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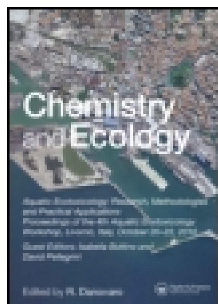

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Preliminary assessment of Ostreopsis cfr. ovata acute toxicity by using a battery bioassay

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Preliminary assessment of *Ostreopsis* cfr. *ovata* acute toxicity by using a battery bioassay

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Ostreopsis cfr. *ovata* toxicity was estimated through acute bioassays using four crustacean species (*Artemia franciscana*, *Tigriopus fulvus*, *Corophium insidiosum* and *Sphaeroma serratum*). The epiphytic dinoflagellate showed significant toxicity towards all tested crustaceans, which have usually exhibited the highest mortalities with increasing the dinoflagellate cell concentrations. Furthermore, our results evidenced a higher sensitivity of *A. franciscana* larvae to *Ostreopsis*, compared with the other species. The results of our bioassays highlighted the usefulness of employing a base-set of different species rather than one derived from a single species, in order to obtain more reliable information on the algal toxicity.

Keywords: bioassays; crustaceans; toxicity; *Ostreopsis* cfr. *ovata*

1. Introduction

In recent years, recurrent human health problems related to toxins produced by some species of microalgae have led to investigations into the occurrence of harmful species and to the development of fast, accurate and easy methodologies for toxin detection. Among the harmful algal species are epiphytic dinoflagellates belonging to the genera *Ostreopsis*, which have expanded their distribution during the last decade, and produce water-soluble toxins [1–4].

The genus *Ostreopsis* Schmidt (1901) belongs to the family Ostreopsidaceae Lindeman (1928) and has a worldwide distribution [5], including the Mediterranean Sea [5–12]. The origins of this genus are tropical and subtropical regions, where it usually forms assemblages with other benthic organisms [13]. In the Mediterranean Sea, the genus *Ostreopsis* includes species that are toxin producers and are now the object of study by many researchers. The increase in the incidence of problems associated with harmful and toxic microalgae suggests the need to establish adequate surveillance programmes, which are currently expensive and time-consuming. Thus, knowledge of the effects of these toxins on aquatic organisms is important for the establishment of water quality criteria.

Multiple detection methods, both biological and chemical, have been developed for the palytoxins and related compounds, and biosensors are also in development. However, none of these

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methods has been validated [14–16]. If sensitive chemical techniques that provide low (parts per billion) detection limits are needed, new inexpensive methods with high throughput would be preferred for regulatory monitoring of algal toxins. Compared with the above-mentioned methods, bioassays usually have the advantages of being simple, quick, sensitive and inexpensive. Indeed, the use of test organisms that can be easily maintained for laboratory studies all year around may prove a valuable contribution to scientific advances in detecting harmful algae toxicity [17]. However, the EU Water Framework Directive (2000/60/EC) [18] and the current Italian Legislation D. Lgs. 152/99 [19] require the use of ecotoxicological tests to assess water quality.

Previous studies have shown that each species and test procedure has its own sensitivity pattern to toxicants [20], and no single species is sensitive to all chemicals [21,22]. Accordingly, the use of test batteries [22–25] is becoming more common.

These methods require the simultaneous use of different test species, representing various habitats and sensitivity to toxicants, taking into account that a single testing species may over- or underestimate the potential toxicity of a particular substance [20,25–27]. Crustaceans are usually preferred in ecotoxicology for their ease of handling in the laboratory and because their toxic response is well documented and likely to be representative of harmful effects produced by different toxicants [24,28,29]. The aim of this study was to detect the acute toxicity of an Ionian strain of *Ostreopsis* cfr. *ovata* on biota, by using four test crustacean species characterised by different habitats and life cycles. The chosen crustaceans were the nauplii of *Artemia franciscana* Kellogg, 1906 (Anostraca) and *Tigriopus fulvus* Fischer, 1860 (Harpacticoida), and juveniles of *Corophium insidiosum* Crawford, 1937 (Amphipoda) and *Sphaeroma serratum* Fabricius, 1787 (Isopoda).

Taking into account that *Ostreopsis* cfr. *ovata* has been detected in the Gulf of Taranto [9], this study was carried out to establish baseline data and assess the potential toxicity of this microalga for the aquatic environment.

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2. Materials and methods

2.1. Ostreopsis *cfr.* ovata *sampling and growth in culture*

Cells of *O*. cfr. *ovata* were isolated from macroalgae collected along the coastline of the Gulf of Taranto (Mediterranean Sea). In the laboratory, individual cells were subsequently dispensed separately into tissue culture plates (16-well polystyrene plates) containing f/2 media [30], prepared in filtered, sterile seawater collected from the area in which the cells had been previously isolated. Clonal cultures were established and grown at $20 \pm 2\,^{\circ}$C with a 14L:10D photoperiod and illumination at $\sim$100 μmol photons$\cdot$m$^{-2}\cdot$s^{-1}. At the beginning of each experiment, the tested alga was diluted to the desired densities in the tubes.

2.2. *Crustacean test species*

Artemia franciscana, a non-autochtonous species, commercially available (Artemia Gold Argentemia) was used at II–III stage nauplii, according to the standard IRSA ISSN:0392-1425 protocol [31].

Approximately, 1–2 mL of cysts of brine shrimp *Artemia salina* were incubated in 12 mL standard artificial seawater (Instant Ocean®) in a Petri dish, at 25 °C for 24 h. The hatched larvae (instar 1) were transferred to a new Petri dish with fresh medium and incubated at 25 °C for 24 h. Forty-eight hours after the start of the incubation, all larvae had moulted to the instar 2–3 stages.

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Tigriopus fulvus is a meiobenthic, euryaline (2–125 PSU) and eurythermal (0–35 °C) copepod species, widely distributed in the Mediterranean [28,32,33]. In this study, a natural population of *T. fulvus* from the Tyrrhenian Sea (Livorno, Italy) was used. The toxicity tests were carried out using nauplii originating from a synchronised culture (24–48 h) of ovigerous females reared in a massive culture, according to ISI/FDSI 14669 [34], modified according to Faraponova et al. [28].

The amphipod *C. insidiosum* is a tube-building species living in brackish and estuarine water of the infralittoral zone, where it is widely distributed and available in large numbers. This species feeds on both sediment and suspended particulate matter. Previous studies demonstrated its tolerance to non-contaminant variables (biotic and abiotic) and sensitivity to toxicants [35–38].

The isopod *S. serratum* is recognised as an omnivorous species living in brackish and estuarine waters of the supra-infralittoral zone [29,39–41]. It eats benthic microalgae, filamentous algae, macroalgae, detritus, small invertebrates and even its conspecifics.

Both *C. insidiosum* and *S. serratum* were collected from an unpolluted site, away from sources of contamination, along an intertidal area of the Second Inlet of Mar Piccolo. Small quantities of sediment were sieved through a 0.5-mm mesh sieve to select the recommended size of animals (2–4 mm body length), avoiding mature females and juveniles. Experimental organisms were acclimated for 3–4 days before the beginning of the tests.

The selection of test species was based on their standardisation and frequent use in toxicity testing, and reported sensitivity to a wide range of pollutants[21,33,34].

2.3. *Bioassay and exposure conditions*

For each test species, testing was performed in two stages. A preliminary range finding test was conducted to determine the range of concentrations to be used during the definitive test. In fact, the toxicity levels of the crustaceans tested is actually unknown. Eleven different concentrations of *O.* cfr. *ovata* and one control were performed in two replicates.

In the definitive and last test, a new series of cell concentrations was prepared, based on results obtained in the preliminary tests. All definitive testing was conducted at least four times.

Six microalgae concentrations (cell·mL^{-1}), prepared with artificial seawater, and one control were performed in three replicates.

In particular, the tests with *A. franciscana* and *T. fulvus* were carried out by exposing 10 nauplii at six concentrations of *O.* cfr. *ovata* (from 2 to 12 cells·mL^{-1} and from 2.5 to 80 cell·mL^{-1}, respectively) in 10 mL artificial seawater (Instant Ocean®). The tests were carried out in conventional 12-multiwell testing plates to ensure a large water surface and enough air, the plate was covered and placed in incubator at 20 ± 1°C in continuous dark. During the exposure period (48 h for *A. franciscana* and 96 h for *T. fulvus*), the nauplii were not fed and the water was not renewed. Mortality of nauplii was noticed as the endpoint. All tests were accompanied by a negative control which measured the response of the organisms in the absence of *O.* cfr. *ovata* and under the best possible exposure conditions. The negative control consisted of cultured cells of the non-toxic species *Tetraselmis suecica* at the highest concentration of 2×10^4 cell·mL^{-1}. For each concentration and control, three replicates were carried out. At the end of the tests, the multiwell plate was placed under a microscope and the total numbers of dead nauplii were counted for each concentration to determine the mortality rate. The nauplii were considered dead if no movement of the appendages was observed within 10 s.

The test experiment with *C. insidiosum* and *S. serratum* was carried out in a 500-mL glass beaker containing filtered natural seawater. Briefly, 20 individuals (randomly selected) were exposed to geometric concentrations of *Ostreopsis* from 2.5 to 40 cell·mL^{-1} for *C. insidiosum* and from 10 to 320 cell·mL^{-1} for *S. serratum*, plus a control with *Tetraselmis suecica* [36]. Four replicates of each *Ostreopsis* cfr. *ovata* concentration were carried out. The beakers were kept at a constant

temperature (18 ± 2 °C), in continuous dark. No food was added to the test chambers and aeration was supplied without disturbing the animals, maintaining the dissolved oxygen levels >70% of air saturation. At the end of the test (96 h), the survivors were counted, apparently dead individuals were considered living if movement was exhibited after gentle stimulation, missing organisms were considered dead.

Concurrently with the acute tests with *O*. cfr. *ovata*, a positive control was performed as quality control test. This determines the sensitivity of the animals when exposed to a single reference toxicant under repeatable conditions and can be employed to verify whether the sensitivity of the adult animals is consistent among experiments. The positive control consisted of a water-only exposure to copper chloride. Animals for the controls were selected from the same population as the test animals.

2.4. *Statistical data analysis*

In order to assess the crustaceans' sensitivity to $CuCl_2$ and *Ostreopsis* cfr. *ovata*, the mean lethal concentration 50 (LC_{50}) values with associated 95% confidence limits were determined with a Trimmed Spearman–Karber [42]. The tests were considered valid if the percentage mortality in the negative control with *Tetraselmis suecica* did not exceed 10% [43,44] and if the calculated LC_{50} obtained in the quality control with the reference toxicant (copper chloride) was <15%. Test results that were significantly different from negative controls (ANOVA; $P < 0.05$) indicated that *O*. cfr. *ovata* was toxic.

3. Results

Mean percentage survival in the negative controls was > 85% in each test, meeting the acceptability criteria established for the tests with these species.

Concerning the response to the reference contaminant copper chloride, the crustaceans used showed the following LC_{50} values: 14.5 (7.9–16.3) mg·L^{-1} for *A. franciscana*, 0.14 ± 0.03 mg·L^{-1} for *T. fulvus*, 1.06 ± 0.17 mg·L^{-1} for *C. insidiosum* and 5.35 ± 0.44 mg·L^{-1} for *S. serratum*. Mean LC_{50} values and their 95% confidence limits for each test species toward *O*. cfr. *ovata* are summarised in Table 1.

As regards the acute toxicity test, species exhibited the highest mortalities with the increase in the *Ostreopsis* cell concentration (Figure 1). The results showed that *A. franciscana* larvae are

Table 1. Results of *O. ovata* acute toxicity test on *A. franciscana*, *T. fulvus*, *C. insidiosum* and *S. serratum*.

Test species	Test no.	LC50 (cell·mL^{-1})	Confidence limits	LC50 mean value (cell·mL^{-1})	SD
A. franciscana	1	1.02	0.42–2.47	1.63	0.54
	2	1.81	0.92–3.56		
	3	2.06	1.02–4.15		
T. fulvus	1	10.03	6.88–14.63	10.11	0.96
	2	9.19	5.68–13.58		
	3	11.11	7.85–16.37		
C. insidiosum	1	12.45	8.56–15.46	11.81	0.73
	2	11.01	7.88–14.42		
	3	11.97	6.85–15.43		
S. serratum	1	219.79	127.03–380.30	214.81	4.36
	3	211.65	137.90–324.85		
	3	213.01	125.51–350.40		

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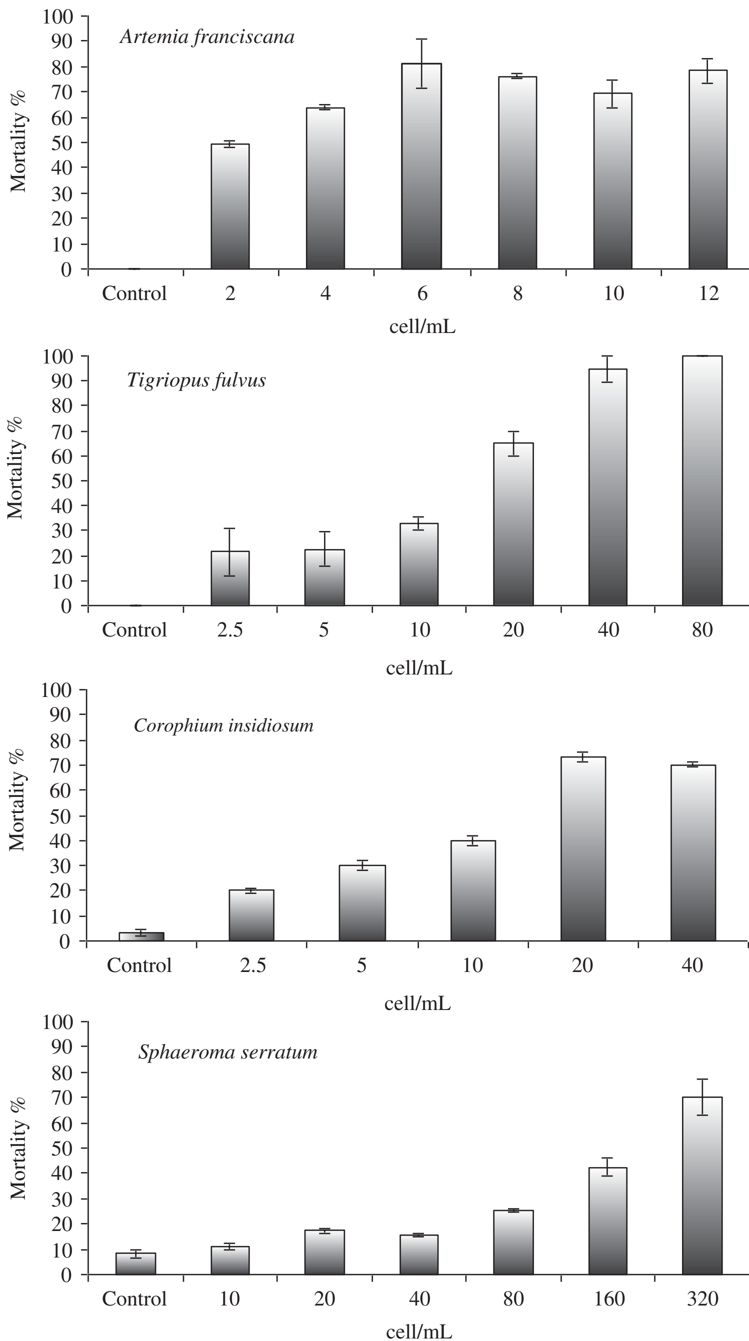

Figure 1. Percentage mortality (%) obtained during the exposure of the tested crustaceans to different concentrations of *Ostreopsis* cfr. *ovata* ($cell{\cdot}mL^{-1}$).

more sensitive to *Ostreopsis* cfr. *ovata* than the other crustaceans tested (ANOVA; $P < 0.05$). By contrast, *S. serratum* appeared to be the most tolerant crustacean species towards the *Ostreopsis* cells. *T. fulvus* and *C. insidiosum* showed similar sensitivity to *O.* cfr. *ovata* (ANOVA; $P < 0.05$) with mean LC_{50} values of 10.11 ± 0.96 and 11.81 ± 0.73 $cell{\cdot}mL^{-1}$, respectively.

Statistical analysis showed that for each test species there were significant differences between the dinoflagellate cell concentrations and controls, therefore the Ionian strain of *Ostreopis* cfr. *ovata* can be classified as a toxic species (ANOVA; $P < 0.05$). *T. fulvus* and *C. insidiosum* showed similar sensitivity to *O. ovata* (ANOVA; $P < 0.05$) with mean LC_{50} values of 10.11 ± 0.96 and 11.81 ± 0.73 cell·mL^{-1}, respectively. Statistical analysis also confirmed the toxicity of the dinoflagellate for these crustaceans (ANOVA; $P < 0.05$).

4. Discussion

Ostreopsis species are producers of palytoxins and palytoxin analogues [3,45,46], which are among the most potent natural non-protein compounds known, exhibiting extreme toxicity in mammals [47].

In tropical and subtropical regions, intoxication due to palytoxin is characterised by very severe symptoms. Several cases of death in humans have been recorded in the Philippines and Singapore after the ingestion of fish, crabs and other seafood contaminated by palytoxin [48]. In fact, palytoxin in these regions does not show any negative effect on marine organisms such as in crabs, various fish and a sea anemone [3,49–54], which are consumed, resulting in numerous cases of human poisoning and death. Recently, in Italian waters (Tyrrhenian, Ligurian and South Adriatic Seas), summer blooms of these species have affected tourist health, causing problems such as rhinorrea, cough, fever, bronchoconstriction with mild dyspnea and wheezing [55]. Furthermore, the *Ostreopsis* cfr. *ovata* blooms in Italian seas have led to alterations in water quality, as well as the death of benthonic invertebrates [56,57]. The mortality observed in these invertebrates might be due to the presence of a palytoxin analogue, ovatoxin-a and its analogues [56].

The brine shrimp (*Artemia* sp.) test is considered to be a useful tool for preliminary assessment of lethality or toxicity of harmful algae [58]. This test has been previously used for *Ostreopsis siamensis* strains from New Zealand [59] and for *O. ovata* strains from the Adriatic and Tyrrhenian Seas (Mediterranean) [60]. In this study, the tested crustaceans evidenced a high lethal effect. Therefore, they can be considered as good candidates to detect *Ostreopsis* toxicity. In addition, they highlighted the usefulness of employing a base-set of different species rather than a single species in ecotoxicological tests, in order to obtain more reliable information for the evaluation of toxicity and potential hazards due to the release of a specific compound in the marine environment. The results evidenced high sensitivity of *A. franciscana* towards *Ostreopsis* cells, in accordaqnce with results obtained by Guerrini et al. [60]. Also *T. fulvus* and *C. insidiosum* showed low LC_{50} values, which make them good and convenient test species to detect the toxicity of *Ostreopsis*. The acute toxicity test with *S. serratum* was lower than that of the other crustaceans utilised, in fact it showed higher LC_{50} values. But this crustacean showed symptoms of toxic effects such as aggressiveness and cannibalism, not observed in controls or with the use of chemical toxicants [36]. The resistance of *S. serratum* might be associated with the feeding habits of the species, which usually fed on benthic macroalgae and sediments, natural habitats of *Ostreopsis*. Although we do not know when *Ostreopsis* cfr. *ovata* became established in Ionian waters, we can hypothesise that over the years, *S. serratum* might have developed a defence mechanism towards this toxic microalga.

5. Conclusions

These results lead us to think that the application of crustaceans in routine monitoring will help us to better understand the possible effects of *Ostreopsis*, on both marine life and environment.

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Such an ecotoxicological approach will be implemented further in future studies by performing an assessment of the chronic toxicity of *Ostreopsis*. In a chronic toxicity test, the organisms will be exposed to toxicants for a long period of their lifetime, and the possible effects on different stages of their life cycles (embryonic development, fecundity and growth rates) evaluated.

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References

[1] S. Lenoir, L. Ten-Hage, J. Turquet, J.P. Quod, C. Bernard, and M.C. Hennion, *First evidence of palytoxin analogues from an* Ostreopsis mascarenensis *(Dinophyceae) benthic bloom in the southwestern Indian Ocean*, J. Phycol. 40 (2004), pp. 1042–1051.
[2] R. Munday, *Occurrence and toxicology of palytoxins*, in *Seafood and Freshwater Toxins. Pharmacology, Physiology, and Detection*, L.M. Botana, ed., 2nd edn, CRC Press, Boca Raton, FL, 2008, pp. 693–713.
[3] R. Munday, *Palytoxin toxicology: Animal studies*, Toxicon 57 (2011), pp. 470–477.
[4] L. Rhodes, *World-wide occurrence of the toxic dinoflagellate genus* Ostreopsis *Schmidt*, Toxicon 57(3) (2011), pp. 400–407.
[5] M.Vila, E. Garcés, and M. Masò, *Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean*, Aquat. Microb. Ecol. 26 (2001), pp. 51–60.
[6] M. Vila, J. Camp, E. Garcés, M. Masò, and M. Delgado, *High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean*, J. Plankton Res. 23 (2001), pp. 497–514.
[7] A. Penna, M. Vila, S. Fraga, M.G. Giacobbe, F. Androni, P. Riobò, and C. Vernesi, *Characterization of* Ostreopsis *and* Coolia *(Dinophyceae) isolates in the Western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8S rDNA sequences*, J. Phycol. 4 (2005), pp. 212–225.
[8] K. Aligizaki and G. Nikolaidis, *The presence of the potentially toxic genera* Ostreopsis *and* Coolia *(Dinophyceae) in the North Aegean Sea, Greece*, Harmful Algae 5 (2006), pp. 717–730.
[9] R. Congestri, A. Penna, and A. Zingone, *BENTOX-NET, a research and management initiative on* Ostreopsis *spp. and other benthic microalgal blooms on the Italian coast*, Harmful Algal News 32 (2006), pp. 11–12.
[10] M. Monti, M. Minocci, A. Beran, and L. Iveša, *First record of* Ostreopsis *cfr.* ovata *on macroalgae in the Northern Adriatic Sea*, Mar. Pollut. Bull. 54 (2007), pp. 598–601.
[11] L. Mangialajo, R. Bertolotto, R. Cattaneo-Vietti, M. Chiantore, C. Grillo, R. Lemee, N. Melchiorre, P. Moretto, P. Povero, and N. Ruggieri, *The toxic benthic dinoflagellate* Ostreopsis ovata*: Quantification of proliferation along the coastline of Genoa, Italy*, Mar. Pollut. Bull. 56 (2008), pp. 1209–1214.
[12] C. Caroppo and A.P. Bisci, *First data on the benthic assemblages of harmful microalgal species in the Gulf of Taranto (Northern Ionian Sea)*, Rapp. Commun. Int. Mer Méditerr. 39 (2010), p. 341.
[13] J.W. Bomber and K.E. Aikman, *The ciguatera dinoflagellates*, Biol. Oceanogr. 6 (1989), pp. 291–311.
[14] M.A. Quilliam, *Liquid chromatography–massspectometry: A universal method for analysis of toxins*?, in *Harmful Algae*, B. Reguera, J. Blanco, M.L. Fernández, and T. Wyatt, eds., IOC UNESCO, Xunta de Galicia, 1998, pp. 509–514.
[15] M. LeDoux and S. Hall, *Proficiency testing of eight French laboratories in using the A.O.A.C. mouse bioassay for paralytic shellfish poisoning: Interlaboratory collaborative studies*, J. AOAC Int 83 (2000), pp. 305–310.
[16] K. Jørgensen, and L.B. Jensen, *Distribution of DSP toxins in consignments of blue mussels*, Food Addit. Contam. 2 (2004), pp. 341–347.
[17] T. Yan, Y. Wang, L. Wang, Y. Chen, G. Han, and M. Zhou, *Application of rotifer* Brachionus plicatilis *in detecting the toxicity of harmful algae*, Chin. J. Oceanol. Limnol. 27 (2009), pp. 376–382.
[18] Directive 2000/60/Ec, *Directive of the European Parliament and the Council of 23 October 2000 establishing a framework for community action in the field of water policy*: OJ, L327, 22 December 2000, pp. 1–72.
[19] D. Lgs. n.152 11/5/1999, *Testo aggiornato del Decreto Legislativo 11 maggio 1999, n.152, 'Disposizioni sulla tutela delle acque dall'inquinamento e recepimento della direttiva 91/676/CEE relativa alla protezione delle acque dall'inquinamento provocato dai nitrati provenienti da fonti agricole.* A seguito delle disposizioni correttive ed integrative di cui al Decreto Legislativo 18 agosto 2000, N°258′, G.U. della Repubblica Italiana, Suppl. Ord. N. 246. 20/10/2000, SerGen, Rome.
[20] B.J. Dutka and K.K. Kwan, *Battery of screening tests approach applied to sediment extracts*, Toxic. Assess. 3 (1988), pp. 303–314.
[21] M.W. Toussaint, T.R. Shedd, W.H. Van der Schalie, and G.R. Leather, *A comparison of standard acute toxicitytests with rapid-screening toxicitytests*, Environ. Toxicol. Chem. 14 (1995), pp. 907–915.
[22] G.C. Castillo, I.C. Vila, and E. Neild, *Ecotoxicity assessment of metals and wastewater using multitrophic assays*, Environ. Toxicol. 15 (2000), pp. 370–375.

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[23] P. Fochtman, A. Raszka, and E. Nierzedska, *The use of conventional bioassays, microbiotests, and some 'rapid' methods in the selection of an optimal test battery for the assessment of pesticides toxicity*, Environ. Toxicol. 15 (2000), pp. 376–384.

[24] L. Mariani, D. De Pascale, O. Faraponova, A. Tornambè, A. Sarni, S. Giuliani, G. Ruggiero, F. Onorati, and E. Magaletti, *The use of a battery test in marine ecotoxicology: The acute toxicity of sodium dodecyl sulfate*, Environ. Toxicol. 21 (2006), pp. 373–379.

[25] M. Narracci, R.A Cavallo, M.I. Acquaviva, E. Prato, and F. Biandolino, *A test battery approach for ecotoxicological characterization of Mar Piccolo sediments in Taranto (Ionian Sea, Sothern Italy)*, Environ. Monitor. Assess. 148 (2009), pp. 307–314.

[26] P. Matthiessen, S. Bifield, F. Jarret, M.F. Kirby, R.J. Law, W.R. McNinn, D.A. Sheahan, J.E.Thain, and G.F.Whale, *An assessment of sediment toxicity in the river Tyne estuary, UK by means of bioassay*, Mar. Environ. Res. 45 (1998), pp. 1–15.

[27] M. Nendza, *Inventory of marine biotest methods for the evaluation of dredged material and sediment*, Chemosphere 48 (2002), pp. 865–883.

[28] O. Faraponova, D. De Pascale, F. Onorati, and M.G. Finora, Tigriopus fulvus *(Copepoda, Harpacticoida) as a target species in biological assays*, Meiofauna Mar. 14 (2005), pp. 91–94.

[29] E. Prato and F. Biandolino, Monocorophium insidiosum *(Crustacea, Amphipoda) as a candidate species in sediment toxicity testing*, Bull. Environ. Contam. Toxicol. 77 (2006), pp. 1–8.

[30] R.R.L. Guillard, *Culture of phytoplankton for feeding marine invertebrates*, in *Culture of Marine Invertebrates. Selected Readings*, C.J. Berg Jr, ed., Hutchinson Ross, Stroudsberg, PA, 1983, pp. 108–132.

[31] L. Guzzella, *Saggio di tossicità acuta con* Artemia sp., IRSACNR, Rome, 1996 (Notiziario dei Metodi Analitici sulle Acque).

[32] A. Carli and M.A. Fiori, *Sviluppo larvale del* Tigriopus fulvus *Fischer*, Atti IX Cong. Soc. Ital. Biol. Mar. (1977), pp. 181–190.

[33] A. Carli, G.L. Mariottini, and L. Pane, *Reproduction of the rockpools Harpacticoid copepod* Tigriopus fulvus *(Fisher 1860) suitable for aquaculture*, XII Congrès International d'Aquariologie, 1988, pp. 295–300.

[34] International Organization for Standardization (ISO), *Water Quality – Determination of Acute Lethal Toxicity to Marine Copepods (Copepoda, Crustacea)*, (1999), ISO/DIS 14669, 1999.

[35] E. Prato and F. Biandolino, Gammarus aequicauda *(Crustacea: Amphipoda): A potential species test in marine sediment toxicity assessment*, Aquat. Ecosyst. Health Manage. 8 (2005), pp. 475–482.

[36] E. Prato, F. Biandolino, and C. Scardicchio, *Test for acute toxicity of copper, cadmium, and mercury in five marine species*, Turk. J. Zool. 30 (2006), pp. 285–290.

[37] E. Prato, F. Biandolino, and C. Scardicchio, *The effects of salinity on the toxicity of cadmium to* Corophium insidiosum, Biol. Mar. Mediterr. 14 (2006), pp. 216–218.

[38] E. Prato, N. Bigongiari, C. Barghigiani, and F. Biandolino, *Comparison of amphipods* Corophium insidiosum *and* C. orientale *(Crustacea: Amphipoda) in sediment toxicity testing*, J. Environ. Sci. Health A 45 (2010), pp. 1461–1467.

[39] M.E. Nicotri, *Factors involved in herbivores food preference*, J. Exp. Mar. Biol. Ecol. 42 (1980), pp. 13–26.

[40] A.I. Robertson and K.H. Mann, *The role of isopods and amphipods in the initial fragmentation of eelgrass detritus in Nova Scotia, Canada*, Mar. Biol. 59 (1980), pp. 63–69.

[41] H.D. Franke and M. Janke, *Mechanisms and consequences of intra- and interspecific interference competition in* Idotea baltica *(Pallas) and* Idotea emarginata *(Fabricius) (Crustacea: Isopoda): A laboratory study of possible proximate cause of habitat segregation*, J. Exp. Mar. Biol. Ecol. 227 (1998), pp. 1–21.

[42] M.A. Hamilton, R.C. Russo, and R.V. Thurston, *Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays*, Environ. Sci. Technol. 11 (1977), pp. 714–719.

[43] P. Vanhaecke and G. Persoone, *Standardised short term toxicity test with* Artemia nauplii, (ARC-test) INSERM, 106 (1981), pp. 370–376.

[44] S. Lera, S. Macchia, L. Dentone, and D. Pellegrini, *Variations in sensitivity of two populations of* Corophium orientale *(Crustacea: Amphipoda) towards cadmium and sodium laurylsulphate*, Environ. Monitor. Assess. 136 (2008), pp. 121–127.

[45] T. Ukena, M. Satake, M. Usami, Y. Oshima, H. Naoki, T. Fujita, Y. Kan, and T. Yasumoto, *Structure elucidation of Ostreocin D, a Opalytoxin analog isolated form the dinoflagellate* Ostreopsis siamensis, Biosci. Biotechnol. Biochem. 65 (2001), pp. 2585–2588.

[46] S. Taniyama, O. Arakawa, M. Terada, S. Nishio, T. Takatani, Y. Mahmud, and T. Noguchi, Ostreopsis *sp., a possible origin of palytoxin (PTX) in parrotfish* Scarus ovifrons, Toxicon 42 (2003), pp. 29–33.

[47] J.S. Wiles, J.A. Vick, and M.K. Christensen, *Toxicological evaluation of palytoxin in 13 several animal species*, Toxicon 12 (1974), pp. 427–433.

[48] C.H. Tan and C.O. Lau, *Chemistry and detection of palytoxin*, in *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, L.M. Botana, ed., Marcell Dekker, New York, 2000, pp. 533–548.

[49] T. Yasumoto, D. Yasumura, Y. Ohizumi, M. Takahashi, A.C. Alcala, and L.C. Alcala, *Palytoxin in two species of xanthid crab from the Philippines*, Agr. Biol. Chem. 50 (1986), pp. 163–167.

[50] C.O. Lau, C.H. Tan, H.E. Khoo, R. Yuen, R.J. Lewis, G.P. Corpuz, and G.S. Bignami, Lophozozymus pictor *toxin: A fluorescent structural isomer of palytoxin*, Toxicon 33 (1995), pp. 1373–1377.

[51] Y. Hashimoto, N. Fusetani, and S. Kimura, *Aluterin: A toxin of filefish,* Alutera scripta*, probably originating from a zoantharian* Palythoa tuberculosa, Bull. Jpn Soc. Sci. Fish. 35 (1969), pp. 1086–1093.

[52] M. Fukui, M. Murata, A. Inoue, M. Gawel, and T. Yasumoto, *Occurrence of 21 palytoxin in the trigger fish* Melichtys vidua, Toxicon 25 (1987), pp. 1121–1124.

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[53] A.M. Kodama, Y. Hokama, T. Yasumoto, M. Fukui, S.J. Manea, and N. Sutherland, *Clinical and laboratory findings implicating palytoxin as cause of ciguatera poisoning due to* Decapterus macrosoma *(mackerel)*, Toxicon 27 (1989), pp. 1051–1053.

[54] V.M. Mahnir, E.P. Kozlovskaya, and A.I. Kalinovsky, *Sea anemone* Radianthus macrodactylus – *a new source of palytoxin,* Toxicon 30 (1992), pp. 1449–1456.

[55] P. Ciminiello, C. Dell'Aversano, E. Fattorusso, M. Forino, G. Silvana Magno, L. Tartaglione, C. Grillo, and N. Melchiorre, *The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean* Ostreopsis ovata *by a new liquid chromatography tandem mass spectrometry method*, Anal. Chem. 78 (2006), pp. 6153–6159.

[56] G. Sansoni, B. Borghini, G. Camici, M. Casotti, P. Righini, and C. Rustighi, *Fioriture algali di* Ostreopsis ovata *(Gonyaulacales: Dinophyceae): Un problema emergente*, Biol. Amb. 17 (2003), pp. 17–23.

[57] P. Ciminiello, C. Dell'Aversano, E. Fattorusso, M. Forino, L. Tartaglione, C. Grillo, and N. Melchiorre, *Putative palytoxin and its new analogue, ovatoxin-a, in* Ostreopsis ovata *collected along the Ligurian coasts during the 2006 toxic outbreak*, J. Am. Soc. Mass. Spectrosc. 19 (2008), pp. 111–120.

[58] A. Demaret, K. Sohet, and G. Houvenaghel, *Effect of toxic dinoflagellates on the feeding and mortality of* Artemia franciscana *larvae*, in *Harmful Marine Algal Blooms Technique and Documentation*, P. Lassus, G. Arzul, E. Erard, P. Gentien, and C. Marcaillou, eds., Lavoisier Intercept Ltd., Andover, UK, 1995, pp. 427–432.

[59] L.L. Rhodes, J. Adamson, T. Suzuki, L. Briggs, and I. Garthwaite, *Toxic epiphytic marine dinoflagellates* Ostreopsis siamensis *and* Coolia monotis *(Dinophyceae) in New Zealand*, NZ J. Mar. Freshwater Res. 34 (2000), pp. 371–384.

[60] F. Guerrini, A. Feller, L. Pezzolesi, V. Sangiorgi, I. Bianco, P. Ciminiello, C. Dell'Aversano, M. Forino, L. Tartaglione, E. Fattorusso, and R. Pistocchi, *Growth and toxicity characteristics of two strains of* Ostreopsis ovata *(Dinophyceae)*, Biol. Mar. Mediterr. 15 (2008), pp. 32–33.

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