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Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevigata* Donovan) at high water temperature

David A.J. Stone [a,b,c,*], Matthew S. Bansemer [b], Brett Lange [c], Elise N. Schaefer [b], Gordon S. Howarth [c], James O. Harris [b]

[a] South Australian Research and Development Institute, Aquatic Sciences Centre, Marine Innovation Southern Australia, 2 Hamra Ave, West Beach, 5024 South Australia, Australia
[b] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, 5001 South Australia, Australia
[c] The University of Adelaide, School of Animal and Veterinary Sciences, Roseworthy, 5371 South Australia, Australia

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ABSTRACT

Summer mortality (SM), a disease caused by an interaction between biotic and abiotic environmental factors at high water temperatures (>22 °C), impacts health, growth and mortality (up to 50%) of larger (≥60 mm) cultured abalone in southern Australia. We aimed to determine if dietary intervention could alleviate mortality demonstrated by abalone at high water temperatures. As this issue is most relevant to farm production, we aimed to demonstrate that different mortality patterns are evident for 2- and 3-year-old greenlip abalone (*Haliotis laevigata* Donovan), irrespective of reproductive state, and to provide potential, practical solutions to this issue. Growth rate, feed intake and haemolymph variables were measured. To test if dietary intervention could minimise mortality at high water temperatures, we selected a commercial diet routinely fed on-farm when SM occurred, as a negative survival control, and live macroalgae (*Ulva lactuca* Linnaeus) as a positive survival control. In Experiment 1, 30 mm 2-year-old and 70 mm 3-year-old abalone were subjected to water temperatures of 18, 22 and 26 °C for 36 days. In Experiment 2, 60 mm 3-year-olds were subjected to 22 and 26 °C for 38 days. In Experiment 1, survival was >95% for all treatments at 18 and 22 °C ($P > 0.05$); whereas, survival was significantly reduced by 35% in 3-year-olds fed the commercial diet at 26 °C compared to all other treatments ($P < 0.05$). In Experiment 2, there was no mortality at 22 °C. At 26 °C, survival was significantly reduced by 50% ($P < 0.05$) for the commercial diet, whereas, survival was >97% for the *U. lactuca* diet. We demonstrated that dietary intervention reduced mortality in larger abalone at 26 °C. We also demonstrated a pattern of mortality in response to high water temperatures that differed for age classes. This information is invaluable for further systematic research to alleviate on-farm abalone mortality associated with high summer water temperatures, particularly in the areas of nutritional or therapeutic intervention.

1. Introduction

Greenlip abalone (*Haliotis laevigata* Donovan) in southern Australia are predominantly cultured in land-based systems in water temperatures that can fluctuate from 10 °C in winter to 25 °C in summer (Stone et al., 2013). Abalone cultured in southern Australia spend a large period of the summer months in water temperatures above their preferred optimum. Optimal water temperatures for growth in this species are size- and strain-dependent and have been recently reported to be 22 °C for 25 mm shell length (SL) animals originating from South Australian waters (Stone et al., 2013), whereas 18.9 °C has been reported to be the preferred temperature for 82 mm SL animals originating from the cooler waters of Tasmania (Gilroy and Edwards, 1998).

When water temperatures exceed 22 °C, increased health problems and significant levels of mortality occur on abalone farms in southern Australia. Small (<60 mm SL) abalone typically show little mortality during summer water temperatures. In contrast, mortality patterns with larger (>60 mm SL) 3-year-old stock during these events are typically sporadic between culture units and have been reported to range from 15 to 50% (Dang et al., 2011a; Vandepeer, 2006), although there is no peer-reviewed published information quantifying these levels. Discussions with abalone farm managers indicate that these figures are an accurate representation of the mortality patterns each summer (Australian Abalone Growers' Association, pers. comm.). In Australia, this mortality event observed is referred to as "summer mortality" (SM) (Dang et al., 2011a; Handlinger et al., 2005; Vandepeer, 2006). A similar condition, "summer immune depression", associated with high summer water temperatures, spawning processes, low immune status,

* Corresponding author at: South Australian Research and Development Institute, Aquatic Sciences Centre, Marine Innovation Southern Australia, 2 Hamra Ave, West Beach, 5024 South Australia, Australia. Tel.: +61 8 8207 5350; fax: +61 8 8207 5481.
E-mail address: david.stone@sa.gov.au (D.A.J. Stone).

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and an increased susceptibility to *Vibrio* spp. infections (Nicolas et al., 2002; Travers et al., 2008, 2009a) has been reported in cultured and wild abalone (*H. tuberculata*) in France.

Currently the aetiology of SM in abalone is hypothesised to be related to a complex interaction between the physiology and metabolism of abalone and a range of abiotic and biotic environmental factors at elevated summer water temperatures (Dang et al., 2011a,b; Handlinger et al., 2005; Vandepeer, 2006), contributing to mortalities caused by bacterial infection (Travers et al., 2009a,b). Additionally, as the condition is prevalent in larger 3-year-old stock, maturation and spawning processes may also be involved (Travers et al., 2009a), however, handling stresses are also implicated (Cardinaud et al., 2014). Nutritional factors have also been implicated in the aetiology of SM (Dang et al., 2011b; Vandepeer, 2006).

In Australia, abalone cultured in land-based systems are fed formulated compounded diets composed predominantly of terrestrial plant, and to a much lesser extent, marine ingredients. In contrast, in their natural environment, larger abalone have a distinct dietary preference for macroalgae, particularly species of the red (*Gracilaria* spp.) and green (*Ulva* spp.) taxa (Shepherd, 1973). Macroalgae possess a range of attributes that are beneficial to the environment, and potentially to the organisms consuming them. Benefits include the removal of nitrogenous wastes and carbon dioxide from the environment to produce oxygen, they also contain a range of bioactive compounds that exhibit antimicrobial and antioxidant properties (Abirami and Kowsalya, 2011; Silva et al., 2013; Tierney et al., 2010), that when consumed may enhance the immune response of the abalone (Dang et al., 2011b).

The aim of this study was to determine if dietary intervention could alleviate mortality demonstrated by abalone at high water temperatures. As this issue is most relevant to farm production, we aimed to demonstrate that different mortality patterns are evident for 2- and 3-year-old abalone, irrespective of reproductive state, and to provide potential, practical solutions to this issue.

2. Methods

2.1. Experimental animals

Greenlip abalone, not previously used for any other experiments, were purchased from South Australian Mariculture at Boston Point, Port Lincoln, South Australia. Upon arrival at the SARDI SAASC the abalone were transferred to flow through 5000-L cylindro-conical tank seawater systems, held at ambient photoperiod and water temperatures, and fed a 5 mm commercial abalone diet [Eyre Peninsula Aquafeed Pty Ltd (EPA), Lonsdale, South Australia, Australia] prior to stocking into the experiments. Experiment 1 used 2-year-old [spawned September 2010; initial weight, 5.95 ± 0.01 g abalone^{-1}; shell length (SL), 38.54 ± 0.01 mm; condition factor (CF), 0.60 ± 0.01] and 3-year-old abalone (spawned September 2009; initial weight, 36.27 ± 0.02 g abalone^{-1}; SL, 63.23 ± 0.03 mm; CF, 0.83 ± 0.01). Experiment 2 used 3-year-old abalone (spawned September 2010; initial weight, 26.83 ± 0.63 g abalone^{-1}; SL, 57.07 ± 0.42 mm; CF, 0.81 ± 0.01).

2.2. Experimental design

In this study the interactive effects of water temperatures, abalone sizes, and different diet types on the survival of greenlip abalone were investigated. Both 2-year-old and 3-year-old abalone at 18, 22 and 26 °C fed the EPA 5 mm commercial abalone diet (negative control diet) or the live *Ulva lactuca* Linnaeus (positive control diet) were trialled for 36 days in Experiment 1. To confirm our findings and to provide more information about feed intake and water quality changes, Experiment 2 was run at 22 and 26 °C with only 3-year-old abalone fed the commercial abalone diet (negative control diet) or the *U. lactuca* (positive control diet) for 38 days.

2.3. Experimental system

The temperature (20 ± 1 °C) and photoperiod [12 h low intensity fluorescent lighting at 3.4 lx (equates to dark limit of civil twilight under a clear sky): 12 h dark] controlled experimental facility consisted of identical temperature controlled salt water systems (Exp. 1 used three systems; Exp. 2 used two systems) described in Stone et al. (2013) supplied with 30 μm sand-filtered, UV treated seawater. Each system supplied sixteen (Exp. 1) or six (Exp. 2) 12.5-L blue plastic culture units (Nally IH305, Viscount Plastics Pty Ltd.; length, 39.2 cm; width, 28.8 cm; 11.0 cm depth; bottom surface area of 1129 cm^2). The culture units were each provided with temperature controlled flow-through water from the reservoir by gravity feed at a rate of 300 mL min^{-1}. Water level was set at 5 cm in each culture unit using a standpipe with a mesh screen (0.8 mm nominal mesh size) on the outlet to retain uneaten food.

2.4. Experimental stocking

For Experiment 1, ten 2-year-old, or ten 3-year-old abalone were weighed, measured and stocked, using systematic interspersion, into each of the four replicate culture units per treatment combination. For Experiment 2, twelve 3-year-old abalone were stocked into each of the three replicate culture units per treatment. A one week acclimation period was used to slowly raise the water temperature (~1 °C day^{-1}) to the desired temperatures. For each experiment, dead abalone were recorded, measured, weighed and in an attempt to keep stocking densities equal, replaced with tagged abalone of a similar weight and size that had been held at the same treatment water temperature and fed their respective diets.

2.5. Diets and feeding

The nutrient composition of the diets used in Experiments 1 and 2 is presented in Table 1. *U. lactuca* were collected from the Outer Harbour area of Gulf St Vincent, South Australia and cultured in sand-filtered seawater supplied with aeration in 4000-L parabolic tanks under ambient photoperiod and temperature at SARDI SAASC. In Experiment 1 natural, non-enriched *U. lactuca* was used, whereas, in Experiment 2 the *U. lactuca* was nitrogen enriched using an altered Guillard's f/2 nutrient medium (Guillard, 1975; Guillard and Ryther, 1962) [sodium nitrate component substituted with ammonium chloride (4.75%)] prior to feeding. Previous work in our laboratory has shown that enriched *U. lactuca* provides a superior nutritional source to non-enriched *U. lactuca*, and this represents a refinement of our experimental design. The same 5 mm EPA commercial negative control diet was used in both experiments. Feed rates for the commercial diet were 4.0% body weight day^{-1} (% bw d^{-1}) for the 2-year-olds and up to 1.2% bw d^{-1} for the 3-year-olds in Experiments 1 and 2. *U. lactuca* feed rates were 6.0% bw d^{-1} for Experiment 1 and 2.5% bw d^{-1} for Experiment 2. Rations were based on stocking biomass and adjusted on mortality weight, and were in excess of the animal's daily intake. Feeding was carried out at 4:00 pm daily until the end of each trial. Cleaning and collection of food waste occurred at 8:30 am daily and were done by sieving the entire tank contents through a fine mesh. The wet uneaten feed was weighed and stored frozen at −20 °C. Uneaten commercial diet was dried in an oven at 105 °C for 16 h. Uneaten *U. lactuca* was dried in an oven at 60 °C for 48 h. The proportion of uneaten feed that was lost through leaching and the collection net, without animals in the tank was determined; and the correction factor was used to calculate the corrected apparent feed intake. Feed intake was determined daily over an 8 day period prior to the end of Experiment 1. In Experiment 2, daily feed intake (reported as g kg^{-1} abalone day^{-1}) and the proportion of days where no food consumption occurred in each tank (%) were determined.