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Histopathology and haemolymph biochemistry following anaesthesia and movement in farmed Australian abalone (*Haliotis rubra* × *Haliotis laevigata*)

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ABSTRACT

Haemolymph and tissue samples of *Haliotis rubra* × *laevigata* hybrid abalone were taken during a routine stock movement procedure on an Australian abalone farm to look for biochemical and histological changes associated with anaesthesia and/or manual movement of the abalone, both of which are used in commercial stock movement operations. Sections of the left kidney, the gill and the surface of the foot were examined under light microscopy and a scoring system was used to measure observed changes and compare treatment groups to controls. The left kidney of anaesthetised abalone contained less protein and haemocytes than control or manually moved abalone on the day of anaesthesia ($p < 0.05$), but returned to baseline levels within 1 day. The foot muscle of anaesthetised and moved abalone had areas of denuded epithelium 5 days after anaesthesia ($p < 0.05$). Protein, phosphate, magnesium, sodium, chloride, calcium and potassium levels were measured in the cell-free haemolymph. The protein levels were significantly elevated compared to controls on the day after anaesthesia and manual movement ($p < 0.05$), but declined to baseline levels within 3 days. There were no significant differences in the other measured ions. Benzocaine anaesthesia, with or without subsequent movement, led to greater histological changes in tissues of abalone, than manual movement without anaesthetic. Comparison of physiological and histopathological changes with haemolymph immune assays may show whether the non-destructive haemolymph sampling can provide early warning of stressors that cause tissue damage. The methods used can be applied to scientifically assess any husbandry procedures with a view to improve stock management and productivity.

1. Introduction

Commercial land based abalone farming requires periodic movement of stock between tanks for such reasons as harvesting, grading into similar sizes and to reduce stocking density (Hooper et al., 2011). Abalone clamp their foot muscle tightly to the tank floor, making them hard to detach. They are removed either mechanically with a spatula (called chipping) or after applying anaesthetic to the tank (Hooper et al., 2011). It is not practical to shift entire tanks containing tens of thousands of stock via chipping, so anaesthetics are used for large scale movement procedures. Chipping is used to move abalone when they are near harvesting size to avoid the problem of residues in the meat. Manual removal can cause physical damage to the foot, leading to subsequent infection (Genade et al., 1988) and is labour intensive.

Many anaesthetics have been evaluated for the purpose of handling abalone, but these studies have concentrated on the effectiveness of the anaesthetic, recovery time, subsequent mortality and growth (Aquilina and Roberts, 2000; Bilbao et al., 2011; Burke et al., 2001; Sharma et al., 2003; White et al., 1996), rather than effects on physiology or tissue damage. Benzocaine dissolved in ethanol is commonly used by farmers to remove abalone from tanks, due to the effectiveness of the anaesthesia, rapid recovery and low mortality (Edwards et al., 2000) and is the anaesthetic method used on the farm where this research was carried out.

Benzocaine (ethyl p-aminobenzoic acid) is a local anaesthetic that works by blocking sodium channels (Pinto et al., 2005; Wang et al., 1997). Benzocaine, like other anaesthetics, leaves residues in the flesh with clearance efficiency dependent on factors such as salinity and water temperature (Chang et al., 2012; Stehly et al., 1998). Levels in

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fish decrease to below detectable levels within 24 h (Allen, 1988), but there are no equivalent studies in molluscs.

Previous investigation into the effects of benzocaine anaesthesia on abalone haemolymph indicated that the procedure results in acute depression of phagocytosis, antibacterial activity and lysosomal neutral red retention time, as well as elevated haemocyte density (Hooper et al., 2011). Observed effects were greatest when abalone were moved after having been anaesthetised, with lesser effects seen with anaesthesia alone. No significant differences were seen when abalone were removed from the tank by chipping when compared to the control group. Recovery generally occurred within one day for all treatments. This suggests a short period of vulnerability to pathogenic infection in abalone after anaesthesia and movement, due to immunosuppression. The potential for more chronic effects of anaesthesia and/or movement on organ systems or homeostasis also requires investigation.

There are only rare reports on the effect of anaesthetics on molluscan physiology. Nembutal anaesthesia in the gastropod *Biomphalaria glabrata* results in lower haemolymph glucose concentrations, with less variation in well-anaesthetised snails versus poorly-anaesthetised snails (Liebsch et al., 1978). The authors speculated that this difference was due to stress of handling on the poorly anaesthetised snails. Benzocaine induced a decline in metabolic rate in abalone (measured as oxygen consumption) that returned to normal after clearance of benzocaine (Chacon et al., 2003). Edwards et al. (2000) reported that chipping of abalone and benzocaine anaesthesia both caused suppression of oxygen uptake, with recovery one day after chipping and 3–5 days after benzocaine anaesthesia. Both chipped and anaesthetised abalone had lower growth rates than control animals, with no significant differences between these two treatments (Edwards et al., 2000). The results of this laboratory study by Edwards et al. (2000) suggest that, although the anaesthetic may have a more profound effect in the acute stage, in the long term it may not be more detrimental to abalone health than chipping. These studies were all done within the laboratory and the situation on farms may differ. The development of opportunistic infections is probably more likely on farms and our previous immune study (Hooper et al., 2011) suggests this is more likely with anaesthesia than with manual chipping. There are no published reports of the effect of benzocaine anaesthesia on histological changes in the tissues, haemolymph protein, nor ion concentrations in invertebrates.

Histopathology is used in disease investigation on abalone farms (Handlinger et al., 2006; Mouton, 2003) and can be used to characterise the lesions present as infectious (Hooper et al., 2007; Moore et al., 2000) or non-infectious (Elston, 1983; Harris et al., 1998). It is important to identify the range of background lesions that can occur in abalone tissues due to husbandry stressors such as anaesthesia and chipping, so that the background lesions associated with farming are not mistaken for lesions due to a disease outbreak. There are a few reports on the effect of on-farm husbandry stressors on abalone histopathology, but these are primarily descriptive rather than quantitative (Elston, 1983; Harris et al., 1998, 1999; Mouton, 2003).

The purpose of this investigation was both to examine the haemolymph biochemistry and quantify histological changes in abalone after anaesthesia and/or manual movement and to compare these with previous results on immune function changes (Hooper et al., 2011). Comparing the physiological and histopathological changes with haemolymph immune assays may show whether the non-destructive haemolymph sampling can provide early warning of stressors that cause tissue damage. One of the main purposes of this paper is to show farmers scientific methods to evaluate their current husbandry methods.

2. Methods

2.1. Animals and experimental design

The experimental design was as described in Hooper et al. (2011). Two year old hybrids of male *Haliotis laevigata* × female *Haliotis rubra*, weighing 30.1 g +/− SE 1.5, (N = 130), were housed in a shed containing 96 concrete flow-through slab tanks 20 m long and 1.8 m wide. Animals were held at a stocking density of 10,000 per tank (8.36 kg/m^2) prior to movement. Three tanks were assigned for each treatment including controls and two abalone sampled from each tank per day. In three control tanks abalone were left undisturbed until they were sampled; in three tanks abalone were chipped; and in three tanks abalone were anaesthetised prior to movement of stock in the routine manner done on this farm. Manual chipping was done using a metal spatula to scoop up the abalone from the tank floor, taking care to avoid cutting the foot. These chipped abalone were tagged with plastic 10 × 3 mm tags attached to the shell with superglue, then left out on a plastic tray for 45 min prior to being replaced into the same tanks. The tanks selected for anaesthesia on the day of movement had the in-flow seawater taps turned off, then were flooded with seawater to which benzocaine had been added (70 g benzocaine in a litre of ethanol per 1000 l of seawater). A fresh mixture was made up prior to anaesthesia of each tank. The abalone were moved once they had ceased adhering to the concrete floor of the tank (approximately at 20–25 min). The farm workers wearing long gloves used their hands to manually sweep these abalone together into small piles and then scoop them up in handfuls and put them into plastic trays. The trays were weighed and then the stock poured from the trays into new tanks or into the same tanks at two thirds of the original stocking density. Thirty of these abalone were randomly sampled from the trays, tagged and replaced into the same three tanks. These abalone were out of the water for approximately 40–50 min. Thirty abalone were deliberately left behind untouched in the tanks and these were tagged where they lay at the time the anaesthetised water was draining out, leaving the shells exposed to air. Fresh seawater, free of anaesthetic, was then pumped back into these tanks, within 20–30 min after the water containing anaesthetic had drained out.

This experiment started the day prior to a routine farm movement procedure (baseline data; day 1). Abalone were subsequently sampled 4 h (day 2), 24 h (day 3) and 72 h (day 5) after treatments. Sampling was standardised across all treatments and controls. Haemolymph was withdrawn from the pedal sinus into a 1 ml sterile plastic syringe with a 26-gauge needle within one minute after an abalone was removed from the tank. Sampled abalone were then euthanized. No abalone were returned to the tanks after sampling and no abalone were sampled more than once.

2.2. Biochemistry

Haemolymph from three groups only were examined; the controls, anaesthesia without movement (A) and chipping (CHIP) treatment groups. No haemolymph was available from the anaesthesia plus movement (A&M) treatment for biochemical analysis. Haemolymph samples were collected into eppendorf tubes, pelleted in a centrifuge (15 min. at 13G) and the cell free plasma stored at −80 °C until being run on a biochemistry multichannel analyzer (Roche Modular SW version 7.5). Abalone samples from healthy farm stock were checked to determine the appropriate equipment settings for each analyte prior to the experiment. Some of the abalone analytes were run on serum settings (calcium and phosphorus) and some were run on urine settings (chloride, potassium, sodium, magnesium), depending on the appropriate range for a given analyte. The within-laboratory precision (coefficient of variation) for each variable was as follows: Serum settings: calcium, 1.5%; phosphorus, 2.7%. For urine settings: chloride 5.6%; potassium 6.3%; sodium 5.5%; calcium 7% and magnesium 7.3%.

2.3. Histopathology

After weighing and taking haemolymph samples, the abalone were euthanized by complete transverse section with a scalpel blade just below the level of the cerebral ganglia. Soft tissues were removed