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Swimming and escape behavior in two species of calanoid copepods from nauplius to adult

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Subject to high predation risk, all developmental stages of copepods depend on evasive behaviors for survival in pelagic environments. Swim and escape behaviors were investigated in copepods from early nauplius to adult using 3D high-speed micro-cinematography. *Parvocalanus crassirostris* and *Eurytemora affinis* are two common estuarine species with broad geographic ranges. The early naupliar stages were mostly immobile, whereas the copepodids and adults spent most of the time actively swimming. Escapes and/or behavioral freezes were elicited by a predator mimic, an abrupt hydromechanical stimulus created by the rapid vertical movement of a 3-mm sphere, and video-recorded at 500 frames-per-second. All developmental stages of planktonic copepods responded with evasive behaviors and responses decreased with distance from the sphere. Maximum response distances were greater and response latencies were shorter in copepodids than in nauplii. Maximum escape speeds increased with copepod size, while the duration of the escape response decreased with the developmental stage. Maximum escape speeds scaled to body length as a power function from early nauplius to adult. Species-specific patterns in escape trajectories were apparent at the first nauplius (N1). These results start to differentiate between performance differences that result from size and design constraints, and those that are due to species-specific behavioral patterns.

KEYWORDS: *Eurytemora affinis*; *Parvocalanus crassirostris*; maximum speed; hydromechanical stimulus

INTRODUCTION

Marine pelagic food webs are characterized by a wide variety of predators consuming a diverse community of prey in an environment with few barriers or hiding places (review: Buskey *et al.*, 2012). Evasive behaviors are thus fundamental to prey survival in this habitat. To successfully evade a predatory attack, an escape requires the timely detection and response to the threat and a motor response that distances the prey from the predator fast enough and far enough to avoid capture. Copepods, small planktonic crustaceans, are among the most successful inhabitants of the pelagic realm. Escapes produced by adult copepods are exceptional in the timeliness of their reactions and remarkable in their motor response (acceleration, force production and maximum velocities) (Strickler, 1975; Svetlychnyy, 1987; Lenz and Hartline, 1999; Lenz *et al.*, 2004).

The threat of predation affects all life stages of these planktonic organisms (Eiane *et al.*, 2002; Ohman *et al.*, 2004). Six naupliar and five copepodid stages need to be considered in addition to the adult stage. Nauplii and early copepodids can be highly abundant, and they are a major food item for many small predators (Eiane *et al.*, 2002; Turner, 2004; Sampey *et al.*, 2007; Llopiz and Cowen, 2009). The developmental stages, in particular nauplii, may not be as capable in escaping from predators as the adults either through lesser detection abilities, escape performances or both (Buskey *et al.*, 1993; Bradley, 2009; Gemmell and Buskey, 2011). Copepodids have much the same body form and locomotory mechanisms as do adults: they use two different sets of appendages to produce normal swimming (rhythmic beating of feeding appendages; Storch, 1929) and escapes (fast succession of multiple power strokes of pereiopods; Storch, 1929). In contrast, the nauplii have a different "bauplan" with three pairs of appendages, the antennules (A1), antennae (A2) and mandibles (Mauchline, 1998). The propulsive force in the crustacean nauplius is produced primarily by the rhythmic beating of the antennae (A2) (Williams, 1994). This raises the question as to how the escape performance in the naupliar stages scales in comparison with the copepodid stages.

The behavioral competence of the nauplius is clearly an important factor in the success of copepods. Studies that have focused on the behavior of copepod nauplii have reported diverse behavioral patterns, including escape responses even in the earliest developmental stages (Yen and Fields, 1992; Paffenhöfer *et al.*, 1996; Titelman, 2001; Green *et al.*, 2003; Titelman and Kiørboe, 2003a, b). Indeed, these escapes are effective in evading predatory attacks from early larval fish stages (von Herbing *et al.*, 2001; Jackson, 2011). However, the limited temporal and/or spatial resolution in these studies has hindered quantitative comparisons of escape performance between developmental stages and species. The proximate cue that triggers escape reactions to a sudden predatory attack is the rapidly rising water deformation produced by the predator (e.g. Holzman and Wainwright, 2009). Such deformations have been shown to trigger escapes with high sensitivity both in adult copepods (Hartline *et al.*, 1996; Lenz and Hartline, 1999; Burdick *et al.*, 2007) and, as will be shown in the present study, in nauplii. Here, we examine the behavioral sensitivity and motor characteristics of nauplii responding to an artificial, well-characterized, reproducible rapidly rising deformation, comparing them with copepodid stages of two species of calanoid copepod: *Eurytemora affinis* and *Parvocalanus crassirostris*. Responses were recorded using a novel high-speed videography set-up with a single camera to obtain sequences of frames containing two dark-field images (front and side view, respectively) produced by the two intersecting matched filter optical pathways (Strickler, 1998; Strickler and Hwang, 1999). This has allowed us to quantify swimming patterns and escape responses. The detailed analysis starts to separate species-specific behavioral patterns from design and size constraints arising from the nauplius form.

METHODS

Copepod collection, culturing and experimental conditions

Two species of calanoid copepod were collected from their natural environment and cultured in the laboratory. *Eurytemora affinis* adults were collected near Northeast Creek in Frenchman Bay off Mount Desert Island (44°25.7′N, 66°11.8′W), Maine, and transferred into 7 L containers. Cultures were established and fed on *Tetraselmis* paste (Reed Mariculture) for 5 days and then shipped to Port Aransas, TX, USA. Adult females were isolated from the stock culture, transferred into

finger bowls and incubated overnight. In the morning, nauplii were removed and transferred into smaller finger bowls (11 cm × 6 cm; diameter × height), in order to raise cohorts of nauplii. *Parvocalanus crassirostris* adults were isolated from sub-surface net collections taken in Kane'ohe Bay, the island of O'ahu (21°26.3′N, 157°47.0′W), Hawaii, and maintained in stock cultures following protocols developed for another paracalanid species (VanderLugt and Lenz, 2008). Females were isolated from the cultures, incubated overnight in finger bowls, and eggs and newly hatched nauplii (*P. crassirostris*) were collected the following morning. These were transferred into smaller finger bowls (11 cm × 6 cm; diameter × height). Copepodid cohorts for both species were established by transferring newly molted copepodites (C1) into separate culture dishes. All cultures were maintained at the experimental temperatures and salinities (*E. affinis*: 20°C, 28 ppt; *P. crassirostris*: 23−25°C, 35 ppt) and fed *ad libitum*. *Eurytemora affinis* were fed on mixtures of live phytoplankton (*Isochrysis galbana*, *Heterocapsa* sp. and *Thalassiosira* sp.), and *P. crassirostris* on *Isochrysis galbana*. Prior to experimentation, nauplii were sorted by stage and 50−100 individuals were added to the experimental container (dimensions: 4.5 × 1.25 × 1.25 cm). Fewer copepodids were added to the experimental chamber (C1−C3: 25−50; C5−C6: 10−20 individuals), and adult *E. affinis* were filmed in a larger experimental container (dimensions: 6 × 4.5 × 4.5 cm). Experimental densities (7−14 nauplii L^{-1}) were comparable with culture densities (10−50 ind L^{-1}), and typically fewer than three individuals were within the volume of interest (VOI) at any given time. No phytoplankton was added to the experimental chambers, although some algal cells would have been transferred during sorting. The animals were acclimated to the experimental vessel for at least 15 min before filming for 1 h or less. The early nauplii of these two species ranged in length from 80 to 100 μm. Adults ranged from ca. 0.43 (*P. crassirostris*) to 1.4 (*E. affinis*) mm total length. The stage-specific sizes of the two species are shown in Fig. 1.

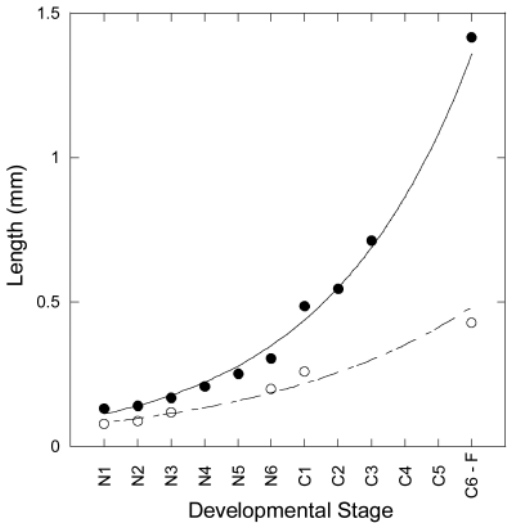

Fig. 1. Total length versus developmental stage for *Eurytemora affinis* (closed circles) and *Parvocalanus crassirostris* (open circles). Data were fitted to an exponential curve—*E. affinis*: $y = 0.0905e^{0.226x}$ ($r^2 = 0.996$); *P. crassirostris*: $y = 0.0721e^{0.159x}$ ($r^2 = 0.964$), where y = length in millimeters, and x = developmental stage from 1 to 12.

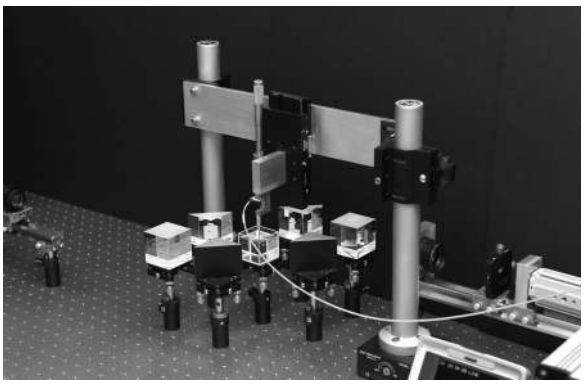

Fig. 2. Photograph of 3D set-up in the Marine Science Institute (Port Aransas, TX) showing video camera on the right, infrared laser light on the left. The hydromechanical stimulus is suspended from the bar supported by vibration dampening posts. The larger experimental chamber (dimensions: 6 × 4.5 × 4.5 cm^3) is shown in the center surrounded by two beam splitters and four 90° prisms. A similar set up was used at the Pacific Biosciences Research Center (Honolulu, HI).

Experimental set-up

Copepod behavior was observed using a high speed video setup which centered on a volume of 4.5 cm^2, 6 cm deep for adults, and 1.25 cm^2, 4.5 cm deep for nauplii, using an optical system similar to one described in Strickler (Strickler, 1998), and scaled down for this study (Fig. 2). This resulted in a single high-resolution image with two views, the front (x, z) and the side (y, z), of copepods within the VOI. These modifications resulted in a novel set-up with three major improvements over previous ones: (i) single image to track individual copepods entering the VOI that allowed precise capture of behavioral sequences in 3D through a camera trigger; (ii) spatial and temporal resolution that provided clear views of nauplii to adults, as well as appendage movements and (iii) the addition of a hydromechanical stimulus into the experimental chamber (see below).

The 3D views were recorded using high-speed digital video cameras (in Texas by a Photron FastCam Super 10K series, in Hawaii by a Kodak Motioncorder SR-3000) at 500 frames per second. The cameras were

attached to external processors controlling speed, resolution and trigger position. The captured sequences stored in the memory buffer of the cameras were reviewed and recorded with a High Definition Sony Video Walkman digital cassette recorder with the high-speed video played back at 30 frames per second.

Behavioral responses were elicited using a hydromechanical stimulus (Lenz and Hartline, 1999; Lenz *et al.*, 2004, 2005). The stimulus was produced by the vertical motion of a 3-mm diameter sphere attached to a rigid metal shaft and suspended into the sample vessel (Fig. 2). An electric signal was generated (HP 8003A Pulse Generator, Hewlett Packard) and amplified (DSM VF-500 Voltage Follower/Linear Amplifier with Sine Wave Generator Function, Dynamic Structures), driving a piezoelectric transducer (DSM LPA 100, Dynamic Structures) that drove the vertical displacement of the sphere Figs 2 and 3. The water displacement was calculated in polar coordinates:

$$d_r = Da^3 \cos\theta \cdot r^{-3} \quad (1a)$$

$$d_\theta = \frac{1}{2} Da^3 \sin\theta \cdot r^{-3} \quad (1b)$$

where D is displacement of the sphere, a is the radius of the sphere, θ is the angle from the axis of movement of the sphere and r is the distance between the center of the sphere and the copepod (van Bergeijk, 1967; Kalmijn, 1988; Gassie *et al.*, 1993; Lenz and Hartline, 1999). Stimulus voltage was set at the beginning of the experimental series, and waveform amplitude and frequency were checked on an oscilloscope prior to starting the experiments. The single step wave generated a hydromechanical stimulus that started at a discrete time and was abrupt (Fig. 3). The deformation rate (Δ_r) at median (threshold) and maximum response distances from the stimulus was computed using the formula:

$$\Delta_r = -3Da^3 r^{-4} \quad (2)$$

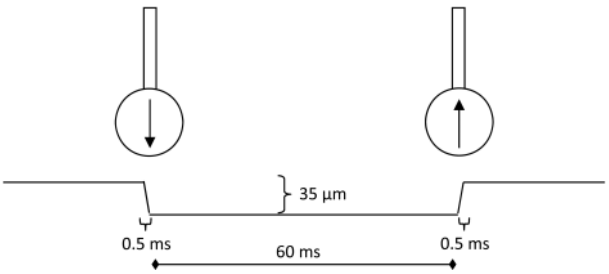

Fig. 3. Diagram of the hydromechanical stimulus. The 3-mm diameter sphere moved down 35 µm in 0.5 ms, then remains stationary for 60 ms prior to returning (upward movement) to its original position, again 35 µm movement completed in 0.5 ms (diagram not to scale).

where D, a and r are the same as in equations (1a) and (1b).

Data analysis

Handling of the digital videos was performed with Adobe Premiere (CS and CS3). The interval between frames was 2 ms, and each animal in the VOI was registered twice on each frame. Thus, each animal had two sets of 2D coordinates, one corresponding to the front view image (x, z) and the other the side view one (y, z). The optical path allowed us to distinguish between the two images by introducing a one-pixel difference in the z-axis between the two images for controlled experiments. Tracking the coordinates on the images was done using a manual mouse-click program (Track-it, v 2.0) and the more automatic Expertvision Cell-Trak video-computer motion analysis system. The data were imported to Microsoft Excel for further evaluation. From the data (x, y, z, time) for each animal in the VOI, we calculated net movement, 3D direction and swimming and sinking speeds.

Durations of escape behaviors, number of power strokes and orientation of the animals were extracted from the video footage and entered into a Microsoft Excel database. Response distances, R_R, were calculated using the coordinates of the organism at the time the stimulus was triggered ($t = 0$) and subtracting this from the coordinates of the center of the sphere,

$$R_R = \sqrt{(x_{ball} - x_0)^2 + (y_{ball} - y_0)^2 + (z_{ball} - z_0)^2}, \quad (3)$$

where x_{ball}, y_{ball} and z_{ball} are the x, y and z coordinates at the center of the stimulus sphere, and x_0, y_0 and z_0 are the x, y and z coordinates, respectively, of the experimental organism at $t = 0$. Specifically, the location of the copepod was measured at the point between the two first antennae on the cephalosome. Two measurements were made for jump distances: (i) displacement, the straight line between initiation and cessation of motion, (L_D); and (ii) integrated distance (L_I), the summed distance of the entire path taken. For total integrated distance, L_I, the length of the entire path of the escape jump was measured

$$L_I = \sum \sqrt{(x_i - x_{i-1})^2 + (y_i - y_{i-1})^2 + (z_i - z_{i-1})^2}, \quad (4)$$

where x_i, y_i and z_i are the x, y and z coordinates, respectively, of the anterior of the experimental organism at any given frame and x_{i-1}, y_{i-1}, and z_{i-1} are x, y and z coordinates, respectively, of the anterior of the

experimental organism in the previous frame. For each interval, swimming speeds were calculated to obtain temporal sequences and to determine maximum speeds achieved during an escape.

For displacement, L_D, the length of the line connecting the start and finish of the escape jump was measured

$$L_D = \sqrt{(x_{end} - x_0)^2 + (y_{end} - y_0)^2 + (z_{end} - z_0)^2}, \quad (5)$$

where x_{end}, y_{end} and z_{end} are x, y and z coordinates, respectively, of the anterior of the experimental organism at the time of the end of the escape.

Data were analyzed using STATISTICA (StatSoft 6.0) software. For the quantitative data on the escape parameters, both escape and escape freeze responses were compiled unless found to be significantly different using Student's *t*-test. Only data from the response to the initial stimulus (1st movement of sphere) were included in the general analyses. Responses to the second stimulus (return movement of sphere) solely or in addition to the first were noted. All escape parameters (delays to onset of escape and maximum speed, distance of animal from stimulus when responding, maximum speed, duration of escape, total integrated jump distance, jump displacement, and stroke frequency) were compared among stages with analyses of variance (one-way ANOVA). Post-hoc analyses were performed to find stage groupings. Comparisons between species were done for each parameter at each stage, or group of stages where appropriate, using Student's *t*-test.

RESULTS

Swimming pattern and activity

High-quality images were obtained for all stages as shown in a single frame taken from a video recording of *P. crassirostris* individuals (Fig. 4A). Both *E. affinis* and *P. crassirostris* swim in a "hop-and-sink"-type locomotory pattern with net movements in adults being much greater than in the earlier stages (Fig. 4B). *Parvocalanus crassirostris* nauplii and copepodids swam in a spiral pattern with a diameter of 1.5−2.5 mm (Fig. 4B, D and C). The average duration of the swim-sink cycle in the nauplii ranged from 0.2 to 0.7 s in *E. affinis*, and from 0.3 and 0.4 s in *P. crassirostris*. Early nauplii were least active and spent most of the time sinking (stage N1, *E. affinis*: 75%; *P. crassirostris*: 88%; Fig. 5). Swimming activity increased with stage, and copepodids tended to swim for longer durations often exceeding 1 s. Adult males and females of *E. affinis* swam nearly continuously (90%) interrupted by short pauses averaging 0.21 ± 0.02 s. In contrast, *P. crassirostris* adults were less active, females spent only 33% of the time swimming, while the non-feeding adult males spent 65% of the time swimming.

Swimming speeds of nauplii and copepodids of both species were typically below 3 mm s^{-1} (Fig. 6A). The adult males of both species had the highest swimming speeds at 3.2 ± 0.1 mm s^{-1} for *E. affinis* and 3.9 ± 0.3 mm s^{-1} for *P. crassirostris* (not included in Fig. 6). *Parvocalanus crassirostris* swam more slowly than *E. affinis* at all stages ($P < 0.05$, *t*-test) except for the adult males. Scaled swimming speeds (BL s^{-1}) showed a steep decline with length, and at all lengths swimming speeds of *P. crassirostris* were lower than those of *E. affinis* (Fig. 6B). Sinking speeds of *E. affinis* and *P. crassirostris* nauplii and copepodids increased with total copepod length and were similar between species (Fig. 6C).

Behavioral responses to the hydromechanical stimulus

The hydromechanical stimulus elicited a change in behavior in nauplii and copepodids. Responses included initiation of an escape jump, freeze behavior (cessation of motion) or a combination, an escape jump followed by a freeze. These behaviors were distinguished from normal swimming behavior by swimming speed and/or the duration of the freeze. During freeze behavior, the animal ceased movement of all appendages for a period that exceeded the length of motionless behavior observed during normal swimming. An exception to this occurred in *E. affinis* adult males and copepodids, which had very short freeze durations. In these cases, the responses were categorized as freezes based on the timing of the response (<20 ms after stimulus presentation, or immediately following an escape). In addition, adult males and females of this species oriented their bodies vertically during freezes, a marked difference from normal swimming.

Thresholds for freezes, escape freezes and escapes were similar and the three types of responses were observed in nauplii and copepodids of both species (Table I). The relative occurrences of escapes, escape freezes and freezes were highly variable (Table I). *Eurytemora affinis* copepodids responded primarily with escapes (> 90%), while responses in nauplii and adults typically included a freeze either by itself or following an escape (20−80% of responses; Table I). Responses in *P. crassirostris* nauplii and copepodites included all three behaviors and escapes only ranged from 40 to ca. 75% of all responses (Table I). The adult males were an

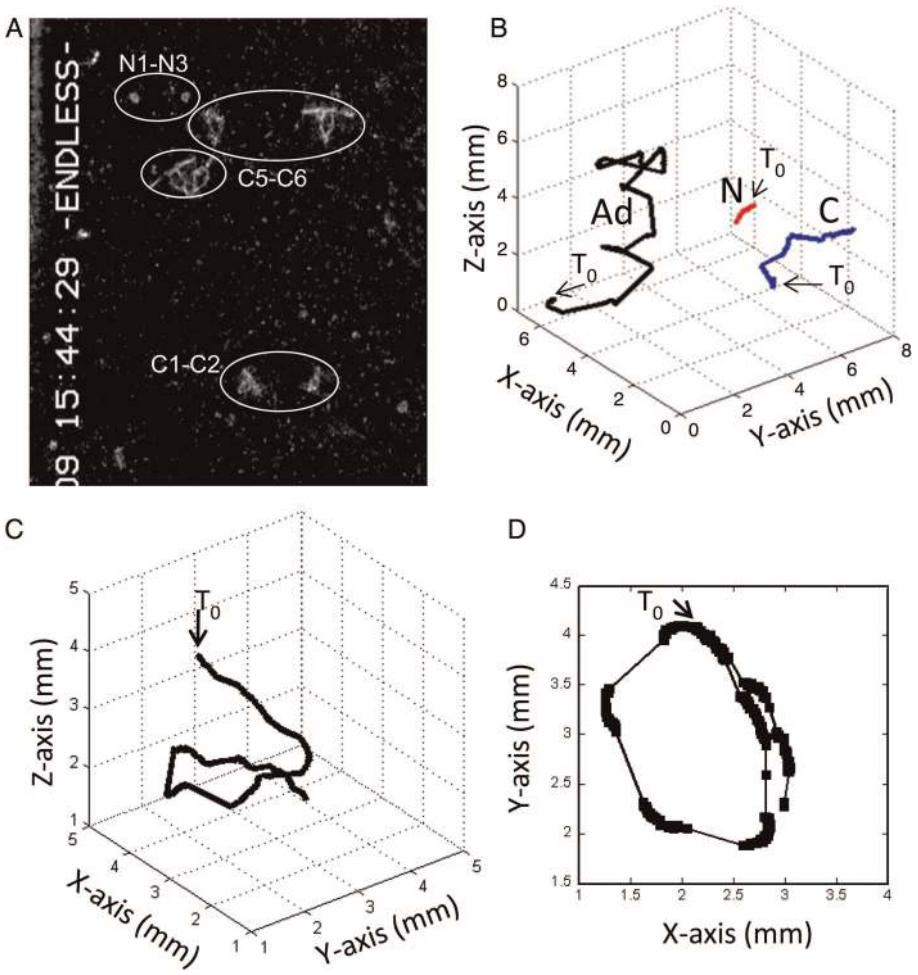

Fig. 4. (**A**) Video image of four *Parvocalanus crassirostris* showing *x*,*z* and *y*,*z* views. Paired views have been circled in white. The individuals shown include one early nauplius (N1–N3), one early copepodid (C1 or C2) and two late stage copepodids (C5 or C6). Video image taken in the presence of phytoplankton cells (*Isochrysis galbana*). (**B**) 3D swim trajectories of *P. crassirostris* for an early nauplius (N), a young copepodid (C) and an adult (Ad). Arrows indicate initial position ($t = 0$). *X*, *y* and *z* coordinates were measured for 215 frames spanning 7.2 s (video recorded at 30 fps). Trajectories shown did not occur simultaneously, however, they were from a single recording session that lasted approximately 7 min. (**C**) 3D swim trajectory of an early nauplius of *P. crassirostris* over a 50 s period. Arrow indicates initial position of nauplius. (**D**) x and y projection of nauplius shown in C.

exception to this pattern, and their responses comprised mostly of escapes (97%; Table I).

Escape responses

During an escape, individuals moved several body lengths from their initial location at stimulus onset, as shown by the video images with paired views of a single *P. crassirostris* adult (Fig. 7). The first image taken at time zero corresponds to the time the sphere moved down. Four milliseconds later, the escaping copepod can be seen as two streaks. The last image taken at 36 ms shows the copepod returning to its normal swimming position. Escapes of *E. affinis* nauplii and copepodids were characterized by an initial reorientation, followed by a rapid swim, and a final vertical movement (Fig. 8A and B). In the copepodids, the initial reorientation was achieved by lifting the urosome, causing a backward movement of the cephalosome. This was immediately followed by the power stroke of the swimming legs, which resulted in forward motion. *Parvocalanus crassirostris* nauplii and copepodids jumped in helical patterns (Fig. 8C and D), which were clearly visible in the copepodids (Fig. 8D). In the nauplii, the spiral was very tight (Fig. 8C). These spiral trajectories were the result of a continuous change in swimming orientation during the course of the escapes.

Escapes were characterized by rapid changes in swimming velocity over time as shown for *E. affinis* and *P. crassirostris* nauplius (N2) and copepodid (C1) each

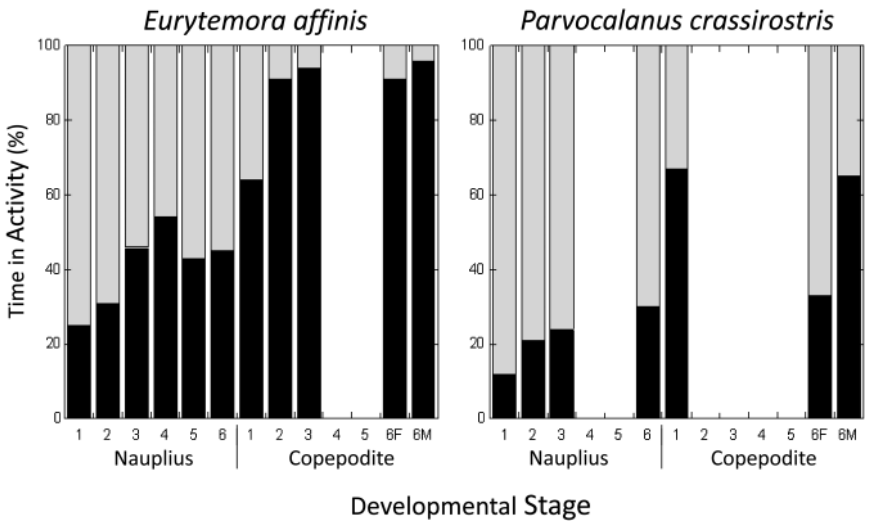

Fig. 5. Activity pattern in developmental stages in *Eurytemora affinis* (**A**) and *Parvocalanus crassirostris* (**B**). Naupliar and copepodid stages indicated, adult female and male activity patterns shown separately. Solid black: proportion of time spent swimming. Hatched: proportion of time spent sinking. Number of observations: *E. affinis*—N1: 17: N2: 101; N3: 46; N4: 18; N5: 41; N6: 81; C1: 107; C2: 65; C3: 26; C6 Females: 32; C6 Males: 78; and *P. crassirostris*—N1: 55; N2: 103; N3: 37; N6: 94; C1: 80; C6 Females: 79; C6 Males: 49.

(Fig. 9A–D). Each peak in velocity corresponds to a power stroke, whereas the troughs are the result of the return stroke of the appendages. In the examples shown, *E. affinis* nauplius and copepodid responded to both the down and up movement of the sphere (arrows) with 3–5 power strokes (Fig. 9A and B). The nauplius and copepodid reached maximum velocities of ca. 90 and 150 mm s^{-1}, respectively. *P. crassirostris* nauplius and copepodid responded to the first stimulus with a long series of power strokes (Fig. 9C and D). Maximum speeds for the nauplius and the copepodid were ca. 45 and 75 mm s^{-1}, respectively. In both species, the maximum escape speeds in these examples were 40–45-fold (nauplius) and 100–150-fold (copepodid) higher than normal swimming (see Fig. 5). Stroke frequencies were similar in both species and ca. 100 Hz in nauplii and copepodites, with the exception of *P. crassirostris* adults which had an average stroke frequency closer to 50 Hz.

Behavioral sensitivity and response latencies

The likelihood of an escape response triggered by the stimulus declined with distance from 80 to 100% response frequency at 3 mm or closer to the center of the sphere to 0% at distances >5.5 mm (Fig. 10A and B). Copepodids (solid bars) responded at greater distances than nauplii (hatched bars) and this was particularly apparent in *P. crassirostris* (Fig. 10B). Average and maximum response distances for the two species by stage are summarized in Table II. Computed water displacements and deformation rates at threshold and maximum sensitivities observed are summarized in Table III. Threshold distances, defined as the distance with 50% of individuals responding to the stimulus, ranged from 2.8 to 3.8 mm, with the smallest and greatest threshold values observed in *P. crassirostris* (Table III).

Average response latencies, the time between the initiation of the hydrodynamic stimulus and the first sign of an escape jump on the videotape, ranged from 2 to 5 ms, and were shorter in copepodids (2–3 ms) than in nauplii (4–5 ms; Table II). This difference was significant in both *E. affinis* ($P = 0.006$, Student's *t*-test) and *P. crassirostris* ($P < 0.0001$, Student's *t*-test). There was no significant difference between the two species for response latencies (Student's *t*-test). Maximum escape speeds were reached more quickly in the copepodids (4–6 ms) than in the nauplii (> 8 ms; Table II). *Eurytemora affinis* adult females were an exception to this trend, and their maximum speeds were reached on average at 10 ms.

Escape speeds

At each stage, nauplii and copepodids of *E. affinis* had significantly greater maximum escape speeds than *P. crassirostris* ($P < 0.05$, *t*-test for each stage, Fig. 11A). Maximum speeds of all *E. affinis* naupliar stages were not significantly different from one another ($P > 0.30$,

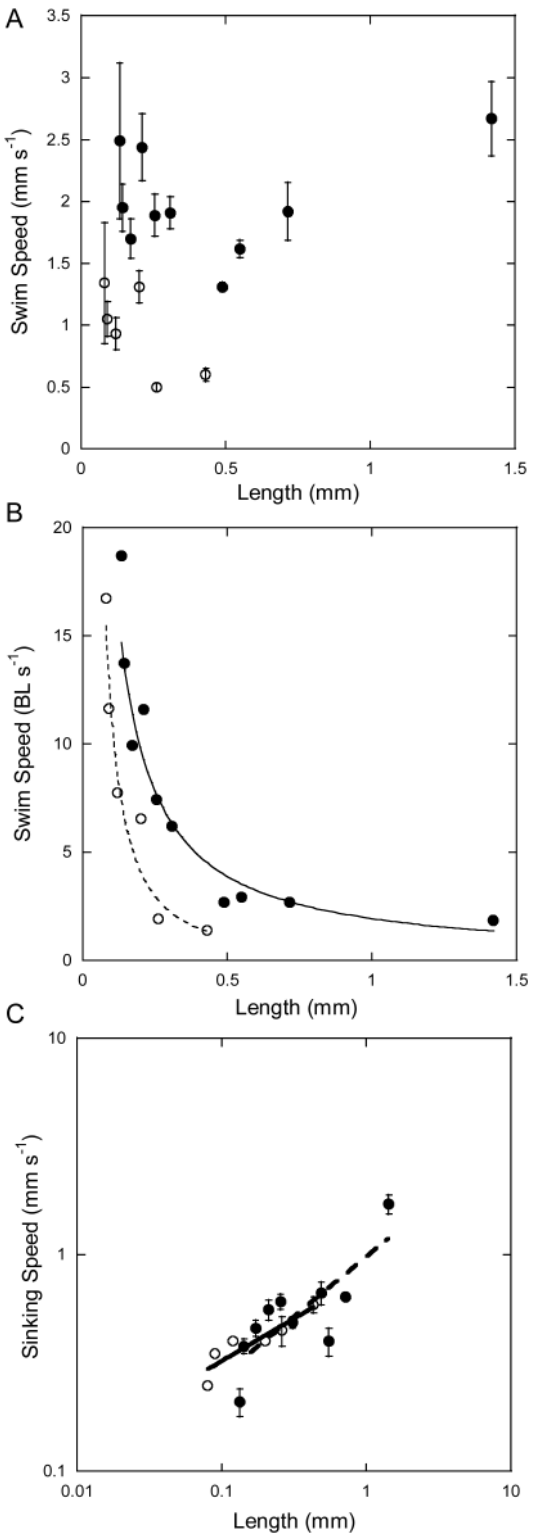

Fig. 6. Swimming and sinking speeds as a function of length for *Eurytemora affinis* (closed circles) and *Parvocalanus crassirostris* (open circles). (**A**) Normal swimming speeds in mm s^{-1} (linear scale). (**B**) Swimming speeds scaled to total body length (BL s^{-1}). Data fitted to power functions: *E. affinis*—$y = 1.94 \times x^{-1.005}$ ($r^2 = 0.929$), and *P. crassirostris*—$y = 0.39 \times x^{-1.453}$ ($r^2 = 0.937$), where y is swimming speed in BL s^{-1} and x = total length in mm. (**C**) Average sinking speeds versus copepod length on a log-log scale. Regression equations fitted to data—*E. affinis*: $y = 0.97 \times x^{0.59}$ ($r^2 = 0.742$); *P. crassirostris*: $y = 0.81 \times x^{0.40}$ ($r^2 = 0.878$), where y = sinking speed in mm s^{-1} and x = length in mm. Error bars are standard errors.

one-way ANOVA) and averaged 80 ± 19 mm s^{-1}. The first three naupliar stages of *P. crassirostris* had similar maximum escape speeds ($P > 0.34$, one-way ANOVA), averaging 38 ± 9 mm s^{-1}, which was significantly slower than the N6 stage (54 ± 19 mm s^{-1}, $P \leq 0.01$; Tukey's unequal n HSD test). Escape speeds nearly doubled between the first copepodid (C1) and the adult (C6) stages (Fig. 11A). Scaled to body length, maximum speeds decreased with nauplius stage remaining nearly constant from the late naupliar to the adult stages ($P > 0.05$, one-way ANOVA; Fig. 11B). Computed Reynolds (Re) numbers at maximum speeds increased with copepod size and swim speeds from Re numbers below 25 in the nauplii, and 400 in *E. affinis* adults.

Escape durations and distance jumped

Pronounced differences between the two species occurred in escape durations and the distances of the escape jumps (Table IV). The duration of the escapes was longer in *P. crassirostris* than in *E. affinis* for all stages ($P < 0.001$, two-way ANOVA). This was particularly apparent in the early nauplii (N1 and N2) with escape durations that exceeded 100 ms in *P. crassirostris*. In *E. affinis* nauplii, escape durations were short enough that nearly 50% of escaping animals responded to both the upward and downward movement of the hydromechanical stimulus (e.g. Fig. 9A).

Escape jump distances, both the integrated distances and net displacements, were greater in *P. crassirostris* than in *E. affinis* nauplii, while the escape trajectories of *E. affinis* copepodids were slightly longer than those of *P. crassirostris* (Table IV). Average escape jump lengths increased with size in *E. affinis* nauplii and copepodids, while escape durations stayed constant (Table IV, Fig. 12). In contrast, net displacements in *P. crassirostris* declined with stage within the nauplii and the copepodids (Table IV, Fig. 12). In this species, net displacements correlated with durations of the escape jump, and the earliest nauplii (N1 and N2) had the greatest net displacements (Fig. 12). Scaled to body length, net displacements in *E. affinis* were <5 body lengths for both the copepodids and nauplii (Table IV). In both species, net displacements in the adults were approximately 1 body length.

Table I: Type and frequency of behavioral responses elicited by the hydromechanical stimulus for nauplii and copepodids of Eurytemora affinis *and* Parvocalanus crassirostris

	Eurytemora affinis					*Parvocalanus crassirostris*				
	Escape %	Escape-Freeze %	Freeze %	*n*	Freeze duration (s)	Escape %	Escape-Freeze %	Freeze %	*n*	Freeze Duration (s)
N1	80	0	20	10	2.5 ± 1.5	59	24	17	29	1.9 ± 0.2
N2	37	34	29	59	3.5 ± 0.1	40	36	24	55	1.9 ± 0.1
N3	20	37	43	35	3.3 ± 0.2	45	30	25	20	2.0 ± 0.1
N4	36	27	36	11	2.1 ± 0.4					
N5	54	42	4	26	3.5 ± 0.2					
N6	57	27	16	44	2.7 ± 0.2	73	12	15	26	1.5 ± 0.3
C1	93	4	3	101	3.3 ± 0.3	51	43	6	49	1.9 ± 0.1
C2	98	2	0	46	0.3					
C3	98	2	0	46	0.1					
C6 F	53	0	47	15	1.4 ± 0.7	67	16	18	51	1.4 ± 0.2
C6 M	51	26	23	43	0.3 ± 0.1	97	3	0	32	0.1

Mean duration of freezes and standard errors are also given, and they were computed for both the freeze only and escape-freeze responses. Frequency of responses given as a percent; *n* = number of responses observed for each stage.

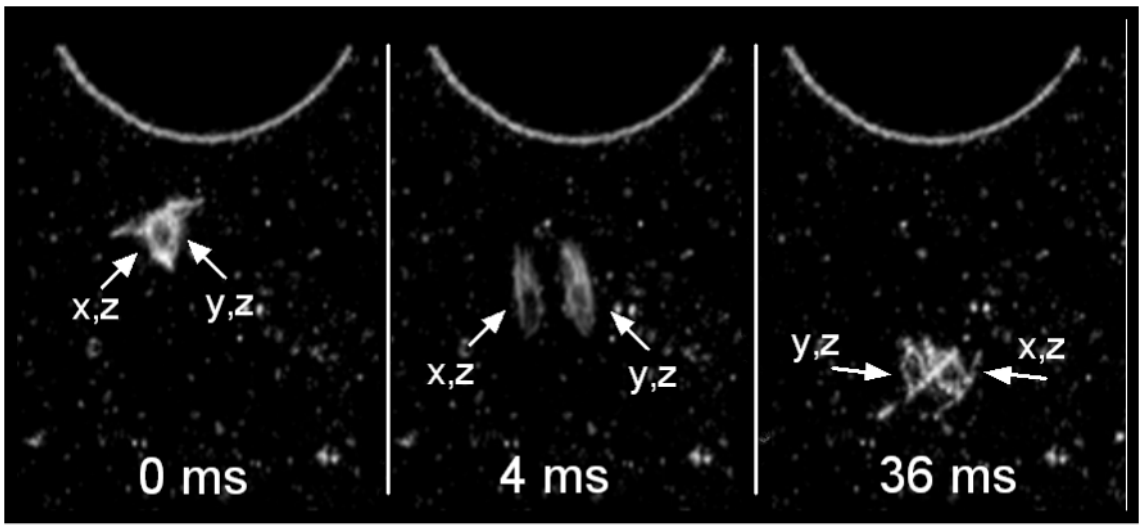

Fig. 7. Three video images taken during an escape sequence of a *Parvocalanus crassirostris* adult using the 3D set-up. The first frame labeled time zero indicates the time of the activation of the stimulus shown by the white outline. The two copepod images are superimposed as indicated by the $x-z$ and $y-z$ arrows. The second frame at 4 ms and the third frame at 36 ms show two separate images ($x-z$ and $y-z$) of the copepod during and at the end of the escape swim. Video images taken in the presence of phytoplankton cells (*Isochrysis galbana*).

DISCUSSION

This study focused on a systematic analysis of swimming activity and escape responses of nauplii and copepodites in two estuarine calanoid copepods using a new 3D filming set-up with high temporal and spatial resolutions. While it has been well established that the earliest nauplii respond to threats with an escape (Buskey *et al.*, 1993; Titelman, 2001; Green *et al.*, 2003), and that these escape responses are sufficient to successfully evade larval fishes (von Herbing and Gallager, 2000; Jackson, 2011), few studies have examined the changes in the kinematics of the escape with developmental stage at the resolution provided by the current set-up. This type of study starts to answer questions about behavioral constraints and plasticity that might be critical in determining encounter rates and predator–prey outcomes by developmental stage and by species.

Swimming behavior

The early naupliar stages of *E. affinis* and *P. crassirostris* swam so infrequently (N1 < 25%) that they could be considered hiding in plain sight. This phrase is typically applied to transparent organisms with minimal motion

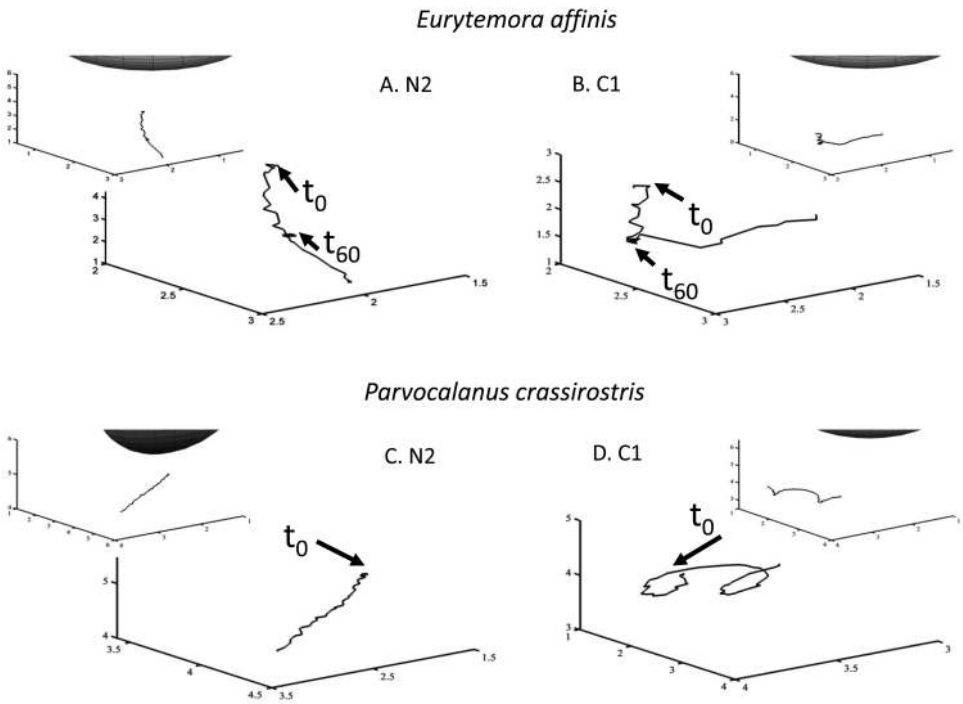

Fig. 8. Examples of 3D escape trajectories for nauplii and copepodids of *Eurytemora affinis* and *Parvocalanus crassirostris*. (**A**) *E. affinis* nauplius stage N2. (**B**) *E. affinis* copepodid stage C1. (**C**) *P. crassirostris* nauplius stage N2. (**D**) *P. crassirostris* copepodid stage C1. Note: *E. affinis* nauplius and copepodid responded with two separate escape response (t_0 and t_{60}) to the two movements of the sphere. *P. crassirostris* nauplius and copepodid responded with a single long duration escape jump at t_0.

(Johnsen, 2001). The N1 and N2 stages are non-feeding, with undeveloped or absent mouths (Peterson, 2001), and may therefore be conserving energy by limiting motion, while also minimizing their hydrodynamic signals. Limited activity levels reduce the conspicuousness of copepod nauplii to both visual and hydrodynamic predators (Buskey *et al.*, 1993), thus effectively decreasing encounter rates with potential predators. Low activity in early nauplii (N1 and N2) has been observed in other species, such as in *Acartia tonsa*, *Calanus helgolandicus*, *E. affinis* and *Temora longicornis* (Titelman and Kiørboe, 2003a), suggesting that this strategy may be common among non-feeding calanoid nauplii. While there was a common trend of increased activity with development, species-specific swimming patterns, such as sink–swim frequency and trajectories were observed at all developmental stages. This agrees with observations by Paffenhöfer *et al.* (Paffenhöfer *et al.*, 1996) and Titelman and Kiørboe (Titelman and Kiørboe, 2003a), and might be related to differences in feeding strategies (Wu *et al.*, 2011). Published naupliar swimming speeds are highly variable, but in general appear to decline from ca. 15 to <5 BL s^{-1} between the N1 and the N6 stages (Fig. 13). In contrast, scaled swimming speeds in the copepodids are similar across species and stages ($\sim$2.5 BL s^{-1}; Fig. 13).

Responses to the hydromechanical stimulus

Escape responses in early nauplii have been observed in many species, using an artificial stimulus (suction: Titelman, 2001; Titelman and Kiørboe, 2003b) and natural predators (copepod: Yen and Fields, 1992; Titelman, 2001; mussel: Green *et al.*, 2003). In response to a hydromechanical stimulus produced by the vertical movement of a sphere, we found that the most common responses were either an escape or an escape followed by a freeze. Decreased swimming is associated with a decrease in detection by a mechanoreceptive predator, especially if the prey is small (Kiørboe and Visser, 1999; Titelman, 2001; Titelman and Kiørboe, 2003a, b; Jiang and Paffenhöfer, 2004; Yamaguchi *et al.*, 2004; Irigoien and Harris, 2006). However, this strategy may also be effective in lowering risk from visual predators in turbid environments, such as those inhabited by *E. affinis* (Arula *et al.*, 2012).

Behavioral sensitivity and response delays

Detection and a timely response to a potential threat can affect predator-prey outcomes as shown by the inverse correlation between predator ingestion rates and response distances of prey (Titelman, 2001). Fish strikes on prey include strategies to delay onset of the abrupt

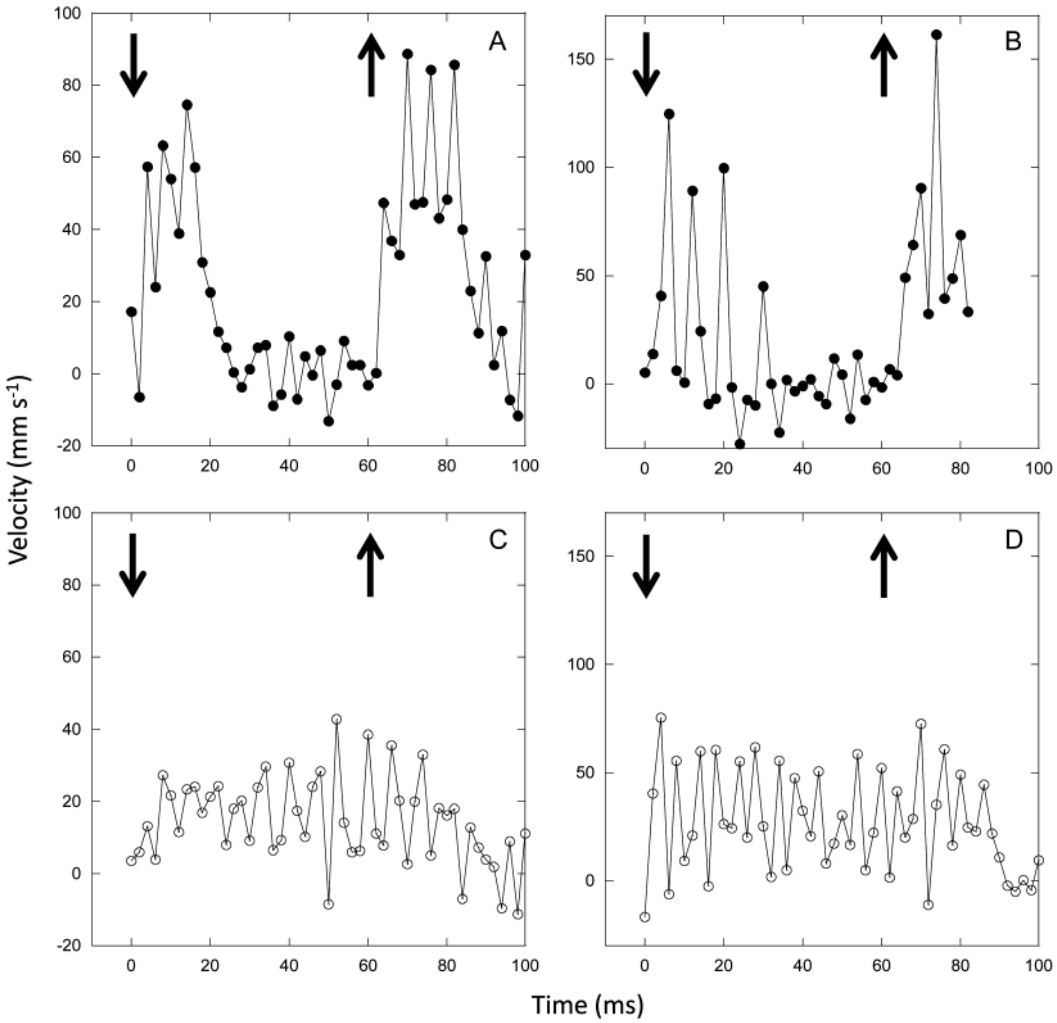

Fig. 9. Swimming velocity over time during escapes. Escapes: (**A**) *Eurytemora affinis* nauplius (stage N2); (**B**) *Eurytemora affinis* early copepodid (stage C1); (**C**) *Parvocalanus crassirostris* nauplius (stage N2); (**D**) *Parvocalanus crassirostris* early copepodid (stage C1). Arrows in A, B, C, D indicate time and direction of stimulus. Scale of y-axis differs between the nauplius (A, C) and copepodid (B, D) graphs. X-axis scale is the same for all graphs.

ambush on prey to minimize the time available for an escape response (e.g. Holzman and Wainwright, 2009; Gemmell, 2011). The hydromechanical stimulus used here is by necessity simple compared to the cues generated by a real predator, but it contains the key feature of a sudden attack. The movement of the sphere produces a rapidly-rising deformation which is the salient component of the ambush strike of many planktivorous fishes (Holzman and Wainwright, 2009).

Planktonic copepods, living in a fluid with no fixed point of reference, are sensitive to deformation rates rather than bulk water movement (Visser, 2001). Thus, if sensory capability is constant, the sensitivity of the copepod to hydromechanical signals increases with the distance between its center of mass (friction) and its sensor, which in turn is typically correlated with size (Kiørboe and Visser, 1999). This suggests that mechanosensitivity should increase with developmental stage as well as with the size of the species. Based on this relationship, and assuming no difference in intrinsic sensor sensitivity, we would predict lowest mechanosensitivity for *P. crassirostris* early nauplii and the highest one for adult female *E. affinis* in our experiments.

The nauplii responded behaviorally to the movement of the sphere, in spite of their small size, and the fact that the first antenna (A1) is poorly developed (Boxshall and Huys, 1998). The maximum response distances observed in nauplii were lower than in the copepodids,

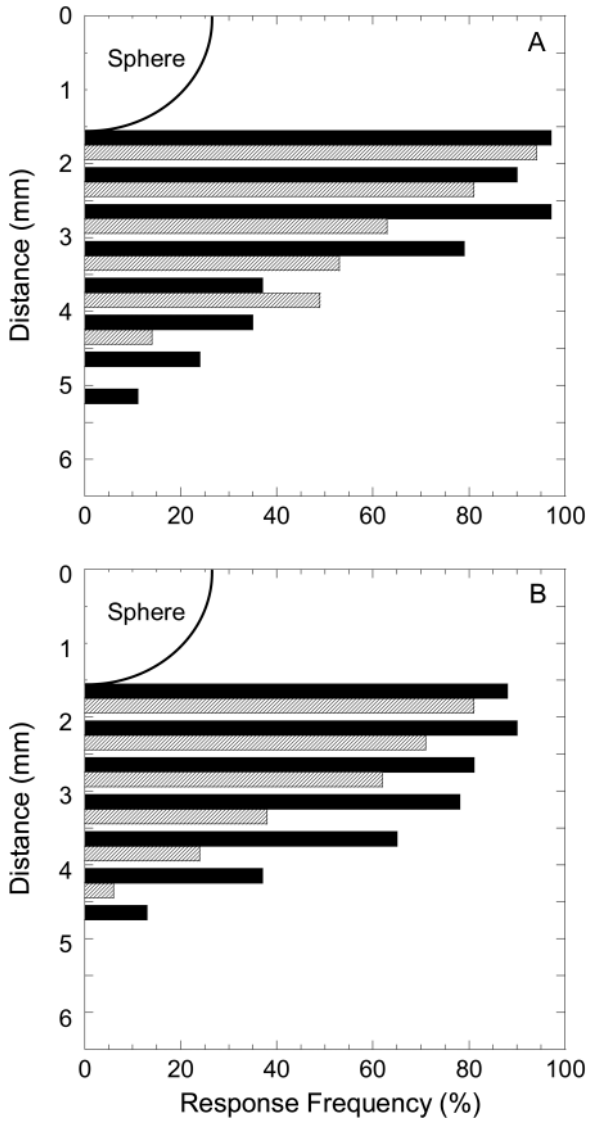

Fig. 10. Proportion of responding nauplii (hatched) and copepodid (solid) individuals with distance from the hydrodynamic stimulus (sphere). (**A**) *Eurytemora affinis* nauplii (stages N1–N6 combined) and copepodids (stages C1–C3 and C6). Number of observation in each bin from nearest to sphere (1.5 to 2 mm) to furthest (>5 mm) were 16, 85, 76, 58, 41, 14, 7, 7 for nauplii and 33, 58, 64, 78, 60, 34, 21, 18 for copepodids. (**B**) *Parvocalanus crassirostris* nauplius (stages N1-N3 and N6) and copepodids (stages C1 and C6). Number of observation in each bin from nearest to sphere (1.5 to 2 mm) to furthest (> 5 mm) were 16, 65, 69, 61, 17, 18, 8, 26 for nauplii and 8, 32, 47, 40, 26, 19, 16, 17 for copepodids.

which is consistent with a lower mechanosensitivity in the nauplii of both species. However, threshold distances in nauplii and copepodids in *E. affinis* were similar, which may relate to the state of motivation of the copepodids, and not detection ability (e.g. Domenici, 2001). Behavioral sensitivities to shears of $1.9–2.6\ s^{-1}$ were reported for *E. affinis* nauplii escaping from a suction stimulus (Titelman and Kiørboe, 2003a, b) which suggest a higher sensitivity to a slowly-rising (suction) than a rapidly-rising deformation (threshold shear: $3.0\ s^{-1}$, Table III). These results are consistent with Burdick *et al.* (Burdick *et al.*, 2007), who found that calanoids appear to be less sensitive to an abrupt hydromechanical stimulus than to a suction stimulus.

One of the advantages of the vertically moving sphere stimulus is that it provides a measurement for the time delay between the stimulus and the behavioral response. In the present study, temporal resolution was limited to 2 ms (video recording at 500 fps), and latencies for the copepodids (2–3 ms) were comparable with those reported by Burdick *et al.* (Burdick *et al.*, 2007). A comparison between nauplii and copepodids in both species showed a longer delay in the nauplius than in the copepodid responses. In addition, the time delays to maximum speeds in the nauplii were two to four times longer than in the copepodids (Table II), which might lower the effectiveness of their escape response. An exception to this pattern was observed in the adult *E. affinis* females, which might have been the result of low motivation to respond behaviorally to the stimulus. This is consistent with the observation of reactive distances that were less than expected for *E. affinis* adult females in our experiments.

Scaling of escape speeds

An empirical analysis of scaling between organism length and maximum escape velocities found that both calanoid copepods and caridean shrimp outperform fishes and other aquatic organisms (Lenz *et al.*, 2004). In adult calanoid copepods, maximum escape velocities and length scale to the $\frac{2}{3}$ power ($L^{\frac{2}{3}}$), and they are nearly an order of magnitude higher than maximum velocities of fishes of the same length (Lenz *et al.*, 2004; Burdick *et al.*, 2007). The current study provides data on maximum escape speeds for copepodids ranging between 0.26 and 1.4 mm and they scale similarly to speeds measured in adult calanoids (Fig. 14). Over the range in size and swimming speeds for *E. affinis* and *P. crassirostris* copepodids, calculated Reynolds numbers were between 20 and 400, a range where both viscous and inertial forces are important.

The nauplius escape data extend the size range for copepods to 0.08 mm. However, the nauplius "bauplan" is very different from the copepodid form. Crustacean nauplii row through the water using primarily their second antennae (A2) to generate propulsive force (Williams, 1994). Swimming speeds are determined by the size of the nauplius, length of appendages,

Table II: Reactive distances and response latencies for nauplii and copepodids of Eurytemora affinis and Parvocalanus crassirostris

		Reactive Distance			Response times	
Species	Stage	Mean reactive distance (mm)	Furthest reactive distance (mm)	Tested range (mm)	Mean latency to 1st motion (ms)	Delay to maximum speed (ms)
Eurytemora affinis	N1	2.1	3.7	1.9–7.5	3 ± 1	8 ± 1
	N2	2.1	3.8	1.7–6.2	4 ± 1	9 ± 1
	N3	1.7	2.3	1.7–3.4	4 ± 1	8 ± 1
	N4	1.8	2.6	1.7–3.1	5 ± 1	11 ± 1
	N5	2.3	4.0	2.2–6.6	5 ± 1	16 ± 1
	N6	2.6	4.0	2.0–5.4	4 ± 1	10 ± 1
	C1	2.6	5.0	1.8–6.3	2 ± 1	6 ± 1
	C2	2.4	3.7	1.5–7.0	3 ± 1	5 ± 1
	C3	2.6	5.5	1.8–7.4	2 ± 1	5 ± 1
	C6M	3.0	4.6	1.6–7.7	2 ± 1	5 ± 2
	C6F	2.2	3.0	1.8–5.5	4 ± 1	10 ± 3
Parvocalanus crassirostris	N1	2.4	3.0	1.5–6.4	4 ± 1	20 ± 2
	N2	2.6	4.4	1.7–5.9	4 ± 1	19 ± 3
	N3	2.6	3.5	2.0–4.9	4 ± 1	13 ± 2
	N6	2.7	3.3	1.7–6.0	4 ± 1	15 ± 1
	C1	2.8	4.3	1.5–5.6	3 ± 1	6 ± 2
	C6M	2.8	3.8	1.8–5.2	3 ± 1	4 ± 1
	C6F	3.1	4.7	1.7–6.5	3 ± 1	6 ± 1

Resolution of response latencies was limited to 2 ms due to video recording speed (500 fps).

Table III: Summary table for threshold (50% response) and maximum behavioral sensitivities for Eurytemora affinis *and* Parvocalanus crassirostris *nauplii (all stages investigated) and copepodids (all stages investigated) given as distance from center of sphere, computed water displacement and shear*

	Eurytemora affinis		*Parvocalanus crassirostris*	
	Nauplii	Copepodids	Nauplii	Copepodids
# Observations	304	286	280	204
Threshold: 50% response				
Distance (mm)	3.3	3.3	2.8	3.8
Water displacement (μm)	3.29	3.29	5.38	2.15
Shear (s^{-1})	3.0	3.0	5.8	1.7
Maximum sensitivity				
Distance (mm)	4.0	5.5	4.4	4.7
Water displacement (μm)	1.85	0.71	1.39	1.14
Shear (s^{-1})	1.4	0.4	0.9	0.7

excursion of the antennae (up to 180°) and beat frequency (10 to 200 Hz) (Williams, 1994). During an escape swim, copepod nauplii use all three appendages with a maximum excursion and high beat frequency (Williams, 1994; Gemmell, 2011; this study; Lenz and Hartline, unpublished). This produces maximum escape speeds that scale similarly to the copepodids and exceed those of other aquatic organisms (Fig. 14).

Duration and distance jumped

Duration of the escapes and the distance jumped vary among copepod species, and with the type of stimulus (Burdick *et al.*, 2007; Waggett and Buskey, 2007). In the current study, a species-specific pattern for the escape duration and trajectory was already apparent in the nauplii. At all stages, *P. crassirostris* had longer escape durations than *E. affinis*. In the nauplii, this also corresponded to a greater net displacement in *P. crassirostris* compared with *E. affinis*, and it correlated with a lower behavioral sensitivity. This trade-off was also observed in other nauplii: the behaviorally less sensitive *Temora longicornis* nauplii only responded after contact with the predator, but jumped much further than *Acartia tonsa* nauplii (29 versus 17 BL) in response to an attack by *Centropages typicus* (Titelman, 2001). In contrast, jump distances in *E. affinis* (nauplii and copepodids) and *P. crassirostris* copepodids and N6 (integrated and net; Table IV) were short (<5 body lengths), and comparable with the short escape trajectories reported for *Tortanus discaudatus*, *Centropages hamatus*, *Acartia hudsonica* and *T. longicornis* in response to a similar stimulus (vertically moving bar) (Burdick *et al.*, 2007).

Ecological implications

The two species investigated here are calanoids that inhabit estuaries and shallow bays. *Eurytemora affinis* is a widespread euryhaline species which can be very abundant in temperate estuaries in North America and

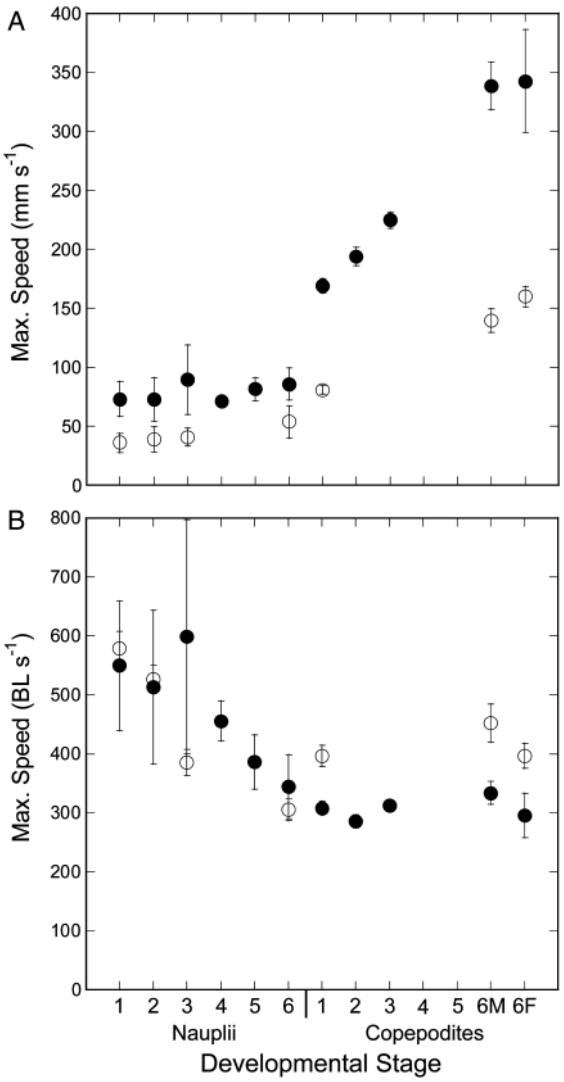

Fig. 11. (**A**) Average maximum escape speeds (mm s^{-1}) measured during escapes elicited by the hydrodynamic stimulus for different developmental stages of *Eurytemora affinis* (closed symbols) and *Parvocalanus crassirostris* (open symbols). (**B**) Data from A recalculated as mean maximum escape speeds in body lengths (BL) per second. Error bars are standard errors. Adult females (AF) and adult males (AM) shown separately.

Europe (Turner, 1981; David *et al.*, 2007). *P. crassirostris* is a subtropical species that is common in embayments such as Kane'ohe Bay, Hawaii (Hoover *et al.*, 2006), Kingston Harbour, Jamaica (Hopcroft *et al.*, 1998) and along the Australian coast (McKinnon and Ayukai, 1996; Duggan *et al.*, 2008). These communities are dynamic and periods of top-down control of copepods and other zooplankton have been suggested (Kimmerer, 1984; Hoover *et al.*, 2006). Interestingly, in Kane'ohe Bay, *P. crassirostris* nauplii may be protected from predation by their small size given that the highly abundant chaetognath, *Sagitta enflata* prefers larger copepodids (Hoover *et al.*, 2006). An escape swim that lasts over 100 ms and moves the nauplius by 15 to 60 body lengths away from the stimulus as observed in the early nauplius stages of *P. crassirostris* suggests the opposite. Jackson (Jackson, 2011) found that pre-flexion larval clownfish (*Amphiprion ocellaris*) attacked nauplii preferentially when presented with a mixture of nauplii, early copepodids and adult *P. crassirostris* as prey in laboratory experiments. Nevertheless, the early nauplii (N1-N3) successfully escaped from this visual predator 50 to 70% of the time (Jackson, 2011), demonstrating that this escape strategy works well for this type of predator. In contrast, the size hypothesis may be supported for the results we obtained for *E. affinis*: a species that inhabits waters characterized by high turbidity (David *et al.*, 2006, 2007), where immobility and short jumps may be effective for evading visual predators.

SUMMARY

Swimming and escape behaviors were investigated in two calanoids, *Eurytemora affinis* and *Parvocalanus crassirostris*. Escapes were elicited using an abrupt hydromechanical stimulus, and behavioral responsiveness, swim speeds and trajectories were quantified in nauplii and copepodids. In addition to escape swims, freezes and escapes followed by freezes were observed in both species, and were most common in the nauplii. Copepodid escape performance exceeded that of the nauplii: (i) copepodids responded at greater distances; (ii) copepodid response delays were shorter and (iii) they reached maximum escape speeds faster. Maximum escape speeds scaled with size across developmental stage and species. Species-specific patterns in escape behavior were present even in the earliest developmental stages. Duration and escape trajectories in *P. crassirostris* were longer than in *E. affinis* at each stage. The spiral trajectories characteristic of *P. crassirostris* escapes may be an adaptation for predator evasion in clear water environments. In contrast, *E. affinis*, an inhabitant of high turbidity environments had escapes characterized by an initial reorientation, short trajectories, and freezes in the nauplii.

ACKNOWLEDGEMENTS

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Table IV: Durations and distances of escape jumps by nauplii and copepodids of Eurytemora affinis *and* Parvocalanus crassirostris *in response to a hydromechanical stimulus*

Species	Stage	Duration (ms)	Integrated Distance (mm)	Net Displacement (mm)	Scaled Net Displacement (BL)
Eurytemora affinis	N1	18 ± 4	0.5 ± 0.2	0.3 ± 0.2	3 ± 1
	N2	24 ± 6	0.8 ± 0.2	0.5 ± 0.2	4 ± 1
	N3	23 ± 3	0.6 ± 0.1	0.4 ± 0.1	3 ± 1
	N4	22 ± 3	0.9 ± 0.1	0.5 ± 0.2	4 ± 1
	N5	31 ± 8	1.0 ± 0.3	0.7 ± 0.3	4 ± 1
	N6	26 ± 7	0.9 ± 0.3	0.7 ± 0.2	3 ± 1
	C1	38 ± 2	2.0 ± 0.1	1.2 ± 0.1	2.2 ± 0.2
	C2	22 ± 1	1.4 ± 0.1	0.9 ± 0.1	1.4 ± 0.2
	C3	21 ± 2	2.0 ± 0.2	1.3 ± 0.1	1.7 ± 0.2
	C6M	17 ± 1	1.8 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
	C6F	10 ± 1	2.1 ± 0.2	1.5 ± 0.4	1.3 ± 0.3
Parvocalanus crassirostris	N1	134 ± 68	2.1 ± 1.3	1.4 ± 0.8	62 ± 8
	N2	108 ± 49	1.8 ± 0.9	1.2 ± 0.6	34 ± 5
	N3	55 ± 21	1.1 ± 0.4	0.7 ± 0.4	14 ± 7
	N6	55 ± 28	1.2 ± 0.8	0.8 ± 0.6	5 ± 1
	C1	64 ± 5	1.8 ± 0.2	1.0 ± 0.1	4.7 ± 0.6
	C6M	34 ± 4	1.2 ± 0.1	0.6 ± 0.1	1.8 ± 0.2
	C6F	33 ± 2	1.3 ± 0.1	0.5 ± 0.1	1.3 ± 0.1

Integrated distances (L_I) and net displacements (L_D) are given for each species and stage in millimeters (mm). Scaled net displacements are given in body lengths (BL). Errors are standard errors of the mean.

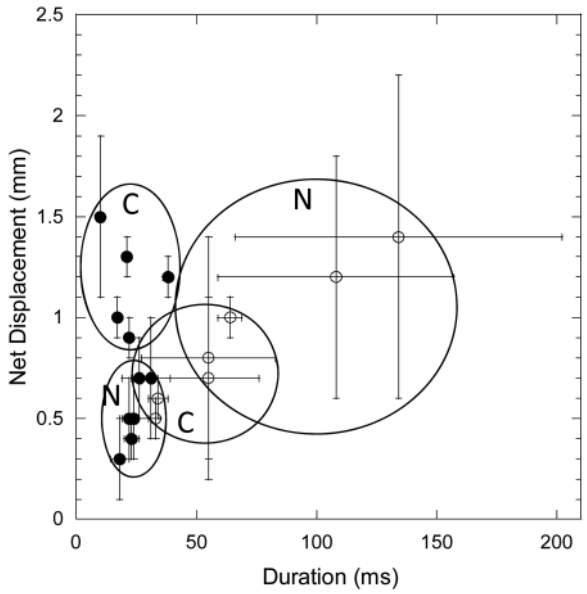

Fig. 12. Average net displacement versus average duration of escape responses for different developmental stages for *Eurytemora affinis* (closed symbols) and *Parvocalanus crassirostris* (open symbols). Error bars are standard errors.

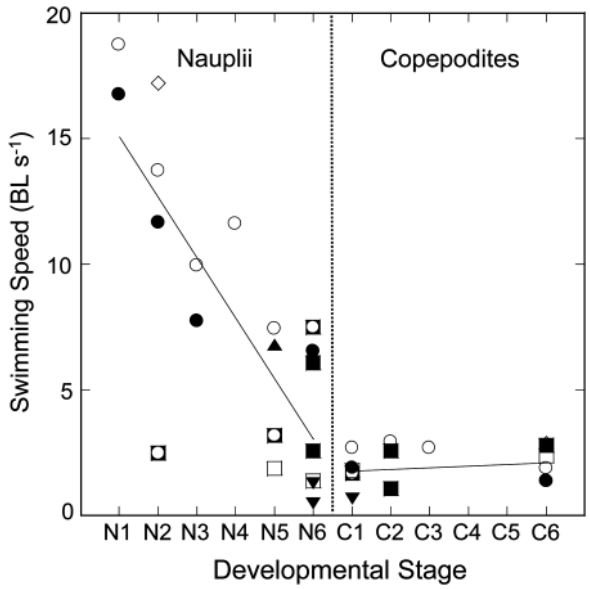

Fig. 13. Swimming speeds in body lengths s^{-1} for nauplii and copepodids by stage. Symbols: open circles, diamonds: *Eurytemora affinis* (present study, Titelman and Kiørboe, 2003b; Seuront, 2006), closed circles: *Parvocalanus crassirostris* (present study), triangles: *Calanus helgolandicus* (Titelman and Kiørboe, 2003b), inverted triangles: *Eucalanus hyalinus* and *E. pileatus* (Paffenhöfer *et al.*, 1996), open squares: *Centropages typicus* (Titelman and Kiørboe, 2003b) and *C. velificatus* (Paffenhöfer *et al.*, 1996), squares with filled circle inside: *Paracalanus* aculeatus, *P. quasimodo* (Paffenhöfer *et al.*, 1996), *P. parvus* (Waggett and Buskey, 2007), and squares with open circle inside: *Temora longicornis* (Titelman and Kiørboe, 2003a, b, van Duren and Videler, 2003), *T. stylifera* (Paffenhöfer *et al.*, 1996), *T. turbinata* (Waggett and Buskey, 2007). Swimming speeds that were comparable to published escape (or jump) speeds were not included in the graph. Lines were fitted separately for nauplii and copepodids.

providing start up phytoplankton cultures, L Ryckman for assistance with microphotography; C. Hyatt for assistance with cultures and staging of nauplii and copepodids. These experiments complied with the US laws governing animal experimentation.

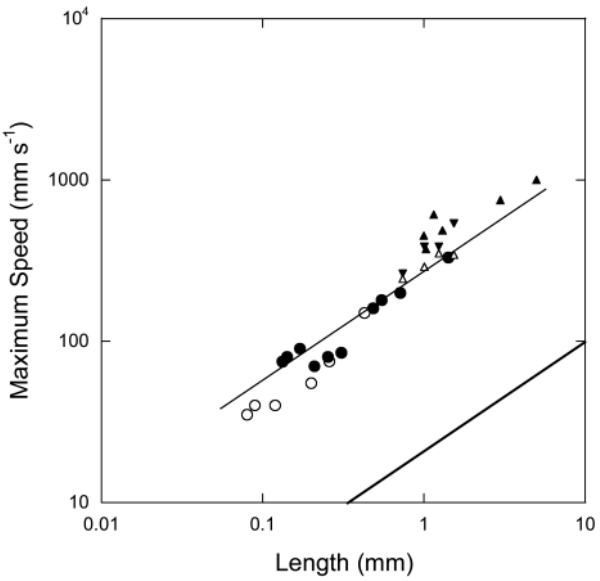

Fig. 14. Log-log plot of maximum escape speeds and copepod lengths for *Eurytemora affinis* (closed circles), *Parvocalanus crassirostris* (open circles). Published data (Burdick *et al.*, 2007) included responses to a rapidly moving bar stimulus (inverted closed triangles), suction (open triangles) and other (closed triangles) stimuli. Maximum escape speeds as a function of length for fishes shown by lower line (Lenz *et al.*, 2004).

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