

Zooplankton avoidance behaviour as a response to point sources of hydrocarbon-contaminated water

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Abstract. Hydrocarbon contamination is a pernicious threat for marine ecosystems as non-lethal effects on the plankton propagate through the food chain and accumulate in the tissues of top predators, ultimately putting human health at risk. The swimming behaviour of the calanoid copepods *Eurytemora affinis* and *Temora longicornis* was investigated in relation to point-source contamination by five different-sized patches of the water-soluble fraction of diesel oil diluted at 1 : 100, 1 : 1000 and 1 : 10 000 in estuarine and coastal waters. Both species consistently showed avoidance of the contaminated patches, irrespective of their size and concentration. Specifically, *E. affinis* exhibited similar sensory abilities irrespective of contaminant concentrations. In contrast, *T. longicornis* more efficiently identified high-density contaminated patches than low-density ones, and exhibited a negative exponential density dependence of its sensing abilities to the intensity of the chemical cues. Although the conclusions from this experiment need to be generalised to a variety of hydrocarbon contaminants, the present work indicates that zooplankton organisms have the potential to avoid hydrocarbon-contaminated waters, and also suggests that zooplankton swimming behaviour could potentially be used as an endpoint for a toxicity bioassay to assess the presence of toxic chemicals in estuarine and coastal waters.

Additional keywords: Diesel fuel oil, *Eurytemora affinis*, pollution, *Temora longicornis*, toxicity bioassay.

Introduction

Estuaries and coastal waters are increasingly impacted by a variety of anthropogenic activities, such as industry, marine transportation, fisheries and tourism. Consequently, estuarine and coastal ecosystems are threatened by a variety of pollutants. One of the most dramatic contaminations of estuarine and coastal waters is related to accidental crude oil spills, such as *Torry Canyon* (1967), *Amoco Cadiz* (1978), *Exxon Valdez* (1989), *Aegean Sea* (1992), *Nakhodka* (1997), *Erika* (1999) and *Pres-tige* (2002), that severely impacted coastal marine communities. However, leaks from ships, petroleum transport, refining and intentional spills are, among others, important sources of polycyclic aromatic hydrocarbons (PAHs) in the ocean (Fernandes *et al.* 1997; Cachot *et al.* 2006), particularly in coastal and shelf waters (Witt 1995; Doval *et al.* 2006).

Mass zooplankton mortalities have occasionally been related to oil slick episodes (e.g. Guzmán del Prío *et al.* 1986; Dale 1987). However, a more pernicious consequence of PAHs resides in their sublethal effects on zooplankton, in particular, the fractions of spilled oil that form: (i) oil slicks that spread quickly over the surface water and disperse into seawater; and (ii) water-soluble emulsions that affect zooplankton physiology, feeding and fecundity (e.g. Cowles and Remillard 1983; Barata *et al.* 2002; Calbet *et al.* 2007). However, despite the widely acknowledged acute chemosensory abilities of copepods reported to induce behavioural stress and subsequent adaptive responses

to a variety of chemical cues related to feeding, mate seeking and predator avoidance (Weissburg *et al.* 1998; De Meester and Weider 1999; Barry 2000), no attempts have been made to assess the effects of hydrocarbons on copepod behaviour.

Hence, the objectives of the present study were to assess the abilities of copepod species representative of estuarine and coastal waters to detect and potentially avoid contaminated waters. Specifically, given the acute chemosensory abilities of zooplankton organisms and the threat that hydrocarbon contamination represents for marine ecosystems, it is hypothesised that zooplankton swimming behaviour may be used as an indicator of sublethal contamination of estuarine and coastal waters by the water-soluble fraction of diesel oil. Zooplankton individuals should then avoid and escape contaminated patches more often than uncontaminated ones, and spend less time in contaminated than uncontaminated patches. The present study considers the effect of patch size (1–7 cm in diameter) and I hypothesised that zooplankton would avoid more and spend less time in patches of increasing contaminant concentration (i.e. 1 : 10 000, 1 : 1000 or 1 : 100 dilutions of filtered *in situ* water).

Materials and methods

Test organisms: the calanoid copepods *Eurytemora affinis* and *Temora longicornis*

To ensure the generality and ecological relevance of the present work, the species used in the experiments were two of the most

common zooplankton species found in estuarine and coastal waters of the northern hemisphere, the calanoid copepods *Eurytemora affinis* (Poppe 1880) and *Temora longicornis* (Müller, 1792). *Eurytemora affinis* is one of the most abundant zooplankton species in the brackish part of northern hemisphere estuaries; individuals are usually localised around the Maximum Turbidity Zone (Soetaert and Van Rijswijk 1993). This species plays a significant role in estuarine food webs as an important food supply for many fishes, shrimps and mysids (Fockede and Mees 1999). Similarly, *T. longicornis* is very abundant in coastal temperate waters of the northern hemisphere and plays a critical role in coastal ecosystem structure and function. It represents from 35 to 70% of the total population of copepods in the Southern Bight of the North Sea (Daan 1989) and is able to remove up to 49% of the daily primary production (Dam and Peterson 1993). Its naupliar stages contribute significantly to the diets of larval fish (Ringuette *et al.* 2002).

Eurytemora affinis individuals were collected from the Seine estuary using a WP2 net (200- μm mesh size; KC, Silkeborg, Denmark) at a temperature of 16°C in the low salinity (4) zone at low tide near the 'Pont de Normandie' (49°28'26"N, 0°27'47"W). *Temora longicornis* were collected with a WP2 (200- μm mesh size) from the inshore surface waters of the eastern English Channel (50°40'75"N, 1°31'1"E) at a temperature of 17°C and a salinity of 32. For both species, specimens were gently diluted in 30-L isotherm tanks using *in situ* estuarine water and seawater, respectively, and transported to the laboratory (Station Marine of Wimereux, France) where adult females were sorted by pipette under a dissecting microscope. Adult females of *E. affinis* and *T. longicornis* were then acclimated for 12 h at *in situ* temperature in two aquaria filled with natural estuarine and coastal waters filtered on Whatman GF/C glass-fibre filters (porosity 0.45 μm ; Whatman International Ltd., Maidstone, Kent, UK).

Hydrocarbon-contaminated stock solutions

The product considered as a potential contaminant of estuarine and coastal waters was commercial diesel fuel oil. The water-soluble fraction of diesel oil (i.e. WSF) was prepared according to the following procedure. For each behavioural experiment, 1.8 L of filtered *in situ* estuarine water and seawater (GF/C Whatman filter; 0.45- μm mesh size) was stirred with 0.2 L of commercial diesel fuel oil for 2 h at 100g. The mixed solution was allowed to stand for 24 h without stirring to separate the oil layer from the oil-saturated water. Using a siphon system, WSF stock solutions were gently drained into autoclaved, acid-rinsed glass containers and diluted with uncontaminated estuarine water and seawater at 'low' (1 : 100), 'medium' (1 : 1000) and 'high' (1 : 10 000) dilutions.

Avoidance test

The behavioural assay developed in the present work is based on the ability of *E. affinis* and *T. longicornis* adult females to avoid a patch of contaminated water occurring in natural, uncontaminated estuarine and marine water respectively. Behavioural experiments were conducted in a 3.375-L (15 cm \times 15 cm \times 15 cm) glass chamber. Patches of contaminated water were created in the centre of the chamber (i.e. the centre of the patch was located 7.5 cm from the walls of the chamber and 7.5 cm

from the surface of the water) as a contaminant point source by dispensing contaminated fluid from a fine pipette (Eppendorf) to flow down into a 5-mm diameter permeable sphere (20- μm pore size) that was suspended in the experimental container. Patches were created through the slow injection (<0.5 mL min⁻¹) of contaminated water in the porous sphere. Before the behavioural experiments, the amount of contaminated water to be injected to create the patches of different diameters (i.e. 1, 2, 4, 5.5 and 7 cm) was determined through the injection of fluorescently stained GF/C-filtered and autoclaved seawater and estuarine water into the experimental container. After injection, the 3-D properties of the fluorescent patches were recorded at a rate of 25 frames s⁻¹ using two orthogonally oriented and synchronised digital cameras (DV Sony DCR-PC120E; Tokyo, Japan) facing the experimental chamber. The spherical character of the patches was ensured by the non-significant differences in diameters estimated in the $x-y$, $y-z$ and $x-z$ planes for 10 patches (Kruskal-Wallis test, $P > 0.05$). In addition, the isotropic distribution of fluorescein within the patches was tested for each patch size by comparing the fluorescence intensities of 10 concentric rings of increasing diameters. For each patch size, 10 distinct patches were created and no significant differences were found between the concentric rings (Kruskal-Wallis test, $P > 0.05$). In addition, no significant differences in fluorescence intensities were found between patch sizes (Kruskal-Wallis test, $P > 0.05$). Finally, no significant increases in patch diameter were observed during a series of 1-h observations conducted immediately after the initial injection for low, medium and high dilutions of contaminated waters (Kendal τ , $P > 0.05$, $n = 10$). This is consistent with the changes in patch size resulting from molecular diffusion (i.e. <0.2 cm) during a 1-h experiment, assuming that the diffusion coefficient of the contaminated waters was 10⁻⁵ cm² s⁻¹ (Jackson and Kjørboe 2004).

Video set-up and behavioural experiments

For each WSF treatment, behavioural experiments were conducted in triplicate for each patch size, and an equivalent amount of natural water was injected into the experimental chambers for control observations. Preliminary experiments conducted with and without the injecting porous sphere did not exhibit any significant difference in the occurrence of *T. longicornis* and *E. affinis* in the middle of the experimental chamber (χ^2 -test, $P > 0.05$). Behavioural experiments were conducted in a temperature-controlled dark room. Before each experiment, 30 individuals of either *E. affinis* non-ovigerous adult females or *T. longicornis* adult females were transferred to the experimental filming set-up, filled with uncontaminated estuarine or coastal water and the corresponding contaminated patch, and allowed to acclimatise for 15 min (Seuront 2006). For both species, a new group of 30 females was used for each treatment.

Three-dimensional trajectories of *E. affinis* and *T. longicornis* adult females were recorded at a rate of 25 frames s⁻¹ using two orthogonally oriented and synchronised infrared digital cameras (DV Sony DCR-PC120E) facing the experimental chamber. Six arrays of 72 infrared light-emitting diodes (LEDs), each mounted on a printed circuit board about the size of a business card (i.e. 9.3 cm long \times 4.9 cm wide) connected to a 12-V DC power supply, provided the only light source from the bottom of the chamber. The cameras overlooked the experimental

chamber from the side, and the various components of the set-up were adjusted so that the copepods were adequately resolved and in focus. The two cameras represented the $x - y$ and the $y - z$ planes of the experimental chamber; 3-D swimming paths were obtained by combining information from the 2-D views.

Each experiment lasted 60 min, after which valid video clips were selected for analysis. Valid video clips consisted of pathways in which the animals were swimming freely, at least two body lengths away from any chamber walls or the surface of the water (Seuront 2006). All experiments were conducted at 16°C in the dark and at night to avoid any potential behavioural artefact related to the diel cycle of the copepods. The treatments (i.e. WSF concentration and patch size) were randomised.

Image analysis and behavioural analysis

Selected video clips were captured (DVgate Plus; Sony, Tokyo, Japan) at 25 frames s⁻¹ as MPEG movies and converted into QuickTime TM movies (QuickTime Pro; Apple, Cupertino, CA, USA); the x , y and z coordinates of the swimming pathways were automatically extracted and subsequently combined into a 3-D picture using LabTrack software (DiMedia, Kvistgård, Denmark). The time step was always 0.04 s, and output sequences of x , y , z coordinates were subsequently used to characterise motion behaviour.

The behavioural response of *E. affinis* and *T. longicornis* adult females to patches of contaminated water was first expressed as the percentage of individuals that avoided contaminated patches (i.e. patch avoidance behaviour) or escaped contaminated patches (i.e. patch escape behaviour) after entering them. In the present study, avoidance was identified as a sharp change in the direction of travel or the combination of a sharp change in swimming direction and an increase in swimming speed. Patch avoidance behaviour was specified by the distance at which individuals avoided contaminated patches, the avoidance turning angle $\alpha_{i,j}$ and avoidance velocity $u_{i,j}$ for a contaminated patch of diameter i ($i = 1, 2, 4, 5.5$ and 7 cm) and concentration j ($j = 1 : 100, 1 : 1000$ or $1 : 10\,000$). Patch escape behaviour was quantified by the escape turning angle $\beta_{i,j}$ and escape velocity $v_{i,j}$ for a contaminated patch of diameter i and concentration j . The significance of turning angles $\alpha_{i,j}$ and $\beta_{i,j}$ and velocities $u_{i,j}$ and $v_{i,j}$ was assessed inferring if $\bar{\alpha}_{i,c} < \bar{\alpha}_{i,j}$ and $\bar{\beta}_{i,c} < \bar{\beta}_{i,j}$, and if $\bar{u}_{i,c} < \bar{u}_{i,j}$ and $\bar{v}_{i,c} < \bar{v}_{i,j}$, where $\bar{\alpha}_{i,c}$ and $\bar{\beta}_{i,c}$ and $\bar{u}_{i,c}$ and $\bar{v}_{i,c}$ are the mean turning angles and swimming speed estimated outside and inside uncontaminated control patches of diameter i . No significant differences were observed between $\alpha_{i,c}$ and $\beta_{i,c}$ (Wilcoxon–Mann–Whitney U -test, $P > 0.05$) or between $\bar{u}_{i,c}$ and $\bar{v}_{i,c}$ (Wilcoxon–Mann–Whitney U -test, $P > 0.05$, $n = 288$), that is, $\bar{\alpha}_{i,c} = \bar{\beta}_{i,c}$ and $\bar{u}_{i,c} = \bar{v}_{i,c}$. The swimming speed was defined from the x , y , z coordinates as: $v_t = f_{sr} \times [(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2 + (z_{t+1} - z_t)^2]^{1/2}$, where (x_t, y_t, z_t) and $(x_{t+1}, y_{t+1}, z_{t+1})$ are the positions of a copepod at time t and $t + 1$ respectively. The turning angle between two successive moves was defined as (Fig. 1):

$$\theta = \arccos \left(\frac{\vec{A} \cdot \vec{B}}{\|\vec{A}\| \|\vec{B}\|} \right) \tag{1}$$

$$\theta_t = 180 - \frac{180}{\pi} \theta \tag{2}$$

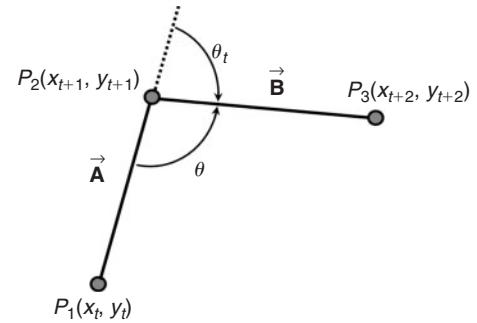


Fig. 1. Two-dimensional illustration of the calculation for turning angle between three locations (P_1 , P_2 and P_3), where \vec{A} and \vec{B} are the vectors between the points $P_1(x_t, y_t)$ and $P_2(x_{t+1}, y_{t+1})$, and between the points $P_2(x_{t+1}, y_{t+1})$ and $P_3(x_{t+2}, y_{t+2})$. The turning angle θ_t is estimated from Eqn (2).

where \vec{A} and \vec{B} are the vectors between the points $P_1(x_t, y_t, z_t)$ and $P_2(x_{t+1}, y_{t+1}, z_{t+1})$ and between the points $P_2(x_{t+1}, y_{t+1}, z_{t+1})$ and $P_3(x_{t+2}, y_{t+2}, z_{t+2})$, and $\|\vec{A}\|$ and $\|\vec{B}\|$ are the lengths of the vectors \vec{A} and \vec{B} , that is, $\|\vec{A}\| = [(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2 + (z_{t+1} - z_t)^2]^{1/2}$ and $\|\vec{B}\| = [(x_{t+2} - x_{t+1})^2 + (y_{t+2} - y_{t+1})^2 + (z_{t+2} - z_{t+1})^2]^{1/2}$.

The avoidance of contaminated patches was further quantified as the ratio $f_{i,j}$:

$$f_{i,j} = 100 \times \frac{N_{i,c}}{N_{i,j}} \tag{3}$$

where $N_{i,c}$ is the number of individuals that entered an uncontaminated patch of diameter i during the 1-h control experiment, and $N_{i,j}$ is the number of individuals that encountered a contaminated patch of diameter i and concentration j ($j = 1 : 100, 1 : 1000$ or $1 : 10\,000$) during the 1-h behavioural experiment. Finally, the patch residence time was estimated as the time that individual copepods spent in uncontaminated and contaminated patches.

Statistical analysis

The distribution of the behavioural parameters was significantly non-normal (Kolmogorov–Smirnov test, $P < 0.01$), even after log₁₀ or square-root transformations. Thus, non-parametric statistics were used throughout the present study. Comparisons between behavioural parameters inside and outside patches were conducted using a Wilcoxon–Mann–Whitney U -test (WMW test; Zar 1996). The effects of the size of the uncontaminated control patches were compared using a Kruskal–Wallis test (KW test; Zar 1996). The effects of contaminated patch size and concentration were investigated using the Scheirer–Ray–Hare extension of the Kruskal–Wallis test (SRH test; Sokal and Rohlf 1995). Appropriate multiple comparison procedures were subsequently used to test for differences between patch diameter and patch concentration. Correlation between variables was investigated using Kendall’s coefficient of rank correlation (τ ; Kendall and Stuart 1966).

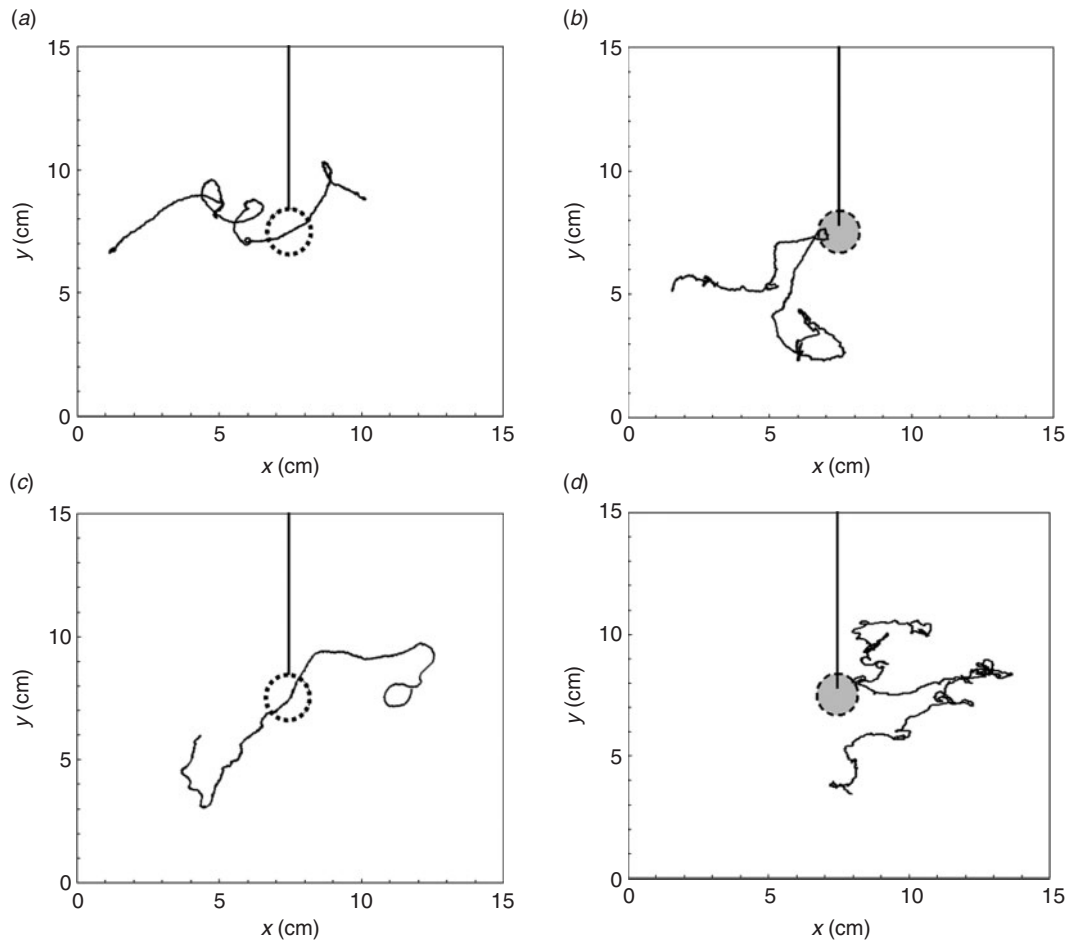


Fig. 2. Two-dimensional views of (a, b) *Eurytemora affinis* and (c, d) *Temora longicornis* swimming paths for 1 cm in diameter (a, c) uncontaminated and (b, d) contaminated patches. Behavioural responses to contaminated patches include (b) patch escape behaviour and (d) patch avoidance behaviour. The contaminated patches correspond to a 1 : 100 dilution of the water-soluble fraction of diesel oil in filtered (a, b) estuarine water and (c, d) coastal seawater.

Results

Escape and avoidance of hydrocarbon-contaminated patches

The swimming paths of *Eurytemora affinis* and *Temora longicornis* adult females were not affected by uncontaminated control patches (Fig. 2a, c), whereas both species consistently avoided the contaminated patches (Fig. 2b, d), for the different patch sizes considered (Fig. 3). The distances at which individuals exhibited patch avoidance were not affected by patch size (SHR test, $P > 0.05$) or patch concentration (SHR test, $P > 0.05$), but were significantly higher ($P < 0.05$) for *E. affinis* (2.2 ± 0.2 mm, $\bar{x} \pm$ s.d.) than for *T. longicornis* (1.7 ± 0.1 mm).

In the control experiments, no avoidance and no escape behaviours were observed for the different sizes of uncontaminated patches (Fig. 2a, b). In contrast, the percentages of individuals that exhibited patch avoidance behaviour (Fig. 2d) were not significantly different between patch sizes and concentrations ($P > 0.05$) for either *E. affinis* ($95.2 \pm 2.1\%$) or *T. longicornis* ($97.3 \pm 1.4\%$). For both species, 100% of the

females that entered a patch exhibited an escape behaviour (Fig. 2b). Turning angles estimated inside the contaminated patch were consistently significantly higher than those estimated outside the contaminated patch (WMW test, $P > 0.05$). In addition, the mean turning angle and swimming speed of both species were not affected by the size of the uncontaminated patch (KW test, $P > 0.05$). *Temora longicornis* turning angles ($43.2 \pm 5.3^\circ$) were, however, significantly higher than *E. affinis* turning angles ($29.7 \pm 2.4^\circ$). The avoidance turning angle $\alpha_{i,j}$ and avoidance velocity $v_{i,j}$ did not differ with the size or the concentration of the contaminated patches (SHR test, $P > 0.05$) for *E. affinis* ($\alpha_{i,j} = 65.2 \pm 2.3^\circ$ and $\bar{v}_{i,j} = 8.5 \pm 0.6$ mm s $^{-1}$) or *T. longicornis* ($\alpha_{i,j} = 68.3 \pm 1.7^\circ$ and $\bar{v}_{i,j} = 12.5 \pm 0.8$ mm s $^{-1}$). For both species, the avoidance turning angle α_i and velocity v_i were significantly higher ($P < 0.01$) than the mean turning angle $\bar{\alpha}_{i,c}$ and swimming speed $\bar{v}_{i,c}$ recorded for the uncontaminated patches, that is, $\bar{\alpha}_{i,c} = 37.3 \pm 1.2^\circ$ and $\bar{v}_{i,c} = 1.7 \pm 0.3$ mm s $^{-1}$ for *E. affinis* and $\bar{\alpha}_{i,c} = 42.1 \pm 1.1^\circ$ and $\bar{v}_{i,c} = 2.5 \pm 0.4$ mm s $^{-1}$ for *T. longicornis*.

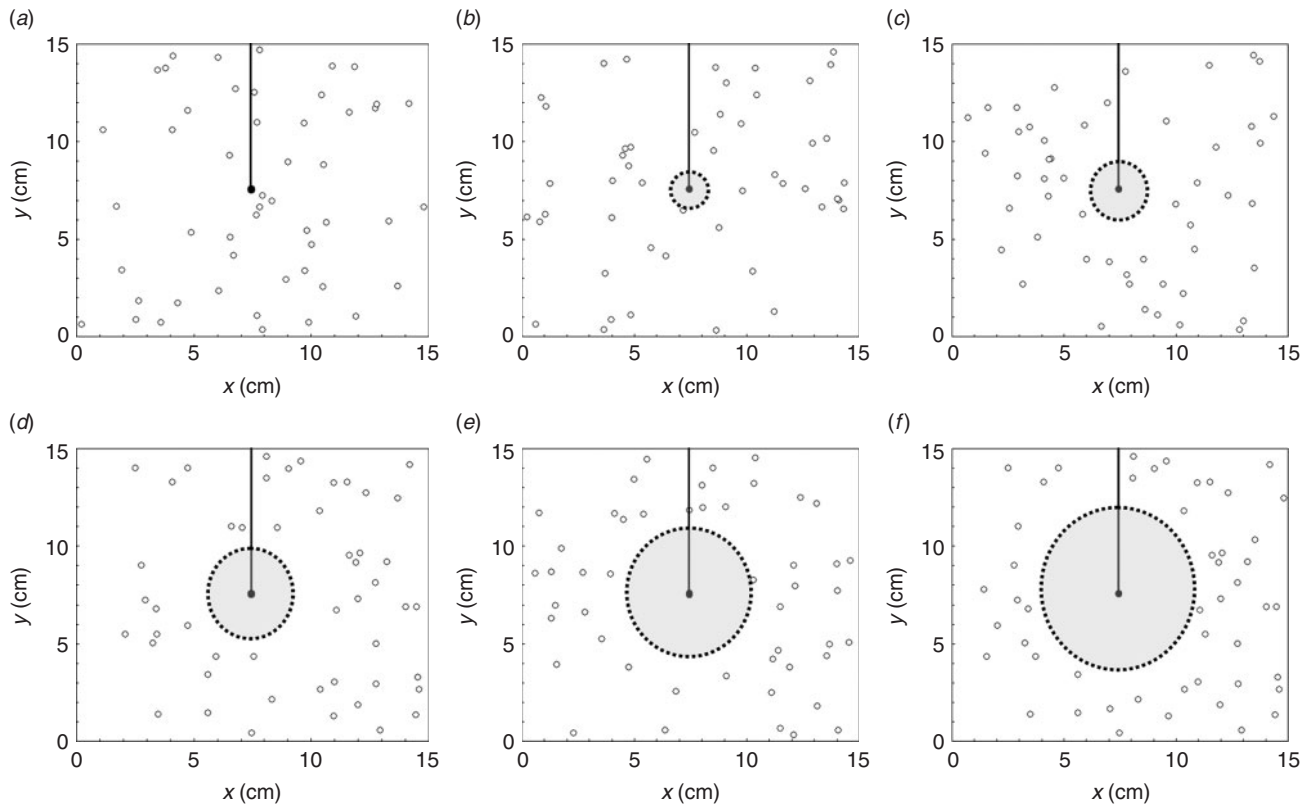


Fig. 3. Two-dimensional snapshots of the position of 50 *Temora longicornis* adult females in relation to the position of (a) the point-source injection system and (b–f) contaminated patches of increasing size. The contaminated patches correspond to a 1 : 1000 dilution of the water-soluble fraction of diesel oil in filtered seawater.

The ratio $f_{i,j}$ was not affected by patch size (SRH test, $P > 0.05$) or patch concentration (SRH test, $P > 0.05$) for *E. affinis* (Fig. 4a), with $f_{i,1:100} = 10.3 \pm 0.6\%$, $f_{i,1:1000} = 10.2 \pm 1.3\%$ and $f_{i,1:10000} = 10.4 \pm 1.4\%$. In contrast, the percentage of *T. longicornis* entering the contaminated patches (Fig. 4b) was significantly affected by the patch concentration (SRH test, $P < 0.01$), but not by the patch size (SRH test, $P > 0.05$), leading to $f_{i,1:10000} = 4.4 \pm 0.9\%$, $f_{i,1:1000} = 13.2 \pm 1.1\%$ and $f_{i,1:100} = 26.0 \pm 1.3\%$. For each patch size, the frequency $f_{i,j}$ increases exponentially with increasing patch concentration ($r^2 > 0.98$, $P < 0.01$).

Residence time in uncontaminated v. hydrocarbon-contaminated patches

The residence time (t) of both *E. affinis* and *T. longicornis* in the uncontaminated control patches significantly increased with the size of the patch (Kendall τ , $P < 0.05$; Fig. 3). *Eurytemora affinis* adult females (Fig. 5b) did not show any significant differences in patch residence time between patch sizes or among patch sizes for different contaminant concentrations (SHR test, $P > 0.05$). When examined by contaminant concentration, the patch residence time of *T. longicornis* was not affected by the size of the contaminated patch (SHR test, $P > 0.05$). However, it did differ among patch sizes (SHR test, $P < 0.05$), and the residence time significantly increased with contaminant concentration, that is, $t_{1:100} = 0.61 \pm 0.02$ s,

$t_{1:1000} = 0.70 \pm 0.02$ s and $t_{1:10000} = 0.83 \pm 0.03$ s ($P < 0.05$; Fig. 5b).

Discussion

Eurytemora affinis and *Temora longicornis* avoidance behaviour

Eurytemora affinis and *Temora longicornis* exhibited systematic behavioural responses (i.e. avoidance and escape behaviours) to the contaminated patches, whereas the uncontaminated patches had no effect on their behaviour (Fig. 2). As hypothesised, this clearly indicates that *E. affinis* and *T. longicornis* avoid and escape contaminated patches more often than uncontaminated ones and spend less time in contaminated than uncontaminated patches. It also ensures the relevance of the present approach to infer the effect of a contaminant point source on copepod swimming behaviour. Behavioural alterations were observed for concentrations of the water-soluble fraction of diesel oil ranging from 0.01 to 1%. This demonstrates the very acute chemosensory abilities of both *E. affinis* and *T. longicornis*, and generalises previous work showing behavioural changes elicited by a variety of chemical cues. *Eurytemora affinis* and *T. longicornis* adult males have the ability to locate a remote female based on her pheromone trail (Katona 1973; Doall *et al.* 1998; Weissburg *et al.* 1998; Yen *et al.* 1998) or to modify their behaviour in the presence of a female's pheromones (Yen *et al.* 2004). More recently, both *T. longicornis* and *E. affinis* have been shown to alter their

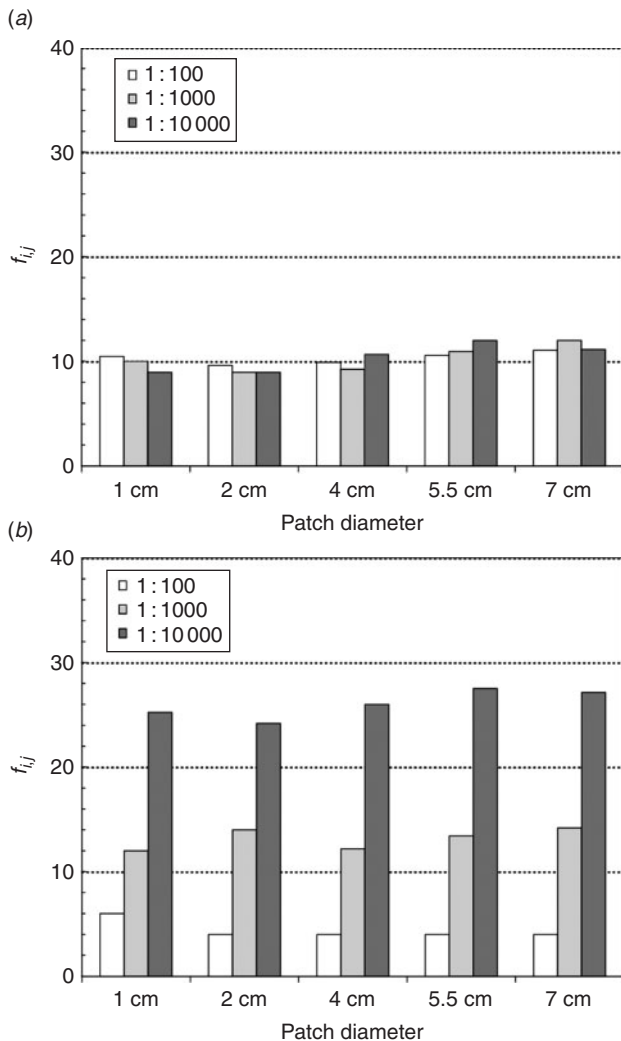


Fig. 4. The ratio $f_{i,j}$ between the number of individuals that entered an uncontaminated patch of diameter i during the 1-h behavioural experiment and the number of individuals that entered a contaminated patch of diameter i and concentration j during the 1-h behavioural experiment for (a) *Eurytemora affinis* in estuarine water and (b) *Temora longicornis* in coastal seawater. The contaminated patches correspond to 1:100, 1:1000 or 1:10000 dilutions of the water-soluble fraction of diesel oil in filtered water samples in each experiment.

motion behaviour in the presence of a chemical gradient related to salinity, phytoplankton and phytoplankton exudates (Woodson *et al.* 2007a, 2007b).

However, in the present work, *E. affinis* and *T. longicornis* exhibited specific behaviours as a function of the size and the concentration of contaminated patches. The proportion of *E. affinis* adult females entering the contaminated patches remained very low (i.e. $\sim 10\%$) and did not exhibit any significant differences as a function of the size and the concentration of the contaminated patches (Fig. 4a). In contrast, the proportion of *T. longicornis* adult females entering a contaminated patch was dependent on the patch concentration, but not on the patch size (Fig. 4b). This resulted in a decrease in the frequency of individuals entering contaminated patches of increasing concentration

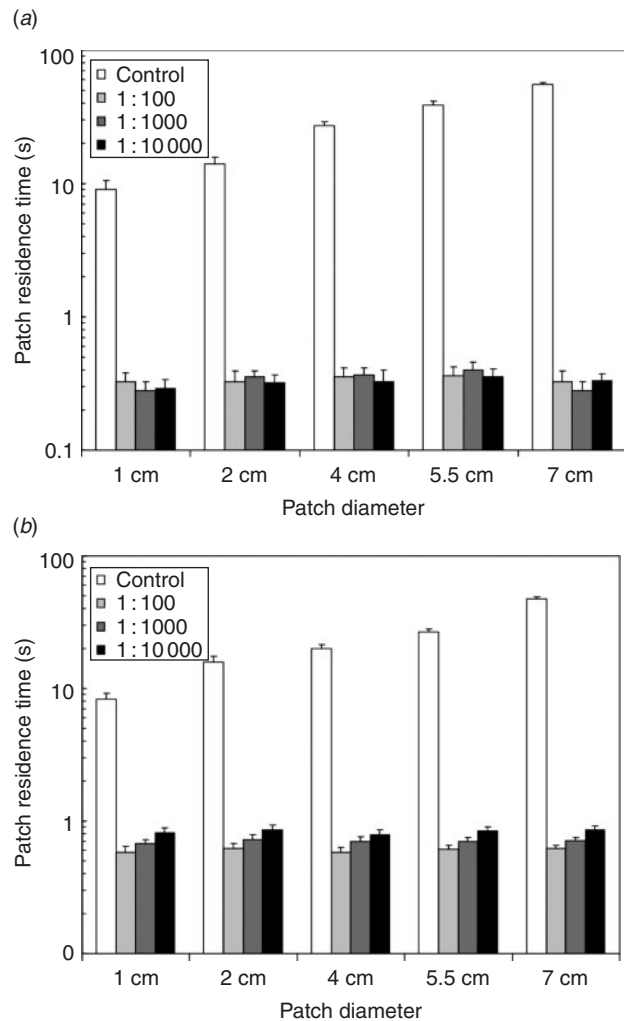


Fig. 5. Time spent by (a) *Eurytemora affinis* in estuarine water and (b) *Temora longicornis* in coastal seawater in uncontaminated control patches (white bars) and in contaminated patches of increasing diameter. The contaminated patches correspond to 1:100, 1:1000 or 1:10000 dilutions of the water-soluble fraction of diesel oil in filtered water in each experiment.

from $\sim 26.0\%$ to 4.5% for patches contaminated at the 0.01 and 1% levels respectively. This suggests that *E. affinis* has the same sensory abilities irrespective of the concentrations of the contaminant, whereas *T. longicornis* more efficiently identifies high-density contaminated patches than low-density ones. Thus, the highly significant exponential decay observed in the frequency $f_{i,j}$ with decreasing contaminant concentration might indicate a negative exponential density-dependence of the sensing abilities of *T. longicornis* to the intensity of chemical cues. This is also consistent with the significantly longer residence time of *T. longicornis* adult females in contaminated patches (Fig. 5a, b), and with the increase in the time spent by *T. longicornis* in patches of decreasing contaminant concentration (Fig. 5b). As hypothesised, zooplankton individuals should then spend less time in contaminated than uncontaminated patches. This has been generalised considering the effect of patch size (1–7 cm in diameter) and hypothesising that zooplankton would avoid

more and spend less time in patches of increasing contaminant concentration (i.e. 1 : 10 000, 1 : 1000 or 1 : 100 dilutions of filtered *in situ* water). As originally hypothesised, the avoidance and escape behaviours of *E. affinis* and *T. longicornis* are more pronounced for patches of high-contaminant concentrations, whereas their patch residence time significantly decreases with the concentration of the contaminant.

Implications for plankton communities and water-quality assessment

The two copepod species considered in the present study, *E. affinis* and *T. longicornis*, exhibited unambiguous patch avoidance strategies for concentrations of the WSF of commercial fuel oil ranging from 0.01 to 1%. These results have potentially critical ecological and practical consequences. The ability of copepods to acutely detect and avoid hydrocarbon patches at very low concentrations might suggest that they may escape contaminated areas following massive and localised oil spills, such as surface oil slicks, and as such, minimise the ecological effects of incidental pollution. A direct consequence of the observed avoidance strategy would be to decrease the long-term cumulative effect of hydrocarbon exposure, its propagation through the food chain and its accumulation in the tissues of top predators. Although the swimming behaviour of *Daphnia* sp. has repeatedly been suggested to be an indicator of exposure to toxic chemicals (Goodrich and Lech 1990; Dodson *et al.* 1995; Baillieul *et al.* 1998; Piao *et al.* 2000), most marine bioassays still rely on exposure times of 24–48 h to determine: (i) the concentration of a test chemical at which 50% of neonates die or are immobilised; or (ii) the number of individuals that died (Calbet *et al.* 2007). The avoidance test presented here suggests that the short-term (i.e. 1 h) swimming behaviour of copepods could be used to assess the presence of toxic substances in estuarine and marine environments, and to prevent long-term detrimental effects on the ecosystem.

Given the large variety of micro-pollutants impacting estuarine and coastal waters, including PAHs (Cachot *et al.* 2006), organochlorines (Chevreuil *et al.* 1996), polychlorinated biphenyls (Carpentier *et al.* 2002; Meybeck *et al.* 2004), phytoestrogens (Pawlowski *et al.* 2003, 2004) and heavy metals (Grosbois *et al.* 2006), it is acknowledged that further work is needed to ensure the generality of zooplankton behaviour susceptibility to pollutants. In addition, the present study uses precise patch structures and no phytoplankton were present in the experiments, whereas in the real world contaminant patches would be stretched and dispersed by turbulence and a range of phytoplankton species would be present. As a hierarchical use of different cues has been demonstrated for *T. longicornis*, but not for *E. affinis* (Woodson *et al.* 2007b), the potential hierarchy of contaminants and phytoplankton in triggering behavioural responses needs to be thoroughly investigated.

In conclusion, the short exposure time and low concentration for detection of hydrocarbon-contaminated patches demonstrated in the present work suggest that changes in zooplankton swimming behaviour might be taken as an endpoint for an acute toxicity bioassay. This could be particularly useful in the framework of long-term observations that provide a very detailed quantitative description of zooplankton swimming behaviour as a function of a range of naturally occurring biotic and abiotic cues

(e.g. Seuront and Vincent 2008). Any significant divergence to the baseline behaviours could act as the first stage of an early warning system for seawater contamination.

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References

- Baillieul, M., de Wachter, B., and Blust, R. (1998). Effect of salinity on the swimming velocity of the water flea *Daphnia magna*. *Physiological Zoology* **71**, 703–707.
- Barata, C., Baird, D. J., Medina, M., Albalat, A., and Soares, A. M. V. M. (2002). Determining the ecotoxicological mode of action of toxic chemicals in meiobenthic marine organisms: stage specific short tests with *Tisbe battagliai*. *Marine Ecology Progress Series* **230**, 183–194. doi:10.3354/MEPS230183
- Barry, M. J. (2000). Effects of endosulfan on *Chaoborus*-induced life-history shifts and morphological defenses in *Daphnia pulex*. *Journal of Plankton Research* **22**, 1705–1718. doi:10.1093/PLANKT/22.9.1705
- Cachot, J., Geffard, O., Augagneur, S., Lacroix, S., Le Menach, K., Peluhet, J., Couteau, J., Denier, X., Pottier, D., and Budzinski, H. (2006). Evidence of genotoxicity related to high PAH content of sediments in the upper part of the Seine estuary (Normandy, France). *Aquatic Toxicology (Amsterdam, Netherlands)* **79**, 257–267. doi:10.1016/J.AQUATOX.2006.06.014
- Calbet, A., Saiz, E., and Barata, C. (2007). Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod *Paracartia grani*. *Marine Biology* **151**, 195–204. doi:10.1007/S00227-006-0468-0
- Carpentier, S., Moilleron, R., Beltran, C., Hervé, D., and Thévenot, D. (2002). Quality of dredged material in the river Seine basin (France) II micropollutants. *The Science of the Total Environment* **299**, 57–72. doi:10.1016/S0048-9697(02)00278-4
- Chevreuil, M., Blanchard, M., Teil, M.-J., Carru, A.-M., Testard, P., and Chesterikoff, A. (1996). Evaluation of the pollution by organochlorinated compounds (polychlorobiphenyls and pesticides) and metals (Cd, Cr, Cu and Pb) in the water and in the zebra mussel (*Dreissena polymorpha*) of the river Seine. *Water, Air, and Soil Pollution* **88**, 371–381. doi:10.1007/BF00294112
- Cowles, T. J., and Remillard, J. F. (1983). Effects of exposure to sublethal concentrations of crude oil on the copepod *Centropages hamatus*. 1. Feeding and egg production. *Marine Biology* **78**, 45–51. doi:10.1007/BF00392970
- Daan, R. (1989). Factors controlling the summer development of the copepod populations in the southern bight of the North Sea. *Netherlands Journal of Sea Research* **23**, 305–322. doi:10.1016/0077-7579(89)90051-3
- Dale, T. (1987). Oil pollution and plankton dynamics. 2. Abundance pattern of ciliates inside and outside enclosures and the responses of ciliates to oil during the 1980 spring bloom in Lindaaspollene Norway. *Sarsia* **72**, 197–202.
- Dam, H. G., and Peterson, W. T. (1993). Seasonal contrast in the diel vertical distribution, feeding and grazing impact of the copepod *Temora longicornis* in Long Island Sound. *Journal of Marine Research* **51**, 561–594. doi:10.1357/0022240933223972
- De Meester, L., and Weider, L. J. (1999). Depth selection behaviour, fish kairomones, and the life histories of *Daphnia hyalina-galeata* hybrid clones. *Limnology and Oceanography* **44**, 1248–1258.

- Doall, M. H., Colin, S. P., Strickler, J. R., and Yen, J. (1998). Locating a mate in 3D: the case of *Temora longicornis*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **353**, 681–689. doi:10.1098/RSTB.1998.0234
- Dodson, S. I., Hanazato, T., and Gorski, P. R. (1995). Behavioral responses of *Daphnia pulex* exposed to carbaryl and *Chaoborus* kairomone. *Environmental Toxicology and Chemistry* **14**, 43–50. doi:10.1897/1552-8618(1995)14[43:BRODPE]2.0.CO;2
- Doval, M. D., Morono, A., Pazos, Y., Lopez, A., Madrinan, M., Cabanas, J. M., and Maneiro, J. (2006). Monitoring dissolved aromatic hydrocarbons in Rias Baixas embayments (NW Spain) after Prestige oil spills: relationship with hydrography. *Estuarine, Coastal and Shelf Science* **67**, 205–218. doi:10.1016/J.ECSS.2005.11.013
- Fernandes, M. B., Sicres, M.-A., Boireau, A., and Tronczynski, J. (1997). Polyaromatic hydrocarbon (PAH) in the Seine River and its estuary. *Marine Pollution Bulletin* **34**, 857–867. doi:10.1016/S0025-326X(97)00063-5
- Fockede, N., and Mees, J. (1999). Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. *Journal of Marine Systems* **22**, 207–228. doi:10.1016/S0924-7963(99)00042-1
- Goodrich, M. S., and Lech, J. J. (1990). A behavioral screening assay for *Daphnia magna*: a method to assess the effects of xenobiotics on spatial orientation. *Environmental Toxicology and Chemistry* **9**, 21–30. doi:10.1897/1552-8618(1990)9[21:ABSAFD]2.0.CO;2
- Grosbois, C., Meybeck, M., Horowitz, A., and Ficht, A. (2006). The spatial and temporal trends of Cd, Cu, Hg, Pb and Zn in the Seine river floodplain deposits (1994–2000). *The Science of the Total Environment* **356**, 22–37. doi:10.1016/J.SCITOTENV.2005.01.049
- Guzmán del Prío, S. A., Chávez, E. A., Alatríste, F. M., de la Campa, S., Gómez, L., Guadarrama, R., Guerra, A., Mille, S., and Torruco, D. (1986). The impact of Ixtoc-1 oil spill on zooplankton. *Journal of Plankton Research* **8**, 557–581. doi:10.1093/PLANKT/8.3.557
- Jackson, G. A., and Kjørboe, T. (2004). Zooplankton use of chemoreception to find and eat particles. *Marine Ecology Progress Series* **269**, 153–162. doi:10.3354/MEPS269153
- Katona, S. K. (1973). Evidence for sex pheromones in planktonic copepods. *Limnology and Oceanography* **18**, 574–583.
- Kendall, M., and Stuart, A. (1966). 'The Advanced Theory of Statistics.' (Hafner: New York.)
- Meybeck, M., Horowitz, A., and Grobois, C. (2004). The geochemistry of the Seine River Basin particulate matter distribution patterns of an integrated metal pollution index. *The Science of the Total Environment* **328**, 219–236. doi:10.1016/J.SCITOTENV.2004.01.024
- Pawlowski, S., Ternes, T., Bonerz, M., Kluczaka, T., van der Burg, B., Nau, H., Erdinger, L., and Braunbeck, T. (2003). Combined *in situ* and *in vitro* assessment of the estrogenic activity of sewage and surface water samples. *Toxicological Sciences* **75**, 57–65. doi:10.1093/TOXSCI/KFG162
- Pawlowski, S., Ternes, T., Bonerz, M., Rastall, A. C., Erdinger, L., and Braunbeck, T. (2004). Estrogenicity of solid phase-extracted water samples from two municipal sewage treatment plant effluents and river Rhine water using the yeast estrogen screen. *Toxicology In Vitro* **18**, 129–138. doi:10.1016/J.TIV.2003.08.006
- Piao, M., Okamura, H., Luo, R., and Aoyama, I. (2000). Interactive effect of heavy metals and pesticide on *Daphnia magna*. *Japanese Journal of Environmental Toxicology* **3**, 23–32.
- Ringuette, M., Castonguay, M., Runge, J. A., and Gregoire, F. (2002). Atlantic mackerel (*Scomber scombrus*) recruitment fluctuations in relation to copepod production and juvenile growth. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 646–656. doi:10.1139/F02-039
- Seuront, L. (2006). Effect of salinity on the swimming behaviour of the estuarine calanoid copepod *Eurytemora affinis*. *Journal of Plankton Research* **28**, 805–813. doi:10.1093/PLANKT/FBL012
- Seuront, L., and Vincent, D. (2008). Impact of a *Phaeocystis globosa* spring bloom on *Temora longicornis* feeding and swimming behaviours. *Marine Ecology Progress Series* **363**, 131–145. doi:10.3354/MEPS07373
- Soetaert, K., and Van Rijswijk, P. (1993). Spatial and temporal patterns in the Westerschelde estuary. *Marine Ecology Progress Series* **97**, 47–59. doi:10.3354/MEPS097047
- Sokal, R. R., and Rohlf, F. J. (1995). 'Biometry: the Principles and Practice of Statistics in Biological Research.' (Freeman: San Francisco.)
- Weissburg, M. J., Doall, M. H., and Yen, J. (1998). Following the invisible trail: kinematic analysis of mate tracking in the copepod *Temora longicornis*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **353**, 701–712. doi:10.1098/RSTB.1998.0236
- Witt, G. (1995). Polycyclic aromatic hydrocarbons in water and sediments of the Baltic Sea. *Marine Pollution Bulletin* **31**, 237–248. doi:10.1016/0025-326X(95)00174-L
- Woodson, C. B., Webster, D. R., Weissburg, M. J., and Yen, J. (2007a). Cue hierarchy and foraging in calanoid copepods: ecological implications of oceanographic structure. *Marine Ecology Progress Series* **330**, 163–177. doi:10.3354/MEPS330163
- Woodson, C. B., Webster, D. R., Weissburg, M. J., and Yen, J. (2007b). The prevalence and implications of copepod behavioral responses to oceanographic gradients and biological patchiness. *Integrative and Comparative Biology* **47**, 831–846. doi:10.1093/ICB/ICM091
- Yen, J., Weissburg, M. J., and Doall, M. H. (1998). The fluid physics of signal perception by a mate-tracking copepod. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **353**, 787–804. doi:10.1098/RSTB.1998.0243
- Yen, J., Prusak, A. C., Caun, M., Doall, M., Brown, J., and Strickler, R. (2004). Signaling during mating in the pelagic copepod *Temora longicornis*. In 'Handbook of Scaling Methods in Aquatic Ecology: Measurement, Analysis, Simulation'. (Eds L. Seuront and P. G. Strutton.) pp. 149–160. (CRC Press: Boca Raton.)
- Zar, J. H. (1996). 'Biostatistical Analysis.' (Prentice-Hall International: Englewood Cliffs.)

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