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Can ecological history influence immunomarker responses and antioxidant enzyme activities in bivalves that have been experimentally exposed to contaminants? A new subject for discussion in "eco-immunology" studies

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ABSTRACT

Numerous studies have demonstrated that environmental parameters affect bivalve immunomarkers. In the present study, we tested the hypothesis that clams (*Venerupis philippinarum*) collected in sites with different environmental conditions respond differently to experimental contaminant exposure. Clams were collected at two sites within the Lagoon of Venice that are influenced differently by both anthropogenic impact and natural conditions: Marghera, which is characterised by relatively high contamination levels and restricted clam fishing, and Chioggia, which is inside a licensed clam culture area that is characterised by lower contamination levels. Total haemocyte count, haemocyte diameter and volume, lysozyme activity in both haemocyte lysate and cell-free haemolymph, superoxide dismutase and catalase activities in gills and digestive glands were measured at time 0 (clam sampling time), after 7 days of acclimation in the laboratory and after 1, 3 and 7 days of copper exposure. Interestingly, statistical analyses (three-way ANOVA and Canonical Correlation Analysis) revealed persistent differences in the biological responses of clams from the two sampling sites before and after copper exposure. Conversely, the influence of copper on cellular and biochemical parameters was negligible. Overall, the results obtained indicated that animals with a different ecological history respond differently to experimental contaminant exposure. In addition, this study suggested that immunomarkers and other biomarkers might be used to determine the origin of fishing products.

1. Introduction

Numerous studies have demonstrated that environmental factor variations such as temperature, salinity, oxygen, nutrients, and contaminants can strongly affect immune parameters in bivalve molluscs. In this context, immunomarkers have been proposed to be sensitive tools in eco-immunology studies to detect signs of impaired bivalve health [1–5].

However, interpretation of the results obtained in laboratory-controlled conditions is often difficult because of the lack of a clear relationship between experimental conditions tested and bivalve immunomarker responses [6,7]. Donaghy et al. [8] proposed three hypotheses to explain inconsistencies between what is expected and what is observed in eco-immunology studies, and these were reported in their manuscript as follows:

(i) haemocyte parameters measured, methods used, and/or experimental timing might not be the most relevant;
(ii) high inter-individual variation might hide potential effects, therefore not allowing demonstration of statistically significant contrasts;
(iii) haemocytes might not be as sensitive to environmental variations because haemocytes maintain bivalve homoeostasis and integrity.

We have one additional hypothesis to explain these unexpected results that is related to the ecological history of the organisms. We hypothesise that animals experiencing different environmental

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field conditions at length respond differently to contaminant exposure under laboratory-controlled conditions. In this context, surprising results have also been recorded in the environment, where variations in environmental parameters (mainly temperature, salinity and oxygen) can strongly influence biological responses in the animals. For example, we recently demonstrated that peculiar environmental features of two sampling sites within the Lagoon of Venice (a landward site and a seaward site close to the Lagoon inlet) rather than pollutants (sediment contamination levels were similar at the two sites) influenced biomarker and immunomarker responses in the clam *Ruditapes philippinarum* [7], now *Venerupis philippinarum*.

To test our hypothesis regarding the influence of ecological history on bivalve biomarkers in this study, *V. philippinarum* were collected at two different sites within the Lagoon of Venice: Marghera, which is close to the inner Lagoon border and is characterised by relatively high industrial contamination levels where clam fishing is restricted, and Chioggia, a licensed area for clam culture that is close to the Cà Roman lagoon inlet that is characterised by low contamination levels [9,10]. Clams were then acclimated in the laboratory for 7 days, which is generally applied as an acclimation period in ecotoxicological studies before copper exposure for 7 more days. Copper was chosen as a model contaminant because it alters bivalve haemocyte parameters and antioxidant enzyme activities [11–18]. Total haemocyte count, haemocyte diameter and volume, lysozyme activity in both haemocyte lysate (HL) and cell-free haemolymph (CFH) were selected as immunomarkers. In addition, superoxide dismutase (SOD) and catalase (CAT) activities in both gills and digestive glands were measured.

2. Materials and methods

2.1. Experimental plan

The experimental plan is depicted in Fig. 1. At least 250 clams with a mean shell length of 3.7 ± 0.4 cm were collected per site in January 2012 from two sites within the Lagoon of Venice. The clams were collected from Marghera, a polluted area (45°24′36″ latitude N, 12°15′12″ longitude E, 8.8 °C, 28.39 psu salinity and pH 7.68) and Chioggia, a reference site (45°15′ latitude N, 12°16.4′ longitude E, 9 °C, 32 psu salinity and pH 8.3).

The experiments were performed during the winter to avoid periods of sexual maturity for the clams, which avoided spawning and reduced possible additional stress during the experiments. After sampling, clams were transferred to the laboratory in refrigerated boxes. Thirty clams per site were immediately used (T0) for tissue collection, whereas the remaining clams were kept in the laboratory for 7 days in two different tanks with a sandy bottom and aerated seawater (31 ± 1 psu salinity, 8 ± 0.5 °C, 8.1 pH) and were fed microalgae (*Isochrysis galbana*). The seawater was renewed every other day. After the 7-day acclimation period (T0 accl.), 30 clams per tank (=site) were used for tissue collection, whereas the remaining clams were exposed to 50 μg Cu l^{-1} (as $CuSO_4$) for 7 more days, which is an environmentally realistic concentration [10,18]. In this case, the clams were maintained in glass aquaria (without sediment) containing aerated seawater (1 l per animal). The seawater was changed daily, and copper and microalgae (*I. galbana* at an initial concentration of approximately 150,000 cells l^{-1}) were added.

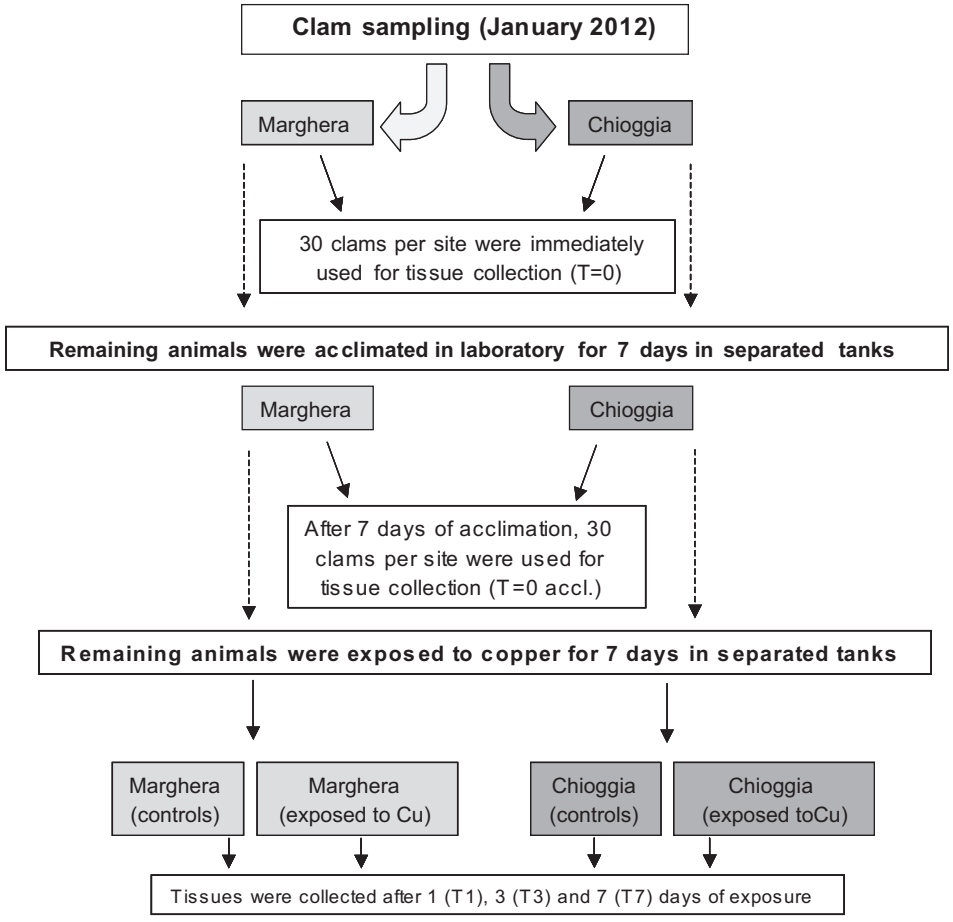

Fig. 1. The experimental plan. See M&M section for details.