The prevalence and implications of copepod behavioral responses to oceanographic gradients and biological patchiness

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Synopsis Several species and developmental stages of calanoid copepods were tested for responses to environmental cues in a laboratory apparatus that mimicked conditions commonly associated with patches of food in the ocean. All species responded to the presence of phytoplankton by feeding. All species responded by increasing proportional residence time in one, but not both, of the treatments defined by gradients of velocity or density. Most species increased swimming speed and frequency of turning in response to the presence of chemical exudates or gradients of velocity. Only one species, *Eurytemora affinis*, increased proportional time of residence in response to gradients in density of the water. Responses of *E. affinis* to combined cues did not definitively demonstrate a hierarchical use of different cues as previously observed for *Temora longicornis* and *Acartia tonsa*. A simple foraging simulation was developed to assess the applicability in the field of the behavioral results observed in the laboratory. These simulations suggest that observed fine-scale behaviors could lead to copepod aggregations observed *in situ*. The present study demonstrates that behavioral response to cues associated with fine-scale oceanographic gradients and biological patchiness is functionally important and prevalent among copepods and likely has significant impacts on larger-scale distributional patterns.

Introduction

Fine-scale biological patchiness and physical gradients in the water column have been documented throughout the coastal Atlantic and Pacific Oceans (Tiselius et al. 1994; Cowles 2004; McManus et al. 2005). Copepods have been observed to aggregate near oceanographic discontinuities (Mackas and Louttit 1988; Holliday et al. 1998), and some species are believed to utilize physical properties to maintain position in the water column (Harder 1968; Genin et al. 2005; Seuront 2006). Additionally, vertical distributions of copepods are influenced by the spatial and temporal variability of food patches, which are often associated with gradients in physical properties (Franks 1995; Leising and Franks 2002). Further, there is increasing evidence that copepod behavior and foraging strategies have an important impact on the distributions of organisms at fine oceanographic scales (centimeters to meters) (Castro et al. 1991; Tiselius et al. 1994; Folt and Burns 1999).

Behavioral responses by copepods to cues associated with physical gradients and chemical presence have been linked to several ecological functions (Tiselius 1992; Yen and Fields 1992; Yen et al. 1998; Kiørboe et al. 1999). Copepods are known to employ velocity gradients or shear during foraging, predator avoidance, and position maintenance (Field and Yen 1997; Genin et al. 2005; Woodson et al. 2005). Responses to density gradients have been demonstrated to influence position maintenance near preferred habitats and to cue the depth of diel vertical migrations (Harder 1968; Mauchline 1998; Seuront 2006). Chemical cues induce swarming or increased activity in some species (Poulet and Ouellet 1982) and this response is terminated if food particles are contacted (Woodson et al. 2007). *Acartia tonsa* and *Temora longicornis* employ multiple cues in a hierarchy to locate resource patches (Woodson et al. 2007). These observations, however, are limited to a few well-studied species.

Observed behavioral responses to fine-scale gradients of oceanographic properties and mimics of gradients in laboratory experiments by some species of copepods (Tiselius 1992; Genin et al. 2005; Woodson et al. 2005, 2007) raise questions about the general significance of these behaviors and how they

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may influence observed distributions in the field. First, how prevalent are responses to cues associated with fine-scale oceanographic gradients among copepods? Second, how variable are responses to such cues among copepod species? Third, can observed behaviors at fine-scales (e.g., centimeters to meters; seconds) lead to distributional patterns observed in the field at larger spatial and temporal scales (e.g., meters to kilometers; hours to days)? The present study begins to address these questions by focusing on direct responses to cues associated with fine-scale oceanographic structure. The objectives are (1) to survey responses of a variety of copepods and nauplii from various habitats and trophic levels, in order to determine if there are general patterns in the behavioral responses to mimics of fine-scale oceanographic gradients and biological patchiness, and (2) to develop a model to assess field-scale impacts of the behaviors observed in the laboratory experiments. We devoted particular attention to the behavior of the estuarine copepod *Eurytemora affinis*, as it became clear during the course of our experiments that this species displayed different reactions to fine-scale cues compared to the other tested species, in which responses were generally consistent with previous observations on *A. tonsa* and *T. longicornis*. Our data suggest that the estuarine copepod *E. affinis* may have a different weighting of cues than the other species examined, possibly as a result of differences in the physical environment in estuarine versus coastal or open ocean conditions.

Materials and methods

Behavioral experiments

Adults of five species of calanoid copepods (*Calanus finmarchicus*, *Calanus pacificus*, *E. affinis*, *Metridia pacifica*, and *Neocalanus plumchrus*) were exposed to isolated treatments of flow-velocity gradient (*V*), fluid density change (*D*, as a change in salinity), presence of biological activity (*C*, *Fd*, as a layer of chemical exudates from phytoplankton or phytoplankton cells, respectively), and a control with no cue present. *Eurytemora affinis* was also exposed to combined treatments [velocity gradient–density change (*VD*), velocity gradient–chemical exudates (*VC*), density change–chemical exudates (*DC*), velocity gradient–density change–chemical exudates (*VDC*), and a treatment consisting of a density change with chemical exudates mixed homogenously in the tank (D–AC) to evaluate the potential interaction of cues in *E. affinis* behavior. Naupliar stages (NII-NIII) of *A. tonsa*, *C. finmarchicus*, and *T. longicornis* also were exposed to the isolated cue treatments. Average adult size, water temperature, and salinity at the time of collection, as well as the location of collection are given in Table 1. Copepods were sampled and shipped overnight to Atlanta, Georgia (USA), where they were transferred to holding tanks, fed, and allowed to acclimate for a minimum of 48 h prior to experiments. Using size selective filtering (50–250 μm), nauplii were sorted prior to each experiment.

Table 1 Copepod species, average sizes, ambient temperatures, and collection locations. Ambient salinity and temperature are those occurring at the time of collection

Species	Adult size (mm)	Ambient temp. (°C)	Ambient salinity (ppt)	Collection location
Calanus finmarchicus	3–4	12	31	Gulf of Maine
Calanus pacificus	2–3	12	32	Monterey Bay, CA, USA
Eurytemora affinis	~1	12	26	Boothbay Harbor, ME, USA
Metridia pacifica	3–4	5	31	Gulf of Alaska
Neocalanus plumchrus	4–5	5	31	Gulf of Alaska

A detailed description of the methods used to create and quantify the various treatments used in the experiments, including calibration data and a sketch of the apparatus, is provided by Woodson et al. (2005, 2007). All experiments were conducted in a plane jet flume apparatus ($100 \times 30 \times 30\,cm^3$) designed to mimic environmental cues associated with high-resource patches. The environmental cue in all treatments was present in a narrow vertical region at the center of the apparatus with uniform control conditions present above and below that region. The experimental setup was housed in an environmental room at the ambient temperature of the particular species (Table 1). A laminar plane jet was used to mimic oceanographic shear layers (i.e., velocity gradients). Discontinuities in density were created by adding artificial seawater to the tank at a density appropriate for each species, then slowly adding water of higher density (+ 2 ppt salinity, same temperature). The water was added through the drain fitting to minimize mixing at the fluid interface. Chemical and biological treatments were created by advecting a solution across the tank using the laminar plane jet, then turning off the jet flow and allowing the momentum to dissipate. Concentrations of the exudate and food (*Tetraselmis* sp., Chlamydomonadaceae) were calculated using

carbon content conversions and cell counts per Menden-Deuer and Lessard (2000).

Each cue was tested in isolation at a predetermined strength or concentration to determine specific responses. Cue strength was selected to match reported *in situ* observations (Dekshenieks et al. 2001; Cowles 2004). The jet velocity (U_j) was 6.7 mm s^{-1} for the velocity-gradient treatment, the salinity change was 2 ppt for the density-change treatment, the concentration was 253 μg C l^{-1} for the chemical-exudate and food treatments. Resulting strain rates for the velocity-gradient treatment were below escape-response thresholds (Woodson et al. 2005). All treatments were contained within a boundary identified by a 0.025 s^{-1} strain-rate threshold (from the velocity-gradient treatment). The thickness of the treatment region ranged from 34 to 54 mm between 50 and 150 mm downstream of the nozzle and covered 39% of the $10 \times 10\,cm^2$ observation region (Woodson et al. 2005). Combined treatment experiments were conducted for *E. affinis* only and followed the methods of Woodson et al. (2007) and of the general procedures outlined earlier.

Groups of either 30 or 70 adult mixed-sex copepods (depending on the size of the individuals and adjusted to prevent overcrowding and artificially high animal densities) were added to the test section of the experimental tank and aggregated at the water surface using a fiberoptic light source. Two replicates were performed for each treatment. Each treatment group was allowed to acclimate in this setup for 2 h. At the end of the acclimation period, the fiberoptic light source was removed. Recording of data began ~5 min after the light source was removed. Light-induced swimming behavior was not observed after this period in preliminary experiments. Additional experiments with the light source at the bottom of the tank showed no differences in behavior. Individuals were allowed to freely interact with the treatment, and observations were recorded on videotape via a closed coupled device (CCD) video camera (Pulnix TM-745, 768×494 pixels with a Nikon Micro-Nikkor 60 mm lens). During recording, infrared diodes provided illumination at wavelengths that did not influence copepod behavior.

Preliminary observations suggested that most, if not all, individuals contacted the treatment region at least once during the 2 h experiment. In each experiment, between 20 and 95 individual paths were recorded onto videotape. Direct observations during initial studies were used to develop protocols, in order to minimize the probability of repeatedly sampling the same individual (Woodson et al. 2005).

Twenty or 50 individual paths (corresponding to experiments with 30 or 70 individuals, respectively) were digitized from videotape using ExpertVision (MotionAnalysis Corporation) at 15 Hz for analysis. A detailed description and validation of the 15 Hz recording rate and resolution can be found in a paper by Woodson et al. (2005). Only individuals that contacted the region of the treatment while in the field of view were used for analysis. Paths were typically 1–2 min in duration (range from 30 s to several minutes). Proportional residence time (PRT), swimming speed, and frequency of turning were calculated from the digitized path data.

The PRT was defined as the ratio of the time in the treatment region to the total time in the $10 \times 10\,cm^2$ observation region. Proportional residence times were compared across all treatments (including the control) for each species, using a single-factor analysis of variance (ANOVA) followed by a *post hoc* Student–Newman–Keuls test (S–N–K). This analysis was employed for species exposed only to isolated treatments (all species except *E. affinis*). Isolated and combined treatment experiments for *E. affinis* were analyzed using a fully nested, fixed factor ANOVA with PRT as the response, and replicate (two per level, 50 paths each) nested within each cue (*V*, *D*, *C*; Woodson et al. 2007). Replicate effects were not significant and thus a full factorial ANOVA with three fixed factors and interactions was conducted using the pooled error variance ($n = 800$). Data were not transformed because they fit normal distributions, and results of a similar analysis with arc-sine transformation yielded statistically similar results in all cases. Thus, we followed the recommendation of Zar (1999) and report the results from the analysis of untransformed data.

Swimming speed and frequency of turning were calculated from positional data for each individual and classified as either precontact or postcontact with the treatment region. The local fluid-velocity vector was subtracted from the animal trajectory; thus, an increase in swimming speed was due to a change in behavior. Comparisons between pre-contact and post-contact values were made using repeat-measures ANOVA, in order to alleviate differences between treatments arising from varying sex ratios or changes in physiological condition of the organisms. Changes in behavior to different treatments were only statistically compared within a species or life-stage, so we applied a *post hoc* correction to reduce the type-1 error rate separately for each repeated-measures analysis. A false discovery rate correction (FDR; Benjamini and Hochberg 1995) was employed to remove the potential for

false significance that could result from the high number of statistical tests within each species or life-stage comparison.

Model development and simulation

To compliment the experimental study, a simple individual-based model (IBM) was developed to assess the impacts of the observed behaviors at field scales. These simulations specifically examined how the responses to the various individual physical and chemical cues may alter the distribution of copepods at field scales. Three simplifying assumptions were made in the development of the model. (1) Transport of model organisms was assumed not to be influenced by flow velocity and turbulence. (2) Other behaviors such as diel vertical migration and responses to conspecifics were specifically neglected to test the effect of observed responses of individuals to fine-scale oceanographic structure only. (3) Model organisms responded to an environmental cue only in the manner observed in the laboratory experiments. Thus, the model specifically addresses only one question: can the fine-scale behaviors observed in the laboratory experiments potentially lead to aggregations at the scales of habitats and populations? The potential impacts of the assumptions, results, and other behaviors are addressed in the ''Consequences and limitations of modeling results'' section.

The model simulation provided information about the location of individuals at each time-step, the number of organisms in the treatment region at each time step, the amount of time spent in the treatment region by each organism, and the average time in the treatment region by all organisms. All model development and simulations were run in MatLab on an Apple (Macintosh) computer.

Two simulation regions were examined. The first region matched the scale of the laboratory experiments (i.e., $10 \times 10 \times 10\,cm^3$), in order to calibrate model parameters to experimental results. The second region was defined to be consistent with the scales of observations in the field (25 m vertical $\times$ 5 m horizontal $\times$ 1 m horizontal). Model organisms initially were placed randomly in the simulation region to match the sample populations from experiments or to achieve densities based on field estimates of the target species (Mauchline 1998). The random initial positions were projected onto a vertical plane to simplify the simulation (Leising 2001). This procedure created a simulation field that was consistent with the relatively 2D nature of the ocean [e.g., vertical gradients occur at scales (meters) much smaller than horizontal gradients (tens of kilometers)]. Thus, the projected dimensions for the laboratory-scale simulation were 10 cm vertical $\times$ 10 cm horizontal, and that for the projected field-scale simulation was 25 m vertical $\times$ 5 m horizontal. The position and motion of organisms were simulated in these planar fields. Individuals did not interact with other model organisms, but merely altered swimming kinematics in accordance with the simulated environmental cue. Individuals that exited the field were replaced by a new organism placed randomly at the edge of the field so that the number of organisms was constant over the simulation duration.

Movements for each organism were specified by speed and direction of swimming, defined separately. Swimming speed was determined at each time step by a Gaussian random number generator (MatLab randn function) with the mean and standard deviation (SD) determined from the laboratory data. Swimming direction was determined in a similar fashion, with the mean direction coinciding with the direction of previous movement and the SD calibrated using our behavioral data. We determined the appropriate SD that produced a frequency of turning of the simulated organisms that matched the frequency of turning observed in laboratory experiments. Direct comparisons showed that this formulation created model paths representative of laboratory observations.

The model simulation algorithm for employing the fine-scale swimming kinematic data from the laboratory is shown in Fig. 1. When an individual model organism was located outside of the treatment region, the mean and standard deviation for swimming speed and rate of turning were defined based on precontact data of swimming (Table 2). For an individual in the treatment region, swimming speed and frequency of turning were altered to simulate observed responses to environmental structure. One of the challenges of interpreting the laboratory data is that the observation period was relatively short; hence, we lack direct information about the longevity of the behavioral response of individual copepods to environmental cues. To account for the fact that altered swimming speed and frequency of turning may not be maintained over long periods of time (i.e., 24 h), a response threshold was defined in the model simulation. The response threshold (γ, range from 0 to 1) defined the propensity of an individual to move, based on the presence of a particular cue, in accordance with the postcontact data on swimming speed and frequency of turning within the treatment region. As described

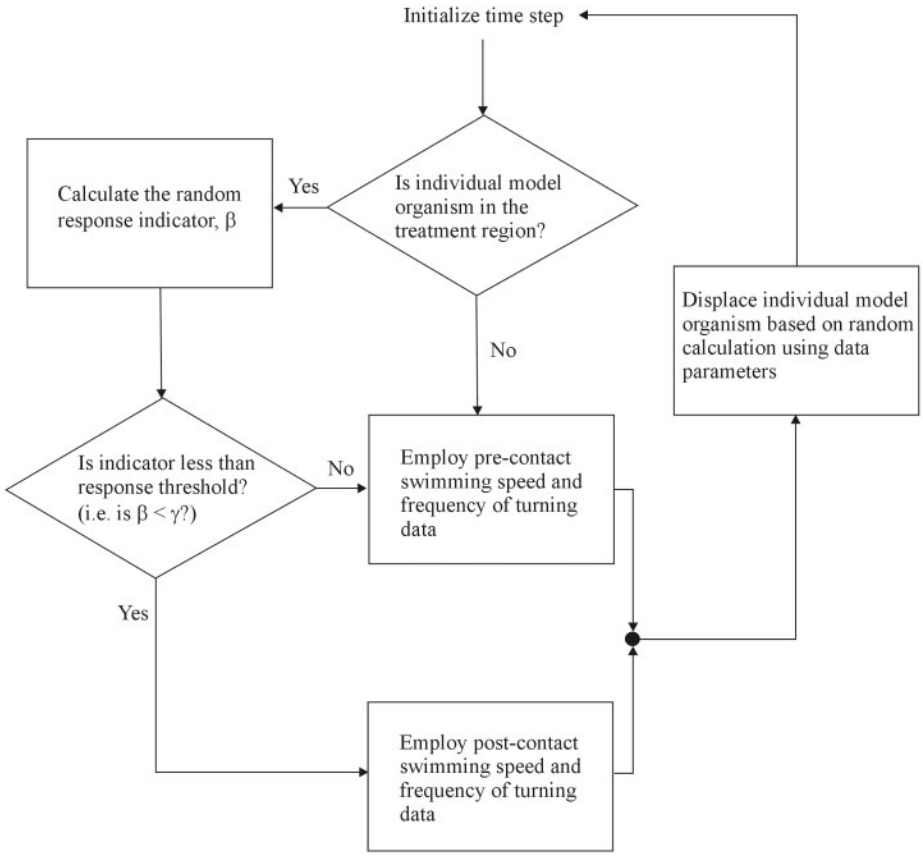

Fig. 1 Flow chart of the model simulation algorithm.

Table 2 Input parameters for swimming speed and frequency of turning in the model simulation for adults of *A. tonsa*

	Swimming speed [mm s^{-1}] (SE)		Frequency of turning [turns ind^{-1} s^{-1}] (SE)	
Environmental cue	**Precontact**	**Postcontact**	**Precontact**	**Postcontact**
Control	2.23 (0.17)	2.15 (0.15)	0.05 (0.02)	0.07 (0.02)
Velocity gradient	3.08 (0.17)	3.68 (0.19)	0.09 (0.02)	0.24 (0.05)
Density change	1.82 (0.07)	1.71 (0.06)	0.06 (0.02)	0.08 (0.03)
Chemical exudates	1.91 (0.18)	2.57 (0.14)	0.02 (0.02)	0.20 (0.04)
Food	2.85 (0.19)	1.97 (0.17)	0.06 (0.04)	0.10 (0.03)

Data are taken from Woodson et al. (2005, 2007).

subsequently, the response threshold was calibrated via the simulations performed at laboratory scale. At each time step, individual behavior was determined by comparing a behavioral indicator value, β, to the calibrated response threshold. The behavioral indicator was randomly-generated via the Mersenne Twister algorithm with uniform distribution between 0 and 1 (Matsumoto and Nishimura 1998). The Mersenne Twister is recommended for uniform random distributions, since the MatLab default pseudo-random number generator shows non-random patterns (Savicky 2006). If the behavioral indicator value was smaller than the calibrated response threshold (i.e., $\beta < \gamma$), then postcontact data were used for swimming speed and frequency of turning (Table 2) in the Gaussian random number generator. Otherwise, precontact data for swimming speed and frequency of turning were used in the Gaussian random number generator. At each time step for model organisms in the treatment region, a new value of β was generated and compared to γ to determine whether precontact or postcontact data were

employed (Fig. 1). This comparison of a randomly generated number to a behavioral threshold is simply a conditional argument to shift between two behavioral states and has no direct biological significance. The response threshold, however, is an important aspect of the model simulation because it accounts for the fact that altered behavior may not be maintained by an individual indefinitely despite the continued presence of an environmental cue. Although this formulation acts as a correction for behavioral duration, further studies are needed to assess the decay of the observed behaviors during prolonged exposure to the various treatments.

Calibration of the behavioral model based on individuals was accomplished by running 50 simulations at spatial and temporal scales that matched the laboratory experiments (e.g., $10 \times 10\,cm^2$ field, 2 h duration, 1 s time-step). The treatment region was defined to be consistent with the laboratory arrangement for the calibration simulations. Mean PRT in the treatment layer was calculated based on the response of individuals in the model population, using response threshold (γ) values between 0 and 1 at increments of 0.01, and the results of the 50 runs at a given response threshold value were averaged to create a response curve. The ''calibrated'' response threshold, γ, was then selected based on the value that best matched the PRT in laboratory experiments with the specified copepod species and environmental cue.

To test the sensitivity of the model to behavioral parameters, the calibration procedure was repeated with mean swimming speed for postcontact values set at (1) the observed mean values of the laboratory experiments, (2) the mean values $\pm$ 1 standard error, and (3) the mean values $\pm$ 2 standard errors. Fifty calibration runs were performed for each value of the input mean swimming speed, and the mean PRT from the runs was calculated as described earlier. The mean PRTs were compared to assess the sensitivity of PRT to the magnitude of the change in swimming speed. A similar series of tests of PRT sensitivity to frequency of turning was also conducted.

Field-scale simulations were run for a 24 h duration with a time-step of 1 s. The model domain consisted of a 25 m water column with a treatment region 1 m thick located at 8 m depth (constituting 4% of the domain) (McManus et al. 2005). Model organisms were randomly distributed at the beginning of each simulation and input swimming behaviors were set to match laboratory results. Swimming speeds of model organisms ($\sim$2–5 $mm\,s^{-1}$) insured that each individual could cover the entire model domain within a small fraction ($\sim$5–15%) of the total duration of the simulation (86,400 s). The algorithm for determining model organism behavior was the same as in the calibration simulations (Fig. 1). In the field-scale simulations, the number of organisms in the treatment region (a value corresponding to abundances and distributions at a given time) was calculated for each time step, which is not the experimental equivalent of PRT for each individual.

Results

Behavioral experiments

All adult species tested increased proportional residence time in the presence of one or more of the treatments (*C. finmarchicus*, df = 99, $F = 23.07$, $P < 0.001$; *C. pacificus*, df = 99, $F = 10.20$, $P < 0.001$; *E. affinis*, df = 799, $F = 35.69$, $P < 0.001$; *M. pacifica*, df = 79, $F = 16.39$, $P < 0.001$; *N. plumchrus*, df = 57; $F = 8.86$, $P < 0.001$). Proportional residence time in the velocity-gradient treatment significantly increased compared to that of controls for all species except *E. affinis* (Fig. 2A; minimum significant difference [MSD]; *C. finmarchicus*, difference = 0.296, $MSD_{0.05,99,5} = 0.127$; *C. pacificus*, difference = 0.258, $MSD_{0.05,99,5} = 0.133$; *E. affinis*, Table 8; *M. pacifica*, difference = 0.232, $MSD_{0.05,60,4} = 0.120$; *N. plumchrus*, difference = 0.264; $MSD_{0.05,60,3} = 0.153$; other species previously reported by Woodson et al. 2005). Changes in proportional residence time were not due to entrainment in the flow as shown by the lack of a significant increase in this parameter by recently dead, intact *C. ethiopica* (Fig. 2A; Woodson et al. 2005). Behaviors for all species leading to increased proportional residence time in the velocity-gradient treatment included increased swimming speed and frequency of turning (Table 3). *Eurytemora affinis*, the one copepod that did not increase proportional residence time in the velocity-gradient treatment, also did not significantly alter swimming speed or frequency of turning (Table 3).

In contrast, *E. affinis* was the only tested species in which adults significantly increased proportional residence time in the presence of a density-change treatment (Fig. 2B; *C. finmarchicus*, difference = 0.004, $MSD_{0.05,99,5} = 0.096$; *C. pacificus*, difference = 0.110, $MSD_{0.05,99,5} = 0.130$; *E. affinis*, Table 8; *M. pacifica*, difference = 0.008, $MSD_{0.05,99,5} = 0.102$; *N. plumchrus*, difference = 0.116, $MSD_{0.05,60,3} = 0.130$; other species previously reported by Woodson et al. 2005). Similar to responses by other species to the velocity-gradient treatment, *E. affinis* increased swimming speed ($P = 0.026$) in the density-change treatment. Frequency of turning also increased with a strong

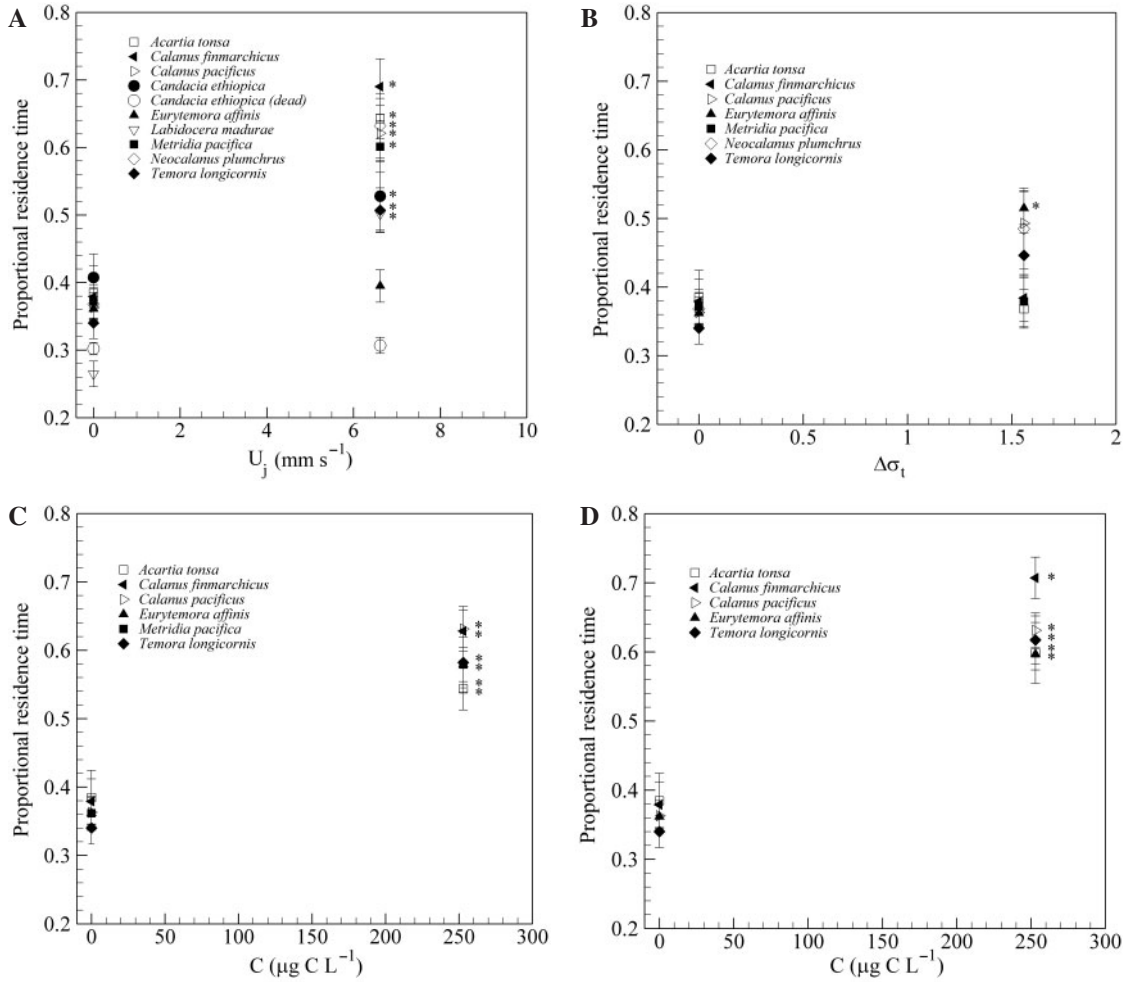

Fig. 2 Proportional residence time for adult copepods in the treatment region for the (**A**) velocity-gradient treatment ($U_j = 6.7\ mm\ s^{-1}$), (**B**) density-change treatment ($\Delta\sigma_t = 1.6$), (**C**) phytoplankton chemical exudates treatment ($C = 253\ \mu g\ C\ l^{-1}$), and (**D**) food treatment (e.g., phytoplankton, $C = 253\ \mu g\ C\ l^{-1}$). Control experiments correspond to no cue in the treatment region [e.g., (A) $U_j = 0\ mm\ s^{-1}$, (B) $\Delta\sigma_t = 0$, (C) absence of exudates ($0\ \mu g\ C\ l^{-1}$), and (D) absence of food]. *Indicates significant differences between treatment and control ($P < 0.05$). Data for adults of *Acartia tonsa*, *Candacia ethiopica*, *Labidocera madurae*, and *Temora longicornis* are taken from Woodson et al. (2005, 2007).

Table 3 Behavioral responses (swimming speed and frequency of turning) of various species of copepod to the velocity-gradient treatment ($U_j = 6.7\ mm\ s^{-1}$)

Species		Swimming speed [mm s^{-1}] (SE)				Frequency of turning [turns $ind^{-1}\ s^{-1}$] (SE)		
Adult copepods	***n***	**Precontact**	**Postcontact**	***P***	***n***	**Precontact**	**Postcontact**	***P***
Calanus finmarchicus	16	3.61 (0.51)	5.63 (0.60)	0.025*	16	0.03 (0.01)	0.10 (0.03)	0.032*
Calanus pacificus	17	6.65 (0.59)	8.18 (0.52)	0.068	17	0.05 (0.03)	0.17 (0.03)	0.014*
Eurytemora affinis	39	4.79 (0.40)	4.39 (0.66)	0.609	26	0.12 (0.03)	0.10 (0.03)	0.691
Metridia pacifica	18	2.57 (0.57)	4.39 (0.43)	0.020*	13	0.09 (0.04)	0.25 (0.06)	0.041*
Neocalanus plumchrus	17	2.61 (0.41)	4.11 (0.47)	0.036*	15	0.03 (0.02)	0.12 (0.03)	0.042*
Nauplii								
Acartia tonsa	28	2.93 (0.44)	2.71 (0.34)	0.708	36	0.09 (0.02)	0.09 (0.02)	0.848
Calanus finmarchicus	16	5.36 (0.44)	5.10 (0.64)	0.781	15	0.16 (0.04)	0.16 (0.05)	0.976
Temora longicornis	31	2.20 (0.14)	4.44 (0.36)	0.001*	34	0.06 (0.01)	0.08 (0.02)	0.374

*Denotes *P*-values <0.05 between precontact and postcontact values by repeated measures ANOVA with a *posthoc* correction for multiple tests. Corresponding data for adults of *Acartia tonsa*, *Candacia ethiopica*, *Labidocera madurae*, and *Temora longicornis* are reported by Woodson et al. (2005).

Table 4 Behavioral responses (swimming speed and frequency of turning) by various species of copepod to the density-change treatment ($\Delta\sigma_t = 1.6$)

Species		Swimming speed [mm s^{-1}] (SE)				Frequency of turning [turns ind^{-1} s^{-1}] (SE)		
Adult copepods	***n***	**Precontact**	**Postcontact**	***P***	***n***	**Precontact**	**Postcontact**	***P***
Calanus finmarchicus	16	5.85 (0.45)	5.77 (0.48)	0.896	16	0.04 (0.02)	0.10 (0.03)	0.128
Calanus pacificus	13	5.29 (0.51)	6.62 (0.66)	0.134	13	0.06 (0.02)	0.08 (0.03)	0.643
Eurytemora affinis	30	2.81 (0.20)	3.52 (0.21)	0.026*	27	0.05 (0.01)	0.14 (0.04)	0.087
Metridia pacifica	20	4.70 (0.81)	4.76 (0.88)	0.960	15	0.14 (0.05)	0.13 (0.05)	0.878
Neocalanus plumchrus	16	2.77 (0.28)	2.91 (0.22)	0.717	17	0.06 (0.02)	0.08 (0.03)	0.723
Nauplii								
Acartia tonsa	31	2.99 (0.46)	2.35 (0.30)	0.251	35	0.07 (0.02)	0.14 (0.03)	0.031*
Calanus finmarchicus	18	4.55 (0.41)	4.64 (0.42)	0.890	16	0.12 (0.04)	0.15 (0.03)	0.536
Temora longicornis	31	3.33 (0.24)	3.87 (0.28)	0.175	34	0.08 (0.03)	0.19 (0.05)	0.051

*Denotes *P*-values <0.05 between precontact and postcontact values by repeated measures ANOVA with a *posthoc* correction for multiple tests. Corresponding data for adults of *Acartia tonsa* and *Temora longicornis* are reported by Woodson et al. (2005).

Table 5 Behavioral responses (individuals crossing the density-gradient region) of various species of copepod to the density-change treatment ($\Delta\sigma_t = 1.6$)

Species		Control (without density-gradient region)		Treatment (with density-gradient region)		
Adult copepods	***n***	**Crossed**	**Did not cross**	**Crossed**	**Did not cross**	***P***
Calanus finmarchicus	20	14	6	6	14	0.031*
Calanus pacificus	20	11	9	4	16	0.056
Eurytemora affinis	40	22	18	20	20	0.823
Metridia pacifica	20	13	7	6	14	0.029*
Neocalanus plumchrus	20	14	6	7	13	0.059
Nauplii						
Acartia tonsa	40	24	6	11	29	0.002*
Calanus finmarchicus	20	14	6	8	12	0.123
Temora longicornis	40	29	11	10	30	0.001*

*Denotes *P*-values <0.05 between precontact and postcontact values by Fisher's exact tests. Corresponding data for adults of *Acartia tonsa* and *Temora longicornis* are reported by Woodson et al. (2007).

trend (Table 4). Adults of the other species did not significantly alter swimming speed or frequency of turning in response to the density-change treatment (Table 4), but did show an aversion to crossing the density-gradient region (Table 5, significant for *C. finmarchicus* and *M. pacifica*; strong trend for *C. pacificus* and *N. plumchrus*; Fisher's exact test). Again, in contrast, a significant difference was not observed for *E. affinis* crossing the density-gradient region compared to the control (Table 5). For adults of each species except *E. affinis*, the behaviors elicited by the density-gradient treatment were similar to previously reported behaviors for *T. longicornis* and *A. tonsa* (Harder 1968; Woodson et al. 2005, 2007).

Adults of all species increased proportional residence time in response to the presence of chemical exudates at 253 μg C l^{-1} (Fig. 2C; *C. finmarchicus*, difference = 0.248, $MSD_{0.05,99,5} = 0.127$; *C. pacificus*, difference = 0.269, $MSD_{0.05,99,5} = 0.145$; *E. affinis*, Table 8; *M. pacifica*, difference = 0.276, $MSD_{0.05,99,5} = 0.135$; other species reported by Woodson et al. 2007). Similarly, adults of all species increased swimming speed and frequency of turning in response to the chemical-exudates treatment (Table 6), although the differences in swimming speed and frequency of turning for *C. pacificus* and in swimming speed for *E. affinis* were strong trends. The food treatment also led to increased proportional residence time for adults of each species tested (Fig. 2D; *C. finmarchicus*, difference = 0.248, $MSD_{0.05,99,5} = 0.116$; *C. pacificus*, difference = 0.296, $MSD_{0.05,99,5} = 0.155$; *E. affinis*, df = 99, F = 61.56, P < 0.001; other species reported by Woodson et al. 2007). Increases in proportional residence time in the food-treatment region, however,

Table 6 Behavioral responses (swimming speed and frequency of turning) of various species of copepod to the presence of chemical-exudates ($C = 253\ \mu g\ C\ l^{-1}$)

Species		Swimming speed [mm s^{-1}] (SE)				Frequency of turning [turns $ind^{-1}\ s^{-1}$] (SE)		
Adult copepods	***n***	**Precontact**	**Postcontact**	***P***	***n***	**Precontact**	**Postcontact**	***P***
Calanus finmarchicus	16	2.58 (0.39)	4.19 (0.54)	0.029*	13	0.02 (0.01)	0.09 (0.03)	0.043*
Calanus pacificus	16	4.71 (0.61)	6.68 (0.76)	0.056	15	0.06 (0.02)	0.16 (0.03)	0.053
Eurytemora affinis	33	4.87 (0.20)	5.56 (0.33)	0.093	28	0.05 (0.02)	0.16 (0.04)	0.007*
Metridia pacifica	15	2.25 (0.66)	4.40 (0.76)	0.048*	15	0.06 (0.02)	0.15 (0.03)	0.030*
Nauplii								
Temora longicornis	31	3.07 (0.71)	2.89 (0.68)	0.499	39	0.09 (0.03)	0.13 (0.03)	0.369

*Denotes *P*-values <0.05 between precontact and postcontact values by repeated measures ANOVA with a *posthoc* correction for multiple tests. Corresponding data for adults of *Acartia tonsa* and *Temora longicornis* are reported by Woodson et al. (2007).

Table 7 Behavioral responses (swimming speed and frequency of turning) of various species of copepod to the presence of food (e.g., phytoplankton, *Tetraselmis* sp., $C = 253\ \mu g\ C\ l^{-1}$)

Species		Swimming speed [mm s^{-1}] (SE)				Frequency of turning [turns $ind^{-1}\ s^{-1}$] (SE)		
Adult copepods	***n***	**Precontact**	**Postcontact**	***P***	***n***	**Precontact**	**Postcontact**	***P***
Calanus finmarchicus	12	5.60 (0.61)	4.28 (0.57)	0.136	13	0.08 (0.04)	0.16 (0.03)	0.134
Calanus pacificus	15	5.70 (0.49)	4.41 (0.59)	0.118	15	0.05 (0.02)	0.11 (0.03)	0.117
Eurytemora affinis	33	3.89 (0.18)	2.97 (0.26)	0.008*	24	0.07 (0.02)	0.13 (0.04)	0.229
Nauplii								
Temora longicornis	33	3.08(0.34)	2.37 (0.13)	0.049*	33	0.05 (0.01)	0.11 (0.02)	0.034*

*Denotes *P*-values <0.05 between precontact and postcontact values by repeated measures ANOVA with a *posthoc* correction for multiple tests. Corresponding data for adults of *Acartia tonsa* and *Temora longicornis* are reported by Woodson et al. (2007).

Table 8 *Eurytemora affinis* responses to combined layers

Factor	df	SS	MS	F	P
Total	799				
Velocity (*V*)	1	0.004	0.004	0.169	0.682
Density (*D*)	1	1.747	1.747	69.039	<0.001**
Chemical (*C*)	1	3.147	3.147	124.370	<0.001**
Velocity × density	1	0.002	0.002	0.084	0.773
Velocity × chemical	1	0.029	0.029	1.144	0.285
Density × chemical	1	1.381	1.381	54.55	<0.001**
Velocity × density × chemical	1	0.010	0.010	0.383	0.536
Error	792	20.046	0.025		

Full-factorial ANOVA with three main factors (velocity, density, and chemical exudates) and interactions with proportional residence time as the dependent factor. Each main factor had two fixed levels in the analysis (present or not present). **Indicates significance at $P < 0.001$.

were a result of lower swimming speeds associated with increased feeding behavior for adults of each species tested (Table 7). Note in this case that only one significant difference was observed.

Eurytemora affinis significantly increased proportional residence time in all isolated and combined treatments except the isolated velocity-gradient treatment (Fig. 3). Responses to the isolated density-change and chemical-exudates treatments were significant. A significant interaction occurred between the density-change and chemical-exudates treatments indicating that the simultaneous presentation of both cues produced a different effect than did either one presented individually (i.e., effects were not additive, Table 8). Behavioral changes in response to the combined treatments were

qualitatively similar to the responses to the isolated density-change and chemical-exudate treatments and included increased swimming speed and frequency of turning (Table 9). Further, a treatment consisting of the density-change region embedded in a homogenous concentration of exudates ($253\,\mu g\,C\,l^{-1}$) revealed significant differences in PRT compared to controls (PRT = 0.529 for treatment compared to PRT = 0.374 for control, df = 99, $F = 12.53$, $P = 0.007$). The swimming speed and frequency of turning increased significantly compared to the precontact value for that treatment (Table 9).

Naupliar stages of *A. tonsa*, *C. finmarchicus*, and *T. longicornis* did not significantly alter proportional residence time in response to any of the treatment constituents, as tested by single-factor ANOVA (Fig. 4; *A. tonsa* nauplii, df = 149, $F = 1.53$, $P = 0.221$; *C. finmarchicus* nauplii, df = 59, $F = 0.88$, $P = 0.421$; *T. longicornis* nauplii, df = 237, $F = 0.723$, $P = 0.577$). Path kinematic parameters, however, did show changes between precontact and postcontact with the treatment region in a few situations. *Temora longicornis* nauplii significantly increased swimming speed after entering the velocity-gradient treatment region, but did not increase their frequency of turning (Table 3). Other species in naupliar stages did not respond to the velocity-gradient treatment according to the path-kinematic parameters employed here. *Acartia tonsa* and *T. longicornis* nauplii altered frequency of turning in the density-gradient treatment (significant and strong trend, respectively), which appears to be a behavioral response of not crossing the density-gradient region (Tables 4 and 5). Nauplii of *C. finmarchicus* showed a similar aversion to crossing the density-change region, but this trend fell short of statistical significance (Table 5). *Temora longicornis* nauplii did not significantly alter their behavior in response to the chemical exudate treatment (Table 6); however, nauplii of this species did respond to the food treatment by decreasing swimming speed and increasing frequency of turning (Table 7).

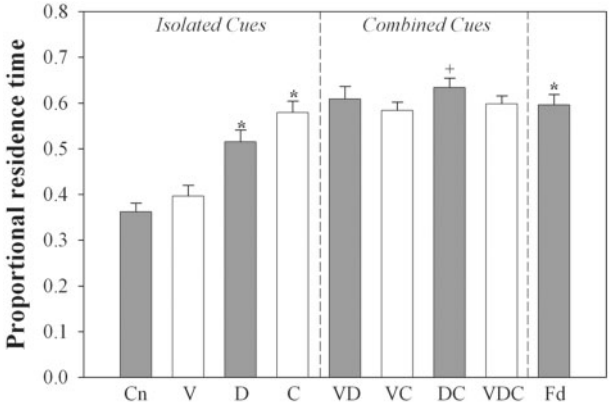

Fig. 3 Proportional residence time for *Eurytemora affinis* in the presence of individual and combined cues. Treatments are defined as: Cn = none; V = velocity gradient only; D = density change only; C = chemical exudates only; VD = velocity gradient and density change; VC = velocity gradient and chemical exudates; DC = density change and chemical exudates; VDC = velocity gradient, density change, and chemical exudates; and Fd = phytoplankton only. *Indicates a significant effect of single treatments relative to the control as determined by a multi-way ANOVA ($P < 0.05$). For combined layers, +indicates significant interactive effects between the constituents of the layer.

Modeling and simulation

We arbitrarily selected adults of *A. tonsa* as the model organism for the simulation, and it should be noted that the other species were qualitatively similar in their response to various treatments, but with quantitative differences due to variation in the data on path kinematics reported in Tables 3–7. Model input parameters for the swimming speed and frequency of turning for adults of *A. tonsa* are shown in Table 2. Individual calibration runs with differing initial (random) distributions showed minimal variation in PRT results (e.g., for $\gamma = 0.20$, PRT = 0.453 ± 0.0005). Mean and standard error of PRT in the calibration simulations converged after

Table 9 *Eurytemora affinis* responses to combined layers

		Swimming speed [mm s^{-1}] (SE)			Frequency of turning [turns $ind^{-1}\,s^{-1}$] (SE)		
Layer	*n*	Precontact	Postcontact	*P*	Precontact	Postcontact	*P*
Velocity–Density	34	3.42 (0.23)	4.01(0.13)	0.017*	0.07 (0.02)	0.11 (0.05)	0.230
Velocity–Chemical	41	3.66 (0.31)	4.43 (0.40)	0.024*	0.05 (0.02)	0.13 (0.03)	0.168
Density–Chemical	38	3.98 (0.62)	4.64 (0.71)	0.028*	0.06 (0.03)	0.17 (0.02)	0.030*
Velocity–Density–Chemical	31	3.32 (0.28)	3.88 (0.16)	0.048*	0.06 (0.02)	0.18 (0.06)	0.024*
Density–All Chemical	28	3.54 (0.33)	4.12 (0.24)	0.049*	0.07 (0.03)	0.15 (0.03)	0.037*

Swimming speed and frequency of turning data for the combined layer treatments. Note that swimming speed and frequency of turning data for the isolated cue treatments are shown in previous tables. *Indicates significance at $P < 0.05$.

approximately 30 runs for all treatments. Proportional residence time in the laboratory-scale simulations was insensitive to changes in input swimming speed and frequency of turning; PRT varied less than 0.02, when the mean values of path kinematics varied by up to ± 2 standard errors. Altering the mean values did, however, influence the rate at which model organisms appeared to aggregate in the treatment region. No significant interactions occurred between swimming speed and frequency of turning. Overall, the results of the calibration for this simplified model were considered robust, and sensitivity to input parameters was not considered further.

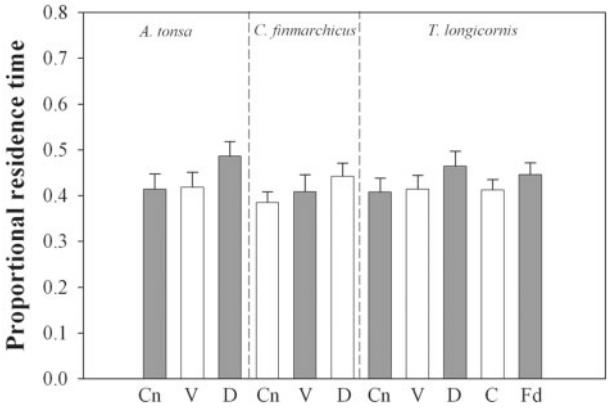

Fig. 4 Proportional residence time for nauplii in the presence of individual cues. Layer treatments are defined as: Cn = none; V = velocity gradient only; D = density change only; C = chemical exudates only; and Fd = phytoplankton only. No significant differences were observed.

Variation of PRT as a function of γ for the laboratory-scale calibration simulations is shown in Fig. 5A (average of all 50 calibration runs). The response for the chemical-exudates treatment was quantitatively similar to the response to the velocity-gradient treatment and hence is not shown for either laboratory-scale or field-scale simulations. The general trend is that increasing the capacity of the simulated individual to respond to physical or chemical features (increasing response threshold) resulted in an increasing tendency to occupy the treatment region. For small values of γ ($\gamma < 0.1$), the proportional residence time reflects random movement in the domain with proportional residence time near 0.39 (the ratio of the area of the treatment region to the area of the model domain). The proportional residence time increases steeply over a middle range of γ ($0.2 < \gamma < 0.7$). The PRT at large values of γ ($\gamma > 0.7$) is dependent on the environmental cue, due to differences in the input parameters for swimming speed and frequency of turning (Table 2). The differences in PRT at large γ between treatments (and consequently input variables) suggest that the fine details of an organism's behavior can produce widely different results both in the laboratory and in the field.

A calibration response threshold of $\gamma = 0.57$ in Fig. 5A best matched the results of the laboratory experiments for *A. tonsa* exposed to an isolated velocity-gradient treatment (PRT = 0.64). Similarly, response thresholds of $\gamma = 0.37$ and $\gamma = 0.31$ matched the measured PRTs for the density-gradient treatment and food treatment, respectively, for *A. tonsa*. Values of γ estimated from the calibration simulations were used subsequently for the

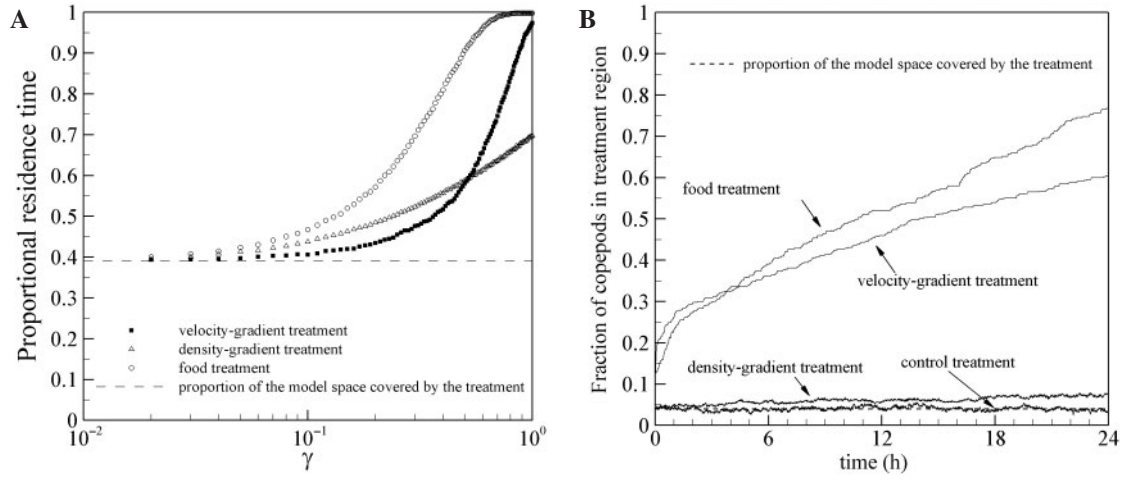

Fig. 5 Population of adults of *Acartia tonsa* in the velocity-gradient, density-change, and food treatments based on the model simulation. (**A**) Model calibration curve showing PRT as a function of the response threshold (γ) for simulations at laboratory scale, and (**B**) 24 h model simulations at field scale showing the fraction of the total simulated population located in the treatment region at each time step.

simulation corresponding to larger spatial and temporal scales.

Simulations for scales in time and length relevant to field conditions ($25 \times 5\,m^2$ field, 24 h period, 1 m thick treatment region, and 1 s time step) demonstrated that aggregations can occur as a result of fine-scale behavioral responses to environmental structure. Figure 5B shows the increase in number of *A. tonsa* individuals within the treatment region over a 24 h period. The initial fractions of copepods in the treatment region were equivalent to a random distribution in the field (i.e., 0.04, which equals the proportion of the model space covered by the treatment region). The control treatment (i.e., no fine-scale response in the treatment region) remains roughly equal to 0.04 over the 24 h period. For the velocity-gradient and food treatments, the fraction of copepods in the treatment region was roughly nine to ten times greater within 6 h. Food treatments led to the densest aggregation in the modeling study, followed by associational cues (e.g., velocity-gradient and chemical-exudates treatments), which was similar to results observed in the laboratory experiments. In contrast, the population for the density-change treatment did not increase substantially within the treatment region compared to the velocity-gradient and food treatments.

Discussion

Responses to cues associated with oceanographic gradients and biological patchiness

Adults of all species of copepods in this study responded to one, but not both, of the physical-gradient (velocity or density) treatments by increasing proportional residence time in the treatment region, swimming speed, and frequency of turning. Most of the tested copepod species, except for *E. affinis*, responded to the velocity-gradient treatment. Behavioral responses to the velocity-gradient treatment may be inherently linked to the mechanisms of patch formation in the coastal ocean, where frontal movements and shearing create distinct patches of phytoplankton within the water column (Epstein and Beardsley 2001; Stacey et al. 2007). Velocity gradients may provide more prevalent cues that are independent of patch thickness or food concentration, and therefore may provide a general cue for the presence of resource-rich zones (Woodson et al. 2007). For the species that responded to a velocity-gradient treatment, the response to a density-gradient treatment was quite different. All tested species, except *E. affinis*, showed an aversion to crossing between bodies of water with disparate densities (equivalent to ~2 ppt salinity change) (Harder 1968) and did not significantly change swimming speed or frequency of turning in response to the cue. In contrast with the behavior of the other species, *E. affinis* often crossed the boundary defined by a change in fluid density and further increased proportional residence time and changed swimming patterns in response to a gradient in density (salinity).

Species of predominantly predatory copepods (*C. ethiopica*, and *Labidocera madurae*) responded to the velocity-gradient treatment in a fashion similar to responses by predominantly herbivorous species (Woodson et al. 2005). These behaviors suggest that predators also may capitalize on the associations between biological activity and physical gradients in the ocean. Phytoplankton patchiness associated with layered bodies of water can lead to aggregations of primary consumers. Based on observations of similar behaviors by larger predatory species, it appears that the next level of the trophic web may also employ cues associated with physical properties of the water column. Thus, regions of strong oceanic gradients (fronts and clines) may be areas where material and energy are transferred across a wide range of trophic levels or functional groups (Healey et al. 1990).

Woodson et al. (2007) observed that *T. longicornis* and *A. tonsa* respond to a hierarchy of cues starting with excited, area-restricted search behavior upon encountering a velocity gradient, which leads to an increase in the time spent in a region of interest. The excited, area-restricted search response is prolonged if concentrations of chemical exudates (primary and secondary metabolites) within the patch are above threshold levels. Finally, physical contact with food particles initiates a feeding response (e.g., decreased swimming speeds) through either mechanosensory or contact chemosensory pathways. Most species tested in the present study appeared to respond in a manner similar to the cue hierarchy established for *T. longicornis* and *A. tonsa*. As in our previous observations of *T. longicornis* and *A. tonsa*, our current experiments show that (1) the velocity-gradient and chemical-exudates treatments elicited increased copepod swimming speed and higher frequency of turning, (2) animals slowed down when they encounter food cells, and (3) gradients in the density of the water present a physical barrier that copepods generally did not cross. However, conclusions about individual species await final verification because a firm declaration of hierarchy requires determining the responses to combined cues in addition to isolated ones (Woodson et al. 2007). Other mechanisms, such as habitat partitioning and

maintenance of position may also lead to the observed responses (Mackas et al. 1993; Seuront 2006). In addition, responses to temperature gradients were not examined and may be important cues in many habitats (e.g., upwelling systems; McManus et al. 2005). For example, *E. affinis* is known to employ salinity gradients to maintain position within productive regions in estuaries (Seuront 2006). Other species may employ similar behaviors in response to temperature in regions where salinity is relatively constant and temperature variations drive density gradients. The present results do, however, show that fine-scale oceanographic gradients provide ecologically-relevant information that can be exploited by a variety of copepods, although environmental conditions that elicit behavioral changes may vary among species, developmental stages, and physiological states.

Cue hierarchy and *E. affinis*

The general pattern of the behavioral response by *E. affinis* is analogous to the previously reported hierarchy of cues for *T. longicornis* and *A. tonsa*, as indicated by the PRT analysis. Hierarchical responses to environmental cues in foraging strategies of *T. longicornis* and *A. tonsa* start with area-restricted search responses (increased PRT, swimming speed, and frequency of turning) to velocity gradients and culminate in feeding responses once food patches are located. When exposed to a velocity-gradient treatment embedded in a homogeneous chemical-exudates field, these species did not respond to the physical gradient, suggesting that velocity gradients, chemical exudates, and food are ordered in a hierarchical search strategy (Woodson et al. 2007). The alternative hierarchy for *E. affinis*, however, starts with excited area-restricted search behavior in response to a density-gradient cue rather than to a velocity-gradient cue. Unlike *T. longicornis* and *A. tonsa*, *E. affinis* consistently responded to density gradients by increasing swimming speed regardless of the presence of other cues. *Eurytemora affinis* also consistently turned more frequently in all treatments that included chemical cues. These behaviors appeared to be a fundamental shift in the form of the behavioral hierarchy. However, experiments designed to determine the hierarchy of cues used by *E. affinis* were not conclusive because this species still responded to a density gradient cue in the presence of a homogenous concentration of chemical exudate.

These results suggest that *E. affinis* may use density gradients in a cue hierarchy, and that density gradients also may be employed as cues in other life processes. *Eurytemora affinis* is a predominately estuarine copepod, where salinity changes can be immense due to inputs of freshwater from rivers and changes in salinity due to tidal motions (Lee 1999). *Eurytemora affinis* also prefers turbid, mixed regions in estuaries that are characterized by high primary productivity and by strong gradients in salinity and has been shown to modify behavior in the presence of salinity gradients (Seuront 2006). Salinity gradients in estuarine habitats may provide cues more closely associated with prey items, or velocity gradients may not be as reliable due to freshwater inputs, the stronger influence of currents associated with tides, or the lack of well-defined velocity structure in the water column. Alternatively, density gradients may be associated with other non-foraging, but potentially aggregating behaviors such as position maintenance within preferred habitats (Seuront 2006). In a non-foraging context, the presence of homogeneous chemical exudates would not override the response to a density-gradient cue, which is, in fact, the behavior that we observed (Table 9). Thus, linkages in estuaries between strong salinity and prey availability may drive behavioral patterns in *E. affinis*, in much the same fashion as velocity gradients do in other species (Seuront 2006; Stacey et al. 2007; Woodson et al. 2007).

Nauplii and oceanographic structure

Naupliar stages of the three species examined in this study showed no increase in PRT in response to the velocity-gradient treatment. The increased swimming speeds observed for *T. longicornis* nauplii may result from an active response, but PRT did not increase. Naupliar stages, like adults, preferred not to cross a discontinuity in density and therefore remained in the original body of water. Chemical-exudate treatments also did not appear to elicit intense search behavior in nauplii of *T. longicornis*. Some began feeding in the presence of food cells, although the population response was still relatively weak.

Many species of calanoid copepods do not begin feeding until reaching the third naupliar stage (NIII; Landry 1983). The lack of responses to cues that are important for foraging adults of the same species suggests that the use of proximal cues during foraging may be ontogenetic and develops in late-stage nauplii or in copepodid stages, or that nauplii have higher thresholds for response (Titelman 2001). Higher thresholds for responses by nauplii to exudates and food are not likely, since our experiments were conducted at the upper range of

field observations. Thus, ontogenetic changes in foraging behavior appear to be the most parsimonious explanation for our observations. Regardless of the mechanism, the existence of life-stage-specific (and species-specific) responses to environmental cues suggests that the composition of aggregations around patches of the ocean defined by gradients in physical properties or biological activity will vary as a function of the available pool of zooplankton.

Consequences and limitations of modeling results

Fine-scale observations in well-defined laboratory experiments guided by *in situ* oceanographic structure have revealed intricate behaviors in foraging copepods. However, the question remains whether fine-scale behaviors expressed in the laboratory are of importance in the field because the laboratory experiments are isolated and controlled and cannot replicate the temporal and spatial scales of the field. Thus, modeling techniques provide a useful tool for assessing how the results of controlled experiments may translate to observations of organisms *in situ* (Leising and Franks 2002). The simulations in this study suggest that intricate foraging behaviors involving multiple cues can potentially lead to observed *in situ* aggregations (Holliday et al. 1998; McManus et al. 2005). Specifically, the change in swimming kinematics in response to the presence of velocity gradients, chemical exudates, and food can lead to aggregation in the field.

Caution should be taken when interpreting these results for several reasons. The laboratory studies were limited due to the small field of view necessary for high-resolution imaging. Consequently, the duration of responses to various treatments was not determined, although the model addressed this issue by employing a response indicator design that was evaluated at each time step. A few additional considerations about the observed behaviors are important for assessing the potential contribution of laboratory data to patterns observed in the field. (1) Observed fine-scale responses to velocity gradients in the context of foraging may not lead to *in situ* aggregations if behaviors are relatively short-lived, e.g., because the high energetic expenditures associated with increased activity (e.g., swimming speed and turning) cannot be sustained. Even if behavior within a cue region decays over time, however, the resulting potential for aggregation may not be affected if organisms regain behavioral responsiveness quickly upon exiting the layer. Given that our present assumptions show a strong potential for individual behaviors to produce aggregations, one benefit of our model, even in this simple form, is to suggest the importance of longer-term experiments or *in situ* studies of copepod behavior at these scales. (2) Copepods that employ velocity gradients in a cue hierarchy may leave a velocity-gradient region or chemical-exudates region in the absence (or at concentrations below feeding thresholds) of food after a brief search period. However, continued responses induced by chemical exudates and the presence of food should lead to dense, persistent aggregations. (3) If, on the other hand, responses are associated with maintenance of position (Genin et al. 2005; Seuront 2006), such behaviors also may lead to aggregation. Further experiments to examine changes in energetic expenditure, the duration of responses associated with foraging, and the objective of certain behaviors for individual species at fine scales will be necessary to quantify the large-scale effects of the observed behaviors.

The simple model simulations were conducted without considering other behaviors such as diel vertical migration. Periodic movements through the water column will likely shorten the time it takes to form an aggregation by insuring that most individuals contact a particular cue region or patch. For instance, imposing diel vertical migration on the model population will insure that each individual contacts the treatment region located at 8 m depth, whereas in the present simulations, there is no guarantee that a model organism will contact such a region. The simulations of foraging also possess other simplifications, such as the lack of physical drivers (currents and turbulence) and the absence of periodic and conspecific behavioral responses. Many of these factors are likely to influence the response of organisms during conditions that support fine-scale oceanographic gradients and biological patchiness (i.e., low to moderate turbulence and high stratification) (McManus et al. 2003, 2005). For example, if turbulence intensity is low, then most zooplankton will be able to actively aggregate and counteract vertical velocities associated turbulent mixing (Mackas et al. 1993; Gallager et al. 2004; Yen et al. 2007).

Conclusions

The results of this study indicate that at field scales behavior may have extensive effects on predator–prey dynamics and distributional patterns in the ocean. *In situ* observations of aggregations of zooplankton support these claims (Mackas and Louttit 1988; Holliday et al. 1998; Gallager et al. 2004;

McManus et al. 2005). These and other results from field, laboratory, and modeling studies suggest that the smaller members of marine communities are dynamic, responsive players at multiple scales. Consequently, exciting new directions in biological–physical coupling are emerging in which the behaviors of zooplankton are intricately linked at both ecological and evolutionary scales to the physical mechanisms driving patchiness of prey. Do higher trophic levels employ similar cues that lead them to well-defined regions of intense biological activity? Field observations of zooplankton aggregations at oceanographic gradients and biological patchiness suggest that this is possible, or even likely, with increased biological activity ranging from primary production to fishes to mammals, including fishermen, concentrated near oceanographic gradients (Healey et al. 1990; Genin 2004).

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