Chapter 5
Hydrodynamics of Sniffing by Crustaceans

Mimi A.R. Koehl

Abstract Chemical signals are dispersed in aquatic environments by turbulent water currents. The first step in smelling these signals is the capture of odor molecules from the water around an organism. Olfactory antennules of crustaceans are used to study the physical process of odor capture because they are external organs protruding into the water where researchers can measure how they interact with their fluid environment. The antennules of lobsters, crabs, and stomatopods, which bear chemosensory hairs ("aesthetascs"), flick through the water. For any array of small hairs, there is a critical velocity range above which the array is "leaky" and fluid can flow between the hairs, and below which fluid barely moves through the spaces between the hairs. When antennules flick they move faster than the critical velocity and water flows into the spaces between aesthetascs. In contrast, during the return stroke the antennule moves more slowly than the critical velocity and the water sampled during the flick is trapped between the aesthetascs until the next flick. Odorant molecules in the water trapped between the aesthetascs during the return stroke and interflick pause diffuse to the surfaces of the aesthetascs, before the next flick traps a new parcel of water. Therefore, each antennule flick is a "sniff," taking a discrete sample of the odor plume in space and time.

5.1 Introduction

Many animals communicate via chemical signals (odors) released into the surrounding fluid (water or air). The first step in smelling chemical signals is the capture of odor molecules from the fluid around an organism. Therefore, to understand how organisms capture odors, scientists need to investigate how olfactory organs interact with the water or air around them. The olfactory antennules of

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crustaceans provide useful systems for studying the physical process of odor capture because they are external organs protruding into the water where researchers can see how they interact with their fluid environment.

The olfactory organs of malacostracan crustaceans (e.g., lobsters, shrimp, crabs, stomatopods) are the lateral branches (called "lateral filaments") of the antennules, which bear chemosensory hairs (called "aesthetascs") (Fig. 5.1a, c, e) (reviewed by

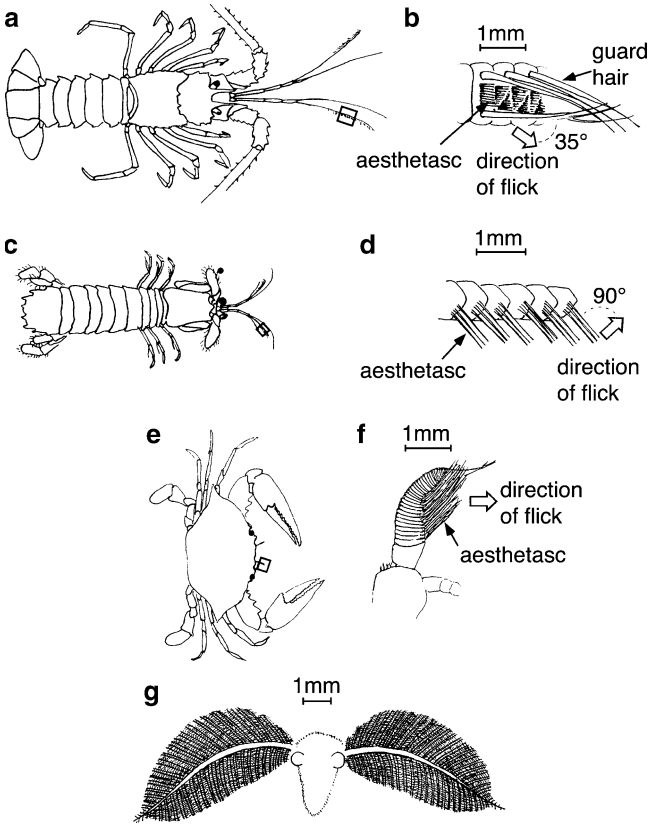

Fig. 5.1 Examples of arthropods with hair-bearing olfactory appendages. The *boxes* around antennules in (**a**, **c**, and **e**) indicate the region of the antennule diagrammed in (**b**, **d**, and **f**), respectively. (**a**) Spiny lobster, *Panulirus argus*. (**b**) Magnified view of a section of the lateral filament of a *P. argus* antennule. The lateral filament flicks downward, with the aesthetascs at an angle of ~35° to the direction of motion (Gleeson et al. 1993). (**c**) Stomatopod ("mantis shrimp"), *Squilla empusa*. (**d**) Magnified view of a section of the aesthetasc-bearing filament of a stomatopod antennule, *Gonodactylus mutatus*. The antennule flicks laterally, with the aesthetascs perpendicular to the direction of movement (Mead et al. 1999). (**e**) Blue crab, *Callinectes sapidus*. (**f**) Magnified view of the tip of the antennule of a *C. sapidus*. The antennule can flick in many directions, but the aesthetascs point in the direction of motion during a flick (Martinez, Lee, and Koehl, unpublished data). (**g**) Head of a male silkworm moth, *Bombyx mori,* showing the olfactory antennae. When the male fans his wings, air moves from front to back across the antennae (Loudon and Koehl 2000) (figure reprinted from Koehl 2001a, with kind permission of Springer Science+Business Media)

Ache 1982; Koehl 2006) (see Hallberg and Skog, Chap. 6). Although nonaesthetasc chemosensory hairs on antennules or legs of some species can also be involved in olfaction (reviewed in Koehl 2006), I will focus here on the aesthetasc-bearing lateral filaments of the antennules to explain the physics of odor capture. Odor molecules in the water around an animal must reach the surfaces of those aesthetascs to be sensed, so understanding the fluid mechanics of arrays of hairs is critical to deciphering how these crustaceans catch chemical signals.

The diversity of antennule morphology and deployment is intriguing. For example, the arrangement of aesthetascs on the lateral filaments differs between species, ranging from the complex arrays on lobster antennules (Fig. 5.1b) to the simple rows on stomatopod antennules (Fig. 5.1d) and the dense brushes on crab antennules (Fig. 5.1f). Do these differences in morphology affect odor capture? Furthermore, many malacostracans flick the lateral filaments of their antennules through the surrounding water (Fig. 5.2). How does flicking affect water motion around the aesthetascs, and thus odor capture?

My interest in the hydrodynamics of molecule capture by flicking crustacean antennules grew from our studies of the hydrodynamics of hair-bearing suspension-feeding appendages and the physical mechanisms they use to capture food particles from the surrounding water (reviewed in Koehl 1995). Those studies, which revealed how difficult it is to get water to flow between tiny hairs, sparked my curiosity about how the chemosensory hairs on insect antennae (Fig. 5.1g) (Loudon and Koehl 2000) and crustacean antennules (Fig. 5.1b, d, f) (Koehl 2001a) can capture molecules from the surrounding fluid. The basic physical rules we discovered about how arrays of hairs interact with fluids (Cheer and Koehl 1987a; Koehl 1992) predicted that the size and spacing of aesthetascs as well as the velocity of antennule flicking should make a big difference to the flow of odor-bearing water into arrays of these chemosensory hairs (Koehl 1996).

Another research path also led me to crustacean antennules. Years of field research in coastal marine habitats made me realize the importance of understanding the physical environment of an organism on the spatial and temporal scales

Fig. 5.2 Diagram of the spiny lobster, *Panulirus argus*, flicking the aesthetasc-bearing lateral filaments of its olfactory antennules. Drawing by Jorge A. Varela Ramos

experienced by that organism (which are not necessarily the scales at which we humans experience the environment). For example, the hydrodynamic forces that can rip a sea anemone off a wave-swept shore depend on the instantaneous water velocities and accelerations it encounters just a few centimeters above the substratum as it sits among its neighbors, not on the much faster freestream water flow across the habitat (Koehl 1977). The waterborne chemical signals that benthic crustaceans encounter in their natural habitats are dispersed from odor sources by messy turbulent water currents. Early models of how animals search for the source of a chemical signal assumed that such turbulent odor plumes are diffuse clouds that become wider and more dilute with distance from the source (reviewed in Koehl 2006). My field experience studying flow microhabitats, however, led me to ask what patterns of odor concentration are actually intercepted by crustaceans navigating in marine habitats. To answer this question I would have to figure out what the *instantaneous* odor concentrations are *in the small slices of water sampled by the olfactory antennules* as they flick in natural environments.

5.2 Physical Mechanisms of Odor Capture

What are the physical mechanisms that olfactory antennules use to capture chemical signals from the surrounding water? Odor molecules diffuse in a fluid via Brownian motion. The time required for molecules to travel through a fluid by Brownian motion increases as the square of the distance (Vogel 1994), therefore molecular diffusion is only important in moving odors over very short distances (e.g., from the water surrounding an aesthetasc to the receptors). Turbulent water currents in the environment transport chemical signals from a source to a crustacean's olfactory antennule, while small-scale water motion near the aesthetascs carries signal-laden water close enough to the surfaces of these chemosensory hairs that odor molecules can diffuse to the olfactory receptors (Koehl 1996, 2001a, b, 2006). Thus, understanding how water samples are moved into the spaces between aesthetascs is an important part of deciphering the process of capturing chemical signals from the environment.

5.2.1 Antennule Flicking

A number of researchers have suggested that when malacostracan crustaceans flick the lateral filaments of their antennules, they increase the penetration of ambient water into the spaces between aesthetascs, and thus bring odor-carrying water closer to the receptor cells in those chemosensory hairs (Snow 1973; Schmitt and Ache 1979; Atema 1985; Gleeson et al. 1993; Koehl 1995, 1996).

Early evidence for this idea was provided by Schmitt and Ache (1979), who found that the response to changes in odor concentration by olfactory receptor neurons in lobster antennules was enhanced if the antennule flicked. The idea that this enhanced response was due to improved water flow into the aesthetasc array was supported by Moore et al. (1991), who found that when they squirted water onto lobster antennules (to mimic flicking), the penetration into the aesthetasc array of tracer molecules carried in the water was increased. How does water flow through an aesthetasc array during a flick, and how does it depend on antennule morphology and motion?

5.2.2 *Fluid Flow Through Arrays of Hairs*

Fluid flow around a hair in an array depends on the relative importance of inertial and viscous forces, as represented by the Reynolds number ($Re = ul\rho/\mu$), where u is velocity, l is hair diameter, ρ is fluid density, and μ is the dynamic viscosity of the fluid (viscosity is the resistance of the fluid to being sheared; a fluid is sheared when neighboring layers of fluid move at different velocities). We humans are big (high l), rapidly moving (large u) organisms who experience high Re turbulent flow dominated by inertia. In contrast, very small (low l) structures such as aesthetascs operate at low Re, where fluid motion is smooth and laminar because viscous forces damp out disturbances to the flow.

Fluid in contact with the surface of a moving body does not slip relative to the body. Therefore, a velocity gradient develops in the fluid next to the body. The lower the Re (i.e., the slower or smaller the body, or the higher the viscosity of the fluid), the thicker this boundary layer of sheared fluid is relative to the size of the body. If the boundary layers around cylinders (i.e., hairs) in an array are thick relative to the gaps between neighboring cylinders, then fluid tends to move around rather than through the array. Using a mathematical model to calculate the velocities of fluid flow around and between cylinders in arrays (Cheer and Koehl 1987a, Cheer and Koehl 1987b; Koehl 1992, 1995, 1996; Koehl 2001a, b), we discovered that hair arrays undergo a transition between nonleaky behavior (where little fluid flows between adjacent hairs) and leaky, sieve-like behavior (where fluid flows between hairs) as Re is increased. Our model predicts that, for very closely-spaced hairs (like aesthetascs on many crustacean antennules), this transition occurs at Re's of about 1, where the leakiness of the hair array is very sensitive to velocity. We found that the sensillae on the antennae of male silkworm moths (*Bombyx mori*) (Fig. 5.1g) operate in this transitional Re range. Although *B. mori* rarely fly, males exposed to female sex pheromone fan their wings. Wing fanning that raises air speed past a walking male moth by 15-fold can increase the velocity through the antennae by a factor of 560 and pheromone interception rates by an order of magnitude (Loudon and Koehl 2000). Similarly, to understand odor capture by aesthetascs in arrays on antennules, their Re's when the antennules flick must be determined.

5.3 Hydrodynamics of Flicking Antennules

We made high-speed videos of flicking antennules of lobsters (Goldman and Koehl 2001), shrimp (Mead 1998), stomatopods (Mead et al. 1999), and crabs (Koehl 2001a). By digitizing the position of an antennule lateral filament in each video frame, we determined its velocity during flicks and return strokes. For all these animals the flick down stroke or outstroke was much faster than the return stroke, and the Re's of the aesthetascs were in the range where the leakiness transition should occur. Our models predicted that water should flow between the aesthetascs during the rapid flick, but not during the slower return stroke.

5.3.1 Dynamically-Scaled Physical Models of Antennules Reveal When Fluid Flows into Arrays of Aesthetascs

We tested these predictions using dynamically-scaled physical models of lobster (Reidenbach et al. 2008), crab (Waldrop, Reidenbach, and Koehl, unpublished data), and stomatopod (Mead and Koehl 2000) antennule lateral filaments. The relative magnitudes of flow velocities measured at different positions in the fluid around a dynamically-scaled model are the same as the relative magnitudes of flow velocities measured at comparable positions around a real antennule (e.g., Koehl 2003). Therefore, we can use dynamically-scaled models to work out the detailed flow velocity maps around and through arrays of aesthetascs (water velocities that would be very difficult to measure around such tiny chemosensory hairs on real flicking antennules). Dynamically-scaled models are geometrically-similar to real antennules and operate at the same Re's. We used large (higher l) models, but operated them at the same Re's as flicking antennules by moving them more slowly (lower u) through mineral oil, a fluid that is more viscous (higher μ) than water.

We used a technique called "particle image velocimetry" (PIV) to determine the flow velocity maps around and through aesthetasc arrays. We marked the oil with neutrally-buoyant particles, and visualized one plane of fluid at a time by illuminating it with a thin sheet of laser light. By moving a video camera at the same speed as a towed antennule model, we could record fluid motion relative to the aesthetascs. Analyzing these videos, we calculated the water velocity vector fields relative to real antennules during their rapid flick and slower return stroke. Such PIV studies of dynamically-scaled physical models revealed that water does flow through the aesthetasc array during the rapid flick down stroke, but not during the slower return stroke for spiny lobsters (Koehl 2001a, b; Reidenbach et al. 2008), stomatopods (Mead and Koehl 2000; Mead and Caldwell, Chap. 11), and crabs (Waldrop, Reidenbach and Koehl, unpublished data).

5.3.2 *Water Flow Through Aesthetasc Arrays of Lobsters and Crabs*

The water velocity profile around the lateral filament of the olfactory antennule of the spiny lobster, *Panulirus argus*, is very different during the flick downstroke than during the return stroke (Fig. 5.3). Although the velocity of the flick is about four times the speed of the return stroke, the water velocity between the aesthetascs during the flick (Fig. 5.3, middle panel), is roughly twenty five times faster than during the return (Fig. 5.3, right panel) because the flick Re is above the Re of the leakiness transition and the return Re is below it (Reidenbach et al. 2008). We found that the flick down stroke lasts just long enough to allow complete replacement of the water in the spaces between the aesthetascs. In contrast, the water between the aesthetascs during the return stroke is essentially trapped there. By working with physical models, we could manipulate the morphology and orientation of an

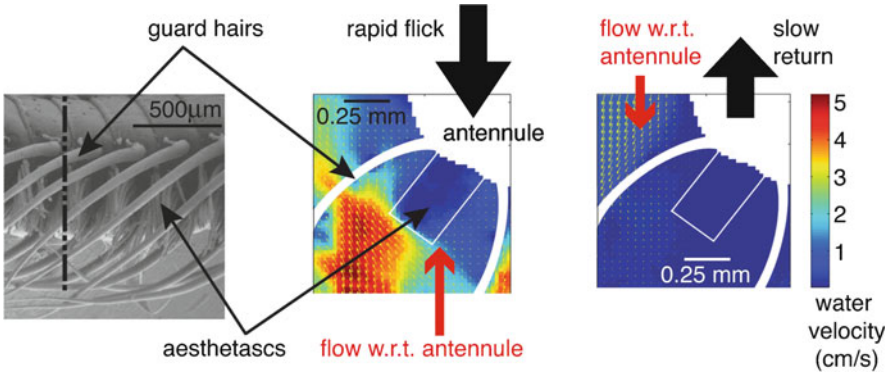

Fig. 5.3 Lateral filament of the antennule of the spiny lobster, *Panulirus argus*. The photograph on the left is a scanning electron micrograph (SEM) of a portion of the lateral filament, showing the chemosensory hairs (aesthetascs) and guard hairs attached to the stalk of the filament (segments of which are visible at the top of the photograph). The *dashed line* shows where a cross-section is taken through the lateral filament, and diagrams of that cross-section are shown in the *middle* and *left panels* of this figure. These diagrams are maps of water velocities *relative to* the lateral filament when it rapidly flicks downward (*middle panel*) and slowly returns upward (*right panel*). The stalk of the lateral filament is labeled "antennule" and the direction of its motion is indicated by the *large black arrow*. The *large red arrow* indicates the direction of water motion relative to the antennule lateral filament. The *white box* outlines the region occupied by the array of aesthetascs, and the position of the guard hairs is labeled. The scale to the right of the diagrams indicates water velocity: *red areas with yellow velocity vectors* show the fastest flow *relative to* the antennule, yellow/green areas with shorter velocity vectors indicate less rapid flow, and *blue areas without velocity vectors* are regions of the slowest water movement. The downstroke is about 4 times faster than the return stroke, but the velocity of the water between the aesthetascs is approximately 25 times faster during the downstroke than during the return stroke (Reidenbach et al. 2008) (SEM by J. Goldman; water velocity maps calculated from PIV measurements around dynamically-scaled physical models of the antennules; modified after Reidenbach et al. 2008)

antennule to explore how such features affect water flow near aesthetascs. These experiments revealed that the complex zigzag arrangement of aesthetascs on the antennules of spiny lobsters (Fig. 5.3, left panel) and their orientation relative to the flicking direction produce uniform flow velocities along the length of the aesthetascs when the antennule flicks.

While a lobster antennule is long and bears a complex array of aesthetascs and nonchemosensory guard hairs, a crab antennule is short and bears a dense cluster of aesthetascs, like a toothbrush (Fig. 5.4, left panel). The aesthetascs on the antennule of a blue crab, *Callinectes sapidus*, are flexible. Ferner and Gaylord (2008) found that if cylinders in a row are flexible and experience fluid flow at right angles to their length at very low Re's (10^{-5}–10^{-3}), then increasing their speed reduces their already low leakiness as the hairs are bent over and moved closer together. What happens to the leakiness of the dense brush of flexible aesthetascs operating at Re's near 1 on a flicking crab antennule? During the rapid flick down stroke when the aesthetascs are on the upstream side of the antennule, they splay apart such that the gaps between neighboring aesthetascs become wider, while during the slower return stroke when the aesthetascs are on the downstream side of the antennule, they are pushed together and the gaps between the hairs become narrower (Koehl 2001a). At Re's near 1 the leakiness of a hair array is very sensitive not only to flow velocity, but also to gap width (Cheer and Koehl 1987a). PIV experiments with dynamically-scaled physical models of *C. sapidus* antennules (Waldrop, Reidenbach and Koehl, unpublished data) showed that water flows through the gaps between aesthetascs during the flick, but not during the return stroke (Fig. 5.4). Because we conducted our experiments with physical models, we could vary the hair spacing, antennule orientation, and antennule speed independently to measure the effects of each. We found that both hair splaying and rapid motion during a flick contribute to the increase in leakiness of the crab aesthetasc array, while both hair clumping and slower motion during the return stroke contribute to the decrease in leakiness of the aesthetasc tuft. As we saw for the lobster, the duration and speed of a crab flick are large enough that much of the water in the aesthetasc array is flushed out and replaced by a new sample of water during the flick (Fig. 5.4, bottom row).

5.3.3 Sniffing

Because these diverse crustaceans flick their antennules in the Re range at which the leakiness of their hair arrays is sensitive to speed, they are able to take fluid samples into their aesthetasc arrays during the rapid down stroke of a flick when the aesthetasc array is leaky. They then retain that captured water within the hair array during the slower return stroke and subsequent stationary pause of the antennule when the aesthetasc array is not leaky. During the next rapid flick down stroke, that water sample is flushed away and replaced by a new one. Therefore, antennule flicking permits these animals to take discrete samples in space and time of their odor environment. In other words, a flick is a sniff (reviewed in Koehl 2006).

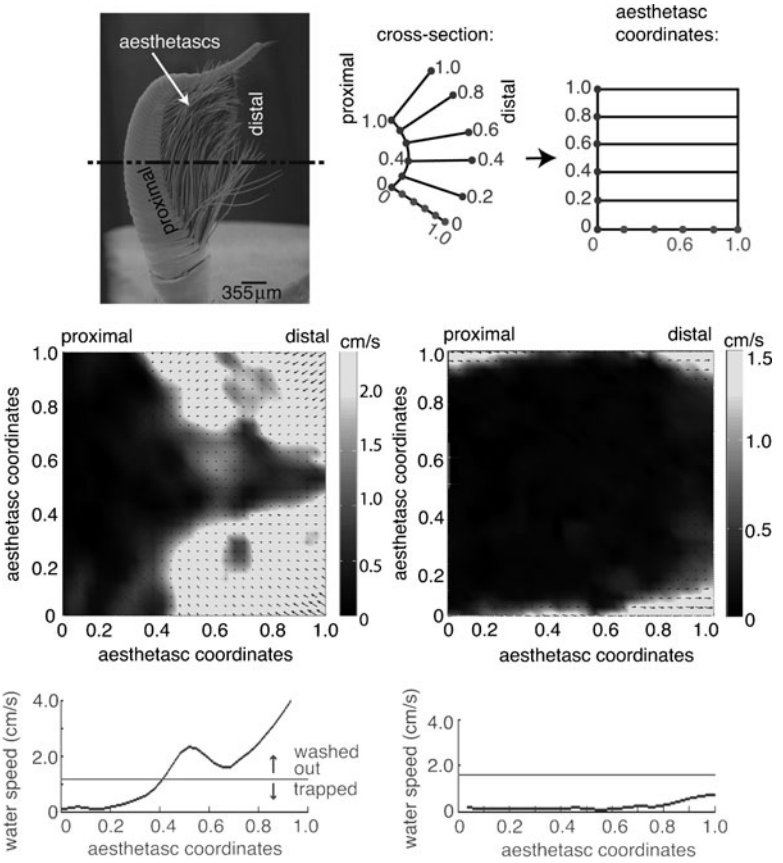

Fig. 5.4 Lateral filament of the antennule of the blue crab, *Callinectes sapidus*. The photograph on the *top left* is a SEM of the lateral filament, showing the chemosensory hairs (aesthetascs) attached to the stalk of the filament. The proximal and distal ends of the aesthetascs are labeled. The *dashed line* shows where a cross-section is taken through the lateral filament, and a diagram of that cross-section is shown just to the *left* of the SEM. Since the aesthetascs splay apart during the rapid flick downstroke and collapse together during the slower return stroke, we have transformed the actual coordinates of the aesthetascs into a rectilinear grid (the "aesthetasc coordinates" shown on the *left* of the *top row*) so that the comparison of the flow between them during the down and return strokes is easier to see. The diagrams in the *middle row* are maps of water velocities *relative to* the lateral filament when it rapidly flicks (*left diagram*) and slowly returns (*right diagram*). These velocity maps are plotted on "aesthetasc coordinates". In the flick downstroke diagram, the antennule is shown moving from left to right, so the water flow *relative to* the antennule is right to left. In the return stroke diagram, the antennule is moving right to left, so the flow relative to the antennule is left to right. The scale to the right of these flow maps indicates water velocity: areas of *white* and *pale gray* show the fastest flow *relative to* the antennule, while the *darkest areas* are regions of the slowest water movement. The graphs at the bottom of the figure show the water velocity relative to the aesthetascs at different positions along the length of the aesthetascs for the flick down stroke (*left*) and return stroke (*right*). The *line* across each graph indicates the water speed necessary for the water in the middle of the aesthetasc array to be washed out of the hair array during the stroke. During the down stroke, most of the water between the aesthetascs is flushed out of the array, whereas during the return stroke, the water is trapped between the aesthetascs (SEM by M. Martinez; water velocity maps calculated from PIV measurements around dynamically-scaled physical models of the antennules by L. Waldrop, M. Reidenbach, and M. Koehl)

5.4 How Odor Plumes are Sampled by Flicking Antennules

When the lateral filament of an antennule flicks, it samples a small slice of the water in a crustacean's environment. What are the patterns of odor concentrations in the water samples captured by flicking antennules as the animals move through habitats exposed to ambient water motion?

5.4.1 Odor Concentrations in Turbulent Odor Plumes

Water currents in aquatic habitats are turbulent. As a chemical signal from an odor source is carried across the environment by a turbulent current, the signal-bearing water is also stirred into the surrounding odor-free water by swirling eddies. Although early models of how animals search for the source of a chemical signal assumed that such odor plumes are diffuse clouds that become wider and more dilute with distance from the source (reviewed in Koehl 2006), scientists are now able to map the fine-scale spatial distribution of odor concentrations in turbulent moving water using a technique called "planar laser-induced fluorescence" (PLIF). If a chemical cue is labeled with a fluorescent dye and allowed to ooze from a source in a flume (a long tank in which water flows), investigators can see how that dye is dispersed by turbulent water currents or waves by illuminating a slice of the odor plume with a sheet of laser light. The laser light makes the dye glow, and the brightness of the dye is proportional to the odor concentration.

Videos of PLIF experiments have revealed that turbulent odor plumes are quite complex and beautiful, and that they are full of holes (i.e., strips of odor-free fluid) (Fig. 5.5). When a turbulent water current flows past an odor source, the water next to the source that contains a high concentration of odor is sheared into filaments. These odor filaments are stretched and rolled up with layers of odor-free water by swirling eddies of various sizes, producing a spatially complex and temporally varying distribution of signal concentrations in an odor plume that becomes wider as it is carried away from the source and meanders across the habitat (reviewed by Weissburg 2000; Moore and Crimaldi 2004; Koehl 2006; Weissburg, Chap. 4). Many shallow coastal marine habitats are subjected to the back-and-forth water flow of waves as well as water currents. Flume PLIF experiments in which waves were superimposed on a water current showed that odor filaments tend to be wider and to be carried to greater heights above the substratum than in the unidirectional current without waves, and that animals navigating near an odor source in wavy flow encounter odor filaments more often than in unidirectional flow (Mead et al. 2003).

In both waves and unidirectional currents, the spatial distribution of odor filaments and odor-free water in a turbulent plume changes with distance from the source of the chemical signal (details reviewed in Koehl 2006; see also Weissburg, Chap. 4). For example, in a plume near the odor source the concentration gradients at the edges of odor filaments are steeper, the concentrations are generally higher,

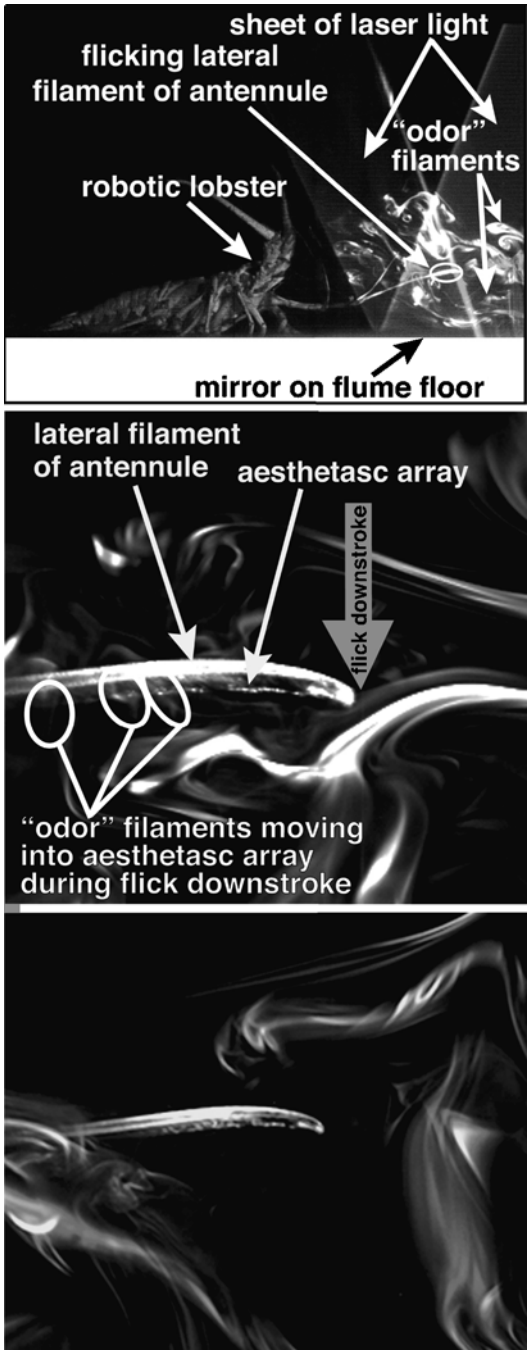

Fig. 5.5 Frames of videos of a robotic spiny lobster, *Panulirus argus*, flicking a real antennule lateral filament at a position 1 m downstream from an "odor" (i.e., fluorescent dye) source in a flume in which a turbulent water current of 0.10 m/s was flowing. The *top image* shows how a sheet of laser light reflected off a mirror on the floor of the flume illuminates the water both above and below a flicking antennule. The light swirls in the water are filaments of dye, and their brightness is proportional to concentration. The *middle* and *lower images* show close-up views of a lateral filament during the rapid flick downstroke. In the *middle image*, filaments of dye can be seen flowing into the aesthetasc array. In contrast, in the lower image, the antennule encounters an odor-free "hole" during the down stroke (frames of videos taken by M. Koehl and J. Koseff)

the odor filaments and the gaps between them tend to be narrower, and the variation in concentration between filaments is greater than they are in that plume at a greater distance downstream from the odor source. Furthermore, the frequency of encounters with odor filaments at the edge of an odor plume is lower than along its midline, although the odor concentrations can be similar. Therefore, the fine-scale patterns of odor concentration in the water contain information about position relative to the source of that odor. Can a flicking antennule capture these fine-scale aspects of odor plume structure?

5.4.2 *Patterns of Odor Concentrations Captured by Flicking Antennules*

A flicking antennule samples only the thin slice of water through which it sweeps. If that slice of water can be illuminated by a sheet of laser light, PLIF can be used to measure the pattern of odor concentrations in the water in the aesthetasc array of the lateral filament of a crustacean antennule. A challenge to this approach is getting a crustacean to flick its antennule in the plane of laser light. We overcame this challenge by using a robotic lobster to flick real antennule lateral filaments in a sheet of laser light shining through a turbulent dye plume in a flume (Koehl et al. 2001) (Fig. 5.5, top). We used fresh antennules from spiny lobsters, *P. argus*, and the robot flicked them using the kinematics we had measured for antennules of that species (Goldman and Koehl 2001). We made high-speed videos of the robot-flicked antennules, and in each video frame we measured the brightness of dye within the aesthetasc array to determine how those dye (i.e., odor) concentrations changed over time.

Our PLIF experiments using the robotic lobster revealed a number of surprises. For example, since an odor plume is full of aroma-free holes, the flicking antennule of a lobster standing in the middle of a plume sometimes encounters filaments of chemical signal (Fig. 5.5, middle), and sometimes it does not (Fig. 5.5, bottom). Furthermore, when a flicking antennule does run into odor filaments, water and the fine filaments of dye (i.e., odor) it carries flow through the spaces between aesthetascs during the rapid down stroke without being stirred up (Fig. 5.5, middle). Then the spatial pattern of odor concentration peaks and valleys that happen to be in the aesthetasc array at the very end of the down stroke are trapped there during the slow return stroke and the stationary pause before the next flick. The odor filaments in the plume around the antennule flow past the lateral filament in the ambient current, but the stripes of chemical signal and of odor-free water within the aesthetasc array stay in place until the next flick, when the old water sample is flushed away and a new sample is trapped between the aesthetascs (Koehl et al. 2001; Koehl 2006). PLIF measurements of the odor samples captured by flicking stomatopod antennules yielded similar results (Mead et al. 2003; Caldwell and Mead, this volume). These experiments indicate that each time the lateral filament of an antennule

flicks, it captures a snapshot of the fine-scale odor concentration patterns in a small slice of the odor plume.

5.5 Flux of Odorant Molecules to Aesthetasc Surfaces

Chemical signals in the water trapped in an aesthetasc array disperse across the small distances between these chemosensory hairs via molecular diffusion. Calculation of the diffusion of odorant molecules carried in the water between aesthetascs on spiny lobster antennules during the slow return stroke (Fig. 5.3, right panel) and interflick pause indicate that the duration of the return stroke and the pause before the next flick is long enough for odor molecules in that water sample to diffuse to aesthetasc surfaces (Reidenbach et al. 2008). Similarly, a mathematical model of the advection (i.e., transport by moving water) and diffusion (i.e., Brownian motion) of odorant molecules in aroma filaments encountered by flicking stomatopod antennules showed that the flux (number arriving per area per time) to aesthetasc surfaces of molecules in odor filaments that have been carried into the spaces between the aesthetascs is high (Stacey et al. 2002). In contrast, the model showed that the flux of signal molecules from odor filaments carried past antennules in the ambient current during the slow return stroke (when water does not flow between the aesthetascs) is very low. These calculations, in combination with the PLIF measurements described above, indicate that a sample of the odor plume is captured within the aesthetasc array during the flick down stroke, and the odorant molecules in that trapped sample diffuse to the chemosensory aesthetascs before that sample is shed and the next sample taken by the subsequent flick.

5.6 Effects of Ambient Flow, Locomotion, and Size on Odor Sampling

Since the leakiness of an array of chemosensory hairs depends on Re, odor sampling by antennules can be affected by the fluid velocity (u) relative to the antennule and by the diameter of the aesthetascs (l). Therefore, ambient water flow and animal locomotion (both affecting u), as well as body size (affecting l) can influence odor capture by antennules.

The ways in which crustaceans deploy their antennules in ambient currents can affect water flow through the aesthetasc arrays. In our flume experiments with lobsters (Koehl et al. 2001), an ambient water current of 10 cm/s did not force water and odor filaments into the aesthetasc arrays on antennules held parallel to the flow direction, whereas water and odor samples did move into the arrays during flick down strokes of 6 cm/s (during a down stroke where the water flow relative to the antennule is perpendicular to the long axis of the antennule). This suggests that if

crustaceans hold their antennules perpendicular to ambient currents with the aesthetascs facing upstream, water should penetrate into the hair array if the ambient flow is fast enough. Are there ambient current velocities above which crustaceans cease antennule flicking because the water motion in the environment drives fluid through their aesthetasc arrays? If so, can the animals track an odor plume to its source as well as they do when they can sniff (i.e., take a discrete odor sample in space and time with each antennule flick)?

Water also moves relative to the antennules of crustaceans when they run (e.g., crabs, 11 cm/s, Martinez et al. 1998) or swim (e.g., mysids, 10–18 cm/s, Cowles and Childress 1988; amphipods 4–14 cm/s, Sainte-Marie 1986; isopods, 8–30 cm/s, Alexander and Chen 1990) through the water. What are the orientations of the antennules when crustaceans locomote, and how does the water movement relative to them affect the leakiness of their arrays of aesthetascs?

Since the leakiness of a hair array depends on size (l), an intriguing aspect of odor capture by crustacean antennules is the ontogeny of sniffing. Malacostracan crustaceans grow from microscopic larvae into large adults. How do antennule morphology and kinematics change during the ontogeny of a crustacean as it changes size, and how does that affect how they take odor samples from the surrounding water? Comparison of different sizes of stomatopods (Mead et al. 1999) and lobsters (Goldman and Koehl 2001) revealed that small animals have larger aesthetascs relative to body size than do big animals, and move rapidly enough during the flick downstroke that they can sniff. Future research should extend these studies to smaller sizes to explore how the morphology and kinematics of the antennules of microscopic larvae and tiny juveniles affect how they sample their odor environment. Is there a lower limit to antennule size for sniffing to be possible?

Our predictions about how Re affects flow through arrays of chemosensory hairs, and thus odor capture, suggest other comparative studies. For example, how do the fluid dynamics of odor sampling by the olfactory organs of the larvae and juveniles mentioned above compare with those of other small crustaceans, such as the planktonic copepods that follow scent trails to find mates (e.g., Weissburg et al. 1998), or the deep sea amphipods that use odors to locate carrion (e.g., Premke et al. 2003)? How do their swimming behaviors or feeding currents affect flow across their olfactory organs, and do they flick? Antennule "sweeps" have been reported from lysianassid amphipods and it was suggested that sweeps facilitate water exchange around the aesthetasc-bearing callynophores (Kaufmann 1994).

5.7 Crustaceans as Model Systems to Study Odor Capture

There are a number of advantages of using crustaceans as systems to study the physical process of odor capture. Antennules are olfactory organs that protrude into the environment, so their interactions with the surrounding odor-bearing fluid are much easier to study than are the fluid mechanics of chemosensory surfaces hidden

within internal nasal passages, such as those of vertebrates. Furthermore, the diversity of antennule morphologies shown by different species of crustaceans enables us to explore the functional consequences of different "designs" of external olfactory organs. Many other types of invertebrate animals have external chemosensory organs that bear hair-like sensillae (reviewed in Koehl 2001a, 2006), so the principles about odor capture learned by studying crustacean antennules can be useful for understanding the function of these other "noses" as well. Another advantage of the species of crustaceans that we have been using as study organisms (e.g., lobsters, crabs, stomatopods) is that they live in accessible shallow marine habitats where we can measure the hydrodynamic conditions that they experience in their natural habitats and that disperse the chemical signals they encounter. Such information enables us to design biologically relevant laboratory studies of antennule function.

Since crustaceans are used to study the neurobiology of olfaction and the behavioral uses of chemical signaling, they provide a system with the potential of enabling us to relate the biophysics of odor capture to how animals process that information, and to how they react to the spatio-temporal patterns of odor information they capture.

5.8 Future Directions

Several important questions about crustacean odor capture that remain unanswered provide promising directions for future research. One such question is whether crustaceans "use" the fine-scale spatio-temporal information their antennules are physically able to capture when they flick in turbulent odor plumes. This question can be addressed at the neurobiological level (Do fine-scale patterns of odor concentrations captured by the antennules affect patterns of neuron firing in the olfactory lobe?) and at the behavioral level (Do fine-scale patterns of odor concentrations captured by the antennules affect plume-searching behavior?). For example, to determine whether different spatio-temporal odor-concentration patterns alter neuron firing, the standard olfactometers (e.g., Y-tube flow set-ups; see also Fig. 10.2 in Thiel, Chap. 10) used to deliver odors to neurobiological preparations could be replaced by odor-delivery systems that mimic the realistic spatial and temporal patterns of odor delivery that antennules experience when flicking at different positions in an odor plume. Similarly, video records of the movements of crustaceans searching in odor plumes visualized by PLIF (Fig. 5.5) enable us to correlate the fine-scale patterns of concentrations of signal captured by right and left antennules on each flick with the subsequent behaviors of the animals (Mead et al. 2003; Mead and Caldwell, Chap. 11). Such studies of *what antennules actually sample* as animals navigate in odor plumes should also enable us to work out search algorithms that were not possible to recognize when whole plumes were visualized and flicking was ignored.

While the olfactory antennules of large malacostracan crustaceans have been used as research systems to elucidate mechanisms by which chemical signals in the environment are sampled, less is known about the hydrodynamics of the chemosensory hairs on other parts of crustacean bodies. The approaches used and the principles elucidated by studying flow through arrays of aesthetascs on antennules can also shed light on how the morphology of other types of chemosensory hairs, as well as their arrangement in arrays and their positions on the body affect their odor-capturing performance. Another important avenue for future neurobiological and behavioral research is to explore how information from chemosensors on the body and legs is coupled with information sampled by the antennules to inform an animal's behavior.

Comparisons of the morphology and kinematics of aquatic olfactory organs with those that operate in air (such as the antennules of terrestrial hermit crabs or the antennae of insects) would provide an interesting test of our ideas about mechanisms of sniffing. The density (ρ) of water is about 1,000 times higher than that of air, but the viscosity (μ) is only about 56 times greater (Vogel 1994), so a structure of a given size must move about 18 times faster in air than in water to achieve the same Re. Therefore, I would expect the leakiness transition for an array of chemosensory hairs of a given size to occur at higher speeds in air. A more striking difference between water and air, however, is that the diffusivity (D, a molecule's propensity to diffuse in a particular fluid) of molecules in air is about 10,000 times greater than in water (Vogel 1994). Because odors can move a given distance via molecular diffusion through air much more rapidly than through water, the filaments in odor plumes are wider (reviewed in Koehl 2006), and the time required for odor molecules to diffuse from sampled air to the surfaces of chemosensory sensillae is much shorter in air than in water. Analysis of the aerodynamics of odor capture by the antennules of intertidal or terrestrial crustaceans (e.g., brachyuran or hermit crabs) could be done using approaches similar to those described in this chapter to determine how antennule morphology and kinematics determine if and how they sniff.

5.9 Summary and Conclusions

Chemical signals are dispersed in aquatic environments by turbulent water currents. The lateral filaments of the antennules of malacostracan crustaceans, which bear arrays of chemosensory hairs (aesthetascs), are an important research system for studying the physics of how olfactory organs bearing hair-like olfactory sensilla capture such chemical signals from the environment. On the scale of an antennule an odor plume is not a diffuse cloud, but rather is a series of fine filaments of aroma-bearing water swirling in scent-free water; the spatio-temporal patterns of these filaments depend on distance from the odor source. When a lobster, stomatopod, or crab flicks the lateral filament of its antennule, water and any odor filaments it is carrying flow through the spaces between the aesthetascs during the rapid down stroke, but not during the slower return stroke or during the stationary pause before

the next flick. For any array of small hairs, there is a critical velocity range above which the array is "leaky" and fluid can flow between the hairs, and below which fluid barely moves through the spaces between the hairs. The striking difference in flow through aesthetasc arrays during the down stroke vs. the upstroke occurs because the down stroke velocities are above and the return stroke velocities are below the speeds at which this transition in leakiness occurs. Odorant molecules in the water trapped between the aesthetascs during the return stroke and pause diffuse to the surfaces of the aesthetascs, before the next flick traps a new parcel of water. Therefore, each antennule flick is a "sniff," taking a discrete sample of the odor plume in space and time. Our work has shown that the size and arrangement of chemosensory hairs on olfactory organs like antennules or antennae, as well as their velocity relative to the surrounding fluid, affect the temporal patterns of odor delivery to the chemosensory hairs. Thus, the physics of odor capture provides the first step in filtering olfactory information from the environment.

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