Profiles of paralytic shellfish toxins in bivalves of low and elevated toxicities following exposure to Gymnodinium catenatum blooms in Portuguese estuarine and coastal waters

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HIGHLIGHTS

- PST profiles in four bivalve species from Portuguese waters were examined.
- Toxin profiles varied between low and elevated bivalve toxicities in the period 2007–2012.
- Molar ratios R1 = (C1+2):B1 were higher in elevated than in low cockle toxicities.
- R2 = [(dcSTX) + (dcGTX2+3)]:[(C1+2) + (B1)] were higher in low wedge clam toxicities than in mussels and cockles.
- Changes in R2 are presumably due to biotransformation after exposure to bloom.

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ABSTRACT

Profiles of paralytic shellfish toxins (PSTs) were examined in 405 composite samples of Mytilus spp., Cerastoderma edule, Donax trunculus and Spisula solida collected between 2007 and 2012 from natural production areas in two estuaries (Aveiro and Mondego), two coastal lagoons (Óbidos and Formosa), and three open coastal areas (Aguda, Comporta and Culatra). Toxin concentrations were obtained from the biotoxin monitoring programme database. Episodes of PST toxicity in Portugal have been associated with Gymnodinium catenatum blooms. Toxin profiles for each species showed no trend over the surveyed years. In general, profiles differ only slightly among areas, except for Óbidos. However, toxin profiles in bivalves varied between low and elevated toxicities, corresponding to below and above the PST regulatory limit, respectively. The ratio R1 = (C1+2):B1, which were the main toxins produced by G. catenatum cells, decreased considerably between elevated and low cockle toxicities, indicating the elimination of C1+2 or conversion of compounds into B1. R2 = [(dcSTX) + (dcGTX2+3)]:[(C1+2) + (B1)], which represents the ratio of minor to major toxins in G. catenatum cells, increased substantially in wedge clams (D. trunculus) of low toxicity and less markedly in cockles (C. edule) and mussels (Mytilus spp.). These differences are interpreted as the predominance of a biotransformation phase after exposure to the algal bloom. The toxin profile of surf clams (S. solida) was dominated by decarbamoyl compounds, reflecting intense biotransformation during exposure to blooms. The higher ratio R2 in low toxicity samples suggests that elimination of the produced decarbamoyl toxins was slower than biotransformation.

1. Introduction

Paralytic shellfish toxins (PSTs) comprise a suite of more than 30 natural tetrahydropurine derivatives produced in the marine environment by toxic phytoplankton such as Gymnodinium catenatum, Alexandrium spp. and Pyrodinium bahamense (Wiese et al., 2010). During blooms of these species, bivalve filter feeders such as mussels, cockles, and clams ingest toxic cells and tend to exhibit transient high toxin concentrations. PSTs are of great concern as they are potent neurotoxins that block the voltage-gated sodium channels in excitable cells, suppressing ion permeation (Kao, 1966). This action has long been documented as a potential cause of serious illness in consumers of contaminated bivalves (Sommer and Meyers, 1937). The better-known toxins are included in the following groups in decreasing order of toxicity: carbamate...

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(saxitoxin-STX, neosaxitoxin-NEO and gonyautoxins-GTX1 to GTX4), decaharmamoyl (dcGTX1 to dcGTX4, dcSTX and dcNEO) and N-sulfocarbamoyl (B1, B2, C1 to C4) (Oshima, 1995a; EFSA, 2009). Other compounds with substituent side chains such as hydroxyl, hydroxybenzoate, or acetate have been identified and structurally described (Wiese et al., 2010).

Studies of PSTs in dinoflagellate cells collected in different regions of the world have pointed to strain-specific profiles (Oshima et al., 1993; Franco et al., 1994; Krock et al., 2007). However, stresses such as alterations in physico-chemical properties and physiology may change toxin profiles of dinoflagellate cells (Etheridge and Roesler, 2005; Poulton et al., 2005; Band-Schmidt et al., 2010). When bivalves ingest toxic algae the accumulated toxins are selectively metabolized and eliminated. The toxin profile in each bivalve species thus varies with time and differs progressively from the algae from which the toxins originate (Oshima et al., 1990; Samsur et al., 2006). For example, mussels and oysters mirror the toxin composition of the causative plankton cells better than clams, which exhibit different proportions of ingested toxins (Bricelj and Shumway, 1998). Specific toxin profiles of bivalves result mainly from different uptake, elimination/retention or epimerization mechanisms (Cembella et al., 1994; Yu et al., 2007; Botelho et al., 2010a). In addition, the occurrence of hydrolysis, chemical and/or enzymatic transformation pathways (Shimizu and Yoshioka, 1981; Oshima, 1995b), as well as bacterial degradation processes (Donovan et al., 2008) may also induce specific PST profiles.

Although toxin concentrations are routinely measured in bivalves of toxicity below the PST regulatory limit in national monitoring programmes, comparison of toxin profiles for different toxicity values are scarce. Turner et al. (2014) reported the variability of occurrence of PSTs in bivalves from the United Kingdom and found no correlation between profiles and total PST content of shellfish. The present work compares PST profiles in bivalve molluscs from Portugal of toxicity below and above the PST regulatory limit caused by *G. catenatum* blooms. Differences were tested for 405 composite samples of *Mytilus* spp., *Cerastoderma edule*, *Donax trunculus* and *Spisula solida* from seven harvesting areas between 2007 and 2012.

### 2. Material and methods

#### 2.1. Bivalve toxicity database

Values of bivalve toxicity by PSTs used in the present study were obtained from the database of the Portuguese biotoxin monitoring programme (BMP). Between 2007 and 2012, toxicity values were obtained through chemical determinations, although two analytical methodologies were used sequentially in this period of time: from 2007 to 2008, PST concentrations were determined using a LC method with pre-column oxidation based on Lawrence et al. (1995) modified by Vale and Sampayo (2001); from 2009 to 2012, the toxins C1+2, B1, dcSTX, dcGTX2+3, GTX2+3 and STX were quantified using the official AOAC pre-column oxidation method by liquid chromatography with fluorescent detection (LC-FLD) (AOAC, 2005). In the first method, the concentrations of the so-called dcSTX, dcGTX2+3, GTX2+3 and STX were determined through the areas of chromatographic peaks that eventually included unresolved peaks, corresponding to other toxins like NEO and GTX1+4. To prevent misunderstanding, concentrations of those toxins were designated by “dcSTX”, “dcGTX2+3”, “GTX2+3” and “STX”. Due to the use of different methodologies, molar proportions of C1+2, B1, dcSTX, dcGTX2+3, GTX2+3 and STX calculated in the period 2007–2012 (method A – AOAC, 2005) cannot be compared to values of 2007–2008 (method B – Vale and Sampayo, 2001).

Bivalve toxicity values were estimated in terms of µg STX di-HCl equivalents per kg, multiplying the toxin concentration by the toxicity equivalence factor (TEF) of each individual compound. After June 2010, toxicity values were estimated following the recommendations of the EU Reference Laboratory for Marine Biotoxins. This results in the duplication of the TEF used to dcSTX, from 0.5 (Oshima, 1995a) to 1 (EFSA, 2009). For the purpose of data consistency in this study, all toxicity values originally determined using Oshima TEF for dcSTX were re-calculated using EFSA. In the case of isomeric pairs as GTX2+3 and C1+2, the highest TEF was used for each pair. The regulatory limit (RL) for PSTs is 800 µg STX di-HCl equivalents per kg. This value was obtained by the sum of the detection limits of dcGTX2+3, dcSTX, C1+2, B1, GTX2+3 and STX multiplied by the corresponding TEFs. A detailed description of the BMP, location of the natural production areas of bivalves in Portugal, analytical methodologies used, and toxicity calculation are described in Vale and Sampayo (2001), Vale et al. (2008), and Botelho et al. (2010b).

#### 2.2. Selection of bivalve species and harvesting areas

Four bivalve species were used in this study, taking into account their geographic distribution, abundance and commercial value. The mussel *Mytilus* spp. and cockle *C. edule* were considered due to their abundance in the areas of Aveiro, Mondego and Óbidos. Cockles were also collected in the Formosa lagoon. Mussels were sampled in hard substrates of the inlet channels, and cockles in sandy areas of inner zones. The wedge clam *D. trunculus* and the surf clam *S. solida* were chosen because they are commercially harvested from natural production areas in the west coast (Aguda and Comporta) and south coast (Culatra) (Fig. 1). Habitats and ecology are described in Gaspar et al. (1999) and Jaquim et al. (2008).

Coordinates of the harvested areas have changed slightly during the multi-annual surveyed period, as the abundance of bivalves varied with time (Rufino et al., 2008). The three systems Aveiro, Mondego and Óbidos are located in the central west coast, and Formosa in the southeastern area. In addition, there are morphological and hydrological differences among these systems. Whereas the Mondego is a tubular estuary, Aveiro, Óbidos and Formosa have broad inner areas and narrow connections to the sea, are generally classified as lagoons (Bettencourt et al., 2004). The western systems (Aveiro, Mondego and Óbidos) are characterised by recurrent episodes of bivalve toxicity by PST (Vale et al., 2008). The Formosa coastal lagoon was selected due to the large quantities of bivalves grown in the tidal flats (Ferreira et al., 2014).

#### 2.3. Number of samples and bivalve toxicity values

Table 1 gives the number of samples of surf clams, wedge clams, cockles and mussels used in this work. This study is based on toxin concentrations of 405 composite samples of bivalves (each one a pool of 30 specimens) obtained between 2007 and 2012 in seven harvest areas. The number of annual samples with toxicity below and above the PST regulatory limit, as well as the corresponding harvest area, is presented. Samples below the regulatory limit showed a total toxicity between 55 and 800 µg STX di-HCl equivalents per kg, while samples above this limit presented a broad variation of toxicity values for the various blooms and areas surveyed. Table 2 shows the median, minimum and maximum of PST toxicity ratios (total toxicity value/regulatory limit for PSTs) of surf clams, wedge clams, cockles and mussels collected between 2007 and 2012 in the corresponding harvest areas.
2.4. Sampling of seston and determination of PSTs

In addition to bivalve toxicity data, samples of the toxin-producing algae were collected to assess the toxin profile in the cells ingested by bivalves. Between 5 and 10 L of surface water samples were collected during a bloom of *Gymnodinium catenatum*, from 23rd June to 21st July 2008, in coastal waters adjacent to Óbidos (9 samples). Water samples for phytoplankton species identification were preserved in Lugol’s iodine and the cells of *G. catenatum* identified under an inverted microscope after sedimentation. Water samples for toxin analysis passed through a GF/C glass filters (porosity 1.2 μm, 150 mm Ø), under light vacuum pressure (100 mmHg), and the material retained in the filters were frozen in 0.1 M acetic acid at −80 °C until analysis.

Toxins were extracted from the seston retained on filters by freeze/thaw cycle, followed by probe sonification in an ice bath for 30 s at 60% amplitude and 20 W (Vibra Cell, Sonics & Materials Inc.) (Botelho et al., 2012). Cell debris after probe sonification was examined under an inverted microscope and revealed full disruption of algal cells in selected samples. The pH of the extracts was adjusted to 6.5 with 0.2 M NaOH. The extracts were filtered (0.2 μm) and diluted to exactly 1 mL.

The determination of PSTs was based on the AOAC pre-column oxidation method by LC-FLD (Anon, 2005; Botelho et al., 2010b). Aliquots of seston extracts were used for oxidation of PSTs with peroxide and periodate oxidant prior to LC-FLD analyses. A similar procedure for both oxidations was followed, substituting the oxidant reagent by water in order to detect naturally fluorescent...
compounds. The quality control of the results was assured through the use of the certified reference materials C1&2, STX-e, dcSTX, GTX5-b (B1), dcGTX2&3, dcNEO-b, GTX1&4-b, GTX2&3-b and NEO-b, from the Institute for Marine Biosciences, National Research Council Canada. Evaluation of linear ranges for PSTs and instrumental limits of detection are described in Botelho et al. (2010b). Instrumental detection limits (nmol L\(^{-1}\)) were 3.9 (C1+2), 4.0 (B1), 4.0 (STX), 4.9 (dcSTX), 8.2 (dcGTX2+3), 8.5 (GTX2+3), 25 (dcNEO), 30 (GTX1+4) and 31 (NEO). The LC system consisted of a Hewlett–Packard/Agilent Model 1050 quaternary pump, Model 1100 in-line degasser, autosampler, column oven, and Model 1200 fluorescence detector. The Hewlett–Packard Chemstation software performed data acquisition and peak integration. The PST oxidation products were separated using a reversed-phase Supelcosil LC-18, 150 × 4.6 mm id, 5 μm column (Supelco) equipped with a guard column Supelguard Supelcosil C18, 20 × 4.0 mm id, 5 μm (Supelco). The column was kept in an oven at 30°C. The mobile phase gradient used 2 mobile phases: A (0.1 M ammonium formate, pH = 6) and B (0.1 M ammonium formate in 5% acetonitrile, pH = 6). The elution gradient consisted of 0–5% B in the first 5 min, 5–70% B in the next 4 min and back to 0% B in the next 5 min. Flow rate was 1 mL min\(^{-1}\) and the injection volumes were 50 μL and 100 μL, for the oxidation products of peroxide and periodate reaction, respectively. The excitation and emission wavelengths for fluorimetric detection were set at 340 nm and 395 nm, respectively.

2.5. Statistical analyses

Prior to statistical analyses, toxin molar proportions were tested for normality and equality of variances. The Mann–Whitney U test was used to evaluate the existing differences between toxin molar

| Table 1 | Annual number of samples, between 2007 and 2012, with toxicity values below and above the regulatory limit for PSTs; surf clam (Aguda-AG, Comporta-CO and Culatra-CU), wedge clam (Comporta-CO and Culatra-CU), cockle (Aveiro-AV, Mondego-MO, Óbidos-OB and Formosa-FO) and mussel (Aveiro-AV, Mondego-MO and Óbidos-OB); 2007–2008 – method B; 2009–2012 – method A. |
| Species | Area | Surf clam | Wedge clam | Cockle | Mussel |
| | | AG | CO | CU | AG | CO | CU | AV | MO | OB | AV | MO | OB |
| Toxicity below RL | | | | | | | | | | | | | |
| 2007 | 3 | 3 | 7 | – | 4 | – | – | 3 | 3 | 4 |
| 2008 | – | – | – | 9 | 3 | 20 | – | – | 18 | 6 | – |
| 2009 | – | – | – | – | – | – | – | 8 | 8 | 14 | – |
| 2010 | – | – | – | – | – | 62 | 3 | 3 | 3 | 21 | 3 | – |
| 2011 | – | – | – | – | – | – | – | 7 | – | – | – |
| 2012 | – | – | – | – | – | 3 | – | – | – | – | – |
| Toxicity above RL | | | | | | | | | | | | | |
| 2007 | 3 | – | – | 7 | – | 13 | 4 | 9 | – | 13 | 7 | 8 |
| 2008 | – | 7 | – | 9 | – | 7 | 8 | 19 | – | 17 | 13 | 14 |
| 2009 | 3 | – | 3 | – | 3 | – | – | 6 | 7 | 4 | – |
| 2012 | – | – | 3 | – | 3 | – | – | – | – | – | – |

| Table 2 | Median and the interval of minimum and maximum of PST toxicity ratios (total toxicity value/regulatory limit) between 2007 and 2012; surf clam (Aguda-AG, Comporta-CO and Culatra-CU), wedge clam (Comporta-CO and Culatra-CU), cockle (Aveiro-AV, Mondego-MO, Óbidos-OB and Formosa-FO) and mussel (Aveiro-AV, Mondego-MO and Óbidos-OB); 2007–2008 – method B; 2009–2012 – method A. |
| Species | Area | Median toxicity ratio (minimum–maximum) |
| | | 2007 | 2008 | 2009 | 2012 |
| Surf clam | AG | 1.7 | – | 2.0 | (1.4–2.0) |
| | CO | – | 6.0 | – | (3.4–13) |
| | CU | – | – | 3.1 | (2.4–3.3) |
| Wedge clam | CO | 23 | 7.0 | – | (2.5–42) |
| | CU | – | – | 2.9 | (1.7–3.3) |
| Cockle | AV | 9 | 3.9 | – | (2.7–20) |
| | MO | 22 | 3.4 | – | (1.4–32) |
| | OB | 8.9 | 2.8 | – | (2.2–118) |
| | FO | – | – | 1.9 | (1.3–9.0) |
| Mussel | AV | 23 | 5.0 | 11 | (1.5–32) |
| | MO | 11 | 5.0 | 11 | (1.5–32) |
| | OB | 40 | 33 | – | (4.1–114) |
proportions in bivalves from selected areas. The significant tests were performed using the STATISTICA 6.0 Statistical Software System.

3. Results

3.1. PST composition in bivalve species

Median, maximum, minimum, 75th and 25th percentiles of molar fractions of the quantified paralytic shellfish toxins relatively to the total quantified toxins in samples of surf clams, wedge clams, cockles and mussels that have toxicity values above the PST regulatory limit are shown in Fig. 2. The proportion of each toxin was calculated for those bivalve species, encompassing data from different harvest areas (Table 1) for the two periods of time: 2007–2008 (method B) and 2009–2012 (method A). A salient aspect is the negligible contributions of C1+2 and B1 (<0.7%) to the toxin profile of surf clams. These contributions contrast to the median proportions of C1+2 and B1 in wedge clams (10% and 63%), cockles (39% and 32%) and mussels (57% and 40%). The molar fractions of dcGTX2+3 and dcSTX were significantly (p < 0.05) higher in surf clams than in the other bivalve species. The disparity between the two groups of compounds was found in data obtained by both methods (A and B), although with different meanings between dcGTX2+3, dcSTX and “dcGTX2+3”, “dcSTX” (Fig. 2a and b). The compounds GTX2+3 and STX had minor contributions to the toxin profiles of all species. The proportion of C1+2 was significantly (p < 0.05) lower in wedge clams than in cockles and mussels, and the opposite was observed for B1.

3.2. Temporal and spatial variability of toxins

To assess whether variability of toxin proportions was influenced by blooms (temporal variation between 2007 and 2012) or harvest areas (spatial variation), significance tests were done for each species, considering two situations: bivalves from the same area exposed to different blooms, and bivalves exposed to blooms occurring in the same period at different areas.

The proportions of dcSTX and dcGTX2+3 in surf clams harvested in Culatra during two Gymnodinium catenatum blooms, August–October 2009 and August–November 2012, were not significantly (p > 0.05) different. Values of those toxins were also not statistically different (p > 0.05) between samples collected in Aguda and Culatra during August–October 2009. Wedge clams from Comporta exposed to blooms in October–November 2007 and July–August 2008 showed different (p < 0.05) proportions of the major toxins, C1+2, B1 and “dcSTX”. Comparison of different blooms in Culatra was not carried out due to the low number of samples (Table 1). The proportions of all toxins quantified in cockles and mussels from Aveiro, Mondego and Óbidos showed no significant (p > 0.05) differences between October–November 2007 and June–August 2008. Otherwise, proportions of C1+2 and B1 in cockles from Óbidos differed significantly (p < 0.05) from specimens from Aveiro and Mondego, both in 2007 and 2008 blooms. The proportions of C1+2 and B1 in mussels from Óbidos in the 2007 bloom differed significantly (p < 0.05) from values found for Aveiro and Mondego.

3.3. PST composition in bivalves with different toxicity values

Fig. 3 shows the median, maximum, minimum, 75th and 25th percentiles of the molar fractions of the toxins quantified in two sets of samples of surf clams, wedge clams, cockles and mussels that presented toxicity values above and below the PST regulatory limit. Elevated toxicity values were registered in summer or autumn and attributed to blooms of G. catenatum that reached the harvest areas (Moita et al., 2003). Bivalves with low toxicity values were collected in late winter and spring.

The toxins dcGTX2+3 and dcSTX were the major ones in surf clam samples of elevated and low toxicity. Furthermore, the molar proportions of each compound did not differ significantly (p > 0.05) between the two sets of samples. The other toxins quantified, C1+2, B1, GTX2+3, and STX, remained as minor components, although significantly (p < 0.05) higher in surf clams of low toxicity. Toxin proportions in samples of wedge clam analysed by method A showed considerable differences between low and elevated toxicities (n = 3 and 6, respectively). The comparison using a more representative dataset (n = 12 and 16 for the method B) pointed to no significant differences between low and elevated toxicity, except for “dcSTX” and “GTX2+3”. Cockles of low toxicity showed significantly (p < 0.05) lower proportions of C1+2 than the elevated toxicity samples. Proportions of B1 varied inversely with the bivalve toxicity values, which is clearer defined considering method A. Significant (p < 0.05) differences were also obtained for dcGTX2+3. In mussels, significant (p < 0.05) differences were found for C1+2, dcSTX, GTX2+3 and STX (method A) and for C1+2, B1, “GTX2+3” and “STX” (method B).

3.4. Toxin profile of PSTs in G. catenatum

Between 28th June and 21st July 2008, the toxicity by PSTs of wild mussels from the NW coast (inlet of the Óbidos lagoon,
Fig. 3. Median, maximum, minimum, 75th and 25th percentiles of molar fractions of quantified PSTs (%) in two sets of samples of surf clam, wedge clam, cockle and mussel presenting toxicity values above and below the PST regulatory limit (RL) between 2007 and 2012; (a) C1+2, B1, dcSTX, dcGTX2+3, GTX2+3 and STX (method A); (b) C1+2, B1, “dcSTX”, “dcGTX2+3”, “GTX2+3” and “STX” (method B).
force the observations that *G. catenatum* cells in the Iberian coast are characterised by the high production of N-sulfocarbamoyl and decarbamoyl compounds. However, the possibility of slight variations in toxin composition should be considered. To search differences on profiles of *G. catenatum* cells from the NW coast, two ratios have been calculated: \( R_1 = \frac{(C1+2)}{B1} \), representing the proportion between the major toxins, and \( R_2 = \frac{[(dcSTX) + (dcGTX2+3)]}{[(C1+2) + (B1)]} \), the proportion between minor and major toxins. These ratios were calculated for the current data (2008) and for the 2007 results (Costa et al., 2010). Values of \( R_1 \) and \( R_2 \) differed slightly in 2008 and 2007: 3.0, 3.9 and 0.10, 0.36 respectively. These differences may be related to the physico-chemical conditions associated with the algal bloom. Although *G. catenatum* blooms are triggered under similar oceanographic conditions in the NW coast of Portugal (Moita et al., 2003; Pitcher et al., 2010), different nutrient availability may exist, which could explain the modifications registered in the toxin ratios \( R_1 \) and \( R_2 \). Nutrient concentrations or composition have been shown that can modify the toxin profiles of cultivated *G. catenatum* cells (Band-Schmidt et al., 2010). Alterations in toxin ratios may also be explained by the different development stages of blooms due to changes in cell size, chain length and toxin leakage (Granéli and Flynn, 2006).

### 4.2. Alteration of major ingested PSTs by bivalves

The ratios \( R_1 \) and \( R_2 \) were also calculated for the bivalve species under two environmental conditions: (i) bivalves exposed to *G. catenatum* blooms in summer or autumn showing toxicity values above the PST regulatory limit, and (ii) bivalves under low abundance of toxic cells in winter/spring, and consequently presenting toxicity values far below the regulatory limit (Fig. 5).

The ratios \( R_1 \) = \( (C1+2)/B1 \) in surf clams could not be determined accurately because their concentrations were below the limit of detection in 90% of the samples. Medians of this ratio in wedge clams, cockles and mussels were lower than in *G. catenatum* cells collected in the NW coast during the blooms of 2007 and 2008 (Fig. 5). Lower ratios in bivalves suggest the reduction of \( C1+2 \), most likely due to biotransformation to decarbamoyl toxins (Cho et al., 2008) or elimination, as well as the conversion of other compounds into \( B1 \) that counterbalanced its elimination or conversion. A plausible explanation for the increase of \( B1 \) is the possibility that this compound is a metabolic product of degradation of \( B2 \) due to their similar chemical structures (Oshima, 1995b). It should not be discarded the possibility of \( C1+2 \) to be converted into \( B1 \) through the elimination of O–sulfate (OSO$_3^-$) in the R2/R3 position, as reported for GTX2 and GTX3 (Oshima, 1995b). However, to the best of our knowledge, that conversion has not been reported in the literature.

The ratios \( R_1 \) in wedge clams were significantly \((p < 0.05)\) lower than in cockles and mussels with elevated toxicity. This difference points to more intense processes involving those toxins in wedge clams during the period that was exposed to *G. catenatum* bloom than in cockles and mussels. The comparable ratio \( R_1 \) registered in wedge clams during winter/spring, after a long period of natural depuration, supports this hypothesis. Conversely, the ratios in low toxicity (winter) cockle and mussels decreased significantly \((p < 0.05)\), which can be interpreted to mean that transformation or elimination processes involving \( C1+2 \) and \( B1 \) in those species are more prolonged than in wedge clams.

### 4.3. Prevalence of decarbamoyl derivates in surf clams

Since decarbamoyl derives contribute approximately 10% to the toxin profile of *G. catenatum* cells (Fig. 4), the ratio \( R_2 \) = \( [(dcSTX) + (dcGTX2+3)]/[(C1+2) + (B1)] \) may be considered as a footprint of PSTs biotransformation in bivalves. The differences...
among the four species, and between low and elevated bivalve toxicity, are shown in Fig. 5. It should be noticed the extreme values of $R^2$ for surf clams (median 72) in comparison to the other species analysed (0.2–0.4) and to $G$. catenatum cells (0.1). Those values reflect the low concentrations of $C_{1+2}$ and $B_1$ in surf clams and their almost entire conversion into decarbamoyl analogues. These conversions have been reported in previous works (Artigas et al., 2007; Vale et al., 2008; Turner et al., 2013). The undetected values of these compounds in surf clams are explained by the rapid transformations of N-sulfocarbamoyl and carbamate toxins into their corresponding decarbamate analogues. Using in vitro experiments, Artigas et al. (2007) and Turner et al. (2013) showed that conversion of N-sulfocarbamoyl toxins occurs within a short time-scale of one hour. A longer period of time was necessary for the carbamate toxins (Turner et al., 2013). In accordance, the profile of surf clams exposed to $G$. catenatum cells observed in the current work was dominated by dcSTX and dcGTX2+3, which reflects the rapid biotransformation of the ingested toxins. This type of profile persisted in periods of low toxicity, $R^2$ in surf clams being significantly ($p < 0.05$) different from other bivalve species (Fig. 5). The lack of differences in the toxin proportion of surf clams among harvest areas is in line with the high biotransformation of the ingested toxins. Presumably, the broad difference registered in the ratio $R^2$ between elevated and low toxicity of surf clams results from elimination of decarbamoyl derivatives after blooms. Since inter-toxin conversion superimposes to other steps of the bioaccumulation process, the major contributors to the toxin profile of surf clams stand independently of the abundance of toxic cells to which the specimens are exposed.

5. Conclusions

Differences of PST profiles between bivalves of elevated toxicity (exposed to $G$. catenatum bloom) and bivalves of low toxicity (winter or spring with low abundance of toxic algae) are better illustrated through the molar ratios $R^1 = (C_{1+2}):B_1$ and $R^2 = [(dcSTX) + (dcGTX2+3)]:(C_{1+2} + B_1)$. The ratio $R^1$ decreased considerably between elevated and low toxicity cockles, indicating the elimination of $C_{1+2}$ associated with the ingested toxic cells or conversion of compounds into $B_1$. The ratio $R^2$ increased considerably in wedge clams of low toxicity and less markedly in cockles and mussels. This change is interpreted as the predominance of a biotransformation phase after exposure to algal blooms. The toxin profile of surf clams is dominated by decarbamoyl compounds, reflecting intense biotransformation during exposure to blooms. This profile remains after that, although the ratio $R^2$ decreased considerably, which may indicate the elimination of the produced dcSTX and dcGTX2+3.

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