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ADVANTAGES AND DISADVANTAGES OF PERFORMING ECOTOXICOLOGICAL BIOASSAYS WITH LARVAE OF POLYCHAETES BELONGING TO THE *Capitella capitata* SPECIES-COMPLEX

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ABSTRACT. The deposit feeder polychaete *Capitella capitata* contributes to the elimination of toxic substances from sediments. The taxon comprises a sibling species-complex that differs in their reproductive modes. It is known that larvae from this species-complex can delay or inhibit metamorphosis to the juvenile stage in the presence of toxic substances in the environment. The incorporation of these substances can delay the recruitment of individuals into natural populations. The advantages of performing bioassays to test effects on metamorphosis of *C. capitata* larvae are that these organisms are cosmopolitan, and are easy to collect, transport and culture without feeding. In addition, each brood can produce a high number of known-aged larvae, which are sensitive to different toxicants, especially when in solution. Nevertheless, the lack of knowledge about their life cycles and experimental protocols, the requirement of daily observations, the intraspecific individual variability, and their small body size and motility can difficult the experimental work. The use of *C. capitata* larvae to perform ecotoxicological studies in laboratory is recommended.

Keywords: Trochophores, Metatrochophores, metamorphosis, laboratory experiments.

Ventajas y desventajas de realizar bioensayos ecotoxicológicos con larvas de poliquetos pertenecientes al complejo de especies *Capitella capitata*

RESUMEN. El poliqueto detritívoro *Capitella capitata* contribuye a la eliminación de tóxicos del sedimento. Este taxón constituye un complejo de especies crípticas que difieren en sus estrategias reproductivas. Las larvas de este complejo de especies son sensibles a la presencia de tóxicos en el ambiente, al grado de retrasar o inhibir la metamorfosis al estado juvenil. La incorporación de estos tóxicos al medio ambiente puede afectar el reclutamiento de los individuos a las poblaciones naturales. Las ventajas de realizar bioensayos para evaluar los efectos sobre la metamorfosis de larvas de *C. capitata* son que dichas larvas son cosmopolitas, y son fáciles de recolectar, transportar y cultivar sin alimento. Además, en cada desove se produce un número elevado de individuos de edad conocida, los cuales son sensibles a diferentes tóxicos, especialmente en solución. Sin embargo, el trabajo experimental puede dificultarse debido al desconocimiento de los ciclos reproductivos y de los protocolos experimentales, al requerimiento de observaciones diarias, a la variabilidad individual intraespecífica, y a su pequeña talla y movilidad. Se recomienda el uso de larvas de *C. capitata* para realizar estudios ecotoxicológicos en laboratorio

Palabras clave: Trocóforas, Metatrocóforas, metamorfosis, experimentos de laboratorio.

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INTRODUCTION

Aquatic environments have been considered as the sink of contaminants with ecotoxicological implications for most organisms inhabiting these ecosystems. Several marine invertebrates have been used as ecotoxicological test species to evaluate demographic parameters, as well as bioavailability, bioaccumulation and biomagnification of pollutants. Some polychaete species have been considered as good candidates for toxicological studies in laboratory due to their relatively easy handling, culturing and transportation. Moreover, some polychaete species have short life cycles that make them ideal organisms to study the effects of toxicants on reproduction and survivorship (Reish, 1980). Results from ecotoxicological bioassays in the laboratory could reflect

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natural behaviour with similar toxicant concentrations.

The endobenthic deposit feeder polychaete *Capitella capitata* (Fabricius, 1780) is considered a universal indicator of organic pollution in marine sediments (*i.e.*, Bellan, 1967; Pearson & Rosenberg, 1978). *Capitella capitata* consists of a complex of morphologically similar species which do not crossbreed; they have been distinguished by enzyme and general protein patterns (through electrophoretic studies), by developmental and reproductive features, and by ecophysiological characters, such as tolerance to abiotic factors and respiration rates (Grassle & Grassle, 1974; Grassle & Grassle, 1976; Zhang & Wu, 1988; Wu *et al.*, 1991; Pearson & Pearson, 1991; Gamenick & Giere, 1994; Gamenick *et al.*, 1998a, Gamenick *et al.*, 1998b, Linke-Gamenick *et al.*, 2000a; Linke-Gamenick *et al.*, 2000b).

A high variability has been observed in the developmental mode within the *C. capitata* species-complex (Méndez *et al.*, 2000). The life cycle consists of several stages with a variable duration according to the sibling species. Females construct brood tubes where fertilized eggs are incubated until their transformation into trochophore larvae (Fig. 1). The development of the gut and 13 segments indicates the transformation into the metatrochophore stage. Depending on the species, larvae may remain inside the brood and hatch as trochophores or metatrochophores, and in some cases, as juveniles. After a swimming period, metatrochophore larvae settle into the sediment and metamorphosis into the juvenile sta-

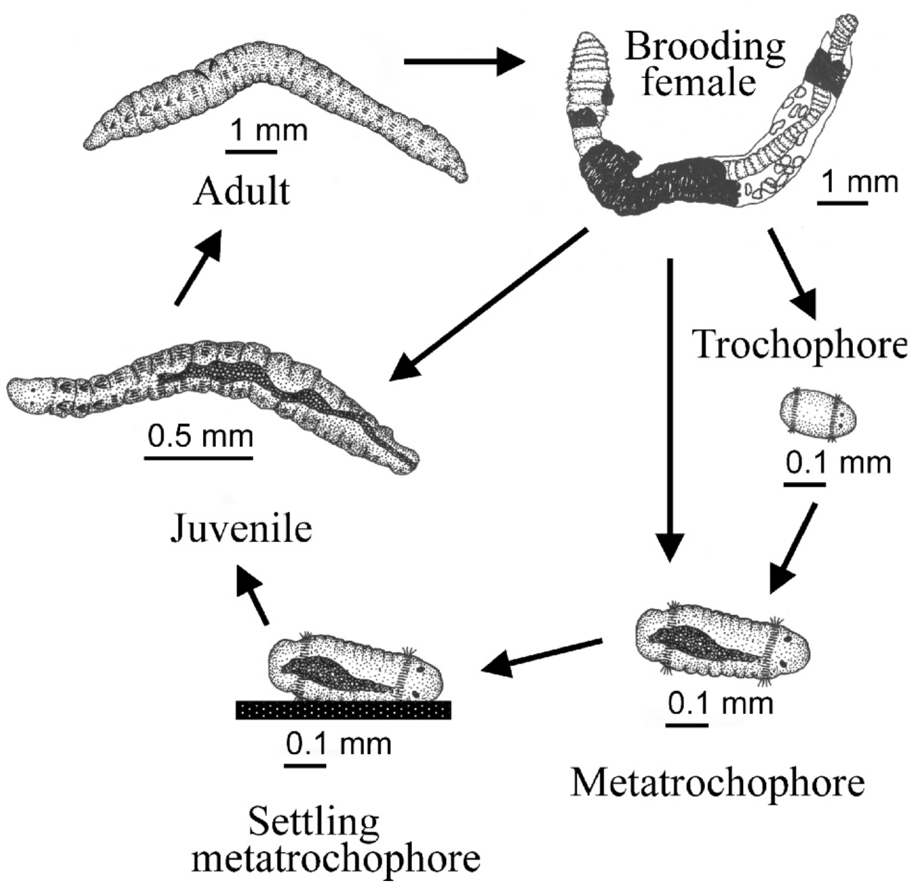

Figure 1. Life cycle of *Capitella capitata*.

Figura 1. Ciclo de vida de *Capitella capitata.*

ge takes place. Once settled, juveniles burrow into the sediments, where they remain for the rest of their life. The adult stage is reached with the apparition of sexual structures and the proliferation of segments (Méndez *et al.*, 2000).

It has been demonstrated that some toxicants produce significant effects on larvae metamorphosis of several *C. capitata* sibling species. Reish *et al.* (1974) observed abnormal *C. capitata* larvae of the second generation after exposure to copper and zinc. These larvae possessed two posterior ends and were capable of swimming, but failed to develop beyond the eight-segmented metatrochal stage. Bellan *et al.* (1972) found that the detergent polyethylene-glycol fatty acid can produce an increase in the time necessary to complete a particular developmental stage in *C. capitata*. Hill & Nelson (1992) concluded that exposure to the pesticide lindane incorporated into seawater delays or prevents metamorphosis and settlement of *Capitella* sp. I larvae. It was recently observed that larval metamorphosis was delayed in *Capitella* sp. B members exposed to cadmium (Méndez, 2002a) and teflubenzuron (Méndez, 2005) incorporated into sediments and this process was totally inhibited in *Capitella* sp. Y specimens exposed to cadmium and copper in solution (Méndez & Green-Ruiz, 2006). These adverse effects on larval metamorphosis could provoke eventual alterations of the population dynamics in nature and, consequently, the ecological services from the *C. capitata* species-complex could be reduced.

Adverse effects of toxicants in solution on larval development of members belonging to the *C. capitata* species-complex have important ecological implications on recruitment of individuals into natural populations. These organisms constitute an important species-complex in polluted environments because, when juveniles and adults, they contribute to the turnover of organic matter and removal of toxic substances from the sediment through their deposit feeding activity (Méndez & Green-Ruiz, 2006).

The compilation of data from the above mentioned studies indicate that *C. capitata* larval metamorphosis can be a good endpoint to evaluate adverse effects of toxicants in the environment. Nevertheless, we have observed that the use of larvae of this species-complex to perform this kind of studies shows several advantages as well as disadvantages. Thus, the aim here is the establishment of such advantages and disadvantages based on previous experiences in our laboratory with several *C. capitata* sibling species.

ADVANTAGES

1) *Capitella capitata* is a cosmopolitan species-complex, which is often associated to organically enriched sediments (Pearson & Rosenberg, 1978). Thus, this species can be easily found in high abundances in different regions of the world.

2) The collection of adult specimens for further culture in the laboratory is relatively easy. It implies the collection of sediment, which has to be sieved through a 0.5 mm or 1 mm mesh. The organisms that are retained in the mesh can be easily collected with forceps.

3) Because *C. capitata* has a high tolerance to low oxygen concentrations, adult specimens can be kept in small containers with sediment (for feeding and burrowing) without aeration for long periods of time (two-three days). This ability, as well as their small size, permits their transportation from the field to the laboratory for further culture.

4) Compared with other benthic species (e.g., shrimps), *C. capitata* is easy to culture in the laboratory in a small space. Worms can be reared in the laboratory prior to the experiments in aquarium tanks (e.g., 20 cm x 30 cm) containing a 2 cm to 4 cm layer of sediment previously sieved to less than 250 µm particle size (Linke-Gamenick *et al.*, 2000b) and 1 L of UV-filtered seawater. Stock cultures are normally maintained in aerated seawater in the dark. Moreover, organisms can be fed weekly with 0.5 g/L of a mixture of equal parts of fish food, baby cereal and dried spinach (Forbes *et al.*, 1996). The culture procedure can be applied to all members of the *C. capitata* species-complex and only temperature and sali-

nity must be adapted to the environmental conditions of each species.

5) Many *C. capitata* sibling species produce a high number of larvae per brood, like *Capitella* cf. *capitata* with a planktotrophic development, which can produce up to 8000 trochophores per brood (Zhang & Wu, 1988) or *Capitella* sp, with a lecithotrophic development, which hatch up to 671 metatrochophores per brood (Qian & Chia, 1992). This permits the performance of bioassays with several replicates of known-aged specimens. To do that, it is recommended to isolate several brooding females (15 to 20 individuals) with developing embryos inside the tubes from the stock cultures and to place them in dishes containing 1 g wet weight of <250 mm sediment, 0.01 g dry weight of artificial food (weekly addition) and seven ml of seawater. They have to be maintained in the previously established culture conditions. Daily observations have to be carried out until hatching using a stereoscopic microscope (Méndez, 2002a, 2002b, 2005; Méndez & Green-Ruiz, 2006). Once hatched, larvae needed for the experiments have to be collected, counted and placed in the experimental dishes. The bioassays must be designed according to the developmental mode of each species.

6) Metatrochophore larvae do not feed on sediments during the swimming and settling periods (Wilson, 1991). Depending on the duration of these periods, relatively long experiments (up to 30 days) can be designed without the incorporation of food into the experimental dishes, which could alter the toxicant concentrations.

7) *Capitella capitata* larvae are sensitive to some toxicants, such as polyethylene-glycol fatty acid, zinc, cadmium, copper and teflubenzuron (Bellan *et al.*, 1972; Reish *et al.*, 1974; Méndez, 2002a, 2005; Méndez & Green-Ruíz, 2006). Moreover, Reish *et al.* (1976) observed that the younger stages of *C. capitata* are less tolerant to metals than adults. The sensitivity exhibited by *C. capitata* larvae to toxicants makes them good test organisms for bioavailability studies.

8) In natural conditions, swimming *C. capitata* larvae are always in direct contact with the surrounding water, while their contact with sediments is sporadic. For this reason, it is recommended to study the effects of toxicants incorporated into solutions. The spiking sediment technique takes a longer period of time and is more complicated than the incorporation of toxicants into marine water. Moreover, the techniques to determinate toxicants in sediments are more sophisticated than those applied for solution analyses.

DISADVANTAGES

1) More than 40 *C. capitata* sibling species with different reproductive mode have been recognized (summary in Méndez *et al.*, 2000). The design of ecotoxicological bioassays requires the previous knowledge of the life cycle of the test species. In some cases, the life cycle studies are long processes, like that of *Capitella* sp. Y from Estero del Yugo, Mazatlan, which median survival time was estimated at 6 months, but some individuals survived up to 2 years and 2 months (Méndez, 2006). Moreover, the experiments have to be designed according to the developmental mode of each species. For instance, *Capitella* sp. Y has a lecithotrophic development with metatrochophore hatching, which implies that only this developmental stage can be analyzed during the experiments. On the contrary, *Capitella* sp. B from Barcelona, is a poecilogonic species with the production of both, trochophore and metatrochophore larvae, which hatch non-simultaneously (Méndez, 2002b). In this case, to test the effects of toxicants on this species, the experiments were designed taking into account the two kinds of larvae (Méndez, 2002a, 2005).

2) Few ecotoxicological studies have been performed with *C. capitata* larvae; thus, no standardized laboratory protocols have been developed. The life cycle variability exhibited by this species-complex makes difficult the standardization of ecotoxicological protocols to work with larvae. Nevertheless, disadvantages 1 and 2 can be minimized using always the same sibling species when perfor-

ming this kind of studies. When possible, it is recommended the use of native species.

3) In order to evaluate the effects of toxicants on larval metamorphosis, the duration of the bioassays must be at least one month, as has been observed using *Capitella* sp. B, which can delay metamorphosis up to 31 days in the presence of cadmium in sediments (Méndez, 2002a). Moreover, larval stages of most of the described *C. capitata* sibling species have short duration. Thus, it is essential to perform daily observations over the experiments.

4) Most of the species from the complex have shown intraspecific individual variability in size and duration of the different developmental stages (Méndez *et al.*, 2000), which can interfere with statistical interpretation. Thus, the experimental design must consider a high number of replicates.

5) The body size is normally too small. For instance, trochophores from *C. capitata* Type 2 measure from 0.04 mm to 0.21 mm (Pearson & Pearson, 1991), and metatrochophores from *Capitella* sp. III only reach 0.05 mm (Grassle & Grassle, 1976). The observation under the microscope of moving small and transparent larvae is difficult. Thus bioassays have to be designed with a small number of larvae per experimental dish, increasing the number of replicates for statistical purposes. Moreover, bioaccumulation and biomagnification studies cannot be performed using such small individuals.

6) The scarcity of literature referring to the effect of toxicants on *Capitella* spp. larvae difficults data interpretation and comparisons between actual and previous ecotoxicological studies.

CONCLUSIONS

Due to the ecological implications of this species-complex and the current status of pollution in the marine environment, further long-term experiments with larvae are strongly recommended. The information discussed here indicates that the performance of bioassays with *C. capitata* larvae have some disadvantages. Nevertheless, the relevance of this species-complex in the marine and estuarine environments, as well as the advantages mentioned here, make these larvae ideal candidates for toxicity tests. Therefore, it is recommended to analyse the effects of relevant environmental concentrations of toxicants in order to extrapolate data from the laboratory to the field.

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