Comparison of short-term toxicity of 14 common phycotoxins (alone and in combination) to the survival of brine shrimp Artemia salina

Yuting Zhang1, 2, Shanshan Song1, Bin Zhang3, Yang Zhang1, Miao Tian1, 2, Ziyi Wu1, Huorong Chen1, Guangmao Ding1, Renyan Liu1, 2*, Jingli Mu1, 2*

1 Institute of Oceanography, College of Geography and Oceanography, Minjiang University, Fuzhou 350108, China
2 Fuzhou Institute of Oceanography, Fuzhou 350108, China
3 Department of Marine Chemistry, National Marine Environmental Monitoring Center, Dalian 116023, China
4 Fujian Marine Environment and Fishery Resource Monitoring Center, Fuzhou 350003, China

Received 26 January 2022; accepted 19 September 2022

© Chinese Society for Oceanography and Springer-Verlag GmbH Germany, part of Springer Nature 2023

Abstract
Toxic harmful algal blooms (HABs) can cause deleterious effects in marine organisms, threatening the stability of marine ecosystems. It is well known that different strains, natural populations and growth conditions of the same toxic algal species may lead to different amount of phycotoxin production and the ensuing toxicity. To fully assess the ecological risk of toxic HABs, it is of great importance to investigate the toxic effects of phycotoxins in marine organisms. In this study, the short-term toxicity of 14 common phycotoxins (alone and in combination) in the marine zooplankton Artemia salina was investigated. The 48 h LC50 of the 14 phycotoxins varied from 0.019 3 μg/mL to 2.415 μg/mL. The most potent phycotoxin was azaspiracids-3 (AZA3; with a LC 50 of 0.019 3 μg/mL), followed by azaspiracids-2 (AZA2; 0.022 6 μg/mL), penecatotoxin-2 (PTX2; 0.046 0 μg/mL) and dinophysistoxin-1 (DTX1; 0.081 8 μg/mL). For the binary exposure, okadaic acid (OA) induced potential additive effects with DTX1, probably due to their similar structure (polyether fatty acid) and mode of action (attacking the serine/threonine phosphoprotein phosphatases). On the other hand, OA showed potential antagonistic effects with PTX2, which might be accounted for by their activation on the detoxification activity of cytochrome P450 activity. In addition, DTX1 induced potential synergetic effects with saxitoxin (STX), yessotoxin (YTX) or PTX2, suggesting the hazard risk. These results provide valuable toxicological data for assessing the impact of phycotoxins on marine planktonic species and highlight the potential ecological risk of toxic HABs in marine ecosystems.

Key words: LC50, harmful algal blooms, binary exposure, ecological risk

Citation: Zhang Yuting, Song Shanshan, Zhang Bin, Zhang Yang, Tian Miao, Wu Ziyi, Chen Huorong, Ding Guangmao, Liu Renyan, Mu Jingli. 2023. Comparison of short-term toxicity of 14 common phycotoxins (alone and in combination) to the survival of brine shrimp Artemia salina. Acta Oceanologica Sinica, 42(2): 134–141, doi: 10.1007/s13131-022-2120-3

1 Introduction
The frequency, scale and magnitude of harmful algal blooms (HABs) have increased in the past decades, due to overfishing, coastal eutrophication, global climate change and invasive species dispersal (De Rijcke et al., 2016). For instance, it is reported that the frequency of HABs along Chinese coast has increased at a rate of 40%±4% per decade from 1970 to 2015 (Xiao et al., 2019). HABs can be classified in two categories, according to the mechanisms underlying the negative impacts: (1) non-toxic HABs, which lead to deterioration of water quality by an excessive increase of turbidity and dissolved oxygen consumption; (2) toxic HABs, which synthesize powerful phycotoxins negatively impacting aquaculture, ecological stability and even public health (Simões et al., 2015). Phycotoxins are natural metabolites produced by micro-algae, including dinoflagellates, phytoplankton, cyanobacteria and etc., that inhabit marine, brackish, or freshwater bodies or soils (Quilliam, 1999). It has been well documented that phycotoxins produced by toxic algae species can lead to acute illness in humans (Turki et al., 2014). For example, diarrheic shellfish poisoning (DSP) is mainly due to the phycotoxins (such as okadaic acid and dinophysistoxin) produced by toxic strains of Prorocentrum spp. and Dinophysis spp. (Dickey et al., 1990; Yasumoto, 1990; Bravo et al., 2001), and paralytic shellfish poisoning (PSP) is predominantly linked to the phycotoxins (such as saxitoxin) by toxic strains of Alexandrium spp. (Hallegraeff, 1993; Abdenadher et al., 2012; Anderson et al., 2012).

Besides human-health concerns, phycotoxins produced by toxic HABs can cause deleterious effects in many aquatic organisms, threatening ecological health and stability (Suganuma et al., 1988; Durbin et al., 2002; Zhang et al., 2009; Faassen et al., 2012). Zooplanktons, channeling primary production to higher trophic levels, play a crucial role in marine ecosystems. It is doc-
umented that when toxic HABs occur, the produced phycotoxins would induce adverse effects on zooplankton, resulting in a re-
duction of species diversity (Jonsson et al., 2009; Xu et al., 2017). The responses of zooplankton to toxic HABs vary significantly,
mainly depending on the species of toxic algae (Turner, 2014; Xu
et al., 2017). However, when exposed to the same species of toxic
alga, copepods may give distinct responses (Xu et al., 2017). One
possible reason is that different strains, natural populations and
growth conditions of the same algal species lead to different
amount of phycotoxin production and the ensuing toxicity (Xu
et al., 2017). Therefore, to fully conclude the toxicity of HABs and to
well compare the potential toxicity of different toxic alga species
in zooplankton, it is necessary to include the use of phycotoxins.
However, toxicological data for the toxic effects of phycotoxins in
aquatic organisms are limited. To date, the median-lethal con-
centration (LC50) of okadaic acid (OA); dinophysistoxin-1
(DTX1), saxitoxin (STX), brevetoxin-2 (PBtx2) and brevetoxin-3
(PBtx3) in aquatic organisms have been documented (O’Ors
et al., 2014; Figueroa et al., 2020; Kirkpatrick et al., 2004; Shaw et al.,
1997), but not for other common phycotoxins such as pecteno-
toxin-2 (PTX2), yessotoxin (YTX), homo-yessotoxin, (hYTX), 13-
desethyl spirolide C (SPX1), gymnodimine (GYM), azaspir-
acids-1 (AZA1), azaspiracids-2 (AZA2) and azaspiracids-3
(AZA3). In addition, phycotoxins do not only occur singly but
also as mixtures in the real-world environment, as subordinate
species might also occur during a bloom event (Smayda, 1997;
Eckford-Soper and Daugbjerg, 2017), a succession from one
dominant specie to another is often observed (Höglund et al.,
2004) and some species can produce different analogues of phy-
cotoxins (Alarcan et al., 2018). It is of great importance to study
the toxic effects of phycotoxins, alone and in combination, in
aquatic organisms, for better understanding the ecological risk of
HABs.

In this study, the toxicity of 14 common phycotoxins (includ-
ing OA, DTX1, PTX2, YTX, hYTX, SPX1, GYM, AZA1, AZA2, AZA3,
STX, dcSTX, PbTx2 and PbTx3) on the survival of brine shrimp
(Artemia salina) was investigated by assessing the LC50 for 48 h.
Furthermore, the combined effect (additive, antagonistic or syn-
ergistic) of two different phycotoxins on the survival of A. salina
was also investigated. The overall aim of this study was to provide
valuable toxicological data for evaluating the toxicity of phyc-

cotoxins in zooplankton and to help better understand the eco-

2 Materials and methods

2.1 Phycotoxins

Certified reference standards for OA, DTX1, PTX2, YTX, hYTX,
SPX1, GYM, AZA1, AZA2, AZA3, STX and dcSTX were purchased
from the National Research Council Halifax, Canada. PbTx2 and
PbTx3 were obtained from Taiwan Renyu Company. The stock
solutions of STX was dissolved in 3 mmol/L HCl, while others in
methanol. The working solutions of phycotoxins were freshly
prepared by serial dilution (3 mmol/L HCl for STX; methanol for
the other phycotoxins) 30 min prior to each experiment.

2.2 Brine shrimp bioassay

The brine shrimp (Artemia salina) assay was carried out fol-

owing the previous technique with slight modification (Lincoln
et al., 1996; Hisem et al., 2011). One gram of dried A. salina cysts
were hatched in filtered artificial seawater (FASW) with gentle
aeration for 24 h under a 12 h light: 12 h dark cycle at (25±1)°C.
The bacteria-free FASW was prepared by dissolving sea salt in tap
water (salinity, 30±1; dissolved oxygen, (6.98±0.17) mg/L), fol-

lowing by filtering (pore size, 0.22 μm). Newly hatched larvae
were collected using a Pasteur pipette after a 24 h incubation and
washed with FASW in a petri dish. After washing, A. salina
were transferred to a 24-well plate (10 individuals for each well) using
a pipette with a 200 μL tip. In general, at each transfer, 2−4 A. sa-


lin with approximately 5 μL carrying solution were added to the
well. The total volume of carrying solution for each well was
less than 20 μL. For the preparation of exposure medium, 20 μL
of phycotoxin working solutions or their respective solvent
(3 mmol/L HCl for STX; methanol for the other phycotoxins) was
transferred into a 24-well microtiter plate with 1.98 mL FASW
and 10 A. salina individuals per well. Each group had three rec-

Plates. During the exposure experiment, A. salina was not fed.

The dissolved oxygen after 48 h exposure was (6.97±0.21) mg/L.

The mortality was counted at 48 h using a stereomicroscope
(Olympus IX71). The death of an individual was defined as fol-


2.3 Statistical analysis

Bioassay data for artemia mortality were analyzed using IBM
SPSS Statistics 21 software. Phycotoxin concentrations (μg/mL)
that resulted in 10% and 50% mortality (i.e., LC10 and LC50 values)
were estimated using log-probability curves with 95% confidence
intervals. LC10 and LC50 values were determined by probabilistic
regression models generated. For the binary exposure, the differ-
ences among the treatments were tested using one-way analysis
of variance (ANOVA) with specific mean comparisons per-
formed by Fisher’s least significant difference (LSD) post hoc test.

Prior to ANOVA analyses, Shapiro-Wilk and Bartlett’s tests were
used to test for normality and homogeneity of variances, respect-
ively. All data were presented as means ± standard error of the
mean (SEM).

3 Results

3.1 Effect of each phycotoxin

The mortality-concentration curves, LC10 and LC50 values for
OA, DTX1, PTX2, PbTx2, PbTx3, YTX, hYTX, STX, dcSTX, GYM,
SPX1, AZA1, AZA2 and AZA3 in A. salina were shown in Fig. 1
and Table 1. On the basis of 48 h LC50, the order of toxicity in
artemia was AZA3>AZA2>PTX2>DTX1+hYTX>AZA1>SPX1>
STX–GYM=OA>dcSTX>STX–PbTx3>PbTx2. Among the tested 14
phycotoxins, the LC50 value of AZA3 in artemia was the lowest
(0.019 3 μg/mL), while PbTx2 showed the least toxic effect with a
LC50 value of 2.415 μg/mL.
Fig. 1. The 48 h mortality-concentration curves of OA (a), DTX1 (b), PTX2 (c), YTX (d), hYTX (e), SPX1 (f), GYM (g), AZA1 (h), AZA2 (i), AZA3 (j), STX (k), dcSTX (l), PbTx2 (m) and PbTx3 (n) in *Artemia salina*, where Y-axis is mortality and X-axis is the log_{10} concentration (unit: ng/mL) of phycotoxins (lg C). The insets are plots of probit transformed responses, where the Y-axis is probit and the X-axis is lg C.

3.2 Combined effect of two phycotoxins

3.2.1 Combination of OA with DTX1, PTX2 or STX

The artemia from the OA+DTX1 group exhibited higher mortality than those from the OA group (*p*=0.001 0), but did not show significantly higher mortality than the DTX1 treated artemia (Fig. 2A). No significant difference in the mortality was found among the OA, PTX2 and OA+PTX2 groups (Fig. 2B). Similarly, the mortality of the artemia from the OA+STX group was close to that from the OA alone group and the STX alone group (Fig. 2C).
Table 1. The 48 h LC50 and LC10 values of marine phycotoxins in Artemia salina (n=3)

| Phycotoxins | LC50/(μg·mL⁻¹) | LC10/(μg·mL⁻¹) |
|-------------|---------------|---------------|
| OA          | 0.372 [0.287–0.746] | 0.124 [0.074 8–0.153]| |
| DTX1        | 0.081 8 [0.046 0–0.139] | 0.029 9 [0.003 27–0.100 7] |
| PTX2        | 0.046 0 [0.035 2–0.057 3] | 0.021 2 [0.009 57–0.029 4] |
| YTX         | 0.171 [0.097 5–0.208] | 0.061 2 [0.039 7–0.133] |
| hYTX        | 0.085 9 [0.068 9–0.237] | 0.048 0 [0.035 9–0.054 8] |
| GM          | 0.191 [0.102–1.667] | 0.054 5 [0.042 7–0.102] |
| SPX1        | 0.118 [0.091 0–0.345] | 0.069 2 [0.061 7–0.087 9] |
| AZA1        | 0.106 [0.032 4–10⁻⁶] | 0.021 9 [0.013 2–5.701] |
| AZA2        | 0.022 6 [0.017 2–0.038 5] | 0.008 89 [0.007 53–0.010 5] |
| AZA3        | 0.019 3 [0.014 5–0.036 8] | 0.008 55 [0.007 24–0.010 3] |
| STX         | 0.899 [0.469–16.520] | 0.288 [0.222–0.716] |
| deSTX       | 0.376 [0.281–0.962] | 0.194 [0.171–0.242] |
| PbTx2       | 2.415 [2.056–3.499] | 0.893 [0.423–1.161] |
| PbTx3       | 1.279 [1.208–1.355] | 0.811 [0.719–0.887] |

Note: The 95% confidence interval are given in brackets.

3.2.2 Combination of DTX1 with PTX2, STX, YTX or hYTX

Relative to the mortality for the DTX1 alone group and the PTX2 alone group, the mortality of the DTX1+PTX2 treated artemia was elevated by 2.6-fold (p<0.001) and 10-fold (p<0.001) respectively (Fig. 2D). The DTX1+STX treated artemia showed significantly higher mortality than those exposed to individual phycotoxin (DTX1 alone or STX alone) (Fig. 2E). Similarly, significant increases (1.9-fold, p<0.000 1) and 11-fold (p<0.000 3) compared to that for the PTX2 group and the hYTX group, respectively (Fig. 2F).

3.2.3 Combination of PTX2 with SPX or hYTX

For the binary exposure to PTX2 and SPX1, no significant difference in the mortality was observed among three groups (Fig. 2H). Differently, the mortality for the PTX2+hYTX group was increased by 4.9-fold (p=0.0015) and 32-fold (p<0.0001) respectively in the mortality were observed in the artemia exposed to DTX1+YTX relative to the artemia from the DTX1 alone group and the YTX alone group (Fig. 2F). In contrast, the artemia from the DTX1+hYTX group did not exhibit higher mortality than those from the DTX1 alone group (Fig. 2G).

4 Discussion

The toxicity of phycotoxins has received increasing attention with the increase of frequency, scale and magnitude of toxic harmful algal blooms (HABs) in recent years (De Rijcke et al., 2016). In this study, AZA3 (with a LC50 of 0.022 6 μg/mL) was the most toxic phycotoxin in artemia, followed by AZA2 (with a LC50 of 0.026 6 μg/mL). AZAs (including AZA1, AZA2, AZA3, AZA4, AZA5 and etc.) are a group of phycotoxins produced by Azadinium spinosum (Ferreiro et al., 2016). In this study, AZA3 (with a LC50 of 0.019 3 μg/mL) showed higher toxicity than AZA1 (with a LC50 of 0.106 μg/mL). Similarly, a study in mice shows that after intraperitoneal administration, AZA2 (with a minimum lethal dose of 110 μg/kg) and AZA3 (140 μg/kg) are more toxic than AZA1 (150 μg/kg) (Toyofuku, 2006; Twiner et al., 2008). These results reinforce the concept that the toxicity of analogues might vary significantly.

To prevent human intoxications, the European Union (EU) has set regulatory limits of phycotoxins in shellfish mainly based on the toxicity on mice (Alarcan et al., 2018). The limits of OA, AZA, PTX, STX and YTX in 1 kg shellfish meat are 160 μg, 160 μg, 160 μg, 800 μg and 1 mg respectively. This suggest that YTX might be the least toxic phycotoxin among the five phycotoxins, followed by STX. In the present study, YTX (with a LC50 of 0.171 μg/mL) is found to be more toxic than STX (with a LC50 of 0.899 μg/mL) and OA (with a LC50 of 0.372 μg/mL) in artemia. This suggests that the toxic effects of phycotoxins in mammals (like mice) and in zooplankton (like A. salina) might be distinct. Therefore, besides of the human-health concerns, the investigation of deleterious effects of phycotoxins on marine food webs also requires attention.

The mechanism of action of phycotoxins have been studied for many years. OA and DTX1 belong to the polynuclear aromatic aldehydes (PAs), in particular PP2A, and as secondary targets, PP1 and PP2B (Farabegoli et al., 2018). GYM and SPX1, belonging to the cyclic imine group, can block nicotinic and muscarinic acetylcholine receptors in the nervous system and the neuromuscular junction, inducing acute toxicity (Marrouchi et al., 2013). The voltage-gated sodium channel (VGNC) is the recognized receptor of both STX and PbTXs, and the binding to VGNC probably results in disorders of ion homeostasis (Rossini and Hess, 2010). AZA is chemically characterized by a cyclic amine group, a carbonyl acid and a unique tri-spiro ring (Rossini and Hess, 2010). Although the mode of action of the AZAs has not been fully elucidated, AZAs are found to inhibit endocytosis (Sala et al., 2013) and to induce cytoskeleton disorganization (Twiner et al., 2005). Exposure of primary cultured neurons to AZA1 increases nuclear levels of phosphorylated (active) c-Jun-N-terminal kinase (JNK), and an inhibitor of JNK could prevent the cytotoxic effect of AZA1, suggesting that the mechanism of action of AZAs might be associated with JNK production (Vale et al., 2007). YTX is a polycyclic ether compound. Three major responses triggered by YTXs in cultured cells have been reported, i.e., a general alteration, an increase in intracellular Ca²⁺ concentration and a disruption of E-cadherin system (Rossini and Hess, 2010). It is recognized that PTXs can interact with F-actin, leading to alterations in the ultrastructure and functioning of cellular cytoskeleton (Terao et al., 1986; Spector et al., 1999).

As some phycotoxins with a similar mechanism of action might work synergistically and phycotoxins probably occur as mixtures in the real-world environment (Smayda, 1997; Högländer et al., 2004; Ferron et al., 2016a; Eckford-Soper and Daugbjerg, 2017; Alarcan et al., 2018), it is of importance to clarify the combined effects of two phycotoxins. In this study, additive effects were observed in OA+DTX1. As OA and DTX1 share a similar mode of action, the observed potential additive effects of OA and DTX1 in artemia are probably due to the "dose addition". On the other hand, the combination of OA and PTX2 exhibited potential antagonistic effects. Similarly, a recent study in human intestinal Caco-2 cells shows that the combination of OA with PTX2 results in reduced toxicity (including, the ROS production, IL-8 release and γ-H2AX phosphorylation) at low concentrations (Alarcan et al., 2018).
al., 2019). It is reported that OA can interact with regulatory nuclear receptors such as PXR (Fidler et al., 2012; Ferron et al., 2016b), which regulate the expression of some cytochrome P450 enzymes (Wang et al., 2012). PTX2 is believed to interact with the AhR and induce P450 1A protein in hepatic cells (Alarcan et al., 2017, 2019). Therefore, one possible explanation is that the mixture of OA and PTX2 might induce cytochrome P450 activity and efflux transporter expression, resulting in higher detoxification/excretion of toxins and thus decreased toxic effects (Alarcan et al., 2019).

In this study, the binary exposure to DTX1+STX, DTX1+YTX or DTX1+PTX2 dramatically elevated the mortality in artemia, compared to the individual exposure, suggesting that DTX1 can interact with STX, YTX and PTX2, and then induce greater effects than additive. The synergistic effects of two phycotoxins have been documented. For instance, the mixture of AZA1 and YTX shows synergism in human intestinal cell models (Caco-2 cells) and the human intestinal epithelial crypt-like (Ferron et al., 2016b). The combination of YTX and OA with a ratio of 1:26.5 exhibits synergistic effects in the human intestinal epithelial crypt-like cells (Ferron et al., 2016b). Our results further highlight the hazard potency of the mixtures of DTX1 and other phycotoxins (like STX, YTX and PTX2) with regard to the ecological risk. It is worth mentioning that this study was conducted under laboratory conditions. In the real-world environment, the fluctuated temperature, solar radiation and bacterial communities might influence the degradation of phycotoxins (Alfonso et al., 2008; Donovan et al., 2008; Pan et al., 2020). Although lipophilic phyco-
cotoxins (including OA, DTX1, PTX2, YTX, hYTX, SPX1, GYM, AZA1, AZA2, AZA3, PbTx2 and PbTx3) have excellent stability and the half-life of the water soluble phycotoxin STXs is about 9 to 28 days in river water (Jones and Negri, 1997; Chen et al., 2018), the possibility of degradation cannot be excluded. In the future, field studies should be conducted to fully assess the ecological risk of phycotoxins.

In summary, this study demonstrates the individual toxicity of 14 phycotoxins in A. salina. On the basis of 48 h LC50, the order of toxicity in artemia is AZA3 (with a LC50 of 0.019 3 μg/mL) > AZA2 (0.022 6 μg/mL) > P-PTX2 (0.046 0 μg/mL) > DTX1 (0.081 8 μg/mL) > hYTX (0.085 9 μg/mL) > AZA1 (0.106 μg/mL) > SPX1 (0.118 μg/mL) > YTX (0.171 μg/mL) > GYM (0.191 μg/mL) > OA (0.372 μg/mL) > dC-CTX (0.376 μg/mL) > STX (0.899 μg/mL) > PbTx3 (1.279 μg/mL) > PbTx2 (2.415 μg/mL). These data would contribute to a more accurate calculation of predicted no effect concentration (PNEC) in assessing the ecological risk of phycotoxins and HABs using species sensitivity distributions (SSDs). Furthermore, the combination of two phycotoxins exhibits potential additive (OA+DTX1; OA+DTX1), antagonistic (OA+PTX2; OA+STK) or synergistic (DTX1+STX; DTX1+YTX; DTX1+PTX2; PTX2+hYTX) effects with regard to the mortality of A. salina. The findings enrich our understanding on the ecological risk of phycotoxins and HABs in zooplankton and marine ecosystems, especially when two or more phycotoxins occur simultaneously.

References
Abdenaderh M, Hamza A, Fekih W, et al. 2012. Factors determining the dynamics of toxic blooms of Alexandrium minutum during a 10-year study along the shallow southwestern Mediterranean coasts. Estuarine, Coastal and Shelf Science, 106: 102–111
Alarcan J, Barbé S, Kopp B, et al. 2019. Combined effects of okadaic acid and pectenotoxin-2, 13-desmethyliploride C or yessotoxin in human intestinal Caco-2 cells. Chemosphere, 228: 139–148, doi: 10.1016/j.chemosphere.2019.04.018
Alarcan J, Biré R, Le Hégarat L, et al. 2018. Mixtures of lipophilic phyctoxins: exposure data and toxicological assessment. Marine Drugs, 16(2): 46, doi: 10.3390/md16020046
Alarcan J, Dubreil E, Huguet A, et al. 2017. Metabolism of the marine phycotoxin P-PTX-2 and its effects on hepatic xenobiotic metabolism: activation of nuclear receptors and modulation of the phase I cytochrome P450. Toxins, 9(7): 212, doi: 10.3390/toxins9070212
Alfonso C, Rehmann N, Hess P, et al. 2008. Evaluation of various pH and temperature conditions on the stability of azaspiracids and their importance in preparative isolation and toxicological studies. Analytical Chemistry, 80(24): 9672–9680, doi: 10.1021/ac801506d
Anderson D M, Alpermann T J, Cembella A D, et al. 2012. The globally distributed genus Alexandrium: multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae, 14: 10–35, doi: 10.1016/j.hal.2011.10.012
Braivo I, Fernández M L, Ramilo I, et al. 2001. Toxin composition of the toxic dinoflagellate Prorocentrum lima isolated from different locations along the Galician Coast (NW Spain). Toxicon, 39(10): 1537–1545, doi: 10.1016/S0041-0101(01)00126-X
Chen Junhui, Han Tongzhu, Li Xiaotong, et al. 2018. Occurrence and distribution of marine natural organic pollutants: Lipophilic marine algal toxins in the Yellow Sea and the Bohai Sea, China. Science of the Total Environment, 612: 931–939, doi: 10.1016/j.scitotenv.2017.08.304
De Rijcke M, Van Acker E, Nevejan N, et al. 2016. Toxic dinoflagellates and Vibrio spp. act independently in bivalve larvae. Fish & Shellfish Immunology, 57: 236–242
Dickey R W, Bobzin S C, Faulkner D J, et al. 1990. Identification of okadaic acid from a Caribbean dinoflagellate, Prorocentrum concavum. Toxicon, 28(4): 371–377, doi: 10.1016/0041-0101(90)90074-H
Donovan C J, Ku J C, Quilliam M A, et al. 2008. Bacterial degradation of paralytic shellfish toxins. Toxicon, 52(1): 91–100, doi: 10.1016/j.toxicon.2008.05.005
D’ors A, Bartolomé M C, Sánchez-Fortún S. 2014. Risk associated with toxic blooms of marine phytoplankton functional groups on Artemia franciscana. Journal of Coastal Life Medicine, 2(8): 625–631
Durbin E, Teegarden G, Campbell R, et al. 2002. North Atlantic right whales, Eubalaena glacialis, exposed to paralytic shellfish poisoning (PSP) toxins via a zooplankton vector, Calanus finmarchicus. Harmful Algae, 1(3): 243–251, doi: 10.1016/S1568-9883(02)00046-X

Table 2. List of the recent toxicological data about the toxicity of phycotoxins in aquatic organisms

| Phycotoxin | Species | Time | LC50(μg·mL−1) | Reference |
|-----------|---------|------|--------------|-----------|
| OA        | Tigriopus californicus | 24 h | 41.7         | Shaw et al. (1997) |
| Artemia franciscana | 24 h | 6.270*         | D’ors et al. (2014) |
| Danio rerio larvae | 24 h | 10              | Figueroa et al. (2020) |
| Danio rerio larvae | 48 h | 8.5             | Figueroa et al. (2020) |
| Danio rerio larvae | 72 h | 7               | Figueroa et al. (2020) |
| Daphnia magna | 48 h | 42.1           | Rambha-Alegría et al. (2018) |
| Daphnia magna | 96 h | 0.003        | Rambha-Alegría et al. (2018) |
| Artemia salina | 48 h | 0.728        | this study |
| DTX1      | Danio rerio larvae | 24 h | 7              | Figueroa et al. (2020) |
| Danio rerio larvae | 48 h | 5.5            | Figueroa et al. (2020) |
| Danio rerio larvae | 72 h | 5              | Figueroa et al. (2020) |
| Daphnia magna | 48 h | 29             | Rambha-Alegría et al. (2018) |
| Daphnia magna | 96 h | 0.008         | Rambha-Alegría et al. (2018) |
| Artemia salina | 48 h | 0.081 9       | this study |
| STX       | Artemia franciscana | 24 h | 4.060*        | D’ors et al. (2014) |
| Artemia salina | 48 h | 1.042 32      | this study |
| PbTx      | Bambusia affinis | 24 h | 0.000 011    | Kirkpatrick et al. (2004) |
| Ornayas latipes | 24 h | 0.015–25    | Poli (1988) |
| PbTx2     | Artemia salina | 48 h | 2.415         | this study |
| PbTx3     | Artemia salina | 48 h | 1.239         | this study |

Note: * represents the calculated equivalent.

Alarcan J, Alarcon J, Barbé S, Kopp B, et al. 2019. Combined effects of okadaic acid and pectenotoxin-2, 13-desmethyliploride C or yessotoxin in human intestinal Caco-2 cells. Chemosphere, 228: 139–148, doi: 10.1016/j.chemosphere.2019.04.018
Eckford-Soper L K, Daugbjerg N. 2017. Interspecific competition study between *Pseudochattonella farcimini* and *P. verruculosa* (Dictyochophyceae)—Two ichthyotoxigenic species that co-occur in Scandinavian waters. Microbial Ecology, 73(2): 259–270, doi: 10.1007/s00248-016-0650-z

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2010. Scientific Opinion on marine biotoxins in shellfish—Cyclic imines (spirolidone, gymnodimines, pinnatoxins and peritoxins). EFSA Journal, 8(6): 1628

Faassen E J, Barkema L, Begeman L, et al. 2012. First report of *homo*janatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. Toxicon, 60(3): 378–384, doi: 10.1016/j.toxicon.2012.04.335

Faragello F, Blanco L, Rodríguez L P, et al. 2018. Phycotoxins in marine shellfish: origin, occurrence and effects on humans. Marine Drugs, 16(6): 186, doi: 10.3390/md16060188

Ferreiro S F, Villarín N, Carrera C, et al. 2016. Subacute cardiovascular toxicity of the marine phycotoxin azaspiracil-1 in rats. Toxicological Sciences, 151(1): 104–114, doi: 10.1093/toxsci/kfu025

Fern P J, Dumazeau K, Beauchard F J, et al. 2016. Combined effects of lipophilic phycotoxins (okadaic acid, azaspiracil-1 and yessotoxin) on human intestinal cells models. Toxins, 8(2): 50, doi: 10.3390/toxins8020050

Ferron P J, Hoogeven K, De Sousa G, et al. 2016b. Modulation of CYP3A4 activity alters the cytotoxicity of lipophilic phycotoxins in human hepatic HepaRG cells. Toxicology in Vitro, 33: 136–146, doi: 10.1016/j.tiv.2016.02.021

Fidler A E, Holland P T, Reschly E J, et al. 2012. Activation of a tunicate xenobiotic receptor inhibits the maturation of cathepsin D in mammalian cells. Chemical Research in Toxicology, 26(3): 444–455, doi: 10.1021/tx300511z

Shaw B A, Andersen R J, Harrison P J. 1997. Feeding deterrent and toxicity effects of apo-fucoxanthinidin and phycotoxins on a marine copepod (*Tigriopus californicus*). Marine Biology, 128(2): 273–280, doi: 10.1007/s002270050095

Simões E, Vieira R C, Schramm M A, et al. 2015. Impact of harmful algal blooms (*Dinophysis aspersa*) on the immune system of oysters and mussels from Santa Catarina, Brazil. Journal of the Marine Biological Association of the United Kingdom, 95(4): 773–781, doi: 10.1017/S0025315414001707

Smyda T J. 1997. What is a bloom? A commentary. Limnology and Oceanography, 42(5part2): 1132–1136, doi: 10.4319/lo.1997.42.5_part_2.1132

Spector I, Brata E, Shochet N R, et al. 1999. New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. Microscopy Research and Technique, 47(1): 18–37, doi: 10.1002/(SICI)1097-0029(19991001)47:1<18::AID-JEMT3>3.0.CO;2-E

Suganuma M, Fujihi H, Suguri H, et al. 1988. Okadaic acid: an additional non-phorbol-12,13-diacetate-13-acetate-type tumor promoter. Proceedings of the National Academy of Sciences of the United States of America, 85(6): 1768–1771, doi: 10.1073/pnas.85.6.1768

Terao K, Ito E, Yanagi T, et al. 1986. Histopathological studies on experimental marine toxin poisoning. I. Ultrastructural changes in the small intestine and liver of suckling mice induced by dinophysistoxin-1 and pectenotoxin-1. Toxicon, 24(11–12): 1141–1151

Toyofuku H. 2006. Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report). Marine Pollution Bulletin, 52(12): 1735–1745, doi: 10.1016/j.marpolbul.2006.07.007

Turki S, Drub A, Fertouna-Bellakhal M, et al. 2014. Harmful algal blooms (HABs) associated with phycotoxins in shellfish: what can be learned from five years of monitoring in Bizerte Lagoon (southern Mediterranean Sea)? Ecological Engineering, 67: 39–47

Turner J T. 2014. Planktonic marine copepods and harmful algae. Harmful Algae, 32: 81–93, doi: 10.1016/j.hal.2013.12.001

Twiner M J, Hess P, Dechauroy M Y B, et al. 2005. Cytotoxic and cytoskeletal effects of azaspiracil-1 on mammalian cell lines. Toxicin, 45(1–2): 39–72, doi: 10.1016/j.toxicon.2005.02.015

Twiner M J, Rehmann N, Hess P, et al. 2008. Azaspiracid shellfish poisoning: a review on the chemistry, ecology, and toxicology with an emphasis on human health impacts. Marine Drugs, 6(2): 39–72, doi: 10.3390/md6020039

Vale C, Gómez-Limia B, Nicolau K C, et al. 2007. The c-Jun-N-terminal kinase is involved in the neurotoxic effect of azaspiracid-1. Cellular Physiology and Biochemistry, 20(6): 957–966, doi: 10.1159/000110456

Wang Yueming, Ong S S, Chai S C, et al. 2012. Role of CAR and PXR in the maturation of cathepsin D in mammalian cells. Chemical Research in Toxicology, 26(3): 444–455, doi: 10.1021/tr300511z

Xiao Xi, Agustí S, Pan Yaqiu, et al. 2019. Warming amplifies the frequency of harmful algal blooms with eutrophication in Chinese coastal waters. Environmental Science & Technology, 53(22): 13031–13041
Xu Jiayi, Hansen P J, Nielsen L T, et al. 2017. Distinctly different behavioral responses of a copepod, *Temora longicornis*, to different strains of toxic dinoflagellates, *Alexandrium* spp. Harmful Algae, 62: 1–9, doi: 10.1016/j.hal.2016.11.020

Yasumoto T. 1990. Marine microorganisms toxins—an overview. In: Granéli E, Sundström B, Edler L, et al., eds. Toxic Marine Phytoplankton. New York: Elsevier, 3–8

Zhang Xue, Hu Hongying, Men Yujie, et al. 2009. Feeding characteristics of a golden alga (*Poterioochromonas* sp.) grazing on toxic cyanobacterium *Microcystis aeruginosa*. Water Research, 43(12): 2953–2960, doi: 10.1016/j.watres.2009.04.003