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Effects of methamidophos on sediment processing and body mass of *Capitella* sp. Y from Estero del Yugo, Mazatlán, Mexico

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ABSTRACT

The cosmopolitan species-complex *Capitella,* a deposit-feeding polychaete, is widely used as an indicator of organic pollution and plays an important role in sewage waste cycling in marine and estuarine ecosystems. The organophosphorous insecticide methamidophos is currently employed in agriculture fields to control insect infestations. Its occurrence could pose a hazard to infauna. A bioassay to investigate the effects of exposing *Capitella* sp. Y to sediment spiked with methamidophos (0.008, 0.016, 0.032, 0.064, 0.130 and 0.260 mg/g dry wt sediment) is described. Increasing methamidophos concentrations significantly reduced faecal pellet production and body mass. Some specimens exhibited morphological abnormalities and behaviour changes, which could be attributed to toxic effects of methamidophos not severe enough to stop feeding activity. Results using *Capitella* sp. Y suggest that methamidophos could affect the rate of sediment processing by polychaetes or other benthic invertebrates in zones subjected to the influence of such insecticide.

1. Introduction

The endobenthic deposit feeder *Capitella capitata* (Fabricius, 1780) is a cosmopolitan polychaete that is considered a universal indicator of organically enriched marine sediments (e.g. Bellan, 1967; Pearson and Rosenberg, 1978; Reish, 1959). *C. capitata* consists of a complex of non-interbreeding but morphologically similar sibling species which are distinguishable by protein variation, developmental and reproductive features, ecophysiological characters, such as tolerance to abiotic factors (i.e., organic matter in sediments, anoxia) and respiration rate (Grassle and Grassle, 1976; Linke-Gamenick et al., 2000a,b; Wu et al., 1991).

A previous study about the development of the deposit feeder *Capitella* sp. Y from Estero del Yugo, Mazatlán, Mexico (Méndez, 2006b) indicated that this species exhibits lecithotrophic development with the production of ciliated metatrochophore larvae. High variation in size and duration of developmental stages was observed, as is the case in most *Capitella* species described (summary in Méndez et al., 2000). The *Capitella* sp. Y population consists of males, females and hermaphrodites. Adults measure about 14-24 mm long, and have a median lifespan estimated to be approximately 6 months; growth stops at day 139 (Méndez, 2006b).

Methamidophos (O,S-Dimethyl Phosphoramidothioate) (MET) is an organophosphorous insecticide widely employed in the state of Sinaloa (Mexico) and other countries in the formulations "Tamaron ®" or "Monitor" to control insect infestations of cotton, potato, tomato and soya cultures (CICOPLAFEST, 1998). MET ($C_2H_8NO_2PS$) is soluble in water at 20 °C at concentrations higher than 2 kg/l (The Royal Society of Chemistry, 1987). There is no evidence of the occurrence of MET in marine sediments. Due to its high solubility in water, it is reasonable to assume that this insecticide reaches marine ecosystems through run-off from agricultural activities and can be deposited in sediments. Since MET is water soluble, it can be suggested that toxicity may be through contamination of pore water. MET has a half-life of 309 d in water at a pH=5, of 27 d at pH=7 and of 3 d at pH=9 and is degraded in the presence of sunlight with a half-life of 90 d at pH=5 (U.S. Environmental Protection Agency, 1989).

MET inhibits the enzyme acetylcholinesterase, which interrupts the transfer of the nervous impulses due to the accumulation of acetylcholine on the colinergic synapses (World Health Organization, 1986; Jones et al., 1999; Spassova and Singh, 2001). Studies have demonstrated that crustaceans and earthworms exposed to MET initially show hyperactivity, which is followed by paralysis and death (Anguas, 2001; Bautista, 2001; Booth and O'Halloran, 2001; Nath and Kumar, 1999; Reinecke et al., 2002).

Few studies have tested the effect of MET on mortality and behaviour of marine invertebrates. Juárez and Sánchez (1989) calculated a 24 h LC_{50}=0.16 mg/l of MET for larvae of the shrimp *Penaeus stylirostris* and observed that metamorphosis was delayed. Bautista (2001) determined a 96h LC_{50}=1.67 mg/l for juveniles of the shrimp *Litopenaeus vannamei*. Leyva-Cota (2004) demonstrated that the

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detritivorous harpacticoid copepod *Tisbe monozota* exhibited a 24h LC_{50}=0.011 mg/g in the presence of food and a 24h LC_{50}=0.0069 mg/g without food, with a decrease in the swimming activity and a slight alteration of the feeding activity.

The purpose of this study was to determine the effects of MET on sediment processing and body mass of *Capitella* sp. Y under laboratory conditions. Physical abnormalities and behaviour changes were also examined. Sediment processing by the *Capitella* species-complex may have important ecological implications in the recovery of polluted sediments via sediment turnover (Selck et al., 1998; Selck et al., 1999; Méndez et al., 2001; Méndez and Baird, 2002; Méndez, 2006a). It was calculated that the *Capitella* population as a whole, could process from 56 to 1368 g of sediment per day m^2 (Méndez 2006a and Méndez and Baird, 2002, respectively). The possible damage produced by MET on invertebrates inhabiting sediments subjected to this insecticide influence (i.e., some ecosystems in the Sinaloa state) could have ecological significance.

2. Materials and methods

2.1. Test organisms

Animals were collected during January 2002 in the Estero del Yugo estuary in muddy sediment with an organic matter content of 6.5 ±2.5% (n=4; loss on ignition method of Dean, 1974). This estuary is located north of Mazatlán on the Pacific coast of Mexico (23°17′54″N, 106°29′01″W) and is episodically connected to the sea. The average bottom temperature was 23 °C.

Worms were maintained for three mo in stock cultures under laboratory conditions in aquarium tanks (20 X 30 cm) containing a 2 to 4 cm layer of sediments (from the supralittoral zone of the sandy beach in front of Hotel Playa, which had previously been sieved to a grain size less than 250 μm in diameter and frozen to kill fauna), 1.5 l of aereated seawater (34‰ salinity) and were maintained at 24±1 °C. Animals were fed weekly with 0.5 g per culture of food consisting of equal parts of commercial fish food (SERA VIPAN), and dried spinach (Forbes et al., 1996).

2.2. Experimental sediments

Since *Capitella* spp. is a detritivorous species-complex, MET was introduced to sediment in order to observe effects this compound may have on its sediment processing activity. The 16 d LC_{50} for *Capitella* sp. Y exposed to MET was previously calculated in our laboratory as 0.54 mg/g dry weight sediment (actual concentration; Anguas, 2004). This value was used when selecting the experimental concentrations. Sediments were collected from the Yugo Estuary and sieved through a 63 μm mesh using distilled water to kill the infauna. They were re-salted with UV filtered seawater (34‰ salinity). The organic matter content of the <63 μm fraction was 15±2% (loss on ignition method of Dean, 1974). MET solutions were added to known volumes of sediment that were homogenised by shaking for 18 h at 24±1 °C (U.S. Environmental Protection Agency, 1998). The contaminated sediments were divided into 0.5 g portions and were frozen until use in the experiment to avoid possible degradation.

Actual MET concentrations in sediments were quantified with previously frozen samples like those used in experiments through gas chromatography. MET extraction was performed following the techniques proposed by the International Atomic Energy Agency (1997), modified according to the characteristics of our experimental sediments. Lyophilised (freeze-dried for 48 h) sediments were extracted in a Soxhlet apparatus using 250 ml of ethylacetate:acetone (1:1) for 8 h. The organic extracts were then concentrated (RapidVap-79100-00, Labconco) to approximately 10 ml. To purify the extracts, sulphur compounds from the sediments were eliminated with activated copper. Then extracts were passed through a glass column with deactivated florisil (5% water HPLC degree) and eluted, first with 30 ml of hexane:ethylacetate (3:1) and then with 30 ml of hexane: ethylacetate (1:1). Both fractions were combined and evaporated to 500 μl under a light nitrogen current. Finally, the extract was transferred to an injection vial and reduced to a volume of 1 ml.

One microlitre of each sample was injected into the gas chromatograph. The measurements were made using an Agilent (HP 6890) equipped with a nitrogen phosphorous detector. The column was an HP-5, 0.25 μm film thickness, 30 m length, 320 μm inner diameter. The gas chromatograph was operated at 60 °C ramped by 20 °C/min up to 200 °C for 8 min. The carrier gas was helium at a flow of 4.4 ml/min and nitrogen was used as auxiliary gas at a flow of 15.3 ml/min. Detector and injector were set at 200 and 300 °C, respectively. The quantification was based using a calibration curve with different concentrations of standard methamidophos. Finally, the actual MET concentrations were calculated by interpolation to obtain the following actual concentrations: 0, 0.008, 0.016, 0.032, 0.064, 0.130 and 0.260 mg/g dry wt sediment methamidophos (=mg/g MET).

2.3. Sediment processing and body mass bioassay

Mature *Capitella* sp. Y individuals, bearing ovaries in the mid region (females), genital spines between the 8th and 9th setigers (males) or both structures (hermaphrodites) were selected. The bioassay was designed according to the techniques proposed by Méndez et al. (2001), Méndez and Baird (2002) and Méndez (2006a). Five individuals, whose size was randomly selected, were placed in each of four replicate, 4-cm diameter dishes containing 0.5 g wet weight of freshly contaminated sediment (0, 0.008, 0.016, 0.032, 0.064, 0.130 and 0.260 mg/g dry weight sediment MET) previously dispersed using a glass rod and a pipette, and 15 ml of seawater (34‰ salinity). The dishes were capped to prevent evaporation. These sediment portions (15% organic matter) contained food in excess and were not completely processed from one census day to the next, as previously tested during a 20 d experiment with the same number of individuals per dish. The dishes were maintained at 24±1 °C in the dark for an experimental period of 15 d. Every five days (census days 5, 10 and 15), worms were removed from the treatments and the remaining bulk sediment, containing pellets, tubes and mucus was fixed in 75% ethyl alcohol for 24 h for further analyses (Selck et al., 1998). Worms were transferred to fresh contaminated sediment and water. The replacement ensured the exposure to the initial MET concentrations avoiding a possible decrease in concentrations due to the high dissolubility of the compound in seawater. This procedure was repeated until day 15. The specimens were observed for a period of 10 min during each census day in order to record the possible abnormalities and behaviour changes.

For sediment processing analyses, fixed bulk sediment samples were sieved through a 130 μm mesh to retain the faecal pellets. Tubes and mucus without attached pellets were removed under the microscope. Faecal pellets were lyophilised (Labconco free zone ® 6 litre freeze dry system) and weighed with a 0.0001 mg precision microbalance (Mettler, model MT5). Pellet production was defined as mg of pellets produced by 5 individuals over the 5-d observation period.

Individual body mass was estimated by image analysis at the beginning of the bioassay, on each census day, and before transfer to new contaminated sediments. Worms were photographed out of their tubes. Body mass was expressed in terms of mg dry weight (to be compatible with pellet production units) using the power function $Y=0.1337X^{1.5445}$ (R^2=0.702; n=24; p<0.001; Y=dry weight (mg), X=area (mm^2)). This function was previously calculated by taking into account 24 individuals whose areas had been calculated using this technique, lyophilised, and weighed. Resolution and magnification were standardized between the two studies.