

ONTOGENIC SHIFTS IN CHEMICAL DEFENSES OF THE
NORTHWEST MEDITERRANEAN SEA *EUPOLYMNIA*
NEBULOSA (POLYCHAETA, TERESELLIDAE)

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ABSTRACT

Polychaetes are known to produce different compounds with biological activities. Most of these compounds appear to be species-specific and may serve either to deter predators, to keep clean the inner tube-surface or to inhibit settlement of potential competitors. In this paper, toxic and deterrent properties of the terebellid *Eupolymnia nebulosa* (Montagu) from the Catalan Sea in the northwest Mediterranean were analyzed for: (1) different stages of their life cycle (i.e., coelomic oocytes, eggs inside the egg-masses, swimming larvae, early benthic stages and adults), (2) external structures (tubes and egg-masses), (3) sexes and (4) adult's body sections (tentacles, thorax and abdomen). Toxicity was assessed by measuring decreases of bioluminescence of the bacterium *Photobacterium phosphoreum* (Microtox® bioassay) following its exposure to polychaete crude extracts. Feeding deterrence assays were also conducted using sympatric generalist predators such as fish (*Coris julis*, *Aidablennius sphinx*, *Parablennius incognitus*, *Lepadogaster* sp.) and crustaceans (*Centropagurus timidus*, *Pisidia longimana*, *Alpheus* sp.). The jelly egg-masses, brooded eggs and coelomic oocytes were non-toxic. All remaining life cycle stages and tube were toxic. No differences were found between sexes and body sections. Operative deterrent effects occurred for: (1) egg-masses (probably physically mediated, linked to consistence of the mucus), and (2) early life cycle stages (i.e., swimming larvae and juveniles) and adult tentacles (probably a chemically mediated unpalatability). However, the results support that both types of deterrence effectively serve to protect against potential predators.

Many benthic sessile invertebrates are defended from predation by means of physical structures and/or chemical substances (e.g., sponges, Uriz et al., 1996; corals, Van Alstyne et al., 1994). Defensive roles have been attributed to physical structures such as sclerites (Uriz et al., 1996) or tubes in adult invertebrates, whereas cocoons have been traditionally considered as protective structures for the earliest life-history stages (Pechenik, 1979; Giangrande et al., 1994). On the other hand, chemical defenses (e.g., toxic and unpalatable compounds) have been widely documented for both adult invertebrates and larvae (Hay, 1996; Lindquist and Hay, 1996; Uriz et al., 1996) and cocoons (Pawlik et al., 1988).

Previous studies on physical and chemical defenses in sessile benthic invertebrates cover a wide range of taxa. However, information on polychaetes is scarce and consisted of the following: (1) chemical bioactivity against embryos and larvae of marine invertebrates in *Hermione hystrix* (Martin and Uriz, 1993), (2) species-specific bromophenols showing anti-microbial properties in *Arenicola cristata*, *Lanice conchilega*, *Notomastus lobatus*, *Thelepus extensus* and *T. setosus* (Higa and Scheuer, 1975; Weber and Ernst, 1978; Woodin et al., 1987; Goerke and Weber, 1990, 1991; Goerke et al., 1991), and (3) recruitment inhibitory bromophenols in *T. crispus* and *N. lobatus* (Woodin et al., 1993, 1997).

The widely distributed terebellid polychaete *Eupolyornia nebulosa* (Montagu) is an obligatory tubicolous species, which lives in shallow water in mixed soft and hard substrates. In the northwest Mediterranean Sea, larvae are incubated in gelatinous egg-masses (Duchêne and Nozais, 1992; Bhaud et al., 1995; Nozais et al., 1997). It has been hypothesized that cocoons and adult tubes play a protective role. The existence of chemical cues in *E. nebulosa* has been postulated since this species maintains symbiotic relationships with the polynoids *Harmothoe areolata* and *Polynoe scolopendrina* which are able to recognize *E. nebulosa* as their host (Davenport, 1953; Martin and Britayev, 1998). Recent laboratory experiments support the existence of an attractant cue in sediment marked by the mucus released by adults which triggers positive responses in both larvae and early benthic juveniles (Duchêne and Martin, unpubl. data). In fact, after occasional nocturnal displacements of adults, the worms also use mucus trails deposited on the sediment to relocate their own tube openings (Duchêne, pers. observ.).

Intra-specific variation in chemical defenses has been widely reported as a function of environmental factors for seaweeds, corals, ascidians and sponges (Becerro et al., 1995; Hay, 1996). A differential distribution of toxic metabolites in the different parts of the body has only been reported for polychaetes in *T. extensus* (Goerke et al., 1991). Information on feeding deterrence at different stages of the life history of benthic invertebrates is scarce (Lindquist et al., 1992; Paul and Hay, 1996; Uriz et al., 1996), and it is lacking for polychaetes. However, recent laboratory observations seemed to support the existence of anti-predatory deterrent mechanisms. First, early benthic stages of *E. nebulosa* were refused by young carnivorous polychaetes (Martin, unpubl. data). Second, in experimental aquaria, neither crabs (e.g., *Carcinus maenas*) nor starfish (e.g., *Echinaster sepositus*) consumed *E. nebulosa* cocoons (Medernach, 1995).

In this paper, the possible toxic and deterrent properties of *E. nebulosa* were examined using two different approaches. Inter-specific differences in toxicity were analyzed as a function of: (1) sex, (2) body region (i.e., tentacles, thorax, abdomen), and (3) life cycle stage (i.e., coelomic oocytes, fertilized eggs inside the egg-mass, swimming larvae, early benthic juveniles, adults). The toxicity of the external structures built by the worm (i.e., tubes and egg-masses) was also analyzed. The predation deterrence of cocoons, early life cycle stages (i.e., swimming larvae, 1-mo old benthic juveniles) and adult body regions was analyzed using sympatric generalist predators. This twofold approach allowed assessing the relationships between generic toxicity and its ecological relevance.

MATERIALS AND METHODS

TOXICITY ASSAYS.—Specimens of *E. nebulosa* were collected by SCUBA diving during early May 1998 at a depth between 3 to 6 m in Blanes Bay in the Catalan Sea, northwest Mediterranean, 41°40'N, 2°48'E. Specimens were maintained in 25-L tanks filled with filtered native seawater and fed weekly with molded commercial vacuum dried fish food (SERA Granumarin®). Three adults were placed in individual 2-L tanks filled with filtered native seawater and a fine layer of native sediment (200–250 µm in grain size) previously cleaned and sterilized. They were allowed to build their tubes for 3 d. They were then removed from their tubes and isolated. Six more mature worms (three of each sex) were gently anaesthetized at 4°C prior to dissection. Abdomens, thoraxes and tentacles were dissected. Then, they were isolated, as well as coelomic oocytes of females.

Three tanks containing both mature males and females mixed together were used to obtain fertilized egg-masses, eggs, larvae and juveniles. Three 10-h old egg-masses and three 5-d old egg-masses were collected and immediately frozen. Once frozen, the external layer of each egg-mass

(i.e., containing only mucus) was separated from the inner part (i.e., containing both mucus and fertilized eggs) under a dissecting stereomicroscope. The remaining egg-masses were observed daily. As soon as larval release started, three egg-masses were relocated to individual 2-L tanks filled with filtered native seawater. From each, 200 swimming larvae were collected with a pipette. The larvae released from three other egg-masses were placed in three 25-L tanks containing a fine layer of native sediment (200–250 μm in grain size) to facilitate larval settlement and tube building. From each of these tanks, two successive groups of 35 1-mo old juveniles and 35 2-mo old juveniles were removed from their tubes and collected with a pipette.

Samples were isolated in sterile containers, frozen immediately and kept at -23°C until they were vacuum-dried and weighed to the nearest 0.001 mg. Freeze-dried samples were double extracted (10 and 15 min) with different volumes of acetone (1 sample vs 2 solvent volume proportion) in an ultrasonic bath. The solvent/extract mixtures were poured into sterile glass tubes. The solvent was then evaporated to dryness and the crude extracts were kept at 4°C until the toxicity assays were run.

Toxicity was assessed by the Microtox[®] model 500 analyzer from Microbics Corporation. This analyzer measures the decrease of bioluminescence (i.e., fluorescence) caused by the target crude extracts in living cell suspensions of the deep-sea bacterium *Photobacterium phosphoreum* [= *Vibrio fisheri*] (Becerro et al., 1995). Four decreasing concentrations of each crude extract diluted with artificial seawater (a dilution factor of 2 was used) were put in contact with the bacterial strains and their fluorescence (in gamma units) was compared with controls of pure bacterial strains. The data, expressed in logarithmic form were fitted to linear regressions. To allow comparisons, toxicity was extrapolated to gamma units per mg of dry weight (DW) biomass using the regression equations. A value of 0.5 gamma mg DW⁻¹ was taken to predicate a toxic effect based on comparative sea-urchin assays (Martin and Uriz, 1993) and Microtox[®] assays run previously (Uriz, unpubl. data).

DETERRENCE OF EGG-MASSSES IN THE FIELD.—Field experiments were carried out by SCUBA diving over a bottom of mixed soft sediment and boulders (the typical Mediterranean habitat for *E. nebulosa*), located at a depth of about 6 m in Blanes Bay. Generalist predators, mostly the rainbow wrasse *Coris julis* (Linné) (Pisces, Labridae) were attracted to the experimental area using pieces of mature sea urchins (*Paracentrotus lividus* Lamark). Some of these pieces were offered without any treatment (controls), while some others were covered with disaggregated egg-masses of *E. nebulosa* collected in the vicinity of the experimental area. The color of eggs was similar in both cases so that visual cues were minimized. Additional entire egg-masses were also offered to the fish. Effective attacks were those implying real ingestion of each potential 'prey'.

DETERRENCE OF SWIMMING LARVAE AND EARLY BENTHIC JUVENILES.—Laboratory experiments were first carried out using the hermit crab *Centropagurus timidus* (Roux) as potential predator. Small specimens of the hermit crab (<1 cm) were collected by SCUBA diving under boulders and aggregates of calcareous algae in Blanes Bay near an area inhabited by *E. nebulosa*. The hermit crabs were kept isolated in plastic multi-well containers (10 ml of filtered native seawater per well, six wells per container, each well measuring 4 cm in diameter and 2 cm in height) and were fed with pellets of SERA Granumarin[®] until the start of the experiments. Trials consisted of introducing five cultured individuals of *E. nebulosa* (i.e., swimming larvae or 1-mo old juveniles devoid of tubes, respectively) per well (six replicates). The number of both larval and juvenile survivors was recorded after 3, 24 and 96 h. Control wells without hermit-crabs revealed null mortalities in all cases.

Deterrence of 1-mo old *E. nebulosa* juveniles was also assessed by direct observation and video tape recording under a stereomicroscope using newly collected sympatric predators. These observations were conducted with the hermit crab *C. timidus*, the snapping prawn *Alpheus* sp. and the porcelain crab *Pisidia longimana* (Risso). The behavior was classified as tentative attacks (i.e., followed by rejection), effective attacks (i.e., followed by ingestion), and null attacks (i.e. direct rejection). After each trial with *E. nebulosa* juveniles, one specimen of another edible polychaete (i.e., syllid, eunicid or hesionid) was offered to the predators to assure that the possible rejections observed were not due to stress or lack of appetite.

DETERRENCE OF ADULT BODY SECTIONS.—Five juvenile specimens (3–5 cm) of three different fish species (i.e., the blennids *Aidablennius sphinx* (Valenciennes) and *Parablennius incognitus* (Bath) and the gobiid *Lepadogaster* sp.) were used as potential predators of adult *E. nebulosa*. Fish specimens were collected by SCUBA diving near an area inhabited by *E. nebulosa* in Blanes Bay. Each fish was kept in individual 2-L tanks filled with filtered native seawater. The tanks were provided with a fine layer of native sand and a piece of brick as a refuge to avoid fish stress. Fish were acclimated of about 1 wk. Pellets of SERA Granumarin[®] were offered daily to the fish with a pipette. The experiments were started only after all fish had accepted food directly from the pipette.

Two series of experiments were carried out. In the first, pieces of *E. nebulosa* were offered directly to the fish. In the second, potential food items were offered according to the following protocol: (1) two pellets of SERA Granumarin[®], (2) the target piece of *E. nebulosa*, (3) mixed pellets of SERA Granumarin[®] and pieces of *E. nebulosa*, (4) several more pellets of SERA Granumarin[®]. This protocol served both to prevent the initial instinctive reaction of hungry fish to the pipette and to ensure that the possible rejection of *E. nebulosa* was not caused by the fish being already replete.

STATISTICS.—The toxicity of external structures and developmental stages was analyzed by one-way ANOVA, whereas that of sex and body regions was assessed by two-way ANOVA. The differences in survivorship between swimming larvae and 1-mo old juveniles were analyzed by analysis of covariance (ANCOVA), using time as covariate (Zar, 1984).

Assumptions of normality and homoscedasticity, required for parametric analysis (Zar, 1984), were met after log-transformation of the data. These assumptions were checked using the Kolmogorov-Smirnov test (Lilliefors probability) and the Bartlett test, respectively. The Tukey honest significant difference (Tukey HSD) multiple comparisons post-hoc test was used to assess the groups responsible for the significant effects obtained in the different ANOVAs (Zar, 1984). All analyses were carried out using the Systat (ver. 5.2, copyright SYSTAT inc., 1990) statistical package. No analysis was necessary for the predators' behavior experiments since the results were consistently either 100% eaten or 100% rejected.

RESULTS

TOXICITY ASSAYS.—The mucus-built external structures (i.e., egg-masses and sandy tubes) showed highly significant differences in toxicity (one-way ANOVA, F ratio = 27.0, P = 0.001). Tube toxicity was higher than that of both 10 h old and 5-d old egg-masses which always showed a toxicity much lower than the 0.5 gamma mg DW⁻¹ W threshold (Fig. 1A).

All body sections from both adult males and females showed toxicity higher than 0.5 gamma mg DW⁻¹ (Fig. 1B). There were non-significant differences in mean toxicity both between body regions and between sexes. However, toxicity levels of both thorax and tentacles were always higher than 0.5 gamma mg DW⁻¹, while some abdomen pieces had a lower toxicity (i.e., slightly higher than 0.4 gamma mg DW⁻¹).

The developmental stages showed highly significant differences in toxicity (one-way ANOVA, F ratio = 23.0, P = 0.00001). The Tukey HSD test showed that toxicity was significantly higher (P < 0.01) for more advanced developmental stages (i.e., from swimming larvae to adults) than for the coelomic oocytes and fertilized eggs inside the egg-mass, which always showed a toxicity much lower than the 0.5 gamma mg DW⁻¹ threshold (Fig. 1C).

DETERRENCE OF EGG-MASSSES IN THE FIELD.—No effective attacks of the labrid *C. julis* on the untreated egg-masses were observed in any of the experiments carried out in the field. Disaggregated egg-masses effectively protected the pieces of mature sea urchins from

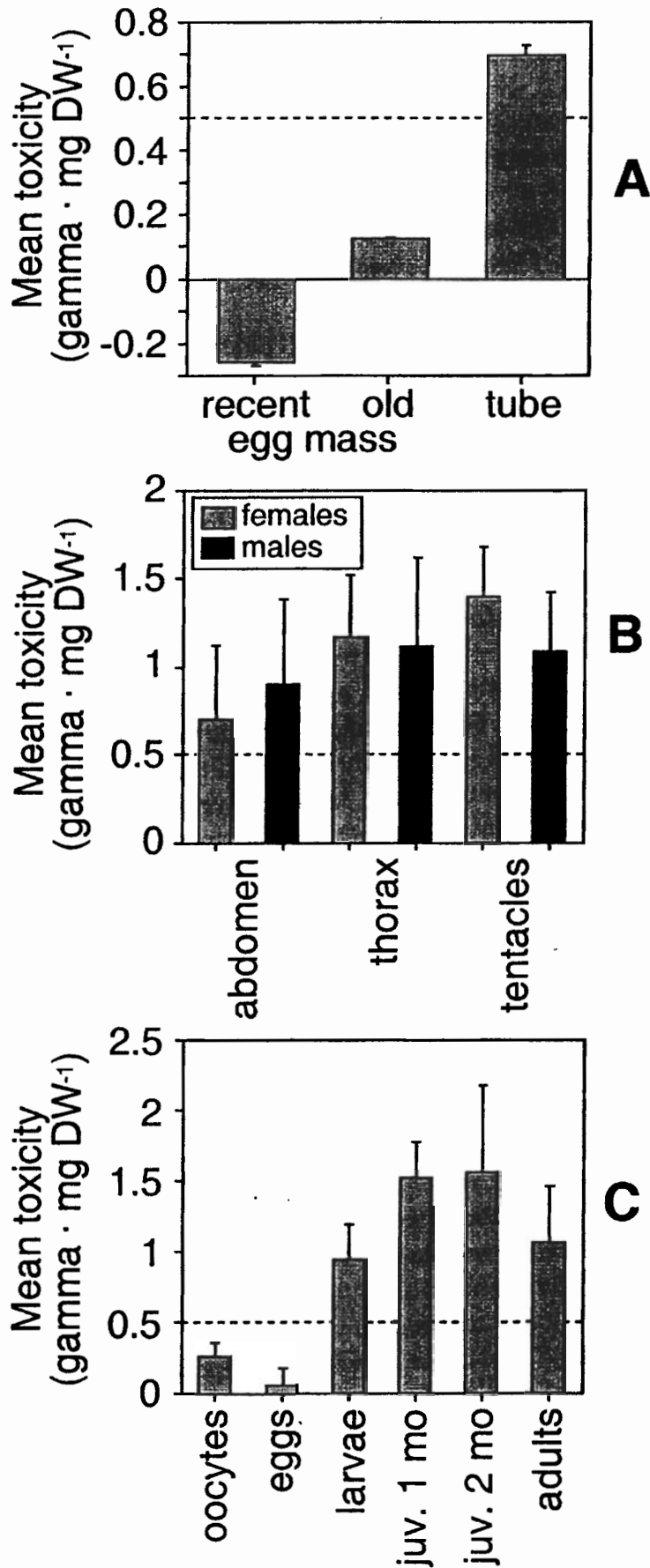


Figure 1. Variation in toxicity of *Eupolymnia nebulosa* as measured by the Microtox® analyzer. A. External structures built by the worm. B. Sexes and body sections. C. Life-cycle stages. Dotted line: ecologically relevant toxicity level.

being consumed by the fish while control pieces (i.e., not covered by the egg-masses) were always eaten.

DETERRENCE OF SWIMMING LARVAE AND EARLY BENTHIC JUVENILES.—There were non-significant differences in the final number of survivors for both swimming larvae and 1-mo old juveniles confined with the hermit crab. However, the survivorship of both stages differed significantly through time (ANCOVA, F ratio = 12.2, $P = 0.001$). In fact, more swimming larvae than 1-mo old juveniles disappeared from the cups at t_3 and, particularly, at t_{24} (Fig. 2).

The behavioral pattern was consistent for all potential predators under study using both swimming larvae and 1-mo old juveniles in the trials. The recently collected hermit crabs, which, presumably, have had no previous contact with *E. nebulosa*, made one (rarely two) effective attacks and then, they ignored all newly offered *E. nebulosa*. Conversely, all attacks were effective if the specimen offered belonged to any of the other polychaete species tested. One day later, all attacks on *E. nebulosa* were tentative and soon they became all null, whereas other polychaetes and pellets of commercial fish food offered were ingested by the hermit crabs. The other two crustaceans observed behaved slightly different from one another. *Alpheus* sp. showed exactly the same behavior as the hermit crab. However, it was much more sensitive to any source of stress and all attacks became null after the first effective one. *Alpheus* sp. uses a pair of appendages to search for any possible food on the bottom. The null attacks include those where prawns picked up the worms with their appendages and quickly discarded them far from their position in the cups. *P. longingimana* did not undertake any effective attack. All were null except for a very few tentative attacks. Conversely, both *Alpheus* sp. and *P. longingimana* fed on other polychaete species and on pellets of commercial fish food after rejecting *E. nebulosa*.

DETERRENCE OF ADULT BODY SECTIONS.—Fish fed directly with pieces of *E. nebulosa* always showed the same behavior. The first attack was effective, regardless of what body section was offered. All successive attacks were: (1) effective for abdomen pieces, (2) the second (rarely the third) tentative and all remaining null for the tentacles and (3) some effective and some tentative with undefined pattern for the thorax. Taking into account that the fish were hungry and that they were conditioned to receive food from the pipette, these first experiments were considered irrelevant to the fish behavior in their natural habitat. It should be pointed out, however, that all fish consumed pellets of commercial fish food after the trials with *E. nebulosa* with exception of those which ate tentacles. In these cases, after an effective attack, the fish refused all kinds of food for the next 24 h to 48 h. Moreover, both effective and tentative attacks triggered a characteristic spasmodic behavior in the fish which resembled a human sneeze.

In the second series of experiments, fish were first fed with fish food and, then, with the target pieces of adult *E. nebulosa*. However, independently of the number of trials carried out, the specimens of *Lepadogaster* sp. (which were always hidden under the piece of brick) behave exactly as above, including the response to the ingestion of tentacles. Conversely, the two blennid species showed a common but different behavior. These fish were either swimming in the tanks or hidden under the pieces of brick. After first being fed with the commercial fish food pellets, they quickly hide themselves under the bricks, with only their heads visible and waiting for the next incoming food. If the incoming food was a piece of *E. nebulosa* abdomen alone, the blennids always attacked it, independent of the number of trials. If the abdomen was mixed with fish food, they ate both with no marked preferences either for the worm or for the fish food. Concerning the

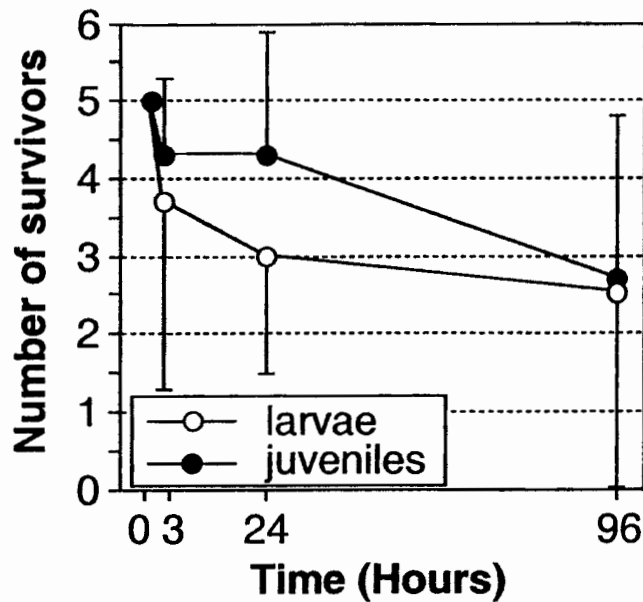


Figure 2. Time course of the survivorship for *Eupolymnia nebulosa* swimming larvae and one-month old juveniles incubated in the presence of a potential predator, the hermit crab *Centropagurus timidus*.

thorax alone, there was an inter-individual variability. Some blennids ate the pieces, but some did not. When mixed with pellets of fish food, all blennids preferred the pellets. However, the thorax was occasionally ingested provided the pellets had been totally consumed. No adverse reaction to the ingestion of thorax pieces was observed. In the case of *E. nebulosa* tentacles, only the first attack (rarely the second) of the first trial was effective. All successive trials where tentacle pieces were offered alone initially triggered tentative attacks. When mixing tentacle with fish food, the blennids initially tried to feed on the pellets, but contact with the tentacle caused a rejection, frequently of both food and tentacles. After a few trials, all offers including tentacle (either mixed or not with fish food) triggered null attacks with the blennids keeping themselves far from the tentacles or, even, hiding under the pieces of brick. Moreover, in all cases, a behavioral spasmodic reaction to either the ingestion of, or the contact with, tentacles was observed. All blennids fed normally on fish food pellets after each trial with the exception when tentacles were ingested.

DISCUSSION

The results support the idea that *E. nebulosa* is a polychaete with field relevant toxicity and anti-predatory properties. Both were present throughout the life cycle of the species, although some differences were evident at the different phases of the life cycle, in the external structures built by the worms and between regions of the body.

Growing evidence supports the idea that many marine larvae (particularly brooded lecithotrophic larvae) are unpalatable to sympatric generalist predators (Lindquist and Hay, 1996). However, this property may not be constant all through the life cycle as has previously been reported for ascidians (Lindquist et al., 1992) and sponges (Uriz et al., 1996). In *E. nebulosa*, a clear transition characterized the ontogeny. The earliest phases (i.e., coelomic oocytes and fertilized eggs inside the egg-masses) did not show significant

anti-bacterial toxicity. Conversely, all phases after release from the egg-mass proved to be toxic. Swimming larvae tended to be as toxic as adults with early benthic juveniles showing the greatest toxicity.

The old egg-masses (i.e., 5–10 d after deposition) usually harbored many different organisms (viz copepods, nematodes, flat worms, protozoans, and some small polychaetes), which seemed to feed on the fertilized eggs (authors, pers. observ.). Although partially protected by the cocoon (see below) brooded embryos were certainly palatable. There are no data on the process of acquisition of the ecologically relevant unpalatability by the brooded embryos (e.g., gradually as they grow, suddenly when they were ready to be released), nor on its relationships with the temperature-dependent differences in size at larval release reported for the species (Nozais et al., 1997).

Both swimming larvae and juveniles were unpalatable. However, some worms disappeared from the experimental cultures in both cases, the survivorship being initially lower in the former than the latter. In agreement with the toxicity results, this may result from a lower concentration of compounds in swimming larvae than in juveniles. According to the behavioral observations, this mortality could be interpreted as a training process for the predators which systematically rejected both larvae and juveniles after the initial successful attacks. Larval mortality as a consequence of the predator's manipulation during unsuccessful attacks was higher than juvenile mortality probably because juveniles were less delicate. However, no differences in survival between both attacked or spared larvae and juveniles after a single trial were observed as has been reported for other unpalatable larvae (Lindquist and Hay, 1996). After various attacks, a worm may be severely damaged or killed. However, such repeated attacks would probably not occur in the field. After realizing the unpalatability of *E. nebulosa*, the predator would presumably find a wide range of potentially edible preys in the habitat.

The two main external structures built by *E. nebulosa* are also thought to have a protective role. However, the gelatinous egg-masses, which are attached to the female's tube openings and exposed, were non-toxic, but the tubes, which are made of mucus-aggregated sand grains and are found under boulders, showed antibacterial activity.

The existence of chemically defended cocoons was reported for the nudibranch *Hexabranhus sanguineus* (Pawlik et al., 1988). In polychaetes, it was suggested that the antiseptic bromophenols produced by terebellids of the genus *Thelepus* may be transferred to the mucous cocoons, which are formed by the tentacles as in *E. nebulosa*, although no studies have specifically determined whether this transfer does occur (Goerke et al., 1991). In *E. nebulosa*, generalist macrobenthic predators, such as crabs or starfish, did not consume the cocoons in experimental aquaria (Medernach, 1995), nor did the labrid *C. julis* disturb the cocoons in the field. Moreover, a layer of disaggregated egg-masses prevented these fish from feeding on sea urchins which are normally consumed. There are two possible explanations for this apparent contradiction. First, deterrence and toxicity in *E. nebulosa* could be based on different compounds. In fact, it has been stated that chemical unpalatability is not necessarily associated with toxicity (Schulte and Bakus, 1992; Pawlik et al., 1995). Although antibacterial compounds might be expected to protect the embryos from bacteria, they might not be present in the egg-masses while deterrent ones would. However, chemical defences are costly in metabolic terms (Fagenström et al., 1987; Turón et al., 1996) so that the production of two different groups of compounds would imply double costs.

The second hypothesis that a physical deterrent is linked to the gelatinous consistency of the mucous egg-masses appears more plausible. As mentioned above, the old egg-masses harbor many meiofaunal organisms which move actively throughout the cocoon. Although the possibility that specific adaptations to the cocoon as a habitat (i.e., commensalism) exists may not be totally discarded, most of these organisms were species that may be found living outside the cocoons. Moreover, there is no reason to assume that chemical deterrents would not affect these small organisms as they affect fish predators. Conversely, a physical deterrence could be effective against large macrofaunal predators while the small meiofaunal ones would remain unaffected. In that case, the cocoon could be a satisfactory habitat having a density only slightly different from that of the seawater. This second possibility also seems more functional. Although there is no evidence, we might assume that cocoon materials (i.e., water plus some molecules of muco-polysaccharides) are less metabolically costly than toxics while they simultaneously perform two relevant tasks: aggregation of larvae to prevent dispersion (Bhaud et al., 1995) and anti-predatory protection.

In the case of the tubes, the toxic activity detected may be an additional anti-predatory mechanism. However, the tubes of *E. nebulosa* are hidden under boulders. Thus, metabolic expense to increase their anti-predator defenses appears to be redundant. In fact, besides predator deterrence (Green, 1977) other ecologically relevant roles have been reported for toxic activities: viz antifouling mechanisms (Becerro et al., 1994) and competition for space (Uriz et al., 1992). Thus, it is more reasonable to assume an anti-epibiont role for this toxic activity which would allow the worms to keep clean both the inner and outer surfaces of the tube. Similar roles have been suggested for antimicrobial compounds such as bromophenols found in tubicolous worms including arenicolid and other terebellid polychaetes (Sheikh and Djerassi, 1975; King, 1986; Woodin et al., 1987).

An ecological role as antiseptics has been also suggested for the antimicrobial compounds present in terebellids (Goerke et al., 1991). In the species of *Thelepus*, these compounds were more abundant in the most distal parts of the body, the tentacles and the pygidium. These distal parts are more exposed to injury by predators as they protrude from worm's tube for feeding and defecation. Thus, it was considered that antiseptic compounds would be more efficient there. *E. nebulosa* also showed the highest toxicity in the tentacles whereas the posterior end was the least toxic part of the body and the thorax was intermediate. Although this antiseptic task could also be expected for the antibacterial compounds found in *E. nebulosa*, it did not explain the differences found between the two extremes of the body. The highest toxicity level could be related to the feeding deterrence shown by the tentacles in the anti-predatory experiments while the lowest toxicity of the abdomen could be related to another mechanism of defense occurring in this species: autotomy. When disturbed or forced to leave their tubes, the worms often release a portion of the abdomen which then starts to move actively. This may attract the attention of a threatening predator to the autotomized piece allowing the head end of the worm to escape. The autotomized region is then regenerated (authors, pers. observ.). Correspondingly, the abdomen portions were always fed in the anti-predatory experiments. The thorax, however, did not show clear deterrent pattern. In fact, some pieces were eaten normally while others triggered immediate rejection by the fish, exactly as the tentacles did. This suggests a differential location of the toxicants in the thorax, perhaps related to their production in one of the three main glandular tissue zones of *E. nebulosa*. A first zone was located in the ventral anterior end and produced the cement used to aggregate

the sand during tube building. Two other zones occurred laterally around the thoracic parapodia and produced the cocoon materials. The ventral zone must be active all year round in both sexes (authors, pers. observ.) and could be related with the toxicity detected in tubes. The two lateral zones were only active in females during the reproductive period (authors, pers. observ.). Thus, as there were not significant differences in toxicity between males and females, and the present experiments were carried out during the reproductive period, we suggest that the lateral zones were not related to the production of toxic compounds.

In conclusion, our results did not allow us to determine whether *E. nebulosa* was toxic to the extent that their ingestion might affect the predators. However, they demonstrated that some stages and body regions were unpalatable and, thus, defended against some of their potential predators. Two different deterrent effects could be proposed as being operative in *E. nebulosa*. (1) A more likely physically mediated unpalatability for the egg-masses, linked to their gelatinous consistence. (2) A more-likely chemically-mediated unpalatability for early life cycle stages (i.e., swimming larvae and juveniles) and adult tentacles, which is related to their high levels of antibacterial toxicity. The coincidence of trends in toxic activities and deterrent behavior supports a close relationship between both mechanisms of chemical defense, suggesting a possible common origin.

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LITERATURE CITED

- Becerro, M. A., N. I. López, X. Turón and M. J. Uriz. 1994. Antimicrobial activity and surface bacterial film in marine sponges. *J. Exp. Mar. Biol. Ecol.* 179: 195–205.
- _____, M. J. Uriz and X. Turón. 1995. Measuring toxicity in marine benthic environments: critical appraisal of three commonly used methods. *Experientia* 51: 414–418.
- Bhaud, M., J. H. Cha, J. C. Duchêne, D. Martin and C. Nozais. 1995. Larval biology and benthic recruitment: New prospect on the role of egg-masses and modelling life-cycle regulation. *Sci. Mar.* 59: 103–117.
- Davenport, D. 1953. Studies in the physiology of commensalism. IV. The polynoid genera *Polynoe*, *Lepidasthenia* and *Harmothoe*. *J. Mar. Biol. Ass. U.K.* 32: 273–288.
- Duchêne, J. C. and C. Nozais. 1992. Étude de l'émission des stades larvaires précoces d'*Eupolyornia nebulosa* (Polychaeta: Terebellidae). Pages 15–12 in P. Nival, J. Boucher and M. Bhaud, eds. IIIe Colloque du Programme National sur le Déterminisme du Recrutement. *Annls. Inst. Océanogr. Monaco* 68.
- Fagenström, T., S. Larsson and O. Tenow. 1987. On optimal defence in plants. *Func. Ecol.* 1: 73–81.
- Giangrande, A., S. Geraci and G. Belmonte. 1994. Life-cycle and life-history diversity in marine invertebrates and the implications in community dynamics. *Oceanogr. Mar. Biol. Ann. Rev.* 32: 305–333.
- Goerke, H. and K. Weber. 1990. Locally-dependent concentrations of bromophenols in *Lanice conchilega* (Polychaeta: Terebellidae). *Comp. Biochem. Physiol.* 97B: 741–744.

- _____ and _____. 1991. Bromophenols in *Lanice conchilega* (Polychaeta, Terebellidae): the influence of sex, weight and season. *Bull. Mar. Sci.* 48: 517–523.
- _____, R. Emrich, K. Weber and J. C. Duchêne. 1991. Concentrations and localization of brominated metabolites in the genus *Thelepus* (Polychaeta, Terebellidae). *Comp. Biochem. Physiol.* 99B: 203–206.
- Green, G. 1977. Ecology of toxicity in marine sponges. *Mar. Biol.* 40: 207–215.
- Hay, M. E. 1996. Marine chemical ecology: what's known and what's next? *J. Exp. Mar. Biol. Ecol.* 200: 103–134.
- Higa, T. and P. J. Scheuer. 1975. Constituents of the marine annelid *Thelepus setosus*. *Tetrahedron* 31: 2379–2381.
- King, G. M. 1986. Inhibition of microbial activity in marine sediments by a bromophenol from a hemichordate. *Nature (London)* 323: 257–259.
- Lindquist, N. and M. E. Hay. 1996. Palatability and chemical defense of marine invertebrate larvae. *Ecol. Monogr.* 66: 431–450.
- _____, _____ and W. Fenical. 1992. Defense of ascidians and their conspicuous larvae: adult vs. larval chemical defenses. *Ecol. Monogr.* 62: 547–568.
- Martin, D. and T. A. Britayev. 1998. Symbiotic polychaetes: Review of known species. *Oceanogr. Mar. Biol. Ann. Rev.* 36: 217–340.
- _____ and M. J. Uriz. 1993. Chemical bioactivity of Mediterranean benthic organisms against marine embryos and larvae of marine invertebrates. *J. Exp. Mar. Biol. Ecol.* 173: 11–27.
- Medernach, L. 1995. Rôle du cocon muqueux dans la protection des larves d'*Eupolymnia nebulosa* (Annélide Polychète). Rapport de Maîtrise de BOP thesis, Univ. Pierre et Marie Curie, Banyuls-sur-Mer.
- Nozais, C., D. Martin, J. H. Cha, J. C. Duchêne, F. Charles and A. Grémare. 1997. Dynamics of egg production in Mediterranean populations of the terebellid polychaete *Eupolymnia nebulosa*. *J. Mar. Biol. Ass. U.K.* 77: 1027–1043.
- Paul, V. J. and M. E. Hay. 1996. Palatability and chemical defense of marine invertebrate larvae. *Ecol. Monogr.* 66: 431–450.
- Pawlik, J. R., M. R. Kernan, T. F. Molinski, M. K. Harper and D. J. Faulkner. 1988. Defensive chemicals of the Spanish Dancer nudibranch, *Hexabranchnus sanguineus* and its egg ribbons: macrolides derived from a sponge diet. *J. Exp. Mar. Biol. Ecol.* 119: 99–109.
- _____, B. Chanas, R. J. Toonen and W. Fenical. 1995. Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrence. *Mar. Ecol. Prog. Ser.* 127: 183–194.
- Pechenik, J. A. 1979. Role of encapsulation in invertebrate life histories. *Am. Nat.* 114: 859–870.
- Schulte, B. A. and G. J. Bakus. 1992. Predation deterrence in marine sponges: laboratory versus field studies. *Bull. Mar. Sci.* 50: 205–211.
- Sheikh, Y. M. and C. Djerassi. 1975. 2,6-Dibromophenol and 2,4,6-tribromophenols - antiseptic secondary metabolites of *Phoronopsis viridis*. *Experientia* 31: 265–266.
- Turón, X., M. A. Becerro and M. J. Uriz. 1996. Seasonal patterns of toxicity in benthic invertebrates: the encrusting sponge *Crambe crambe* (Poecilosclerida). *Oikos* 75: 33–40.
- Uriz, M. J., D. Martin and D. Rosell. 1992. Relationships of biological and taxonomical characteristics to chemically mediated bioactivity in Mediterranean littoral sponges. *Mar. Biol.* 113: 287–297.
- _____, X. Turón, M. A. Becerro and J. Galera. 1996. Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. *J. Exp. Mar. Biol. Ecol.* 205: 187–204.
- Van Alstyne, K. L., C. W. Wyllie and V. J. Paul. 1994. Antipredator defenses in tropical soft corals (Coelenterata: Alcyonaria). II. The relative importance of chemical and structural defenses in three species of *Sinularia*. *J. Exp. Mar. Biol. Ecol.* 178: 13–34.
- Weber, K. and W. G. Ernst. 1978. Occurrence of brominated phenols in the marine polychaete *Lanice conchilega*. *Naturwissenschaften* 65: 262.

- Woodin, S. A., M. D. Walla and D. E. Lincoln. 1987. Occurrence of brominated compounds in soft-bottom benthic organisms. *J. Exp. Mar. Biol. Ecol.* 1987: 209–217.
- _____, R. L. Marinelli and D. E. Lincoln. 1993. Allelochemical inhibition of recruitment in a sedimentary assemblage. *J. Chem. Ecol.* 19: 517–529.
- _____, S. M. Lindsay and D. E. Lincoln. 1997. Biogenic bromophenols as negative recruitment cues. *Mar. Ecol. Prog. Ser.* 157: 303–306.
- Zar, J. H. 1984. *Biostatistical analysis*. Prentice Hall International, New Jersey. 718 p.

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