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ScienceDirect
Aquaculture 264 (2007) 82–91
Aquaculture
www.elsevier.com/locate/aqua-online

Effect of starvation on biological factors related to immunological defence in the Sydney rock oyster (*Saccostrea glomerata*)

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Received 13 September 2006; received in revised form 5 December 2006; accepted 21 December 2006

Abstract

The effects of starvation on various factors related to immunological defence in Sydney rock oysters (*Saccostrea glomerata*) were tested in controlled laboratory experiments. A range of parameters was assessed, including total haemocyte counts, and phenoloxidase (PO), superoxide, acid phosphatase and peroxidase activities. Condition indices were also monitored as an indicator of oyster fitness. We found that the frequency of haemocytes and phenoloxidase activity decreased by 25% and 14% respectively when oysters were fed half-satiation rations for three weeks. These decreases became statistically significant when oysters were starved for three weeks. Superoxide and peroxidase activities also decreased significantly when oysters were starved for two to four weeks, while phenoloxidase and acid phosphatase activities were significantly inhibited after oysters had been starved for at least three weeks. All of the immunological parameters returned to at least their original levels after starved oysters were fed to satiation for six days. Phenoloxidase activities over-compensated during the recovery response, so that levels post-recovery were substantially higher than those evident before starvation. There were no significant changes in condition indices during any of the starvation treatments. These data indicate that starvation compromises immunological activity, and that the duration and intensity of starvation differentially affect immune responses.

Keywords: Oyster; *Saccostrea glomerata*; Immunological response; Starvation

1. Introduction

Outbreaks of infectious QX disease among Sydney rock oysters (*Saccostrea glomerata*) on Australia's east coast have devastated some oyster growing areas over the past thirty years. The common occurrence of the causative agent for QX disease, *Martelia sydneyi*, in many estuaries is contrasted by the sporadic geographic and temporal distribution of QX epizootics. This suggests that other factors such as environmental conditions affect the susceptibility of oysters to QX disease. A number of environmental changes affect oyster immune function (Hegaret et al., 2004; Gagnaire et al., 2006). By impairing the immune system, environmental stressors may leave oysters vulnerable to protozoan pathogens like *M. sydneyi* (Anderson et al., 1996; Fisher et al., 1999). Stressors that have been shown to affect immunological activity include mechanical disturbance (Lacoste et al., 2002; Ballarin et al., 2003), salinity (Fisher et al., 1987), temperature (Soudant et al., 2004) and chemical pollutants (Pipe

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doi:10.1016/j.aquaculture.2006.12.031

and Coles, 1995; Oliver et al., 2001; Chu et al., 2002). We have shown that low salinity inhibits immunological activity in *S. glomerata*, specifically phenoloxidase activity which decreases significantly when salinity falls below 20 ppt (Butt et al., 2006). However, low salinity is not characteristic of all QX disease outbreaks, and the contribution of other potential stressful factors, like starvation, remains poorly understood.

Most work on poor nutrition in marine invertebrates has focused on the effects of dietary limits on growth in aquaculture systems. For instance, dredge oyster (*Ostrea chilensis*) larvae and spat whose parents had been starved exhibited significantly higher mortality rates than the offspring of fully fed parents (Wilson et al., 1996). Similarly, starvation in juvenile flat oysters, *Ostrea edulis,* and the abalone, *Haliotis discus hannai*, affects energy reserves and limits subsequent growth and development (Millar and Scott, 1967; Du and Mai, 2004).

The effects of starvation on immune function have only been investigated recently. Eastern oysters (*Crassostrea virginica*) maintained on nutritionally deficient diets had significantly lower phagocytic activities and hyalinocyte viabilities than oysters fed on algae of a high nutritional value. These effects on haemocyte activities were compounded when nutritional deficiencies were combined with high-temperature shocks (Hegaret et al., 2004). Haemocyte activities, including circulating haemocyte frequencies and phagocytic and oxidative activities, were similarly affected by dietary restrictions in Pacific oysters, *Crassostrea gigas*, and the clam, *Ruditapes philippinarum* (Delaporte et al., 2003, 2006). Zhang and Li (2006) showed that *C. gigas* starved for 42 days had significantly lower mean condition indices and haemocyte neutral red retention (NRR) times than fed oysters. NRR time is an indicator of lysosomal membrane integrity, a crucial component of cellular immune defence.

Many measures of immunological activity have been used to assess the potential susceptibility of molluscs to invasive pathogens. Here, we test a broad range of indicators, including total haemocyte counts (THC) and phenoloxidase, superoxide, acid phosphatase and peroxidase activities, as well as condition indices. The number of circulating haemocytes provides a measure of cellular defensive capacity. Phenoloxidase is an enzyme that catalyses a cascade of reactions to form the defensive pigment melanin, creating numerous fungistatic and antibacterial intermediaries in the process (Soderhall and Cerenius, 1998; Sorrentino et al., 2002; Decker and Jaenicke, 2004). Superoxide and peroxide are enzymatically generated reactive oxygen species involved in cytotoxic respiratory bursts associated with phagocytic activity (Bramble and Anderson, 1997; Anderson, 1999; Whitten and Ratcliffe, 1999), while acid phosphatase is a key lysosomal enzyme that is also induced by phagocytosis (Xia et al., 2000; Chen et al., 2007; Jing et al., 2006b). Condition indices are a common measure of oyster fitness (Smith et al., 2000).

Our interest in the effects of starvation on immunological function in *S. glomerata* comes from anecdotal evidence that a recent outbreak of QX disease in the Hawkesbury River, NSW, was associated with unusually low algal loads in the river. Here we report on laboratory trials that were used to determine the effects of dietary restriction on biological factors related to immunological defence in *S. glomerata*. Understanding these effects will assist future efforts to monitor *S. glomerata*'s susceptibility to disease.

2. Materials and methods

2.1. Experimental design

Two trials were carried out to monitor the effects of starvation on immunological activity. The initial trial was conducted at the New South Wales Department of Primary Industries (NSW DPI) Port Stephens Fisheries Centre using 'Bistro' grade oysters, between 50 mm and 60 mm in length. The oysters were collected from Port Stephens, NSW, Australia. Oysters were acclimated in a recirculating conditioning system at 24 °C for one week. A random sample of ten oysters was then used to estimate a satiation feed rate. These oysters were held without feed for 24 h. Each oyster was then placed in a separate 10 L aquarium with 1×10^5 algal (Tahitian *Isochrysis*, clone T-ISO) cells mL^{-1}. The oysters were then left to feed for 4 h. The number of algal cells remaining after this time was estimated microscopically using an Improved Neubauer haemocytometer. The maximum algal cell consumption rate over 24 h was estimated to be 5.94×10^9 algal cells per oyster per day.

After determining the satiation feed rate, all remaining oysters were allocated to one of three different treatments. In the first treatment oysters were fed a mixed diet of T-ISO, *Pavlova lutheri* and *Chaetoceros muelleri* at the satiation feed rate. The second treatment included oysters fed the same diet at half of the satiation ration (2.97×10^9 cells day^{-1}), while the third treatment incorporated oysters maintained in 1 μm filtered seawater without algae. This final treatment severely restricted nutrition. Aquaria were maintained at 24 °C and 35 ppt seawater for the duration of the experiment. Each treatment used three replicate aquaria, each