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Combined effect of temperature and nutritional regime on the elimination of the lipophilic toxin okadaic acid in the naturally contaminated wedge shell *Donax trunculus*

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HIGHLIGHTS

- *D. trunculus* harvested in Portugal exhibits high OA values for long periods.
- Contaminated individuals were depurated at 16 and 20 °C at three nutritional regimes.
- Depuration rate was faster at 20 °C under non-toxic diets than at 16 °C.
- Wedge clams with OA 3 times above the regulatory limit were depurated in 6 days.

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ABSTRACT

The influence of nutritional regime and water temperature on depuration rates of OA-group toxins in the wedge shell *Donax trunculus* was examined by exposing naturally contaminated specimens to three nutritional regimes (microalgae, commercial paste of microalgae, and starvation) for 14 days at 16 °C and 20 °C. Total OA was quantified in the whole soft tissues of the individuals collected in days 2, 4, 6, 8, 10, 12 and 14. Mortality, dry weight, condition index, gross biochemical composition and gametogenic stages were surveyed. Low variation of glycogen and carbohydrates during the experiments suggest that wedge shells were under non-dramatic stress conditions. Wedge shells fed with non-toxic diets showed similar depuration rates being 15 and 38% higher than in starvation, at 16 and 20 °C, respectively. Depuration rates under non-toxic diets at 20 °C were 71% higher than at 16 °C. These results highlight the influence of water temperature on the depuration rate of total OA accumulated by *D. trunculus*, even when the increase is of only 4 °C, as commonly observed in week time scales in the southern Portuguese coastal waters. These results open the possibility of a faster release of OA in harvested wedge shells translocated to depuration systems when under a slight increase of water temperature.

1. Introduction

Okadaic acid (OA) and its analogues, namely dinophysistoxin-1 (DTX-1) and dinophystoxin-2 (DTX-2), constitute the OA-group toxins (Carmody et al., 1996; Lee et al., 1988; Yasumoto et al., 1985). These toxins are naturally produced by various dinoflagellates of the genus *Prorocentrum* and *Dinophysis* (Morton et al., 2009; Reguera et al., 2012; Yasumoto et al., 1980). During bloom events of these species, bivalves and other marine filter feeders tend to accumulate those toxic compounds in concentrations that can easily exceed the regulatory limit for human consumption (EC, 2004). Uptake of those lipophilic thermo stable polyethers (McCarron et al., 2008) may inhibit serine/threorine protein phosphatases, leading to the diarrhetic shellfish poisoning syndrome (DSP) (Bialojan and Takai, 1988; Yasumoto et al., 1978).

The amount of OA-group toxins retained in the whole soft tissues of a bivalve depends on the balance involving ingestion of toxic cells, transformation of the uptake OA-group toxins namely during the digestive process, and toxin excretion mainly as fecal

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particles (Blanco et al., 2005, 1999; Bricelj and Shumway, 1998). When bivalves do contain OA-group toxins above the regulatory limit, bivalve's harvesting is halted for species as the mussels *Mytilus edulis* and *M. galloprovincialis*, the cockle *Cerastoderma edule*, the oyster *Crassostrea virginica,* the clams *Spisula solida* and *Mya arenaria*, and the wedge shell *Donax trunculus* (Deeds et al., 2010; Hattenrath-Lehmann et al., 2013; Reguera et al., 2014; Vale et al., 2008). In general, the closure periods varies from weeks to months, according to the intensity and duration of the toxic bloom and the ability of each bivalve species has to eliminate the toxins uptake (Vale et al., 2008).

In order to minimize social and economic impact of those closures, few attempts have been made to accelerate the depuration of OA-group toxins in contaminated bivalves. For example, transference of contaminated mussels to areas free of toxic cells may result in the enhancement of the toxin depuration rate in short periods depending on favorable environmental conditions (Blanco et al., 1999; Haamer et al., 1990; Poletti et al., 1996). However, the practicability of this procedure is highly questionable especially if a large quantity of bivalves is to be transferred to a clean area. Experiments performed by Bauder et al. (2001) and Blanco et al. (1999) showed higher release of toxins when larger quantities of non-toxic phytoplankton cells were added to the scallop *Argopecten irradians* and the mussel *M. galloprovincialis*, respectively. Results also suggested that the metabolic fecal loss was the main route for the elimination of OA-group toxins. The positive effect on depuration rates by increasing the amount of non-toxic feed to bivalves has also been confirmed in controlled microcosm experiments with *M. edulis* (Marcaillou-Le Baut et al., 1993; Marcaillou et al., 2010). Notwithstanding, a less clear relation between feed quantity and toxin elimination has been observed by Svensson (2003). It is well documented that temperature enhances the metabolic activity in bivalves and subsequently the toxin depuration rates (Bricelj et al., 2014; Hawkins and Bayne, 1992; Shumway et al., 1995). Increments on water temperature may trigger other changes leading to unexpected results. For example, an increase of temperature caused a breakdown of lipids in an experiment with *M. edulis* and, despite the lipophilicity of the OA-group toxins, depuration rate did not increase (Svensson and Förlin, 2004).

Exploitation of *D. trunculus* in soft beds constitutes one of the most important artisanal fisheries in south of Portugal, as well as in other southern European regions (Gaspar et al., 1999; Pereira et al., 2016). Annual official landings in 2015 was of 308 ton with the sales value of 875 k€ (INE, 2016). However, *D. trunculus* exhibits recurrently elevated concentrations of OA-group toxins, between spring and autumn, related with the proliferation of *Dinophysis acuminata* and *D. acuta* (Vale, 2006; Vale et al., 2008; Vale and Sampayo, 2002). Toxins accumulated in *D. trunculus* often exceed 5–7 times the values found in the clam *S. solida* collected in the same area, as well as of other bivalves harvested in the surrounding area (Vale, 2006). Furthermore, the residence time of OA-group toxins in wedge shells is longer than in other bivalve species. As a consequence, the closure periods on harvesting exceed largely those of other bivalve species exposed to the same toxic algal bloom (Vale et al., 2008) causing economic constraints on the regular harvesting of *D. trunculus* (Oliveira et al., 2015).

In view of this, the aim of this work is to examine whether changes on nutritional regime and water temperature influence the depuration rates of OA-group toxins in wedge shells. This hypothesis was tested by exposing for 14 days, at 16 °C and 20 °C, naturally contaminated *D. trunculus* to three nutritional regimes: microalgae *Isochrysis aff. galbana*, concentrated paste of *Tetraselmis suecica* and starvation. Concentrations of OA, DTX-1 and DTX-2 were determined in whole soft tissues of wedge shells, accomplished by the survey of condition index, protein, total lipids, carbohydrates, glycogen and gametogenic stages.

2. Material and methods

2.1. Collection of wedge shells

Approximately 8900 specimens of *D. trunculus* were harvested using a hand-dredger in Culatra island (Olhão) at the south coast of Portugal (Fig. 1). Bivalves were collected on 11th June 2015 during a bloom of several species of *Dinophysis*, which led to the accumulation of OA-group toxins in wedge shells. Data were obtained from the monitoring programme of toxic phytoplankton and biotoxins run by the Portuguese Institute for the Sea and the Atmosphere (IPMA). After discarding dead or damaged individuals, wedge shells were maintained at 10 °C and transported to the laboratory at Tavira, located 30 km from the harvesting area.

2.2. Experimental design

Laboratory experiments consisted of exposing *D. trunculus,* naturally contaminated with toxins of the okadaic acid group, for 14 days to three nutritional regimes: microalgae *Isochrysis* aff. *galbana* (T-iso) with an average of 3×10^9 cells L^{-1}, commercial concentrated paste of *Tetraselmis suecica* "*Phytobloom Ice Tetraselmis*" (PIT) with an average of 2×10^9 cell g^{-1} ww, and starvation (no feed added). The high nutritional diets used in the experiments are currently applied for bivalve production, namely of *D. trunculus* (Martínez-Pita et al., 2012; Ruíz-Azcona et al., 1996). The microalgae T-iso culture added in the experiments was grown in a temperature controlled room at 20 ± 2 °C under continuous illumination (9900 lux) in aerated seawater enriched with f/2 medium (Guillard, 1975) and a salinity of 33 ± 1. Cells of T-iso were counted regularly in a standard algal cell counter (Büker chamber) and harvested in the late exponential growth phase. Concentrated paste PIT was purchased from Necton, S.A., Olhão, Portugal (http://phytobloom.com/aquac_microalgae_tetraselmis/).

The feeding experiments run at 16 ± 1 °C and 20 ± 1 °C (referred herein as 16 °C and 20 °C, respectively), which comprises the temperature interval commonly found in the sampled waters during June (IPMA data). Furthermore, temperature of the intertidal sands may vary daily 2–3 °C due to the fluctuation of solar radiation and water height, as reported by Falcão and Vale (2003) for sand banks of *Ruditapes decussatus* production at Ria Formosa, south of Portugal.

Six experiments, corresponding to three diets at two temperatures, run in triplicate. Each experiment was initially performed with 420 individuals (23.7–25.6 mm shell length; 0.058–0.128 g, dw of whole soft tissues) placed into each 15-L plastic tank

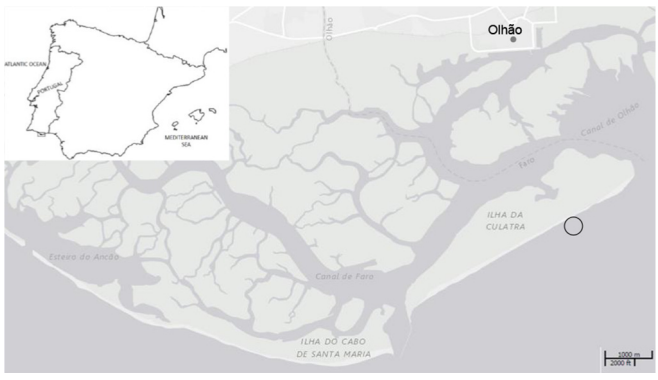

Fig. 1. Wedge shell *Donax trunculus* harvesting area (Olhão) in south coast of Portugal.