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## Determination of oxygen consumption of Donkey's ear abalone *Haliotis asinina* under laboratory conditions fed with varying micro-algae seaweeds

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### Abstract

*Haliotis asinina*, the seawater abalone with different sizes i.e. small (1-2 cm in shell-length) medium (3-4 cm in shell-length) and large (5-6 cm in shell-length) were selected and further tested for determination of changes in the rate of oxygen consumption for 24 hours period. Three Feeding treatments included the seaweeds species *Gracilariopsis heteroclada*, *Caulerpa lentillifera* and unfed treatments were tested. The abalones for the unfed treatment were originally fed *G. heteroclada*, and not fed 24 h prior the experiment. The highest oxygen consumption levels were recorded for the starved juveniles (small size, 1-2 cm SL) *H. asinina* and lowest in juveniles fed *Caulerpa lentillifera*, followed by juveniles fed *Gracilariopsis heteroclada*. Starved sub-adult (medium size, 3-4 cm SL) abalone registered oxygen consumption levels between *Caulerpa lentillifera* and *Gracilariopsis heteroclada* fed abalone. Further, caloric requirements ranged from 28.32 cal•gm<sup>-1</sup> (i.e. 5-6 cm SL) to 59.15 cal•gm<sup>-1</sup> (i.e.1-2 cm SL) for the *C. lentillifera* treatment and from 26.39 cal•gm<sup>-1</sup> (i.e.5-6 cm SL) to 124.79 cal•gm<sup>-1</sup> (i.e.1-2 cm SL) for the *G. heteroclada* treatment. The starvation treatment has corresponding higher (for 1-2 cm SL) and lower (for 5-6 cm SL) caloric requirements, respectively. The results are discussed in the light of metabolic processes in sea-water mollusks. The relationship between the rate of oxygen consumption and dry body weight were established in the experimental animals. Regression analysis was made and  $\{R^2\}$  was calculated; the values of  $[R^2]$  were obtained to be 0.90 and 0.72 and 0.40 for the starved, *Gracilariopsis heteroclada* and *Caulerpa lentillifera* fed, respectively.  $[R^2]$  values were highly significant in logarithmic relationship.

**Keywords:** *Haliotis asinina*, different sizes, *G. heteroclada*, *C. lentillifera*, oxygen consumption

### 1. Introduction

Abalone is gastropods belonging to the family *Haliotidae* <sup>[1, 2]</sup> and sub class *Archaeogastropoda* <sup>[1, 3]</sup>. It is possible that seaweed grazers are selective in their diet <sup>[4]</sup>. Researches on seaweed preferences in abalone have been investigated in a variety of countries. The Australian green lip abalone *H. laevigata* shows strong preference for red over brown seaweeds <sup>[5]</sup>. Conversely, the Japanese abalone *H. discus hannai* prefers the brown seaweed <sup>[6]</sup>. *H. cracherodii*, *H. corrugata* *H. folgeri* and *H. rufescens* from California also prefer brown seaweeds <sup>[7]</sup>, and the South African abalone *H. midae* prefers a variety of brown seaweed <sup>[1]</sup>. Moreover, the European abalone *H. tuberculata* shows strong preferences for a certain variety of seaweed <sup>[8]</sup>, depending on the energy content. In addition, *H. tuberculata* tends to optimize its food sources <sup>[9]</sup>.

Measurement of the rate of metabolic heat production may result of fixed ratios between the quantities of oxygen consumed, carbon dioxide produced and heat produced from the synthesis of carbohydrate, protein and lipid <sup>[10]</sup>. The relationship between energy metabolism and the rate of oxygen consumption it may be reliably used for both short term measurements of resting energy expenditure (over periods of less than an hour) and measurements of total energy expenditure over longer periods (up to 24 hours or more).

*Haliotis asinina* is the largest among the tropical abalone species and occurs throughout the Indo-Pacific. Abalone inhabit near-shore reef, and usually are very patchily distributed <sup>[11]</sup>. They are primary nocturnal feeders <sup>[12]</sup>. The tropical abalone *H. asinina* has been harvested extensively and become commercially important fisheries product for export market. However, studies on the biology are limited and as well as for aquaculture.

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Therefore, the main objective of this study is to describe the metabolic requirements of the donkey's ear abalone *Haliotis asinina* in terms of its oxygen consumption in response to size differences fed with varying macro algae seaweeds.

## 2. Materials and Methods

The seawater *Haliotis asinina* abalone with varying in body sizes were collected from South East Aquaculture fisheries development Center (SEAFDEC) Tigbauan, Iloilo city - Philippines. Three size classes of abalone were selected and reared in six (forty Liter) aquaria with shelter and aeration. Immediately after brought to the laboratory, the shells of the animals were brushed and washed with seawater in order to remove the algal biomass, mud and other waste materials. The cleaned animals were divided into three size groups of shell length i.e. small (1-2 cm), medium (3-4 cm) and large (5-6 cm). Each group comprises fifteen animals. After measuring length, they were set for 2 weeks acclimatization period in laboratory conditions, under constant aeration. The physico-chemical parameters of water i.e. Temperature, pH and dissolved oxygen contents were also measured. Feeding treatments included the seaweed species *Caulerpa lentillifera* and *Gracilariopsis heteroclada*, and an unfed treatment, respectively. The abalone for the unfed treatment were originally fed *G. heteroclada*, and not fed one day before the respiration experiment was conducted.

**Respiratory chambers:** The respiration chamber consisted of eight 2.781 L acrylic respiratory chambers placed in temperature-controlled water bath in a completely flow-through recirculating system. The experimental design consisted of three treatments – animal fed with *Caulerpa lentillifera* or animal fed with *Gracilariopsis heteroclada* or starved abalone with four replicates each. Prior to the experiment, initial dissolved oxygen (DO) concentrations of water in all the chambers (without the abalone) and the water bath were determined. It was established that DO in the chambers and in the water bath were similar. Three runs were conducted for the feeding treatments and each run lasted for 24 hours. At the end of each run, the dry weight (g) of the abalone was determined. The rate of oxygen consumption of animals was determined according to Winkler's modified method [13]. Acrylic respiratory 2.781 L capacity for each size group with an inlet and outlet were used to determination of oxygen consumption of abalone. Four pieces of small size, two pieces of medium size, and one piece of large size abalones were placed in 2.781 L airtight plastic chambers with four replicates size<sup>-1</sup> class, respectively. They were kept in continuous circulation of water inside the chamber. After 3 hours, 300 ml of water sample from each chamber was drawn to find out the oxygen content.

**Experimental Design:** Oxygen concentration was analyzed titrimetrically and expressed as mg O<sub>2</sub> L<sup>-1</sup> h<sup>-1</sup>. Each run was monitored for 24 h with 3 h interval water sampling. In each run namely as abalone with different sizes fed with *Caulerpa lentillifera*, abalone fed with *Gracilariopsis heteroclada* while abalone in the starved group were not fed for 24 h prior to the experiment, respectively. Shell length (cm), wet weight without shell (gm), and dry weight (gm) after oven drying to constant weight, and flow rate of water sample collection were also recorded. Three 24 hours runs were done for the three feeding treatments. Oxygen consumption was computed as:

$$\frac{\text{mgO}_2 \cdot \text{L}^{-1} * \text{Flow rate}}{\text{Weight} * \text{time}}$$

Total expenditure of metabolic energy day<sup>-1</sup> was calculated by multiplying the average metabolic rate by 24 h. Energy units were based on the conversion of 1 ml O<sub>2</sub> = 4.8 calories.

**2.1 Statistical Analysis:** The mean values of three size groups of the abalone were used for statistical analysis. All measurements were made at ambient water temperature and photoperiod to best reflect natural conditions. Second order polynomial regression analyses were used to generate correlation coefficients (R<sup>2</sup>).

## 3. Results

The highest oxygen consumption levels were recorded for the starved juveniles (small size, 1-2 cm SL) *H. asinina* and lowest in juveniles fed *Caulerpa lentillifera*, with juveniles fed *Gracilariopsis heteroclada* intermediate. Starved sub-adult (medium size, 3-4 cm SL) abalone registered oxygen consumption levels intermediate to *Caulerpa* and *Gracilariopsis* fed abalone. The same pattern progressed with the adult abalone (large size, 5-6 cm SL) where the oxygen consumption curve is lowest in starved abalone compared to the fed abalone.

During Starvation experiment, the rate of oxygen consumption of the small size group (1-2 cm shell length) ranged from 7.65 to 14.48 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, while for the medium size group, the oxygen consumption ranged between 3.09 to 6.41 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>. Moreover, and for large size group, rate of oxygen consumption in ranged between 0.82 to 3.74 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, the highest value was observed in first sampling and the lowest found in fourth sampling.

Further, and during *Caulerpa lentillifera* experiment, the oxygen consumption of the small size group ranged from 1.07 to 7.87 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, while for the medium size group, the rate of oxygen consumption ranged between 1.10 to 3.11 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, and for the large size group, the rate of oxygen consumption ranged between 1.04 to 1.98 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, respectively. For animals fed with *Gracilariopsis heteroclada* seaweed, the oxygen consumption of the small sizes, ranges from 2.92 to 15.95 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, and for the medium sizes the oxygen consumption ranges between 2.53 to 5.66 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>. While for large sizes, oxygen consumption in ranges between 0.74 to 1.80 mlO<sub>2</sub>•g<sup>-1</sup>•h<sup>-1</sup>, the figure below clearly illustrates the pattern of oxygen consumption by the *Haliotis asinina* under starvation, *Caulerpa lentillifera* and *Gracilariopsis heteroclada* treatments, respectively (Figure 1).

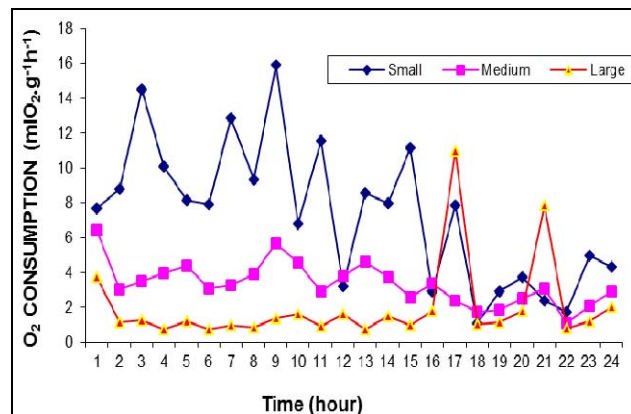
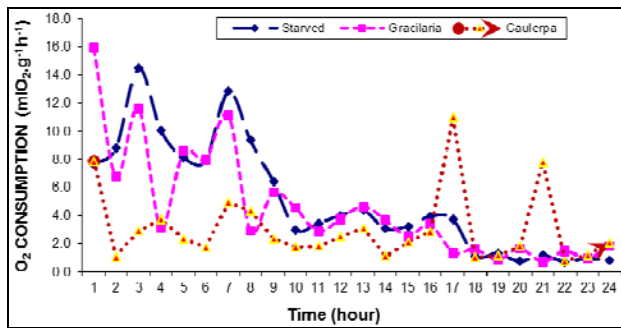


Fig 1: Oxygen consumption over a 24 h period as size differences tested of abalone *Haliotis asinina*.

Abalone in the field usually forage more intensively during the night [14] and organisms from this study were fed strictly according to the natural period; food was available from 20:00 h until 08:00 h. Respiration rates by oxygen consumption measured during the night tend to be higher than the rates during the day, in all treatments and groups. Data from the experiment suggested that abalone were in average more active during night time than the daytime. The results of measurement of oxygen consumption for *Haliotis asinina* showed differences in the energy expended through respiration between starved, *Gracilariopsis* and *Caulerpa* diets measured during 24 hours experiments (Figure 2).

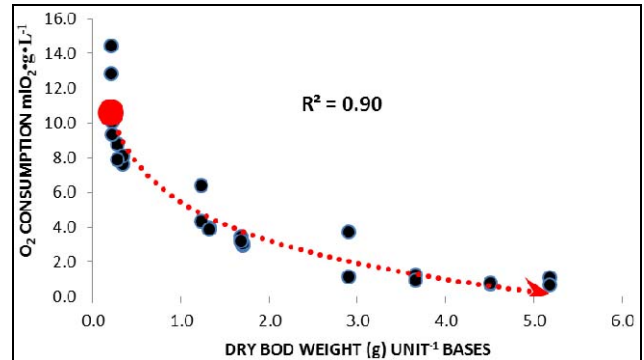


**Fig 2:** Oxygen consumption as a food type over a 24 h period of abalone *Haliotis asinina* fed seaweeds *Gracilariopsis heteroclada*, *Caulerpa lentillifera* or starved for 24 h.

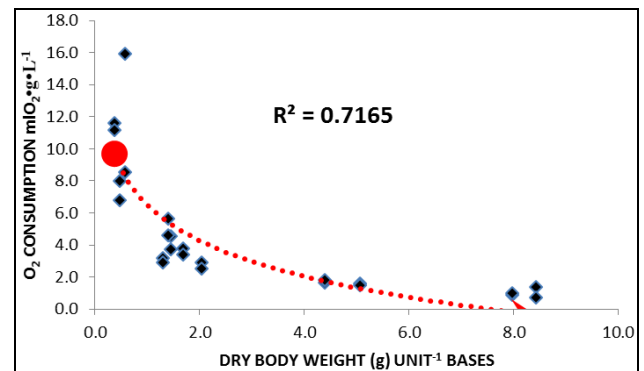
The highest oxygen consumption levels were recorded for the starved juveniles (small size, 1-2 cm SL) *H. asinina* and lowest in juveniles fed *Caulerpa lentillifera*, with juveniles fed *Gracilariopsis heteroclada* intermediate. Starved (medium size, 3-4 cm SL) abalone registered oxygen consumption levels intermediate to *Caulerpa* and *Gracilariopsis* fed abalone. The same pattern progressed with the adult abalone (large size, 5-6 cm SL) where the oxygen consumption curve is lowest in *Caulerpa* abalone compared to the starved abalone. From the highest oxygen consumption curves of starved juvenile *H. asinina* followed by levels for fed juveniles, the pattern progresses to intermediate values in sub-adult sizes between the lowest curve generated by *C. lentillifera* representing the basal or minimum respiration, and the *G. heteroclada* having the highest curve representing the active metabolism. The proportion of energy used in respiration increased dramatically at starvation when size increased, although some of the starvation effects are affected by differences in animal size. When abalone were maintained and fed on seaweeds *Gracilariopsis* and *Caulerpa* a significant increment in oxygen consumption with mean maximum of 9871.35 cal animal $\cdot$ day $^{-1}$  and 1894.97 cal animal $\cdot$ day $^{-1}$ , and highest for those abalone starved for 24 h as maximum of 11460.94 animal $\cdot$ day $^{-1}$  as size increased.

Although the size of the animals used was varied for each treatment (Starved, *Gracilariopsis* and *Caulerpa* seaweeds diets) the energy consumed was higher in starved animals compared with fed *Gracilariopsis* and *Caulerpa*, respectively. Comparisons of respiration rates between animals fed on the two different seaweeds and the starved animals showed nutritional influence on the energy expended through oxygen consumption. The effect of body size on rates of oxygen consumption was determined from data of the day and night respiration at the same feeding and also between groups of each size treatment. The logarithmic relationship results based on dry body weight revealed higher values of the constant  $R^2$

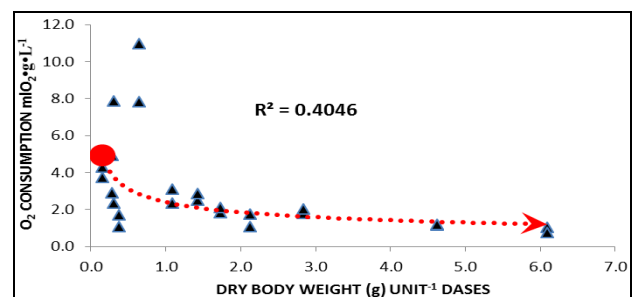
for the three different sizes of abalone fed with varying seaweeds ranging from 0.898 to 0.716 and 0.404, for the starved, *Gracilariopsis* and *Caulerpa*, respectively (Figures 3, 4 and 5).



**Fig 3:** Logarithmic relationship of abalone *Haliotis asinina* during starvation for 24 h and oxygen consumption rates unit $^{-1}$  dry body weight.



**Fig 4:** Logarithmic relationship of abalone *Haliotis asinina* fed seaweed *Gracilariopsis heteroclada* for 24 h experiment and oxygen consumption rates unit $^{-1}$  dry body weight.



**Fig 5:** Logarithmic relationship of abalone *Haliotis asinina* fed seaweed *Caulerpa lentillifera* for 24 h and oxygen consumption rates unit $^{-1}$  dry body weight.

Generally, caloric requirements for abalone *Haliotis asinina* ranged from 28.32 cal $\cdot$ gm $^{-1}$  (i.e. 5-6 cm SL) to 59.15 cal $\cdot$ gm $^{-1}$  (i.e. 1-2 cm SL) for the *C. lentillifera* treatment and from 26.39 cal $\cdot$ gm $^{-1}$  (i.e. 5-6 cm SL) to 124.80 cal $\cdot$ gm $^{-1}$  (i.e. 1-2 cm SL) for the *G. heteroclada* treatment. The starvation treatment has corresponding higher (for 1-2 cm SL) and lower (for 5-6 cm SL) caloric requirements, respectively.

#### 4. Discussion

The metabolic rate is strongly dependent on body size, it is necessary to introduce weight specific correlation comparison between animals of different sizes. It is known that weight

specific rate of oxygen consumption is lower in larger organisms than in smaller ones. This generalization applies in comparisons between mollusks of different sizes as well as belongs to same species or different ones.

In the present study, the daily respiration energy expenditure in the three size groups tested with different seaweeds diets decreased with size and diet (i.e. starvation) also affected these rates. Animals fed on *Gracilariopsis* and *Caulerpa* diets spent less energy through respiration than those starved, even at the same sizes. The findings obtained showed clearly that the size and diet have an effect on oxygen consumption rates. Energy used for respiration was higher during the night time and it was significantly different from that spent for organism fed *Gracilariopsis* and *Caulerpa* diets, respectively. Since any changes in external temperature would result in changes of oxygen consumption (i.e. metabolic rate), therefore, in case of *Haliotis asinina* oxygen consumption was positively correlated with temperatures between 18 °C and 21 °C during night time. At 28 °C, oxygen consumption was lower during daytime.

The rate of oxygen consumption and the respective exponent values from this study were strongly dependent upon both body size and diets. The respiratory exponents of 0.90 to 0.72 are quite comparable with those found for *H. tuberculata* when fed on seaweed with values of 0.76 [9], moreover, comparable data were found on the exponent of oxygen consumption for *H. kamtschatkana* which ranged from 0.74 to 0.78 [15].

However, the respiratory exponents found in this study, with values 0.898 for starved animals were slightly the same with those reported for *H. midae* [1], with values ranging from 0.83 to 0.94, and also for grazing gastropods [16]. Oxygen consumption for *Haliotis asinina* from this study was considerably elevated when starved.

In the present study on *Haliotis asinina*, the size specific oxygen consumption followed a general trend of acceptance i.e. higher values of oxygen consumption for smaller sized than larger sized. Nevertheless, several studies stated that the body size are important implication hence bivalves and mollusks populations that are dominated by older and large individuals have a lowest value than those composed of small individuals [16, 17]. It is also showed that the energy flow through small individuals of species may be much greater than larger ones. The rate of oxygen uptake increased in small sized animals because small individuals with relatively small glycogen reserves, which increases considerably their protein catabolism, whereas larger ones to a great extent on their relatively large glycogen storage [16].

## 5. Conclusion

In conclusion, the relationship between oxygen consumption and size differences, food sources and temperature has been attributed to physiological processes and reactions taking place in the animal's body and that attributed to give no uniform level of respiration rates.

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