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AN OPEN-SYSTEM RESPIROMETER FOR MEASURING STEADY-STATE OXYGEN UPTAKE BY AQUATIC ANIMALS APPLIED TO THE SCALLOPS *Pecten Maximus L.*

Djoko Suprapto[*)]
Faculty of Fisheries and Marine Science Diponegoro University
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ABSTRACT

An open-system respirometer for measuring steady-state oxygen uptake rate of aquatic animals is described. The principle of the measurement was to calculate the difference between the oxygen concentrations of the water entering the respiratory chambers with that out from the respiratory chambers. This difference indicated the oxygen consumed by the animals for their respiration. The measuring system was tested to calculate the oxygen consumption related to dry body weight of scallop Pecten maximus L. collected from different populations. This correlation was expressed by allometric equation as: $Y = aX^b$. The equation was then discussed with the finding of several authors.

Key words: Respirometer, Oxygen uptake, Scallop, Dry body weight.

[*)] **Correspondence**: Phone: 024-7474698, Fax.: 024-7474698

INTRODUCTION

Two different methods of measuring oxygen consumption are used at present, both of which are based on oxygen concentration measurement using polarographic oxygen electrodes. In the classical method, as used amongst others by Bayne (1971a), Vahl (1972) and Widdows (1978) the oxygen uptake is determined in a closed system with the oxygen electrode directly inserted into the respiration cells.

In this present study the calculation of oxygen uptake is based on a differential measurement of oxygen concentration between inlet and outlet water flowing through the respiration chambers. Both methods have disadvantages. In the closed method, the oxygen is depleted during the experiment and therefore, the animal will experience a concentration of oxygen that decreases gradually due to their respiration activity. Consequently, the value of respiration rate of the experimental animal is not constant and considered not to represent the real respiration activity. The flow-through method requires simultaneous calibration of the oxygen electrodes. The open-system, however, is preferable because the concentration of oxygen in the water medium could be kept in a constant value.

The purpose of the present work was to develop a respirometer for steady-state oxygen uptake in the normal range of oxygen concentration by using the open-system principle. The measuring system was tested by measurement of oxygen consumption of scallop related to their dry body weight.

MATERIAL AND METHODS

Description of Apparatus

The measuring system apparatus is diagrammatically presented in figure 1. Water from the reservoir tank (1), well oxygenated until the saturation point was pumped (2) and was simultaneously passed through an oxygenator column (3), the respiration chambers (4), Clark oxygen electrode (5) (Radiometer, Copenhagen), a peristaltic pump (6) and back to a reservoir tank where a thermostat (7) was placed to control the water temperature during the experiment.

Beyond the flowing system mentioned above, there was water coming from reservoir tank directly to the electrode. The concentration of oxygen measured from this water represent the concentration of oxygen before taken by the experimental animal. The oxygen electrode current was amplified by a digital acid-base analyzer (8) (Radiometer, PHM 71). The electrode signal was continuously monitored on a potentiometric recorder (9) during the experiment. An electric programmer (10) makes an individual (one by one) sampling from the respiratory chamber possible.The different components of the flowing circuit in the measuring system were connected by nylon tubes which were impermeable to oxygen. One problem in obtaining a good value of oxygen uptake measurement was the presence of air bulbs in front of the electrode membrane. The most important finding from this present study is the finding of the instrument called **bulb air trap** (11) and is presented in figure 2, as well as the design of a **respiratory chamber** for the **big size** animal and the **small one** (figure 2).

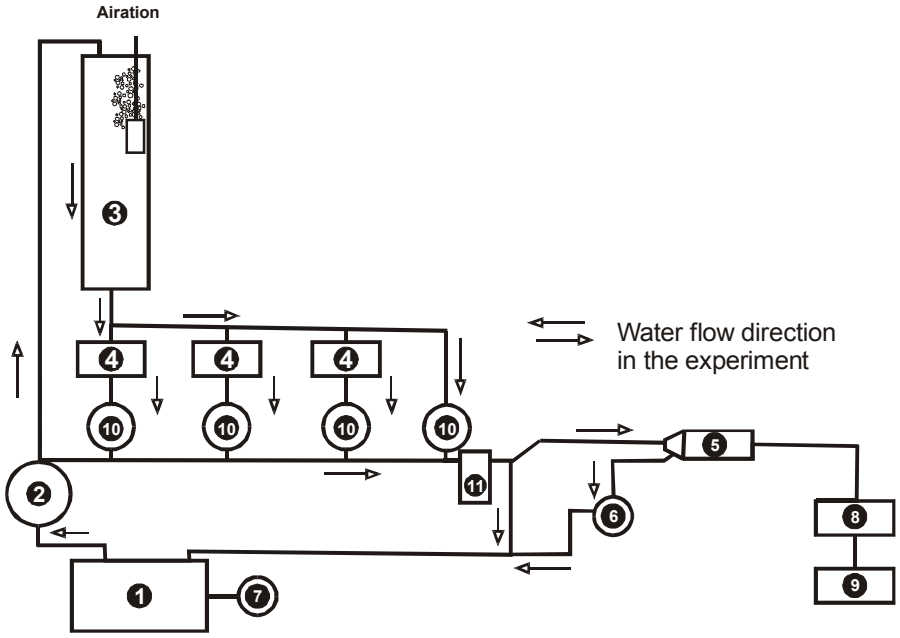

Figure 1. Schematic outline of the respirometer

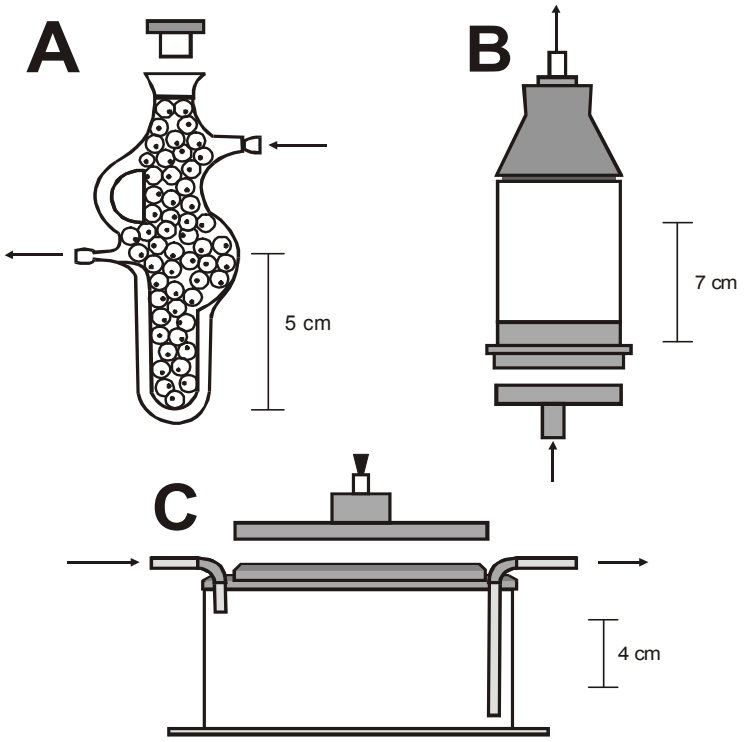

Figure 2. Water direction during the experiment in the respiration chamber.
A. Bulb-air trap
B. Respiration chamber for small size animal.
C. Respiration chamber for bigger size animal.

Calculation of Oxygen Uptake

The oxygen consumption was calculated by measuring oxygen concentration of the water entering the respiration chamber (CO_2e) and that which comes out of the respiration chamber (CO_2o). Then the oxygen consumption of the experimental animal ($\dot{V}O_2$) was calculated by the following equation:

$$\dot{V}O_2 = (CO_2e - CO_2o)Q$$

Where Q is the debit of the water flowing. Meanwhile the calculation of the concentration of oxygen in the water was based on Henry's erquation, namely:

$$CO_2 = \alpha PO_2$$

Where:

- α = Solubility of oxygen in the water, **(µmol /l torr/h)** Readable in the table established by Dejours (1981)
- PO_2 = Partial pressure of oxygen in the water **(torr.)**

Finally, the formula to calculate the oxygen consumption could be written as follows (**µmol/h**):

$$\dot{V}O_2 = \alpha(PO_2e - PO_2o)Q$$

The method of calculation of partial pressure of oxygen in the water was calculated using the following equation:

$$PO_2 = (Patm. - PH_2O)fO_2$$

Where:

- **P atm.** = Atmospheric pressure (read in the barometer)
- **PH_2O** = Pressure of water vapor (depending on the temperature and read in the table)
- **fO_2** = Concentration of oxygen in the air (20,93%)

Measurement Method of $\dot{V}O_2$ in Scallop Acclimatisation

In every alteration of the water quality the the medium will force the animal to make an effort to adapt. This condition will influence energetical balance of the animal and consequently will take more oxygen (Bayne, 1976) for providing energy needed. The duration of acclimatisation depends on many factors, including the life history of the animal. The indicator that the animal arrived in well adapted condition was when the valves open steadily and conduct respiration and ventilation actively.

Temperature

During the experiment the water temperature was kept constant (12°C). Otherwise the different temperature will influence either the oxygen uptake of the experiment animal or coefficient of solubility of oxygen. Therefore to overcome the problem the room in which the experiment was conducted should be well isolated from the exterior. Besides, the water should be thermostated to assure the constant temperature of the water medium.

Partial Pressure of Oxygen

The influence of partial pressure of oxygen to oxygen uptake, especially in bivalves have been studied by several authors: Bayne (1971, 1973), Taylor and Brand (1975), Shumway (1981) and Brand and Morris (1984). Animals have been characterized as either oxygen conformers, (i.e., $\dot{V}O2$ varies in indirect proportion to PO2) or oxygen regulators (i.e., is more or less independent of PO2). The point at which oxygen consumption ceases to be oxygen independent and become oxygen dependent is known as critical oxygen tension and is not always easy to determine precisely. Shumway (1981) indicated that bivalves have the capacity to maintain a relatively constant oxygen uptake when the partial pressure of oxygen is above the critical point. Suprapto (1986) maintains that the critical point of oxygen consumption of scallop is located between 70–80 torr.

Therefore, during the experiment partial pressure of oxygen should be kept above the critical point by giving intensive airation in the column of water before being distributed to the measurement system.

Method

Each experiment of oxygen uptake of the scallop is repeated ten times which represented 400 minutes (six hours 40 minutes). The result obtained was in **µmol/h** and then transferred to **ml/h**, to make comparison with findings of other authors much easier. As mentioned above, this measuring system is tested by the measurement of oxygen related to dry body weight:

$$\mathbf{Y = aXb^b}$$

Where:
- **Y** = Oxygen consumption (ml/h)
- **X** = Dry weight of animal (gr)
- **a** and **b** = The slope and intercept of the log. y and log. x regression respectively.

The oxygen intake of a number of scallops collected from different origins (Brest and Scotland) have been measured in the form of $\dot{V}O_2$ standard and $\dot{V}O_2$ active. The latter was used to measure the

animals fed with *Dunaliella primolecta* (5 millions cells per liter).

The dry body weight was calculated by drying the meat of the experimental animal (dissected after the experiment was finished) in the oven (80°C, 48 hours) until the constant weight was obtained.

RESULTS AND DISCUSSION

The results of measurement of oxygen uptake related to dry body weight either standard or active are presented in table 1 as well as in the figure 3 and 4.

Table 1. Allometric equation of the relationship of oxygen consumption with dry body weight of *Pecten maximus L.* from Brest and Scotland.

Population	N	Type of $\dot{V}O_2$	Equation $\dot{V}O_2 = aW^b$	r	SD.
Brest	32	standard	0.70 $W^{0.7}$	0.89	0.07
Brest	32	active	0.76 $W^{0.98}$	0.85	0.11
Scotland	30	standard	0.87 $W^{0.58}$	0.96	0.03
Scotland	30	active	2.19 $W^{0.53}$	0.95	0.03

In this present study the value of **(a)** was 0.70 – 2.11. The range of this value was very wide and indicated that the capability of the response either specific or individual to the nutritive availability was different. Based on the result of **(a)** value some notes can be stated here - the existence of feed in the water medium increased the value of **(a)**, meaning that the value of **(a)** of the $\dot{V}O_2$ active was larger than the value of **(a)** of the $\dot{V}O_2$ standard. The degree of changes of the **(a)** value depend to origin of the population. This finding is in agreement with the study of Navarro and Winter (1982) on *Mytilus chilensis.*

The value of coefficient **(b)** also depends on the population studied and availability of feed in the medium of experiment. The results of the measurement of the oxygen uptake (table 1), showed that the value of **(b)** ranged from 0.48 – 0.98. For the bivalves, in general the value of **(b)** varies from 0.38–0.98. According to Shafee (1980) and Bayne et.al., (1976) the value of **(b)** was between 0.4-0.9. Kennedy and Mihursky (1982) found the value of **(b)** between 0.31–0.95. Therefore the result of the present study which used the measurement system was in agreement with the findings of these authors. The correlation of the weight with the oxygen consumption was positive, meaning that the bigger the animal, the more the oxygen uptake.

Eventhough the value of **(b)** in this experiment was similar to the finding of some authors, namely Krueger (1960), Kennedy and Mihursky (1982), Bayne et.al.(1976) who stated that to obtain a good result of **(b)** value, the experiment should be measured in animals with large ranges of weight (1 – 50 units). The biological meaning of the **(b)** value was still not clear. In fact, the value of **(b)** obtained from this experiment came from individuals having the same age class and of course having limited ponderal distribution. Furthermore in interpreting the value of **(b)** one should be extremely careful, since this value is influenced by temperature (Verrannan, 1972, 1974), aquatic condition (Toulmond, 1987), nutritive condition (Widdows, 1978), starvation (Marsden et.al., 1973; Newell et.al., 1976), season and food level

(Newell and Roy, 1973 . and Widdows, 1978).

One of the primary endogenous factors affecting oxygen uptake in bivalves is shell valve movement. Any factor which affects the degree to which shell valves are, or remain open will affect water flow and necessarily affect oxygen consumption rate.

The effect of shell valve activity on oxygen consumption rates have been noted by several authors and they stated that the degree to which

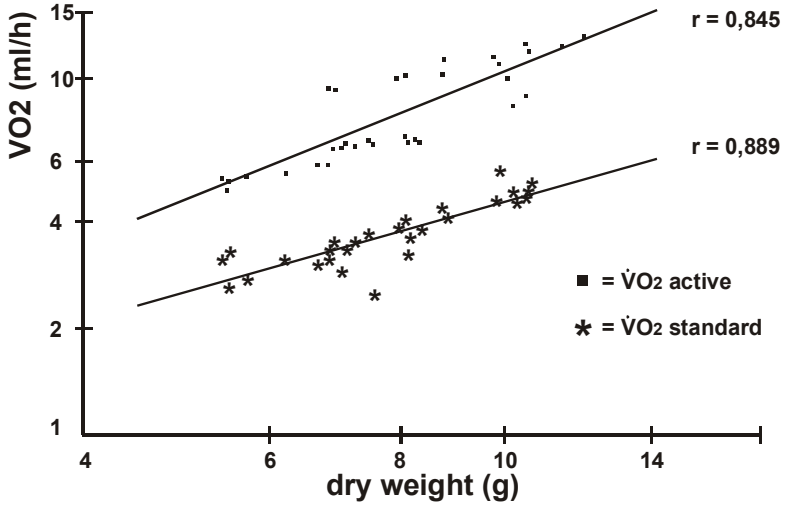

Figure 3. Relationship between $\dot{V}O_2$ with dry body weight of the Brest population

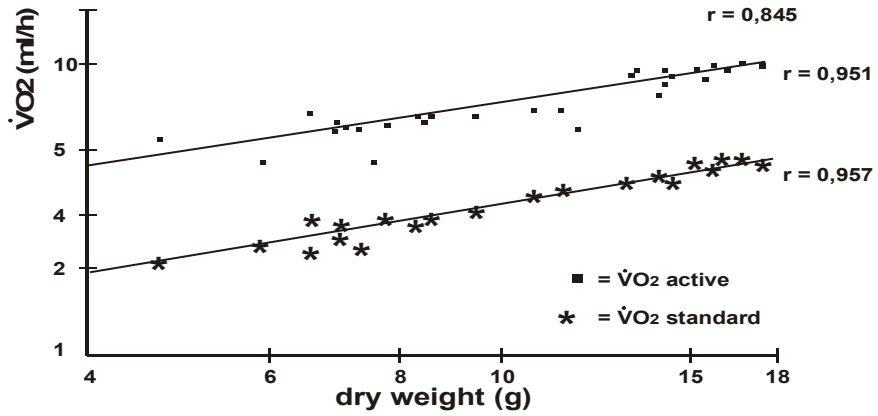

Figure 4. Relationship between $\dot{V}O_2$ with dry body weight of the Scotland population of the shell with its valve open will influence the oxygen consumption rate.

Shell valve activity, pumping rate and cilliary activity can physically restrict the amount of oxygen made available of bivalve. In addition, these activities along with other physiological processes (e.g. feeding, digestion and excretion) are

energetically costly and the level of activity will also affect the oxygen consumption (Shumway, 1981).

The present study showed that the active oxygen consumption for both populations was higher than the standard of oxygen consumption which is in agreement with the findings of authors mentioned above, since the experimental animals open their valves for a longer time so that the rate of oxygen consumption will automatically become higher.

CONCLUSION

- The measuring system set up in this experiment showed a good result in the measurement of oxygen consumption.
- The rate of oxygen consumption is strongly influenced by the body weight and the presence of feed.
- To produce a good value of **(b)** the numbers of experimental animal should have a very wide range of weight (1 –50 units).

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