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Sanitary problems related to the presence of *Ostreopsis* spp. in the Mediterranean Sea: a multidisciplinary scientific approach

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Abstract. The increased presence of potentially toxic microalgae in the Mediterranean area is a matter of great concern. Since the end of the last century, microalgae of the genus *Ostreopsis* have been detected more and more frequently in the Italian coastal waters. The presence of *Ostreopsis* spp. has been accompanied by the presence of previously undetected marine biotoxins (palytoxins) into the ecosystem with the increased possibility of human exposure. In response to the urgent need for toxicity characterization of palytoxin and its congeners, an integrated study encompassing both *in vitro* and *in vivo* methods was performed.

Key words: Ostreopsis, palytoxin (PLTX), acute toxicity, myotoxicity, cutaneous toxicity.

Riassunto *(Problemi sanitari relativi alla presenza di* Ostreopsis *spp. nel Mar Mediterraneo: un approccio scientifico multidisciplinare)*. La sempre maggiore presenza di microalghe potenzialmente tossiche nell'area mediterranea è motivo di grande preoccupazione. Dalla fine del secolo scorso, microalghe appartenenti al genere *Ostreopsis* sono state isolate con sempre maggiore frequenza nelle acque costiere italiane. La presenza di specie di *Ostreopsis* è stata accompagnata dalla comparsa di biotossine marine (palitossine), mai isolate prima nell'ecosistema, con conseguente aumento della probabilità di esposizione umana. In risposta al bisogno urgente di caratterizzazione della tossicità della palitossina e dei composti strutturalmente correlati, è stato scelto un approccio di studio integrato tra metodiche *in vitro* e *in vivo*.

Parole chiave: Ostreopsis, palitossina (PLTX), tossicità acuta, miotossicità, tossicità cutanea.

INTRODUCTION

Microalgae of *Ostreopsis* spp. are unicellular epiphytic benthic dinoflagellates [1, 2]: originally, they were thought to colonize only tropical and sub-tropical areas, but they are now being detected more and more frequently in temperate seas [3, 4], suggesting their geographic spread in the benthic environment. Concern about the distribution of *Ostreopsis* is motivated by its high potential for toxicity [4]. The entrance of potentially toxic dinoflagellates into the ecosystem can have impacts at several levels. In general, sanitary and economic consequences, often tightly connected, are of the greatest concern. In the Mediterranean region, appearance and proliferation of *Ostreopsis* spp. were first recorded in the late 1970s and 1980s [5, 6]. In Italy, the presence of *Ostreopsis ovata* was recorded for the first time in 1994, along the coasts of the Lazio region [7]. Since then, the presence of *Ostreopsis* spp., most often *O.* cf. *ovata*, has been recorded several times along the Italian coastline [8-21], including the northern areas, such as the Gulf of Genoa [19, 22, 23] and the Gulf of Trieste *(Figure 1)* [19, 24, 25].

Globally, attention shifted toward *Ostreopsis* in 1995, when Dr. Takeshi Yasumoto's group isolated and characterized PLTX-like compounds from *O. siamensis* [26-27]. Until this time, the origin of palytoxins was thought to be soft corals of the genus Palythoa [28]. Since then, there has been increasing consensus in the scientific community regarding microalgae as at least one producer of the toxins, even though the biosynthetic pathways are still unclear and represent a field of open and ongoing research. In addition to *O. siamensis*, putative PLTX and analogues have been isolated from O. mascarenensis [29] and *O. ovata* [30-35]. Extensive studies on *Ostreopsis* samples has led to the identification of several PLTX congeners *(Figure 2)*, including ostreocin-D [26, 27], mascarenotoxins [29-34] and several ovatoxins, denoted ovatoxin-a, -b, -c, -d, -e [30-34] and ovatoxin-f [35].

Palytoxin is considered among the most toxic compounds of natural origin ever isolated. It impairs the function of the Na^+/K^+-ATPase [36-39], whose physiological activity is of crucial importance for eukaryotic cells. Since 2006, our research group at

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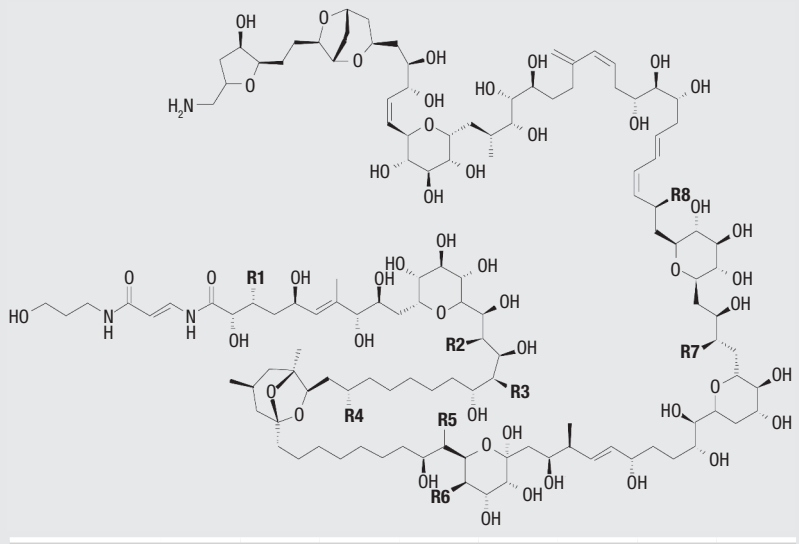
Fig. 1 | *Schematic representation of the sites of* Ostreopsis *blooms along the Italian coasts. (Data sources: [7-21]; http://arpa.sicilia.it and A. Penna, personal communication).*

the University of Trieste has followed the PLTX-phenomenon and added the study of PLTXs to the ongoing research on other algal toxins. This mini-review is mainly focused on the results obtained by our research group on this topic. Considering the potential different route of exposure to these toxins and the possible scenarios of intoxication, starting from the available data, an integrated *in vivo* and *in vitro* approach, including toxicological, physiological, cellular biology and biochemical studies, was adopted. This mini-review mainly summarizes the results of these studies, aimed to characterize PLTXs toxicity, and to identify the target organs and the mechanism at the basis of the toxic effects, useful also to provide a suitable therapeutic approach.

Potential exposure to Ostreopsis *spp. and the related toxins into marine aerosols and seawater: possible health effects*

In the Mediterranean area, the increasing proliferation of *Ostreopsis* spp. along the coastlines was accompanied by the occurrence of human intoxication [10, 40-43]. In particular, human exposure to marine aerosol and/or seawater concomitantly to *Ostreopsis* proliferations was associated with an illness in which symptoms involved mainly the upper respiratory tract [40]. The cause-effect correlation between the cases of malaise and the involvement of algal toxins has not been completely clarified: in fact palytoxins were never detected in marine aerosol so far, even though these toxins were quantified in field algal samples [31]. Furthermore, although *Ostreopsis* cells concentrations were determined in seawater, these data are not predictive for human risk since dinoflagellates do not always produce the same amount of toxins, if any [25]. *Ostreopsis* cell debris can be also present in the marine aerosol and their contribution to the ef-



Toxin	R1	R2	R3	R4	R5	R6	R7	R8
PLTX	CH_3	OH	OH	CH_3	H	OH	OH	OH
OST-D	H	OH	H	H	OH	H	OH	OH
OVA-a	CH_3	H	OH	CH_3	OH	H	H	OH
42-OH-PLTX	CH_3	OH	OH	CH_3	OH	OH	OH	OH

Fig. 2 | *Structure of palytoxin (PLTX), ostreocin-D (OST-D), ovatoxin-a (OVA-a) and 42-hydroxy-palytoxin (42-OH-PLTX).*

fects on human health cannot be excluded. Anyway, the recurrence of sanitary problems associated with *Ostreopsis* blooms suggests a relationship between these phenomena [43].

Along the Italian coastlines, the first documented health problems associated with *Ostreopsis* blooms were described as general malaise in people exposed both to seawater and/or marine aerosol in Tuscany [9] and Apulia [8]. Later, symptoms such as rhinorrhea, cough, dyspnea and fever, associated with blooms along the Bari coast, were described in more detail by Gallitelli, *et al.* [10]. Similar symptoms were observed along the Spanish and French Mediterranean coasts, accompanied by ocular irritation, headache and, in some cases, by fever [41, 42]. Other anecdotal descriptions of respiratory problems following marine aerosol exposure during *Ostreopsis* blooms have also been reported along the Mediterranean coast [25, 43, 44]. The most serious sanitary problems occurred on the Liguria coast in summer of 2005 [22, 40] and repeated, with a lower intensity, in 2006 [40]. In July 2005, more than 200 people enjoying the Genoa beach and promenade suffered an unusual influenza-like syndrome, characterized by a wide spectrum of symptoms such as fever, sore throat, cough, dyspnea, headache, nausea, rhinorrhea, lacrimation, vomiting and dermatitis. Approximately 20% of patients required hospitalization (1-3 days), and some of them needed the intensive care unit of the local hospitals [40]. This occurrence represents the most severe incident described to date in terms of both the number of people affected and for the severity of the symptoms.

In addition to the problems at the respiratory level, skin irritation was frequently observed after aerosol exposure and/or seawater contact during *Ostreopsis* blooms. Indeed, in summer 2005, concomitant with marine aerosol exposure during *Ostreopsis ovata* blooms in Genoa (Northern Italy), the incidence of dermatitis was 5% [40]. Erythematous dermatitis was also observed by Gallitelli (personal communication) in patients exposed to marine aerosols during *Ostreopsis* blooms, along Apulia coasts (Southern Italy) [43].

Although the actual cause of this dermatitis has not yet been unequivocally determined, dermal toxicity has been associated to PLTX-like molecules contaminating other marine organisms [43, 45]. For instance, skin toxicity has been reported after handling zoanthids *(Palythoa)* used as aquarium decorative corals: persistent signs of dermotoxicity and perioral paresthesia were attributed to PLTX in a patient with intact skin [46]. Another case of skin toxicity due to handling PLTX-containing zoanthid corals (*Parazoanthus* spp.) involved a patient who cut his fingers while cleaning his aquarium. Dermal distress with swelling, paresthesia and numbness around the site of injury, as well as systemic symptoms, were recorded [47]. Despite the reports on human dermal toxicity attributed to PLTXs, very little data regarding skin toxicity are presently available in the scientific literature. Our group contributed to the elucidation of the cutaneous effects of the parent compound PLTX: an *in vitro* toxicity study was carried out using the human HaCaT keratinocytes [48], a predictive model for the evaluation of skin toxicity and an ideal model for first-round screening of dermotoxic agents [49]. The cytotoxicity of PLTX on HaCaT cells was investigated after a short time exposure (4 h) and different cellular endpoints were evaluated. PLTX reduced mitochondrial activity (MTT assay), cell viability (sulforhodamine B assay) and plasma membrane integrity (LDH leakage), albeit with different potencies (EC_{50} = 6.1 ± 1.3 x 10^{-11} M, 4.7 ± 0.9 x 10^{-10} M and 1.8 ± 0.1 x 10^{-8} M, respectively). These data suggest that the sequence of intracellular events following the interaction of PLTX with its molecular target includes mitochondrial damage, causing a reduction in cell viability and plasma membrane rupture, with resulting leakage of LDH. Moreover, mitochondrial dysfunction was tentatively associated with oxidative stress, since PLTX induced superoxide anion accumulation after only 1 h exposure [48]. All these effects were inhibited by the presence of ouabain, a cardiac glycoside that inhibits PLTX binding to its molecular target (Na^+/K^+-ATPase). These data demonstrated the dependency of PLTX cytotoxicity on the interaction with the pump, which is transformed by PLTX into a non-selective cationic pore [37-39, 50]. The main consequence of this interaction seems to be a sustained intracellular overload of Na^+, followed by an overload of Ca^{2+} [51-54]. Consequently, the mechanism of PLTX cytotoxicity was investigated, with particular attention to the ionic imbalance induced by the toxin. On HaCaT cells, removal of Na^+ from the cell medium almost completely abolished: *i)* PLTX-induced oxidative stress, *ii)* impairment of mitochondrial activity and *iii)* appearance of morphological changes, demonstrating that intracellular Na^+ accumulation is the first and crucial step in mediating PLTX-induced early cell damage. By contrast, Ca^{2+} withdrawal did not affect PLTX-induced oxidative stress or cell morphology, confirming the Na^+-dependency of these effects on HaCaT keratinocytes [48].

Potential exposure to Ostreopsis *spp. and related toxins via seafood: possible health effects*

The presence of toxic *Ostreopsis* species into the ecosystem is related to the entrance of their toxins into the food web. The accumulation of biotoxins in the food web is a common, naturally occurring phenomenon [55], but it may lead to significant concentrations of toxic compounds in edible organisms and represent a potential threat to human health. Palytoxins are no exception and have been detected in several species of crustaceans, fish, mollusks and echinoderms, even if the consequences of their accumulation seem to differ from area to area [56-65]. In tropical and subtropical areas, accumulation of PLTXs in fish and crustacean lead to some cases of intoxication, even with lethal outcomes [43, 57-60]. In Madagascar, for instance, a lethal human intoxication

has been reported resulting from ingestion of PLTX-contaminated sardine sharing habitat with *Ostreopsis* [59]. In the Mediterranean area, contamination involved mainly mollusks and echinoderms, such as shellfish and sea urchins [19, 61-66] but, to date, no case of human intoxication has been described. In Italy, palytoxins were detected mainly in the frame of monitoring programs [19, 64]. The presence of ovatoxin-a (303-625 μg/kg) has been reported mainly in wild mussels collected along the rocky Italian coasts [64]. Similarly, in France, contamination reached 450 μg PLTX equivalents/kg of total flesh of sea urchins and 230 μg PLTX equivalents/kg of total flesh of mussels [65]. PLTXs and related toxins are not routinely tested, because no regulation at Italian or European level currently include them in the monitoring programs. Moreover, in Mediterranean Sea, the most abundant PLTXs detected in seafood are ovatoxins, and in particular ovatoxin-a, not yet available in sufficient amounts for oral toxicological studies, that are necessary for regulatory purposes.

Anyway, EFSA suggested 30 μg PLTXs (sum of PLTX and ostreocin-D)/kg shellfish meat as maximum level in shellfish [63]. However, this value is based on the limited available toxicological studies carried out in mice with the pure toxins after oral, sublingual and intratracheal exposure, the last one being not representative in humans for this evaluation. Other toxicological studies on PLTX were initially performed immediately after its discovery: no effects were observed in rats after oral administration of 40 μg/kg of a compound, which molecular weight was 3300 Da [67], nearly 500 Da higher than that currently reported for PLTX. More than thirty years later, an LD_{50} = 510 μg/kg was estimated for PLTX in mice, evaluating only lethality as endpoint of toxicity [68]. Subsequently, Ito and Yasumoto [69] reported tissue damages induced by the oral administration of PLTX or ostreocin-D (200 or 500 μg/kg). To implement the toxicological characterization, the acute oral toxicity of PLTXs in mice was evaluated increasing the dosages and expanding the panel of endpoints (*i.e.* histological and hematoclinical analyses). The toxicity was initially evaluated, within 24 h after the administration, on the parent compound PLTX and it was found to be strictly dose-related [70]. Later, the study was repeated on 42-hydroxy-PLTX [71], chemically characterized in 2009 [72] *(Figure 2)*. Similar in structure, PLTX and 42-hydroxy-PLTX also resulted in similar toxicity and symptoms. During the observation period, some of the mice presented scratching, jumping, paralysis of the hind limbs, respiratory distress, occasionally accompanied by cyanosis and died within 24 h from the administration of the toxins. Histological analysis revealed decreased glycogen content in hepatocytes. Mice that survived the treatment exhibited several degrees of inflammation of the mucosa in the forestomach [70, 71].

In animals treated from the dose of 600 μg/kg, hematochemical analysis revealed alteration in plasma levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and aspartate transaminase (AST), suggesting involvement of the muscular tissue in the toxicity of PLTX and 42-hydroxy-PLTX [70, 71]. In animals treated with PLTX, dose-dependent ultrastructural alterations of skeletal and cardiac muscle were also observed. The identification of skeletal muscle as one of the targets for PLTX was in agreement with the epidemiological data [43], which revealed that, in the majority of human cases, muscular problems and myalgia were reported as distinctive features [57, 58, 60]. For this reason, cultures of mouse skeletal muscle cells were chosen as a suitable model for the deeper investigation of the mechanism of action of both PLTX [73] and 42-hydroxy-PLTX (Del Favero *et al.*, in preparation). As mentioned above, PLTX is known to impair the activity of the Na^+/K^+ pump, with dramatic consequences on cellular ionic homeostasis [36-39, 50]. As well as high toxicity, quite common to all the cells models tested so far [74], PLTXs triggered an uncontrolled intracellular calcium ($[Ca^{2+}]_i$) increase [72, 73] and morphological alterations [73]. These events seemed to be strictly related to the development of the toxic insult [73]. The $[Ca^{2+}]_i$ increase consisted of a transitory Ca^{2+} response (transient phase) followed by a slower and more sustained $[Ca^{2+}]_i$ increase (long-lasting phase). The transient phase was sustained by the *i)* activation of voltage-dependent Ca^{2+} channels, *ii)* Na^+/Ca^{2+} exchanger (reverse mode) and *iii)* Ca^{2+} release from intracellular stores with no influence on the PLTX-mediated toxicity. The long-lasting phase seemed to be sustained by the activation of stretch-activated channels and represents a crucial step in the development of the myotoxic insult [73]. PLTXs did not only severely impair cellular viability, but also altered the functional properties of skeletal muscle cells, such as the ability to respond to physiological stimuli [73] (Del Favero *et al.*, in preparation). On the whole, the skeletal muscle cell cultures allowed the characterization of the ionic disequilibrium triggered by PLTXs, opening new insight into the mechanism of action of PLTX at the single cell level.

The alterations at the muscular level observed in mice after acute PLTX oral exposure, together with the epidemiological observations in humans (lethality, muscle cramps, myalgia, and cardiac alterations) suggest PLTX absorption in the gastrointestinal tract after oral exposure. Thus, information on the gastrointestinal absorption of PLTX seemed to be pivotal for a rational risk assessment. Since no toxicokinetic data on PLTX were available, an *in vitro* study was carried out for the evaluation of PLTX absorption through the intestinal barrier: to this aim the human Caco-2 cell line was used. However, Caco-2 cells are one of the most sensitive cell models for PLTX, presenting reduced viability at the sub pico-molar range (EC_{50} = 8.9 ± 3.7 x 10^{-12} M after 4 h exposure, MTT assay). Unfortunately, the high sensitivity of this model precluded the possibility of evaluating PLTX absorption [75].

DISCUSSION AND CONCLUSIONS

The relative rapidity that characterizes the entrance of new species of potentially harmful microalgae in the Mediterranean ecosystems represents an immense challenge from the scientific and regulatory point of view. The data necessary for the evaluation of real toxicological hazard beneath naturally occurring phenomena, such as algal blooms, require time and resources. Thus, even though *Ostreopsis* appeared in Mediterranean waters over 30 years ago [5], the toxicological consequences of exposure to its suite of toxins is still an open research field.

A multidisciplinary scientific approach for the toxicological characterization of PLTXs based on literature and epidemiological data was followed. Initially, wider *in vivo* acute toxicity studies allowed to individuate the skeletal muscle as one of the main targets of PLTXs toxicity, in agreement with human symptoms. Although no structural alterations were observed in mice, the sharp increase in CPK, K^+ and LDH plasma levels suggested the skeletal muscle involvement, subsequently confirmed by ultra-structural changes. Further *in vitro* studies on skeletal muscle cells contributed to the elucidation of PLTX effects at functional level and to the characterization of its mechanism of action, opening new perspectives.

The lack of toxicokinetic data on PLTX and the difficulty of predicting absorption and distribution in the body is still a challenge for the comprehension of the hazard associated with PLTXs in the food web. Moreover, the accumulation of the toxins in several edible marine species [19, 56-65] opens the possibility of repeated human exposure through contaminated seafood collected in the same area.

Considering exposure routes different from the oral one, the cutaneous toxicity was characterized using an *in vitro* approach. The high toxicity of PLTX on skin keratinocytes [48] raises valid concerns about the potential human exposure to PLTX-related toxins in seawater and needs to be further investigated. These data will help the prevention of toxicity for people exposed to seawater during *Ostreopsis* blooms, either professionally or recreationally. Toxicological evaluation of PLTX-like compounds after inhalational exposure remains one of the most crucial issues, and should be addressed as soon as possible by the scientific community.

In conclusion, sanitary problems related to *Ostreopsis* spp. could be due to the entrance of previously absent toxins into the food web and to human exposure to marine aerosols and/or seawater during large algal blooms. A multidisciplinary approach to the problem should be further adopted for their complete exploitation and evaluation. The number of known toxins is constantly increasing, requiring additional efforts for toxicity characterization. Studies are urgently needed to evaluate the effects of these toxins after repeated oral exposure. Another crucial point is the characterization of the oral toxicity of the new ovatoxin analogues as well as of their inhalational toxicity.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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References

1. Schmidt J. Peridiniales. *Bot Tidsskr* 1901;24:212-21.
2. Fukuyo Y. Taxonomical study of benthic dinoflagellates, collected in coral reef. *Bull Jap Soc Sci Fish* 1981;47:967-78. http://dx.doi.org/10.2331/suisan.47.967
3. Shears NT and Ross PM. Blooms of benthic dinoflagellates of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 2009;8:916-25. http://dx.doi.org/10.1016/j.hal.2009.05.003
4. Rhodes L. World-wide occurrence of the toxic dinoflagellate genus *Ostreopsis* Schmidt. *Toxicon* 2011;57:400-7. http://dx.doi.org/10.1016/j.toxicon.2010.05.010
5. Taylor FJR. A description of the benthic dinoflagellate associated with maitotoxin and ciguatoxin, including observations on Hawaiian material. In: Taylor DL, Seliger HH (Eds.). *Toxic dinoflagellate blooms.* North-Holland, New York: Elsevier; 1979. p. 71-6.
6. Abboud-Abi Saab M. Les dinoflagelleés des eaux côtières libanaises-espèces rares ou nouvelles du phytoplankton marin. *Lebanese Science Bulletin* 1989;5:5-16.
7. Tognetto L, Bellato S, Moro I, Andreoli C. Occurrence of *Ostreopsis ovata* (Dinophyceae) in the Tyrrhenian Sea during summer 1994. *Bot Mar* 1995;38:291-5. http://dx.doi.org/10.1515/botm.1995.38.1-6.291
8. Di Turi L, Lo Caputo S, Marzano MC, Pastorelli AM, Pompei M, Rositani L, *et al.* Ostropsidiaceae (Dynophyceae) presence along the coastal area of Bari. *Biol Mar Mediterr* 2003;10:675-8.
9. Sansoni G, Borghini B, Camici G, Casotti M, Righini P, Rustighi C. Fioriture algali di *Ostreopsis ovata* (Gonyaulacales: Dinophyceae): un problema emergente. *Biologia Ambientale* 2003;17:17-23.
10. Gallitelli M, Ungaro N, Addante LM, Gentiloni Silver M, Sabbà C. Respiratory illness as a reaction to tropical algal bloomsoccurringinatemperateclimate.*JAMA*2005;293:2599-600. http://dx.doi.org/10.1001/jama.293.21.2599-c
11. Penna A, Vila M, Fraga S, Giacobbe MG, Andreoni F, Riobò P, *et al.* 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* 2005;41:212-25. http://dx.doi.org/10.1111/j.1529-8817.2005.04011.x

12. Penna A, Fraga S, Battocchi C, Casabianca S, Giacobbe MG, Riobó P, *et al.* Phylogeographical study of the toxic benthic dinoflagellate genus *Ostreopsis* Schmidt. *J Biogeogr* 2010;37:830-41. http://dx.doi.org/10.1111/j.1365-2699.2009.02265.x
13. Zingone A, Siano R, D'Alelio D, Sarno D. Potentially toxic and harmful microalgae from coastal waters of the Campania region, Tyrrhenian Sea, Mediterranean Sea. *Harmful Algae* 2006;5:321-37. http://dx.doi.org/10.1016/j.hal.2005.09.002
14. Barone R. Behavioral trait of *Ostreopsis ovata* (Dinophyceae) in the Mediterranean rock pools: the spider strategy. *Harmful Algal News* 2007;33:1-3.
15. Totti C, Cucchiari E, Romagnoli T, Penna A. Bloom of *Ostreopsis ovata* on the Conero riviera (NW Adriatic Sea). *Harmful Algae News* 2007;33:12-3.
16. Totti C, Accoroni S, Cerino F, Cucchiari E, Romagnoli T. *Ostreopsis ovata* bloom along the Conero Riviera (Northern Adriatic Sea): Relationships with environmental conditions and substrata. *Harmful Algae* 2010;9:233-9. http://dx.doi.org/10.1016/j.hal.2009.10.006
17. Guerrini F, Pezzolesi L, Feller A, Riccardi M, Ciminiello P, Dell'Aversano C, *et al.* Comparative growth and toxin profile of cultured *Ostreopsis ovata* from the Tyrrhenian and Adriatic Seas. *Toxicon* 2010;55:211-20. http://dx.doi.org/10.1016/j.toxicon.2009.07.019
18. Accoroni S, Romagnoli T, Colombo F, Pennesi C, Di Camillo CG, Marini M, *et al. Ostreopsis* cf. *ovata* bloom in the northern Adriatic Sea during summer 2009: ecology, molecular characterization and toxin profile. *Mar Pollut Bull* 2011;62:2512-9. http://dx.doi.org/10.1016/j.marpolbul.2011.08.003
19. Istituto Superiore per la Protezione e la Ricerca Ambientale. *Fioriture algali* di *Ostreopsis ovata lungo le coste italiane*. ISPRA (ISPRA Atti, 2011).
20. Pagliara P, Caroppo C. Toxicity assessment of *Amphidinium carterae, Coolia* cfr. *monotis* and *Ostreopsis* cfr. *ovata* (Dinophyta) isolated from the northern Ionian Sea (Mediterranean Sea). *Toxicon* 2012;60:1203-14. http://dx.doi.org/10.1016/j.toxicon.2012.08.005
21. Istituto Superiore per la Protezione e la Ricerca Ambientale. *Monitoraggio di Ostreopsis ovata ed altre microalghe potenzialmente tossiche lungo le coste italiane nel triennio 2007-2009.* ISPRA (ISPRA Rapporti, n. 127/2010).
22. Brescianini C, Grillo C, Melchiorre N, Bertolotto R, Ferrari A, Vivaldi B, *et al. Ostreopsis ovata* algal blooms affecting human health in Genova, Italy, 2005 and 2006. *EuroSurveill* 2006;11(36):3040.
23. Mangialajo L, Bertolotto R, Cattaneo-Vietti R, Chiantore M, Grillo C, Lemee R, *et al.* The toxic benthic dinoflagellate *Ostreopsis ovata*: quantification of proliferation along the coastline of Genoa, Italy. *Mar Poll Bull* 2008;56:1209-14. http://dx.doi.org/10.1016/j.marpolbul.2008.02.028
24. Monti M, Minocci M, Beran A, Iveša L. First record of *Ostreopsis* cfr. *ovata* on macroalgae in the Northern Adriatic Sea. *Mar Poll Bull* 2007;54:598-601. http://dx.doi.org/10.1016/j.marpolbul.2007.01.013
25. Honsell G, De Bortoli M, Boscolo S, Dell'Aversano C, Battocchi C, Fontanive G, *et al.* Harmful dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo: detection of ovatoxins in field samples and cell immunolocalization using antipalytoxin antibodies. *Environ Sci Technol* 2011;45:7051-9. http://dx.doi.org/10.1021/es201373e
26. Usami M, Satake M, Ishida S, Inoue A, Kan Y, Yasumoto T. Palytoxin analogs from the dinoflagellate *Ostreopsis siamensis*. *J Am Chem Soc* 1995;117:5389-90. http://dx.doi.org/10.1021/ja00124a034
27. Ukena T, Satake M, Usami T, Oshima Y, Fujita T, Kan Y, *et al.* Structure elucidation of Ostreocin-D, a palytoxin analog isolated from the dinoflagellate *Ostreopsis siamensis*. *Biosci Biotechnol Biochem* 2001;65:2585-8. http://dx.doi.org/10.1271/bbb.65.2585
28. Moore RE, Scheuer PJ. Palytoxin: a new marine toxin from a coelenterate. *Science* 1971;172:495-8. http://dx.doi.org/10.1126/science.172.3982.495
29. Lenoir S, Ten-Hage L, Turquet J, Quod PJ, Bernard C, Hennion MC. First evidence of palytoxin analogues from an *Ostreopsis mascarenensis* (Dinophyaceae) benthic bloom in southwestern Indian Ocean. *J Phycol* 2004;40:1042-51. http://dx.doi.org/10.1111/j.1529-8817.2004.04016.x
30. Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Magno GS, Tartaglione L, *et al.* The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. *Anal Chem* 2006;78(17):6153-9. http://dx.doi.org/10.1021/ac060250j
31. Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Tartaglione L, Grillo C, *et al.* 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 Spectrom* 2008;19:111-20. http://dx.doi.org/10.1016/j.jasms.2007.11.001
32. Ciminiello P, Dell'Aversano C, Dello Iacovo E, Fattorusso E, Forino M, Grauso L, *et al.* Complex palytoxin-like profile of *Ostreopsis ovata*. Identification of four new ovatoxins by high-resolution liquid chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2010;24:2735-44. http://dx.doi.org/10.1002/rcm.4696
33. Ciminiello P, Dell'Aversano C, Dello Iacovo E, Fattorusso E, Forino M, Grauso L, *et al.* Isolation and structure elucidation of Ovatoxin-a, the major toxin produced by *Ostreopsis ovata*. *J Am Chem Soc* 2012;134:1869-75. http://dx.doi.org/10.1021/ja210784u
34. Rossi R, Castellano V, Scalco E, Serpe L, Zingone A, Soprano V. New palytoxin-like molecules in Mediterranean *Ostreopsis* cf. *ovata* (dinoflagellates) and in *Palythoa tuberculosa* detected by liquid chromatography-electrospray ionization time-of-flight mass spectrometry. *Toxicon* 2010;56:1381-7. http://dx.doi.org/10.1016/j.toxicon.2010.08.003
35. Ciminiello P, Dell'Aversano C, Iacovo ED, Fattorusso E, Forino M, Tartaglione L, *et al.* Unique toxin profile of a Mediterranean *Ostreopsis* cf. *ovata* strain: HR LC-MS(n) characterization of ovatoxin-f, a new palytoxin congener. *Chem Res Toxicol* 2012;25(6):1243-52. http://dx.doi.org/10.1021/tx300085e
36. Habermann E. Palytoxin acts through Na^+, K^+-ATPase. *Toxicon* 1989;27:1175-87. http://dx.doi.org/10.1016/0041-0101(89)90026-3
37. Kim SY, Marx KA, Wu CH. Involvement of the Na,K-ATPase in the induction of ion channels by palytoxin. *Naunyn Schmiedeberg's Arch Pharmacol* 1995;351:542-54. http://dx.doi.org/10.1007/BF00171047
38. Wu CH. Palytoxin: membrane mechanism of action. *Toxicon* 2009;54:1183-9. http://dx.doi.org/10.1016/j.toxicon.2009.02.030
39. Rossini GP and Bigiani A. Palytoxin action on the Na(+),K(+)-ATPase and the disruption of ion equilibria in biological systems. *Toxicon* 2011;57:429-39. http://dx.doi.org/10.1016/j.toxicon.2010.09.011
40. Durando P, Ansaldi F, Oreste P, Moscatelli P, Marensi L, Grillo C, *et al. Ostreopsis ovata* and human health: epidemi-

ological and clinical features of respiratory syndrome outbreaks from a two year syndromic surveillance, 2005-2006, in north-west Italy. *Euro Surveill* 2007;12(23).

41. Kermarec F, Dor F, Armengaud A, Charlet F, Kantin R, Sauzade D, *et al.* Health risks related to *Ostreopsis ovata* in recreational waters. *Env Risques Santé* 2008;7:357-63.
42. Tichadou L, Glaizal M, Armengaud A, Grossel H, Lemée R, Kantin R, *et al.* Health impact of unicellular algae of the *Ostreopsis* genus blooms in the Mediterranean Sea: experience of the French Mediterranean coast surveillance network from 2006 to 2009. *Clin Toxicol* 2010;48:839-44. http://dx.doi.org/10.3109/15563650.2010.513687
43. Tubaro A, Durando P, Del Favero G, Ansaldi F, Icardi G, Deeds JR, *et al.* Case definitions for human poisonings postulated to palytoxins exposure. *Toxicon* 2011;57:478-95. http://dx.doi.org/10.1016/j.toxicon.2011.01.005
44. Pfannkuchen M, Godrijan J, Mariić Pfannkuchen D, Iveša L, Kružić P, Ciminiello P, *et al.* Toxin-producing *Ostreopsis* cf. *ovata* are likely to bloom undetected along coastal areas. *Environ Sci Technol* 2012;46:5574-82. http://dx.doi.org/10.1021/es300189h
45. Deeds JR, Handy SM, White KD, Reimer JD. Palytoxin found in *Palythoa* sp. Zoanthids (Anthozoa, Hexacorallia) sold in the home aquarium trade. *PLoS One* 2011;6:1-9. http://dx.doi.org/10.1371/journal.pone.0018235
46. Nordt SP, Wu J, Zahller S, Clark RF, Cantrell FL. Palytoxin poisoning after dermal contact with zoanthid coral. *J Emerg Med* 2009;40(4):397-9. http://dx.doi.org/10.1016/j.jemermed.2009.05.004
47. Hoffmann K, Hermanns-Clausen M, Buhl C, Buchler MW, Schemmer P, Mebs D, *et al.* A case of palytoxin poisoning due to contact with zoanthid corals through skin injury. *Toxicon* 2008;51:1535-7. http://dx.doi.org/10.1016/j.toxicon.2008.03.009
48. Pelin M, Zanette C, De Bortoli M, Sosa S, Della Loggia R, Tubaro A, *et al.* Effects of the marine toxin palytoxin on human skin keratinocytes: role of ionic imbalance. *Toxicology* 2011;282:30-8. http://dx.doi.org/10.1016/j.tox.2011.01.010
49. Gibbs S. *In vitro* irritation models and immune reactions. *Skin Pharmacol Physiol* 2009;22:103-13. http://dx.doi.org/10.1159/000178869
50. Artigas P, Gadsby DC. Ion channel-like properties of the Na^+/K^+ pump. *Ann NY Acad Sci* 2002;976:31-40. http://dx.doi.org/10.1111/j.1749-6632.2002.tb04711.x
51. Frelin C, Van Renterghem C. Palytoxin. Recent electrophysiological and pharmacological evidence for several mechanisms of action. *Gen Pharmacol* 1995;26:33-7. http://dx.doi.org/10.1016/0306-3623(94)00133-8
52. Ares IR, Cagide E, Louzao MC, Espina B, Vieytes MR, Yasumoto T, *et al.* Ostreocin-D impact on globular actin of intact cells. *Chem Res Toxicol* 2009;22:374-81. http://dx.doi.org/10.1021/tx800273f
53. Sheridan RE, Deshpande SS, Adler M. Cytotoxic action of palytoxin on aortic smooth muscle cells in culture. *J Appl Toxicol* 2005;25:365-73. http://dx.doi.org/10.1002/jat.1080
54. Schilling WP, Snyder D, Sinkins WG, Estacion M. Palytoxin-induced cell death cascade in bovine aortic endothelial cells. *Am J Physiol Cell Physiol* 2006;291:657-67. http://dx.doi.org/10.1152/ajpcell.00063.2006
55. Mebs D. Occurrence and sequestration of toxins in food chains. *Toxicon* 1998;36:1519-22. http://dx.doi.org/10.1016/S0041-0101(98)00143-3
56. Yasumoto T, Yasumura D, Ohizumi Y, Takahashi M, Alcala Ac, Alcala LC. Palytoxin in two species of xanthid crab from the Philippines. *Agric Biol Chem* 1986;50:163-7. http://dx.doi.org/10.1271/bbb1961.50.163
57. Noguchi T, Hwang DF, Arakawa O, Daigo K, Sato S, Ozaki H, *et al.* Palytoxin as the causative agent in the parrotfish poisoning. In: Gopalakrishnakone P, Tan CK (Eds.). *Progress in venom and toxin research: Proceedings of the first Asia-Pacific Congress on Animal, Plant and Microbial Toxins*. Singapore: Faculty of Medicine, National University of Singapore; 1987. p. 325-35.
58. Alcala AC, Alcala LC, Garth JS, Yasumura D, Yasumoto T. Human fatality due to ingestion of the crab *Demania reynaudii* that contained a palytoxin-like toxin. *Toxicon* 1988;26:105-7. http://dx.doi.org/10.1016/0041-0101(88)90142-0
59. Onuma Y, Satake M, Ukena T, Roux J, Chanteau S, Rasolofonirina N, *et al.* Identification of putative palytoxin as the cause of clupeotoxism. *Toxicon* 1999;37:55-65. http://dx.doi.org/10.1016/S0041-0101(98)00133-0
60. Taniyama S, Mahmud Y, Terada M, Takatani T, Arakawa O, Noguki, T. Occurrence of a food poisoning incident by PLTX from a serranid Epinephelus sp. in Japan. *J Nat Toxins* 2002;11:277-82.
61. Aligizaki K, Katikou P, Nikolaidis G, Panou A. First episode of shellfish contamination by palytoxin-like compounds from *Ostreopsis* species (Aegean Sea, Greece). *Toxicon* 2008; 51:418-27. http://dx.doi.org/10.1016/j.toxicon.2007.10.016
62. Aligizaki K, Katikou P, Milandri A, Diogène J. Occurrence of palytoxin-group toxins in seafood and future strategies to complement the present state of the art. *Toxicon* 2011;57:390-9. http://dx.doi.org/10.1016/j.toxicon.2010.11.014
63. EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on marine biotoxins in shellfish; Palytoxin group. *EFSA J* 2009;7(12)1393:38. http://dx.doi.org/10.2903/j.efsa.2009.1393
64. Ciminiello P, Dell'Aversano C, Dello Iacovo E, Fattorusso E, Forino M, Tartaglione L. LC-MS of palytoxin and its analogues: state of the art and future perspectives. *Toxicon* 2011;57:376-89. http://dx.doi.org/10.1016/j.toxicon.2010.11.002
65. Amzil Z, Sibat M, Chomerat N, Grossel H, Marco-Miralles F, Lemee R, *et al.* Ovatoxin-a and palytoxin accumulation in seafood in relation to *Ostreopsis* cf. *ovata* blooms on the French Mediterranean coast. *Mar Drugs* 2012;10:477-96. http://dx.doi.org/10.3390/md10020477
66. Bellocci M, Ronzitti G, Milandri A, Melchiorre N, Grillo C, Poletti R, *et al.* A cytolytic assay for the measurement of palytoxin based on a cultured monolayer cell line. *Anal Biochem* 2008;374:48-55. http://dx.doi.org/10.1016/j.ab.2007.10.033
67. Wiles JS, Vick JA, Christensen MK. Toxicological evaluation of palytoxin in several animal species. *Toxicon* 1974;12:427-33. http://dx.doi.org/10.1016/0041-0101(74)90011-7
68. Rhodes LL, Munday R. Palytoxins: a risk to human health? *In: Proceedings of the 20th Marine Biotoxin Science Workshop.* Wellington New Zealand: New Zealand Food Safety Authorities 23; 2004.
69. Ito E, Yasumoto T. Toxicological studies on palytoxin and ostreocin-D administered to mice by three different routes. *Toxicon* 2009;54:244-51. http://dx.doi.org/10.1016/j.toxicon.2009.04.009
70. Sosa S, Del Favero G, De Bortoli M, Vita F, Soranzo MR, Beltramo D, *et al.* Palytoxin toxicity after acute oral adminis-

tration in mice. *Toxicol Lett* 2009;191:253-9. http://dx.doi.org/10.1016/j.toxlet.2009.09.009

71. Tubaro A, Del Favero G, Beltramo D, Ardizzone M, Forino M, De Bortoli M, *et al.* Acute oral toxicity in mice of a new palytoxin analog: 42-hydroxy-palytoxin. *Toxicon* 2011;57:755-763. http://dx.doi.org/10.1016/j.toxicon.2011.02.009
72. Ciminiello P, Dell'Aversano C, Dello Iacovo E, Fattorusso E, Forino M, Grauso L, *et al.* Stereostructure and biological activity of 42-hydroxy-palytoxin: a new palytoxin analogue from Hawaiian *Palythoa* subspecies. *Chem Res Toxicol* 2009;22:1851-9. http://dx.doi.org/10.1021/tx900259v
73. Del Favero G, Florio C, Codan B, Sosa S, Poli M, Sbaizero O, *et al.* The stretch-activated channel blocker Gd^{3+} reduces palytoxin toxicity in primary cultures of skeletal muscle cells. *Chem Res Toxicol* 2012;25:1912-20. http://dx.doi.org/10.1021/tx300203x
74. Bellocci M, Sala GL, Prandi S. The cytolytic and cytotoxic activities of palytoxin. *Toxicon* 2011;57:449-59. http://dx.doi.org/10.1016/j.toxicon.2010.12.013
75. Pelin M, Sosa S, Della Loggia R, Poli M, Tubaro A, Decorti G, *et al.* The cytotoxic effect of palytoxin on Caco-2 cells hinders their use for *in vitro* absorption studies. *Food Chem Toxicol* 2012;50:206-11. http://dx.doi.org/10.1016/j.fct.2011.10.032