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Limnol. Oceanogr., 51(4), 2006, 1925–1930

Dimethyl sulfide triggers search behavior in copepods

Abstract—The oceans are nutritionally dilute, and finding food is a major challenge for many zooplanktonic predators. Chemodetection is necessary for successful prey-capture, but little is known about the infochemicals involved in the interaction between herbivorous copepods and their phytoplankton prey. We used females of *Temora longicornis* to investigate chemodetection of dimethyl sulfide (DMS) in this calanoid copepod and quantified its behavioral response to plumes of DMS using video-microscopy in combination with laser-sheet particle image velocimetry (PIV). Slow injection of a 1-μmol L^{-1} DMS plume into the feeding current resulted in a characteristic behavioral pattern (''tail-flapping''), a redirection of flow equivalent to 30% of the average current velocity, and changes in the location of flow-induced vortices. In free-swimming individuals, this likely results in somersault-type movements that are associated with search behavior in copepods. In comparison to seawater controls, DMS injections significantly increased the average number of tail-flaps per copepod during the first 2 s after exposure to DMS gradients. Our results demonstrate that copepods can detect and react to plumes of DMS and suggest that this biogenic trace gas can influence the structure and function of pelagic foodwebs.

Calanoid copepods (average size range 0.5–2 mm) show a variety of behavioral patterns related to vertical migration, swarming, feeding, mating, and swimming (Mauchline 1998). Jumping and escape reactions involve fast and rhythmical beating of the swimming legs that propel the copepods at velocities of 10–100 mm s^{-1} (van Duren and Videler 2003). Various swimming modes are found in suspension feeders that create regular feeding currents with their mouth appendages (Fig. 1; Koehl and Strickler 1981; Malkiel et al. 2003) and result in swimming velocities of a few mm s^{-1} when foraging (van Duren and Videler 2003). This linkage between feeding and swimming greatly increases the prospects of encountering prey (Gerritsen and Strickler 1977). Instead of depending on random encounters with other organisms, however, behavioral studies (Poulet and Marsot 1978; Koehl and Strickler 1981; Gill and Poulet 1988), ultrastructural analyses (Gill 1986), and modeling efforts (Moore et al. 1999; Jiang et al. 2002; Jackson and Kiørboe 2004) suggest that copepods use mechanoreception and chemoreception when searching for food.

The responses to sexual attractants over large ranges (centimeters and tens of seconds) provide the basis for our understanding of chemoreception in copepods (e.g., Mauchline 1998). In contrast, small-range behavioral responses to environmental stimuli may last less than a second, and their detailed investigation is challenging. Such responses result in successful detection of mate-related or food-associated odor trails and constitute the immediate reaction of copepods to such compounds. Despite many efforts, little is known about the release of specific compounds by prey algae and their detection by copepods. In general, successful chemoreception of potential food requires the release of diffusible infochemicals by prey, molecular diffusion and advection by fluid motion that disperses the chemical signal in the environment, and the presence of suitable chemoreceptors in the predator (Jiang et al. 2002). Biogenic trace gases such as dimethyl sulfide (DMS) fulfil the first two prerequisites (Steinke et al. 2002), but whether DMS can be detected by copepods has not been tested.

Although many algae produce DMS in an enzymatic reaction from the secondary metabolite dimethylsulfoniopropionate (Stefels and van Boekel 1993; Steinke et al. 1998), the ecological reasons for DMS production are still uncertain, and its evolutionary feasibility lacks support (Simó 2001). The average oceanic DMS concentration is around 5 nmol L^{-1} (Kettle et al. 1999) but it can reach concentrations of tens to hundreds of nmol L^{-1} in coastal ecosystems (van Duyl et al. 1998), phytoplankton blooms (Malin et al. 1993), or polar waters (DiTullio and Smith 1995). Interactions within the microbial foodweb, such as grazing by microzooplankton (e.g., heterotrophic flagellates and ciliates), can greatly enhance DMS production (Wolfe et al. 1997). As a result, microzooplankton provide a point source of DMS and are thought to be surrounded by a diffusion-limited boundary layer or ''active space'' that, in comparison to bulk seawater concentrations, can be enriched with DMS. For example, model calculations on the distribution of DMS on the surface of small cells after brief (5 s) release of DMS indicate concentrations of 15 μmol L^{-1} and 1 μmol L^{-1} DMS at distances from the organism of 1 μm and 20 μm, respectively (Wolfe 2000). This active space is distorted by the laminar feeding current of a predatory copepod and provides a leading-edge of plankton-associated chemicals that could give the copepod advance warning time (Strickler 1982; Moore et al. 1999; Jiang et al. 2002) when approaching DMS-producing prey. Because some copepods selectively feed on the microzooplankton that graze on phytoplankton (Turner and Granéli 1992), the ability to detect DMS would increase foraging success in these copepods. To address this question, we tested for DMS-chemoreception in tethered females of the calanoid copepod *Temora longicornis* and investigated its behavioral response to plumes of DMS by slowly injecting

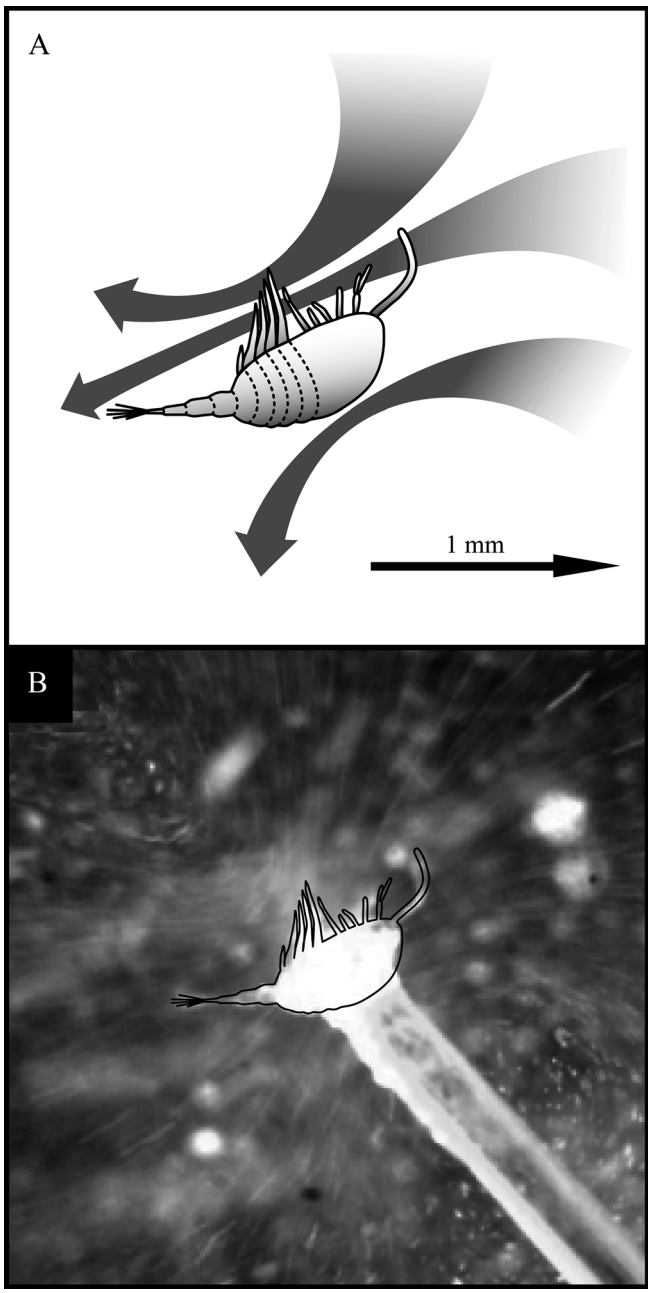

Fig. 1. The calanoid copepod *T. longicornis*. (A) Principal morphology showing six frontal appendages, five swimming legs, and the typical orientation during feeding with an illustration of the laminar feeding current (grey arrows, width indicates flow rate: wide = low, narrow = high). Black arrow shows the scale and swimming direction. (B) Time exposure picture (approximately 1 s) of tethered female in experimental vessel. Image brightness is adjusted to show trajectories of particles in the feeding current. Outline of copepod in (A) is added for clarity to illustrate orientation of the tethered individual.

a plume of a DMS-in-seawater solution or seawater-only control into the laminar feeding current.

Cultivation and tethering of copepods—The omnivorous calanoid copepod *T. longicornis* (O.F. Müller) was grown in 4-liter batch cultures at low light intensity (0.5 μmol m^{-2} s^{-1}; light : dark = 14 : 10 h) and a temperature of 15°C. The phytoplankton prey *Thalassiosira weissflogii* (a low DMS producer) and *Rhodomonas salina* (no DMS) were added to the cultures at approximate densities of 2,500 cells mL^{-1} (300 μg C L^{-1}) and 10,000 cells mL^{-1} (300 μg C L^{-1}), respectively. Prey densities were checked daily with a particle counter (Multisizer 3, Beckmann Coulter). Adult females were selected and transferred into a petri dish. Several drops of a carbon dioxide (CO_2) solution (carbonated mineral water) were added to the dish as an anesthetic before tethering the copepod to the end of a glass capillary (0.37-mm diameter) using 150-mbar suction. The effect of CO_2 disappears within a few minutes after transfer of the tethered copepod to the seawater-filled experimental vessel. Only copepods creating a constant feeding current were used for the experiments.

Preparation and injection of seawater and DMS solution—A primary stock-solution of DMS (1 mmol L^{-1}) was freshly prepared by diluting 73 μL of DMS (Merck-Schuchardt) into 1-liter of cold (4°C), filtered (filter paper, Schleicher & Schuell), and aged natural seawater. The 1-μmol L^{-1} working solution was prepared by diluting 10 μL of primary stock into 10 mL of filtered seawater. Injections of 10-μL plumes were carried out at a rate of 2 μL s^{-1} approximately 3 mm in front of the copepods with a micropipettor (Gilson) that was modified with a piece of tubing and a glass capillary (0.69-mm diameter) attached to an x-y-z micromanipulator. Copepods swim at low Reynolds numbers when feeding (Re $\approx$ 1), and their feeding current is dominated by a laminar flow regime. As a result, the plumes diluted by diffusion only and persisted as a coherent patch during passage of the mouth appendages, which typically lasted for 6 s.

Laser-sheet particle image velocimetry—A tethered copepod was positioned in lateral view in the middle of the experimental vessel using an x-y-z micromanipulator. The laser beam (Coherent Innova K laser, maximum power = 1W) was delivered to the experimental vessel through a protected (armored) glass fiber (Newport Inc.) and transferred into a light sheet through a purpose-built sheet probe (Stamhuis et al. 2002) incorporating converging and cylindrical lenses (Newport Inc.). The sheet plane was positioned horizontally at the level of the tethered copepod. The seawater in the vessel was seeded with 4-μm nylon beads (TSI Inc.). The copepod and the suspended particles in the illuminated plane were imaged using a progressive scan digital camera at 512 $\times$ 512 pixels resolution (Adimec MX12, Eindhoven) and stored uncompressed on a stack of hard disks using a dedicated recording system (Cam2Disk, Dutch Vision Systems). To map the flow, the recorded images were analyzed pairwise for particle displacement applying commercially available DPIV software originally developed in our laboratory (SwiftPIV 4.0, Dutch Vision Systems), using 55 $\times$ 55 pixels interrogation areas with 50% overlap and a center of gravity weighed to grey value peak finder (Stamhuis et al. 2002). This resulted in vector maps and color-coded velocity magnitude and vorticity

plots. Flow fields were evaluated qualitatively as a whole or numerically by comparing sets of velocity vectors from designated areas in the flow field.

Image analysis—Image analysis enabled detailed mapping of the flow field and provided a tool to quantify behavioral responses in relation to injections of DMS and seawater. Images collected at a rate of 25 Hz during each injection were converted into movies (Animation Shop, Jasc Software, Inc.) and analyzed for the period when a seawater or DMS plume passed the copepod's mouth appendages. The plumes are visible in the images since injected fluids lack the reflective particles that were added to the experimental vessel for visualization of the flow when using particle image velocimetry (PIV). Further control recordings were taken to ensure that the copepods created a constant feeding current during periods without the injection of fluids.

Results and discussion—The combination of video microscopy and PIV provided a tool for monitoring the behavior and flow fields surrounding individual copepods. We were able to differentiate between five behavioral patterns: (1) Formation of a laminar feeding current that is driven by the mouth appendages and indicated by anterior-to-posterior flow of water at 2–3 mm s^{-1}. This current results in directed swimming (cruising) when untethered. (2) Brief flicks with antennules and swimming legs that do not significantly alter the flow pattern. (3) Short hops with swimming legs that would move an untethered copepod suddenly forward by a few body lengths. (4) Escape jumps with antennules and swimming legs that highly increase the overall flow and can propel an untethered copepod at 10–100 mm s^{-1}. (5) High-frequency (>25 Hz) dorso-ventral and lateral movements of the urosome (hereafter referred to as tail-flaps) that result in a local redirection of the flow as described in the following.

In the case of DMS, the passing of a plume resulted in an initial up-gradient in DMS concentrations that was followed by a down-gradient in DMS when the plume passed. The first 75 images (equivalent to 3 s) after passage of a DMS gradient were scored for tail-flaps and compared to tail-flap scores when a plume of seawater passed the copepod. We summarized the results into five-frame (0.2 s) intervals and calculated the average number of tail-flaps per injection (seawater $n = 7$, DMS $n = 11$). Tail-flaps occurred only after injecting fluid into the feeding current, but the average number of tail-flaps per copepod was significantly higher with DMS injections (Fig. 2; chi-square distribution, $\chi^2_1 = 7.97$, $p < 0.005$), and the temporal patterns are significantly different between treatments (chi-square distribution, $\chi^2_7 = 21.29$, $p < 0.005$). This suggests that the behavioral response can be triggered by the presence of an infochemical during DMS injections, by gradients such as leading and trailing edges of DMS or seawater plumes, by hydromechanical cues, or by a combination of these that result in a stronger signal. In comparison to seawater, plumes with DMS resulted in three times the average number of tail-flaps per injection over the initial 1-s period and a doubling of these responses

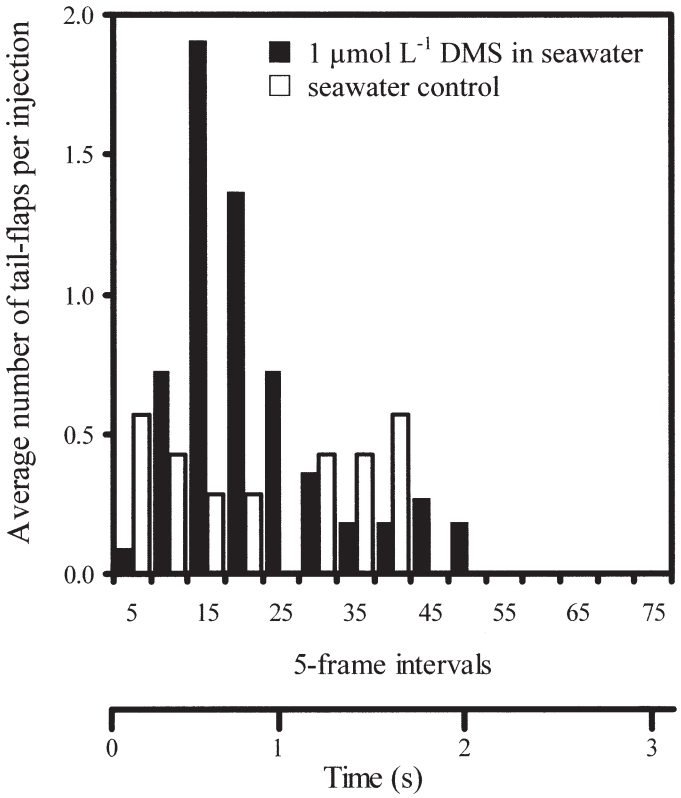

Fig. 2. Tail-flap responses during plume gradients after slow (2 μL s^{-1}) injection of 10 μL of seawater ($n = 7$) or 1 μmol L^{-1} DMS in seawater ($n = 11$). DMS injections result in significantly more tail-flap responses in comparison to seawater controls. Shown is the average number of tail-flaps per injection with time after summarizing the data into five-frame (0.2 s) intervals. No tail-flap responses were recorded for either treatment after 2 s of a plume gradient or in control recordings without fluid injections.

for the 2-s period. Our data provide the first evidence that copepods show a chemosensory response to DMS, suggesting that plumes of this gas can provide the copepods with information about the presence of food. Furthermore, because tail-flaps were associated with up- or down-gradients, it is likely that this behavior is triggered by rapid changes in DMS concentration rather than absolute concentration of this compound. The threshold concentration for this response is currently unknown and requires further investigation.

The two-dimensional PIV analysis of the feeding current during normal feeding shows a laminar flow that produces a jet of water behind the copepod (Fig. 3a). This flow would result in directed swimming in untethered copepods. On the sides of the copepod's length axis, two vortices with opposite rotational sense are visible (Fig. 3b). This flow pattern represents a horizontal cross-section of the three-dimensional converging flow cone (funnel) in front of the copepod and the diverging flow cone behind it, with a vortex-ring structure (torus) around its waist. This flow arrangement changes dramatically during tail-flapping and is marked by a redirection of the current at the ventral side

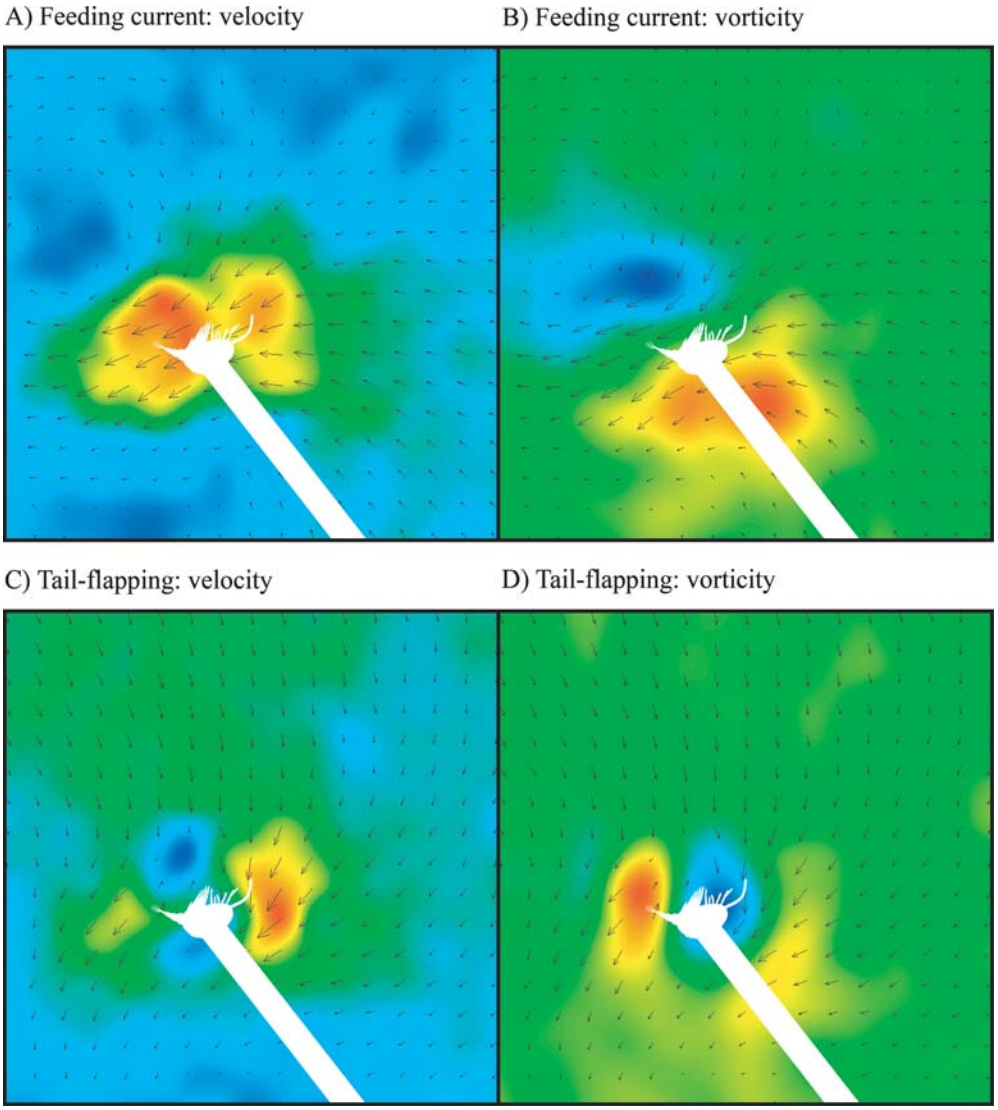

Fig. 3. Laser-sheet PIV with a tethered female of *T. longicornis*. (A and C) Flow velocity (blue = low, red = high), (B and D) vorticity (rate of fluid rotation in the horizontal plane; blue = maximum clockwise direction, red = maximum counter-clockwise direction). Arrows indicate direction and magnitude of flow. (A) Laminar feeding current with jet of water indicated by long arrows in the red area behind the copepod and (B) vortices shown in blue and red on both sides of the feeding current of the copepod. (C and D) Change of flow characteristics during tail-flapping after slow (2 μL s^{-1}) injection of 10 μL of a 1-μmol L^{-1} DMS in seawater solution into the feeding current approximately 3 mm in front of the copepod. Redirection of the feeding current is indicated by altered flow pattern on the ventral side of the copepod (blue area close to the center of the image in C) that results in a clockwise vortex associated with the mouth appendages (blue area in D). Orientation of copepod and position of tether is similar to that in Fig. 1B and shown in white. The image sequences used for these analyses are available as movies in the online appendix to this paper (see Web Appendix 1: http://www.aslo.org/lo/toc/vol_51/issue_4/1925a1.html)

of the copepod, equivalent to 30% of the average current velocity (Fig. 3c). This results in a new distribution of vorticity, a measure for the fluid rotation in the horizontal plane, replacing the two large vortices that are detectable during normal swimming (Fig. 3b) with smaller vortex structures. These smaller vortices are associated with a redirection and recirculation of water along the mouth appendages where chemical detection occurs (Fig. 3d). Hence, in tethered copepods, tail-flapping allows repeated sampling of the DMS-scented water for info-chemical cues.

A description of the behavioral responses in free-swimming copepods is not possible with our experimental design. However, rapid tail-flapping has been noted in free-

swimming copepods (Strickler 1982), and the observed redirection of flow probably results in somersault-type movements that make the copepod repeatedly pass and sample the DMS-scented water. Yen et al. (1998) describe a search behavior with somersaulting when male copepods find or lose a trail of female sexual attractants. These behavioral patterns help copepods to spatially integrate the chemical signals, suggest the existence of short-term memory, and are important for mating success. The tail-flapping response during exposure to DMS may be analogous to this behavior and could aid the copepod in tracing prey trajectories that ultimately help in successful prey capture. Together, this can provide a mechanism for improved characterization of the composition and spatial extension of chemical stimulants in the environment before a new feeding strategy is established.

DMS has been studied extensively for its role in the formation of sulfate aerosol and cloud-condensation nuclei and its influence on climate (Charlson et al. 1987). The so-called ''plankton-climate connection'' has attracted much research attention (e.g., Simó 2001) but the utility of DMS as an aquatic infochemical has been ignored. Seabirds have been shown to react to DMS and can use atmospheric gradients of this gas to detect and locate patches of high biological activity (Nevitt et al. 1995). Furthermore, volatile compounds are recognized for their importance in tritrophic interactions between plants, herbivores, and carnivores in terrestrial ecosystems. For example, attack of the lima bean by herbivorous mites releases several volatile compounds including linalool and methyl salicylate that attract carnivorous mites to an infected plant (Dicke et al. 1993) and result in the activation of five separate defense genes in neighboring, uninfected plants (Arimura et al. 2000). Much less is known about odors that convey environmental information in the sea. Our results indicate that zooplankton react to DMS and, hence, it may be more than a marine trace gas with atmospheric and climatic consequences. Future work should address the threshold concentration of this reaction and the effect of other, related compounds, such as dimethylsulfoniopropionate, acrylate, and dimethyl sulfoxide, or compounds not directly related to algal prey (e.g., dimethyl disulfide or carbon disulfide) to assess their effects. For copepods, DMS is an infochemical and they can react to gradients of this gas with the behavioral response described here. This is an important finding, and chemoreception of DMS in copepods indicates that this trace gas may mediate tritrophic interactions between phytoplankton, herbivorous microzooplankton, and carnivorous zooplankton analogous to the interactions between terrestrial organisms (Steinke et al. 2002). Microbial consumption, photochemical breakdown, and sea-to-air transfer are significant loss factors for DMS, all of which result in a favorable signal-to-noise ratio necessary for infochemicals to be effective. It is likely that DMS affects the structure and function of aquatic ecosystems similar to the effect of volatiles released from terrestrial plants during herbivore attack. Thus, we conclude that DMS and possibly other biogenic volatiles are likely to have an ecological role in facilitating trophic interactions in the plankton.

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References

Arimura, G., R. Ozawa, T. Shimoda, T. Nishioka, W. Boland, and J. Takabyashi. 2000. Herbivory-induced volatiles elicit defence genes in lima bean leaves. Nature **406:** 512–515.

Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature **326:** 655–661.

Dicke, M., J. Bruin, and M. W. Sabelis. 1993. Herbivore-induced plant volatiles mediate plant-carnivore, plant-herbivore, and plant-plant interactions: Talking plants revisited, p. 182–196. *In* J. Schultz and I. Raskin [eds.], Plant signals in interactions with other organisms. American Society of Plant Physiologists.

DiTullio, G. R., and W. O. Smith. 1995. Relationship between dimethylsulfide and phytoplankton pigment concentrations in the Ross Sea, Antarctica. Deep-Sea Res. Pt. I **42:** 873–892.

Gerritsen, J., and J. R. Strickler. 1977. Encounter probabilities and community structure in zooplankton: A mathematical model. J. Fish. Res. Board Can. **34:** 73–82.

Gill, C. W. 1986. Suspected mechanosensory and chemosensory structures of *Temora longicornis* (Copepoda, Calanoida). Mar. Biol. **93:** 449–457.

———, and S. A. Poulet. 1988. Responses of copepods to dissolved free amino-acids. Mar. Ecol. Progr. Ser. **43:** 269–276.

Jackson, G. A., and T. Kiørboe. 2004. Zooplankton use of chemodetection to find and eat particles. Mar. Ecol. Progr. Ser. **269:** 153–162.

Jiang, H. H., T. R. Osborn, and C. Meneveau. 2002. Chemoreception and the deformation of the active space in freely swimming copepods: A numerical study. J. Plankton Res. **24:** 495–510.

Kettle, A. J., and others. 1999. A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month. Global Biogeochem. Cy. **13:** 399–444.

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Acknowledgments

A culture of *Temora longicornis* was provided by Marja Koski. Developmental stages and sex of copepods used in our experiments were determined by Saskia van Veldhuizen. Alastair Grant and Gareth Janacek assisted with the statistical analysis. We thank two anonymous reviewers, Alastair Grant, Thomas Kiørboe, Peter Liss, Gill Malin, and Sue Turner for comments on earlier versions of this paper. M.S. was supported by grants from the U.K. Natural Environment Research Council (NER/I/S/2000/00897 and NE/B500282/1).

Koehl, M. A. R., and J. R. Strickler. 1981. Copepod feeding currents—food capture at low Reynolds-number. Limnol. Oceanogr. **26:** 1062–1073.

Malin, G., S. Turner, P. Liss, P. Holligan, and D. Harbour. 1993. Dimethylsulphide and dimethylsulphoniopropionate in the northeast Atlantic during the summer coccolithophore bloom. Deep-Sea Res. I. **40:** 1487–1508.

Malkiel, E., I. Sheng, J. Katz, and J. R. Strickler. 2003. The three-dimensional flow field generated by a feeding calanoid copepod measured using digital holography. J. Exp. Biol. **206:** 3657–3666.

Mauchline, J. 1998. The biology of calanoid copepods. Academic Press.

Moore, P. A., D. M. Fields, and J. Yen. 1999. Physical constraints of chemoreception in foraging copepods. Limnol. Oceanogr. **44:** 166–177.

Nevitt, G. A., R. R. Veit, and P. Kareiva. 1995. Dimethyl sulphide as a foraging cue for Antarctic Procellariiform seabirds. Nature **376:** 680–682.

Poulet, S. A., and P. Marsot. 1978. Chemosensory grazing by marine calanoid copepods (Arthropoda: Crustacea). Science **200:** 1403–1405.

Simó, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol. Evol. **16:** 287–294.

Stamhuis, E. J., J. J. Videler, L. A. van Duren, and U. K. Müller. 2002. Applying digital particle image velocimetry to animal-generated flows: Traps, hurdles and cures in mapping steady and unsteady flows in Re regimes between 10^{-2} and 10^{5}. Exp. Fluids **33:** 801–813.

Stefels, J., and W. H. M. van Boekel. 1993. Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. Mar. Ecol. Progr. Ser. **97:** 11–18.

Steinke, M., G. Malin, and P. S. Liss. 2002. Trophic interactions in the sea: An ecological role for climate relevant volatiles? J. Phycol. **38:** 630–638.

———, G. V. Wolfe, and G. O. Kirst. 1998. Partial characterisation of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliania huxleyi*. Mar. Ecol. Progr. Ser. **175:** 215–225.

Strickler, J. R. 1982. Calanoid copepods, feeding currents, and the role of gravity. Science **218:** 158–160.

Turner, J. T., and E. Granéli. 1992. Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west coast of Sweden. J. Exp. Mar. Biol. Ecol. **157:** 19–31.

van Duren, L. A., and J. J. Videler. 2003. Escape from viscosity: The kinematics and hydrodynamics of copepod foraging and escape swimming. J. Exp. Biol. **206:** 269–279.

van Duyl, F. C., W. W. C. Gieskes, A. J. Kop, and W. E. Lewis. 1998. Biological control of short–term variations in the concentration of DMSP and DMS during a *Phaeocystis* spring bloom. J. Sea Res. **40:** 221–231.

Wolfe, G. V. 2000. The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. Biol. Bull. **198:** 225–244.

———, M. Steinke, and G. O. Kirst. 1997. Grazing-activated chemical defence in a unicellular marine alga. Nature **387:** 894–897.

Yen, J., M. J. Weissburg, and M. H. Doall. 1998. The fluid physics of signal perception by mate-tracking copepods. Philos. T. Roy. Soc. B **353:** 787–804.

Received: 7 July 2005
Accepted: 14 February 2006
Amended: 21 March 2006