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FEATURE ARTICLE

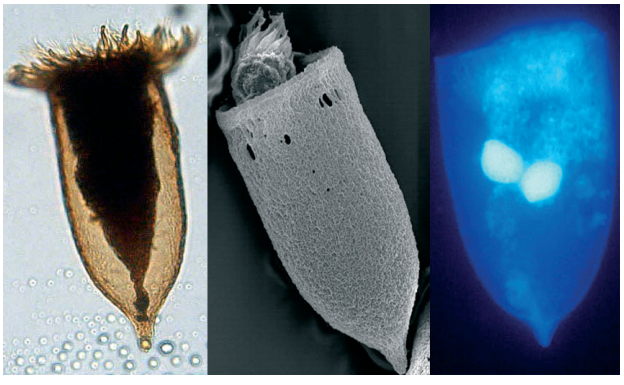
Responses of marine planktonic protists to amino acids: feeding inhibition and swimming behavior in the ciliate *Favella* sp.

Suzanne L. Strom[1,*], Gordon V. Wolfe[2], Kelley J. Bright[1]

[1]Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Rd., Anacortes, Washington 98221, USA
[2]Department of Biological Sciences, California State University Chico, Chico, California 95929-0515, USA

ABSTRACT: Feeding rates of the tintinnid *Favella* sp. on the dinoflagellate *Heterocapsa triquetra* were inhibited by a number of dissolved free amino acids (DFAAs), with inhibition inversely proportional to the size of the amino acid side chain. The most inhibitory compounds (valine, cysteine, proline, alanine, and serine) reduced feeding to <20% of the control rate at a concentration of 20 µM. Inhibition was dose-dependent, with a threshold of ca. 200 nM for proline, and did not depend on ciliate feeding history (well-fed versus starved). Inhibition occurred rapidly (<5 min after exposure) and was partially reversible upon removal of DFAAs. Detailed analysis of swimming did not reveal consistent changes in *Favella* sp. behavior upon exposure to inhibitory amino acids. In contrast to *Favella* sp., the heterotrophic dinoflagellate *Gyrodinium dominans* showed no feeding response to 20 µM DFAAs, while the tintinnid *Coxliella* sp. exhibited reduced feeding (to approximately 50% of control rates) in response to a subset of the amino acids active in *Favella* sp. Our findings, along with the prevalence of some inhibitory compounds at nM concentrations in natural waters, point to a signaling function for these amino acids. Feeding deterrence in *Favella* sp. is, however, contrary to the typical attractant or stimulatory role of DFAAs, which has been documented for organisms ranging from bacteria to metazoans. The information content of the signal remains unclear but may be related to detection of prey quality during suspension feeding by *Favella* sp.

KEY WORDS: Chemical ecology · Signaling · Ingestion · Swimming behavior · Ciliate · Dinoflagellate



Feeding by the tintinnid *Favella* sp., a common coastal planktonic ciliate, is strongly inhibited by certain dissolved free amino acids. Feeding responses and swimming behavior indicate a signaling function for the inhibitory amino acids. Chemical signaling of this type affects predator–prey interactions in the ocean's microbial realm.

Photos: Gordon Wolfe

INTRODUCTION

Signaling among microbes can profoundly affect both individual behavior and community structure and function, as for example in the formation of biofilms (Pasmore & Costerton 2003) or the recently discovered nitric oxide stress response in diatoms (Vardi et al. 2006). However, little is known about the role of signaling in regulating microbial predator–prey interactions in the ocean's planktonic realm (Wolfe 2000). Because much of the ocean's primary production is consumed by microbes, with major consequences for

*Email: stroms@cc.wwu.edu

ocean biogeochemical cycles, it is important to understand processes such as chemical signaling that could regulate microbial interactions in the plankton.

The present research stems from our recent observation that feeding by free-living marine ciliates and heterotrophic dinoflagellates is inhibited by the organic osmolyte dimethylsulfoniopropionate (DMSP), a methionine derivative (Strom et al. 2003, Fredrickson & Strom unpubl.). In addition, the dinoflagellate *Amphidinium longum* showed reduced feeding in response to some structural analogues of DMSP, including glycine betaine and N,N-dimethyl glycine. These results were unexpected since prior work on organisms from bacteria to fish has shown positive (e.g. attractant, feeding stimulant) responses to amino acids (e.g. Poulet & Ouellet 1982, Levandowsky et al. 1984, Valentincic 1985, Miller et al. 2004).

DMSP and glycine betaine are non-toxic and present at high intracellular concentrations in numerous algae. They can be leaked or exuded into the environment by viable cells, particularly during stationary growth phase or when stressed (Matrai & Keller 1994, Wolfe et al. 2002), though information on algal exudation is scarce. DMSP release has also been associated with grazing and viral lysis of DMSP-containing algal cells (Lee et al. 2003, and references therein). Such releases can produce gradients with concentrations reaching the nanomolar to micromolar range (Wolfe 2000). These concentrations reduced feeding by protists, a response that we postulated to be a signaling phenomenon rather than general metabolic inhibition or toxicity (Strom et al. 2003). Based on these findings, we suspected that other compounds with similar functions in the algal cell might also inhibit feeding in ciliates and dinoflagellates. DMSP is known to be an algal osmolyte (Kirst 1989) and is hypothesized to be an antioxidant (Sunda et al. 2002).

In this paper we explore the effects of a range of compounds, especially amino acids, on feeding responses and swimming behavior of protists. While the focus is on the tintinnid *Favella* sp., a genus of relatively large ciliates widely distributed in coastal seas (Pierce & Turner 1993), we also studied an aloricate spirotrich ciliate and a heterotrophic dinoflagellate. We show that amino acids and the structurally related compounds betaine and DMSP inhibited feeding in the ciliates, but not in the dinoflagellate. Evidence that these are signaling compounds comes from numerous aspects of our data. However, the amino acid signaling response in *Favella* sp. is different in fundamental ways from that previously described for aquatic organisms including bacteria, protists, invertebrates, and fishes. The properties of the *Favella* sp. signaling response shed light on its possible adaptive significance.

MATERIALS AND METHODS

Chemical stocks and analyses. DMSP was obtained from Selact BV. All other chemicals were purchased from Sigma-Aldrich and were of the highest purity available. Amino acids were all L-enantiomers. Chemical stock solutions were freshly prepared in ultra-pure water ('Nanopure', purified by reverse osmosis, deionized, 0.2 µm filtered) the day before each experiment and stored overnight at 4°C. Addition volumes were 15 µl unless otherwise indicated; control treatments received an equivalent volume of ultra-pure water.

Dissolved free amino acids (DFAA) were analyzed according to Parsons et al. (1984). All glassware was cleaned by soaking in base solution and combusting before use, then kept clean during the experiment by soaking in 0.25 M NaOH. Samples for DFAA analysis were filtered through precombusted (450°C) glass fiber filters; the first few ml of filtrate from each sample were used to rinse the 20 ml glass sample collection vial, the remaining volume was collected in the vial and stored frozen (–70°C) until analysis. Separate standard curves based on the appropriate amino acid (serine, arginine) were prepared for each experimental treatment; glycine was used as the standard for analysis of DFAA samples from controls.

Culture methods. Heterotrophic protists used in this study included 2 tintinnid ciliates isolated from northern Puget Sound, Washington, USA: *Favella* sp. (SPMC101), and *Coxliella* sp. (SPMC110). The taxonomic status of the genus *Coxliella* is uncertain, since *Favella* sp. will produce *Coxliella*-form loricae when regrowing from the trophont (Laval-Peuto 1981). However, our culture produced exclusively *Coxliella*-form loricae during 4 mo in culture. The heterotrophic dinoflagellate *Gyrodinium dominans* (SPMC103) was isolated from northern Øresund, between Denmark and Sweden (Hansen & Daugbjerg 2004). All were maintained on prey mixtures at 15°C in filtered (0.2 µm), autoclaved seawater (30 psu) with addition of a dilute trace metal mixture (henceforth 'ciliate medium'). Phytoplankton prey cultures were grown in f/2 without added Si, prepared from 30 psu seawater. Phytoplankton were maintained at 15°C and approximately 50 µmol photons m^{-2} s^{-1} in a 12:12 h light:dark cycle. When used for experiments, phytoplankton cultures were in mid to late exponential phase.

Feeding rate measurements (general approach). Protist grazers were pre-starved unless otherwise stated. The ciliates *Favella* sp. and *Coxliella* sp. were separated from their maintenance prey by sieving through a 20 µm mesh, followed by resuspension in fresh ciliate medium for 18 h (*Favella* sp.) or 4 h (*Coxliella*) before the start of the experiment. *Gyrodinium dominans* was starved by allowing the culture

to deplete the prey over time. Immediately before each experiment, samples were taken to determine the 'background' level of prey in food vacuoles. As a percentage of the number of ingested cells per grazer in controls, these background levels were ≤2% for *Favella* sp., 12% for *Coxliella*, and 45% for *G. dominans*. For experiments, protist grazers were combined with fresh ciliate medium in 60 ml polycarbonate bottles (4 per treatment); experimental volumes were 30 ml. Chemicals were added to 20 µM unless otherwise indicated, and grazers were allowed to acclimate for variable amounts of time depending on the experiment. Phytoplankton prey cells were then added at levels known to saturate feeding rates (400 µg C l^{-1}) and grazers were allowed to feed for an appropriate time interval as determined in preliminary experiments (i.e. the mid-point of the vacuole filling time course). A 20 ml aliquot from each experimental bottle was then added to cold 10% glutaraldehyde (final concentration 0.5%) plus 4′6′diamidino-2-phenylindole (DAPI stain, final concentration approx. 0.2 µg ml^{-1}). Preserved samples were stored at 4°C overnight, then filtered (polycarbonate filters: 5 µm pore size for *Favella* sp., 1 µm for other grazer species), slide-mounted, and frozen (–20°C) for epifluorescence microscopy. Slides were analyzed within 2 mo of preparation. Individual protist grazers were located using UV excitation and the DAPI-induced white fluorescence of grazer nuclei and cytoplasm. The number of prey cells contained in each individual's food vacuole(s) was then enumerated using blue light excitation and the red autofluorescence of the ingested phytoplankton prey. The feeding rate reported for a single sample represents the average ingestion of 100 to 200 individuals.

Compound- and dose-dependence of feeding rates. Experiments were conducted under controlled temperature conditions (15°C) by placing incubation bottles in beakers suspended in a water bath. For all experiments, grazers were allowed to acclimate to added chemicals for 15 min before addition of prey cells.

Favella sp. was fed autotrophic dinoflagellate *Heterocapsa triquetra* (Øresund isolate SCCAP K-481): Initial concentrations of *Favella* sp. and *H. triquetra* were 10 to 18 and 620 cells ml^{-1}, respectively. Samples for microscopy were preserved 5 min after prey addition. *Favella* sp. feeding inhibition was studied in 4 separate experiments: 1 examining the effects of DMSP functional analogues including various algal antioxidants and osmolytes; 2 separate experiments examining the effects of various amino acids; and 1 experiment assessing dependence of feeding inhibition on proline dose.

Gyrodinium dominans was fed cryptophyte *Rhodomonas salina* strain #1 (Danish Institute for Fisheries Research strain): Initial concentrations of *G. dominans* and *R. salina* #1 were 150 and 6800 cells ml^{-1}, respectively. Samples for microscopy were preserved 2 h after prey addition. One experiment was conducted to examine the effects of amino acids and related compounds on feeding.

Coxliella sp. fed cryptophyte *Rhodomonas salina* strain #2 (CCMP1319, aka *Pyrenomonas salina*). Initial concentrations of *Coxliella* and *R. salina* #2 were 17 and 11 600 cells ml^{-1}, respectively. Samples for microscopy were preserved 45 min after prey addition. One experiment was conducted as for *Gyrodinium dominans*.

Effects of long-term exposure to chemicals. Two experiments were conducted to determine whether *Favella* sp. could adapt (sensu Machemer & Teunis 1996) to inhibitory chemicals; in other words, would the feeding inhibition effect weaken or disappear after prolonged exposure? The first experiment lasted 4 h and the second lasted 24 h; *Favella* sp. concentrations in the 2 experiments were 19 and 4 cells ml^{-1}, respectively. Both experiments were conducted in a walk-in environmental chamber at 15°C with prestarved *Favella* sp. and *Heterocapsa triquetra* (at 620 cells ml^{-1}) as prey. To initiate the experiments, prestarved *Favella* sp. from a single batch culture were divided into 500 ml polycarbonate bottles (3 per treatment, actual volume 450 ml) and bottles were spiked with chemicals (225 µl addition volume). Treatments in the first experiment were 20 µM proline and an ultra-pure water control; treatments in the second experiment were 20 µM serine (inhibitory), 20 µM arginine (neutral), and an ultra-pure water control. These bottles contained no prey cells. After chemical addition, the 500 ml bottles were immediately subsampled (42 ml) into 60 ml polycarbonate bottles containing *H. triquetra* cells for a 'time zero' determination of feeding rate. A 20 ml subsample of *Favella* sp. plus *H. triquetra* was taken from each 60 ml bottle and used to completely fill a polystyrene slideflask chamber for video-taping of swimming behavior (see section 'Swimming behavior filming and analysis'); the remaining 22 ml was allowed to incubate for 5 min before preservation for epifluorescence examination of food vacuoles as described above. Subsequent samples were transferred from the 500 ml, amino acid-spiked bottles to the smaller bottles for determination of feeding rate and swimming behavior at additional time points.

To determine whether exposure to amino acids increased ciliate mortality, we took additional samples (20 ml) from the 500 ml bottles at 0, 12 and 24 h during the second experiment. These were preserved in acid Lugol's solution (final concentration 10%) for determination of ciliate abundance. Samples were settled and ciliates counted using inverted microscopy. Growth

rates in each bottle were determined from the slope of ln-transformed abundance vs. time relationships. A further set of samples (each 18 ml) was taken at all time points for determination of DFAA concentration in both spiked and control bottles.

Feeding history experiment. We tested whether feeding history (starved vs. well fed) affected the feeding response to added proline, one of the most inhibitory amino acids. Additionally, some starved *Favella* sp. were exposed to filtrate from the dinoflagellate prey culture to determine whether dissolved cues from prey modulated the inhibitory effect of proline. The experiment was performed as above, except that *Favella* sp. in some treatments were not prestarved. All *Favella* sp. were sieved and resuspended in fresh medium 22 h before the experiment. However, *Heterocapsa triquetra* prey cells were immediately added back to one portion of the stock culture (to 620 cells ml^{-1}) so that these *Favella* sp. were well fed before the experiment, while another portion of the stock culture was prestarved. Prey cell density in the well-fed *Favella* sp. culture was estimated and additional prey were added (to 620 cells ml^{-1}) 5 h before the experiment. The prestarved portion was further divided into a portion that was left untreated and a second portion that was combined with filtrate from *H. triquetra* culture 22 h and 5 h before the experiment. Filtrate was prepared using a polycarbonate filter (0.8 µm pore size), and this was added in the same proportions (22 h: 12 to 400 ml; 5 h: 7 to 412 ml) as *H. triquetra* culture to *Favella* sp. stock culture in the well-fed treatment.

Feeding rates with and without added 20 µM proline were measured in 3 treatments: well fed, starved, and starved plus prey culture filtrate. Feeding rates were measured as uptake of fluorescently labeled algae (FLA) over 10 min. FLA were prepared from *H. triquetra* the day before the feeding experiment by staining them with 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein hydrochloride (DTAF) at 65°C according to Rublee & Gallegos (1989). Prey cells were added to all treatments as a 15:85 mixture of FLA : unstained *H. triquetra*. For the well-fed culture, the unstained fraction included the prey cells that remained in the bottle from the previous (22 and 5 h) feedings. For all treatments, total ingestion was calculated by scaling FLA uptake by the FLA:unstained *Heterocapsa triquetra* proportion. Total FLA + unstained *H. triquetra* concentration was 620 cells ml^{-1} in all treatments; *Favella* sp. density was 16 cells ml^{-1}.

Recovery experiment. We determined the time course and extent of *Favella* sp. feeding rate recovery from exposure to proline. Experiments were conducted at 15°C in dim light in a walk-in environmental chamber. Three treatments were used, each in triplicate: control (no chemical exposure, followed by rinsing in clean ciliate medium), proline recovery (exposure to 20 µM proline followed by rinsing in clean medium), continuous proline exposure (exposure to 20 µM proline followed by rinsing in 20 µM proline-enriched medium). Rinsing and resuspension of cells in each bottle ensured that handling effects were the same for all treatments. Prestarved *Favella* sp. at 28 cells ml^{-1} were suspended in 200 ml of ciliate medium that was then spiked with the appropriate chemical (100 µl Nanopure water for controls, 100 µl proline solution for proline-exposed treatments). After 10 min exposure to chemicals, the contents of each bottle were poured through a 20 µm mesh sieve to retain *Favella* sp. cells. These were rinsed in the sieve with 2 approximately 100 ml volumes of the appropriate solution (clean ciliate medium for control and recovery treatments, 20 µM proline-enriched medium for continuous exposure treatment), *Favella* sp. cells were then resuspended (to 200 ml) in the same solutions and immediately subsampled (30 ml) for the first feeding rate determination. Feeding rates in the subsamples were measured on *Heterocapsa triquetra* at 620 cells ml^{-1} over a 5 min incubation, as described in 'Feeding rate measurements (general approach)' (above). Additional feeding rate measurements were made at time intervals up to 1 h after rinsing and resuspension.

Swimming behavior filming and analysis. We videotaped and analyzed *Favella* sp. swimming behavior in conjunction with 4 feeding experiments, as well as in 6 trials without feeding. Although initial tests used a 15°C water bath to maintain temperatures, most experiments were conducted in a walk-in incubator. Cultures were dispensed into 20 ml polystyrene slide-flask chambers, and amino acids were added to final concentration of 2 to 200 µM. Controls received equivalent volumes of Nanopure water. Swimming was filmed with a dissecting microscope (Olympus CO11 with darkfield adapter) for 2 to 3 min intervals at 4 to 6 time points. Light levels were reduced to a minimum, and the focal plane was set near the middle of the flask to avoid tracks that might be influenced by chamber walls. Video (30 frames s^{-1}) was generated with a Sony B/W camera and recorded by VCR. Videos were digitized with a Videum 1000 frame capture board (Winnov) and compressed using Intel Video R3.2 format.

LabTrack 2.0 (Bioras) was used to track cell motion. Typical parameter settings were as follows: threshold : 50 to 100, depending on lighting; min track length: 30 frames; search radius: 10 pixels; min target size: 4 pixels; max target size: 50 pixels; min velocity: 1.00 pixel/frame. To avoid bias by track fragments, only tracks greater than 30 frames (1 s) in duration were retained and analyzed. Pixels were converted to microns from slide micrometer measurements and

saved as Excel-readable output files. Output data for each cell track included time and position for each frame, and velocity (V) acceleration (A), and angle averaged successively (i.e. a running average) over 6 frames. Over 10 experiments, video acquisition ranged from 60 to 385 tracks per tape, each track with 30 to 600 frames, for a total of 2700 to 5000 tracks in 250 to 500 min of video per experiment.

Bulk track statistics were calculated from output files with a PERL script. For each track, we calculated the average velocity and 1 s averaged net-gross-displacement ratio (NGDR), a measure of turning rate. In addition, tracks were analyzed for helical vs. straight swimming patterns using wavelet analysis, which extracts local-frequency information from a time series signal by decomposing it into time–frequency space using periodic functions, and thus determines how the signal changes over time. The y component of the velocity of each track was concatenated into a series of sequential frame numbers and analyzed with a weighted wavelet Z-transform using the program WinWWZ (www.aavso.org/data/software/winwwz.shtml). This analysis produces a running series of local frequency, period, and amplitude averaged around a moving window for each frame. Parameters were as follows: time step = 10, low freq = 0.002, high freq = 0.05, freq step = 0.002, constant = 0.05, and bin width = 3. By inspection, an arbitrary threshold value of the product of frequency and amplitude could be used to determine helical versus straight swimming.

Statistical analyses. Experiments investigating compound- and dose-dependence of feeding rates in *Favella* sp., *Gyrodinium dominans* and *Coxliella* sp. were analyzed using ANOVA followed by Tukey's HSD post-hoc test for multiple comparisons. Data from one of the *Favella* sp. experiments examining the effects of various amino acids did not meet the assumption of equality of variances despite the use of various data transformation methods. Therefore post-hoc multiple comparisons for this experiment used the Games-Howell test. Data from the feeding history experiment were analyzed with the paired samples t-test, while long-term exposure experiments were analyzed with repeated measures ANOVA. All statistical analyses were performed with SPSS v. 14.0 software.

RESULTS

Compound- and dose-dependence of feeding rates

We found no effect of the algal antioxidants ascorbic acid, glutathione, dimethylsulfoxide, or acrylate on *Favella* sp. feeding rate (Table 1). Similarly, the algal osmolytes mannitol and betaine did not affect feeding rates. The osmolytes DMSP and proline, an amino/imino acid, reduced feeding to 63 and 23% of the control rate, respectively (Table 1).

The finding that 20 µM proline was strongly inhibitory led us to investigate other amino acids. These showed a wide range of effects (Fig. 1A). No amino acid was stimulatory (i.e. none promoted a feeding rate significantly higher than the control), and many were inhibitory. Rates in treatments that significantly reduced feeding ranged from 64 to 5% of the control, with the lowest rates caused by addition of valine and cysteine. Other particularly inhibitory amino acids were proline, alanine, and serine, all of which reduced feeding to <20% of the control rate (Fig. 1A). We tested the effects of 2 other low molecular weight compounds as well: sodium pyruvate and ammonium chloride. Neither affected *Favella* sp. feeding rates at a concentration of 20 µM (data not shown).

The strength of the inhibition was inversely proportional to the length of the amino acid side chain, with small side chain amino acids the most inhibitory (Fig. 1B). Glycine, with no side chain, showed only a weak effect. Inhibition was not clearly related to side chain polarity or charge. For example, among the strongly inhibitory compounds, proline, alanine, and valine are non-polar, while serine and cysteine are polar. Among the weakly or non-inhibitory compounds, lysine, arginine and histidine are positively charged, while aspartic and glutamic acids are negatively charged, and trypotophan and isoleucine are neutral.

The dose-response experiment showed that feeding inhibition in *Favella* sp. was dependent on proline concentration (Fig. 2). The lowest tested concentration with an inhibitory effect was 200 nM. With increasing

Table 1. *Favella* sp. fed *Heterocapsa triquetra*. Feeding inhibition (feeding rate as % of control) of the tintinnid in the presence of 20 µM antioxidants and organic osmolytes. Values are averages (n = 4) with 1 SD in parentheses. Rates that are statistically significantly different from controls are in **bold** (DMSP: $p = 0.057$; proline: $p < 0.001$). Control feeding rate averaged 0.41 (±0.10) cells grazer^{-1} min^{-1}

Functional class Compound	Feeding rate (% of control)
Antioxidants	
Glutathione	92 (10)
Ascorbate	102 (11)
DMSO	112 (22)
Acrylate	117 (17)
Osmolytes	
Mannitol	104 (19)
Betaine	88 (14)
DMSP	**63** (7)
Proline	**23** (5)

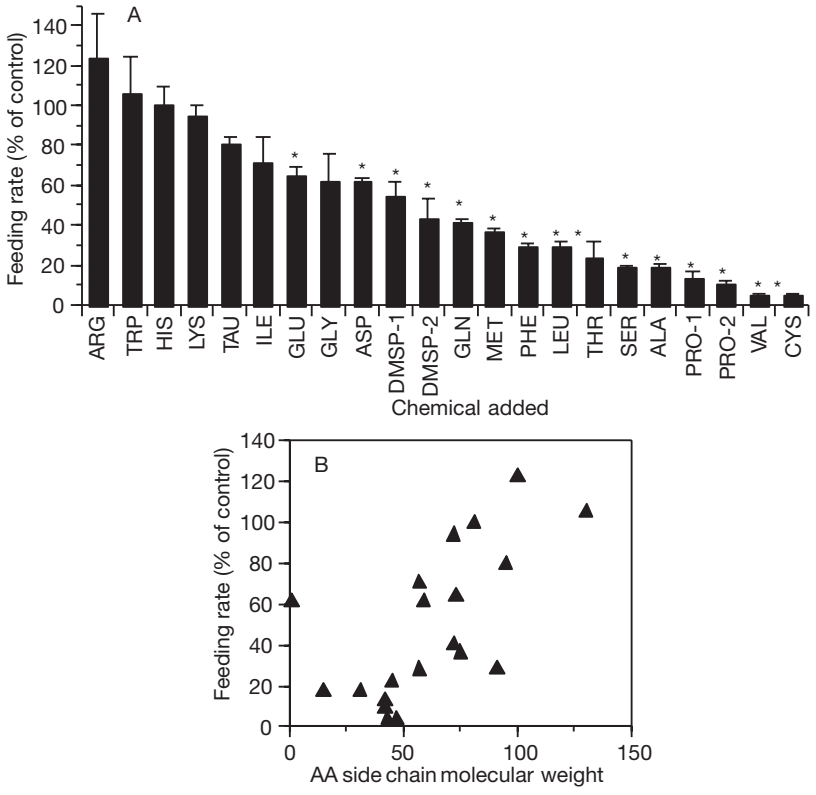


Fig. 1. *Favella* sp. feeding on *Heterocapsa triquetra*. (A) Feeding rates of the ciliate (as % of control rates, mean ± 1 SD, n = 4) in the presence of 20 µM concentrations of 19 amino acids, DMSP and betaine. Combined results of 2 experiments conducted within 2 mo of each other (DMSP and proline data shown for both experiments). Data are arranged from highest to lowest average rates; *rates that are statistically significantly different from controls ($p < 0.05$). Control feeding rates averaged 0.99 ± 0.10 and 0.90 ± 0.02 cells grazer^{-1} min^{-1} for the first and second experiments, respectively. (B) *Favella* sp. feeding rates from (A) as a function of amino acid (AA) side chain molecular weight. arg: arginine; trp: tryptophan; his: histidine; lys: lysine; tau: taurine; ile: isoleucine; glu: glutamic acid; gly: glycine; asp: aspartic acid; DMSP: dimethylsulfoniopropionate; gln: glutamine; met: methionine; phe: phenylalanine; leu: leucine; thr: threonine; ser: serine; ala: alanine; pro: proline; val: valine; cys: cysteine

proline concentration, feeding rate decreased from 63% of the control rate at 200 nM to 10% at 20 µM.

Long-term exposure and feeding history effects

The 2 long-term exposure experiments demonstrate the rapidity of the inhibitory effect, which occurred in <5 min following exposure to the active amino acids. During both experiments we initiated a 5 min feeding assay immediately following addition of 20 µM amino acids. Over the course of the 5 min assay, proline reduced feeding to 20% of control levels in the first experiment, while serine reduced feeding to 48% of control levels in the second experiment (Fig. 3B,D). Adaptation to the added proline (i.e. recovery from feeding inhibition) was not seen over 4 h exposure, with rates remaining consistent at or below 40% relative to the control (Fig. 3A,B). The slight overall increase in all feeding rates during the 4 h time course is consistent with the documented diel cycle of *Favella* sp. (Jakobsen & Strom 2004). In the second experiment, longer exposure to amino acids led to partial adaptation but not to complete recovery. Feeding in the presence of added serine increased from 48 to 84% of control rates over 12 h, after which both absolute and relative rates decreased (Fig. 3C,D). Arginine had no effect on feeding at any time point. DFAA analyses showed that serine and arginine concentrations changed little during the incubations. Serine decreased from 19.10 to 17.60 µM and arginine from 20.45 to 19.67 µM over the 24 h period. Total DFAA concentration in the control bottles averaged 0.35 µM initially and 0.19 µm after 24 h incubation.

These experiments also demonstrate the lack of amino acid toxicity. Long-term exposure to 20 µM serine and arginine had no effect on 24 h growth rates of *Favella* sp. (1-way ANOVA, $p = 0.97$). Rates were variable but low and positive on average, indicating little or no mortality in the bottles. Average growth rates (±1 SD) were 0.22 d^{-1} (±0.26) for the control treatment, 0.21 d^{-1} (±0.41) for the serine treatment, and 0.16 d^{-1} (±0.41) for the arginine treatment.

Feeding inhibition was not dependent on feeding history. *Favella* sp. ingestion

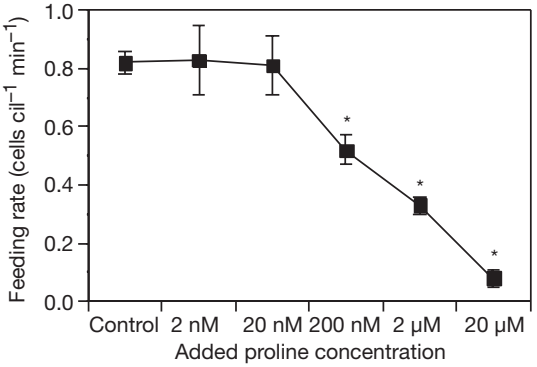


Fig. 2. *Favella* sp. feeding on *Heterocapsa triquetra*. Effect of increasing proline dose on ciliate (cil) ingestion of *H. triquetra* (mean ± 1 SD, n = 4). *Rates that are statistically significantly different from controls ($p < 0.001$)

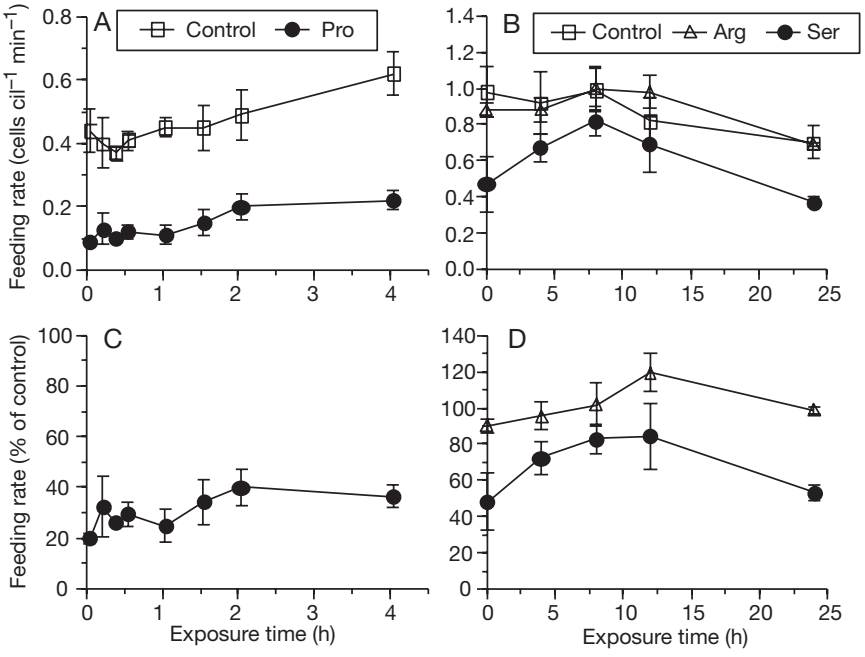


Fig. 3. *Favella* sp. Feeding rates of the ciliate (cil) (mean ± 1 SD, n = 3) during 2 long-term amino acid exposure experiments. (A,B) absolute feeding rates; (C,D) rates in amino acid treatments as % of control rates. (A,C) A 4 h experiment with proline (pro) and control treatments; (B,D) 24 h experiment with serine (ser), arginine (arg), and control treatments. Addition of proline or serine significantly reduced feeding rates (ANOVA, $p < 0.01$)

rate in the presence of 20 µM proline was very low (0.03 to 0.05 cells ciliate^{-1} min^{-1}) regardless of whether the ciliates were previously fed, starved, or starved in the presence of filtrate from the *Heterocapsa triquetra* prey culture (Fig. 4). Control (i.e. no proline) feeding rates were lower in the well-fed culture than in the 2 prestarved treatments (0.26 vs. 0.41 cells ciliate^{-1} min^{-1}, respectively). Thus, the proline inhibitory effects varied when normalized to the appropriate controls, with feeding rates 18% of control rates in the well-fed treatment, and 7 to 8% in the prestarved treatments.

Recovery from amino acid exposure

Partial recovery of *Favella* sp. feeding rates from exposure to proline was very rapid. Observations made immediately after rinsing and resuspension showed that feeding in the proline recovery treatment averaged 48%

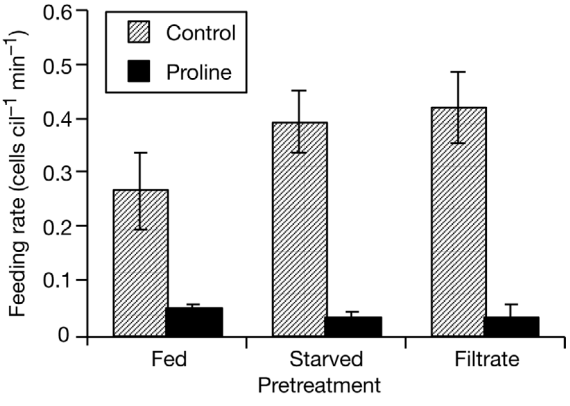


Fig. 4. *Favella* sp. Feeding rates of the ciliate (cil) (mean ± 1 SD, n = 4) in control and 20 µM proline treatments after preincubation in 3 different feeding conditions: fed (*Heterocapsa triquetra*), starved, and starved in the presence of filtrate from *H. triquetra* culture. Rates in all proline treatments were statistically significantly lower than corresponding control rates ($p < 0.01$)

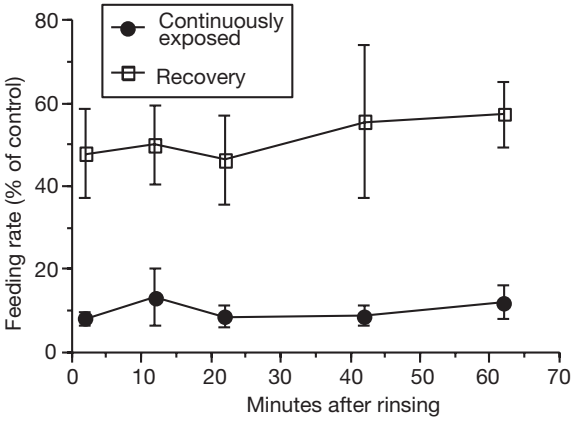


Fig. 5. *Favella* sp. Feeding rates of the ciliate (as % of control, mean ± 1 SD, n = 3) during the recovery experiment. Rates are shown for proline recovery and continuously proline-exposed treatments. Control feeding rates averaged 0.24 ± 0.05 for the first time point and 0.43 ± 0.09 cells grazer^{-1} min^{-1} for all other time points

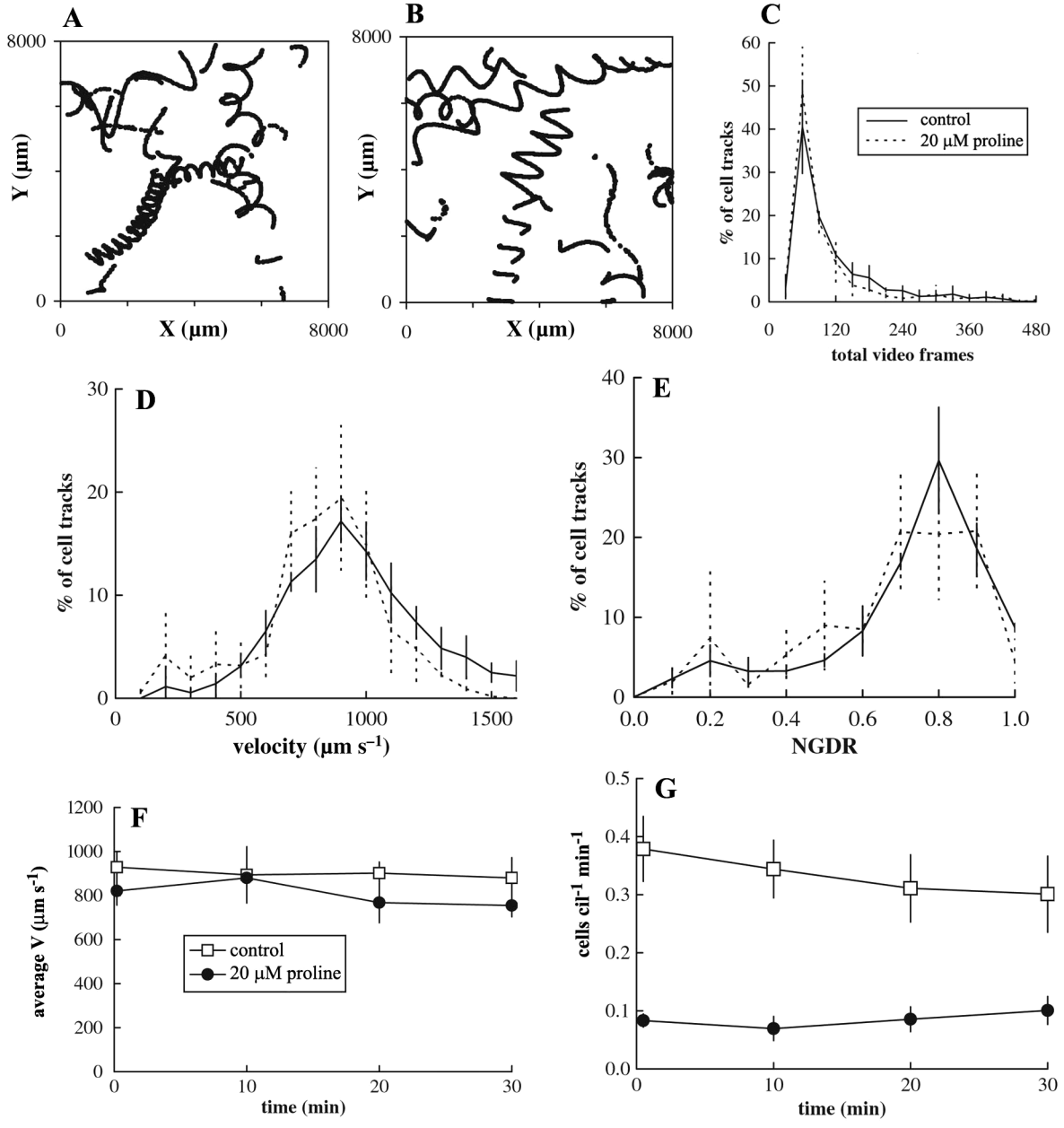


Fig. 6. *Favella* sp. Swimming behavior analysis. Examples of helical swimming (selected tracks) in (A) control at 20 min and (B) 20 µM proline at 30 min. (C) Frames per track showing that most tracks were 2 to 4 s in duration (30 frames s^{-1}), with a small number of tracks up to 15 s. Exposure to proline (20 µM for 20 min) had no effect on distributions of (D) velocity (binned into 100 µm s^{-1} increments) or (E) NGDR (net-gross-displacement ratio, binned into 0.1 increments). Similarly, 20 µM proline did not significantly change (F) average velocity (*V*) or NGDR (not shown) over 30 min. (G) Feeding inhibition was, in contrast, immediate and profound. Data in C to G are mean ± 1 SD (n = 3). Cil: ciliate

of control rates (Fig. 5). In contrast, feeding in the continuously proline-exposed treatment averaged only 8% of control rates. No additional recovery was observed within the 1 h time course of our experiment. Rates remained unchanged, with averages ranging from 46 to 57% of control rates in the recovery treatment, and 8 to 13% in the continuously exposed treatment.

Effects of amino acids on swimming behavior

In control flasks, ciliates swam in a variety of helices (Fig. 6A), which did not change dramatically when amino acids were added (Fig. 6B). Most tracks were 2 to 4 s in duration, although a small number were much longer (Fig. 6C). Although individual cells varied

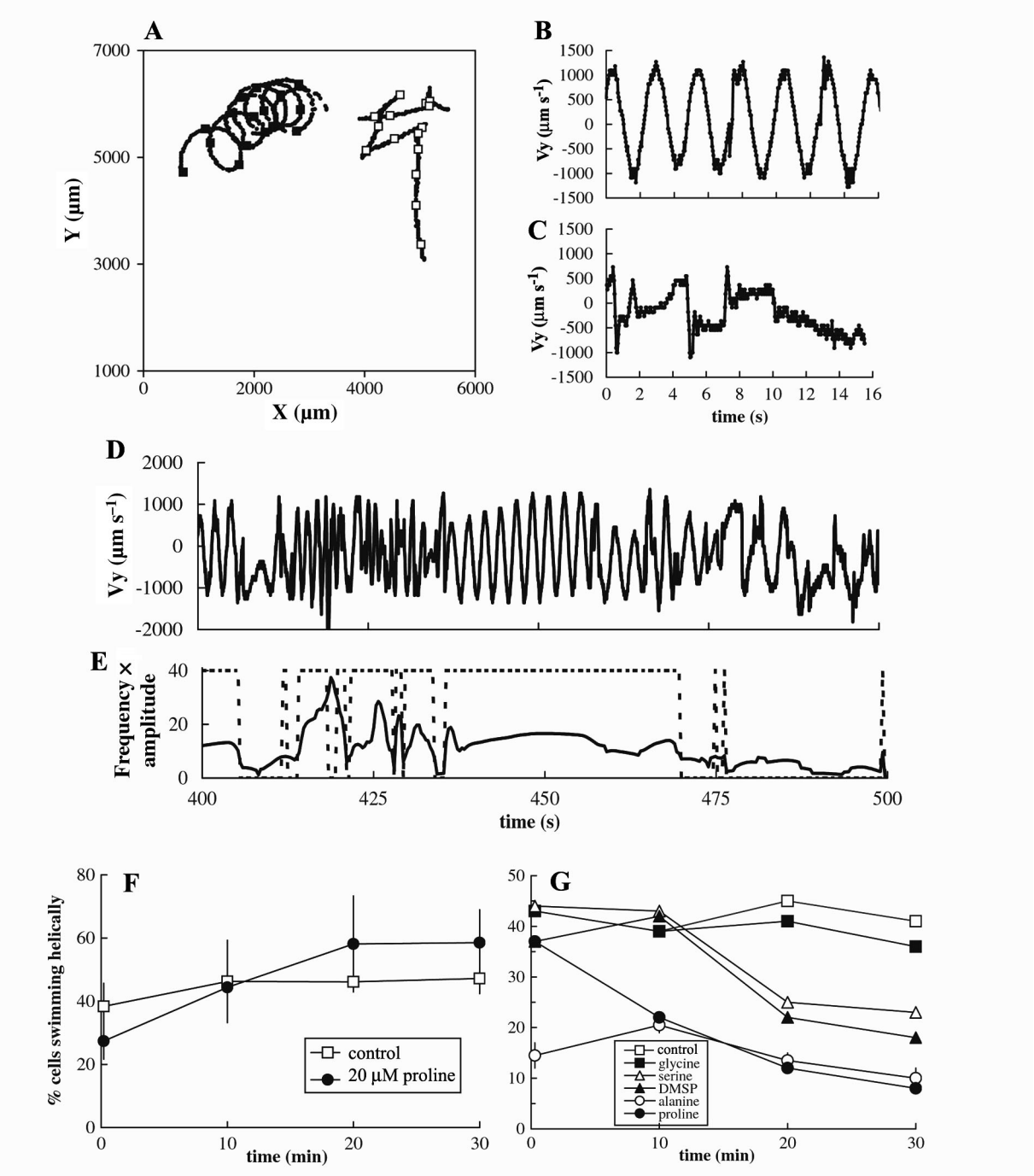


Fig. 7. *Favella* sp. Use of wavelet analysis to estimate fraction of cells exhibiting helical swimming. (A) Selected tracks exhibiting helical (left) or start-stop (right) behavior, with symbols denoting 1 s intervals. (B,C) *Vy* (*y* component of velocity *V*) vs. time for (B) helical and (C) start-stop swimming. (D,E) Example of concatenated track *Vy* and wavelet analysis, showing threshold detection of helices (dotted line in E). (F,G) Change in percent helical swimming over time. (F) Experiment in which feeding was reduced by presence of 20 µM proline (mean ±1 SD, n = 3). Fraction of cells swimming helically increased over 20 min, but not significantly. (G) Experiment testing behavior in presence of 20 µM amino acids or DMSP, in which helical swimming was reduced by some amino acids. n = 1; error bars on alanine denote pseudoreplicate variability of different track populations

considerably, the distributions of *V* and NGDR were unimodal and not significantly changed by amino acid additions (Fig. 6D,E). Average swimming speed ranged from 600 to 1200 µm s^{-1}, with an average NGDR of 0.6 to 0.8. Neither *V* (Fig. 6F) nor NGDR (data not shown) changed over time or with amino acid addition, in sharp contrast to feeding depression, which was consistently immediate (Fig. 6G).

Closer examination of individual swimming tracks showed that a fraction of cells did not swim helically, but in 'start-stop' movements that resulted in zig-zag patterns (Fig. 7A). When the *y* component of velocity (*Vy*) was plotted against time, these cells could be distinguished from those swimming helically (Fig. 7B,C). We concatenated tracks of all cells and used wavelet analysis to determine the fraction of the time cells were swimming helically (Fig. 7D,E). Although we did not see consistent changes in the degree of helical swimming between experiments, there appeared to be subtle trends (Fig. 7F) that occurred over 10 to 30 min. In at least one instance, the fraction of cells exhibiting helical swimming decreased in response to amino acids (alanine, proline, serine) that strongly inhibited feeding (Fig. 7G). However, these results were not consistent among experiments. Helical period and amplitude did not vary significantly among treatments or over time regardless of experiment (data not shown).

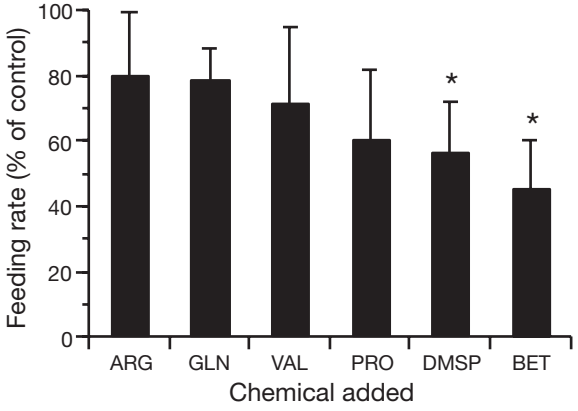


Fig. 8. *Coxliella* sp. feeding on *Rhodomonas salina*. Feeding rates of the ciliate on *R. salina* strain #2 (as % of control rates, mean ±1 SD, n = 4) in the presence of 20 µM concentrations of 4 amino acids, DMSP and betaine (BET). * indicates rates significantly different from controls ($p < 0.05$). Control feeding rates averaged 1.5 ± 0.3 cells grazer^{-1} h^{-1}. Abbreviations as in Fig. 1

Amino acid inhibition of other protist grazers

Experiments with *Coxliella* sp. and *Gyrodinium dominans* demonstrated that the inhibitory effect of amino acids and related compounds on other heterotrophic protists is variable. The heterotrophic dinoflagellate *G. dominans* showed no response to added amino acids, including serine, histidine, cysteine, arginine, lysine, valine, proline, glutamine, and glutamic acid. Nor did *G. dominans* respond to DMSP or betaine (1-way ANOVA, $p = 0.93$; data not shown). Feeding rates averaged 0.4 cells grazer^{-1} h^{-1} across all treatments. In contrast, feeding by the tintinnid ciliate *Coxliella* sp. was moderately inhibited by some tested compounds (Fig. 8). Addition of 20 µM betaine and DMSP reduced feeding to 45 and 56% of control rates, respectively. The data also suggest that proline was an effective inhibitor (to 60% of control rates, $p = 0.082$, Tukey HSD post-hoc comparison). However, *Coxliella* was not affected by glutamine or valine, amino acids that moderately or strongly inhibited *Favella* sp. (Fig. 1A). Unfortunately *Coxliella* was lost from culture before additional compounds could be tested.

DISCUSSION

Amino acids as chemical signals

We found that numerous amino acids inhibited feeding by the tintinnid ciliate *Favella* sp., a geographically widespread coastal genus. The most inhibitory amino acids — valine, cysteine, proline, alanine, and serine — reduced feeding to <20% of the control rate at 20 µM concentration. Several lines of evidence indicate that these amino acids are signaling compounds for *Favella* sp. (1) Long-term (24 to 28 h) exposure to inhibitory amino acids had no effect on growth or mortality of *Favella* sp., demonstrating that the compounds are not toxic. (2) *Favella* sp. responded to proline, one of the more inhibitory DFAAs, at concentrations as low as 200 nM. (3) Removal of amino acids caused immediate partial reversal of inhibition. (4) We observed a clear effect of amino acid side chain size on feeding inhibition, suggesting a specific interaction with a cell surface receptor. DFAAs are released both by phytoplankton (prey for *Favella* sp.) and during grazing by copepods and other zooplankton (potential predators of *Favella* sp.), so the potential 'information content' of these molecules is high. Once released, DFAAs have a short lifetime in seawater, as they are preferred substrates for bacterial uptake and growth. Thus, background concentrations in seawater are low and pulses short-lived (see next section). This is consistent with a signaling function in that the *Favella* sp. feeding response would not be triggered by environmental 'background' DFAA levels, but would be responsive to realistic enhancements associated with localized release of the compounds.

It is well known that free amino acids are signaling compounds for a broad range of aquatic organisms. Extensive research on crustaceans (amphipods, copepods, crabs, lobsters) and fishes has shown that DFAAs can be attractants and feeding stimulants (e.g. Poulet & Ouellet 1982, Carr et al. 1996, Ide et al. 2006). DFAAs

are released from decomposing animal tissue so these responses are adaptive for organisms that search for and consume detritus. Feeding stimulation, neurological responsiveness, and behavioral attraction by DFAAs has also been reported for echinoderms (Valentincic 1985), a nudibranch (Murphy & Hadfield 1997), a mud snail (Zimmer et al. 1999) and a planarian (Miyamoto & Shimozawa 1985). Some larval invertebrates use free amino acids as cues for settlement and metamorphosis, including the oyster *Crassostrea virginica* and the queen conch *Strombus gigas* (Zimmer-Faust & Tamburri 1994, Boettcher & Targett 1998). Among aquatic microbes, amino acids are attractants for abalone sperm (Riffell et al. 2002), numerous bacteria (Miller et al. 2004 and references therein) and protists including the cryptophyte *Chroomonas* sp., the heterotrophic dinoflagellate *Gymnodinium fungiforme*, the nanoflagellate *Pseudobodo tremulans* and the ciliate *Tetrahymena* (Levandowsky et al. 1984, Spero 1985, Sibbald et al. 1987, Lee et al. 1999).

These studies, and many others, demonstrate that DFAAs are generally attractants and stimulants for aquatic organisms, over effective concentrations ranging from 10s of nanomolar to millimolar. This contrasts sharply with our results showing a range of DFAAs to be inhibitors of ciliate feeding. To our knowledge, only a few prior studies have reported inhibitory or deterrent effects of amino acids. Ferner & Jumars (1999) found spionid polychaete feeding was depressed by millimolar concentrations of taruine, threonine and valine; while Hauser et al. (1975) found that several amino acids generated a negative prey-seeking response in the saprophytic dinoflagellate *Crypthecodinium cohnii*.

Could amino acids inhibit grazing in natural waters?

Total DFAA concentrations in the marine euphotic zone can be as high as those eliciting a response from *Favella* sp. in our dose-response experiment. The threshold concentration for feeding inhibition was 200 nM proline. While DFAA levels in oligotrophic waters can be <10 nM (e.g.

Table 2. Average and maximum concentrations of individual dissolved free amino acids (nM) from the upper water column (first 5 locations) and other marine environments (last 3 locations). n = number of sites, dates and/or depths sampled. DFAAs inhibiting *Favella* sp. feeding to <25% of control rates shown in **bold**; ΣI = summed concentration of most inhibitory amino acids. meth = derivatization method for amino acid detection, where opa = *o*-phthaldialdehyde and nin = ninhydrin. Amino acid abbreviations: asp: aspartic acid; glu: glutamic acid; ser: serine; val: valine; gln: glutamine; his: histidine; glyl: glylcine; thr: threonine; arg: arginine; ala: alanine; cys: cysteine; leu: leucine; lys: lysine; pro: proline. Amino acids typically present at low concentration and not strongly inhibitory were not tabulated, including asparagine, tyrosine, methionine, tryptophan, phenylalanine, isoleucine. Blank: not reported; bd: below detection

Location	Depth	n		gly	**ser**	**ala**	asp	**thr**	glu	**val**	lys	**pro**	leu	his	arg	gln	**cys**	ΣI	meth	Source
Pamlico R. estuary	Surface	12	avg	132	75	30	22	21	13	12	11	9	8	7	7			146	nin	Crawford et al. (1974)
			max	540	180	61	52	42	25	29	37	16	14	14	14			328		
NE Atlantic and North Sea	3 m	15	avg	173	115	47	26	34	33	16	18	39	16	20			bd	251	nin	Billen et al. (1980)
			max	426	508	176	100	84	154	48	36	210	44	76			13	1026		
S California coast	Surface	8	avg	22	30	12	8	*	4	7			3					49	opa	Andersson et al. (1985)
			max	42	76	34	16	*	9	12			8					122		
Sargasso Sea	3 to 100 m	8	avg	17	17	10	13	11	17									38	opa	Liebezeit et al. (1980)
			max	30	27	23	19	28	31									77		
Baltic Sea	1 to 60 m	25	avg	12	17	5	6	4	5	1	7		1	3	4	3		26	opa	Mopper & Lindroth (1982)
			max	22	30	11	10	9	11	3	14		3	6	11	5		50		
Antarctic sea ice	0–160 cm	16	avg	2610	3830	950	1320	*	540	510	740		960	2390	690			5290	opa	Yang (1995)
			max	3250	4830	3720	2340	*	870	1020	1610		1610	3400	1190			9570		
Narragansett Bay	Near bottom	5	avg	91	172	131	116		100	1	0		1	1	43	2		304	opa	Rice (1999)
			max	113	201	175	152		135	3	1		2	1	62	3		379		
NW Atlantic coast	Seasurface microlayer	22	avg	137	86	75	27	26	43	17			14	16	11	12		204	opa	Kuznetsova et al. (2004)

* Reported as gly + thr

the Sargasso Sea, Hoch & Kirchman 1995), concentrations in coastal waters typically range from 10s to 100s of nanomoles (Table 2). In blooms or at discontinuities, concentrations can be orders of magnitude higher. For example, Sellner & Nealley (1997) found DFAA levels of 4.2 µM in a Chesapeake Bay dinoflagellate bloom. Concentrations ranged up to 4.5 µM in a copepod-rich layer of the Celtic Sea (Poulet et al. 1991), 3.6 µM in the Delaware Estuary (Hoch & Kirchman 1995), and 27.2 µM in Adriatic Sea aggregates (Simon et al. 2002).

Fewer data are available on the composition of this amino acid pool. However, serine and alanine, 2 of the most inhibitory DFAAs for *Favella* sp., are often major constituents (Table 2). Other amino acids that are often abundant include glycine, glutamic and aspartic acids, lysine, and ornithine. Given typical coastal concentrations of 10s to 100s of nanomoles for total DFAA, environmental concentrations of inhibitory amino acids would generally be too low to elicit a response from *Favella* sp.. On occasions, however, combined concentrations of the most inhibitory DFAAs (ΣI, Table 2) reach levels sufficient to inhibit feeding, perhaps especially in layers, patches, and microzones in the sea.

An important side note is that most measurements of marine DFAA composition have been made using *o*-phthaldialdehyde (OPA) to create fluorescent amino acid derivatives that are quantified after high-performance liquid chromatographic separation (Lindroth & Mopper 1979). OPA reacts weakly and unstably with cysteine and proline (Birwé & Hesse 1991, Jørgensen & Jensen 1997), so these strongly inhibitory amino acids cannot be quantified using the OPA-HPLC method. Very little information is available on the concentrations of dissolved free cysteine and proline in marine environments.

Sources and composition of released DFAA in the marine plankton are difficult to determine due to simultaneous uptake by bacteria and protists. Direct release (exudation) by phytoplankton can be a significant source to the DFAA pool, as phytoplankton can contain substantial quantities of free amino acids (Marsot et al. 1991, Granum et al. 2002). Proline is an osmoregulator in some phytoplankton species and, as such, is present at high intracellular concentrations (Kirst 1989). Intracellular proline concentrations can increase even more in response to osmotic or oxidative stress, such as that imposed by heavy metals (Sharma & Dietz 2006). In these species, a concentrated pool of free amino acids is available for release in response to activation signals, cell disruption, and other processes. Marine bacteria and ciliates also use amino acids for osmoregulation, particularly proline, glycine, alanine, and glutamic acid (Kaneshiro et al. 1969, Csonka & Hanson 1991, Cronkite et al. 1993). In addition to exudation, release of DFAA has been associated with grazing by copepods and protists (Andersson et al. 1985, Nagata & Kirchman 1991, Rosenstock & Simon 2001). Extrapolating from arguments made for total DOC release, protist grazing is likely to be a major pathway for DFAA production under non-bloom conditions when cells are turning over at high rates (Strom et al. 1997).

How do amino acids act?

Studies on model ciliates such as *Paramecium, Tetrahymena*, and *Euplotes* have revealed a range of swimming responses to both attractant and repellent dissolved cues (Van Houten 1978). Intensive study has revealed the physiological and biochemical underpinnings of such behavioral shifts. Electrically excitable cells respond to chemicals that alter membrane potential, either through direct interaction with ion channels or by secondary signaling. This in turn leads to calcium fluxes that change ciliary beating, resulting in aggregate changes in swimming speed, orientation and turning frequency, that lead to either attraction or dispersion responses (Kung & Saimi 1982, Machemer & Teunis 1996). Like these model ciliates, *Favella* sp. has the capability for chemosensory responses. For example, Buskey & Stoecker (1988, 1989) showed that *Favella* sp. decreased swimming speed and increased turning frequency after encountering a prey patch; such changes were also triggered by filtrate from prey cultures in the absence of prey cells. In contrast, our experiments, which used uniformly dispersed signals rather than patches, have not shown any consistent behavioral change in *Favella* sp. upon exposure to inhibitory DFAAs. We recognize the limitations of 2D analysis of 3D helical motions, which can fail to detect subtle changes. However, average helical swimming speeds and NGDR, a measure of turning frequency, were not different in control treatments vs. treatments containing inhibitory amino acids (Fig. 6). Helical swimming appeared in some experiments to be replaced by start-stop motions in response to inhibitory compounds. However, the time course of the behavioral changes was slower than that of feeding inhibition, and the behavioral changes were not consistent among experiments yielding nearly identical feeding responses.

A further contrast with previous work on ciliates is evident in the adaptation to deterrent signals. When initially exposed to chemorepellents such as lysozyme or GTP, *Paramecium* showed an increased frequency of swimming reversals and changes of direction, but recovered fully after 10 to 15 min continuous exposure,

due to desensitization (Kim et al. 1997). In our experiments, *Favella* sp. feeding rate showed only partial adaptation, and that required >4 h rather than minutes to achieve (Fig. 3). Furthermore, removal of inhibitory amino acids only led to partial recovery over 1 h (Fig. 5), indicating the involvement of multiple mechanisms with different recovery times. Taken together, these observations indicate that the amino acid signaling system in *Favella* sp. may be fundamentally different from that in other, better-studied ciliates.

Despite these differences, we suspect that amino acid inhibition may result from specific binding to cell ion channels, or to receptors that indirectly affect membrane potential. Another possibility is that DFAAs bind to membranelles and interfere with chemoreception or capture of prey. For example, DFAA binding might inhibit the ciliary reversals associated with capture of large prey, including *Heterocapsa triquetra*, by *Favella* sp. (Stoecker et al. 1995). We are currently pursuing studies of amino acid binding to further understand the mechanism of inhibition.

Generality and adaptive significance of the inhibitory effect

The generality of the amino acid inhibitory response demonstrated by *Favella* sp. is not clear. The dinoflagellate *Gyrodinium dominans* showed no feeding response to any added amino acid (data not shown). The tintinnid *Coxliella* sp. was moderately inhibited by DMSP, betaine, and probably proline (Fig. 8), but showed no response to 2 other amino acids that inhibited *Favella* sp. However, the *Coxliella* culture at the time of the experiment was not at the peak of health; control feeding rates were low, and the ciliate was lost from culture soon after the experiment. Sensitivity to chemical signals may be related to physiological condition. Alternatively, compared to *Favella* sp., *Coxliella* may be adapted to waters that are chronically higher in DFAA concentration. More research is needed to establish the species-specificity of the inhibitory effect reported here.

One of the most interesting aspects arising from the amino acid response in *Favella* sp. is its adaptive significance. Considerable evidence (widespread response to amino acids among aquatic organisms, lack of toxicity, environmentally relevant response threshold) supports the idea that amino acids are signal molecules for *Favella* sp. What is the information content of the signal? Amino acid pulses might indicate the presence of actively feeding zooplankton, which are potential predators on *Favella* sp. Weissburg et al. (2002) have hypothesized that the use of chemical signals to detect nearby predators is a widespread strategy among potential prey organisms. If warned of predators, however, one would expect a behavioral response from *Favella* sp.: either a reduction in swimming activity to reduce hydromechanical signals that might attract predators, or an active escape response. We saw no consistent indication of a change in swimming behavior upon exposure to inhibitory amino acids. A second possibility is that amino acids signal the proximity of algal (or other) prey, and would attract *Favella* sp. (as they do many other aquatic organisms), given the appropriate behavioral assay. In this case, one would again expect a behavioral response, e.g. the decreases in swimming speed and increases in turning frequency that have been associated with *Favella* sp. exposure to prey in previous studies (Buskey & Stoecker 1988, 1989). Also, it is not clear why feeding would cease in the presence of an attractant. Few, if any, previous studies of chemosensory response in protists have measured feeding rates. Finally, amino acids may signal the presence of low quality or harmful prey. DMSP, betaine, proline, alanine, and possibly other amino acids are produced or accumulated at higher rates under osmotic and oxidative stress (Kaneshiro et al. 1969, Hare et al. 1998, Sharma & Dietz 2006), and leakage of these compounds from cells might indicate loss of membrane integrity. This hypothesis fits our observation of feeding inhibition without an accompanying behavioral response.

CONCLUSIONS

Feeding rates in the tintinnid *Favella* sp. were inhibited by a number of amino acids, with inhibition inversely proportional to the size of the amino acid side chain. Inhibition was dose-dependent, with a threshold in the vicinity of 200 nM of proline, and did not depend on the feeding history (well-fed vs. starved) of *Favella* sp. Inhibition occurred rapidly (<5 min after exposure) and was readily, though not completely, reversible. These findings, along with the prevalence of some inhibitory compounds at nanomolar concentrations in natural waters, point to a signaling function for these amino acids. Amino acid signaling is well known in aquatic systems, but amino acids usually constitute an attractant or stimulatory (rather than inhibitory) cue. Further, in contrast to findings from model ciliates such as *Paramecium* and *Tetrahymena*, *Favella* sp. did not rapidly or completely adapt to the presence of the inhibitory compounds. Nor did the tintinnid show consistent accompanying changes in swimming behavior, although the capacity for behavioral changes in response to chemical cues has been documented in the genus. Data presented here, along with work in progress (Wolfe et al. unpubl. data) indi-

cate involvement of an amino acid receptor in the transmission of the inhibitory signal. The information content of the signal remains unclear but may be related to detection of prey quality during suspension feeding by *Favella* sp.

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LITERATURE CITED

Andersson A, Lee C, Azam F, Hagström Å (1985) Release of amino acids and inorganic nutrients by heterotrophic marine microflagellates. Mar Ecol Prog Ser 23:99–106

Billen G, Joiris C, Wijant J, Gillain G (1980) Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian coastal zone of the North Sea and the English Channel. Estuar Coast Mar Sci 11:279–294

Birwé H, Hesse A (1991) High-performance liquid chromatographic determination of urinary cysteine and cystine. Clin Chim Acta 199:33–42

Boettcher AA, Targett NM (1998) Role of chemical inducers in larval metamorphosis of queen conch, *Strombus gigas* Linnaeus: relationship to other marine invertebrate systems. Biol Bull 194:132–142

Buskey EJ, Stoecker DK (1988) Locomotory patterns of the planktonic ciliate *Favella* sp.: adaptations for remaining within food patches. Bull Mar Sci 43:783–796

Buskey EJ, Stoecker DK (1989) Behavioral responses of the marine tintinnid *Favella* sp. to phytoplankton: influence of chemical, mechanical and photic stimuli. J Exp Mar Biol Ecol 132:1–16

Carr WES, Netherton JC, Gleeson RA, Derby CD (1996) Stimulants of feeding behavior in fish: analysis of tissues of diverse marine organisms. Biol Bull 190:149–160

Crawford CC, Hobbie JE, Webb KL (1974) The utilization of dissolved free amino acids by estuarine microorganisms. Ecology 55:551–563

Cronkite DL, Diekman AB, Lewallen B, Phillips L (1993) Aminotransferase and the production of alanine during hyperosmotic stress in *Paramecium calkinsi*. J Eukaryot Microbiol 40:796–800

Csonka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol 45:569–606

Ferner MC, Jumars PA (1999) Responses of deposit-feeding spionid polychaetes to dissolved chemical cues. J Exp Mar Biol Ecol 236:89–106

Granum E, Kirkvold S, Myklestad SM (2002) Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: diel variations and effects of N depletion. Mar Ecol Prog Ser 242:83–94

Hansen G, Daugbjerg N (2004) Ultrastructure of *Gyrodinium spirale*, the type species of *Gyrodinium* (Dinophyceae), including a phylogeny of *G. dominans*, *G. rubrum*, and *G. spirale* deduced from partial LSU rDNA sequences. Protist 155:271–294

Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21:535–553

Hauser DCR, Levandowsky M, Glassgold JM (1975) Ultrasensitive chemosensory responses by a protozoan to epinephrine and other neurochemicals. Science 190:285–286

Hoch MP, Kirchman DL (1995) Ammonium uptake by heterotrophic bacteria in the Delaware estuary and adjacent coastal waters. Limnol Oceanogr 40:886–897

Ide K, Takahashi K, Nakano T, Sato M, Omori M (2006) Chemoreceptive foraging in a shallow-water scavenging lysianassid amphipod: role of amino acids in the location of carrion in *Scopelocheirus onagawae*. Mar Ecol Prog Ser 317:193–202

Jakobsen HH, Strom SL (2004) Circadian cycles in growth and feeding rates of heterotrophic protist plankton. Limnol Oceanogr 49:1915–1922

Jørgensen NOG, Jensen RE (1997) Determination of dissolved combined amino acids using microwave-assisted hydrolysis and HPLC precolumn derivatization for labeling of primary and secondary amines. Mar Chem 57: 287–297

Kaneshiro ES, Holz GG Jr, Dunham PB (1969) Osmoregulation in a marine ciliate, *Miamiensis avidus*. II. Regulation of intraceullular free amino acids. Biol Bull 137:161–169

Kim MY, Kuruvilla HG, Hennessey TM (1997) Chemosensory adaptation in *Paramecium* involves changes in both repellent binding and the consequent receptor potentials. Comp Biochem Physiol 118A:589–597

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. Annu Rev Plant Physiol Plant Mol Biol 40:21–53

Kung C, Saimi Y (1982) The physiological basis of taxes in *Paramecium*. Annu Rev Physiol 44:519–534

Kuznetsova M, Lee C, Aller J, Frew N (2004) Enrichment of amino acids in the sea surface microlayer at coastal and open ocean sites in the North Atlantic Ocean. Limnol Oceanogr 49:1605–1619

Laval-Peuto M (1981) Construction of the lorica in Ciliata Tintinnina. *In vivo* study of *Favella ehrenbergii*: variability of the phenotypes during the cycle, biology, statistics, biometry. Protistologica 17:242–279

Lee ES, Lewitus AJ, Zimmer RK (1999) Chemoreception in a marine cryptophyte: behavioral plasticity in response to amino acids and nitrate. Limnol Oceanogr 44:1571–1574

Lee PA, Saunders PA, de Mora SJ, Deibel D, Levasseur M (2003) Influence of copepod grazing on concentrations of dissolved dimethylsulfoxide and related sulfur compounds in the North Water, northern Baffin Bay. Mar Ecol Prog Ser 255:235–248

Levandowsky M, Cheng T, Hehr A, Kim J and 6 others (1984) Chemosensory responses to amino acids and certain amines by the ciliate *Tetrahymena*: a flat capillary assay. Biol Bull 167:322–330

Liebezeit G, Bolter M, Brown IF, Dawson R (1980) Dissolved free amino acids and carbohydrates at pycnocline boundaries in the Sargasso Sea and related microbial activity. Oceanol Acta 3:357–362

Lindroth P, Mopper K (1979) High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Anal Chem 51:1667–1672

Machemer H, Teunis PFM (1996) Sensory-motor coupling

and motor responses. In: Hausmann K, Bradbury X (eds) Ciliates: cells as organisms. Gustav Fischer Verlag, Stuttgart, p 379–402

Marsot P, Cembella AD, Colombo JC (1991) Intracellular and extracellular amino acid pools of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae) grown on unenriched seawater in high-cell-density dialysis culture. J Phycol 27:478–491

Matrai PA, Keller MD (1994) Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: intracellular variations. Mar Biol 119:61–68

Miller TR, Hnilicka K, Dziedzic A, Desplats P, Belas R (2004) Chemotaxis of *Silicibacter* sp. strain TM1040 toward dinoflagellate products. Appl Environ Microbiol 70: 4692–4701

Miyamoto S, Shimozawa A (1985) Chemotaxis in the freshwater planarian, *Dugesia japonica japonica*. Zool Sci 2: 389–395

Mopper K, Lindroth P (1982) Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. Limnol Oceanogr 27:336–347

Murphy BF, Hadfield MG (1997) Chemoreception in the nudibranch gastropod *Phestilla sibogae*. Comp Biochem Physiol 118A:727–735

Nagata T, Kirchman DC (1991) Release of dissolved free and combined amino acids by bactivorous marine flagellates. Limnol Oceanogr 36:433–443

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon, Oxford

Pasmore M, Costerton JW (2003) Biofilms, bacterial signaling, and their ties to marine biology. J Ind Microbiol Biotechnol 30:407–413

Pierce RW, Turner JT (1993) Global biogeography of marine tintinnids. Mar Ecol Prog Ser 94:11–26

Poulet SA, Ouellet G (1982) The role of amino acids in the chemosensory swarming and feeding of marine copepods. J Plankton Res 4:341–361

Poulet SA, Williams R, Conway DVP, Videau C (1991) Co-occurence of copepods and dissolved free amino acids in shelf sea waters. Mar Biol 108:373–385

Rice MA (1999) Uptake of dissolved free amino acids by northern quahogs, *Mercenaria mercenaria* and its relative importance to organic nitrogen deposition in Naragansett Bay, Rhode Island. J Shellfish Res 18:547–553

Riffell JA, Krug PJ, Zimmer RK (2002) Fertilization in the sea: the chemical identity of an abalone sperm attractant. J Exp Biol 205:1439–1450

Rosenstock B, Simon M (2001) Sources and sinks of dissolved free amino acids and protein in a large and deep mesotrophic lake. Limnol Oceanogr 46:644–654

Rublee PA, Gallegos CL (1989) Use of fluorescently labelled algae (FLA) to estimate microzooplankton grazing. Mar Ecol Prog Ser 51:221–227

Sellner KG, Nealley EW (1997) Diel fluctuations in dissolved free amino acids and monosaccharides in Chesapeake Bay dinoflagellate blooms. Mar Chem 56:193–200

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726

Sibbald MJ, Albright LJ, Sibbald PR (1987) Chemosensory responses of a heterotrophic microflagellate to bacteria and several nitrogen compounds. Mar Ecol Prog Ser 36: 201–204

Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat Microb Ecol 28:175–211

Spero HJ (1985) Chemosensory capabilities in the phagotrophic dinoflagellate *Gymnodinium fungiforme*. J Phycol 21:181–184

Stoecker DK, Gallager SM, Langdon CJ, Davis LH (1995) Particle capture by *Favella* sp. (Ciliata, Tintinnina). J Plankton Res 17:1105–1124

Strom SL, Benner R, Ziegler S, Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. Limnol Oceanogr 42:1364–1374

Strom SL, Wolfe GV, Slajer A, Lambert S, Clough J (2003) Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethylsulfoniopropionate. Limnol Oceanogr 48:230–237

Sunda WG, Kieber DJ, Kiene RP, Huntsman SA (2002) An antioxidant function for DMSP and DMS in marine algae. Nature 418:317–320

Valentincic T (1985) Behavioral study of chemoreception in the sea star *Marthasterias glacialis*: structure-activity relationships of lactic acid, amino acids, and acetylcholine. J Comp Physiol A 157:537–545

Van Houten J (1978) Two mechanisms of chemotaxis in *Paramecium*. J comp Physiol 127:167–174

Vardi A, Formiggini F, Casotti R, De Martino A, Ribalet F, Miralto A, Bowler C (2006) A stress surveillance system based on calcium and nitric oxide in marine diatoms. PLOS Biology 4:411–419

Weissburg MJ, Ferner MC, Pisut DP, Smee DL (2002) Ecological consequences of chemically mediated prey perception. J Chem Ecol 28:1953–1970

Wolfe GV (2000) The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. Biol Bull 198:225–244

Wolfe GV, Strom SL, Holmes JL, Radzio T, Olson MB (2002) Dimethylsulfoniopropionate cleavage by marine phytoplankton in response to mechanical, chemical, or dark stress. J Phycol 38:948–960

Yang H (1995) Distribution of free amino acids in sea and lake ice cores from Antarctica with special reference to ice biota. Proc Natl Inst Polar Res Symp Polar Biol 8:114–125

Zimmer RK, Commins JE, Browne KA (1999) Regulatory effects of environmental chemical signals on search behavior and foraging success. Ecology 80:1432–1446

Zimmer-Faust RK, Tamburri MN (1994) Chemical identity and ecological implications of a waterborne, larval settlement cue. Limnol Oceanogr 39:1075–1087

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