Marine Environmental Research 77 (2012) 23–29

Contents lists available at SciVerse ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Uptake and release of paralytic shellfish toxins by the clam *Ruditapes decussatus* exposed to *Gymnodinium catenatum* and subsequent depuration

Maria João Botelho [a,*], Carlos Vale [a], Rita Velez Grilo [a], João Gomes Ferreira [b]

[a] IPIMAR, Av. Brasília, 1449-006 Lisbon, Portugal
[b] CMA, Department of Environmental Sciences and Engineering, Faculty of Sciences and Technology, New University of Lisbon, Qta Torre, 2829-516 Monte de Caparica, Portugal

ARTICLE INFO

Article history:
Received 20 October 2011
Received in revised form
11 January 2012
Accepted 11 January 2012

Keywords:
Paralytic shellfish toxins
Ruditapes decussatus
Particulate organic matter
Uptake
Depuration
Mass balance

ABSTRACT

A laboratory experiment was performed with the clam *Ruditapes decussatus*, fed with the toxic dinoflagellate *Gymnodinium catenatum* and the non-toxic algae *Isochrysis galbana* (14 days) and subsequently only with *I. galbana* (15 days). Individual paralytic shellfish toxins were determined by LC-FLD in *G. catenatum* cells, whole clam tissues, and particulate organic matter (POM) produced by clams. The toxins dcSTX and dcGTX2 + 3 in the algae were less abundant than C1 + 2 and B1, but were predominant in clams during both the exposure and depuration phases. The toxin dcNEO was only detected in clams during a short period, indicating conversion from other compounds. The toxin composition of the POM indicated the export of dcSTX as faeces or pseudo-faeces along the entire experiment (2.5–14 nmol mg^{-1}), B1 was present in a short period of the exposure and C1 + 2 and dcGTX2 + 3 absent. A mass balance calculation indicated that approximately 95% of C1 + 2 and 85% of B1 supplied to the clams were converted into other toxins or lost in solution. Conversely, the net gain of 512, 61 and 31 nmol for dcSTX, dcGTX2 + 3 and dcNEO, respectively, suggests the conversion from other assimilated compounds by clams during exposure and depuration phases.

1. Introduction

Paralytic shellfish toxins (PSTs) are a group of neurotoxic alkaloids produced in the marine environment by dinoflagellates like *Alexandrium* spp., *Pyrodinium bahamense* and *Gymnodinium catenatum* (Llewellyn, 2006). The documented PSTs are grouped into three structural families in decreasing order of toxicity (Oshima, 1995a; Anon, 2009): carbamate (saxitoxin-STX, neosaxitoxin-NEO and gonyautoxins-GTX1 to GTX4), decarbamoyl (dcGTX1 to dcGTX4, dcSTX and dcNEO), and *N*-sulfocarbamoyl (B1, B2, C1 to C4). Most of the *Alexandrium* species have a carbamate-dominated toxin profile, while decarbamoyl and *N*-sulfocarbamoyl toxins are dominant components in *G. catenatum* (Cembella et al., 1987; Chou et al., 2004; Ordás et al., 2004; Band-Schmidt et al., 2005; Costa et al., 2010). It is well documented that PSTs are efficiently accumulated by filter-feeders during blooms of toxic phytoplankton species, being causative agents of paralytic shellfish poisoning in humans (Sommer and Meyers, 1937; Gessner and Middaugh, 1980).

Various studies have proved that shellfish exposed to dinoflagellates exhibit different PSTs profiles from the toxin producers (Bricelj et al., 1990; Oshima et al., 1990; Cembella et al., 1993; Samsur et al., 2006). Metabolic interconversion of assimilated PSTs achieved by enzymatic and chemical reactions in shellfish tissues may contribute to those differences (Shimizu and Yoshioka, 1981; Kotaki et al., 1985; Oshima, 1995b; Bricelj and Shumway, 1998). Incubation in vitro of toxic dinoflagellates or purified toxins extracts allowed elucidating the role of enzymatic activities (Sullivan et al., 1983; Fast et al., 2006; Artigas et al., 2007). Different uptake and depuration kinetics of individual PSTs may also contribute to the registered modifications on toxin profiles between the toxic algae and the exposed shellfish (Blanco et al., 2003; Yu et al., 2007; Botelho et al., 2010a).

Despite the low concentration of individual toxins in the dissolved fraction their quantification provides additional information on the elimination of individual PSTs (Bricelj and Shumway, 1998; Sekiguchi et al., 2001). Toxins in particulate organic matter rejected by shellfish are at higher concentrations, but may be difficult to interpret if encompassing particles from different pathways, namely faeces, pseudo-faeces and wasted food (Samsur et al., 2006; Estrada et al., 2007; Samsur et al., 2007).

This work reports the levels of the toxins C1 + 2, B1, dcSTX, dcGTX2 + 3 and dcNEO in whole tissues of the clam *Ruditapes decussatus* and in particulate organic matter rejected by the clams during a 29-day feeding experiment, including exposure to

* Corresponding author. Tel.: +351 21 3027172; fax: +351 21 3015948.
E-mail address: mjoao@ipimar.pt (M.J. Botelho).

0141-1136/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2012.01.002

G. catenatum and subsequent depuration. Toxin composition of toxic algae, clams and particulate fraction in conjunction with mass balance calculations for individual toxins, allowed identifying the toxins exported as faeces or pseudo-faeces, as well as the compounds biotransformed during exposure and depuration phases.

2. Material and methods

2.1. Algal culture

The culture of the dinoflagellate *G. catenatum* (strain C37-07, IPIMAR collection) was maintained in natural seawater enriched with GSe medium (salinity 28, Blackburn et al., 2001) at 18 °C under a 16 h light: 8 h dark photocycle with a light intensity of 15 μmol photons m^{-2} s^{-1}. The strain was then mass cultured in 10-L culture flasks under the same conditions to supply to the clam feeding experiment. Cells of *G. catenatum* were harvested in the late exponential growth phase. The non-toxic microalgae *Isochrysis galbana* was grown in Wallerstein and Miquel medium (Bandarra et al., 2003) at 18 °C in 75-L plastic bags under constant illumination at salinity 25. Cells of the toxic microalgae were counted in Palmer-Maloney chambers under an Zeiss IM 35 inverted microscope and of *I. galbana* in an automatic particle counter (Coulter EPICS XL).

2.2. Clams

A total of 450 clams (*R. decussatus*) were obtained from growth banks at Ria Formosa, a coastal lagoon located in southern Portugal with an annual production of 5000 T. Clams were collected in November 2008, after several years of undetected *G. catenatum* blooms in the lagoon or adjacent coastal zone. Animals were acclimatized during 15 days to the laboratory conditions in a 50-L aerated polyethylene tank with filtered seawater and fed daily with the non-toxic microalga *I. galbana* culture (2×10^9 cells). Whole tissue wet weight and shell length of the clams ranged within 1.3–1.8 g and 2.8–3.9 cm, respectively.

2.3. Feeding experiment

Clams were divided into 42 groups of 10 individuals each and placed into 5-L plastic tanks filled with filtered natural seawater stored in a reservoir. The seawater in the tanks was continuously aerated and renewed daily. Water temperature remained at 12 ± 1 °C. The feeding experiment consisted of two phases schematically illustrated in Fig. 1. During phase I (14 days) each 10-clam group was fed twice a day with 1×10^4 cells of *G. catenatum* culture and 5×10^7 cells of *I. galbana* culture. During phase II (15 days) clams were fed twice a day only with 5×10^7 cells of *I. galbana* culture. No control animals were considered in the experiment, since *I. galbana* does not produce toxins and clam responses to toxin exposure was not studied. Throughout the 29 days of the experiment no clam deaths occurred and changes in the whole tissue weight were negligible.

2.4. Samples

Toxic algae. Aliquots of 400-mL *G. catenatum* culture were harvested at days 3, 9 and 14 of the phase I for toxin analysis. Algal mass was obtained by filtering the culture under light vacuum pressure (100 mmHg) through GF/C glass filters (1.2 μm), and freezing in 0.1 M acetic acid at −80 °C until analysis.

Clams. Specimens from three pools of 10 clams each were sacrificed in days 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29, dissected and composite samples ($n = 10$) of whole soft tissues were prepared and stored at −80 °C until analysis.

Particulate organic matter. Particulate organic matter (POM) produced by clams was collected from the experimental tanks daily after day 3. Seawater of three tanks was transferred to an inverted conic recipient and particles were left settling for 12 h. Material was then collected by filtration through 0.45 μm polycarbonate membranes, and kept frozen in 0.1 M acetic acid at −80 °C until analysis.

2.5. Reagents

All chemicals and solvents used were LC or analytical grade. Sodium hydroxide and hydrogen peroxide were from Merck (Germany). Ultra-pure water was obtained by a *Milli-Q* system Millipore (France). Acetonitrile, acetic acid, methanol and ammonium formate were purchased from Sigma–Aldrich (Germany).

2.6. Toxin extraction and oxidation

Toxic algae. Toxins of *G. catenatum* cells retained on filters were extracted according to the method described in Artigas et al. (2007), with the following modifications. The extraction was done by freeze/thaw cycle followed by ultrasonication in an ice bath for 30 s at 60% amplitude and 20 W (*Vibra Cell*, Sonics & Materials Inc.). Cell debris after ultrasonification was examined under an inverted microscope revealing full disruption of the surveyed samples. The crude extract was centrifuged at 2500 g for 10 min, and then cleanup using a Solid Phase Extraction (SPE) C18 cartridge (*Sep-Pak Light*, Waters, USA). The 130-mg C18 cartridge was conditioned with 1.5 mL methanol and 1.5 mL ultra-pure water. Subsequently an aliquot of 250 μl of supernatant was loaded, then

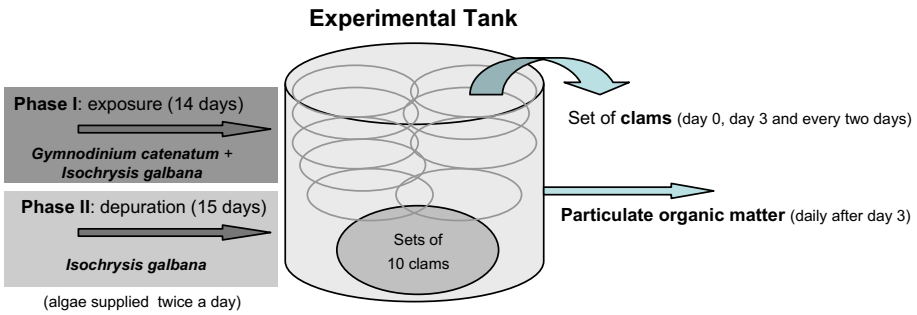

Fig. 1. Schematic representation of the laboratory feeding experiment.