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Effects of severe heat stress on immune function, biochemistry and histopathology in farmed Australian abalone (hybrid *Haliotis laevigata* × *Haliotis rubra*)

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ABSTRACT

High summer temperatures are one of the most stressful environmental problems confronted by the abalone mariculture industry and are commonly associated with outbreaks of infectious disease. We tested the effect of extreme but non-lethal elevated temperatures on abalone immunology, biochemistry and quantitative histology. We subsequently compared the haemolymph results to the histology to gain increased understanding of how heat stress impacts abalone health. Abalone were kept in water that was heated from the ambient 16 °C temperature to 26 °C within 5 h and then held at 26 °C for one week to determine the effects of this acute heat stress on the day of temperature elevation and whether there was acclimatization or deterioration 2 and 7 days later. Antibacterial activity, phenoloxidase activity and neutral red retention times declined significantly with heat and did not recover. The total haemocyte count was elevated significantly during heat stress and was highest on day 1. The phagocytic rate was elevated on day 1 but had recovered by the following day. Acid phosphatase activity, leucine aminopeptidase, haemolymph protein and haemolymph electrolytes (calcium, phosphate, magnesium, sodium, and chloride) were not significantly affected by heat stress. This indicates that severe heat stress causes changes in some, but not all haemolymph parameters. The sublethal immunologic effects seen in haemolymph samples occurred concurrently with histological changes. The digestive gland had significantly increased haemocyte infiltrates in heat stressed abalone. Heat stressed abalone had significantly greater loss of epithelium lining from the gills, with no recovery. The gill goblet cell numbers declined significantly on day 2 and had recovered by day 7. There was no significant change in the volume of fluid or protein concentration of the haemolymph in the gill sinuses between treatment groups. These results indicate that immunosuppression and organ damage are likely to be involved in the increased incidence of bacterial disease reported by abalone farmers during summer.

1. Introduction

Abalone aquaculture is an intensive industry, which is often land-based, with tanks that are stocked at high density. Due to more crowded conditions than in the wild (Goodsell et al., 2006; Shepherd, 1973) and suboptimal environments, abalone in these systems are vulnerable to infection by microorganisms that are often ubiquitous (Handlinger et al., 2005). A link between stress and immunosuppression has been established in abalone, associated with increased susceptibility to disease outbreaks (Cheng et al., 2004a; Hooper et al., 2007a; Wassnig et al., 2009), including outbreaks of vibriosis associated with sharp rises in water temperature during the summer months (Cheng et al., 2004a; Handlinger et al., 2005; Reuter and McOrist, 1999; Travers et al., 2008, 2009).

It would be very useful to the industry to be able to predict periods of heightened stress on-farm that could lead to immunosuppression and disease outbreak. Currently disease outbreaks are investigated after they have occurred. Most diagnostic laboratory work in abalone is based on histopathology and microbiology, either in routine surveillance work to identify problems on a farm or in investigating the cause of disease outbreaks (Handlinger et al., 2006; Hooper et al., 2007b; Mouton, 2003; Pitcher et al., 2001). Prior to a disease outbreak, methods are needed that will reveal stress sufficient to cause immunosuppression

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and thus predispose abalone to opportunistic bacterial infections. Two approaches to this question are studied here with respect to the effects of severe heat stress; haemolymph immune assays and quantitative histology.

In vitro assays done on haemolymph that assess immune function in abalone provide mechanisms for assessing their health status and potential disease resilience (Dang et al., 2011; Hooper et al., 2007a; Travers et al., 2008). Haemolymph sampling via the pedal sinus is a non-lethal method to examine stock and some assays such as haemocyte counts can be done rapidly on the farm (Hooper et al., 2012). There has been a recent body of work on the effect of heat stress on various immune and physiologic parameters in abalone. Haemocyte counts were reported to rise quickly with sudden increased water temperature and then decline as they acclimatized (Dang et al., 2012). Lysosomal membrane stability (NRR assay) decreased with a sudden decline or elevation from the temperature to which the stock had been acclimatized (Wang et al., 2006). Phenoloxidase and phagocytic rates declined after spawning during small temperature fluxes in summer (Travers et al., 2008), but it was not clear what direct effect water temperature had on these immune parameters, relative to spawning stress. Antibacterial activity elevated transiently with heat stress and then declined below baseline levels (Dang et al., 2012). Leucine aminopeptidase (LAP), which is modified by bacterial disease (Paillard et al., 2004), was not affected by temperature (Travers et al., 2008). There have been no reports of the effect of temperature on acid phosphatase (ACP) in abalone. Significant elevation of ACP in abalone and other molluscs infected with bacteria has been found, indicating that it is likely to be important in abalone immunity (Cong et al., 2008; Wang et al., 2004).

The effect of thermal stress has not been tested on haemolymph electrolytes in abalone. Furthermore, there have been only a few reports on environmental stress and osmoregulation in abalone, including decreased water pH (Burke et al., 2001), increased water nitrite concentrations (Harris et al., 1998), increased water salinity (Cheng et al., 2002), and low dissolved oxygen (Cheng et al., 2004b) and these have shown that different stressors have different effects on haemolymph electrolytes.

Histopathology is an essential component of investigating disease outbreaks and knowledge of histological changes seen with a common stressor such as heat is needed. Knowledge of the lesions of heat stress aids in differentiation of changes seen with infectious agents, feed problems, toxic diseases and other causes, from those due solely to heat stress. The reports that describe microscopic lesions associated with environmental stressors include work on transport stress, nutritional stress, overstocking (Mouton, 2003) and increased nitrate in the water (Harris et al., 1998). There are a few reports on the effect of heat stress on histology (Braid et al., 2005; Schaefer et al., 2013; Travers et al., 2008). Heat stress alone leads to digestive gland atrophy in a study extending over one year (Braid et al., 2005). Elevated heat had varying effects on different levels of crop and stomach epithelium, with none of the histological changes noted being associated with altered growth rates (Schaefer et al., 2013).

There is a body of work comparing immune or metabolism assays in haemolymph or tissues with histological changes in key organs in abalone such as gill and digestive gland (Hooper et al., 2011, 2012; Rosenblum et al., 2005, 2006; Zhou et al., 2010) but only Travers et al. (2008) studied the effect of heat stress on histologic changes and compared these with immune assays on haemolymph. Reports on abalone histology that are quantitative (Braid et al., 2005; Friedman et al., 2003; Harris et al., 1999; Moore et al., 2000; Rosenblum et al., 2005, 2006; Vilchis et al., 2005) rather than descriptive, have indicated that scoring allows statistical analysis of the results leading to increased information gained.

Farm records show that the shallow on-farm tanks from which the abalone were sourced, experience relatively rapid temperature fluctuations, hence the rapid elevation of water temperature selected for this experiment of 10 °C within 5 h. The only study of the critical thermal maximum temperature (CTM) or upper lethal temperature in Australian abalone found that 50% of *Haliotis rubra* and *Haliotis laevigata* died at temperatures of 26.9° and 27.5 °C, respectively (Gilroy and Edwards, 1998). The preferred temperatures for these two species were 16.9 °C and 18.9 °C, respectively. Ectotherms acclimatized to higher temperatures will tolerate higher temperature elevations than those acclimatized to a lower temperature (Becker and Genoway, 1979; Cheng et al., 2004a; Portner, 2002). The acclimatization of molluscs to elevated temperatures involves altering their metabolic rate and can occur within 5–8 days (Peck et al., 2002). Water temperatures of 26 °C have been recorded in land based abalone farms in some parts of Australia and temperatures of 24°–25 °C are fairly commonly recorded during summer. Farmers in these locations have reported elevated mortality rates in previous years when the water flowing through their tanks reached at least 24 °C for several consecutive days. In this experiment, abalone were kept in water heated from the ambient temperature of 16 °C to 26 °C for 7 days. The upper temperature and the rapidity of temperature elevation used were slightly above reported temperatures and were selected to produce a severe but non-lethal stress.

The effect of rising water temperature on farms is likely to be complex, involving not only the effect of heat on metabolism but also environmental decline. Increased biofouling of pipes, decreased water quality in the farm tanks, increased bacterial growth rates and up-regulation of the expression of bacterial virulence factors such as adhesins are all affected by rising temperatures (Cheng et al., 2004a; Parveen et al., 2008; Rosenberg and Falkovitz, 2004; Toren et al., 1998). In this experiment we imposed heat stress (and the consequent lower dissolved oxygen) in a controlled laboratory environment that excluded these other potential confounding factors occurring on-farm. This experiment investigated whether abalone immunity, biochemistry and histology were affected by sudden, severe temperature elevation and whether there was acclimation or deterioration over seven days of continued high water temperature.

2. Methods

2.1. Abalone

Juvenile abalone used in this study were two year old hybrids of male *H. laevigata* × female *H. rubra*, sourced from a Victorian land-based farm. Hybrid abalone are commonly farmed in Australia and the work is thus relevant to commercial farmers of this species.

The abalone were acclimatized for two weeks at 16 °C, in 12 tanks that contained 8 l of water and eight abalone. The water was recirculated: after sand biofiltration, seawater was held in an 18,000 l reservoir and passed through a 2 mm mesh filter. The flow rate through each replicate tank was 10 l/h throughout the experiment.

Treatments were applied independently to each tank. Each tank was saturated with oxygen by delivering air from a compressor via a plastic airline to each tank, with the airline opening at the bottom of the tank. The temperature increase will lead to a reduction in dissolved oxygen, but this is what would happen on-farm in hotter conditions, so that heat stress is regarded as involving the effects on abalone of these combined changes. Feeding ceased the day before the experiment in both control and treated tanks and all food and faecal debris were removed. The tanks were covered to keep out light, except at the actual time of sampling.

On day 1 of the experiment, heating rods were placed in 6 of the tanks and over a period of 5 h, the water temperature was raised from 16 to 26 °C. This temperature was monitored twice daily by a thermometer. Two abalone per tank were sampled on day 1, 5 h after the temperature had been raised to 26 °C, followed by two more abalone on day 2 and again on day 7. The abalone were observed as the temperature rose to document any behavioural changes.