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Short communication

Spawning impact on lysosomal stability of the Pacific Oyster, *Crassostrea gigas*

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Abstract

The impact of spawning on the circulating hemocyte density and neutral red retention (NRR) time of lysosome in hemocytes of the Pacific Oyster, *Crassostrea gigas*, were assessed. Significant decreases were observed in both hemocyte density and NRR time in spawned oysters, which could aggravate the health of the oysters. These conditions may, therefore, facilitate summer mortality through increased infection with parasites and pathogens and decreased tolerance to environmental conditions.

Keywords: *Crassostrea gigas*; Hemocyte; Neutral red retention time; Spawning

1. Introduction

Since serious losses of Pacific Oysters were reported from Japanese oyster culture locations (Koganezawa, 1974), summer mortality has threatened the production of oyster farms (Lipovsky and Chew, 1972; Glude, 1975; Mori, 1979; Beattie et al., 1980; Perdue et al., 1981). Although several possible hypotheses have been taken into account to explain these phenomena, e.g., bacterial infection after general weakness (Lipovsky and Chew, 1972), increase of viral infection following temperature elevation (Renault et al., 1994), genetic differences of resistances to the mortality (Beattie et al., 1980) and impact of physico-chemical aspects of seawater in culture area and cultural practices (Caceres-Martinez et al., 1998; Cheney et al., 2000) etc., the cause of summer mortality is still uncertain.

The defense systems of marine bivalves are affected by several factors (Anderson, 1993; Roch, 1999), such as alteration of environmental condition (Fisher and Newell, 1986; Fisher, 1988; Fisher and Tamplin, 1998; Genthner et al., 1999), exposure to xenobiotics (Auffret and Oubella, 1997; Baier-Anderson and Anderson, 1997), toxins from possible food organism sources (Jones et al., 1995) and infection with pathogens and parasites (Ford and Ashton-Alcox, 1998). Among the cellular organelles in marine animals, lysosomes play an important role in defense system against many biotic and abiotic

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stresses, e.g., digestion, reproduction, cell repair and immune response to environmental stress (Camus et al., 2000; Hauton et al., 1998), chemicals (Fernley et al., 2000; Nasci et al., 1998; Lowe et al., 1995a) and metals (Shepard and Bradley, 2000). Domouhtsidou and Dimitriadis (2001) demonstrated that lysosomal and lipid alteration in the digestive gland of mussels, *Mytilus galloprovincialis* (L.) could be used as biomarkers of environmental stress. Also, Hauton et al. (1998) demonstrated that neutral red retention time (NRR) of lysosome in hemocytes of *Ostrea edulis* was highly affected by variation in salinity and water temperature. These environmental stresses can contribute to deleterious structural changes in the lysosomal membrane (Schneider et al., 1984). A possible mechanism postulated by Lowe et al. (1992) was impairment of the lysosomal proton pump. A failure or impairment of membrane Mg^{2+} ATP-dependent H^{+} ion proton pump would lead to a marked increase of the intralysosomal pH and result in a state of equilibrium between two sides of the membrane and free passage of the lysosomal contents (Ohkuma et al., 1982). Thus, structural changes to lysosomal membranes could be a threatening challenge for cell integrity and function.

A few references are available for the seasonal variation of immune-related activities in marine bivalves (Ringwood et al., 2002; Weinstein, 1995; Fisher et al., 1996a, b). But little information is available on the cost of spawning to the immune system, especially in the oyster. Considering the periodical coincidence between summer mortality and spawning period, it has been suspected that spawning is, to some extent, responsible for the summer mortality of farmed oysters.

The objective of this study was, therefore, to investigate the impact of spawning activity on the lysosomal stability using a NRR assay in the Pacific Oyster, *Crassostrea gigas*, and to assess the suitability of this assay as a biomarker for health state.

2. Materials and methods

2.1. Oyster collection and processing

Fully ripened oysters of a similar size (70–80 mm in shell height) were collected from an oyster farm and transported to the laboratory in June 2002. The oysters were acclimated for 2 days to laboratory conditions in a clear plastic tank filled with seawater filtered to 1 μm at 22 °C and aerated. The oysters were fed spray-dried marine phytoplankton (E.S.V. Brooklyn, NY, USA).

After sampling the control (unspawned) oysters, spawning induction was carried out by the introduction of a gamete concentrate collected from other ripe oysters, regardless of sexes. The induced oysters were continually monitored and spawned oysters were immediately removed from the tank and sampled. Oysters that did not spawn were discarded. Control (unspawned) and spawned oysters were sampled by shucking the right valve and removing approximately 0.5ml hemolymph per oyster from the pericardial cavity with syringes pre-washed with temperature adjusted (4 °C) saline (Hauton et al., 2000). The wet and dry weight of the soft tissue was then determined. Because it was difficult to remove hemolymph from the ripe oyster without also getting gametes, one drop of the hemolymph was immediately examined for gametes under the light microscope (Olympus BX-50). If contamination by gametes was detected, the sample was discarded. After confirming that the sample was free of gametes, the hemocytes were further analysed.

2.2. Circulating hemocyte density

The hemolymph was gently mixed to ensure homogeneity and then the density of hemocytes was determined by counting the cells in duplicate sample with a hemocytometer.

2.3. Neutral red retention (NRR) assay

An aliquot (50 μl) of each hemolymph sample was placed on a poly-L-lysine (Sigma Diagnostics, St. Louis, USA)-coated glass slide and incubated in a light proof moisture chamber for 30 min to allow the cells to attach on the slide. Excess hemolymph was gently removed and then 40 μl of neutral red (Sigma Chemical, St. Louis, USA) working solution was added (Lowe et al., 1995a,b). The cells were incubated in the chamber at room temperature for 15 min and examined under the light microscope (total