

# Copepod escape behavior in non-turbulent and turbulent hydrodynamic regimes

Rebecca J. Waggett<sup>1,2,\*</sup>, Edward J. Buskey<sup>1</sup>

<sup>1</sup>The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, Texas 78373-1267, USA

<sup>2</sup>Present address: NOS, NOAA, 101 Pivers Island Road, Beaufort, North Carolina 28516-9722, USA

**ABSTRACT:** Copepods respond to velocity gradients in the ambient fluid generated by the movement of nearby predators. Escape behavior of several species in response to hydrodynamic stimuli has been analyzed under non-turbulent conditions; however, copepods normally experience a flowing or turbulent environment. Two neritic species (*Paracalanus parvus* and *Temora turbinata*) were exposed to a siphon-generated flow field under both non-turbulent and turbulent conditions. Deformation rates of 6.16 and 3.93 s<sup>-1</sup> were required to elicit escape behavior in *P. parvus* and *T. turbinata*, respectively. Copepod jump distances in response to the siphon-generated flow field were >6.8 mm and were not significantly different under non-turbulent and turbulent conditions.

**KEY WORDS:** Copepod · Escape reaction · Flow-field · Hydrodynamic stimuli · Turbulence

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

Copepods use mechanoreception to detect hydrodynamic disturbances generated by approaching predators and employ rapid escape responses to avoid capture. Hydrodynamic disturbances are detected by innervated setae on the first antennae (Yen & Nicoll 1990, Yen et al. 1992, Bundy & Paffenhöfer 1993, Lenz et al. 1996, Weatherby & Lenz 2000). Velocity gradients adjacent to the copepod bend the setae. When sufficient bending occurs, neurophysiological signals are generated through the depolarization of innervating neurons (Gassie et al. 1993, Lenz & Yen 1993, Lenz et al. 2000, Fields et al. 2002). The resulting nerve impulse triggers a motor response in the form of an escape reaction, during which a copepod propels itself forward at speeds >100 body lengths (bl) s<sup>-1</sup>, with accelerations often exceeding 100 m s<sup>-2</sup> (Strickler 1975, Buskey et al. 2002).

Several experiments have been performed and theoretical models developed to identify the specific component of fluid flow that is responsible for triggering copepod escape behavior (Fields & Yen 1996, 1997,

Viitasalo et al. 1998, Kiørboe & Visser 1999, Kiørboe et al. 1999). Fields & Yen (1996) were able to spatially separate the components of the siphon flow field (speed, acceleration, and shear). Their results suggested that changes in fluid shear trigger copepod escape behavior. It was later suggested that the conclusions of Fields & Yen (1996, 1997) were situation-specific and that deformation rate, rather than fluid shear, serves as the primary trigger for copepod escape reactions and provides a more general measure for escape reaction locations (Viitasalo et al. 1998, Kiørboe & Visser 1999, Kiørboe et al. 1999).

Components of copepod feeding and escape behavior may be affected by the presence of small-scale turbulence (Costello et al. 1990, Marrasé et al. 1990, Saiz & Alcaraz 1992, Hwang & Strickler 1994, Clarke et al. 2005). The onset of turbulence may first stimulate a high rate of escape reactions and increased feeding activity (Costello et al. 1990, Saiz & Alcaraz 1992, Hwang & Strickler 1994), but copepods slowly habituate to the turbulent environment (Hwang & Strickler 1994). Clarke et al. (2005) reports that turbulence increases the capture success of spinyhead blenny

\*Email: rebecca.waggett@noaa.gov

*Acantheblemaria spinosa* feeding on *Acartia tonsa*, further suggesting that turbulence interferes with the mechanoreceptive abilities of copepods.

In the present study, the sensitivity and escape behavior of 2 wild-caught, co-occurring species was evaluated under non-turbulent and turbulent hydrodynamic conditions. Copepods were exposed to a siphon-generated flow, and the copepods' response under non-turbulent conditions was used as a baseline for comparing their sensitivities.

## MATERIALS AND METHODS

Copepods were collected from The University of Texas Marine Science Institute pier (27° 50.3' N, 97° 03.1' W) by holding a 0.25 m diameter 153  $\mu\text{m}$  mesh plankton net within the tidal current for approximately 10 min. The contents of the cod end were diluted into a bucket of whole seawater and returned to the laboratory for sorting. Adult copepods of the species *Paracalanus parvus* and *Temora turbinata* (0.66 and 0.74 mm prosome length, respectively) were sorted from the plankton under a dissecting scope using a wide bore pipette. Individual copepods were examined at 50 $\times$  magnification to ensure that only healthy copepods with intact first antennae were chosen for experimentation. Copepods were separated and isolated in 50 ml beakers filled with 0.2  $\mu\text{m}$  porosity filtered seawater.

A 9.1  $\times$  5.0  $\times$  9.7 cm clear acrylic plastic chamber filled to a height of 7.5 cm with 0.2  $\mu\text{m}$  porosity filtered seawater was used for experiments with non-turbulent conditions while a larger 20.5  $\times$  10.0  $\times$  15.0 cm clear plastic chamber containing a turbulence-generating apparatus was substituted to generate turbulent conditions (Gilbert & Buskey 2005). A detailed description and figure of the turbulence chamber can be found in Gilbert & Buskey (2005). Briefly, a small motor powered the oscillation of a 1.0  $\times$  6.0  $\times$  5.5 cm plastic grid with 1.1  $\text{cm}^2$  openings positioned vertically inside the chamber. The grid oscillated between 6 and 8 cm from the siphon tip, at a frequency of 0.8 Hz. The steady state root-mean-square (rms) turbulent velocity was calculated to be 0.08  $\text{cm s}^{-1}$ , using the equations from Hopfinger & Toly (1978).

The siphon was made by heating the middle region of a pipette tip (0.07 cm inner diameter) and bending it to a 90° angle. Tubing connected to the pipette allowed continuous siphoning of water from the chamber into a beaker filled with 800 ml of filtered seawater. To maintain a constant water level and head pressure, a peristaltic pump (Masterflex Model 7553-30) returned this water to the system via flexible tubing inserted into a 2.0 cm diameter PVC pipe with 20  $\mu\text{m}$  mesh attached

to the submerged end. The strength of the flow field remained constant throughout the experiments. A Lasiris, laser sheet (Model 20501003), with a wavelength of 670 nm and power output <5 mW was aligned above the siphon to illuminate a vertical sheet parallel to and directly in front of the siphon tip (Fig. 1). Interactions during experiments and particle tracking could be viewed in a 360° region around the siphon tip.

Experiments were run with ca. 50 individuals of a single species under non-turbulent conditions and >100 copepods during turbulent conditions. Copepods acclimated to the chamber and experimental conditions (turbulence or still water) for 10 min prior to experimentation. Copepods in all experiments were exposed to the siphon flow for 30 min and their escape behavior was recorded with a Cohu camera equipped with a Nikon Nikkor 55 mm lens using standard NTSC video. Experiments were replicated multiple times to observe 25 to 50 escape reactions per species.

Low densities of neutrally buoyant particles, hydrated *Artemia* sp. cysts, were added to each experimental chamber and particle movements were recorded for 30 min. Video recordings were digitized using Motion Analysis VP-110 video-to-digital processor. ExpertVision Cell-Trak software was used to track particles and calculate their velocities. Over 50 particle tracks were analyzed in the non-turbulent regime and 90 in the turbulent regime. Position and speed data were then analyzed using Surfer® v. 8, to create contour plots of the flow speeds surrounding the mouth of the pipette in both hydrodynamic regimes.

The computerized image analysis system, Nikon Metavue®, was used to measure copepod reactive distance (the linear distance between the center of the siphon mouth and the anterior end of the copepod) from the siphon and the total distance of the escape jump. Analysis was limited to copepods swimming parallel to the siphon tip within the vertical plane illuminated by the laser sheet (Fig. 1). Only the first escape jump, consisting of 1 to several uninterrupted thrusts of their pereopods, was examined for each observed copepod. Calculations of deformation rate and signal strength were made using the assumption that the siphon apparatus created a radially symmetric flow field. The response threshold for each species was estimated as the maximum deformation rate,  $\Delta$  ( $\text{s}^{-1}$ ), along the radial axis using Eq. (2) presented by Kjørboe et al. (1999). Signal strength,  $S_\Delta$  ( $\text{cm s}^{-1}$ ), is defined as the velocity difference between a responding copepod and the surrounding fluid (Kjørboe et al. 1999). The signal strength due to fluid deformation was calculated from the deformation rate with an additional equation from Kjørboe et al. (1999).

Statistical analyses were performed with SigmaStat software (v. 3.0). Mann-Whitney rank sum tests were

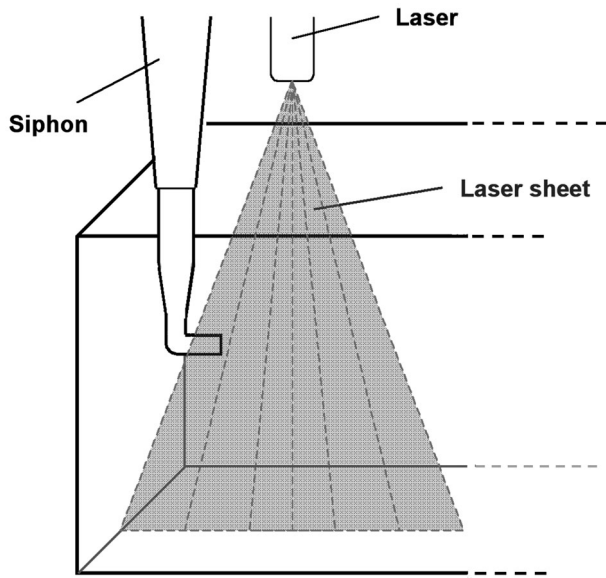


Fig. 1. Area around the siphon tip. Lasiris laser (670 nm, <math><5\text{ mW}</math> power) illuminates a 2 mm thick, 25 mm long vertical sheet in front of the siphon tip

used to compare the escape reaction components of the 2 species and to make intraspecific comparisons of the response to the siphon stimulus in the 2 hydrodynamic regimes.

## RESULTS

Volume flow,  $Q$ , from the siphon was  $0.56 \pm 0.03\text{ ml s}^{-1}$  (mean  $\pm$  SD) in both hydrodynamic regimes. The flow field generated by the siphon in the absence of turbulence was nearly symmetrical, with maximum velocities of  $30\text{ mm s}^{-1}$  at the siphon tip (Fig. 2). With the addition of turbulence, water velocity should, on average, remain constant around the siphon; however, turbulence added random fluctuations to the flow, creating several regions where velocities exceed  $5\text{ mm s}^{-1}$  at distances  $>4\text{ mm}$  from the siphon tip. These regions were not present in the non-turbulent regime.

There were no significant differences in the mean reactive distance of *Paracalanus parvus* and *Temora turbinata* (Fig. 3A, Table 1) or their mean jump distances (Table 1, Mann-Whitney rank sum test,  $p = 0.22$ ). *P. parvus* and *T. turbinata* responded to a large range of deformation rates ( $0.47$  to  $24.05$  and  $0.14$  to  $81.86\text{ s}^{-1}$ , respectively).

*Paracalanus parvus* responded significantly further from the siphon tip than *Temora turbinata* in the turbulent hydrodynamic regime (Fig. 3B, Table 1, Mann-Whitney rank sum test,  $p = 0.001$ ). Reactive distances ranged from  $0.29\text{ mm}$  (*P. parvus*) to  $7.40\text{ mm}$

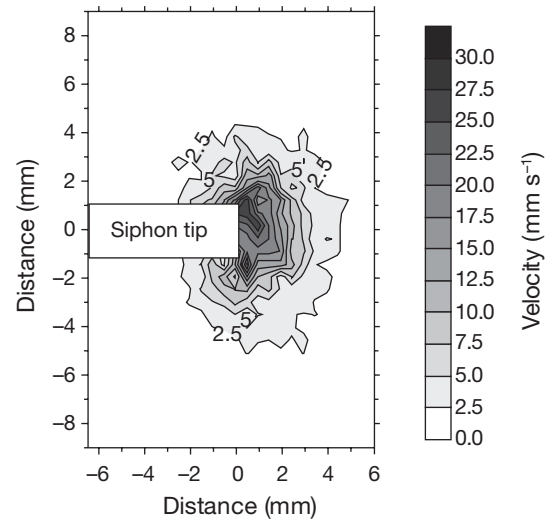


Fig. 2. Velocity profile of water surrounding the siphon tip. Increased darkening of shaded isolines represents increased fluid velocity nearing siphon tip

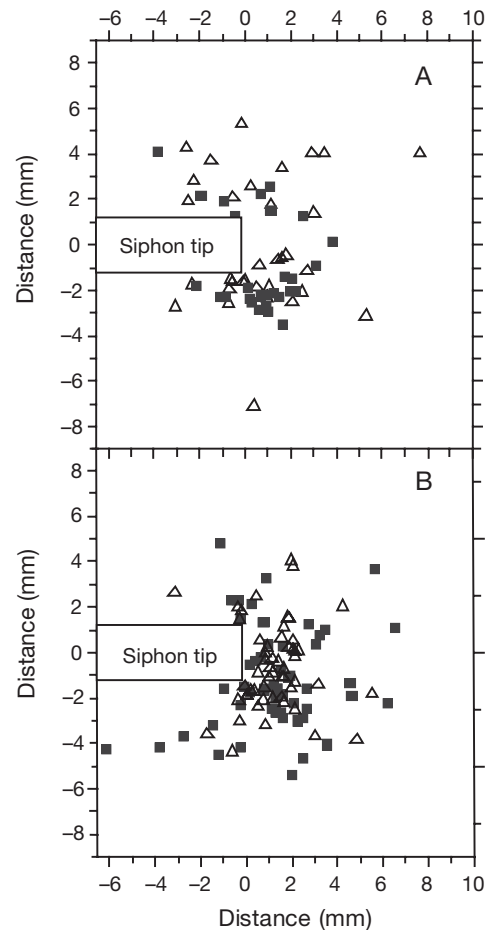


Fig. 3. *Paracalanus parvus* (■) and *Temora turbinata* (Δ). Locations where escape reactions were initiated in response to siphon-generated flow field under (A) non-turbulent and (B) turbulent water conditions

Table 1. *Paracalanus parvus* and *Temora turbinata*. Summary of behavioral characteristics of escape responses to siphon flow in non-turbulent and turbulent hydrodynamic regimes. Values are means (SE). Probability values (p) calculated using Mann-Whitney rank sum tests; \*significant difference,  $\alpha = 0.05$

Behavioral characteristic	Non-turbulent regime			Turbulent regime		
	<i>P. parvus</i> (n = 28)	<i>T. turbinata</i> (n = 34)	p	<i>P. parvus</i> (n = 53)	<i>T. turbinata</i> (n = 54)	p
Reactive distance (mm)	2.44 (0.15)	2.83 (0.29)	0.534	3.07 (0.23)	2.13 (0.17)	0.001*
Jump distance (mm)	8.29 (1.12)	6.81 (0.56)	0.220	7.51 (0.76)	7.62 (0.64)	0.577
Threshold deformation rate (s <sup>-1</sup> )	6.16	3.93	0.534	2.66	10.88	0.001*
Signal strength (mm s <sup>-1</sup> )	2.03	1.51	0.932	0.88	4.19	<0.001*

Table 2. Summary of threshold deformation rates of copepod species exposed to various hydrodynamic stimuli (modified from Kiørboe et al. 1999)

Species	Deformation rate (s <sup>-1</sup> )	Stimulus	Source
<i>Paracalanus parvus</i>	6.16	Siphon	This study
<i>Temora turbinata</i>	3.93	Siphon	This study
<i>Acartia tonsa</i>	2.02	Siphon	Gilbert & Buskey (2005)
<i>Acartia</i> spp.	2.5–12	Cylinder	Buskey et al. (2002)
<i>Temora longicornis</i>			
CI–CII	0.73	Siphon	Titelman (2001)
CII–CIV	0.71		
<i>Acartia tonsa</i>			
Female	0.46	Siphon	Kiørboe et al. (1999)
CII–CIII	0.49		
<i>Eurytemora affinis</i>	1.9	Siphon	Viitasalo et al. (1998)
<i>Temora longicornis</i>	6.5	Siphon	Viitasalo et al. (1998)
<i>Acartia tonsa</i> (adult)	0.38	Siphon	Fields & Yen (1997)
<i>Euchaeta rimana</i>	2.4	Siphon	Fields & Yen (1997)
<i>Pleuromamma xiphias</i>	4.6	Siphon	Fields & Yen (1997)
<i>Labidocera madurae</i>	6.3	Siphon	Fields & Yen (1997)
<i>Oithona</i> sp.	3.8	Siphon	Fields & Yen (1997)

(*P. parvus*). Jump distances did not differ significantly between the species (Table 1, Mann-Whitney rank sum test,  $p = 0.58$ ).

*Paracalanus parvus* had similar reactive distances to the siphon flow field in both the presence and absence of turbulence (Mann-Whitney rank sum test,  $p = 0.17$ ); however, a slight significant difference was found in the same comparison for *Temora turbinata* (Mann-Whitney rank sum test,  $p = 0.05$ ). No significant differences were found when comparing the jump distance of *P. parvus* or *T. turbinata* Mann-Whitney rank sum test,  $p = 0.70$  and  $0.62$ , respectively).

## DISCUSSION

Calculated mean threshold deformation rates in this study for *Paracalanus parvus* (6.16 s<sup>-1</sup>) and *Temora turbinata* (3.93 s<sup>-1</sup>) fell within the range of published values (present Tables 1 & 2; Fields & Yen 1997,

Viitasalo et al. 1998, Kiørboe et al. 1999, Buskey et al. 2002). Behavioral responses of adult *Acartia* spp. have been stimulated at threshold deformation rates as low as 0.38 and 0.46 s<sup>-1</sup> using a siphon configuration (Table 2, Fields & Yen 1997—as calculated by Kiørboe et al. 1999, Kiørboe et al. 1999, respectively) and at deformation rates down to 0.4 s<sup>-1</sup> to the vertical movement of a computer-controlled cylinder (Buskey et al. 2002). Deformation rates calculated for additional copepod species ranged from 0.7 s<sup>-1</sup> for *T. longicornis* copepodites (Titelman 2001) to 6.5 s<sup>-1</sup> for *T. longicornis* adults (Viitasalo et al. 1998) (Table 2). In the present study, both species responded to minimum deformation rates <0.20 s<sup>-1</sup>.

It is understandable that copepod species would vary in their response threshold, but several different values have been reported for a single species, *Acartia tonsa*. Studies by Fields & Yen (1997) and Kiørboe et al. (1999) used cultured copepods, while Buskey et al. (2002) and the present study used individuals within a few hours of collection from the wild. Cultured copepods responded to much lower deformation rates than their wild conspecifics; it is possible that wild copepods have habituated to certain levels of hydrodynamic noise or that the process of culturing has selected for more sensitive copepods.

In their natural habitats, copepods are exposed to varying levels of turbulence. The small-scale turbulence in the present study was calculated to have an rms turbulent velocity of approximately 0.08 cm s<sup>-1</sup> corresponding to dissipation rates of ca. 10<sup>-3</sup> to 10<sup>-4</sup> W m<sup>-3</sup> (Rothschild & Osborn 1988, Yamazaki & Squires 1996) that fall within the range of dissipation values reported for the oceanic mixed layer (10<sup>-3</sup> to 10<sup>-5</sup> W m<sup>-3</sup> or 0.1 to 1 mm<sup>-2</sup> s<sup>-3</sup>; Granata & Dickey 1991).

Copepods used in our experiments can be found in coastal zones, estuaries, and tidal channels. Therefore, they encounter a broad range of turbulence intensities, as high as  $10^{-2}$  W kg<sup>-1</sup> in tidal channels (Lazier & Mann 1989) to lows of  $10^{-5}$  W kg<sup>-1</sup> in surface coastal waters (Oakey & Elliott 1982). Although the addition of low levels of turbulence had no significant effect on the behavior or reactive distances of *Paracalanus parvus*, with the addition of turbulence, the deformation rate at that location would be altered. *Temora turbinata* had a smaller reactive distance in response to the siphon under turbulent conditions, suggesting that turbulence may have a greater effect on their ability to detect hydrodynamic signals such as those produced by a predator. This suggests that the addition of turbulence may reduce the sensitivity of *T. turbinata*, rendering them unable to distinguish environmental turbulence from the approach of a predator. Gilbert & Buskey (2005) exposed the copepod *Acartia tonsa* to a siphon stimulus under similar levels of turbulence as those used in the present study and found a significant decrease in *A. tonsa* sensitivity to hydrodynamic signals with the addition of turbulence. *A. tonsa* often resides in shallow bays and estuaries and commonly experiences higher turbulence intensities than *P. parvus* and *T. turbinata*. The escape behavior of *A. tonsa* to the siphon flow was affected more strongly by the addition of turbulence (Gilbert & Buskey 2005). These combined findings suggest that copepod escape behavior can be significantly affected by the introduction of natural levels of small-scale turbulence.

**Acknowledgements.** We thank P. H. Lenz, L. A. Fuiman, G. A. Holt and P. A. Montagna for critical review of the original manuscript. Funding for this research was provided by the National Science Foundation through Grant OCE-9910608, The University of Texas Marine Science Institute E. J. Lund Fellowship and the Laura Brooks Flawn, M.D. Endowment Fund. This is The University of Texas Marine Science Institute contribution number 1408.

#### LITERATURE CITED

- Bundy MH, Paffenhöfer GA (1993) Innervation of copepod antennules investigated using laser scanning confocal microscopy. *Mar Ecol Prog Ser* 102:1–14
- Buskey EJ, Lenz PH, Hartline DK (2002) Escape behavior of planktonic copepods in response to hydrodynamic disturbances: high speed video analysis. *Mar Ecol Prog Ser* 235: 135–146
- Clarke RD, Buskey EJ, Marsden KC (2005) Effects of water motion and prey behavior on zooplankton capture by two coral reef fishes. *Mar Biol* 146:1145–1155
- Costello JH, Strickler JR, Marrasé C, Trager G, Zeller R, Freise AJ (1990) Grazing in a turbulent environment: behavioral response of a calanoid copepod, *Centropages hamatus*. *Proc Natl Acad Sci USA* 87:1648–1652
- Fields DM, Yen J (1996) The escape behavior of *Pleuromamma xiphias* in response to a quantifiable fluid mechanical disturbance. In: Lenz P, Hartline D, Purcell J, Macmillan D (eds) *Zooplankton: sensory ecology and physiology*. Gordon & Breach, Amsterdam, p 323–339
- Fields DM, Yen J (1997) The escape behavior of marine copepods in response to a quantifiable fluid mechanical disturbance. *J Plankton Res* 19:1289–1304
- Fields DM, Shaeffer DS, Weissburg MJ (2002) Mechanical and neural responses from the mechanosensory hairs on the antennule of *Gaussia princeps*. *Mar Ecol Prog Ser* 227: 173–186
- Gassie DV, Lenz PH, Yen J, Hartline DK (1993) Mechano-reception in zooplankton first antennae: electrophysiological techniques. *Bull Mar Sci* 53:96–105
- Gilbert OM, Buskey EJ (2005) Turbulence decreases the hydrodynamic predator sensing ability of the calanoid copepod *Acartia tonsa*. *J Plankton Res* 27:1067–1071
- Granata TC, Dickey TD (1991) The fluid mechanics of copepod feeding in a turbulent flow: a theoretical approach. *Prog Oceanogr* 26(S3-A):243–261
- Hopfinger EJ, Toly JA (1978) Spatially decaying turbulence and its relation to mixing across density interfaces. *J Fluid Mech* 78:155–175
- Hwang JS, Strickler JR (1994) Effects of periodic turbulent events upon escape responses of a calanoid copepod, *Centropages hamatus*. *Bull Plankton Soc Jpn* 41:117–130
- Kjørboe T, Visser AW (1999) Predator and prey perception in copepods due to hydromechanical signals. *Mar Ecol Prog Ser* 179:81–95
- Kjørboe T, Saiz E, Visser AW (1999) Hydrodynamic signal perception in the copepod *Acartia tonsa*. *Mar Ecol Prog Ser* 179:97–111
- Lazier JRN, Mann KH (1989) Turbulence and the diffusive layers around small organisms. *Deep-Sea Res* 36: 1721–1733
- Lenz PH, Yen J (1993) Distal setal mechanoreceptors of the first antennae of marine copepods. *Bull Mar Sci* 53: 170–179
- Lenz PH, Weatherby TM, Weber W, Wong KK (1996) Sensory specialization along the first antenna of a calanoid copepod, *Pleuromamma xiphias* (Crustacea). In: Lenz P, Hartline D, Purcell J, Macmillan D (eds) *Zooplankton: sensory ecology and physiology*. Gordon & Breach, Amsterdam, p 355–363
- Lenz PH, Hartline DK, Davis AD (2000) The need for speed. I. Fast reactions and myelinated axons in copepods. *J Comp Physiol A* 186:337–345
- Marrasé C, Costello JH, Granata T, Strickler JR (1990) Grazing in a turbulent environment: energy dissipation, encounter rates, and efficacy of feeding currents in *Centropages hamatus*. *Proc Natl Acad Sci USA* 87: 1653–1657
- Oakey NS, Elliott JA (1982) Dissipation within the surface mixed layer. *J Phys Oceanogr* 12:171–185
- Rothschild BJ, Osborn TR (1988) Small-scale turbulence and plankton contact rates. *J Plankton Res* 10:465–474
- Saiz E, Alcaraz M (1992) Free-swimming behavior of *Acartia clausi* (Copepoda: Calanoida) under turbulent water movement. *Mar Ecol Prog Ser* 80:229–236
- Strickler JR (1975) Swimming of planktonic *Cyclops* species (Copepods, Crustacea): pattern, movements and their control. In: Wu TT, Brokaw C, Brennan C (eds) *Swimming and flying in nature*. Plenum Press, New York, p 599–613
- Titelman J (2001) Swimming and escape behavior of copepod nauplii: implications for predator-prey interactions among copepods. *Mar Ecol Prog Ser* 213:203–213
- Viitasalo M, Kjørboe T, Flinkman J, Pedersen L, Visser A (1998) Predation vulnerability of planktonic copepods:

consequences of predator foraging strategies and prey sensory abilities. *Mar Ecol Prog Ser* 175:129–142

Weatherby TM, Lenz PH (2000) Mechanoreceptors in calanoid copepods: designed for high sensitivity. *Arthropod Structure Development* 29:275–288

Yamazaki H, Squires KD (1996) Comparison of oceanic turbulence and copepod swimming. *Mar Ecol Prog Ser* 144:299–301

*Editorial responsibility: Howard Browman (Associate Editor-in-Chief), Storebø, Norway*

Yen J, Nicoll NT (1990) Setal array on the first antennae of a carnivorous marine copepod, *Euchaeta norvegica*. *J Crustac Biol* 10:218–224

Yen J, Lenz PH, Gassie DV, Hartline DK (1992) Mechano-reception in marine copepods: electrophysiological studies on the first antennae. *J Plankton Res* 14: 495–512

*Submitted: January 21, 2006; Accepted: May 11, 2006*  
*Proofs received from author(s): February 28, 2007*