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Disruption of swimming in the hyperbenthic mysid *Neomysis integer* (Peracarida: Mysidacea) by the organophosphate pesticide chlorpyrifos

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Abstract

Mysids are used routinely by regulatory authorities for conducting 96 h LC_{50} toxicity tests to evaluate the potential hazards of pollutants to aquatic ecosystems. Data from these acute tests suggest that the European estuarine mysid *Neomysis integer* (Peracarida: Mysidacea) is comparatively sensitive to trace metals and organophosphate pesticides, and may be a suitable alternative to the frequently-used sub-tropical American mysid *Americamysis* (≡ *Mysidopsis*) *bahia* for testing the toxicity of chemical contaminants to European estuarine biota. There is, however, growing demand for the development of toxicity tests which are more representative of the effects of toxic contaminants on natural populations, and which provide results that are more readily extrapolated to natural ecosystems, than acute tests. Behavioural disruption, particularly of swimming ability, is used increasingly in laboratory toxicity studies as a sensitive endpoint for assessing the effects of contaminants on aquatic biota. This paper describes a sensitive laboratory technique, using an annular flume, to determine the effects of an organophosphate pesticide on the swimming behaviour of *N. integer*. Following 7-day exposure to 0.038 μg chlorpyrifos l^{-1}, mysids became hyperactive and more swam forward into a slow current (3 cm s^{-1}) than control mysids. Despite this hyperactivity, pesticide-exposed mysids were unable to swim faster than 15 cm s^{-1}, whereas control mysids were able to swim faster than 18 cm s^{-1}. Other changes in swimming behaviour following pesticide exposure included fewer mysids maintaining position, and more mysids swimming with the current at high current velocities (18 cm s^{-1}), than control individuals. These responses of chlorpyrifos-exposed *N. integer* are predicted to cause reduced ability of *N. integer* to maintain position in the natural estuarine habitat. Furthermore, the swimming behaviour of *N. integer* was affected at pesticide concentrations below the 7 day LC_{50} (0.084 μg chlorpyrifos l^{-1}), highlighting the importance of using sub-lethal toxicity studies for predicting environmental consequences of pollutant discharge. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Mysids; Swimming ability; Chlorpyrifos; Toxicity test; Annular flume; Rheotaxis

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1. Introduction

In the natural environment, concentrations of individual toxic contaminants are generally much lower than the LC_{50} (median lethal concentration) values estimated in the laboratory (Gaudy et al., 1991). Although acute toxicity tests provide a simple index of contaminant toxicity for regulatory authorities, they are often criticized because they provide little relevant information on the effects of toxicants in the natural environment. Measurement of an organism's behaviour following contaminant exposure provides a better understanding of the likely environmental consequences of toxic contamination than lethal effects, especially if behaviour is disrupted at environmentally-realistic toxicant concentrations. Several behavioural responses have been used to assess toxicant effects on various test organisms, including distance travelled, travel velocity, frequency of direction change, time spent active, response to light and tail-flip escape response (e.g. Farr, 1977; Lang et al., 1981; Sørensen et al., 1995; Bayley and Baatrup, 1996; Wilson, 1996). Swimming speed is perhaps the most frequently used 'behavioural' measure of an aquatic organism's physiological status (e.g. Wilson and Wood, 1992; Wilson, 1996). In particular, many studies of swimming behaviour have focused on swimming capacity or ability and forced swimming. However, most of these studies have been made using teleost fish (e.g. Little and Finger, 1990; Beaumont et al., 1995). Similar information for aquatic invertebrates is limited. Mysids are used frequently in laboratory toxicity tests (e.g. USEPA, 1987; ASTM, 1990) but, despite a large literature on their swimming behaviour (e.g. Zelickman, 1974; Hough and Naylor, 1992; Schlacher and Wooldridge, 1994; Roast et al., 1998a), there are few published data on the effects of toxicant exposure on mysid swimming.

Neomysis integer (Peracarida: Mysidacea) is a common member of the permanent hyperbenthic fauna of the upper reaches of European estuaries (Mees et al., 1994). Recently, the swimming behaviour of *N. integer* (e.g. rheotaxic response, swimming ability and proximity to the substratum) has been examined using an annular flume (Roast et al., 1998a). The hyperbenthic nature and positive rheotaxic response of *N. integer* make this species a good candidate for swimming behaviour studies. Furthermore, acute toxicity tests have revealed that *N. integer* is comparatively sensitive to various toxic contaminants including trace metals [e.g. 96 h LC_{50} 5.3 μg $Cd^{2+}_{(aq)}$ l^{-1} (Wildgust and Jones, 1998)] and pesticides [96 h LC_{50} 0.54 mg dimethoate l^{-1} (Roast et al., 1999)]. Indeed, *N. integer* may be a suitable alternative species to the frequently-used American mysid, *Americamysis* (≡ *Mysidopsis*) *bahia*, for assessing the effects of toxicants on European estuarine biota (Emson and Crane, 1994; Roast et al., 1998b). Most toxicity studies with *N. integer* have been acute (LC_{50}) tests and there are few sub-lethal data for this species. In previous flume studies, the main factor affecting the position maintenance of *N. integer* appeared to be the ability of mysids to swim against a current (Roast et al., 1998a). Following exposure to two organophosphate pesticides, there was a reduction in the maximum swimming speed of *A. bahia* (Cripe et al., 1981). Therefore, similar toxicant-induced reduction in the swimming capacity of *N. integer* is predicted to disrupt its ability to maintain position, possibly leading to mortalities in the natural environment (e.g. due to mysids being swept into unfavourable parts of the estuary). If the swimming behaviour of *N. integer* is affected at toxicant concentrations much lower than LC_{50} estimates, and which are environmentally realistic, comparison of swimming behaviours may prove to be a useful measure of toxicant exposure.

In the present study, an annular flume (Widdows et al., 1998) was used to assess the swimming behaviour of *Neomysis integer* following exposure to the organophosphate pesticide chlorpyrifos (Dursban™, DowElanco). Chlorpyrifos (*O,O*,-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is a widely used, broad spectrum, non-systemic organophosphorothioate insecticide which inhibits the production of acetylcholine esterase at nerve synapses (Barron and Woodburn,

1995; Whitehead, 1997). Chlorpyrifos is comparatively toxic to *N. integer* [96 h LC_{50} 0.14 μg chlorpyrifos l^{-1} (Roast et al., 1999)] and, assuming that cholinergic neural pathways are involved in mysid locomotion, exposure to chlorpyrifos was predicted to have a detrimental effect on the swimming behaviour of *N. integer*. The aim of the present study was to examine the effects of exposure to low concentrations of chlorpyrifos on the swimming behaviour of *N. integer* and to use the results to predict effects of this pesticide on natural mysid populations.

2. Materials and methods

2.1. Collection and maintenance of mysids

Neomysis integer were collected from the southern side of Terras Bridge, East Looe River Estuary, Cornwall (UK) by sweeping an FBA net (1 mm mesh) along the water's edge at low tide (Roast et al., 1998a). Mysids were returned to the laboratory in habitat water (salinity = c. 1) and placed in a 15 l holding tank at a salinity of 10 (± 1) (prepared by combining filtered seawater with double-distilled, de-ionised water) in a constant-temperature room (15 ± 1°C). Under-gravel and Eheim-filters™ were used to maintain water quality. Lighting was provided at ambient laboratory levels by overhead fluorescent lights connected to a time-switch producing a 16 h light:8 h dark photoperiod (Roast, 1997). Mysids were fed *ad libitum* on <48 h old *Artemia* sp. (Great Lakes, Utah) nauplii, hatched in the laboratory from cysts.

2.2. Exposure of mysids to chlorpyrifos

Mysids were exposed to various concentrations of chlorpyrifos for 168 h. The 168 h LC_{50} estimate for *Neomysis integer* is 0.084 μg chlorpyrifos l^{-1} (Roast et al., 1999). Three sub-lethal concentrations (0.038, 0.056, 0.072 μg chlorpyrifos l^{-1}), and one concentration predicted to cause some mortalities (0.100 μg chlorpyrifos l^{-1}), were chosen for the pesticide exposure. Chlorpyrifos is very hydrophobic [log $K_{ow} = 4.7$ (Whitehead, 1997)], therefore, acetone (Distol grade) was used as a carrier for pesticide dosing (Roast et al., 1999). An initial stock of 1 g chlorpyrifos l^{-1} acetone was diluted in series to give a final stock concentration of 1 mg chlorpyrifos l^{-1} acetone. Test solutions were made by stirring 1 l of dilution water (salinity of 10, made by combining filtered seawater with double-distilled, de-ionised water) at high speed in a 2 l conical flask such that a vortex was created. Toxicant stock (76, 112, 144 or 200 μl of 1 mg chlorpyrifos l^{-1}) was then injected via a Gilson pipette into the centre of the vortex and allowed 5 min to mix. The conical flask was filled to the 2 l mark to give the final exposure concentration. Equalising volumes of acetone were injected into each exposure vessel such that all vessels contained the same acetone concentration (i.e. 124, 88 and 56 μl acetone were injected into the 0.038, 0.056 and 0.072 μg chlorpyrifos l^{-1} solutions, ensuring that all exposure solutions contained 100 μl acetone l^{-1}). A solvent control (dilution water and 100 μl acetone) and normal control (dilution water only) were also prepared to assess any effects of acetone on mysid swimming behaviour. 1500 ml of each exposure solution was poured into individual 2 l tall-form glass beakers into which 10 mysids of equal length (12 ± 1 mm from the anterior margin of the rostrum to the tip of the telson) were placed. Ovigerous females and any mysids that were dropped or injured during handling were excluded from the experiments. Chlorpyrifos is comparatively unstable in water [hydrolysis DT_{50} = c. 1.5 days (Whitehead, 1997)], therefore exposure solutions were replaced daily with freshly-made chlorpyrifos solutions. All chlorpyrifos concentrations described hereafter are nominal, however, the pesticide exposure regime was identical to that used by Roast et al. (1999), when gas chromatograph analysis showed that c. 55% of the chlorpyrifos was lost from the exposure vessels within the first 24 h. It is assumed that similar degradation or loss of chlorpyrifos occurred in the present exposure vessels. Mortalities in the exposure vessels were recorded every 24 h and dead or moribund mysids were removed. Mysids were fed

ad libitum on <48 h old *Artemia* sp. nauplii. Following exposure, mysids were placed in the flume for analysis of swimming behaviour. Mysids which showed obvious symptoms of pesticide poisoning (e.g. spiral swimming or repeated, slow escape response tail-flips) were not used in the swimming experiments (see Section 2.4).

2.3. Analysis of mysid swimming behaviour

An annular flume was used to examine the swimming behaviour of *Neomysis integer* (Roast et al., 1998a) (Fig. 1). The flume consisted of a 10 cm wide water channel of up to 35 cm depth (Widdows et al., 1998). A motor-driven, rotating, smooth drive-plate located 5 cm below the water surface created a water current by friction. Current velocity was adjustable from <1 to >50 cm s^{-1}. A full description of the flume and its hydrodynamic properties can be found in Widdows et al. (1998). The flume was filled with pesticide-free water of salinity of 10 (± 1) (prepared by combining distilled water with filtered seawater) and maintained at 15 ± 1°C in a constant-temperature room. In contrast to the method used by Roast et al. (1998a), no sediment was used in the present study, leaving the bare perspex base of the flume as the substratum. Following pesticide exposure, 20 mysids were placed in the flume and allowed 30 min without any water current to acclimate to the flume. After 30 min, a free-stream current velocity of 3 cm s^{-1} was generated. After 5 min acclimation to the new current velocity, mysid swimming behaviour was examined by direct observation and the frequency of each of four behavioural types (see next section) was recorded. The current velocity was then increased to 6 cm s^{-1} and, after a further 5 min acclimation period, mysid swimming behaviour was again recorded. This procedure was repeated, increasing the cur

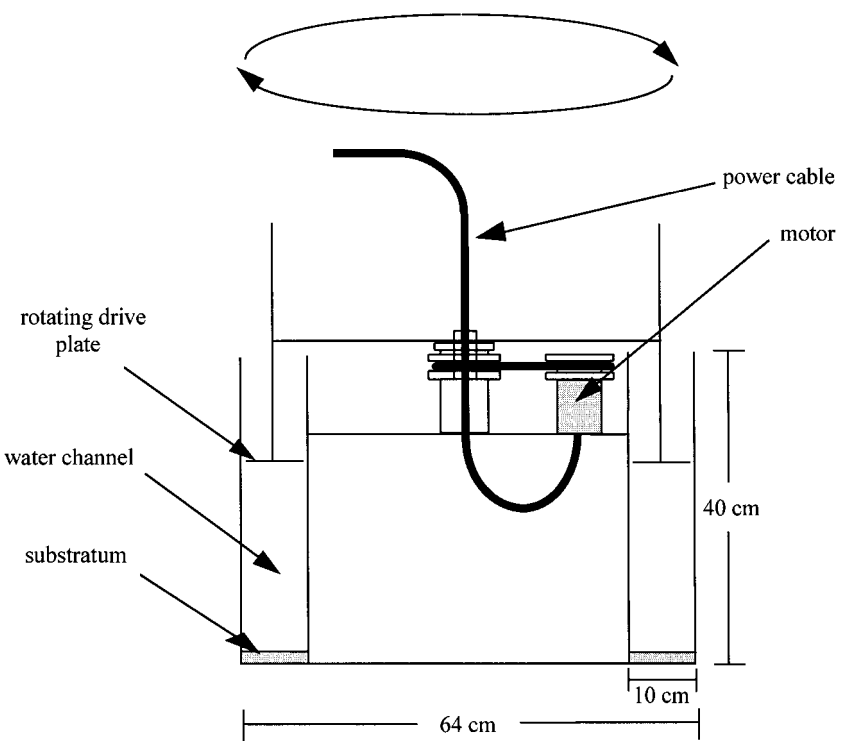

Fig. 1. Cross-section of the annular flume.

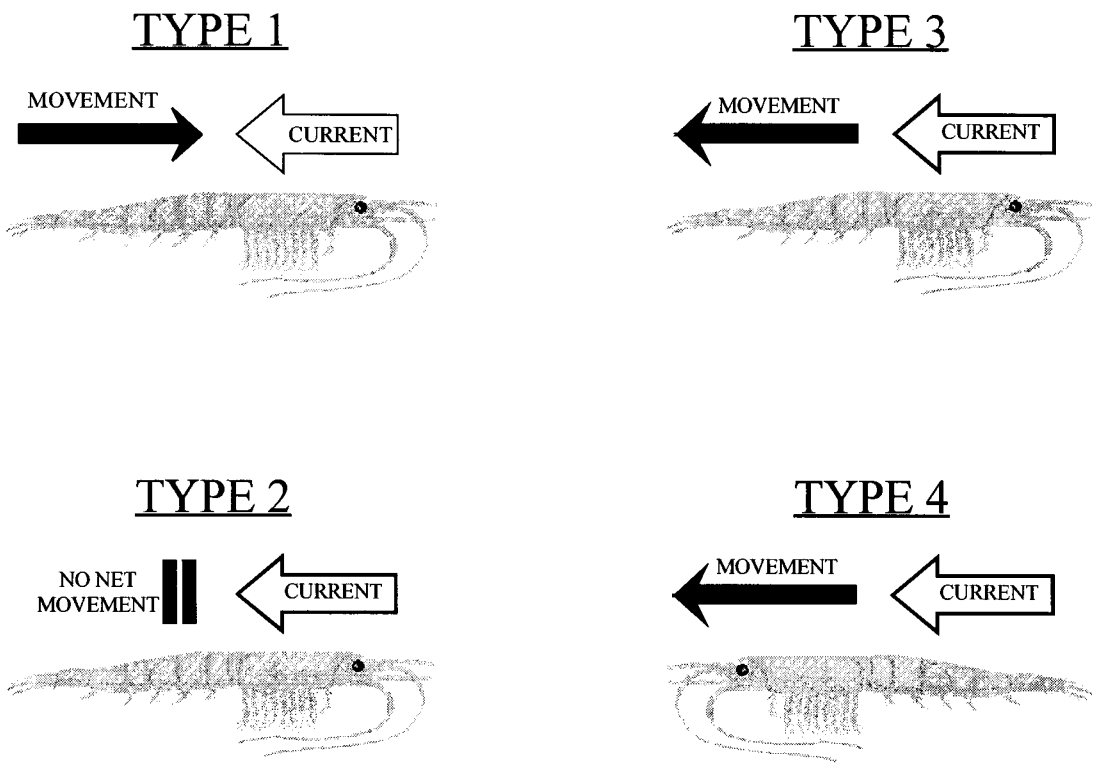

Fig. 2. Behavioural responses, based on direction facing and net movement, recorded in the flume. Detailed descriptions are given in the text (Section 2.4).

rent velocity in 3 cm s^{-1} increments up to 18 cm s^{-1} [most *N. integer* have been shown previously to be unable to swim against a current of 18 cm s^{-1} (Roast et al., 1998a)]. Each experiment was repeated 10 times, such that 200 individual mysids were examined at each pesticide concentration.

2.4. Swimming behaviour categories

Four behavioural types, based on direction facing and swimming activity, were identified (Fig. 2):

Type 1: The mysid was facing into the current, swimming faster than the current velocity and moved forward into the current.

Type 2: The mysid was facing into the current, swimming at the same speed as the current velocity and remained stationary (mysids which 'gripped' scratches in the base or sides of the flume were included in this category).

Type 3: The mysid was facing into the current, swimming slower than the current velocity and was swept with the current.

Type 4: The mysid was facing with the current and was carried with the flow.

The vertical position in the water column of each mysid was also recorded. Mysids swimming > 15 cm above the substratum were grouped into one category (Roast et al., 1998a).

Mysids which showed obvious signs of pesticide poisoning did not fit into any of the four behavioural categories defined and tended to be swept erratically around the flume, colliding frequently with other mysids in the flume. Therefore, these mysids (and mysids which died during the

course of the exposure period) were not included in the flume experiments. When the number of mysids able to maintain position is expressed as a proportion of all mysids exposed to each pesticide concentration, the proportion is lower than when the data are presented as a proportion of only those mysids swimming 'normally'. For example, following 7 day exposure to 0.072 μg chlorpyrifos l^{-1}, 40% of mysids swimming 'normally' were able to maintain position, but if mysids which had died or were unable to swim are included in the analysis, only c. 20% of the mysids exposed to this pesticide concentration would be able to maintain position (Fig. 3). More mysids had died or were showing abnormal swimming behaviour at the higher pesticide concentrations (i.e. there was a concentration response) and this is represented by the difference between the two proportions at each pesticide concentration (Fig. 3). The aim of the present paper was to establish the effects of chlorpyrifos on the swimming behaviour of *Neomysis integer* and not lethal effects [which have been described previously (Roast et al., 1999)], hence all subsequent datasets do not include mysids which died or showed obvious signs of pesticide poisoning. It may be considered, therefore, that the results of the present study are a conservative representation of the effects on natural *N. integer* populations exposed to chlorpyrifos since the data are from those mysids most resistant to chlorpyrifos.

2.5. Statistical treatment of results

Behavioural frequency data were changed into proportions, arcsine square-root transformed and examined statistically by one- and two-way analysis of variance (ANOVA).

3. Results

Swimming behaviour of mysids in the acetone control was not significantly different from those mysids in the normal control (ANOVA, $f = 0.45$, d.f. = 1, $P > 0.05$), indicating that 100 μl acetone

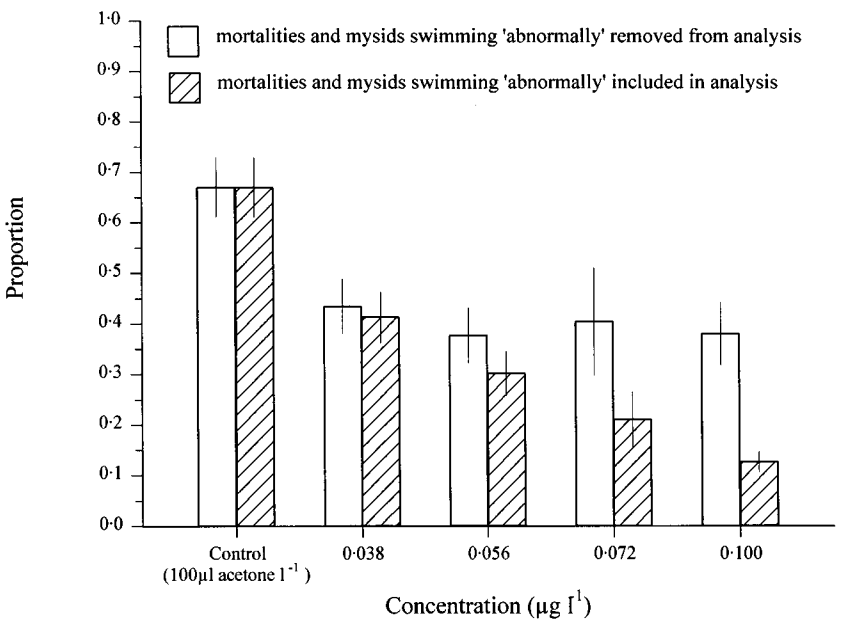

Fig. 3. Comparison of datasets when mysids which died or were swimming abnormally during the exposure period are included (hatched bars) and excluded (open bars). Data for behavioural types 1 and 2 have been combined and thus represent those mysids able to maintain position (all other mysids were, therefore, being swept by the current, see Fig. 2). Current velocity = 12 cm s^{-1}. Error bars correspond to 95% confidence intervals; n = variable (≥ 200).

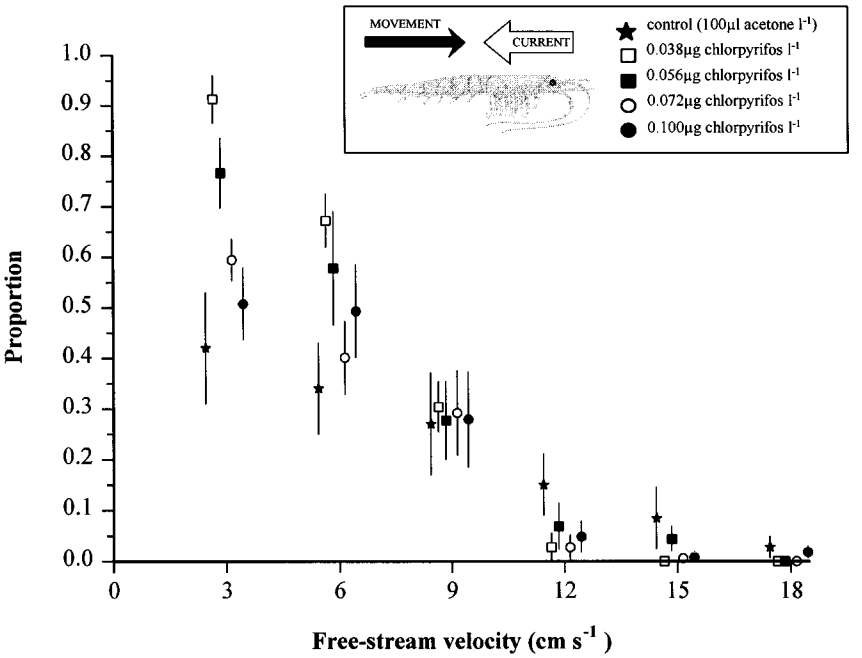

Fig. 4. Effect of chlorpyrifos on proportion of mysids swimming forward into the current (Type 1). Data points are means with corresponding 95% confidence intervals; $n = 200$.

l^{-1} had no significant effect on mysid swimming behaviour. All control data described hereafter are acetone control mysids since these represent the more appropriate control.

The swimming behaviour of *Neomysis integer* was affected significantly by both chlorpyrifos concentration and current velocity [ANOVA, $f \geq 4.1$ (concentration) and ≥ 16.3 (velocity), d.f. = 4 (concentration) and 5 (velocity), $P < 0.01$]. The effects of current velocity on the swimming behaviour of *N. integer* have been reported previously (Roast et al., 1998a) and are not addressed here. There was a significant interaction between pesticide concentration and current velocity (ANOVA, $f = 5.5$, d.f. = 20, $P < 0.01$), demonstrating that the effects of chlorpyrifos on the swimming behaviour of *N. integer* are complex.

3.1. Behaviour type 1

The effect of chlorpyrifos on the proportion of mysids swimming forward into the current was dependent upon current velocity and different responses were recorded at lower (e.g. 3 cm s^{-1}) compared with higher (e.g. 12 cm s^{-1}) velocities (2-way ANOVA, $f = 13.2$, d.f. = 20, $P < 0.01$). At 3 cm s^{-1}, more mysids exposed to ≤ 0.072 µg chlorpyrifos l^{-1} swam forward into the current compared with control mysids (95% confidence intervals, $P < 0.05$) (Fig. 4). A similar response was recorded for mysids exposed to 0.038 and 0.056 µg chlorpyrifos l^{-1} at 6 cm s^{-1} (Fig. 4). The increase in the frequency of this behaviour at low current velocities was concentration specific, with the frequency of mysids swimming forward into the current related inversely to pesticide concentration (Fig. 4). Exposure to 0.10 µg chlorpyrifos l^{-1} had no significant effect on the frequency of this behaviour at 3 or 6 cm s^{-1} (95% confidence intervals, $P > 0.05$) (Fig. 4). At higher current velocities (e.g. 12 cm s^{-1}), exposure to all chlorpyrifos concentrations caused a reduction in the frequency of mysids swimming forward into the current compared with control mysids (95% confidence intervals, $P < 0.05$) (Fig. 4). At current velocities in excess of 15 cm s^{-1}, the effect of flow

velocity appears to have a greater influence on mysid swimming behaviour than pesticide exposure, and there is no significant effect of chlorpyrifos on the frequency of mysids swimming forward into the current (95% confidence intervals, $P > 0.05$) (Fig. 4).

3.2. Behaviour type 2

At each current velocity, pesticide exposure caused significant effects on the proportion of *Neomysis integer* maintaining position. The general response was that fewer chlorpyrifos-exposed mysids maintained position compared with control mysids (1-Way ANOVA, $f \geqslant 5.4$, d.f. $= 4$, $P < 0.01$) (Fig. 5). Chlorpyrifos-related disruption of position maintenance behaviour was dependent upon current velocity (2-Way ANOVA; $f = 7.42$, d.f. $= 20$, $P < 0.01$). In general, exposure to low pesticide concentrations (i.e. 0.038 and 0.056 μg chlorpyrifos l^{-1}) caused fewer mysids to maintain position at lower speeds (e.g. 3–12 cm s^{-1}) compared with control mysids (95% confidence intervals, $P < 0.05$) (Fig. 5). In general, exposure to higher concentrations of chlorpyrifos (i.e. 0.072 and 0.100 μg l^{-1}) had no significant effect on mysid position maintenance at these speeds (95% confidence intervals, $P > 0.05$) (Fig. 5). In contrast, fewer mysids exposed to the two higher pesticide concentrations maintained position at the higher current velocities of 15 and 18 cm s^{-1} compared with control mysids (95% confidence intervals, $P < 0.05$) (Fig. 5).

3.3. Behaviour type 3

Although chlorpyrifos exposure caused significant effects on the proportion of *Neomysis integer* facing into the current but being displaced (2-Way ANOVA, $f = 4.19$, d.f. $= 4$, $P < 0.01$), responses were complex and dependent on current velocity (Fig. 6). Few clear conclusions can be drawn from these data, although at 12 cm s^{-1} a greater proportion of mysids exposed to chlorpyrifos faced into the current and were displaced compared with control *N. integer* (95% confidence intervals, $P < 0.05$) (Fig. 6).

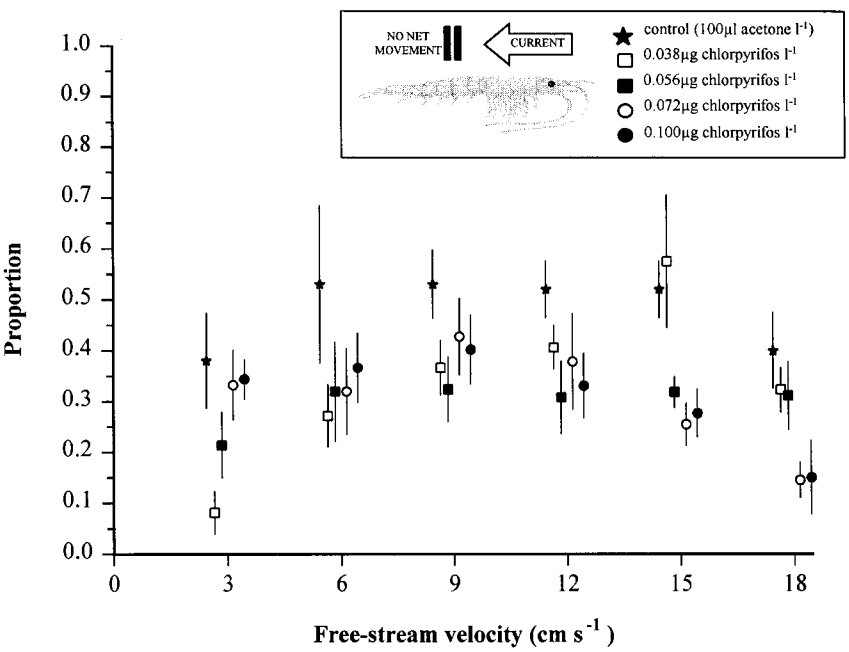

Fig. 5. Effect of chlorpyrifos on proportion of mysids maintaining position (Type 2). Data points are means with corresponding 95% confidence intervals; $n = 200$.

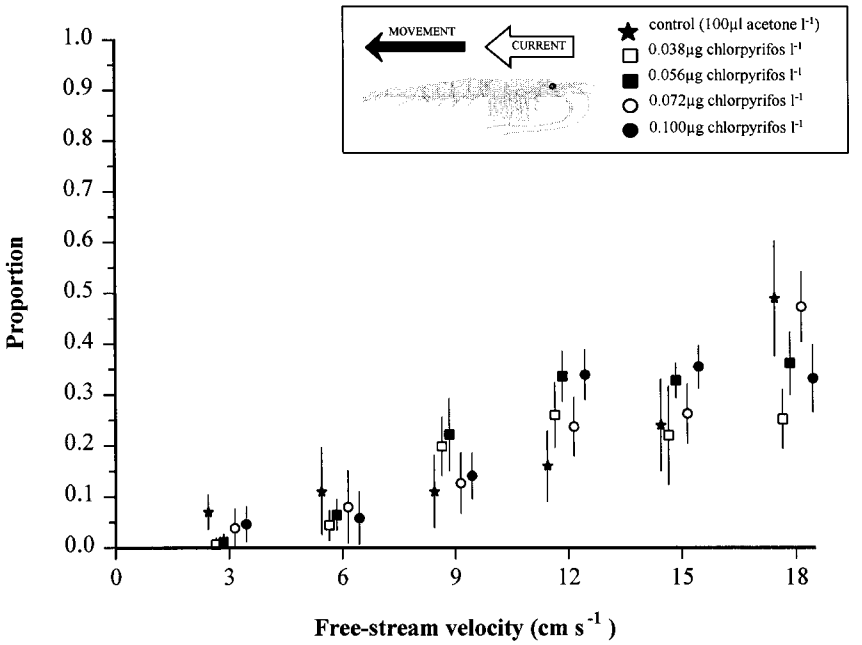

Fig. 6. Effect of chlorpyrifos on proportion of mysids facing into the current but being displaced (Type 3). Data points are means with corresponding 95% confidence intervals; $n = 200$.

3.4. Behaviour type 4

Exposure to chlorpyrifos caused increased numbers of *Neomysis integer* to swim with the current at current velocities ≥ 12 cm s^{-1} compared with control mysids (95% confidence intervals, $P < 0.05$) (Fig. 7). For example, at 18 cm s^{-1} only c. 10% of the control mysids swam with the current, compared with 30–50% of the exposed mysids (95% confidence intervals, $P < 0.05$) (Fig. 7).

3.5. Vertical distribution

Exposure to chlorpyrifos had a variable effect on the vertical distribution of *Neomysis integer* and significant differences due to pesticide exposure were identified only at certain heights. The most obvious differences in vertical distribution occurred at higher current velocities. For example, at 15 cm s^{-1}, exposure to 0.038 and 0.072 µg chlorpyrifos l^{-1} caused significantly more mysids to remain on the substratum compared with control mysids; the latter were more evenly distributed throughout the water column (1-Way ANOVA, $f = 5.4$, d.f. $= 4$, $P < 0.01$) (Fig. 8). In addition, significantly more mysids exposed to 0.100 µg chlorpyrifos l^{-1} swam above the substratum at current velocities ≥ 15 cm s^{-1} compared with control animals (Fig. 7). Significant differences were also recorded in the proportions of mysids swimming at 3, 5, 10, 14 and > 15 cm above the substratum (1-Way ANOVA, $f \geq 2.6$, d.f. $= 4$, $P < 0.01$) although there was no obvious pesticide-related trend.

3.6. Swimming behaviour above the substratum

Since *Neomysis integer* displays a distinct association with the substratum, the effect of chlorpyrifos on the frequency of each behaviour type was analysed after removing data points for mysids swimming in the substratum boundary layer [i.e. those mysids on or within 1 cm of the substratum (Widdows et al., 1998)]. The effect of chlorpyrifos exposure on the swimming behaviour of mysids swimming above the boundary layer was highly variable, and there was no significant difference between pesticide-exposed and control

mysids at many velocities. Although the responses of mysids swimming above the substratum were more variable than mysids in the boundary layer, the same general responses were recorded. For example, at 3 cm s^{-1} more chlorpyrifos-exposed mysids swam forward into the current than control mysids (95% confidence intervals, $P < 0.05$) (Fig. 9). In addition, at higher current velocities (e.g. 18 cm s^{-1}), exposure to chlorpyrifos caused fewer mysids to maintain position (1-Way ANOVA, $f = 9.22$, d.f. $= 4$, $P < 0.01$) and more mysids to swim with the current (1-Way ANOVA, $f = 11.6$, d.f. $= 4$, $P < 0.01$) than control animals.

4. Discussion

Current velocity is an important factor controlling the distribution of *Neomysis integer* in the natural estuarine environment. In the East Looe River Estuary, Cornwall (UK), *N. integer* maintained its position only in areas where the current velocity did not exceed c. 12 cm s^{-1} (Roast et al., 1998a). Furthermore, position maintenance was controlled primarily by the ability of *N. integer* to swim against a current (Roast et al., 1998a). Any disruption of this ability may cause the displacement of mysids to unfavourable regions of the estuary or make them more vulnerable to predation, possibly leading to mysid mortalities.

At comparatively low current velocities (i.e. up to 6 cm s^{-1}), exposure to relatively low chlorpyrifos concentrations (e.g. 0.038 and 0.056 μg l^{-1}) caused increased swimming activity by *N. integer* and led to more mysids swimming forward into the current. Although evidence of the role of acetylcholine in crustaceans is limited (Atwood, 1982), increased activity is consistent with the mode of action of chlorpyrifos, resulting from the accumulation of acetylcholine at neural junctions causing over-stimulation of the peripheral nervous system (WHO, 1986; Barron and Woodburn, 1995). Since chlorpyrifos affected mysid swimming in the present study it appears that cholinergic neural pathways are involved in mysid locomotion. Cholinergic pathways also appear to control locomotion in other arthropods. For example, *Folsomia candida* (Hexapoda: Collembola)

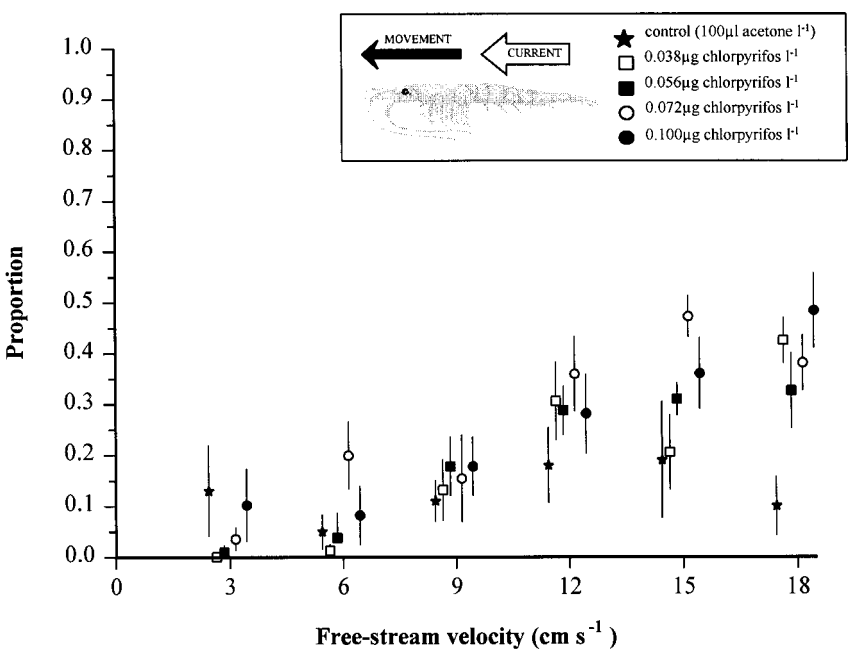

Fig. 7. Effect of chlorpyrifos on proportion of mysids facing with the current (Type 4). Data points are means with corresponding 95% confidence intervals; $n = 200$.

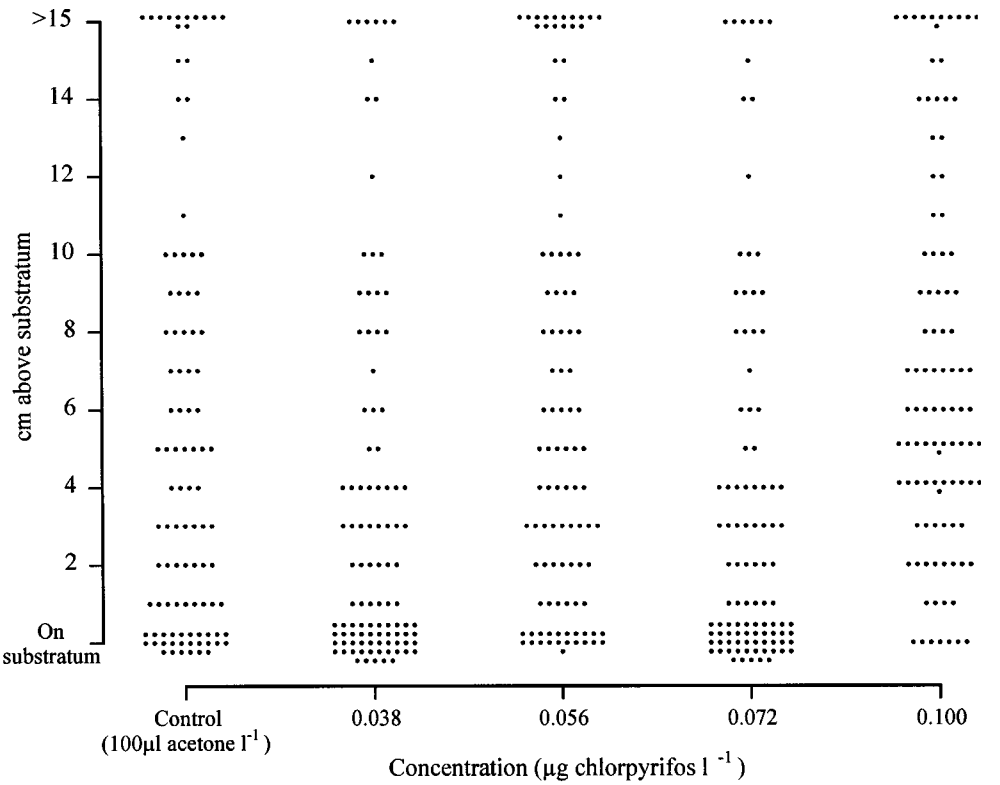

Fig. 8. Effect of chlorpyrifos on the mean vertical distribution of *Neomysis integer* in a current velocity of 15 cm s^{-1}. Each point represents 1% mysid occurrence, irrespective of behavioural type; $n = 200$.

and *Porcellio scaber* (Peracarida: Isopoda) had increased activity following exposure to the organophosphate pesticide dimethoate (Sørensen et al., 1995; Bayley and Baatrup, 1996). Increased activity following pesticide exposure has not been reported previously for mysids, although *Mysidopsis juniae* swam twice as far as control animals following exposure to 5 µl benzene l^{-1} (Martinez et al., 1992). Benzene, however, would not affect the nervous system in the same manner as an organophosphate and the increased activity of *M. juniae* may simply have been an avoidance response. The increased swimming activity recorded in the present study at 0.038 µg chlorpyrifos l^{-1} could have several consequences for mysids in their natural environment. Increased movement might cause the mysids to move into areas of faster flow (leading to increased displacement from optimum conditions) or cause their displacement from regions of optimum feeding [e.g. movement away from the maximum turbidity zone (Fockedey and Mees, 1999)]. Alternatively, increased activity may cause mysids to be more noticeable to predators who hunt by sight, or to move into areas where predator density is increased. All these possible consequences of pesticide exposure would lead indirectly to mysid mortalities in the natural, estuarine environment (defined here as '*ecological death*'). Thus, a chlorpyrifos concentration of 0.038 µg l^{-1} may lead to the ecological death of *N. integer*, even though the pesticide may not cause direct lethal effects at this concentration (Roast et al., 1999). The LC_{50} estimate, used frequently to establish 'allowable' concentrations, for a 7 day exposure to chlorpyrifos is c. 0.084 µg l^{-1} (Roast et al., 1999), i.e. more than twice that which disrupts mysid swimming. This comparison illustrates the importance of sub-

lethal responses to toxicants and highlights further the flaws of using mortality as an end-point in toxicity tests to predict the environmental consequences of pollutant discharge. Even if increased swimming activity does not lead to mortalities of *N. integer*, it will result in increased metabolic activity (i.e. by increased oxygen consumption), leading possibly to the utilization of energy reserves which may be required for other purposes (e.g. gametogenesis). In rainbow trout (*Oncorhynchus mykiss*), the energy required to swim at any given speed was greater in fish exposed to trace metals (Waiwood and Beamish, 1978; Wilson, 1996). Assuming similar energy utilization in pesticide-exposed mysids, *N. integer* exposed to chlorpyrifos would be able to swim for a shorter duration, leading to the disruption of position maintenance. Since our results are expressed as proportions, it is possible to examine the change from one behaviour type to another following exposure to chlorpyrifos, allowing more detailed assessment of the effect of the pesticide on *N. integer*. Increased forward movement of mysids at lower current velocities was due to fewer mysids maintaining position (or vice versa) since there is no pesticide-related difference in the frequency of the other two behaviour types at low current speeds. Thus, control mysids were maintaining position at low current speeds, whereas chlorpyrifos exposure led to mysids swimming forward, confirming that exposure to chlorpyrifos did cause hyperactivity of the mysids, and not other responses.

Generally, pre-exposure to chlorpyrifos caused a reduction in the ability of *N. integer* to swim against a current, such that mysids were unable to swim forward into the current above 12 cm s^{-1}. The ecological implications of a reduction in the swimming ability of *N. integer* are clear, with increased probability of their displacement in the estuary. In the East Looe River Estuary, *N. integer* were distributed permanently in regions where water flow did not exceed 12 cm s^{-1} (Roast et al., 1998a). However, mysids may occasionally

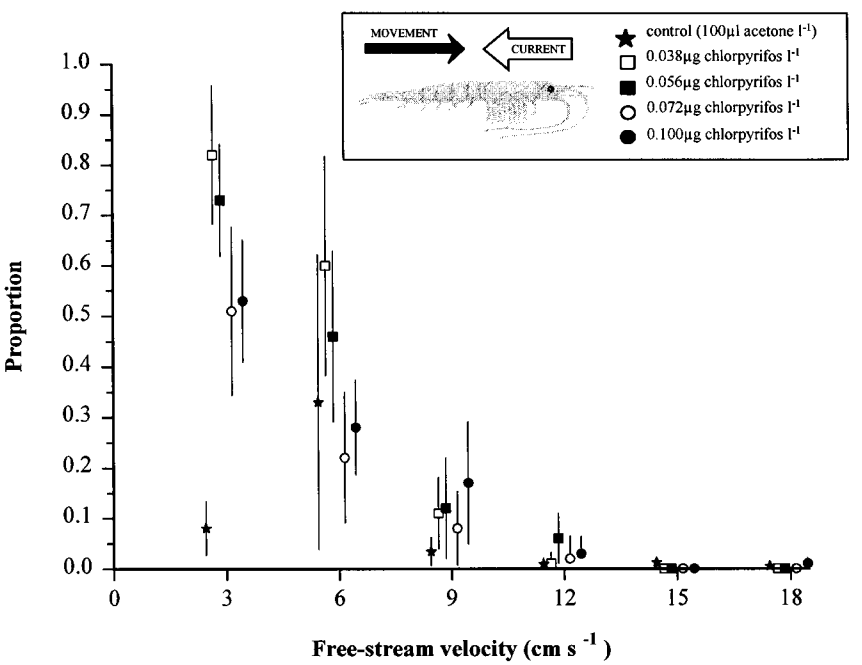

Fig. 9. Effect of chlorpyrifos on the proportion of mysids swimming forward into the current above the boundary layer (Type 1) (i.e. data for mysids swimming on or within 1 cm of the substratum have been removed). Data points are means with corresponding 95% confidence intervals; n = variable.

move into areas of faster flow and therefore need to swim faster than 12 cm s^{-1} to regain their position with the rest of the population. Exposure to chlorpyrifos would reduce the ability of *N. integer* to increase its swimming speed when necessary, thus causing mysids to be swept away from the rest of the population. Again, ecological death is predicted to occur at concentrations below those causing direct lethal effects. Reduction of swimming ability following toxicant exposure has been reported previously for teleost fish (e.g. Wilson and Wood, 1992; Taylor et al., 1996). With particular significance to the present study, Cripe et al. (1981) reported that exposure to two organophosphate pesticides reduced the swimming speed of *Americamysis* (≡ *Mysidopsis*) *bahia*. Following 96-h exposure to phorate and methylparathion, the maximum sustained speed of *A. bahia* was reduced from 12 (in control mysids) to c. 1 cm s^{-1} (phorate) and 7 cm s^{-1} (methylparathion) (Cripe et al., 1981). The effects of chlorpyrifos on *N. integer*, identified in the present study, are similar to those reported for organophosphate poisoning of *A. bahia* (Cripe et al., 1981).

The third noticeable effect of chlorpyrifos exposure on mysid swimming was that significantly more pesticide-exposed mysids swam with the current at high velocities (e.g. 18 cm s^{-1}) compared with control mysids. There are two possible explanations for this response. Exposure to chlorpyrifos may have caused a disruption of the sensory capabilities of *N. integer* leading to a disruption of the usual positive rheotaxic response (Roast et al., 1998a). This appears unlikely since the frequency of this response was only elevated at high velocity (if sensory disruption had occurred, increased swimming with the current would be expected at all current velocities). More likely, pesticide exposure exerted increased energy expenditure on *N. integer*, causing periods of exhaustion and mysids to travel with the current whilst resting. Although chlorpyrifos exposure may have caused increased energy expenditure, this seems to have little relevance in terms of position maintenance in the natural environment since this response was only recorded at high current speeds. For example, at 18 cm s^{-1}, similar proportions of control and pesticide-exposed mysids were swept with the current (only direction facing was different). The data imply, however, that pesticide-exposed mysids would try to swim against a current for a shorter duration than unexposed mysids, which may lead to the displacement problems described earlier.

In the natural environment, *N. integer* shows a strong affinity for the substratum and in previous flume studies mysids have been seen to shelter from high current velocities on the lee-side of substratum unconformities (Roast et al., 1998a). At high current velocities, mysids are swept from the substratum and being unable to re-acquire position on the substratum become more evenly distributed through the water column (Roast et al., 1998a). In the present study, the effect of chlorpyrifos on the vertical distribution of *N. integer* was unclear and since effects were noticeable only at high current velocities (e.g. 15 cm s^{-1}) the significance of such effects in the natural environment are assumed to be small. Furthermore, those mysids swimming above the boundary layer showed the same general type of behaviour at each current velocity as those mysids in the boundary layer.

Chlorpyrifos usage in the UK increased between 1992 and 1994 (Garthwaite et al., 1995). For example, in 1994 an estimated 132 t was applied to 270 302 ha of arable crops, compared with 23 t applied to 35 821 ha in 1992 (Garthwaite et al., 1995). Current European Community (EC) legislation states that no more than 0.1 μg l^{-1} of any one pesticide, and no more than 0.5 μg l^{-1} total pesticide loading, be present in potable water (Council of European Communities, 1980), although few member countries achieve these criteria (Murgatroyd and Patel, 1994). For example, Environmental Quality Standards (EQS) for the protection of aquatic life and potable waters, as defined by the Department of the Environment (UK), state that dimethoate levels should not exceed 1 μg l^{-1} in freshwaters or 0.2 μg l^{-1} in potable waters (Murgatroyd and Patel, 1994). However, there are no EQS values for estuaries or seawater (Murgatroyd and Patel, 1994). Based on these 'allowable' concentrations for freshwater, mysid swimming behaviour is predicted to be disrupted.

5. Summary

Exposure to chlorpyrifos disrupted the swimming behaviour of *Neomysis integer*. At low current speeds, more pesticide-exposed mysids swam forward into the current compared with controls. Fewer pesticide-exposed mysids swam forward into the current at high current velocities than controls. Position maintenance behaviour was less frequent in chlorpyrifos-exposed mysids at all current velocities and more mysids exposed to chlorpyrifos swam with the current at high current velocities. In general, all these responses were recorded following exposure to an environmentally realistic chlorpyrifos concentration (0.038 μg chlorpyrifos l^{-1}; >50% lower than the LC_{50} estimate for the same exposure period). Each of these laboratory responses may be used as a sensitive endpoint for the identification of chlorpyrifos exposure. Responses recorded in the flume imply the behavioural disruption of field populations of *N. integer*. Furthermore, the results may be used to predict the effects of real contamination events in the natural environment, including *ecological death*, as opposed to simply identifying toxicant-induced mortality.

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