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Is the mangrove cockle *Anadara tuberculosa* a candidate for effluent bioremediation? Energy budgets under combined conditions of temperature and salinity

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ABSTRACT

To determine extreme and optimum temperature and salinity for growth in the mangrove cockle *Anadara tuberculosa*, we measured the scope for growth in adult cockles exposed to 16 treatments composed of combinations of four salinities (20, 30, 40, and 50 psu) and four water temperatures (23, 26, 29, and 32 °C). Higher clearance rates were recorded in cockles maintained at 30 and 40 psu and 32 °C. Consistently high clearance rates (0.9 to 1.14 l g^{-1} h^{-1}) were recorded in cockles held at 40 psu, regardless of temperature. Absorption efficiency was higher at 20 psu and lowest at 40 and 50 psu. Absorbed energy increased with decreasing salinity and increasing temperature. Respiration energy was higher at low to moderate salinities (20 to 40 psu) and at intermediate temperatures (26 and 29 °C). The lowest respiration energy and excretion energy were recorded in cockles held at 50 psu, regardless of temperature. Excretion energy was inversely related to temperature and salinity, with high values in cockles held at low salinity (20 and 30 psu) and low temperature (23 and 26 °C). Scope for growth was directly related to temperature and inversely related to salinity. The highest scope for growth occurred in cockles held at 32 °C (120.4 to 327.3 J g^{-1} h^{-1}) and the lowest in cockles held at 23 °C (4.3 to 84.1 J g^{-1} h^{-1}). The mangrove cockle can tolerate a wide range of temperature and salinity and maintain positive scope for growth. This species could be grown under a wide variety of conditions, especially in tropical lagoons receiving fresh water runoff. However it can also be grown in challenging environments such as shrimp ponds effluents and take advance of the cockle tolerance to extreme conditions.

1. Introduction

Energy budget is a description of energy acquisition versus energy expenditure. In a biological system, energy is frequently obtained after food ingestion while energy expenditure can be summarized as metabolic costs (e.g. respiration, excretion, and reproduction). Scope for growth (SFG) is a physiological index that determines the amount of energy that is potentially available for growth and reproduction (Winberg, 1960) and is calculated as the difference between the measures of absorbed energy and respired and excreted energy. SFG has been extensively used in marine bivalves to determine population health (Din and Ahamad, 1995), physiological plasticity of invasive species (Sarà et al., 2008), optimum feeding ranges for aquacultural purposes (Ibarrola et al., 1998; Kesarcodi-Watson et al., 2001a,b; Velasco, 2007; Yukihira et al., 1998), and identifying physical and chemical parameters for optimum growth of aquaculture species (Soria et al., 2007; Yukihira et al., 2000).

The mangrove cockle, *Anadara tuberculosa* (also called pustulose ark clam) are an important commercial species harvested by artisanal fishermen along the Pacific coasts of Mexico and Central and South America (McKenzie, 2001). Cockle species inhabit important intertidal zone sediments in vulnerable areas such as mangrove swamps (McKenzie, 2001) and are exposed to high variation in environmental conditions (temperature, salinity, and dissolved oxygen). Although aquaculture of this species is still not conducted at a commercial scale, cockles have been identified as potential species for aquaculture not only in countries from the Tropical East Pacific Region but also in the United States (Power and Walker, 2001) and Australia (Nell et al., 1994). Spat collection methods have been tried with limited success (Borrero, 1986), and physiological responses to hypoxia and low salinity have been used to determine appropriate culture conditions (Davenport and Wong, 1986). More recently, cockles have been studied as potential species for bioremediation of shrimp farm effluents with positive results (Miranda et al., 2009; Peña-Messina et al., 2009). With the daily removal of ca. 0.8 g of biodeposits per average adult cockle (Miranda et al., 2009), the potential for the

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reduction of particulate matter from aquaculture effluents makes cockles an attractive species for cultivation. Water temperature and salinity in effluent waters of shrimp or fish ponds show a wide range of variation due to slow water movement and low pond depth. The determination of tolerance to extreme temperature and salinity is a key step for the selection of appropriate species for bioremediation purposes.

We investigated the energy budget and calculated SFG in the mangrove cockle *Anadara tuberculosa* (G. B. Sowerby I, 1833) that were exposed to combinations of temperature and salinity to determine optimal ranges of these variable and to determine the extent of the effect of extreme conditions on SFG. Since commercial mono-culture of cockles may not have economic appeal due to their low market value, it is still possible that cockles used for bioremediation could be marketed at the end of the culture cycle of the primary species (Jones and Preston, 1999; Lin et al., 1993; Miranda et al., 2009). The study of the energy budgets of cockles maintained under conditions that are observed in commercial aquaculture farms will assist determining the range at which mangrove cockles can be used for aquaculture effluent bioremediation.

2. Materials and methods

Wild adult cockles with a mean (±SD) shell length of 48 (±6 mm) and dry tissue mass of 1550 (±210 mg) were collected at Teacapán (22.35°N, 105.75°W) in the State of Sinaloa in August 2007 when this species shows the least proportion of ripe specimens and a high proportion of spent specimens. Before the experiment, 144 cockles were cleaned and separated into 16 subgroups of nine cockles each. Each subgroup was maintained for 15 days at the selected combination of temperature and salinity to allow for full acclimation at the most extreme conditions. Acclimation may occur within hours or in few days after the desired ambient conditions are reached (Sicard et al., 1999). Physiological rates and energy budgets were recorded for 16 combinations at four salinities (20, 30, 40, and 50 psu) and four temperatures (23, 26, 29, and 32 °C).

A standard 50 mg l^{-1} suspension of total particulate matter (TPM) was used as diet since this was the mean concentration of particulate matter observed in shrimp farm effluents (De Jesús-Huerta, 2005). The diet was composed of 60% particulate inorganic matter (PIM) and 40% particulate organic matter (POM). PIM was obtained from: A) sediment from a shrimp farm (20 mg l^{-1}). This sediment was dried, crushed, and sieved (55 μm mesh) before reducing the sediment to ash in a furnace at 450 °C, and B) the inorganic fraction of the diatom *Chaetoceros muelleri* (10 mg l^{-1}). For POM, the organic fraction of the diatom *Ch. muelleri* was used (20 mg l^{-1}). A mean organic content of 80% of the total diatom dry weight was used as the basis for POM (Renaud et al., 2002). Mean dry weight of *Ch. muelleri* was 78.7 pg cell^{-1} (Piña-Valdez, 2004). The suspension of total particulate matter was constantly aerated to prevent settlement and no pseudofaeces were observed during the acclimation and the experimental period.

Nine 1 l experimental chambers, each containing a single cockle, were used for each combination of temperature and salinity. An additional chamber for each combination, containing two empty valves of a cockle shell, was used as a control for measuring settling of the suspended particulates.

The experimental chambers received a mean (±SD) flow of water (Q) of 2.4 (±0.6 l h^{-1}). At this rate of flow, TPM in the chambers was always above 80% of the initial concentration (50 mg l^{-1}). Scope for growth was measured (Winberg, 1960) by subtracting respiration and excretion energy from absorbed energy.

Absorbed energy (A; in J h^{-1} g^{-1}) was calculated with the formula

$$A = C \times AE;$$

C is consumed energy (C; in J h^{-1} g^{-1}) and AE is absorption efficiency. Consumed energy was calculated with the formula

$$C = 23(POM_c) \times CR;$$

The number 23 represents the caloric content of 1 mg of POM and POM_c is the concentration of particulate organic matter (mg l^{-1}) (Widdows and Johnson, 1988) The results of the formula have been standardized to reflect the units J h^{-1} g^{-1}. Clearance rate (CR; in l h^{-1} g^{-1}) was calculated with the formula

$$CR = [(D_1 - D_0) / D_1] \times Q / C_{dw};$$

where D_1 and D_0 are particle densities (particles ml^{-1}) at the inlet and outlet of the experimental chamber, Q is the water flow (l h^{-1}), and C_{dw} is the mean dry weight of the cockle (Hildreth and Crisp, 1976). The results of the formula have been standardized to reflect the units l h^{-1} g^{-1}. Particle density was determined every 3 h during an experimental period of 12 h (four times) using a Spectrex Laser PC-2000 particle counter.

Absorption efficiency (AE; in %) was determined (Conover, 1966) by the formula

$$AE = [(F - E) / (1 - E) \times F] \times 100;$$

where F is the proportion of organic content in the feed and E is the proportion of organic content in the feces. Feces were collected from the chambers at the end of the experiment. Feces and feed were placed on separate pre-weighed and previously ashed 47-mm fiberglass filters. Feces were rinsed with 4% ammonium formate to remove salt water and dried at 60 °C for 24 h. The proportion of organic content in the feed and feces was obtained after reducing the samples to ash in a furnace at 450 °C for 6 h.

Respiration energy (R; in J h^{-1} g^{-1}) was calculated as a product of the rate of oxygen consumption (VO_2) and 14.1 J mg^{-1} O_2 (Crisp, 1971). VO_2 (mg O_2 h^{-1} g^{-1}) was obtained by measuring dissolved oxygen with a Microx TX DO meter in each experimental chamber and the control chamber. VO_2 was calculated as

$$VO_2 = [(PO_{2c} \times Q) - (PO_{2e} \times Q)] / DW;$$

where PO_{2c} and PO_{2e} are the dissolved oxygen concentration in the control and experimental chambers, respectively, and DW the dry mass of the cockle.

Excretion energy (U; in J h^{-1} g^{-1}) was calculated as a product of ammonia excretion rate (ER; in mg h^{-1} g^{-1};) and 24.9 J mg^{-1} NH_4–N (Elliot and Davison, 1975). ER was calculated as

$$ER = [(NH_4 - N_e \times Q) - (NH_4 - N_c \times Q)] / DW,$$

where NH_4–N_e and NH_4–N_c are the ammonia concentrations in the experimental and control chambers. Ammonia content was determined with a multi-analyzer (Orion EA 940 equipped with an Orion 95–12 probe). Scope for growth (SFG; J h^{-1} g^{-1}) was calculated as SFG = A − (R + U) defined in Winberg (1960).

The Lilliefors test and Bartlett homoscedasticity test were used to determine the use of parametric or nonparametric statistics. Physiological data were analyzed with two-way ANOVA and multiple regressions to determine main effects and interactions. Where significant differences (P<0.05) within physiological parameters were observed, Tukey's multiple comparison test was used. The second degree polynomial model from Statistica 7.0 was used to obtain the surface response graph for SFG.

3. Results

Significant differences were observed in most physiological parameters in cockles maintained at 50 psu compared to all other salinities (Table 1). Similarly cockles maintained at 32 °C showed