

Key words: chemosensation, olfactory tracking, predation risk, risk assessment, risk sensitivity, trade-offs.

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INTRODUCTION

Nearly every animal uses chemicals to recognize something important for their survival, such as food, predators, mates and other conspecifics, or suitable habitats. Animals in these situations likely are confronted with a mélange of different attractive (e.g. food, mates) and repellent odors (e.g. predator odors), and must be able to discriminate and locate the attractive source, while responding appropriately to the repellant or aversive cue. Many studies indicate that animals respond to the tradeoffs inherent in situations in which they are confronted with conflicting cues. Crustaceans or mollusks challenged with combinations of prey and predator odors may suppress foraging to avoid being eaten (e.g. Hazlett, 2004; Nakaoka, 2000). Male moths use odorants emitted from conspecifics to find mates, but will not track when attractive plumes are intermingled with chemicals characteristic of heterospecific females (Fadamiro et al., 1999; Liu and Haynes, 1992). Behavioral changes (e.g. foraging suppression) of animals in environments with conflicting cues have substantial ecological effects on the focal species, as well as cascading effects on other community members (i.e. trait-mediated interactions, and trait-mediated indirect interactions, respectively) (Preisser et al., 2005). Consequently, understanding the processes that mediate how animals resolve conflicting signals can provide insight into the ecological effects of information conflicts, as well as reveal physiological mechanisms.

The sensitivity of neural systems to spatial and temporal patterns suggests these may be important arbiters of behavioral responses of animals to conflicting odor environments. Odor signals transported in flow often are composed of discrete odor filaments interspersed with clean water (Webster and Weissburg, 2001; Webster and Weissburg, 2009). This implies that plumes from differing sources may mix and intermingle to create a complex spatial and temporal pattern of attractive and aversive odor filaments, rather than a homogenized blend. Unfortunately, the stimulus environment has generally not been characterized or controlled in investigations on the effect of conflicting odor signals. The few studies that have been done with moths suggest fine scale patterns of such signals may strongly affect the response of animals.

Although most investigations examining how spatial and temporal chemical signal structure mediates tracking have done so using single chemical signal sources (e.g. Mead et al., 2003; Moore et al., 1991; Weissburg and Zimmer-Faust, 1993), a few studies have shown that animals fail to navigate to attractive plumes in situations where both attractive and aversive cues are released from different sources but can intermingle. As noted above, male moths will not track to conspecific pheromone plumes when they have been laced with constituents characteristic of heterospecific females. However, these same studies show that tracking can occur when the sources of attractive conspecific and repressive heterospecific odors are spatially distinct (1–50 mm), or when the two odors are emitted alternately from a single source.

These observations in insect studies suggest that the temporal and spatial structure of dual plumes has a significant effect on the behavioral outcome, but leaves a number of issues unresolved. Pheromonal attraction is mediated by a detection system that is highly tuned to a few individual compounds detected by specialized receptors on a single sensory appendage (Arbas et al., 1993; Vickers et al., 1998). It is not known whether more general chemosensory systems, such as those mediating attraction to food, function in the same way. Additionally, because general odorants such as food are sensed *via* multiple and redundant systems (e.g. Derby and Atema, 1982; Horner et al., 2004; Keller et al., 2003), a question arises as to the specificity and identity of the sensory organs that mediate

the decision to track. Finally, do environmentally relevant levels of mixing also alter or affect tracking by animals confronted with conflicting information?

We set out to examine these issues using blue crabs, which track extremely well to metabolites released by prey. This tracking is suppressed when blue crabs are exposed to mixtures of prey metabolites and metabolites from injured blue crabs (Moir and Weissburg, 2009). In these experiments, blue crabs were exposed to a mixture of the two cues introduced through a single source, and avoided this source by moving away from it or remaining quiescent. Responses were similar when crabs were exposed to plumes consisting only of injured blue crab metabolites. The observations indicate that blue crabs interpret the scent of injured blue crabs as indicative of mortal threat, and respond by behaviors that potentially reduce their exposure to predators. Thus, we asked whether blue crabs can disambiguate information about attractive *versus* aversive odor sources and successfully locate the attractive source when confronted with intermingled plumes from both sources. We performed experiments at two different levels of turbulent mixing to examine environmental effects on this process. Blue crabs track food-related cues using sensory input from receptors on the tips of their claws and legs, and aesthetascs (Keller et al., 2003). Either set of sensory appendages is sufficient for animals to track, although both success level and efficiency (e.g. time, distance traveled) decrease when only one is used. Thus, we manipulated sensory input by deafferentation to determine the role of specific sensory appendages in allowing animals to track attractive sources when these cues are intermingled with aversive odor.

MATERIALS AND METHODS

Animal collection and deafferentation protocols

Using baited traps, male and female blue crabs, *Callinectes sapidus* Rathbun 1896, were collected from habitats in Wassaw Sound and surrounding areas near the Skidaway Institute of Oceanography (Savannah, GA, USA). The crabs were shipped to Atlanta, GA, and kept in communal tanks filled with Instant Ocean artificial seawater (ASW) at a concentration of 25–33 p.p.t. and a temperature of 20°C. Crabs were tested within 20 days of collection. The crabs were maintained on a 12 h light:12 h dark cycle (lights were turned on at 06:00 h), and fed freshly thawed shrimp and squid *ad libitum*. We withheld food from the crabs ~12 h prior to testing to ensure that they were not satiated and to standardize the hunger level. All animals were intermolt males and females of 10–14 cm carapace width as measured from spine to spine.

We manipulated the sensory complement of blue crabs to address questions concerning how animals distinguish between attractive and aversive odors. The antennules of intact animals were shaved with a scalpel to remove all aesthetascs and associated hair-like sensilla. During this procedure, blue crabs were affixed dorsal side down to a platform and a ASW-soaked sponge was placed over the mouth and gill region. We viewed the antennules under a dissecting microscope during scraping to confirm all aesthetascs and other hairs were removed at the base. Deafferentation of claws and walking legs was accomplished by soaking the appendages in distilled water for 30 min. Crabs were again affixed to a platform, and syringes containing distilled water were placed over the appendages. Controls for antennule deafferentation consisted of treating crabs as described above, except the antennules were brushed with the blunt side of the scalpel. Controls for deafferentation of claws and walking legs utilized the same procedure as above but with saltwater-filled syringes. Animals with antennule treatments were tested within 3 days of manipulation, whereas blue crabs with claw/walking leg treatments were tested within 2 h of exposure to distilled or ASW treatments. The efficacy of these treatments has been confirmed in previous investigations (Keller et al., 2003).

Stimuli

We used attractive (food-related) and aversive (injured blue crab metabolites) to determine how animals react to these two odor sources when presented simultaneously from different sources. The attractive odorant stimulus was created by soaking intact shrimp in ASW at a concentration of 7.5 g l^{-1} for 1 h. The aversive solution was made from one injured crab (punctured on the dorsal midcarapace with a 5 mm diameter metal rod) soaked for 3.5 h in 3 l ASW. The ASW used for each solution was obtained directly from the flume's sump to avoid density or chemical differences between the solutions and the surrounding water. Fresh attractive and aversive solutions were made daily within 2–3 h of testing, and both have been used previously to examine blue crab chemosensory behavior (Ferner et al., 2005; Keller et al., 2003; Moir and Weissburg, 2009).

Stimuli were delivered at the upstream edge of the working section *via* dual 4.2 mm diameter nozzles connected to a pressure-delivery system with in-line flow meters (Fig. 1). Experimental treatments consisted of attractive (attractive + ASW) and conflicting (attractive + aversive) combinations. Each solution (ASW, aversive or attractive) was introduced at a rate of 20 ml min^{-1}. Thus, both the overall release rate (40 ml min^{-1}) and the concentration of individual solutions were constant. Blue crabs do not respond to metabolites from injured blue crabs (that is, they are aversive) (Moir and Weissburg, 2009). Consequently we did not include aversive-only plume treatments.

Flow environment

We characterized blue crab search behavior in an indoor recirculating flume (12.5 m long×0.75 m wide) in which we could control fluid flow and boundary-layer conditions. This flume has been used extensively for fluid physical and behavioral investigations (e.g. Jackson et al., 2007), and provides stable and equilibrium working boundary layer conditions in the working section. Flow velocity was maintained at 4.9±0.08 cm s^{-1} (mean ± s.d.) with water depth controlled by a vertical tailgate. The bed of the flume was covered in sand with a mean diameter (d_{50}) of ~1 mm. These conditions were chosen to be representative of environments that blue crabs encounter in the field (Finelli et al., 2000; Smee et al., 2010), but

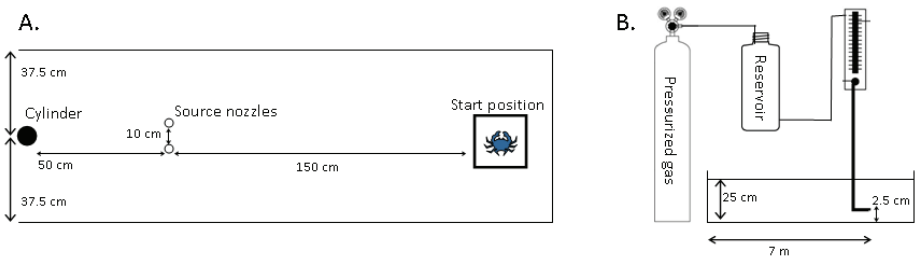

Fig. 1. Schematic diagram of the flow and chemical stimulus set up. Water flow is from left to right. (A) Top view of flow tank showing positions of the 10.1 cm diameter cylinder, the dual source nozzles and the starting cage. (B) Side view of a single source nozzle and pressure delivery system. Drawings not to scale.

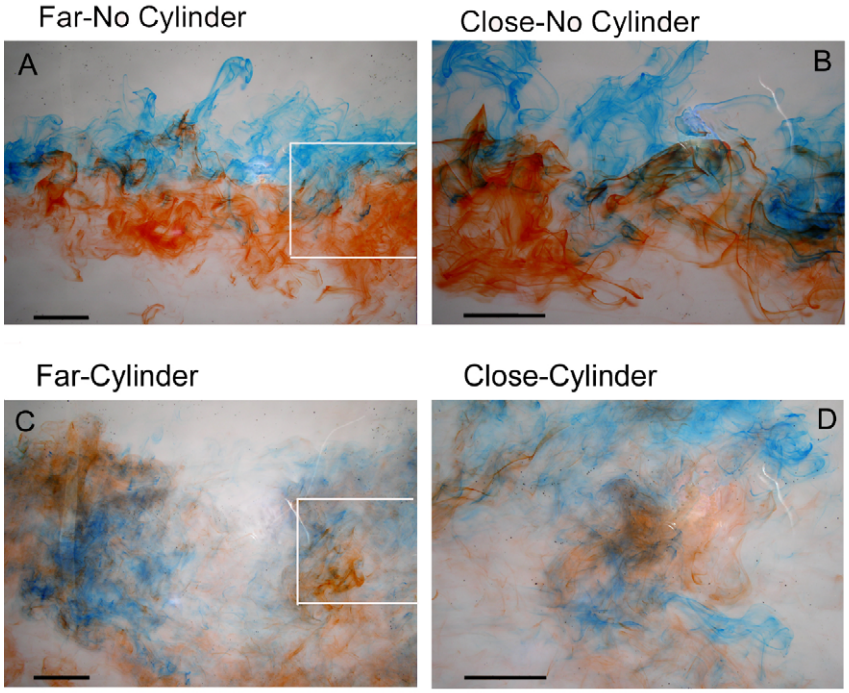

Fig. 2. Dye visualization images of dual plumes in the absence (A,B) and presence (C,D) of the upstream cylinder. The right-hand edge of all views is ~1 cm upstream of the crab starting cage. Flow is from left to right. The white box is the approximate location of the close-up view. Scale bar is 10 cm in A and C, and 7 cm in B and D. Illumination was provided by a slide projector modified to produce a light sheet ~1 cm thick located at the release height of the odor source (25 mm above the bed). Note that the close views represent plume images taken at different times from those of the far views.

which produce a relatively high level of foraging success. Experimental conditions resulted in a boundary layer with a roughness Reynolds number of ~3 and a bed shear velocity (u^*) of 3.01 mm s^{-1} (Jackson et al., 2007). Light levels were lowered during trials to minimize visual cues during navigation and because field observations indicated peaks in foraging activity in near-dark periods of early morning and evening (Clark et al., 1999). Some experiments utilized a cylinder in order to increase turbulent mixing and homogenize the two separate odor streams (Fig. 1). Mixing was achieved using a 10.1 cm cylinder placed upstream of the nozzle, thereby creating a von Karman vortex street in the cylinder wake. The shedding frequency was ~0.1 Hz, which corresponds to a Strouhal number of 0.2 (note that the Reynolds number for the cylinder flow is ~5000). Under normal conditions, the proximity of the two nozzles to each other resulted in considerable intermingling of odor filaments from the two sources as the plumes expanded downstream (Fig. 2A,B). The increased mixing associated with the cylinder causes considerable homogenization of the two odor plumes such that attractive and aversive odors become blended together (Fig. 2C,D).

Behavioral experiments

We examined tracking success and kinematics of normal and deafferented animals in response to attractive (attractive + ASW) and conflicting (attractive + aversive) dual odor plumes using a standard protocol. Tracking behavior was recorded with an overhead video camera and tracks were reconstructed using video-motion analysis (see below). Fluorescent 'glowsticks' (2.5 cm long, 4 mm diameter) were affixed to the dorsal carapace of test animals using a rubberband. Animals were placed in a small cage and acclimated for 30 min. Chemical solutions were released 2 min before the end of the acclimation period to ensure odor plumes reached the crab at the start of the trial. The trial began when the barrier was removed and the animal was allowed to respond freely to the chemical stimulus environment. The trial ended when the crab reached and attempted to grasp a nozzle or moved upstream beyond the nozzle. A trial was ended if 10 min elapsed without the crab traveling out of the start area. At the end of each trial, the tested crab was presented with a small piece of shrimp to confirm its willingness to eat. The trial was not included in the final data if the crab did not grab the shrimp decisively (characterized by a direct movement toward the shrimp and its subsequent capture). The percentage of non-motivated animals was roughly 5–10%, and did not vary significantly across treatments.

We performed three series of experiments. In experiment 1, we examined the response of normal, unmanipulated animals to attractive and conflicting dual plumes in the absence and presence of the cylinder. The goal of these experiments was to determine how environmental conditions affect the ability of animals to respond positively to the attractive source in the presence of an aversive one. These experiments revealed that the cylinder disrupted tracking specifically in the conflicting treatment. Therefore, in experiments 2 and 3, we looked at the response of control (sham) and deafferented animals (antennule and leg/claw, in experiments 2 and 3, respectively) to the conflicting dual plume in the presence of a cylinder. This gave us insight into the sensory basis for the disruption of tracking.

For all experiments, we ran multiple trials each day to randomize treatments as much as possible. We determined the initial side of the attractive source with a coin flip, and then alternated sides for each subsequent trial. We also interspersed conflicting and attractive conditions on a single day, although we generally ran fewer attractive treatments on these days to maximize the number of runs with conflicting plumes and to minimize the number of crabs used to provide metabolite solutions. Thus, there were some experimental days in which we only used attractive plumes. Both sham-treated and deafferented animals were always used as experimental subjects on a given test day during experiments 2 and 3.

Data analysis

We analyzed tracking success as the frequency of animals that successfully found the source. We also analyzed tracking kinematics for each crab that successfully found the odor source. The position of the glow stick was digitized using a motion analysis system

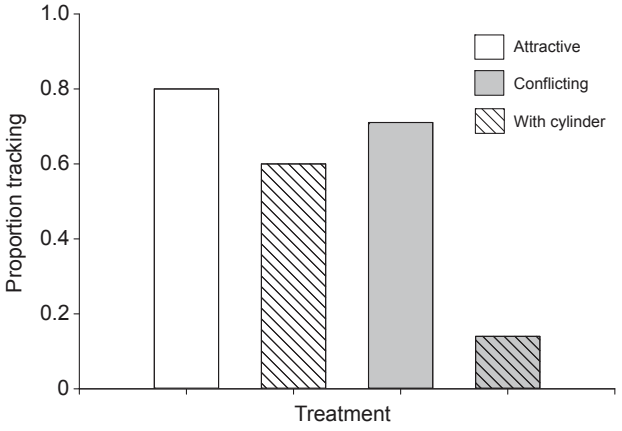

Fig. 3. Proportion of animals that tracked attractive and conflicting dual plumes with (hatching) and without (no hatching) the cylinder. Cylinder presence has no effect on tracking success for the attractive odor treatment (G^2=0.81, d.f.=1, P>0.05), but affects tracking success in the conflicting odor treatment (G^2=7.67, d.f.=1, P<0.01). There is no association between tracking and plume treatment in the absence of the cylinder (G^2=2.59, d.f.=1, P>0.05), but plume type alters tracking success when the cylinder is present (G^2=10.6, d.f.=1, P<0.01). Thus, increased mixing created by the cylinder degrades tracking success to only the conflicting dual plume treatment. N=18 for attractive no cylinder, N=17 for attractive plus cylinder, N=20 for conflicting no cylinder, N=14 for conflicting plus cylinder.

(Motion Analysis VP110, Santa Rosa, CA, USA). Raw data were acquired at 30 Hz during digitization, but path kinematics were calculated with data down-sampled at a final rate of 5 Hz. The digitized data were used to calculate each crab's speed, and path linearity as the net to gross displacement ratio (NGDR). This metric ranges from 0 to 1, where 1 indicates a completely straight path from origin to destination. In addition, we used the digitized records to calculate the proportion of time the animal was on the side of the attractive stimulus. The midpoint between the two source positions defined the interior edge of the 'stimulus side', which then extended all the way to either the left or right flume wall, depending on which source was emitting the attractive compound (i.e. the 'stimulus side' was from the midpoint to the left flume wall when the left-hand source released the attractive compound). Finally, we determined the time it took a successful forager to leave the starting cage.

Tracking success was analyzed using G-tests to examine the frequency of source location for animals as a function of plume type and cylinder presence (experiment 1), or plume type and deafferentation status (experiments 2 and 3). Experiments 2 and 3 were analyzed separately as we were primarily interested in comparisons of sham *versus* deafferented animals of each type. Path kinematics were analyzed using ANOVA, as functions of the same treatments, again with separate analysis for experiments 2 and 3. Experiment 1 was analyzed using a two-way ANOVA, but we could not use this design for experiments 2 and 3, as there were no replicates for one of the experimental conditions. Therefore, we used a one-way ANOVA to examine differences across the remaining three treatments. Tukey–Kramer *post hoc* tests were used to determine significant differences between specific treatment groups.

RESULTS

Blue crabs clearly responded to the presence of the attractive source despite its proximity to the aversive source (Fig. 3). In fact, the presence of the aversive cue (conflicting treatment) caused little decrease in tracking success under normal mixing conditions, and the success rate was similar to that observed in many other experiments of this type without aversive odor (Jackson et al., 2007; Keller et al., 2003). Qualitative odor visualizations confirm that this tracking success in the conflicting treatment occurs despite the intermingling of discrete filaments of attractive and aversive odor cues at the point where the crab initially encounters the plumes, and for some distance upstream (Fig. 2A,B). Although the presence of the cylinder had a small impact on tracking the attractive plume, it produced a dramatic decrease in tracking success for the conflicting treatment where odor filaments from the two different stimuli appear more blended (Fig. 2C,D). The G-test showed a significant effect of plume type and cylinder treatment on tracking frequency (G^2=18.35, d.f.=4, P<0.01), and analysis of sub-tables (i.e. 2-way tables extracted from the full 3-way analysis) showed that cylinder presence had no effect on tracking success for the attractive odor treatment (G^2=0.81, d.f.=1, P>0.05), whereas it did determine tracking success in the conflicting odor treatment (G^2=7.67, d.f.=1, P<0.01). Similarly, there was no association between tracking and plume treatment in the absence of the cylinder (G^2=2.59, d.f.=1, P>0.05), whereas the plume type altered tracking success when the cylinder was present (G^2=10.6, d.f.=1, P<0.01). Thus, increased mixing created by the cylinder degrades tracking success to only the conflicting dual plume treatment.

Kinematic analysis showed that tracking speed ($F_{3,37}$=23.41, P<0.001; Fig. 4A) and exit time from the start cage ($F_{3,37}$=15.31, P<0.001; Fig. 4B) were influenced by both the odor treatment and the degree of mixing imposed by the cylinder. Tracking speed was significantly greater in attractive plumes ($F_{1,37}$=49.85, P<0.001) and when there was no cylinder ($F_{1,37}$=8.31, P<0.001), with no evidence of an interaction ($F_{3,37}$=0.38, P>0.05). Crabs took 2–3 times longer to exit the start cage when exposed to conflicting plumes ($F_{1,37}$=20.23, P<0.001), whereas cylinder presence exerted no significant effect either by itself or in combination with plume type ($F_{1,37}$<1.78, P>0.05 for both comparisons). In general, both conflicting odor plumes and plumes subjected to enhanced mixing were less attractive than plumes composed only of attractive chemicals or treatments that lacked a cylinder.

Odor plume type and the presence of a cylinder affected path linearity ($F_{3,37}$=3.85, P<0.05; Fig. 5A) and position ($F_{3,37}$=3.71, P<0.05; Fig. 5B) of the searcher. Crabs moved along straighter paths in attractive plumes ($F_{1,37}$=6.23, P<0.001) and in the absence of cylinder-induced mixing ($F_{1,37}$=10.68, P<0.01), with a marginally insignificant interaction ($F_{1,37}$=3.90, P=0.06). Although both plume type and cylinder presence were significant, animals moved very directly to the source emitting the attractive shrimp odor even in the presence of the aversive cue, except when cylinder presence was paired with the conflicting plume treatment. Side bias was significantly affected by plume type ($F_{1,37}$=4.73, P<0.05), but not by cylinder presence ($F_{1,37}$=0.09, P>0.5) or its interaction with plume type ($F_{1,37}$=0.36, P<0.5). Crabs in conflicting plumes spent about 75% of their foraging time on the side of the source emitting the attractive shrimp metabolites, but spent roughly equal amounts of time on each side when in the attractive plumes. These analyses indicate crabs are largely able to direct their trajectory towards the attractive shrimp odor even in the presence of aversive crab metabolites, except when the cylinder increases mixing.

Plume type and antennulary deafferentation treatment interacted to determine tracking success for crabs in experiment 2. Animals lacking antennulary sensors showed slightly diminished tracking success overall, but tracked the conflicting plume at a much higher frequency than the sham group (Fig. 6A). A G-test revealed a

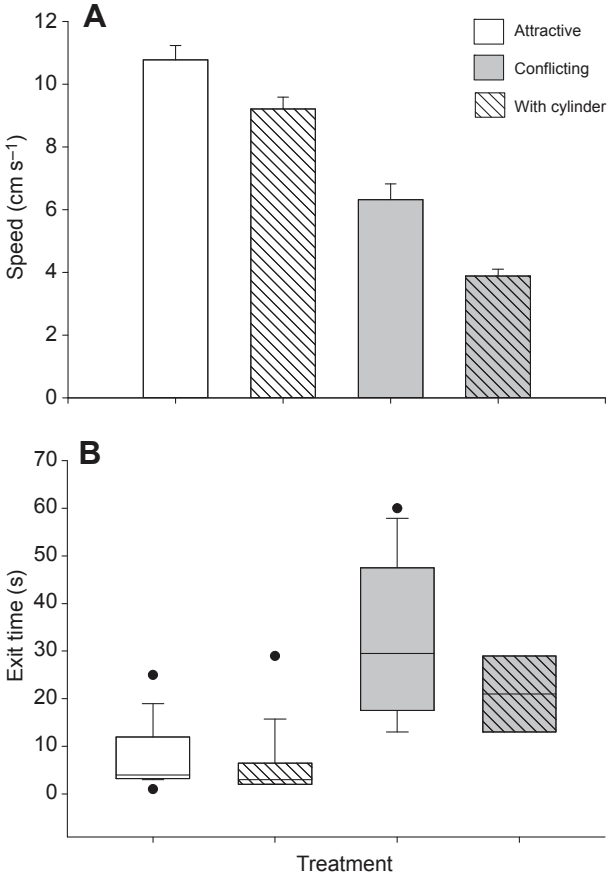

Fig. 4. Speed (A) and exit time from the start cage (B) of crabs that tracked successfully to attractive and conflicting plumes with (hatching) and without (no hatching) a cylinder. (A) Speed (mean and s.e.m.) was significantly greater with attractive plumes ($F_{1,37}$=49.85, P<0.001) and when there was no cylinder ($F_{1,37}$=8.31, P<0.001). (B) Box plot of exit times with median (solid line), 25% and 75% quartiles (box) and 10–90% limits (whiskers), with outliers represented as circles. Exit time was affected by plume type ($F_{1,37}$=20.23, P<0.001), but not by cylinder presence ($F_{1,37}$<1.78, P>0.05). N=15 for attractive no cylinder, N=12 for attractive plus cylinder, N=12 for conflicting no cylinder, N=2 for conflicting plus cylinder. Note that this analysis is for successful tracks only, and so sample sizes represent a subset of those given in Fig. 3.

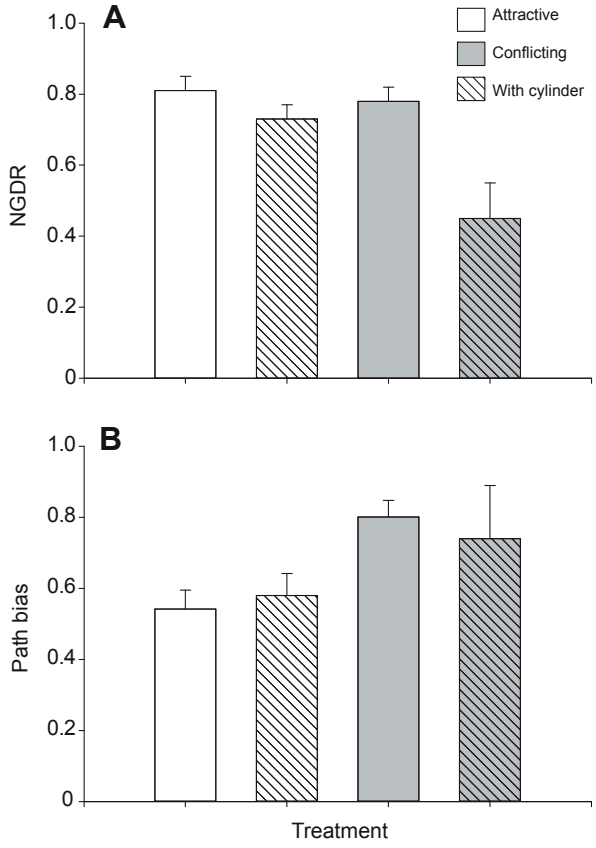

Fig. 5. Net:gross displacement ratio (NGDR; A) and path bias (proportion of the path on the side of the attractive plume source; B) of crabs that tracked successfully to attractive and conflicting plumes with (hatching) and without (no hatching) a cylinder. Means and s.e.m. (A) NGDR is a measure of path linearity ranging from 0 (circular path with no net movement) to 1 (movement in a straight line). Crabs moved along straighter paths in attractive plumes ($F_{1,37}$=6.23, P<0.001) and in the absence of cylinder-induced mixing ($F_{1,37}$=10.68, P<0.01). (B) Bias was significantly affected by plume type ($F_{1,37}$=4.73, P<0.05), but not by cylinder presence ($F_{1,37}$=0.09, P>0.5). N=15 for attractive no cylinder, N=12 for attractive plus cylinder, N=12 for conflicting no cylinder, N=2 for conflicting plus cylinder. Note that this analysis is for successful tracks only, and so sample sizes represent a subset of those given in Fig. 3.

significant association of deafferentation treatment and plume type on tracking frequency (G^2=17.16, d.f.=4, P<0.01), with the analysis of sub-tables showing a significant association between tracking frequency and deafferentation status for conflicting but not attractive plumes (G^2=10.31, P<0.01 and G^2=0.26, P>0.05 for conflicting *versus* attractive plumes, respectively; d.f.=1), and that tracking is associated with plume type for sham-treated animals but is not associated with plume type for deafferented crabs (G^2=10.65, P<0.01 and G^2=0.75, P>0.05 for sham *versus* deafferented animals, respectively; d.f.=1). Thus, removing antennulary sensors caused crabs to respond to attractive and conflicting plumes similarly, but sham-treated animals continued to show suppressed tracking in conflicting plumes.

In contrast to results for animals with antennulary manipulations, deafferentation of legs (experiment 3) did not alter the pattern of tracking in response to different plume types (Fig. 6B). Although the full 3-way matrix was significant (G^2=17.16, d.f.=4, P<0.01), analysis of sub-tables showed a lack of association between deafferentation status and tracking for both attractive and conflicting plumes (G^2<0.21, d.f.=1, P>0.05) and that tracking was associated with plume type for both deafferented and sham-treated groups (G^2>9.18, d.f.=1, P<0.01). Thus, crabs in experiment 3 tracked to attractive but not conflicting plumes regardless of whether they had functioning leg/claw chemosensors.

Kinematic analysis showed patterns that were consistent with the analysis of tracking performance (Table 1). The combination of antennule treatment and plume type had a significant impact on foraging speed ($F_{2,29}$=7.97, P<0.05; recall that we could not perform a full two-way ANOVA because one treatment lacked replicates). Importantly, *post hoc* tests indicated that the speed of the sham-operated animals in the attractive treatment was significantly higher than that of the antennule-deafferented animals in both the conflicting and attractive plume treatments. In other words, although deafferentation affects movement speed, it does so non-specifically, and deafferented crabs exposed to conflicting and attractive plumes behave similarly. Analysis of exit time yielded the same result; exit time was significantly affected by treatment ($F_{2,29}$=17.63, P<0.001), with *post hoc* tests showing that sham-treated animals in attractive plumes had significantly faster exit times than deafferented animals

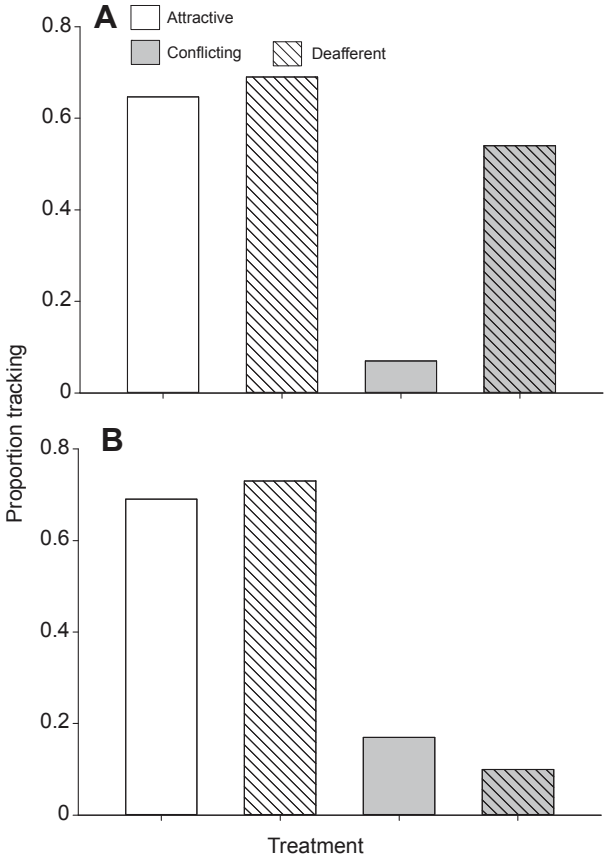

Fig. 6. Proportion of normal (no hatching) and deafferented (hatching) crabs that tracked conflicting dual plumes with and without the cylinder. (A) Antennule-deafferented crabs. There is significant association between tracking frequency and deafferentation status for conflicting but not attractive plumes (G^2=10.31, P<0.01 and G^2=0.26, P>0.05 for conflicting *versus* attractive plumes, respectively; d.f.=1). Tracking frequency is associated with plume type for sham-treated animals but is not associated with plume type for deafferented crabs (G^2=10.65, P<0.01 and G^2=0.75, P>0.05 for sham *versus* deafferented animals, respectively; d.f.=1). Thus, removing antennulary sensors caused crabs to respond to attractive and conflicting plumes similarly, but sham-treated animals continued to show suppressed tracking in conflicting plumes. N=17 for attractive–sham, N=13 for attractive–deafferent, N=15 for conflicting–sham, N=22 for conflicting–deafferent. (B) Leg-deafferented crabs. There is a lack of association between deafferentation status and tracking frequency for both attractive and conflicting plumes (G^2<0.21, d.f.=1, P>0.05). Tracking frequency is associated with plume type for both deafferented and sham treatment groups (G^2>9.18, d.f.=1, P<0.01). Thus, crabs tracked to attractive but not conflicting plumes regardless of whether they had functioning leg/claw chemosensors. Note the difference in scaling of the y-axis relative to Fig. 3.

in either plume type, which did not differ. Neither NGDR nor side bias was significantly affected by treatment ($F_{2,29}$<1.84, P>0.05 for both comparisons).

Deafferentation of leg chemosensors also changed path kinematics, but the patterns were very different to that of the antennule deafferentation experiment. Speed and exit time were affected by treatment ($F_{2,21}$=8.42, P<0.05; $F_{2,21}$=43.95, P<0.001, for speed and exit time, respectively), but the differences were associated with plume type. *Post hoc* tests showed that sham-treated animals tracking to conflicting plumes were significantly slower and took longer to exit than either sham-treated or deafferented animals tracking the attractive plume (we did not include the deafferented–conflicting plume treatment as only one animal tracked). This is the expected pattern if deafferentation of leg chemosensors does not affect the ability of animals to encode attractive *versus* aversive stimuli. Path linearity (NGDR) also was significantly different across treatments ($F_{2,21}$=8.42, P<0.01), but *post hoc* tests showed the effect was due to deafferentation status and not plume type. This observation is consistent with previous experiments showing that leg chemosensors control steering (Keller et al., 2003). Sham-treated animals tracking conflicting plumes spent a greater proportion of their time in the attractive side, although this was not a significant effect ($F_{2,21}$=1.19, P>0.05), possibly because of the low sample size for this group.

DISCUSSION

The results of these experiments provide insight into the mechanisms that permit animals to respond appropriately in situations where both risk (i.e. threat of predation) and reward (food) are present. Animals in such situations will be confronted with multiple cues indicating these different contingencies, and must be able to weigh their relative likelihood. Blue crabs confronted with homogeneous blends of risk and reward cues elect not to forage (Moir and Weissburg, 2009) but, as shown here, will respond positively to food cues in the presence of risk cues as long as odor signals are composed of relatively discrete filaments of each odor. In the absence of the cylinder, crabs successfully navigated attractive plumes with and without aversive plumes at roughly equal levels. Analysis of the tracking kinematics showed that crabs detected both cues even when the cylinder was present. The aversive cue suppresses movement, delays the decision to navigate, and causes the animal to move away from the aversive signal even as it attempts to locate the attractive source. Thus, the decision to track must reflect the fact that foragers are able to determine that the two cues are released separately and judge the spatial separation as representing a situation in which the benefits of obtaining food outweigh the costs. This apparent cost–benefit analysis is reversed and the negative effects are enhanced in the presence of the cylinder; navigation is almost completely suppressed and, when present, tracking speeds are slower. As revealed by the deafferentation experiments, antennulary input is both necessary and sufficient for encoding the presence of the aversive cue and mediating subsequent responses.

The results suggest that spatial separation of attractive and aversive odor sources permits tracking because these plumes contain intermingled filaments that still retain some spatial and temporal discreteness. Such plumes are judged to represent a less risky situation than when odors are well mixed, which might indicate attractive and aversive odors are close enough that responding to the attractive odor exposes the forager to an unacceptable level of risk. Environmentally induced mixing, in our case, produced by a cylinder, homogenizes odors from different sources, such that dual plumes previously considered to represent non-risky situations become blended enough to suppress foraging. Thus, the decision to forage in the presence of conflicting signals will be a function of both the degree of separation of the two odor sources and the level of mixing. Both spatial and temporal separation of filaments may be responsible for preserving navigation in conflicting plumes. Investigators examining suppression of pheromone tracking in moths (see below) have suggested the key factor is a sufficient time interval between the arrival of attractive and agonistic (aversive) compounds. However, our qualitative flow visualization suggests spatial distinctness occurs on scales of one to several millimeters, and separation either along an antennule 1–2 mm in length or perhaps between antennules may also be important in mediating the decision

Table 1. Summary of kinematic performance for sham-operated and antennule and leg deafferention treatments for those animals that successfully tracked to the odor source in attractive and conflicting dual plumes

	Antennules				Legs/claws			
	Attractive–sham	Attractive–deafferent	Conflicting–sham	Conflicting–deafferent	Attractive–sham	Attractive–deafferent	Conflicting–sham	Conflicting–deafferent
Speed (cm s^{-1})	9.84±0.42	7.93±0.46	4.42	7.68±0.41	9.19±0.46	9.48±0.43	4.86±0.89	4.99
Exit time (s)	8.09±1.29	33.22±4.05	48	33.0±4.21	7.36±0.68	6.64±0.68	29.5±7.5	18
NGDR	0.75±0.037	0.62±0.49	0.64	0.74±0.49	0.72±0.02	0.62±0.03	0.74±0.06	0.73
Bias	0.58±0.07	0.44±0.06	0.78	0.56±0.08	0.48±0.04	0.54±0.05	0.72±0.06	0.61
N	11	9	1	12	11	11	2	1

Data are means ± s.e.m. Note that this table shows results only for successful trackers, which is a subset of all behavioral trials, and therefore the sample sizes differ from those reported in Fig. 6.
NGDR, net:gross displacement ratio.

to track. The fact that antennulary input seems to mediate avoidance (i.e. biases animal trajectories towards the attractive side; Fig. 5B) further suggests it is necessary to examine the role of both spatial and temporal discreteness as factors affecting tracking decisions.

Although our experiments suggest the importance of both source separation and mixing, they do not provide an accurate estimate of what inter-source distance typically is 'safe' for animals in the field. Although our flow conditions are representative of the natural habitat, they are at the low end of mixing levels measured in estuaries and salt marshes (Smee et al., 2008). Moreover, source characteristics will also affect both the spatial spread of plumes and the degree of mixing. We suspect that under many field situations, the 10 cm distance we used here would result in plumes that are mixed sufficiently well such that foraging would be suppressed. That is, they are more likely to resemble plumes produced with the cylinder present. Further insight into the physical conditions that suppress or elicit foraging require a more precise quantification of spatial and temporal properties of filaments in these dual plumes, which can obtained using two-color laser-induced fluorescence (LIF) imaging. We are currently developing this capability based on our previous 3D single-color LIF system (Dickman et al., 2009).

This study provides further evidence that physical factors may alter perception of odors to modulate the non-consumptive effects (NCEs) of predators. NCEs occur when prey perceive predators or their activities, and change their behavior, morphology or other traits (Werner and Peacor, 2003). NCEs can cascade to affect other community members, and predators in many systems may influence community structure primarily *via* NCEs as opposed to direct consumption (Preisser et al., 2005). Odor cues are significant in altering animal traits that result in the propagation of NCEs in many systems including those in which crustaceans are either predators or prey (Kats and Dill, 1998), but the ability of prey to perceive predator odors is reduced by flow and mixing (Smee and Weissburg, 2006a; Smee and Weissburg, 2006b). Mixing may therefore reduce NCEs by interfering with prey perceptual abilities. However, the current results indicate that greater mixing may also increase the possibility for NCEs in circumstances where prey are faced with conflicting signals. Mixing-induced homogenization of dual odor plumes containing attractive and aversive cues would suppress foraging, which is a ubiquitous form of NCE. In fact, introducing aversive injured crab metabolites to baited crab traps reduces the number of blue crabs entering these traps relative to the number entering traps containing bait alone (Ferner et al., 2005), indicating the potential for conflicting odor plumes to affect foraging behavior in the field.

Spatial and temporal discreteness of incoming odor signals also affects responses of moths to dual pheromone plumes consisting of attractive conspecific odors and inhibitory components (antagonists) produced by other species (Fadamiro and Baker, 1997; Fadamiro et al., 1999; Liu and Haynes, 1992). The flight frequency of male moths is strongly suppressed when odor sources are co-emitted. As in blue crabs, the presence of the antagonist reduces movement speed, and alters turning behavior such that moths avoid plume contact or move out of the plume (Fadamiro et al., 1999; Vickers and Baker, 1997). Slight to moderate cross-stream separation (1–50 mm) of odor sources or alternating pulses of attractive pheromones (10 Hz combined pulse rate) elicit tracking, sometimes at levels observed in the absence of antagonists. The suppression is thought to be due to the 'simultaneous' arrival of attractive and antagonistic molecules, although the absence of quantitative measurements of arrival time at the animal's sensors prevents estimation of the critical interval length at which successive attractive–antagonistic pulses suppress tracking.

It is unclear whether the neural processes that produce these responses in moths are the same as those governing the behavior in blue crabs. Moth pheromone detection occurs *via* highly tuned chemosensors that are specialized for this role and which project to a specific brain region (Vickers et al., 1998). Antagonists and attractive pheromone components are detected by different receptor neurons, each tuned to the specific chemical, and which may be co-located in the same sensory hair (Fadamiro et al., 1999).

In contrast, foraging suppression in blue crabs occurs in the context of a less specific sensory encoding system. The identity of both the excitatory and inhibitory (aversive) chemicals mediating blue crab responses to food and predation risk are unknown, but attractive molecules, at least, are numerous. Crustaceans are responsive to a wide variety of attractive components in food, including amino acids, sugars, nucleotides and other compounds (Carr and Derby, 1986; Derby and Atema, 1988; Zimmer-Faust, 1989). Although the specific substances that elicit tracking are unknown, the large number of stimulatory molecules suggests attraction is mediated by chemical blends. There are no fully identified water-borne predatory deterrents, although there are a few cases where molecules have been partially characterized and revealed to be fairly uncommon water-borne moieties such as fatty acid-derived sulfated compounds (Yasumoto et al., 2006) or low molecular weight carboxylic acids (Agrawal et al., 1999). It is possible that water-borne predator odors work by central nervous system inhibition as suggested in moths. However, peripheral effects, such as mixture suppression, are a potential hypothesis as well, given the broad nature of attractive molecules and the likely importance of blends as indicators of food. Specific (inhibitory) molecules may interfere with, or modify the binding of, attractive molecules to receptors, thus interfering with food recognition. Aversive chemicals in insects and mammals (Dethier

1976; Formaker and Frank, 1996; Jørgensen et al., 2007) suppress or inhibit the neural activity of receptor neurons that are excited by food-activated cells.

Although both antennulary and leg chemosensory organs are sufficient to mediate orientation to food (Fig. 6) (see also Keller et al., 2003), the chemosensors on the antennules specifically control foraging suppression in response to the metabolites from injured blue crabs. Animals with deafferented antennules tracked dual odor plumes in the presence of the cylinder, whereas animals without leg chemosensors behaved in the same way as intact animals; neither of these two groups tracked conflicting dual plumes with the cylinder. Similarly, the kinematics of tracking performance in deafferented animals shows that chemosensors on the antennules, and not legs, regulate the behavior of animals in the presence of the aversive cue. Antennule-deafferented animals had similar speeds, biases and exit times in conflicting and attractive plumes. This pattern is different from that of both normal and sham-treated antennule groups, which moved more slowly, took longer to exit the start cage, and avoided contact with aversive blue crab cues. In contrast, animals with deafferented claw/leg sensors continued to move more slowly, delayed tracking and avoided the aversive chemical. Antennulary sensors, then, are both necessary and sufficient to suppress tracking.

Although crustaceans possess multiple chemosensor-bearing appendages that have a redundant role in navigating to general food-related stimuli (Horner et al., 2004; Keller et al., 2003), antennular chemosensory input mediates responses to more specialized signals. For instance, aesthetasc chemosensors on antennules mediate attraction to conspecific urine in spiny lobsters (Horner et al., 2008b), and mediate individual recognition in clawed lobsters and crayfish (Horner et al., 2008a; Johnson and Atema, 2005), and responses to female pheromones in blue crabs (Gleeson, 1982). It appears as though these antennulary chemosensors also regulate responses to injured blue crab metabolites that suppress foraging. The reasons behind the division of the chemosensory system into general *versus* specialized detection remain unclear, but may relate to the exigency of encoding spatial and temporal patterns necessary to guide navigation *versus* differentiation of chemical blends.

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