

## THE CONTRIBUTION OF RESPIRATORY PAPULAE AND TUBE FEET TO OXYGEN UPTAKE IN THE SEA STAR *ASTERIAS FORBESI* (DESOR)

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### SUMMARY

Oxygen uptake of 13 *Asterias forbesi* (Desor) was measured at 7.5°C with all surfaces exposed to sea water and when either the aboral or oral surface was covered. Oxygen uptake by the whole animal ranged from 6 to 30  $\mu\text{l O}_2/\text{g}$  per h and was related to body wet weight by the relationship  $\dot{V}_{\text{O}_2(\text{total})} = 79 W^{0.46}$  (or  $\log \dot{V}_{\text{O}_2(\text{total})} = -0.46 \log W + 1.90$ ). The aboral surface (papulae) and oral surface (tube feet) were able to maintain 61 and 52%, respectively, of the total oxygen uptake of the sea star. Therefore, the papulae, which are associated with the perivisceral celom, and the tube feet, components of the water-vascular system, can each serve a major role in oxygen uptake by *A. forbesi*.

Key words: oxygen uptake, sea star, respiratory surfaces, tube feet, papulae, water-vascular system, perivisceral celom.

### INTRODUCTION

Most echinoderms possess more than one respiratory surface (Farmanfarmaian, 1966). The relative importance of the different respiratory surfaces to the echinoderm's total oxygen consumption has been determined for holothuroids (Winterstein, 1909; Newell and Courtney, 1965; Brown and Shick, 1979) and echinoids (Farmanfarmaian, 1959; Steen, 1965; Fenner, 1973; Smith, 1980). Morphological studies suggest that the respiratory surfaces of asteroids (sea stars) are the tube feet and papulae (Tiedemann, 1816; Hyman, 1955; Cobb, 1978). Tube feet are muscular appendages of the oral body wall, connected internally to the water-vascular system. Papulae are evaginations of the body wall, emerging between skeletal ossicles. The lumen of each papula communicates directly with the perivisceral celom. Most of the papulae are on the aboral surface, but some occur on the oral surface.

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Conflicting statements appear in the literature concerning the amount of oxygen uptake via these two respiratory surfaces. Koller and Meyer (1933) and Meyer (1935) attempted to quantify the contribution of the tube feet to the total oxygen consumption of *Asterias rubens*. A series of experiments were performed in which they covered individual arms with rubber bags, ligated the radial canal of the water-vascular system, ligated individual tube feet, and covered the ambulacral grooves, and thus the tube feet, with rubber and adhesive tape. From these experiments, they concluded that the tube feet account for about two-thirds of the sea star's total oxygen uptake. However, these investigators never performed the reciprocal experiment of covering the papulae and leaving the tube feet exposed. Farmanfarmanian (1959) and Steen (1965) directly demonstrated that most of the oxygen uptake in echinoids occurs through the tube foot-ampulla portion of the water-vascular system. By extending these results to asteroids, Farmanfarmanian (1966) agreed with the conclusion of Koller and Meyer (1933) and Meyer (1935) that the tube feet represent the principle respiratory surface in sea stars.

Yet, Binyon (1972) stated that the papulae play a dominant role in the gas exchange of sea stars, and Cobb (1978) reported that the papulae account for 50% of the total respiration in asteroids. Neither author cited new evidence on the respiratory function of papulae to justify their statements. In other studies, Johansen and Petersen (1971) found that the papulae in the nidamental chamber of the cushion star, *Pteraster tessellatus*, are responsible for 90% of the animal's total oxygen uptake, while Shick (1976) discussed the possible respiratory significance of the epi-proctal cone of the mud star, *Ctenodiscus crispatus*. However, the nidamental chamber and the epi-proctal cone are highly specialized structures and are not common asteroid organs.

In light of the previous reports, it is not surprising that recent reviews of echinoderm biology (Meglitsch, 1972; Barnes, 1980) have cautiously attributed equal importance to the papulae and tube feet in the respiration of sea stars. The present study was designed to establish clearly the relative contributions of the papulae and tube feet to the total oxygen uptake of the sea star, *Asterias forbesi* (Desor).

#### MATERIALS AND METHODS

Thirteen *A. forbesi*, ranging from 26 to 100 mm in radius, and from 10 to 158 g in wet weight, were collected from the intertidal zone on the coast of Massachusetts. They were kept in the laboratory in sea water maintained at  $7.5 \pm 0.5$  °C, the normal winter temperature at the collecting sites. The salinity ranged from 33.6 to 37.3‰. The sea stars were fed weekly on pieces of fish.

The oxygen uptake of individual *A. forbesi* was measured in a sea water-filled respirometer constructed from an oxygen impermeable, flexible, plastic container with a screw cap (Fig. 1). A plastic grid with 1 cm<sup>2</sup> openings, fastened across the

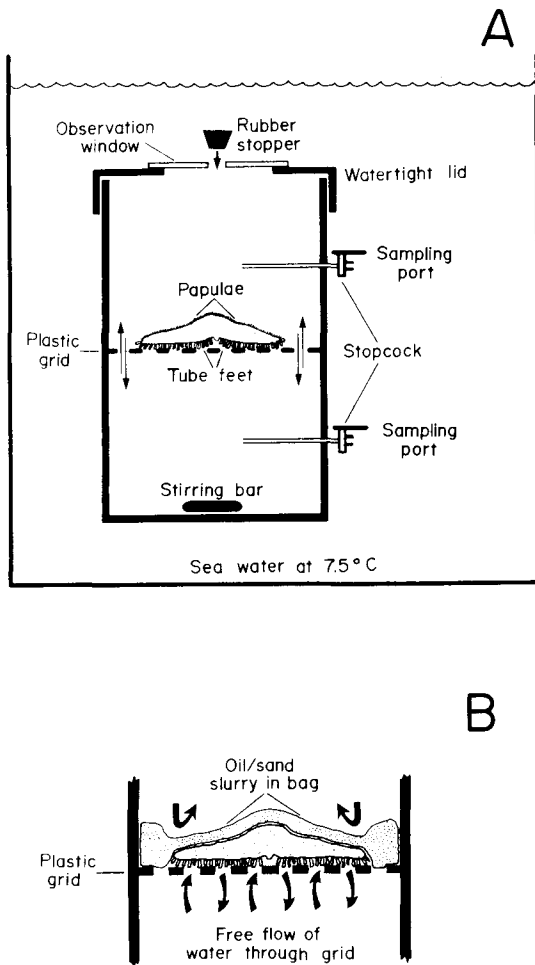


Fig. 1. Schematic diagram of the experimental apparatus. (A) The entire respirometer containing a sea star, as it would appear during the determination of  $\dot{V}_{O_{2(\text{total})}}$ . (B) A more detailed diagram of the sea star covered by the oil-sand slurry bag during the determination of  $\dot{V}_{O_{2(\text{oral})}}$ .

center of the container, divided the respirometer into an upper chamber (vol., 1530 ml) and a lower chamber (vol., 1170 ml). When a sea star was placed on the grid in the upper chamber, its oral surface was freely exposed, through the grid, to the water in the lower chamber. Needles piercing the wall of the respirometer provided water sampling ports in both the upper and lower chambers. A magnetic stirring bar in the lower chamber mixed the water. The container lid had a plexiglass observation window with an 8 mm hole drilled in the center. Any air bubbles could be removed easily through this hole, which was fitted with a rubber stopper during the measurement periods. The entire apparatus was immersed in a sea-water bath at  $7.5 \pm 0.5^\circ\text{C}$ .

Before the following experiments were performed, each sea star was allowed at least 12–24 h to acclimate to the upper chamber of the respirometer, during which time the sea water was aerated.

#### *Whole animal oxygen uptake*

The total rate of oxygen uptake,  $\dot{V}_{O_2(\text{total})}$ , of an acclimated sea star was determined by completely filling the respirometer with sea water and securing the lid. Air bubbles were removed and the respirometer was sealed. The magnetic stirring bar was activated to ensure an even distribution of dissolved oxygen in the respirometer. Water samples (0.5 ml) were taken from both the upper and lower chambers at the start of the experiment, and again after 1–4 h. A Radiometer 27 blood-gas analyzer was used to determine the oxygen partial pressures ( $P_{O_2}$ ) of each sample. The duration of each experiment depended on the animal's mass and oxygen uptake, lasting long enough for the undisturbed sea star to decrease the  $P_{O_2}$  of the sea water in the closed respirometer by 15–20 mm Hg. The  $P_{O_2}$  electrode was equilibrated to the water temperature in the respirometer ( $7.5 \pm 0.5^\circ\text{C}$ ), and was calibrated with air-saturated sea water before each pair of  $P_{O_2}$  readings was taken.

In this series of experiments, water could move freely across the grid between the upper and lower chambers, so the respirometer comprised a single vessel. The average  $P_{O_2}$  of the samples from the upper and lower chambers, between which there was usually no significant difference, was always taken as the  $P_{O_2}$  of the entire respirometer.

Total oxygen consumption, in  $\mu\text{l O}_2/\text{g}$  per h, was calculated as follows:

$$\dot{V}_{O_2(\text{total})} = \frac{\alpha \cdot \Delta P_{O_2} \cdot V}{t \cdot W},$$

where  $\alpha$  is the oxygen solubility of sea water, in  $\mu\text{l O}_2/\text{mm Hg per l}$  at  $7.5^\circ\text{C}$ ;  $\Delta P_{O_2}$ , in mm Hg, is the decrease in  $P_{O_2}$  in the respirometer during the experiment;  $t$  is the time, in hours, that the respirometer was closed;  $W$  is the wet weight, in grams, of the sea star; and  $V$  is the total volume, in liters, of the container minus the volume of the sea star. After all measurements were made on a particular sea star, its volume was determined by water displacement.

#### *Oxygen uptake by the oral surface, $\dot{V}_{O_2(\text{oral})}$*

After  $\dot{V}_{O_2(\text{total})}$  was determined, the sea water in the respirometer was replaced with fresh, air-saturated sea water. A double-layered plastic bag, containing a known volume of mixed sand and mineral oil, was laid over the aboral surface of the sea star and pressed against the sides of the respirometer to form a seal between the upper and lower chambers (Fig. 1B). Thus, a 2- to 3-cm thick barrier of plastic, oil and sand was created between the papulae on the aboral surface of the sea star and the sea water in the upper chamber. The respirometer lid was replaced and air bubbles were removed.

Oxygen uptake from the upper and lower chambers was then measured, as described above. Since only the oral surface was exposed to the sea water in the lower chamber (Fig. 1B),  $\dot{V}_{O_2(\text{oral})}$  could be calculated from the decrease in the  $P_{O_2}$  in that chamber.

Control experiments without a sea star in the chamber were performed to assess the ability of the oil, sand and plastic barrier to isolate the dissolved oxygen in the upper chamber from that in the lower chamber. When  $P_{O_2}$  gradients twice those expected during the actual experiments were established between the upper and lower chambers, there was usually no significant oxygen transfer between these chambers. However, in a few actual experiments, a barely measurable decrease in the  $P_{O_2}$  of the upper chamber was recorded. This was attributed to an inadequate seal between the bag and the respirometer wall, which probably allowed a small amount of oxygen diffusion between the chambers, rather than diffusion through the bag or oxygen uptake by the covered aboral surface. On this assumption, any measurable uptake of oxygen from the upper chamber was added to that from the lower chamber to yield  $\dot{V}_{O_2(\text{oral})}$ .

#### *Oxygen uptake by the aboral surface, $\dot{V}_{O_2(\text{aboral})}$*

In these experiments, the sea star was placed oral side up on the grid in the respirometer. The oil-sand slurry in the plastic bag was laid over the oral surface of the animal and spread to the edges of the respirometer. This completely covered the ambulacral grooves containing the tube feet, so that only the papulae on the animal's aboral surface were exposed to the sea water in the bottom chamber. Measurement of  $P_{O_2}$  and calculation of oxygen uptake by the aboral surface,  $\dot{V}_{O_2(\text{aboral})}$ , was carried out as described above.

#### *Experimental protocol*

After the acclimation period,  $\dot{V}_{O_2(\text{total})}$  was measured, followed immediately by the determination of  $\dot{V}_{O_2(\text{oral})}$  on the same animal. A second determination of  $\dot{V}_{O_2(\text{total})}$  was performed to ensure that covering the aboral surface had not injured or otherwise disturbed the sea star, thus altering its respiratory rate. Then, a second measurement of  $\dot{V}_{O_2(\text{oral})}$  was made.

$\dot{V}_{O_2(\text{aboral})}$  was similarly determined immediately after measuring the  $\dot{V}_{O_2(\text{total})}$  of an acclimated sea star. However, in these experiments a second determination of  $\dot{V}_{O_2(\text{total})}$  or  $\dot{V}_{O_2(\text{aboral})}$  was not made.

Differences between population means were tested with Student's *t*-test (significance level of 0.05).

## RESULTS

$\dot{V}_{O_2(\text{total})}$  was determined twice for each sea star (before and after the measurement

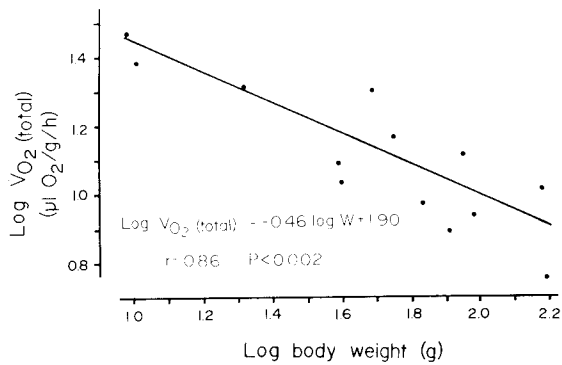


Fig. 2. The relationship between the log body wet weight and log  $\dot{V}_{O_2(\text{total})}$  at 7.5°C, determined from 13 *Asterias forbesi*.

of  $\dot{V}_{O_2(\text{oral})}$ . No significant difference ( $P > 0.10$ ) was found between the two sets of values, so their mean was then taken as representative of each individual, and was used in subsequent analyses.

$\dot{V}_{O_2(\text{total})}$  ranged from 6  $\mu\text{l O}_2/\text{g}$  per h in a 150 g sea star to 30  $\mu\text{l O}_2/\text{g}$  per h in a 10 g sea star at  $7.5 \pm 0.5^\circ\text{C}$ . The relationship between body wet weight and  $\dot{V}_{O_2(\text{total})}$  of 13 *A. forbesi* is given by the equation  $\log \dot{V}_{O_2(\text{total})} = -0.46 \log W + 1.90$  and is presented in Fig. 2.

The two separate measurements of  $\dot{V}_{O_2(\text{oral})}$ , made on each of 8 *A. forbesi*, were not significantly different ( $P > 0.10$ ), so their mean was also taken as representative for each animal. As with  $\dot{V}_{O_2(\text{total})}$ , weight-specific oxygen uptake by the oral surface decreased with increasing body weight. This relationship is described by the equation  $\log \dot{V}_{O_2(\text{oral})} = -0.48 \log W + 1.68$  (correlation coefficient,  $r = -0.96$  and  $P < 0.001$ ).

The linear regression of  $\dot{V}_{O_2(\text{oral})}$  against  $\dot{V}_{O_2(\text{total})}$  for the 8 animals examined (Fig. 3) has a slope of 0.52 and a high correlation coefficient of 0.87. Thus, when only the oral surface of a sea star was exposed to sea water, approximately one-half of its total oxygen uptake occurred, regardless of the animal's total oxygen uptake.

The oxygen uptake by the aboral surface was also dependent on body weight, as described by the equation  $\log \dot{V}_{O_2(\text{aboral})} = -0.72 \log W + 2.13$  ( $r = -0.99$ ,  $P < 0.01$ ). From the 5 sea stars examined, the regression of  $\dot{V}_{O_2(\text{aboral})}$  against  $\dot{V}_{O_2(\text{total})}$  (Fig. 3) has a slope of 0.61, with an  $r$  value of 0.95. Thus, the aboral surface alone achieved more than one-half of the animal's total oxygen uptake.

## DISCUSSION

The total oxygen uptakes by *A. forbesi*, measured at 7.5°C (Fig. 2), compare favorably with total oxygen uptakes of other echinoderms measured at temperatures

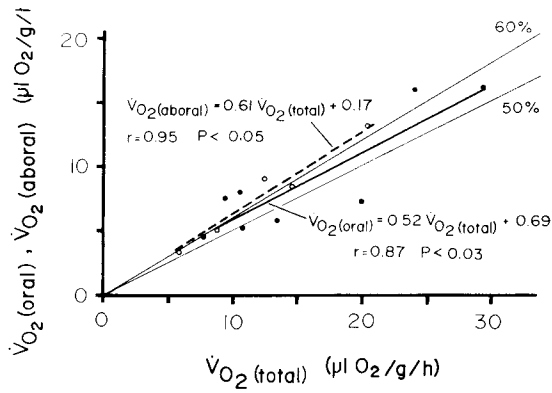


Fig. 3. The relationship between  $\dot{V}_{O_2(\text{total})}$  and  $\dot{V}_{O_2(\text{oral})}$  (—, •) and  $\dot{V}_{O_2(\text{aboral})}$  (----, ○) in *Asterias forbesi*. Also indicated are lines representing 50 and 60% of  $\dot{V}_{O_2(\text{total})}$ .

below 10°C (Belman and Giese, 1974; Webster, 1975). The exponential factor (–0.46) for the inverse relationship between  $\dot{V}_{O_2(\text{total})}$  and body weight of *A. forbesi* is low compared with many invertebrates (Prosser, 1973), but within the range reported for other sea stars (Koller and Meyer, 1933).

The present experiments have temporarily restricted oxygen uptake by either the oral or aboral surface. Since no significant difference ( $P > 0.10$ ) occurred between the values of  $\dot{V}_{O_2(\text{total})}$  measured before and after the determination of  $\dot{V}_{O_2(\text{oral})}$ , our technique of covering one surface of the sea star did not permanently disrupt the normal processes of gas exchange. Moreover, these experimental conditions reflect those in the field. Sea stars are commonly found in very dense masses on mussel beds with their oral surfaces tightly applied to the substrate while feeding. This behavior similarly prevents exposure of the tube feet to sea water. Although other oral or aboral structures may make minor contributions to oxygen uptake, the tube feet and papulae have long been suspected to be the major sites of gas exchange in asteroids (see Hyman, 1955; Farmanfarmanian, 1966). Here, we have demonstrated that in *A. forbesi* either the oral surface (i.e. tube feet) or the aboral surface (i.e. papulae) alone can remove oxygen from sea water at rates exceeding 50% of  $\dot{V}_{O_2(\text{total})}$  (Fig. 3).

Notably, the individually determined  $\dot{V}_{O_2}$  of the oral and aboral surfaces collectively exceed  $\dot{V}_{O_2(\text{total})}$ . Oxygen uptake through one surface is apparently increased when the other surface is deprived of oxygen. An enhanced diffusion, driven by an increased oxygen gradient across the respiratory surface, would occur if the internal fluid  $P_{O_2}$  fell when one respiratory surface was covered. Furthermore, after removing the oil–sand slurry bag at the end of the  $\dot{V}_{O_2(\text{aboral})}$  experiments, the tips of some sea stars' arms were curled through the plastic grid in what may have been an attempt to right themselves. This righting behavior obviously increases the animal's

activity and may explain the slightly higher rates of oxygen uptake determined for the aboral surface.

Although either the aboral or oral surface of *A. forbesi* can account for the major portion of total oxygen uptake, the relative contribution of these surfaces to the oxygen tension in the celomic fluid of sea stars has not been established. Both the perivisceral celom and the water-vascular system have an internal circulation provided by a ciliated epithelium (Gemmill, 1915; Budington, 1942; Fenner, 1973; Cobb, 1978). The perivisceral fluid, bathing the metabolically active pyloric ceca and gonads, circulates directly into the internal lumen of the papulae. Thus, the transport of oxygen from the sea water to the internal tissues after diffusing across the thin membrane of the papulae can be easily visualized. Oxygen diffusion would be aided by low  $P_{O_2}$  levels in the perivisceral fluid, as occurs in the sea star *Pteraster tessellatus* (Johansen and Petersen, 1971) and the sea urchin *Strongylocentrotus purpuratus* (Webster and Giese, 1975). On the other hand, the water-vascular fluid in the tube feet does not directly enter the perivisceral celom. External oxygen must diffuse across two tissue barriers, the thick walls of the tube feet and the walls of the ampullae, before reaching the main body celom. Also, the tube feet are muscular (Hyman, 1955), since they are organs of locomotion. Therefore, a significant proportion of the oxygen uptake attributed to the oral surface may simply reflect the oxygen consumed by tube activity, rather than oxygen uptake for distribution to tissues in the perivisceral celom.

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