

The function of the rhinophore and the tentacles of *Nautilus pompilius* L. (Cephalopoda, Nautiloidea) in orientation to odor

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Abstract

We have previously demonstrated that nautilus can detect and follow odor plumes produced by a distant source from up to 10 m. The use of the rhinophores is necessary for this behavior. Here we examine which structures may be linked to different behavioral stages of the search (“near-field” and “far-field”) to determine their function in the orientation behavior of *Nautilus*. We injected 1 cc of odor onto each of the four structures that nautilus are known to manipulate as they track an odor plume to its source (rhinophore, digital tentacles, preocular tentacles, postocular tentacles; presented in random order; N = 11 animals). The application of odor to the rhinophore (and preocular tentacles to a lesser degree) resulted within 10 s in the distinctive far-field “cone of search” behavior ($P < 0.0001$). Stimulation of the digital tentacles resulted in the animal immediately releasing its lateral digital tentacles and swimming toward the substrate ($P < 0.019$) with medial digital tentacles making contact with the odor source, as observed when nautilus are in proximity to an odor source. Control trials with odor-free stimulus did not elicit either of these postures, but resulted in an overall increase in swimming and rocking behavior, supporting morphological studies demonstrating that these structures may also have an important mechanosensory function. These results indicate that the rhinophores and perhaps the preocular tentacles play a role in far-field odor detection, while the lateral digital tentacles are at least in part responsible for chemosensory food-searching behaviors near (but not in contact with) the odor source.

Keywords: *Nautilus*, *cephalopod*, *chemosensory*, *orientation*, *rhinophore*, *tentacle*

Introduction

The few living species of *Nautilus* are the remainder of a large group of externally shelled cephalopod molluscs that thrived in the oceans from 225 to 65 million years ago. Nautilus may provide insight into the sensory biology and behavioral ecology of similar heavily shelled

inhabitants of ancient oceans. Because of their importance to the fossil record and their unique modern-day ecology, nautilus are of interest to both geologists and biologists. Modern coleoid cephalopods (Octopuses, cuttlefishes, squids) show highly modified morphology and behavior for a fast, visual, and predatory lifestyle. Our experiments examine the chemosensory abilities of *Nautilus*, which differ significantly from those of other cephalopods in both their anatomy and behavior.

Nautilus live among the coral reefs of the Indo-Pacific, remaining in dimly lit waters for most of their lives at depths of up to 300 m, only traveling to shallower, warmer waters (75 m) to forage during nighttime hours (Carlson et al. 1984, Saunders 1984, Saunders and Landman 1987, Ward et al. 1984, Ward 1987, Hanlon and Messenger 1996, Wells et al. 1992). Coupled with their nocturnal habits, at these depths, nautilus are probably more dependent upon chemical and tactile information to solve important tasks than vision. The structure of the large but primitive pinhole eye of *Nautilus* supports the idea that vision is not the most essential sensory system for foraging (Messenger 1981) as it is for other modern cephalopods (Messenger 1991, Munz 1991, O'Dor et al. 1993).

Nautilus possess a pair of rhinophores, one located below each eye and open to the exterior by a narrow pore (Barber and Wright 1969). These putative olfactory structures are similar to the olfactory organs in *Octopus* and other cephalopods but are significantly larger, as are the olfactory lobes in the brain of *Nautilus* (Young 1965). The epithelium of the rhinophores possesses cells that are similar in ultrastructure to chemoreceptors located in the sucker of *Octopus*, the olfactory structure of squids, and the lip of *Sepia* (Graziadei 1964, Emery 1975, 1976, Gilly and Lucero 1992, Lucero et al. 1992). Our previous work (Basil et al. 2000) demonstrates that they are essential for orientation to odor.

While the morphology of the tentacles is well described (Kier 1987), the sensory anatomy of the tentacles was less well known until recently. Preocular and postocular tentacles were proposed to function both in tactile and in chemosensory behavior (Willey 1898, Bidder 1962, Fukada 1980). Taste bud-like cells on the long digital tentacles of *Nautilus macromphalus* have been proposed as contact chemoreceptors (Fukada 1980, 1987). Barber and Wright (1969) described putative mechanoreceptive cells on the postocular tentacles, perhaps serving the function of an eyelid, wiping debris off the lensless eye. While there were numerous behavioral observations proposing sensory function (see Bidder 1962, Basil et al. 2000, 2002), little morphological data existed describing the potential sensory abilities of the preocular and digital tentacles.

Recently, however, Ruth et al. (2002) used electron microscopy to examine the epithelium of the rhinophore, preocular, postocular, and digital tentacles of *Nautilus* in detail. They described numerous ciliated cell types concentrated in these areas indicating that the epithelium of these structures has a sensory, particularly chemosensory and mechanosensory, function. One cell type in particular, with long tufted cilia, (found in pre- and postocular tentacles, the lamellae of the lateral-digital tentacles, and in the olfactory pits of the rhinophores) seems to be suited for far-field chemoreception. Other cell types (found for instance near the base of the rhinophores) are mechanosensory, while the medial-digital tentacles seem to be suited for a contact chemoreception function.

We have previously demonstrated in a naturalistic odor-tracking paradigm, and by selective and reversible olfactory blockage, that *Nautilus* (1) detect odor at a distance of up to 10 m, (2) use odor-plume information to locate the odor source, and (3) rely upon their rhinophores to track an odor to its source (Basil et al. 2000, 2002). When nautilus oriented in turbulent odor plumes, they exhibited a number of distinct behaviors as they moved toward the source of the odor. They began their far-field distant search by spreading their digital tentacles out laterally in a distinctive pattern called

“cone of search” or “cat’s whiskers” (Bidder 1962). They then moved upcurrent, perhaps using mechanosensory and visual input to remain on target while keeping in contact with the odor plume. If contact with the plume was lost, nautilus initiated circling behavior until the odor plume was once again detected. Within a few body lengths of the odor source (near-field), they fully extended their lateral digital tentacles downward, and swam toward the substrate as though they were searching for a buried food item, ultimately using their medial digital tentacles to grasp at the substrate and touch the odor source (Basil et al. 2000).

Here we examine which structures are linked to different behavioral stages of the search (“near-field” and “far-field”) to determine their function in the orientation behavior of *Nautilus*. Based on the patterns of behavior exhibited by orienting animals and supported by the morphology reported by Ruth et al. (2002), we predicted that rhinophore stimulation would elicit the “cone of search” (far-field) posture while lateral digital tentacle stimulation would result in the animal swimming toward the substrate with lateral digital tentacles extended while using its medial digital tentacles (near-field) to search for a food item. We were particularly interested in the behavior of the preocular and postocular tentacles because we had not been able to track their behavior closely during seminaturalistic studies.

Methods

Animals

Experiments were run October–November, 2001. Eleven *Nautilus*, captured in the Philippines, were housed in a 300-gal recirculating system of filtered sea water kept at 17°C. Five and six animals each were kept in separate vertical cylindrical tanks (2.5 m in height, 1.5 m in diameter) to support their natural tendency to swim up and down in the water column. Water traveled from the tanks to the biofilter, into a chiller (Aqua Logic Chiller 1/3 hp AE4), through two 80 W UV filters (Emperor Aquatics Ultraviolet 80 Watt Sterilizer model 02080), and then back into the holding tanks. Both tanks were provided with protein skimmers (Berlin Turbo Protein Skimmer model VS32). Daily checks were made of animal health, nitrogen levels, pH, temperature, alkalinity, and salinity. While home tanks were kept as dark as possible, the animals experienced an ambient light:dark cycle due to the water-quality maintenance and feeding schedules. Animals were fed 1/2 of a *Tilapia* head every 2–3 days. *Tilapia* heads provided both protein and calcium; nautilus under this feeding regimen did not show the dark banding pattern on the shell that captive nautilus often do. Experiments were performed between 19:00 and 22:00, after lights off, when the animals normally become active, on days when they had not been fed.

Flume

Experiments took place in a Plexiglas flow-through flume (1.4 m × 0.4 m × 0.32 m) filled with filtered sea water at ambient temperature (22°C, Figure 1). Before each trial, animals were slowly acclimated to the warmer flume temperature over 1 h. At the end of each trial, animals were again acclimated to home-tank temperature over the course of an hour.

The testing area of the flume was confined to a 0.25 m × 0.4 m × 0.32 m chamber, created from flow-through plastic mesh (80% open area), located 0.4 m from the downstream outlet pipe of the flume (Figure 1). Here water was of consistent directional flow as demonstrated by pre-test dye studies with red food coloring. Flow was slow, about 1 cm/s, so any

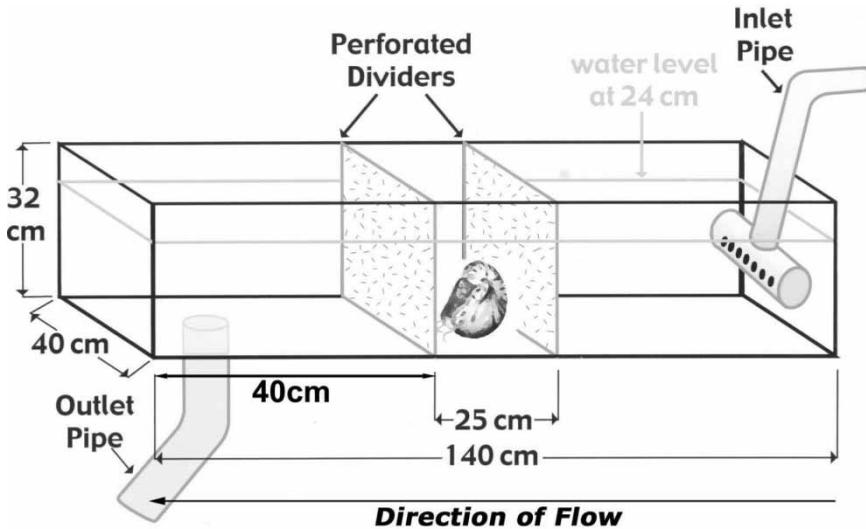


Figure 1. Experimental flume: experiments took place in a Plexiglas flow-through flume ($1.4\text{ m} \times 0.4\text{ m} \times 0.32\text{ m}$) filled with filtered sea water at ambient temperature (22°C). The testing area of the flume was confined to a $0.25\text{ m} \times 0.4\text{ m} \times 0.32\text{ m}$ chamber, created from flow-through plastic mesh (80% open area), located 0.4 m from the downstream outlet pipe of the flume.

odor in the tank remained in one location for a period of time before moving downstream and out. The flume was flushed between stimulus applications.

Habituation

Before a trial began, the temperature-acclimated animal was placed into the chamber for 10 min, and the area was covered with a piece of black plastic to prevent disturbance of the animal from activity in the experimental room and to encourage habituation to the chamber. Animals were considered habituated to the chamber when they were observed to attach with one or two digital tentacles to the plastic mesh (resting position). This generally happened well before 10 min transpired, but nonetheless all animals were given the entire time to habituate.

Experiments

Experiments were performed by at least four experimenters. One experimenter knew the identity of the animal, the experimenter stimulating sensory structures knew the order of organ stimulation during a trial, one experimenter filmed from the side with a stationary camera, and one experimenter filmed the experiment from above with a moving camera. These four experimenters were different from trial-to-trial so no one person knew all outcomes. Videotapes were scored by “blind” observers experienced in analyzing behavior in nautilus, but under different conditions. A within-subject design was used with each animal tested once in the odor condition (all four randomly ordered structure stimulations) and once in the no-odor condition (all four randomly ordered structure stimulations). At least a week transpired between conditions for each animal.

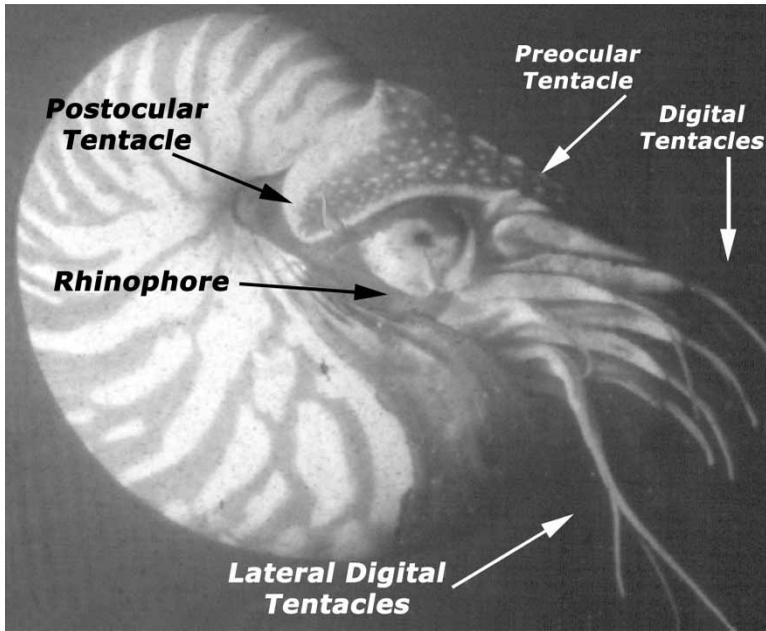


Figure 2. Stimulated structures: one cc of a 1.5% solution of 30 g/l tuna extract mixed with filtered seawater and an inert red food coloring for visualization, was hand-pipetted directly (within 1 cm) onto one randomly chosen of four appendages on one side of the animal only: rhinophore, digital tentacles, preocular tentacle, and postocular tentacle, (see labels). The rhinophore was stimulated by pipetting stimulus 1 cm behind the rhinophore, so that it would be drawn over the rhinophore by the gill current.

Odor-condition trials

Trials were divided into two phases: prestimulation and stimulation.

Prestimulation phase: After habituation, animals were filmed for 10 s in the chamber without any odor stimulation to gather baseline data in the absence of odor.

Stimulation phase: After prestimulation, 1 cc of a 1.5% solution of 30 g/l fish extract (Basil et al. 2000) mixed with filtered sea water and an inert red food coloring for visualization was hand-pipetted directly (within 1 cm, confirmed during video analysis) onto one randomly chosen of four appendages on one side of the animal only (either the rhinophore, digital tentacles, preocular tentacle, and postocular tentacle; Figure 2). Only one side of the animal was stimulated because any asynchrony in bilateral stimulation might complicate the response. While this simplifies the stimulus, our aim was to determine the response of each structure in as isolated a fashion as possible. Filming continued for 60 s after stimulation was complete. Any behaviors that occurred in the entire 70 s time period were recorded by both camera operators.

After stimulation of one randomly chosen structure, the chamber with the *Nautilus* was covered once again with black plastic, and the flume was drained partially until any remaining dyed odor stimulus evacuated the tank. The tank was then refilled upstream for the next stimulation trial. Ten seconds of prestimulation footage was filmed, and the next randomly chosen structure was tested in an identical manner as above. Each animal experienced each structure-stimulation condition once, presented in random order in one filming session to control for order effects. At the end of trials, nautiluses were reacquainted to their home-tank temperature, returned to their home tanks, and fed a piece of *Tilapia* head.

During video analysis, the location of the pipet relative to each structure was confirmed to ensure standardization of application of odor, as was precise stimulation of the structure since the stimulus was dyed.

No-odor controls

Nautilus cannot be maintained in the testing arena for more than 1/2 h without experiencing signs of stress. Stress in *Nautilus* is expressed by a stereotypical “rocking” behavior, where the animal rocks back and forth on its anterior and posterior axis. We, therefore, separated out the no-odor trials into a different set of experiments from the odor trials to shorten overall trial length for each animal to less than 1/2 h. Indeed, when two mixed odor/no-odor trials were performed, stimulating each of the four structures of two animals with filtered seawater and rhodamine only and also with odor (all randomly) to control for hydrodynamic cues (resulting in eight total stimulations), the animals began to exhibit signs of stress over time. The health and well being of the animals was our primary focus, so no-odor controls were performed separately in the same fashion as odor trials. However, the stimulus contained only 1 cc red food dye and filtered seawater. By reducing session length by separating odor and no-odor trials, we would be certain to stimulate all organs within sessions of each type. No animals exhibited rocking behavior in either the odor or the no-odor condition.

Behavioral scoring

Using the footage taken from the stationary and the hand-held cameras, three separate “blind” observers, experienced with observing *Nautilus* behavior under different conditions, scored the following behaviors in 5-s intervals for all trials of a particular subject (Figure 3A–F).

Cone of search (3A): *Nautilus* extends its digital tentacles laterally so that the beak can be seen between them, and swims forward slowly, turning slightly from side to side. Arrow points to downward-oriented siphon; the animal is swimming upward in the water column. The scoring was as follows: 1 = cone of search present in 5-s bin; 0 = cone of search not present in 5-s bin).

Touch bottom: *Nautilus* quickly swims to the bottom of the tank while lateral digital tentacles are extended (Figure 2). Associated with digging behavior by the medial digital tentacles when it reaches tank bottom (3B, G). The scoring was as follows: 1 = Animal Touched Bottom in 5-s bin; 0 = Animal did not touch bottom in 5-s bin.

Post-ocular tentacle extension (3C): *Nautilus* extends post-ocular tentacle out of sheath by at least 1 cm (1 = yes; 0 = no).

Pre-ocular tentacle extension (3C, F): *Nautilus* extends pre-ocular tentacle out of sheath by at least 1 cm (1 = yes; 0 = no).

Digital tentacle extension (3D, E, F): *Nautilus* extends the bulk of their digital tentacles (lateral and medial) in any direction by less than half a shell length (=1) or more than half a shell length (=2). All digitals (Figure 3D, F); lateral digital (Figure 2 and Figure 3E).

Ventilation rate (3G): The number of ventilations (measured by watching the mantle expand and contract) per 5-s bin.

Rocking behavior: *Nautilus* rocks back and forth on its anterior-posterior axis rapidly while ventilating quickly. This is considered to be a stress or irritation response. Once again: 1 = presence of behavior in 5-s bin, 0 = lack of behavior in 5-s bin.

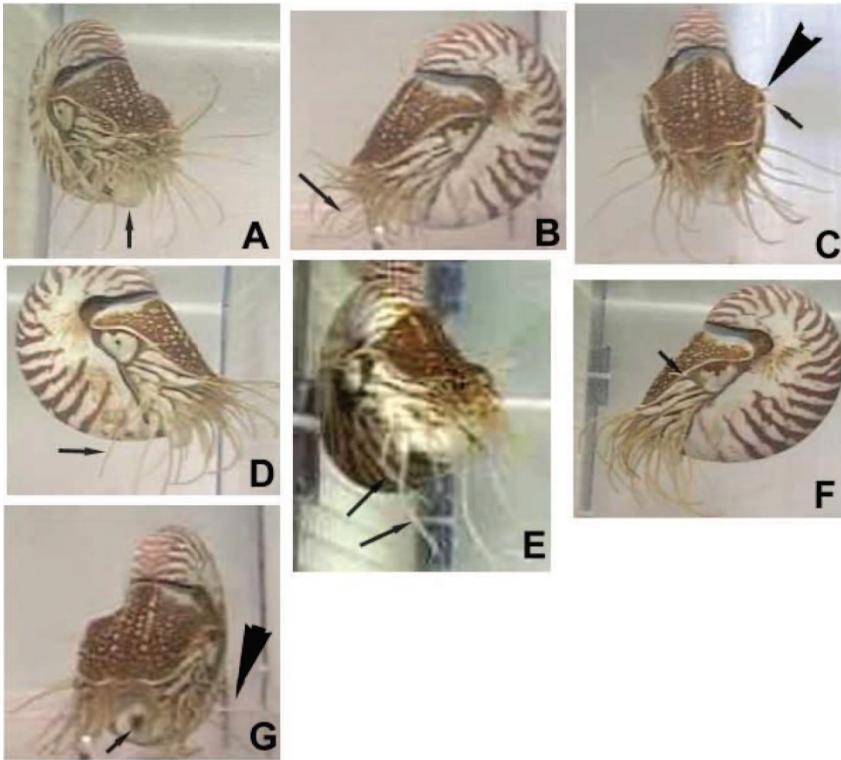


Figure 3. Behavioral Scoring (images taken from videotaped trials): (A) Cone of search posture. Digital tentacles extend laterally, revealing the siphon, which is directed downward so the animal will rise in the water column. (B) Touch bottom posture. *Nautilus* is touching the bottom of the experimental arena with its medial digital tentacles (arrow). Lateral digital tentacles are extended laterally. (C) Preocular (small arrow) and postocular (large arrow) extension. Both tentacles are completely extended from sheaths. Animal is in cone of search posture. (D) Digital tentacle extension. Arrow indicates lateral digital tentacles, which are extended by about 0.5 shell length. (E) Digital drop behavior: Lateral digital tentacles (2 pairs) fully extended as animal moves toward substrate. (F) Preocular tentacle extension (arrow) and digital extension behavior. (G) Touch bottom behavior. Medial tentacles touch substrate extensively while lateral digital tentacles (arrow) are pulled away from the substrate.

Swimming: Animal actively swam in any direction at least 5 cm in a given time bin. Scoring as above.

Turning direction: *Nautilus* either turns toward or away from side of stimulation.

Statistical analyses

Ventilation rate was analyzed using an ANOVA (within-subject repeated-measures design) while all other behaviors were analyzed using the Friedman Ranks test. The mean response before stimulation was compared to the mean response after stimulation with stimulated structures as factors. Overall significance was determined and the structures were then ranked with respect to their contribution to the significance value.

Because the no-odor controls were run separately from the odor trials for the well-being of the animals, we could not directly statistically compare them to the odor tests. We therefore

analyzed them identically (ANOVA, Friedman Ranks test) but separately to see if there were any significant effects at all of structure stimulation in the no-odor condition.

Other analyses

A naïve observer rescored ten percent of trials for each blind observer (above). No significant differences in behavioral scores were found.

Results

Odor trials

Ventilation rate (ANOVA): There was an overall increase in ventilation rate as the trial proceeded $P(F_{2, 13})=0.006$. However, there was no significant effect of the odor stimulation of any particular structure on ventilation rate, except for a slight increase $P(F_{10, 13})=0.07$. No-odor controls did not increase their ventilation rate regardless of what structure was stimulated.

Cone of search (Figure 3A; Friedman Ranks): Nautiluses displayed the cone of search response significantly more often after odor stimulation of the rhinophore (Figure 2) than before stimulation of the rhinophore ($\text{Chi}^2: 18.357, df=3, P<0.0001$, Figure 4). This response generally occurred within 5 s of stimulation and lasted well past the 70 s filming period. Stimulation of the preocular tentacle (Figure 2) also elicited a cone of search response more often after stimulation than before, though the effect was less strong (Figure 4). This response was almost immediate (~ 2 s) and lasted well past the filming period (70 s). No other structure showed such effects. Structures (Figure 2) were ranked as follows with respect to their contribution to the significance value: Rhinophore > Preocular Tentacle \gg Postocular Tentacle > Digital Tentacles.

Touch bottom (Figure 3B, G; Friedman Ranks): After odor was applied to the digital tentacles of *Nautilus* (Figure 2), they extended their lateral digital tentacles and then touched the bottom of the tank/substrate with their medial digital tentacles significantly more often than before stimulation ($\text{Chi}^2=9.2, df=3, P<0.019$, Figure 5). This response occurred

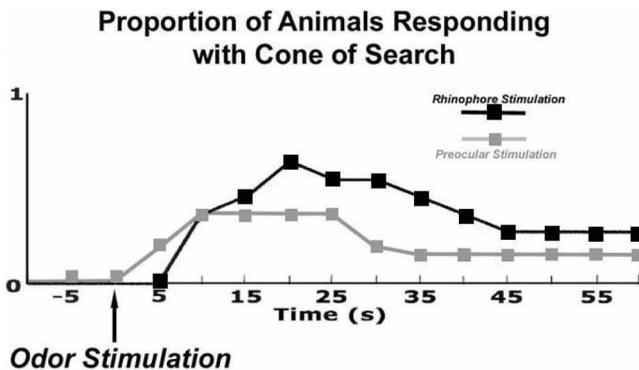


Figure 4. Proportion of animals responding with cone of search. Arrow indicates time of odor stimulation. Rhinophore-stimulated animals respond within 10 s while preocular stimulated animals respond immediately. Cone of search and ocular extension lasts for at least 70 s after stimulation, in keeping with natural orientation behavior. *Separate* no-odor control group did not show any cone of search behavior in response to rhinophore stimulation.

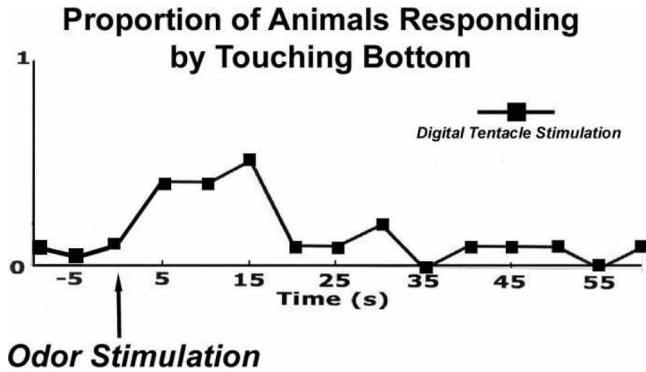


Figure 5. Proportion of animals responding with touch bottom. Animals respond with the touch bottom behavior within 2 s of stimulation of digital tentacles. Behavior lasts for up to 20 s, in keeping with natural orientation behavior. *Separate* no-odor control group did not show any touch bottom behavior in response to digital tentacle stimulation.

within 2 s of stimulation and was of limited duration (20 s). Lateral digital tentacles were pulled posteriorly and did not touch the substrate (Figure 3G). This response was observed for a shorter period of time than cone of search (Figure 3A). Based on ranking, the lateral digital-tentacle stimulation contributed the most to this effect. Preocular tentacles ranked second in this analysis. Structures (Figure 2) were ranked as follows with respect to their contribution to the significance value: Lateral Digital Tentacles > Preocular Tentacle \gg Rhinophore > Postocular Tentacle.

Other behaviors (Friedman Ranks): No other combinations of stimulated structures and resultant behaviors were significant pre- and post-odor application.

No-odor control group

Stimulation with artificial seawater and red food coloring, particularly directed toward the preocular tentacles, resulted in an increase in rocking and swimming behavior overall, but this was not significant ($P < 0.07$). No other behaviors were more likely to occur after stimulation of any structure in the no-odor condition.

Interobserver reliability

There were no significant differences in the trials scored by the naïve observer and the other three observers.

Order effects

Animals tended to habituate to the odor as stimulation-trials proceeded; the last application was generally less likely to elicit a response than the first. However, this effect was not significant because the stimulations were randomized; any small effect it might have had was taken into consideration in analyses.

Discussion

Successfully solving the problem of tracking odor to its source is critical to the survival of many animals (*Nudibranchs*, Willows 1978, *Snails*, Chase and Croll 1981, *Moths*,

Vickers and Baker 1994, *Cephalopods*, Boal 1997, Boal and Marsh 1998, Boal and Golden 1999, *Fishes*, Loekkeborg et al. 2000, *Crustaceans*, Grasso and Basil 2002, *Aplysia*, Painter et al. 2003) including nautiluses. These nocturnal animals, living in dimly lit waters for most of their lives, most likely rely heavily on odor to find food and mates based on naturalistic and laboratory studies (Bidder 1962, O'Dor et al. 1993, Basil et al. 2000, 2002). This chemically guided behavior, supported by bilateral olfactory organs, is similar to that of lobsters, nocturnal animals that occupy similar benthic habitats (Moore and Atema 1991, Basil and Atema 1994, Atema 1996, Grasso and Basil 2002). Since the environment in which they live also carries many hydrodynamic patterns (turbulence, flow), mechanosensory abilities are likely to be involved in solving these important problems as well.

Our study demonstrates that key chemosensory structures, namely the rhinophore and the various tentacles of *Nautilus* (Figure 2; Basil et al. 2000, Ruth et al. 2002), are associated to different extents with specific odor-orientation behaviors that vary as a function of distance from the odor source during natural orientation behavior (Bidder 1962, Basil et al. 2000, 2002, Ruth et al. 2002). Far from the source, nautiluses extend their tentacles laterally and swim forward slowing in a posture called cone of search. Often their ocular tentacles are extended broadly as well in this posture. Closer to the source, nautiluses drop toward the substrate with their four lateral digital tentacles extended, and then reach out to the odor source with their medial digital tentacles. Results from control trials with no odor indicate that odor, in combination with hydrodynamic cues, is necessary for these behaviors. Odor plumes are turbulent and patchy by nature (Murlis and Jones 1981, Bell and Carde 1984, Moore and Atema 1991, Basil and Atema 1994, Atema 1996, Grasso and Basil 2002), most animals often use a combination of flow and chemical information to locate a distant odor source such as a food item or a mate.

We found that stimulation of the rhinophore significantly elicited the cone of search behavior associated with far-field tracking behavior and was the least likely to bring about near-field behaviors like moving toward the substrate and digging. Stimulation of the tentacles had the converse effect, significantly resulting in behaviors that animals exhibit when they are close to the odor source (near field). Taken together, these results support the notion that the rhinophores serve primarily in distance chemoreception during tracking, while the digital tentacles come into play when the animal is locally searching for food after tracking, ultimately digging for the item, searching coral reef crevices, etc., with the medial digital tentacles. Other molluscs (Field and Macmillan 1973, Willows 1978, Chase and Croll 1981, Lemaire and Chase 1998) also demonstrate differences in search behaviors far from and near to the source of important odors in their environment.

Preocular tentacles, when stimulated, are strongly associated with both near-field and far-field behaviors. This is in keeping with behavioral observations (Basil et al. 2000, Ruth et al. 2002) that these tentacles are fully extended laterally the entire time the animal is searching for food. Located on either side of the *Nautilus*, these tentacles may provide constant important "bilateral" sensory information along with the rhinophores (Basil et al. 2000) as the animal orients. Based on our results and the morphological descriptions of Ruth et al. (2002), we posit that they are at least collecting odor information, and probably combining that with hydrodynamic/mechanosensory information at the same time. The role of the postocular tentacles is less clear, although they may have a hydrodynamic function during orientation. Ongoing tentacle-blocking studies may reveal the complete role both sets of ocular structures play in orientation. Future investigation of the role of the tentacles in overall tracking behavior may benefit from research on tentacular function in other molluscs (e.g. Chase and Croll 1981, Lemaire and Chase 1998).

When the rhinophore of a *Nautilus* is stimulated, the animal responds in a delayed fashion; about 10 s elapses before full cone of search posture appears. However, this posture is of long duration, at least 30 s. Considering that the odor stimulus was only 1 cc delivered over a few seconds, and only to one side of the animal, this is a strong response. Animals orienting in naturalistic plumes encounter odor patches of that size far more frequently as they orient toward the source (Moore and Atema 1991, Basil and Atema 1994, Grasso and Basil 2002). The cone-of-search posture lasts proportionately longer as a result in naturalistic conditions. In naturalistic experiments, nautiluses took up to 5 min to locate an odor source 2 m away in Basil et al. (2000), and generally hold their tentacles in the cone-of-search posture until about 15 cm away from the odor source. The digital tentacles slowly extend from their sheaths during this search as well. Thus the role of the rhinophores is most likely to initiate a long-distance search posture that increases the likelihood of encounters with odor patches, which are of lower concentration and are interrupted by patches of odorless water far from the source (Moore and Atema 1991, Basil and Atema 1994, Grasso and Basil 2002) by increasing the surface area and breadth of the tentacles exposed to the odor plume. That nautiluses hold this posture for an extended period of time even after a small exposure to odor supports this proposed odor-plume-search function. Ruth et al. (2002) have described one cell type in the epithelia of the sensory structures of *Nautilus*, with long tufted cilia (found in pre- and postocular tentacles, the lamellae of the lateral-digital tentacles, and in the olfactory pits of the rhinophores) that seems to be suited for far-field chemoreception. The epithelium at the base of each rhinophore in the *Nautilus* has flask-shaped ciliated cells that are similar in ultrastructure of chemoreceptors located in the suckers of *Octopus* spp., the olfactory organs of squids and the lips of *Sepia* spp. (Graziadei 1964, 1965, Emery 1975, 1976, Barber 1987). Rhinophores have also been demonstrated to be instrumental in tracking odor at a distance in *Tritonia diomedea*, a nudibranch that detects its sea-whip prey by following the odor plumes that they generate (Willows 1978).

Stimulation of the digital tentacles caused an immediate and short-term effect. Nautiluses responded by touching bottom within 2 s of odor stimulation and the behavior terminated with the medial tentacles grasping at the substrate 20 s later. Thus it seems that the role of the digital tentacles is for near-field, but not contact, chemosensory behavior. Once the digital tentacles are stimulated at a certain level (whether that be a level of concentration or a rate of encounter of odor patches – a question we are currently examining), the medial tentacles come into play almost immediately to initiate local contact chemosensory search. Ruth et al. (2002) have, in fact, described cell types found for instance near the base of the rhinophores that are mechanosensory while the medial-digital tentacles seem to be suited for a contact chemoreception function. These kinds of near-field feeding behaviors after long-distance tracking have also been observed in crayfishes, American lobsters, spiny lobsters, and crabs (*review*, Grasso and Basil 2002) and in other molluscs (Willows 1978, Chase and Croll 1981).

The Chambered *Nautilus* is armed with numerous tentacles, a pair of rhinophores, and two sets of ocular tentacles possessing tufted sensillae that appear to be adapted for detecting odor at a distance. Coupled with numerous mechanoreceptors and near-field contact receptors, their morphology (along with their three-dimensional searching behavior) is ideal for an animal that inhabits a complex habitat that is mostly devoid of visual information. Since they are not tied to the substrate as are, for instance, lobsters and crabs, nautiluses must solve complex three-dimensional problems as they track an odor source (e.g., *Moths*, Vickers and Baker 1994, *Nautilus*, Basil et al. 2000). They do so using, in various stages and probably in combination, their rhinophores, ocular tentacles, and digital tentacles. Our work

sheds light onto the different functions these structures perform as a *Nautilus* searches for a distant odor source. Future studies will determine how mechanosensory information and odor-patch structure also complement this complex odor-tracking behavior.

Our continuing studies of the sensory biology of *Nautilus*, an animal that has remained relatively unchanged for millions of years (Saunders and Landman 1987, Ward and Saunders 1997), may also provide insight in to the sensory systems that guided the behavior of ancient cephalopods.

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References

- Atema J. 1996. Odor plumes and olfactory landscapes. *Biol. Bull.* 191:129–138.
- Barber VC, Wright DE. 1969. The fine structure of the sense structures of the cephalopod mollusc *Nautilus*. *Zeit. für Zellforsch.* 102:293–312.
- Barber VC. 1987. The sense organ of *Nautilus*. In: Saunders WR, Landman NH, editors. *Nautilus—The biology and paleobiology of a living fossil*. New York: Plenum Press. pp. 223–230.
- Basil J, Atema J. 1994. Lobster orientation in turbulent odor plumes: Simultaneous measurement of tracking behavior and temporal odor patterns. *Biol. Bull.* 187:272–273.
- Basil JA, Hanlon RT, Sheikh SI, Atema J. 2000. Three-dimensional odor plume tracking by the living fossil, *Nautilus pompilius*. *J. Exp. Biol.* 203:1409–1414.
- Basil JA, Lazenby GB, Nakanuku L, Hanlon RT. 2002. Female *Nautilus* are attracted to male conspecifics odor. *Bull. Mar. Sci.* 70:217–225.
- Bell WJ, Carde RT. 1984. Eds. *Chemical Ecology of Insects*. Sunderland: Sinauer Associates, Inc.
- Bidder AM. 1962. Use of tentacles, swimming and buoyancy control in the pearly *Nautilus*. *Nature* 196:451–454.
- Boal JG. 1997. Female choice of males in cuttlefish (Mollusca: Cephalopoda). *Behavior* 143:307–317.
- Boal JG, Golden DK. 1999. Distance chemoreception in the common cuttlefish, *Sepia officinalis* (Mollusca: Cephalopoda). *J. Exp. Mar. Biol. Ecol.* 235:307–317.
- Boal JG, Marsh SE. 1998. Social recognition using chemical cues in cuttlefish (*Sepia officinalis*, Linnaeus, 1758). *J. Exp. Mar. Biol. Ecol.* 230:183–192.
- Carlson BA, McNibben JN, DeGruy MV. 1984. Telemetric investigation of vertical migration of *Nautilus belauensis* in Palau. *Pac. Sci.* 38:183–188.
- Chase R, Croll RP. 1981. Tentacular function in snail olfactory orientation. *J. Comp. Physiol. A* 143, 357–362.
- Emery DG. 1975. The histology and fine structure of the olfactory structure in the squid *Lolliguncula brevis*. *Tissue and Cell* 7:357–367.
- Emery DG. 1976. Observations on the olfactory structure of adult and juvenile *Octopus joubini*. *Tissue and Cell* 8:33–46.
- Field LH, Macmillan DL. 1973. An electrophysiological and behavioral study of sensory responses in Tritonia (Gastropoda, Nudibranchia). *Mar. Behav. Physiol.* 2:171–185.
- Fukada Y. 1980. Observations by SEM. In: Hamada T, Obata I, Okutani T, editors. *Nautilus macromphalus in captivity*. Japanese Expert Consultation on Living Nautilus. Tokyo: Tokai University Press. pp. 23–33.
- Fukada Y. 1987. Histology of the long digital tentacles. In: Saunders WB, Landman N, editors. *Nautilus—The biology and paleobiology of a living fossil*. New York: Plenum Press. pp. 249–256.

- Gilly W, Lucero M. 1992. Behavioural responses to chemical stimulation of the olfactory structure in the squid, *Loligo opalescens*. *J. Exp. Biol.* 162:209–220.
- Grasso FW, Basil JA. 2002. How lobsters, crayfishes, and crabs locate sources of odor: Current perspectives and future directions. *Curr. Opin. Neurobiol.* 12:721–727.
- Graziadei P. 1964. Electron microscopy of some primary receptors in the sucker of *Octopus vulgaris*. *Zeit. für Zellforsch.* 64:510–522.
- Graziadei P. 1965. Sensory receptor cells and related neurons in cephalopods. Cold Spring Harbor Symp. Quant. Biol. 30:45–57.
- Hanlon RT, Messenger JB. 1996. *Cephalopod Behaviour*. Cambridge, UK: Cambridge University Press.
- Kier WM. 1987. The functional morphology of the tentacles musculature of *Nautilus pompilius*. In: Saunders WB, Landman N, editors. *Nautilus—The biology and paleobiology of a living fossil*. New York: Plenum Press. pp. 257–269.
- Lemaire M, Chase R. 1998. Twitching and quivering of the tentacles during snail olfactory orientation. *J. Comp. Physiol. A* 182:81–87.
- Loekkeborg S, Skajaa K, Fernoe A. 2000. Food-search strategy in ling (*Molva molva* L.): Crepuscular activity and use of space. *J. Exp. Mar. Biol. and Ecol.* 247:195–208.
- Lucero MT, Horrigan FT, Gilly WF. 1992. Electrical responses to chemical stimulation of squid olfactory receptor cells. *J. Exp. Biol.* 162:231–249.
- Messenger JB. 1981. Comparative physiology of vision in Molluscs. *Handbook of Sensory Physiology, Vol VII/6C: Comparative Physiology and Evolution of Vision in Invertebrates*, Springer-Verlag.
- Messenger JB. 1991. Photoreception and vision in molluscs. In: Cronly-Dillion, Gregory RL, editors. *Evolution of the Eye and Visual System*. London: Macmillan Press. pp. 364–367.
- Moore PA, Atema J. 1991. Spatial information in the three-dimensional fine structure of an aquatic plume. *Biol. Bull.* 181:408–418.
- Munz WRA. 1991. Anatomical and behavioral studies on vision in *Nautilus* and *Octopus*. *Malacological Bull.* 9:69–74.
- Murlis J, Jones C. 1981. Fine scale structure of odor plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiol. Ent.* 6:71–86.
- O'Dor RK, Forsythe J, Webber DM, Wells J, Wells MJ. 1993. Activity levels of *Nautilus* in the wild. *Nature* 362:626–627.
- Painter SD, Clough B, Black S, Nagle GT. 2003. Behavioral characterization of attracting a water-borne peptide pheromone in the genus *Aplysia*. *Biol. Bull.* 205:16–25.
- Ruth P, Schmidtberg H, Westermann B, Schipp R. 2002. The sensory epithelium of the tentacles and the rhinophore of *Nautilus pompilius* L. (Cephalopoda, Nautiloidea). *J. Morph.* 251:239–255.
- Saunders WB. 1984. The role and status of *Nautilus* in its natural habitat: Evidence from deep-water remote camera photosequences. *Paleobiology* 12:469–486. .
- Saunders WB, Landman N. 1987. *Nautilus—The biology and paleobiology of a living fossil*. New York: Plenum Press. pp. 257–269.
- Vickers NJ, Baker TC. 1994. Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proc. Nat. Acad. Sci. USA* 91:5756–5760.
- Ward PD. 1987. *The Natural History of Nautilus*. London: Allen and Unwin Press.
- Ward PD, Saunders WB. 1997. *Allonautilus*: A new genus of living nautiloid cephalopod and its bearing on phylogeny of the Nautilida. *J. Palaeontol.* 71:1054–1064.
- Ward PD, Carlson B, Weekly M, Brumbaugh B. 1984. Remote telemetry of daily vertical and horizontal movement of *Nautilus* in Palau. *Nature* 309:248–252.
- Wells MJ, Wells J, O'Dor RK. 1992. Life at low oxygen tensions: The behavior and physiology of *Nautilus pompilius* and the biology of extinct forms. *J. Mar. Biol. Assoc. UK* 72:313–328.
- Willey A. 1898. The preocular and postocular tentacles and osphradia of *Nautilus*. *Q. J. Microsc. Sci. N. Ser.* 40:197–201.
- Willows AOD. 1978. Physiology of feeding in *Tritonia* 1. Behavior and mechanics. *Mar. Behav. Physiol.* 5:115–135.
- Young JZ. 1965. The central nervous system of *Nautilus*. *Phil. Trans. Roy. Soc. Lond. B.* 249:1–25.