



Scope for growth, biochemical composition, and antioxidant immune responses of the penshell *Atrina maura* to flow velocity and concentration of microalgae

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ABSTRACT

This study shows the short-term effect of three flow rates (0.8, 1.6, and 2.5 cm s⁻¹) and three ranges of microalgae *Isochrysis galbana* (clone T-Iso) concentrations (80–120, 150–180 and 200–250 cells μL⁻¹) on the scope for growth, biochemical composition, and immune responses of the digestive gland and adductor muscle in the juvenile penshell *Atrina maura*. Significant differences in *A. maura* responses occurred between flow rates. Ingestion rate, absorption rate, and scope for growth (SFG) were highest at 1.6 cm s⁻¹. Respiratory rate increased with the flow rate and decreased with the concentration of microalgae. As T-Iso concentration increased, ingestion rate, absorption rate and SFG increased. The increased ingestion rate, absorption rate and SFG were significantly correlated to the concentration of microalgae at 0.8 and 1.6 cm s⁻¹ and respiratory rate was positively correlated at 2.5 cm s⁻¹ ($P < 0.05$). Antioxidant enzyme immune activity was higher in the adductor muscle, associated with higher metabolic activity as respired energy. Glutathione S-transferase and catalase activities increased with flow rate. Antioxidant enzyme activities, lysozyme, and lipid peroxidation levels differed with tissues. T-Iso concentration was inversely correlated to acetylcholinesterase; lipid peroxidation levels decreased with T-Iso and were associated with energy transfer. The penshell's scope for growth was dependent on the flow and positively related to availability of food. Immune response was also associated with the flow rate.

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1. Introduction

Bivalves are filter feeders, capturing suspended particles in the water column for sustaining growth and producing gametes. They form an important trophic link between primary and secondary production and reflect changes in the environmental conditions of their habitat (Manduzio et al., 2004). Bivalves are subjected to daily oscillations in the energy ingestion from microalgae and suspended particles through water currents and turbulence. Energy gain processes and growth are associated with the flow speed and turbulence (Eckman et al., 1989; Lenihan et al., 1996), differing greatly between siphonate and non-siphonate bivalve species (Grizzle et al., 1992), even within species of the same genus (Ackerman, 1999). Those differences are associated with the mechanism of capturing particulates in passive and active filter feeders (Lesser et al., 1994),

flow velocity and seston concentration (Pilditch and Grant, 1999; Strohmeier et al., 2009).

The physiological status of bivalves can be assessed through the scope for growth model, which is associated with population health. The SFG, or energy available for growth and reproduction, is an index reflecting stress and integrates physiological responses from environmental changes, either natural or from human activity. SFG measures the balance between energy absorption and energy loss processes (respiration and excretion) and has been widely used in environmental monitoring assessments. Negative SFG indicates high stress when the organism uses body reserves for maintenance; and high and positive SFG indicates optimum conditions (Tsangaris et al., 2010). However, the biological performance of the mussel *Mytilus galloprovincialis* along an environmental gradient, depicted significant differences in the antioxidant enzyme tested and no significant change in scope for growth (Fernández et al., 2010).

Bivalve energy reserves are often influenced by changes in seasonal and life cycles (Wilhelm-Filho et al., 2001), environmental variables, food availability and quality. Accumulation of energy occurs under good feeding conditions (Matsui, 2002). However during the process of energy generation, molecular oxygen (O₂) undergoes tetravalent reduction to water and partial reduction of O₂ results in the formation

Abbreviations: A, absorption rate; AChE, acetylcholinesterase; CAT, catalase; CDNB, 1-chloro-2,4-dinitrochlorobenzene; GST, glutathione S-transferase; I, ingestion rate; R, respiratory rate; SFG, scope for growth; TBARS, lipid peroxidation; T-Iso, *Isochrysis galbana* (clone T-Iso); U, excreted energy.

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of reactive oxygen species (ROS) (Mayzaud and Conover, 1988). Antioxidant enzymes such as glutathione S-transferase and catalase eliminate ROS from the cell, even so, some damage occurs as lipid peroxidation at cell membranes (Livingstone, 2003). There is a need to better understand antioxidant activity under a broader range of environmental conditions, but there is certainly information available on these sub-lethal conditions.

The penshell is a bivalve whose adductor muscle has a high economic importance in Latin American and Oriental markets. Since natural beds are over-exploited, there is a growing interest for its cultivation. To our knowledge there is no published information on the effect of environmental variables such as flow and seston concentration (important for site selection in aquaculture ventures), on the physiology, biochemistry and immunology of *A. maura*. Here we aimed to investigate relationships between scope for growth, biochemical composition and immune responses for juvenile penshells kept at three flow rates and three concentrations of the microalgae (*I. galbana*).

2. Materials and methods

2.1. Experimental conditions

Hatchery-reared penshells *A. maura* with a mean ($\pm$ SD) shell length 72.4 mm ($\pm$ 7.7 mm; $n=166$), showing no trace of gonadal development were obtained from a shellfish hatchery in Los Mochis, Sinaloa, Mexico. Penshells were acclimated in 60 l tanks with aerated, 1 μ m filtered seawater (26 °C, 40 psu) and fed once a day with cultured microalgae *I. galbana* clone T-Iso (150×10^3 cells mL⁻¹). This ration is routinely employed for the maintenance of penshells in our hatchery. About 40% of the water was replaced daily. No deaths occurred during the trials.

Experimental chambers (Fig. 1) were made with 5-mm clear plexiglass (12.0 $\times$ 5.0 $\times$ 5.5 cm). The water entered and flowed through screwed 0.5-inch tubing coupled to plastic pipes. The inlet led to two 3-mm gross plexiglass perforated plates with holes of 5 mm diameter, ensuring laminar flow of seawater. The animal section of the chamber had a volume of 233.7 cm³ (8.5 $\times$ 5.0 $\times$ 5.5 cm) to house individual penshells. Bivalves were oriented facing the water current, as described by Eckman et al. (1989). The removable outflow plate was fixed with stainless steel nuts screwed to the chamber, and sealed hermetically with an o-ring to avoid water seepage and fluctuation of flow. The chamber outflow plate had a plastic, screwed pipe connected to a flow valve. The valve was adjusted for flow rates of 0.8, 1.6, and 2.5 cm s⁻¹. The water drawn from the chambers were recorded (mL h⁻¹) for calculating the scope for growth.

A mixture of gently aerated filtered seawater (1 μ m) and cultured T-Iso at their exponential growth phase, was homogeneously maintained with a submersible electrical pump in a plastic 200-l reservoir at 25 °C. Microalgae concentration was adjusted to the desired level with a particle counter (Multisizer 3, Beckman-Coulter,

Fullerton, CA) equipped with a 100 μ m pore size tube. The mixture was gravity-supplied to the experiment chambers from flexible pipes.

For 24 h prior to the trials, each penshell was placed in an individual tank containing its corresponding mixture of aerated filtered sea water and microalgae. Then the bivalves were placed in other tanks containing 1 μ m-filtered seawater and were kept for 24 h to void the digestive tract of food and feces. Nine treatments were tested consisting of a matrix of three ranges of microalgae concentrations (80–120, 150–180 and 200–250 cells μ L⁻¹), and three flows (0.8, 1.6, and 2.5 cm s⁻¹). At these ranges of concentrations, penshells did not produce pseudofeces. Each treatment was tested from 9 to 60 times. The number of tests varied because of our intent to investigate physiological responses at as many algal concentration as possible within each range. Each chamber contained 24, 26, and 37 individuals, depending on the flow (0.8, 1.6, and 2.5 cm s⁻¹, respectively). Variance normality tests were done to ensure that statistical analyses were not affected by differences in treatment test numbers.

Control chambers received the same water supply as their respective experimental treatments, but without any bivalves.

2.2. Physiological measurements

2.2.1. Sample collection

Samples and data were collected after 2 h when microalgae ingestion and respiratory rate attained a steady state. Water samples for dissolved oxygen, ammonia, concentration of microalgal cells, and total particulate matter were collected from running water samples in the chambers. After the data was collected, the penshells were placed in individual filtered seawater containers for sampling feces. Shell length of the penshell was measured with a precision caliper. Specimens were stored at -80 °C for shell length biometric relationships and biochemical analysis.

Dissolved oxygen was recorded with a microelectrode meter (Microx TX2 Oxyview v4.16, PreSens, Regensburg, Germany). Oxygen consumption was calculated as the difference between the oxygen concentration from the control chamber (PO_{2c}) and each experimental chamber (PO_{2i}).

The concentration of microalgae was measured with a counter (Coulter-Beckman) with a 100 μ m pore size tube. Consumption of microalgae (C) was calculated as the difference between cell count from the control (C₁) and experimental chambers (C₂).

Organic content of the microalgae was estimated from collections from low-vacuum filtered seawater, which was passed through preashed GF/C filters (Whatman). Filters were rinsed with 3% isotonic ammonium formate, then dried at 60 °C, and burned in a furnace at 450 °C for 3 h. Total particulate matter, microalgal organic matter, and organic fraction of the food were calculated following Strickland and Parsons (1972). Dissolved ammonia (μ g NH₄g⁻¹ h⁻¹) was determined following the phenol-hypochlorite microplate method (Hernández-López and Vargas-Albores, 2003).

Feces were collected and sieved through a 50- μ m pore size mesh with filtered seawater. Feces were processed, as described for microalgal organic content on pre-weighted Whatman filters, obtaining feces ash free dry weight and feces organic fraction (Strickland and Parsons, 1972).

Organic content of the microalgae was transformed to energy equivalents with the conversion factor of 23.5 (J mg⁻¹ h⁻¹; Widdows and Johnson, 1988). Oxygen was converted to energy units using the conversion factor of 20.2 J mL⁻¹ O₂ (Elliot and Davison, 1975), and ammonia was transformed with the conversion factor of 0.0249 (J mg⁻¹ h⁻¹; Hutchinson and Hawkins, 1992).

2.2.2. Biometric data and allometric relationship of soft tissue

Specimens stored at -80 °C were used for analysis of the shell length-dry weight of soft tissues allometric relationship, biochemical

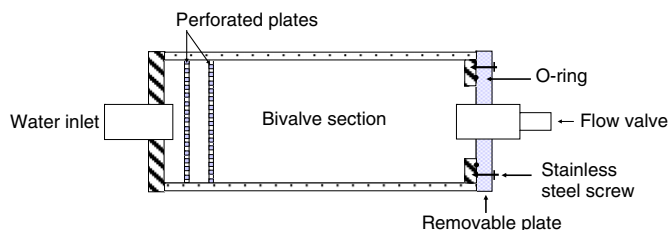


Fig. 1. Flow chamber for ecophysiological measurements. The water oxygen recording sensor was fixed at the outlet port.

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