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Exposure to domoic acid affects larval development of king scallop *Pecten maximus* (Linnaeus, 1758)

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Abstract

Domoic acid (DA) is a highly toxic phycotoxin produced by bloom forming marine diatoms *Pseudo-nitzschia* spp. Bivalves can accumulate this toxin to a high level through their feeding activities, and thus illness or death in can occur in consumers of bivalves. In this study, king scallop, *Pecten maximus*, larvae were exposed to dissolved domoic acid (DA) for 25 d, and the toxin accumulation and effects of harbouring this toxin were investigated. Scallop larvae incorporated DA continuously during the larval culture period and accumulated a maximum DA level of 5.21 pg ind^{-1} when exposed to a solution of 50 ng ml^{-1} dissolved DA. As a result of the DA treatment, larval growth, measured in terms of shell length and the appearance of the eye-spot, and larval survival were significantly compromised. This is the first study on DA incorporation dynamics in *P. maximus* larvae, signifying the potential of using shellfish larvae for the study on mechanisms of phycotoxin accumulation. The negative effect of DA exposure suggests that this toxin could possibly influence natural recruitment in *P. maximus*, and it may be necessary to protect hatchery-cultured scallop larvae from DA during toxic *Pseudo-nitzschia* blooms.

Keywords: King scallop; Larvae; Domoic acid; Development; Survivorship

1. Introduction

Domoic acid (DA) is a potent neurotoxin that can cause illness and memory loss or Amnesic Shellfish Poisoning (ASP) in people who consume DA contaminated shellfish (Todd, 1993; Bates et al., 1989). This phycotoxin is produced by certain species of the marine diatom genus *Pseudo-nitzschia*, and can be accumulated in shellfish that feed on them. A safety limit of 20 mg DA kg^{-1} of shellfish tissue has been imposed to protect the consumer. Several shellfish species caught in the wild have been found to contain DA, and high levels are often found in *Pecten maximus* (Gallacher et al., 2001; Hess et al., 2001).

P. maximus is commercially important in Europe where around 49,000 t (FIGIS, 2001) are landed annually of which 600 t are farmed. Both Scotland and France are major producers. Since 1999, continuous restrictions on harvesting *P. maximus* in Scotland due to high DA contamination have drawn research attention to this species (Gallacher et al., 2001). It was found that *P. maximus* can accumulate DA to a high level and harbour the toxin in its tissue for a long time period (Campbell et al., 2001; Blanco et al., 2002).

There is no evidence that harbouring DA imposes any negative effect on the biology or physiology of *P. maximus*. However, some marine species seem to be sensitive to phycotoxins during their early developmental stage. For example, exposure to *Alexandrium tamarense* cells or cellular fragments decreased egg hatching and larval survivorship of *Chlamys farreri* (Yan et al., 2001); exposure to *A. tamarense* at a cell density of 10,000 cells ml^{-1} negatively affected larval development of *Argopecten irradians concentricus* (Yan et al., 2003); the toxic dinoflagellate, Karenia brevis, also induced larval mortality and affected juvenile feeding in the bay scallop *A. irradians* (Leverone and Blake, 2003); morphological abnormalities and sensorimotor defects were found in larval fish exposed to dis-

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solved saxitoxin (Lefebvre et al., 2004); and organic extracts of the green seaweed *Caulerpa taxifolia* affected survival and development of sea urchin larvae *Paracentrotus lividus* (Pedrotti and Lemée, 1999). Phycotoxins produced by harmful algal blooms were also found to be one of the major reasons for mass mortality in cultivated molluscs (Pan and Liu, 1990).

In this study the accumulation of DA in *P. maximus* larvae exposed to dissolved DA and the impact of harbouring the ASP toxin on larval development and survivorship were investigated. The discovery of negative effects on the larvae could have profound implications for fishery recruitment and resource management. The study provides an insight into the mechanism of phycotoxin accumulation in shellfish, and for the first time investigates the relationship between Amnesic Shellfish Poisoning (ASP) and *P. maximus* larvae.

2. Materials and methods

2.1. Aim of experiment

The aims of the experiment were to study possible DA accumulation in scallop larvae and to investigate any negative effect of dissolved DA on larval development.

2.2. Materials

Broodstock *P. maximus* of 9.8–13.2 cm shell height were collected by SCUBA diving from a depth of 8–14 m in Loch Melfort on the west coast of Scotland on 8 March 2006 and transported for 1.5 h to the Scottish Association for Marine Science (SAMS) Laboratory. All broodstock were kept in 1.5 m^3 circular tanks, with a flow-through sea water system, at a density of 15 individuals per tank for 68 d until spawning. The broodstock were fed with a formulated feed (Spat 1, INVE Aquaculture Nutrition, Belgium) or live microalgae (*Isochrysis* sp. and/or *Pavlova lutheri*) once every 2 d, at a daily ration of 6000 cells (or particles) ml^{-1}. This maintenance diet was used as these wild caught broodstock had already achieved good gonad development, hence the feeding ratio was lower than the 3% dry weight d^{-1} for broodstock conditioning in Millican (1997). Aeration was increased to keep the formulated feed suspended and water flow was stopped for 6 h after each feeding, so that a maximum of feed particles/microalgal cells were filtered by the scallops. The seawater was sand-filtered before passing into the tanks, and the ambient water temperature was between 10 and 12 °C.

Purified crystalline domoic acid ($C_{15}H_{21}NO_6$) was purchased from Sigma–Aldrich Company. ASP direct cELISA kits were produced by Biosense Laboratories, Norway. Microplates from the ELISA test were read on an EL 340 Bio-kinetics microplate reader fitted with a 450 nm filter.

2.3. Methods

2.3.1. General methods

Broodstock were induced to spawn by exposure to air followed by return to seawater and a temperature shock (+4.0 °C) and the eggs artificially fertilized as adapted from Millican (1997). Three scallops spawned on 15 May 2006. Fertilized eggs were collected in 60 l bins and allowed to hatch at a density of 50 individuals ml^{-1}.

After 48 h, D-larvae were transferred to 5 l tanks at an initial density of 5 ind. ml^{-1}. Nine tanks were divided into three treatment groups, and there were three replicates for each treatment. Larvae in each treatment group were cultured in 0, 30, 50 ng ml^{-1} DA solution, and therefore referred to as Control, DA30 and DA50 treatments. These DA solutions were prepared by adding 0, 1.5 or 2.5 ml DA stock solution into each tank filled with 5000 ml seawater, before D-larvae were placed into the tanks and when the seawater was exchanged. The DA stock solution, with a DA concentration of 100 μg ml^{-1}, was prepared by dissolving 5 mg DA in 50 ml Millipore water. The stock solution was kept in a light-proof glass bottle, and stored at 4 °C. DA in the stock solution could be kept stable for a long time, while any decrease in the DA level in the treatment seawater would have been expected to be minimal over the 12 d after the addition of the stock solution (Bates et al., 2003).

Larvae in all treatments were fed with microalgae once a day. *Isochrysis* sp. (Butcher) (Culture Collection of Algae and Protozoa (CCAP) strain number 927/12) was fed to the larvae at a daily ration of 2000 cells ml^{-1} during days 2–23 after fertilization. *Pavlova lutheri* (Droop) Green, 1975 (CCAP 931/1) was fed to the larvae at a daily ration of 2000 cells ml^{-1} during days 12–23 after fertilization. This feeding rate was comparable to that used by Liu et al. (2006) and, although lower than that of Robert et al. (1996) and Millican (1997), was adequate for normal development. Both microalgal species were cultured in Guillard's f/2 medium (Guillard and Ryther, 1962), and harvested for feeding the larvae when in the exponential growth phase.

Tanks were cleaned every 3 d. During cleaning the water and larvae were kept in another container. The water was changed once during the larval culture process (at 12 d after fertilization). No aeration was provided. Five micron filtered seawater was exposed to UV light (two 30 W light tubes) to reduce bacterial numbers before being used during spawning, egg hatching and larval culture. The room air temperature was controlled to 16 °C. Irradiance condition was controlled at ca. 15–24 μmol m^{-2} s^{-1} under a 12 h light/dark regime.

2.3.2. ELISA test for larval DA content

Three replicates of 1500 fertilized eggs were collected immediately after fertilization. Three replicates of 500 newly hatched D-larvae were sampled 48 h after fertilization. Starting from day 5 after fertilization, 1000 larvae were sampled every 5 d from each tank by filtering an adequate amount of seawater (calculated according to the larval density on the day of sampling) in the respective tank through a 45 μm mesh. Each larval sample was soaked in fresh 5 μm filtered seawater for 10 min and washed repeatedly, with the aim of eliminating any external DA, before being collected in the extraction solvent (sea below). The final samples were collected on day 25, and immediately before the observation of the start of settlement in some of the larvae, which had the foot projecting and were seeking attachment surfaces.