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Reduction of recruitment of Acartia pacifica nauplii from benthic resting eggs due to organochlorine pesticides

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Abstract: Many estuarine and coastal planktonic copepods depend on the hatching of benthic resting eggs for recruitment of nauplii to the water column population. The potential effects of two organochlorine pesticides, hexchloriobinzene (HCH) and dichlorodiphenyltrichloroethane (DDT), on the recruitment of Acartia pacifica nauplii from benthic resting eggs in the seabed of Xiamen Bay were experimentally investigated. The abundance of A. pacifica nauplii hatched from the sediment significantly decreased with the increase of pesticide concentration. Trimmed Spearman-Karber analysis gave sediment 96-h LC_{50} values were 84.81 ng/g for HCH, and 157.94 ng/g for DDT. The median AI (AI_{50}) was -0.77, which suggested that the combined effect of HCH and DDT showed a weak effect than individual effects. There was a positive relationship between mortality and exposure time in DDT treatment, while the relationship was not significant in HCH treatment. The results suggest that organochlorine pesticides can reduce recruitment of A. pacifica nauplii from benthic resting eggs to planktonic population.

Keywords: organochlorine pesticide; resting egg; recruitment; Acartia pacifica

Introduction

Many estuarine and coastal copepods actually spend a portion of their life cycle in the sediment as resting eggs (Grice and Marcus, 1981; Marcus, 1996), which is likely to profoundly influence the dynamics of pelagic community (Boero et al., 1996; Hairston et al., 2000; Bell and Weithoff, 2003; Wang et al., 2005a) since benthic resting eggs enable the persistence of such species and are important agents of local recolonization. Copepod resting eggs can be extremely abundant ($10^6/m^2$) in bays and estuaries (Marcus, 1996). They can remain viable in the sediment for months to years (Marcus et al., 1994; Marcus and Lutz, 1998; Jiang et al., 2004), even for centuries (Hairston et al., 1995). The accumulation of resting eggs that can persist for an extended period of time in the sediment thus creates an egg bank analogous to the seed banks of many terrestrial plants (Hairston and De Stasio, 1988; De Stasio, 1989; Marcus et al., 1994; Hairston et al., 1995; Jiang et al., 2004). Egg bank not only can ensure copepods to survive through a harsh period, but can have profound effects on microevolutionary dynamics (Hairston and De Stasio, 1988).

Estuarine and coastal environments are subject to the input of many anthropogenic chemicals that can affect natural communities. Among the anthropogenic chemicals, organochlorine pesticides may cause the most serious problems because of their chemically stable nature, affinity for living systems, bioaccumulative capacity, general toxicity, resistance to degradation, and persistence in the environment (Kennish, 2001). The fine-grained and cohesive nature of estuarine sediments dictates that contaminants are likely to be absorbed onto particles and incorporated within the sediment. As a result, copepod resting eggs may be at special risk of organochlorine pesticides. However, little research has focused on the effects of organochlorine pesticides on copepod benthic resting eggs. Most studies of copepods focus on the survival, growth, reproduction, and genetic adaptation (Forget et al., 1999; Schizas et al., 2001; Staton et al., 2002; Klosterhaus et al., 2003).

In the environment, species are exposed to large number of chemicals, and therefore, the toxicological studies about the adverse effects and potential risk of the mixtures of chemicals is growing. It is not known whether mixtures of toxicological organochlorine pesticides follow a simply additive model (Marking, 1977). Gaining insight into the potential effect of pollution on the benthic resting eggs of planktonic copepods is important since the hatching of these eggs is essential for the seasonal restoration of population (Suderman and Marcus, 2002). In order to address those unknowns, experiments were conducted to investigate the potential effects of organochlorine pesticides on the recruitment of copepod nauplii into planktonic population.

Two organochlorine pesticides were selected for experiments, hexchloriobinzene (HCH) and dichlorodiphenyltrichloroethane (DDT). The species used in the experiment is Acartia pacifica, which is the winter-spring dominant copepod in Xiamen Bay. During the period from November to May only smooth subitaneous eggs are produced, most of which can hatch within 1—2 d at ambient temperature. After the annual maximum in water, the planktonic

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population decreases in size, and simultaneously the females begin to produce diapause eggs, which are spiny and do not hatch immediately. As water temperature increases, the planktonic population is further reduced and completely disappears at the end of June (Wang et al., 1994). The accumulation of viable eggs (subitaneous and diapause eggs) in the seabed of Xiamen Bay constitutes a potential source of recruitment of nauplii into the pelagic population (Jiang et al., 2004; Wang et al., 2005a). The aim of our study was to investigate the potential effects of organochlorine pesticides on the recruitment of copepod nauplii into planktonic population.

1 Materials and methods

The study site (118°2.363′E; 24°26.778′N) was located approximately in the center of Xiamen Bay, China. Previous investigations of the study site measured the sediment background concentrations of organochlorine pesticides were 0.78, and 17.4 ng/g for HCH and DDT, respectively (Zhang et al., 1996). Five kilograms of muddy surface sediments (0—5 cm) were sampled in June, 2003. The sediments were immediately transported to the laboratory, where they were randomly mixed to ensure the homogeneous distribution of resting eggs.

Sediment toxicity tests were performed for HCH, and DDT individually. The test of HCH was composed of five treatments (10, 50, 100, 500, and 1000 ng/g) plus a control (0.78 ng/g). The test of DDT was composed of four treatments (50, 100, 500, and 1000 ng/g) plus a control (17.4 ng/g).

In order to evaluate the effect of the mixture of HCH and DDT, a sum toxic unit (TU) approach was used (Marking, 1977). Simple additive toxicity of the organochlorine pesticides in mixture was assumed as a null hypothesis. The TU of a mixture was calculated by summing the ratios of the concentrations of each organochlorine pesticides in the mixture divided by its LC_{50} when present alone. The 96-h LC_{50} values of HCH and DDT were obtained from the results of the present study single toxicity tests. The mixture test was composed of five treatments (30, 150, 300, 750, and 1500 ng/g) and a control (18.18 ng/g).

Another experiment was conducted to investigate the effect of exposure time on the viability of resting eggs. The test was composed two treatments (50 ng/g HCH; 50 ng/g DDT) and a control. The viability of resting eggs was investigated at six timepoints over a 3-month period (4, 10, 20, 30, 60, and 90 d after the treatment).

Before every test, a range of sediment concentrations was achieved by adding appropriate aliquots of concentrated organochlorine pesticides measured stock solution and stirring sediment enough. Organochlorine pesticides (HCH and DDT, Fluka®) used were purchased in the highest purity. The treatments and the control were kept at 10℃. Resting eggs hatch synchronously if they are chilled and then warmed (personal observation).

Sediment sub-samples of equivalent size were obtained by filling a dish (9.1 cm^3). The mean weight of the content of the dish was 13.1 g, with a SD of 0.4 g (n=10). The contents of sediment were washed through a 200 μm mesh gauze and a 50-μm mesh gauze. The former strains out larger particles in the sediment, A. pacifica eggs and similarly sized particles remained on the latter while fine sediment was washed away. The material remaining was incubated in 30 ml beakers filled with sea water (25%) filtered through a 5-μm mesh gauze at room temperature (24—28℃) to allow viable eggs to hatch. The photoperiod was changed to the ambient outdoor light/dark cycle. The contents of beakers were observed every two days. The filtered sea water over the sediment was renewed. Each nauplii recovered from the supernatant was individually transported to a 50-ml beaker. They were offered a mixed diet of Isochrysis galbana and Phaeodactylum tricornutum until they were identifiable. The abundance of A. pacifica was counted. The incubation lasted for 8 d because most nauplii occurred within 5 d (personal observation). By thorough aeration, cleaning of the sediment and removal of finer particles, this provides a measurement of the maximum hatch in the event of complete resuspension of the eggs in clean seawater (Lindley et al., 1998).

Mortality due to the pesticides was calculated by:

$$M = 100(1 - T/C) \tag{1}$$

where M is the mortality, T is the mean numbers hatched from treatments and C means numbers hatched from the control. Analysis of variance (ANOVA) was used to test the significant differences in effects among treatments. Student's t-test was used to investigate significant differences in hatching nauplii between the treatment and the control. The 96-h LC_{50} values for both the single organochlorine pesticide and mixture tests (Hamilton et al., 1977). The TU resulting in 50% mortality in the mixture test (TU_{50}) was calculated as follows:

$$TU_{50} = (LC_{50\,m}/LC_{50})_{HCH} + (LC_{50\,m}/LC_{50})_{DDT} \tag{2}$$

where $LC_{50\,m}$ is the LC_{50} of HCH or DDT in the HCH-DDT mixed test.

The additive index (AI) resulting in 50% mortality in the mixture test (AI_{50}) was calculated using the following equations (Marking, 1977):

$$AI_{50} = (1/TU_{50}) - 1 \quad \text{for } TU_{50} < 1 \tag{3}$$

$$\text{or } AI_{50} = TU_{50}(-1) + 1 \quad \text{for } TU_{50} > 1 \tag{4}$$

Equations 2 and 3 simply sets the TU scale to zero for simple additivity, and yields negative values for toxicant antagonism or positive values for toxicant synergism (Marking, 1977).

2 Results

The abundance of A. pacifica nauplii hatched from the HCH test is illustrated in Fig.1. There was a significant negative relationship (P<0.01) between the number of nauplii and HCH concentration (Fig.1). An increase of the HCH concentration from 10 to 1000 ng/g reduced the number of hatched nauplii by 15.4% —100% (Fig.1). Few A. pacifica resting eggs could survive under high concentrations (500 and 1000 ng/g).

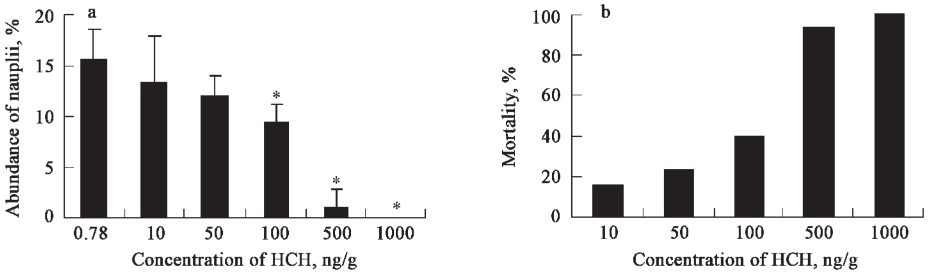

Fig. 1 Abundances of A. pacifica nauplii hatched from the sediments and their mortality in the 96-h HCH toxicity test
Error bars show one standard deviation from the mean; an asterisk denotes a statistically significant difference (P<0.01) from the control

The abundance of A. pacifica nauplii hatched from the sediment in DDT test was presented in Fig.2. The mean abundance of nauplii emerging from the sediment samples decreased markedly with the increase of DDT concentration (P<0.01). An increase of the DDT concentration from 50 to 1000 ng/g, reduced the number of hatched nauplii by 7.69%—100% (Fig.2).

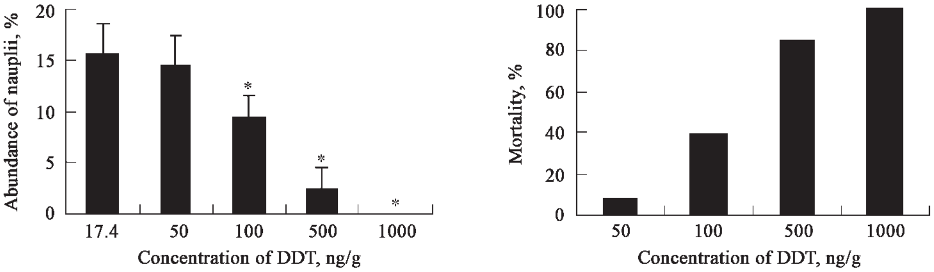

Fig.2 Abundances of A. pacifica nauplii hatched from the sediments and their mortality in the 96-h DDT toxicity test
Error bars show one standard deviation from the mean; an asterisk denotes a statistically significant difference (P< 0.01) from the control

The abundance of A. pacifica nauplii hatched from the HCH-DDT mixed test is illustrated in Fig.3. The number of nauplii significantly declined with the concentration (P<0.01). An increase of the mixed concentration from 30 to 1500 ng/g, reduced the number of hatched nauplii by 7.14%—100% (Fig.3). Few A. pacifica nauplii emerged under high concentrations (1000 and 1500 ng/g).

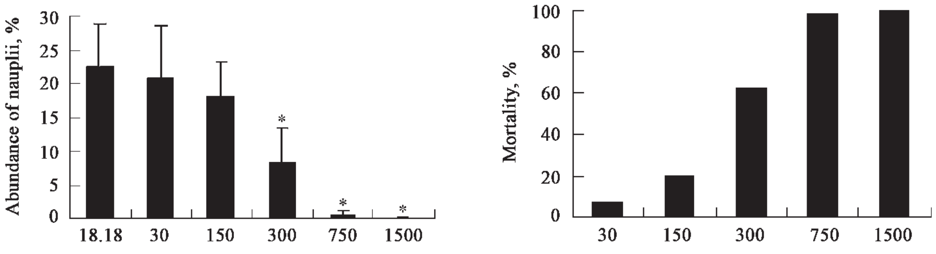

Fig.3 Abundances of A. pacifica nauplii hatched from the sediments and their mortality in the 96-h mixed toxicity test
Error bars show one standard deviation from the mean; an asterisk denotes a statistically significant difference (P< 0.01) from the control

The 96-h LC$_{50}$ values for both the single and mixed tests are presented in Table 1. The 96-h LC$_{50}$ values for resting A. pacifica eggs were 84.81 ng/g for HCH, and 157.94 ng/g for DDT. The median AI (AI$_{50}$) was -0.77, which suggested that the combined effect showed a weak effect than individual effects.

Table 1 The 96-h LC_{50} values drived by the Trimmed Spearman-Karber (SK) model

Pesticide	Trimmed SK LC_{50} pesticide, ng/g dry sediment	95% CIE, ng/g dry sediment	Trim, %
HCH	84.81	71.19—101.04	0.00
DDT	157.94	140.34—177.74	0.00
Mixture	215.26	196.88—235.36	0.00

Mortality of eggs in two treatments (50 ng/g HCH; 50 ng/g DDT) at all timepoints is illustrated in Fig.4. There was a positive relationship ($P<0.01$) between mortality and exposure time in DDT treatment (50 ng/g), while the relationship was not significant ($P=0.067$) in HCH treatment (50 ng/g).

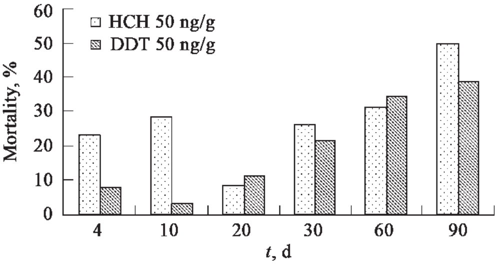

Fig.4 Percent mortality of A. pacifica resting eggs versus exposure time

3 Discussion

Benthic-pelagic coupling refers to a two-way exchange, or flux of matter, between benthic and pelagic environments in aquatic systems. Most studies have focused on the deposition of non-living organic matter to the seabed, resuspension and release of nutrients from the seabed back into the overlying water column, and the effects of benthic organisms in those processes (Graf, 1992; Marcus and Boero, 1998; Raffaelli et al., 2003). However, what must also be taken into account, but is often overlooked, is the reciprocal recruitment of living particles between the benthos and the overlying water body, which is likely to profoundly influence the dynamics of benthic and pelagic communities (Boero et al., 1996; Hairston et al., 2000; Bell and Weithoff, 2003). Thus, it is wise for researchers interesting in the fluctuations of zooplankton population to pay attention not only to features of the pelagic environment, but also the benthic environment. The aim of present study was to investigate the potential effect of organochlorine pesticides on the hatching success of planktonic copepod resting eggs that occur in the seabed of Xiamen Bay. The results clearly indicated that the viability of A. pacifica resting eggs in the sediment was sensitive to organochlorine pesticides. The mortality of resting eggs increased with the increase of pesticide concentration and exposure time. The effects on resting eggs in the sediment due to contaminants, including metal (unpublished data), oil (Suderman and Marcus, 2002), organochlorine compounds (PCP and DCB; Lindley et al., 1999), are particularly important because sediments often sequester high concentrations and the exposure times is long due to dormancy during periods when the pelagic population is absent or rare. Therefore, contamination could diminish the hatching success of resting eggs in the seabed and inhibit recruitment of nauplii into pelagic population, leading to changes in the zooplankton community. Such changes could result in further ecological changes since copepods of the plankton form the first vital link in the food chain that leads from the minute algal cells of the phytoplankton up to the large fishes and mammals.

Resting eggs could be distinguished into two types: subitaneous and diapause eggs. Subitaneous eggs can normally hatch after spawning, but if they are exposed to unsuitable conditions (e.g., low temperature or oxygen), development is delayed and they become quiescent. Unlike subitaneous eggs, diapause eggs must undergo a refractory phase, during which development does not resume even if conditions are suitable (Grice and Marcus, 1981; Marcus, 1996). We did not distinguish resting eggs into subitaneous eggs and diapause eggs since classification of these was very difficult when they were mixed in the sediment. Diapause eggs can survive longer than subitaneous eggs when exposed to adverse environmental conditions such as anoxia, sulfide, extreme temperatures, and even digestion, possibly because of the thick chorion, or outer membrane that protect the eggs (Marcus, 1984; Marcus and Lutz, 1998; Wang et al., 2005b). Even though A. pacifica diapause eggs may be less sensitive to organochlorine pesticides than subitaneous eggs, neither of them can survive when continuously exposed to the high level of pesticide concentration since almost no nauplii emerged under such condition in our experiment.

When mortality of resting eggs was estimated, we assumed that there was no effect of background concentrations of HCH and DDT on the viability of these eggs since it was very difficult to be investigated. The sediment background concentrations were 0.78, and 17.4 ng/g for HCH and DDT, respectively, at the study site (Zhang et al., 1996). The viability of A. pacifica resting eggs, especially subitaneous eggs, may be reduced by the sediment background concentrations. Thus values of estimated percentage mortality may be underestimated, which may result in the overestimate the sediment 96-h LC_{50} values. So further investigation on the effect of sediment background concentration of organochlorine pesticides is needed. Although the sediment background concentrations were investigated several years ago, the assume that the background concentrations of HCH and DDT did not changed significantly is reasonable due to their chemically stable nature, resistance to degradation, and no new input after international prohibition (Kennish, 2001). An exponential decrease between the excess ^{210}Pb activities and depth of sediment was observed at the present study site (Jiang et al., 2004), which suggested that there was no greatly physical and biological distribution. Thus, the concentration of HCH and DDT may be persistent in our study site.

Aquatic organisms in the natural environment often expose to multiple pesticides and toxic chemicals simultaneously. The chemicals have complex effects on the organisms, sometimes being antagonistic or additive, and at other times synergistic (Hanazato, 2001). The TU approach to mixture toxicity assessment has been widely employed in aquatic toxicology, which can provide insight about toxic interaction in the mixture (Marking, 1977). The median AI(AI_{50}) was -0.77, which suggested that HCH and DDT in mixture produced antagonistic effect on the viability of A. pacifica resting eggs. The 96-h LC_{50} values for A. pacifica resting eggs in Xiamen Bay were 84.81 ng/g for HCH, and 157.94 ng/g for DDT, respectively, which appears that resting eggs is less sensitive to DDT than to HCH. Although the mechanism of toxicant antagonism is unknown, it may be good news that the joint effect of HCH and DDT is weaker than individual effects if it is also a case in the field as illustrated in the laboratory.

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