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Toxicity of the purple mucus of the polychaete *Halla parthenopeia* (Oenonidae) revealed by a battery of ecotoxicological bioassays

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Summary: Mucus secretions play a number of functions related to polychaete physiology and ecology. Under stress conditions, the polychaete *Halla parthenopeia* (Oenonidae) produces a purple mucus after mechanical stimulation, whose function is still unknown. Here, we assessed the toxicity of this purple mucus by means of both acute toxicity bioassays on the polychaete *Dinophilus gyrociliatus* and commercial ecotoxicological kits (Microtox®, Rotoxkit® and Artoxkit®). Palatability was also tested with the fish *Oryzias melastigma*. After emitting purple mucus, *H. parthenopeia* quickly moves away and starts releasing transparent mucus. Acute toxicity bioassays showed that the mucus was harmless (transparent), or lethal even when diluted about 1000 times (purple). Purple mucus was toxic at different concentrations, the LC_{50} ranging from 0.7-0.3 g l^{-1} for *D. gyrociliatus* to 76 g l^{-1} for *Artemia franciscana* (Artoxkit®). Freeze-dried brine shrimp coated with transparent or purple muci were both consumed by *O. melastigma*. We hypothesized that the purple mucus is involved in the chemical defence of *H. parthenopeia* against competitors and parasites, and that its colour and toxicity are due to hallachrome, a 1,2-anthraquinone found in the skin of *H. parthenopeia*.

Keywords: chemical defence; mucus; lethal effects; *Halla parthenopeia*; marine invertebrates; ecotoxicology.

La toxicidad del mucus púrpura del poliqueto *Halla parthenopeia* (Oenonidae) revelada por una batería de bioensayos ecotoxicológicos

Resumen: Las secreciones mucosas de los poliquetos desempeñan múltiples roles fisiológicos y ecológicos. El poliqueto *Halla parthenopeia* (Oenonidae) reacciona frente a una estimulación mecánica produciendo un mucus púrpura cuya función resulta, aun hoy en día, desconocida. El presente artículo evalúa la toxicidad de dicha secreción mediante bioensayos toxicológicos agudos (basado en el poliqueto *Dinophilus gyrociliatus*) y mediante kits ecotoxicológicos comerciales (Microtox®, Rotoxkit® and Artoxkit®). Asimismo, se analiza la palatabilidad mediante un test basado en el pez *Oryzias melastigma.* Tras secretar el mucus púrpura, *H. parthenopeia* se aleja rápidamente, al mismo tiempo que produce un mucus transparente. Los bioensayos toxicológicos agudos muestran que dichas secreciones son inocuas (transparente) o letal aun diluido unas 1000 veces (púrpura). El mucus púrpura puede ser tóxico a diferentes concentraciones y en función del test, oscilando su LC_{50} entre 0.7-0.3 g l^{-1} (para *D. gyrociliatus*) y 76 g l^{-1} (para *Artemia franciscana*, Artoxkit®). Las *Artemia* liofilizadas fueron comidas por *O. melastigma* tanto si estaban revestidas de mucus transparente como púrpura. Nuestros resultados nos hacen pensar que el mucus púrpura de *H. parthenopeia* podría estar involucrado en un mecanismo de defensa química contra sus posibles competidores, pero también frente a posibles parásitos, y que, probablemente, tanto su color como su toxicidad tengan relación con la presencia del pigmento hallacromo, una 1,2-antraquinona, descubierto en la piel de esta especie de oenónido.

Palabras clave: defensa química; mucus; efecto letal; *Halla parthenopeia*; invertebrados marinos; ecotoxicología.

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INTRODUCTION

Mucus production constitutes a key morphological and functional feature affecting the survivorship of many polychaetes (Bonar 1972, Giangrande et al. 2013), having numerous physiological and ecological functions such as feeding and tube building (e.g. Lewis 1968, Gaill and Hunt 1986, Mouneyrac et al. 2003). Several hesionid, dorvilleid and terebellid species secrete mucus to produce brood chambers, egg cases or tubes (Storch 1988, Martin et al. 2000, Simonini et al. 2009). In several sabellid species, the mucus is involved in tube building, in defence from pathogenic bacteria and in electrolyte homeostasis (Stabili et al. 2009, Giangrande et al. 2013).

Individuals of the oenonid species *Halla parthenopeia* and *H. okudai* can produce muci with different functions (Imabayashi et al. 1996, Osman et al. 2010a, b). The genus *Halla* includes some large polychaetes (up to 1 m long) living in soft bottoms of coastal temperate and sub-tropical marine habitats (Osman et al. 2010a). In particular, *H. parthenopeia* and *H. okudai* occur, respectively, in the Mediterranean and the northern Red Sea (Osman et al. 2010a, b), and in the western Pacific (Imabayashi et al. 1996, Idris and Arshad 2013). Both are active bivalve predators and produce two types of mucus involved in foraging and feeding (Imabayashi et al. 1996, Kawai et al. 1999, Osman et al. 2010 a, b). These worms feed by wounding their prey and secreting abundant mucus with paralytic activity that forces the bivalve to open its valves. Then, the worm consumes the bivalve soft tissues by suction, after having digest them thanks to a digestive transparent mucus (Kawai et al. 1999, Osman et al. 2010a). Both species produce a third type of transparent mucus (structural) that facilitates locomotion and helps to stabilize and keep open their galleries (Kawai et al. 1999, Osman et al. 2010a).

Under stress conditions, *H. parthenopeia* also secretes an additional type of mucus with a characteristic purple colouration, as known since its original description (Delle Chiaje 1828). Also, fishermen's internet forums often complain about the difficulty in removing the purple colour from the bare hands after the manipulation of the worms. *Halla parthenopeia* is in fact used as fishing bait in Mediterranean countries, where it is referred to as 'Ver de chalut' (France), 'Llobarrero' (Spain) or 'Dragone' (Italy) (Normandie Appats Italia, personal communication). Histological assays demonstrated that the purple mucus is produced by epidermal glandular structures, whose pores are irregularly distributed along the surface of the animal (Bielig and Möllinger 1960).

Within the framework of a project aimed at developing new techniques for the aquaculture of Mediterranean polychaete species (Nesto et al. 2012), we reared some *H. parthenopeia* specimens. Some worms extracted from the sediment and placed in clean tanks with sea water during routinely biometric measurements responded by producing a considerable quantity of transparent mucus similar to that reported by Osman et al. (2010a). Furthermore, individuals most roughly transferred emitted a large amount of purple mucus. Consequently, protozoans and invertebrates that normally occurred in our marine tanks (i.e. *Euplotes* sp., rotifers, copepods and interstitial polychaetes) got trapped within mucus and were either killed (purple) or remained alive (transparent).

The only available information that supports a potentially biological activity of the purple mucus came from research performed in the 1930s. Specimens of *H. parthenopeia* immersed in distilled water immediately turned it purple (Friedheim 1932, 1933). The pigment, extracted with diethyl ether and re-suspended in seawater, caused an increase in the respiratory rates in eggs of the sea urchin *Paracentrotus lividus* and *Sphaerechinus granularis* (Friedheim 1932). However, the function of the purple mucus is still unknown.

In this study, we aimed at filling this gap by examining the behaviour and modalities of secretion of both transparent and purple mucus in *H. parthenopeia*: we proposed an optimized procedure for mucus collection and we assessed their toxicity through acute toxicity bioassays with the dinophilid polychaete *Dinophilus gyrociliatus.* This species well represents small sized invertebrates and larvae/juveniles that may contact the mucus (Simonini et al. 2011). We expected harmful effects only for the purple mucus. In addition, the commercial ecotoxicological tests (Microtox®, Rotoxkit® and *Artoxkit*®) were used to evaluate the toxicity of the purple mucus on different taxa (bacteria, rotifers and crustacean, respectively) and a palatability assay with the teleostean *Oryzias melastigma* was used to test its potential deterrence.

MATERIALS AND METHODS

Rearing of *Halla parthenopeia* and mucus collection

We used 26 specimens of *H. parthenopeia* (5-15 g wet weight, 25-40 cm in length) collected by professional divers in sandy-muddy sea bottoms (about 10 m depth) near Marseille, France.

The specimens were transported individually or in pairs in small plastic boxes containing 1 cm of wet sand, and placed inside containers refrigerated at 12°C-17°C. Once in the laboratory, they were transferred to three 80-L plastic tanks with 7 cm of sand and ca. 50 L of artificial sea water (Reef Crystal, Instant Ocean, salinity 30-35). The worms were equally grouped into three pools (pools 1, 2, and 3), each assigned to a tank covered with a lid of transparent plastic to minimize evaporation. Water circulation was guaranteed by two porous flints at the opposite corners of each tank, to provide sufficient aeration to both water and sediment. During the experimental period, half of the water in each tank was renewed with clean artificial sea water once a week.

During laboratory rearing, the animals were periodically fed on live clams (*Ruditapes philippinarum,* 2-4 cm in length). Every two-three days we replaced the consumed clams (found with open and clean valves with or without traces of digestive mucus) or dead

clams (with open valves and tissues still attached). We maintained a density of one to two living clams per worm and we recorded the consumed preys and the traces of digestive mucus.

The animals of each pool were removed from their tank the day before collecting the two types of mucus, gently transferred in pairs into smaller plastic tanks (40×60×12 cm) with the same type of seawater as in the tank of origin, with a flint ventilation. In the small tanks, the animals settled along the edges and built tubes of transparent mucus mixed with sand, which were removed after a few hours with the aid of absorbent paper.

The transparent and purple mucus for the experiments was collected in three main steps. First, the transparent mucus produced during the night was removed and stored at 4°C. Then, the worms were repeatedly stimulated (with plastic pliers or by transferring them to adjacent low tanks) until they secreted purple mucus, which was collected using plastic tweezers or pipettes and stored at 4°C. Stimulation was repeated three or four times at most, to avoid excessive stress.

The refrigerated samples of transparent and purple mucus were placed separately into 50-mL polypropylene vials and centrifuged (about 2600 g for 5 min, Thermo Scientific PK110). The supernatant water was removed and known volumes of each type of mucus were weighed and homogenized on ice (T8, IKA-Werke). Aliquots of fresh mucus were immediately used for the assays, while the remaining material was divided into aliquots of 0.8-1.2 g, stored in polypropylene vials, and frozen at –80°C.

The whole operation took about 2 h for each pair of worms and was performed after 3, 5 and 8 months of laboratory rearing for pools 1, 2 and 3, respectively.

Acute toxicity bioassays with *Dinophilus gyrociliatus*

Dinophilus gyrociliatus is a small-size (max. 1 mm long) progenetic species with a short life-cycle (10 d between zygote and first reproduction at 24°C) that colonizes coastal hard and soft bottoms (ASTM 2000, Marcheselli et al. 2010) and has been used in water toxicity tests since the 1980s. This species is very sensitive to various toxic compounds (metals, detergents, xenobiotics, ordnance compounds, palytoxin) and can be easily cultured in the laboratory. Acute tests with newborn individuals require small volumes of seawater samples, are easy to set up, cheap, fast (96 h), and give reproducible results (Reish and Gerlinger 1997, Simonini et al. 2011). ASTM (2000) and Marcheselli et al. (2010) reported detailed descriptions and references for culturing and testing procedures with *D. gyrociliatus*. In fact, this species tolerates experimental conditions widely different from those provided in classical acute tests, confirming its usefulness as a test species for assessing the effects of non-conventional matrices, such as seawater samples containing microalgae (Simonini et al. 2011) or mucus suspensions (as in the present study).

The laboratory culture of *D. gyrociliatus* used in this study was established with specimens collected from the Venice Lagoon (Italy) in 2007. Worms were maintained in the laboratory under constant temperature (24°C), photoperiod (12 h light/dark) and salinity (32-37), and fed on fish food TetraMin (Tetra).

For each assay, about 500 juveniles were collected within 24 h from hatching and randomly assigned to an experimental treatment. Each treatment included 5 bowls, each containing 10 mL of the experimental solutions and 10 individuals of *D. gyrociliatus*. Fish food was not provided during the tests. Artificial seawater was used as control. We assessed the level of sensitivity of the *D. gyrociliatus* strain used by performing $LC_{50\ 96\ h}$ tests using $Cu(NO_3)_2$ (Panreac Quimica, analytical grade) as a reference toxic substance before and after the experiments with mucus. Tests with the fresh mucus, transparent or purple, were repeated for each of the three pools of *H. parthenopeia* to obtain a reliable measure of mucus toxicity as well as the possible influence of the duration of the rearing period and ageing.

The experimental mucus solutions were obtained after appropriate dilutions in artificial sea water. The concentrations tested ranged between 0.2 and 2 g l^{-1} (0.02-0.2%) for the purple mucus (fresh and frozen) and between 10 and 500 g l^{-1} (1 and 50%) for the transparent mucus. The tests with the transparent mucus were extended to ten days, to eventually exclude potential long-term toxicity.

Tests with transparent and purple frozen mucus (after 20 d and 90 d at –80°C) from individuals of pool 2 were performed to assess possible differences in toxicity with respect to fresh mucus. Preliminary analyses showed that the toxicity of the purple mucus was not affected by freezing (ANOVA: $F_{2,12}=2.28$, $p>0.12$) and no mortality was observed in the treatments with frozen transparent mucus. Consequently, the commercial ecotoxicity tests were performed with the purple frozen mucus to avoid both logistic problems (e.g. impossibility to perform all tests at the same time) and excessive stressing of the worms.

Ecotoxicological tests with Microtox®, Rotoxkit® and Artoxkit®)

Microtox® liquid phase test is an acute toxicity bioassay based on the reduction of the bioluminescence activity by the marine bacterium *Vibrio fischeri* after 15 min of exposure to a toxic matrix. The test was carried out in the Microtox M500 analyser (Modern Water) according with the ISO 11348-3:2007 protocol. The tested concentrations of purple frozen mucus ranged from 0.3 to 30 g l^{-1}.

The Rotoxkit M® bioassay uses newborns emerging from the cysts of the rotifer *Brachionus plicatilis* that were exposed to concentrations of mucus ranging from 0.3 to 18 g l^{-1}. The Artoxkit M® test uses instar II–III larvae of the anostracan crustacean *Artemia franciscana*. The concentrations of purple frozen mucus tested with Artoxkit M® ranged from 1% to 50%. Artoxkit M® and Rotoxkit M® were run for 24 h and were conducted according to the standard operating procedure (Microbiotests Inc, www.microbiotests.be).

Palatability of *Halla parthenopeia* and its mucus

The marine medaka *Oryzias melastigma*, an emerging model fish for marine toxicological studies (e.g. Wu et al. 2012), was used for the palatability tests. About 60 juveniles, laboratory-reared in aerated artificial sea water (salinity 32) at 28°C in a 14 h/10 h light/dark cycle regimen for a period of two months before experiments, were provided by Aurifish, Italy. Fish were fed three times a day with fragmented fish flakes (Tetra Pro Energy, Tetra) and living *A. franciscana*. Then, eight *O. melastigma* were isolated for one week in 5-L plastic aquaria under the same conditions except for being fed with freeze-dried *A. franciscana* (FDA) (Sera). Standard feeding assays (Kicklighter and Hay 2006) using FDA as control food, were conducted 2 h after morning feeding. Specifically, if FDA was consumed, we offered the fish an FDA coated with the fresh purple mucus. The procedure was repeated with the same fish the next day, using FDA and FDA coated with fresh transparent mucus.

Data analysis

The trimmed Spearman-Karber method was used to obtain LC_{50}/EC_{50} (median lethal/effective concentration) values and their relative 95% confidence intervals (c.i. 95%) for all species/tests. Abbott's correction (Abbott 1925) was adopted in the (rare) cases in which effects were also observed in controls.

For the tests with *D. gyrociliatus*, a one-way ANOVA was used to compare $LC_{50\ 96\ h}$ among pools. A Student-Newman-Keuls (SNK) test was run when significant differences between experimental groups were detected. ANOVA and SNK tests were performed on data that were both normal and homoscedastic. Normality and homoscedasticity were checked using the Jarque-Bera and Cochran tests, respectively. Fisher's exact test was used to assess the frequency of consumer acceptance of control FDA vs. FDA coated with transparent or purple mucus in the palatability experiment.

RESULTS

Halla parthenopeia behaviour during mucus production

In resting conditions and in the absence of sediment, *H. parthenopeia* produced a transparent viscous mucus forming a tube around its body. After removing the mucus, mechanical stimulation induced the emission of purple mucus. Initially, the animal wriggled projecting mucus in the surrounding water, which became rapidly purple. Then, it restarted to crawl and the purple mucus formed a casing around it. Subsequently, the worm quickly produced abundant transparent mucus (Fig. 1) and moved away from the purple secretion. With repeated stimulation, the production of mucus (transparent and purple) visibly decreased, and the worm's movements slowed down.

The density of the transparent and purple mucus were similar to that of the sea water (1.022 g l^{-1}). For each pool, about 80-150 mL of purple mucus was obtained, with an average of 12-13 g per worm.

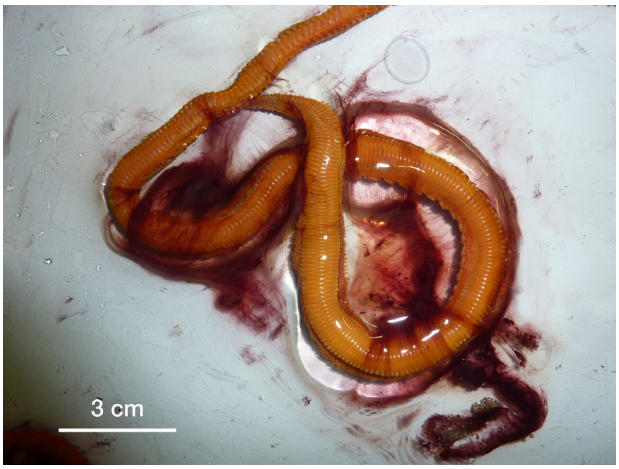

Fig. 1. – *Halla parthenopeia* (40 cm long) a few minutes after mechanical stimulation, surrounded by abundant transparent mucus and, further outward, by purple mucus.

Bioassays with *Dinophilus gyrociliatus*

The fresh transparent mucus had no adverse effects on *D. gyrociliatus*. During the 96-h test, in fact, all *D. gyrociliatus* consumed mucus and survived at the highest concentration tested (50% of transparent mucus in sea water). After one more week, all individuals grew to sexual maturity and reproduced. At this stage, the mucus was massively colonized by the ciliate *Euplotes crassus*, a common inhabitant of healthy *D. gyrociliatus* cultures.

In contrast, the exposure to the purple mucus was quickly lethal at relatively low concentrations (Fig. 2). Tests using purple mucus from pools 1 and 2 gave similar results, while that from pool 3 showed a significantly greater toxicity (ANOVA: $F_{2,12}$=386, p<0.001; SNK test pool 1 = pool 2 ≠ pool 3). The 100% mortality was observed at 1.3 g l^{-1} for pool 1 and 2, and 0.78 g l^{-1} for pool 3.

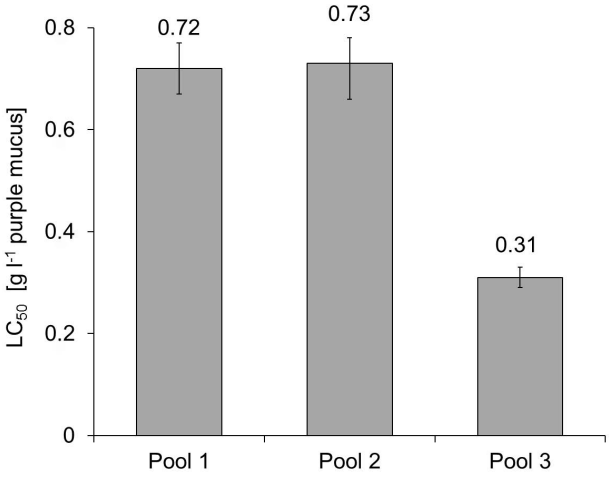

Fig. 2. – Acute toxicity bioassays with *Dinophilus gyrociliatus*. Median lethal concentrations (LC_{50}, with 95% confidence intervals) after 96 h of exposure to the purple mucus of *H. parthenopeia*.

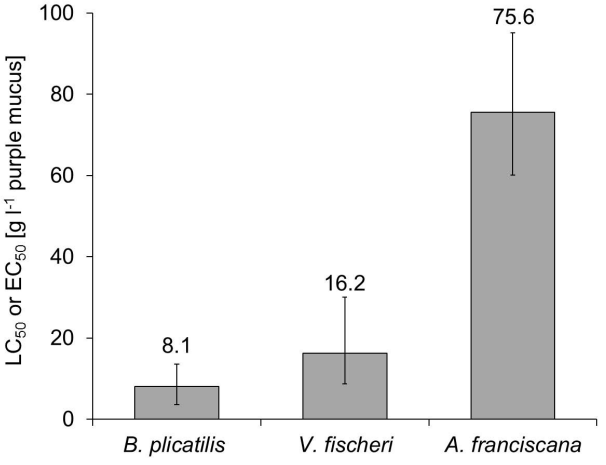

Fig. 3. – Median lethal/effect concentrations (LC_{50}, or EC_{50}, with 95% confidence intervals) estimated for *Brachionus plicatilis* (Rotoxkit®, 24 h), *Vibrio fischeri (*Microtox®*,* 15 min) and *Artemia franciscana* (Artoxkit®, 24 h) after exposure to the frozen purple mucus produced by *Halla parthenopeia* (pool 2).

Tests with $Cu(NO_3)_2$ evidenced a similar sensitivity of the strain of *D. gyrociliatus* for the two trials performed before ($LC_{50\ 96\ h}$= 0.10 mg l^{-1}; c.i. 95%=0.09-0.11 mg l^{-1}) and after ($LC_{50\ 96\ h}$= 0.11 mg l^{-1}; c.i. 95%= 0.10-0.12 mg l^{-1}) the main experiments.

Bioassays with commercial ecotoxicological kits

The purple mucus affected all tested species negatively (Fig. 3). The rotifer *B. plicatilis* was the most sensitive (Rotoxkit®, $LC_{50\ 24\ h}$= 8.1 g l^{-1}, c.i. 95%= 3.6-13.6 mg l^{-1}). The emitted luminescence of *V. fischeri* was also influenced by the exposure to the purple mucus (Microtox®, $EC_{50\ 15\ min}$=16.2 g l^{-1}; c.i. 95%=8.7-30 mg l^{-1}). Finally, lethal effects on *A. franciscana* were observed for relatively high concentrations (Artoxkit®, $LC_{50\ 24\ h}$=76 g l^{-1}; c.i. 95%=60.1-95.1 mg l^{-1}).

Palatability assays

Transparent and purple mucus did not deter fish feeding (Fisher exact test, p=1.000). Control FDA and FDA coated with either transparent or purple mucus were always readily tasted and then eaten by all eight individuals of *O. melastigma*.

DISCUSSION

The transparent mucus of *Halla parthenopeia* produced no toxic effects on *D. gyrociliatus*, which survived to 10-day prolonged exposures using this transparent mucus as a food. In contrast, the purple mucus obtained from the three pools of *H. parthenopeia* was extremely toxic, even a 1000-time dilution causing 100% of mortality after 96 h. The LC_{50} for the first two pools were twice those calculated for the third pool. Several factors may explain the among-pool variability: for instance, the different duration of laboratory rearing, the different weight/age of the worms, the heterogeneous consistency of the mucus when secreted and the consequent difficulty in separating the simultaneously produced purple and transparent mucus. Conversely, the sensitivity of the *D. gyrociliatus* strain may be discarded as a possible source of variability, as the $LC_{50\ 96\ h}$ in the reference tests were similar throughout the experimental phase and consistent with those previously reported (Reish and Gerlinger 1997, Simonini et al. 2011). In parallel, this confirms the reproducibility of the responses of *D. gyrociliatus* to toxicants (Simonini et al. 2011).

The toxicity of the purple mucus did not change after freezing, even after three months of storage. This feature represents a highly functional characteristic to be considered for the design of future ecotoxicological assays with this secretion. The results obtained with frozen mucus in the commercial assays gave further support for its harmful effects, and highlighted the broad-spectrum of its activity on marine invertebrates.

The noteworthy morphological, life-history and phylogenetic differences existing among the test species, as well as the different duration of the assays, did not permit purely quantitative comparisons among them. However, notwithstanding these limitations, two species, *D. gyrociliatus* and *B. plicatilis*, appear to be more sensitive and two, *V. fischeri* and *A. franciscana*, more tolerant. In particular, the LC_{50} obtained with Microtox® and Artoxkit® were, respectively, 20 and 80 times greater than those observed in the acute tests with *D. gyrociliatus.* This pattern is not surprising as *D. gyrociliatus* becomes a very sensitive species when overstressed (e.g. Marcheselli et al. 2010, Simonini et al. 2011). In turn, *Artemia* is often considered as a less sensitive organism (see Nunes et al. 2006). Accordingly, *Artemia*-based assays showed a lower sensitivity when compared with Microtox® and Rotoxkit® under the same experimental conditions (Guerra 2001).

Our study describes for the first time the modality of emission of the purple mucus in *H. parthenopeia*, a noteworthy ability in terms of both quantity and toxicity. A concentration of about 1 g l^{-1} was sufficient to kill all individuals of *D. gyrociliatus* in the acute bioassays. Thus, in an extremely simplified scenario where the mucus can diffuse freely and homogeneously in water, one *H. parthenopeia* producing 12-13 g of purple mucus would exterminate all *D. gyrociliatus* occurring in 10 L of sea water.

After the emission of purple mucus, *H. parthenopeia* produced a thick layer of transparent mucus that created a barrier between the toxic secretion and its body wall (Fig. 1), and then quickly moved away. These findings suggest a protective role of the transparent mucus in limiting the exposure of *H. parthenopeia* to its toxic exudate and merit further investigation.

The emission of secretions, such as ink or mucus, is an anti-predatory strategy adopted by several marine invertebrates. For example, when physically disturbed, the sea hares (Opisthobranchia: Anaspidea) release a purple ink which functions as a deterrent against potential predators and has a shading effect too, acting as a 'smoke screen' and permitting the escape of the animal after its production (Carefoot et al. 1999, Nolen and Johnson 2001). We considered the possibility that

the purple mucus produced by *H. parthenopeia* could have a shading effect, but we discarded it because after its emission most of the purple pigment remains attached to the mucus. The pigment that dissolves in water is not sufficient to create a shadowing effect (the water remains transparent). Finally, the occurrence of a shading strategy seems unlikely in *H. parthenopeia*, a relatively slow-moving worm (even when disturbed) which lives in galleries in the sediment.

The phyllodocid polychaete *Phyllodoce mucosa* exhibits an anti-predatory response via the extrusion of a repulsive mucus, which prevents the ingestion of the worm by several species of fish (Prezant 1980). However, this seems not to be the case of the purple mucus of *H. parthenopeia*. In fact, the results of palatability experiments do not support its function as a predator-deterrent, and are in line with field observations. These worms are normally ingested by fishes such as the seabass *Dicentrarchus labrax* and the gilthead seabream *Sparus aurata* and thus are commonly used as fish baits (Normandie Appats, personal communication). Indeed, preliminary observations under laboratory conditions evidenced that the American lobster *Homarus americanus* consumed pieces or whole individuals of *H. parthenopeia* indiscriminately even when the purple mucus was emitted (Simonini, personal observations). On the other hand, *H. parthenopeia* is a mobile worm living in tubes within unconsolidated sediments (Osman 2010a), which are characteristics often observed in palatable species lacking chemical and mechanical deterrents against predators (Kicklighter and Hay 2006). Moreover, palatability experiments evidenced that polychaete species closely related to Oenonidae, such as *Lumbrineris* sp. *Arabella iricolor* and *Drilonereis filum*, are commonly predated by crabs and fishes (Kicklighter and Hay 2006). Successive tests with multiple large-sized predators may also help to exclude any anti-predatory role of the purple mucus.

In some marine worms, the production of mucous secretion is involved in the defence strategy against competitors, parasites and/or pathogens. For example, the mucoid fluid excreted by the lugworm *Arenicola marina* and the cat-worm *Nephtys hombergii* inhibits the settlement of juvenile and larvae of intra- and interspecific competitors (Hardege et al. 1998). Sabellid species such as *Sabella spallanzanii*, *M. infundibulum* and *S. spectabilis* secrete a high amount of mucus with high lysozyme-like activity, which plays an important role in defending the worms from bacterial attack (Giangrande et al. 2013). Independently from their biological function, exudate emission can be induced through mechanical stimulation of the worms (Hardege et al. 1998; Giangrande et al. 2013). Accordingly, the capacity of *H. parthenopeia* purple mucus to exert toxic effects on organisms that are representative of very different taxonomic groups suggests that it can act as a chemical deterrent against a broad range of potential competitors, parasites and/or pathogens.

It has been hypothesized that different types of natural compounds such as peptides (e.g. the perinerin obtained from *Perinereis aibuhitensis* [Pan et al. 2004] and the lysozyme produced by sabellid species), pigments (the bonellin extracted from *Bonellia viridis* [Giudici 1984]), halogenated metabolites (e.g. Kicklighter et al. 2004), and uncommon amphipathic substances (2-alkylpyrrole sulfamates from the cirratulid *Cirriformia tentaculata* [Kicklighter et al. 2003]; complanine from the fireworm *Eurithoe complanata* [Nakamura et al. 2008]), could defend marine annelids from competitors, pathogens or predators.

At present, we do not know the origin of the toxicity of the purple mucus. The main difference between non-toxic and toxic mucus was the purple colour of the latter. The skin of *H. parthenopeia* contains a red pigment, hallachrome, a 1,2-anthraquinone unsubstituted at positions 9 and 10 (Prota 1971), whose biological activity is unknown. To the best of our knowledge, the only other 1,2-anthraquinone natural products are sinapiquinone and rufoolivacin C and D, three pigments isolated from mushrooms of the genus *Cortinarius* (Gill and Milanovic 1999, Gao et al. 2010). Interestingly, rufoolivacin C and D are toxic to *A. franciscana* (Gao et al. 2010). Thus, we may hypothesize that the hallachrome could be harmful for some marine organisms.

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REFERENCES

Abbott W.S. 1925. A method of computing the effectiveness of an insecticides. J. Econ. Entomol. 18: 265-267.

ASTM 2000. E 1562-00 Standard guide for conducting acute, chronic and life-cycle aquatic toxicity tests with polychaetous annelids. ASTM, West Conshohocken, USA, 21 pp.

Bielig H.J., Möllinger H. 1960. Über hallochrom, den epithhelfarbstoff von *Halla parthenopeia* Costa. H.-S. Z. Physiol. Chem. 321: 276-289. http://dx.doi.org/10.1515/bchm2.1960.321.1.276

Bonar D.B. 1972. Feeding and tube construction in *Chone mollis* Bush (Polychaeta). J. Exp. Mar. Biol. Ecol. 9: 1-18. http://dx.doi.org/10.1016/0022-0981(72)90002-0

Carefoot T.H., Pennings S.C., Danko J.P. 1999. A test of novel function (s) for the ink of sea hares. J. Exp. Mar. Biol. Ecol. 234: 185-197. http://dx.doi.org/10.1016/S0022-0981(98)00153-1

Delle Chiaje S. 1828. Memorie sulla storia e notomia degli animali senza vertebre del Regno di Napoli (Vol. 3). Stamperia della società Tipografica, Napoli, 232 pp.

Friedheim N.A.H. 1932. Sur deux ferments respiratoires accessoires d'origine animale. C. R. Acad. Sci. III-Vie 111: 505-507.

Friedheim N.A.H. 1933. Das Pigment von *Halla parthenopeia*, ein akzessorischer Atmungs-Katalysator. Biochem Z. 259: 258-268.

Gaill F., Hunt S. 1986. Tubes of deep sea hydrothermal vent worms *Riftia pachyptila* (Vestimentifera) and *Alvinella pompejana* (Annelida). Mar. Ecol. Prog. Ser. 34: 267-274. http://dx.doi.org/10.3354/meps034267

Gao J.M., Qin J.C., Pescitelli G., et al. 2010. Structure and absolute configuration of toxic polyketide pigments from the fruiting bodies of the fungus *Cortinarius rufo-olivaceus*. Org. Biomol. Chem. 8: 3543-3551. http://dx.doi.org/10.1039/c002773a

Giangrande A. Licciano M., Schirosi R., et al. 2013. Chemical and structural defensive external strategies in six sabellid worms (Annelida). Mar. Ecol. 35: 36-45. http://dx.doi.org/10.1111/maec.12053

Gill M., Milanovic N.M. 1999. Pigments of Fungi. LVIII. Sinapiquinone and sinapicolone, red pigments from the toadstool *Cortinarius sinapicolor*. Aust. J. Chem. 52: 1035-1040. http://dx.doi.org/10.1071/CH99079

Giudici M.N 1984. Defence mechanism of *Bonellia viridis*. Mar. Biol. 78: 271-273. http://dx.doi.org/10.1007/BF00393013

Guerra R. 2001. Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents. Chemosphere 44: 1737-1747. http://dx.doi.org/10.1016/S0045-6535(00)00562-2

Hardege J.D., Bentley M.G., Snape L. 1998. Sediment selection by juvenile *Arenicola marina*. Mar. Ecol. Prog. Ser. 166: 187-195. http://dx.doi.org/10.3354/meps166187

Idris I., Arshad A. 2013. Checklist of polychaetous annelids in Malaysia with redescription of two commercially exploited species. Asian J. Anim. Vet. Adv. 8: 409-436. http://dx.doi.org/10.3923/ajava.2013.409.436

Imabayashi H., Saito H., Ohmasa C., et al. 1996. Foraging behaviour of the polychaete *Halla okudai* in aquarium. Benth. Res. 50: 11-17.

Kawai K., Kunitake H., Saito H., et al. 1999. Paralytic and digestive activities of jelly-like substances secreted by a Lysaretid polychaete, *Halla okudai*. Benth. Res. 54: 1-7.

Kicklighter C.E., Hay M.E. 2006. Integrating prey defensive traits: contrasts of marine worms from temperate and tropical habitats. Ecol. Monogr. 76: 195-215. http://dx.doi.org/10.1890/0012-9615(2006)076[0195:IPDTCO]2.0.CO;2

Kicklighter C.E., Kubanek J., Barsby T., et al. 2003. Palatability and chemical defense of tropical marine worms: alykylpyrrole sulfamates protect *Cirriformia tentaculata* from fishes. Mar. Ecol. Prog. Ser. 263: 299-306. http://dx.doi.org/10.3354/meps263299

Kicklighter C.E., Kubanek J., Hay M.E.. 2004. Do brominated natural products defend marine worms from consumers? Some do, most don't. Limnol. Oceanogr. 49: 430-441. http://dx.doi.org/10.4319/lo.2004.49.2.0430

Lewis D.B. 1968. Feeding and tube building in the Fabriciinae (Annelida, Polychaeta). Proc. Linn. Soc. Lond. 179: 37-49. http://dx.doi.org/10.1111/j.1095-8312.1968.tb01099.x

Marcheselli M., Conzo F., Mauri M., et al. 2010. Novel antifouling zinc pyrithione: short- and long-term effects on survival and reproduction of the marine polychaete *Dinophilus gyrociliatus*. Aquat. Toxicol. 98: 204-210. http://dx.doi.org/10.1016/j.aquatox.2010.02.010

Martin D., Le Nourichel C., Uriz M.J., et al. 2000. Ontogenic shifts in chimical defenses of the Northwest Mediterranean sea *Eupolymnia nebulosa* (Polychaeta, Terebellidae). Bull. Mar. Sci. 67: 287-298.

Mouneyrac C., Mastain O., Amiard J.C., et al. 2003. Trace-metal detoxication and tolerance of the marine worm *Hediste diversicolor* chronically exposed in their environment. Mar. Biol. 143: 731-744. http://dx.doi.org/10.1007/s00227-003-1124-6

Nakamura K., Tachikawa Y., Kitamura M., et al. 2008. Complanine, an inflammation-inducing substance isolated from the marine fireworm *Eurythoe complanata*. Org. Biomol. Chem. 6: 2058-2060. http://dx.doi.org/10.1039/b803107j

Nesto N., Simonini R., Prevedelli D., et al. 2012. Effects of diet and density on growth, survival and gametogenesis of *Hediste diversicolor* (O. F. Müller 1776) (Nereididae, Polychaeta). Aquaculture 362: 1-9. http://dx.doi.org/10.1016/j.aquaculture.2012.07.025

Nolen T.G., Johnson P.M. 2001. Defensive inking in *Aplysia* spp.: multiple episodes of ink secretion and the adaptive use of a limited chemical resource. J. Exp. Biol. 204: 1257-1268.

Nunes B.S., Carvalho F.D., Guilhermino L.M., et al. 2006 Use of the genus *Artemia* in ecotoxicity testing. Environ Pollut. 144: 453-462. http://dx.doi.org/10.1016/j.envpol.2005.12.037

Osman I.H., Gabr H.R., El-Etreby S. 2010a. Rearing trials of *Halla parthenopeia* under laboratory conditions (Polychaeta: Oenonidae). J. Exp. Mar. Biol. Ecol. 383: 1-7. http://dx.doi.org/10.1016/j.jembe.2009.10.016

Osman I.H., Gabr H.R., Saito H., et al. 2010b. Reproductive biology of the highly commercial polychaetes of the Suez Canal. J. Mar. Biol. Assoc. U.K. 90: 281-290. http://dx.doi.org/10.1017/S0025315409000186

Pan W., Liu X., Ge F., et al. 2004. Perinerin, a novel antimicrobial peptide purified from the clamworm *Perinereis aibuhitensis* Grube and its partial characterization. J Biochem. 135: 297-304. http://dx.doi.org/10.1093/jb/mvh036

Prezant R.S. 1980. An antipredation mechanism of the polychaete *Phyllodoce mucosa* with notes on similar mechanisms in other potential prey. Fish. Bull.-NOAA 77: 605-615.

Prota G., D'Agostino M., Misuraca G. 1971. Isolation and characterization of hallachrome, a red pigment from the sea worm *Halla parthenopeia*, Experientia 27: 15-16. http://dx.doi.org/10.1007/BF02137712

Reish D.J., Gerlinger T.V. 1997. A review of the toxicological studies with polychaetous annelids. Bull. Mar. Sci. 60: 584-687.

Simonini R., Massamba N'Siala G., et al. 2009. Distribution of the genus *Ophryotrocha* (Polychaeta) in Italy: new reports and comments on the biogeography of Mediterranean species. Vie Milieu 59: 79-88.

Simonini R., Orlandi M., Abbate M. 2011. Is the toxic dinoflagellate *Ostreopsis* cf. *ovata* harmful to Mediterranean benthic invertebrates? Evidences from ecotoxicological tests with the polychaete *Dinophilus gyrociliatus*. Mar. Environ. Res. 72: 230-233. http://dx.doi.org/10.1016/j.marenvres.2011.08.009

Stabili L., Schirosia R., Licciano M., et al. 2009. The mucus of *Sabella spallanzanii* (Annelida, Polychaeta): Its involvement in chemical defence and fertilization success. J. Exp. Mar. Biol. Ecol. 374: 144-149. http://dx.doi.org/10.1016/j.jembe.2009.04.016

Storch V. 1988. The ultrastructure of Polychaeta. I. Integument. In Westheide W., Hermans C.O. (eds), Microfauna Marina, Vol. 4. Gustav Fischer Verlag, pp 13-36.

Wu X., Huang Q., Fang C., et al. 2012. PFOS induced precocious hatching of *Oryzias melastigma* – From molecular level to individual level. Chemosphere 87: 703-708. http://dx.doi.org/10.1016/j.chemosphere.2011.12.060