

products of visual function from the illuminated rhabdomere, to take up cast-off rhabdomere membrane, or to sequester hemolymph proteins taken into the receptor cell by pinocytotic vesicles associated with the rhabdomere (11-13). Our results show that the receptor cells can continue to function, though at a reduced level, with the system of multivesicular bodies eliminated. The regulation of diet now gives us a second means (in addition to varying illumination) of manipulating receptor cell ultrastructure.

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6. Retinal action potentials were recorded from intact mosquitoes by means of electrolytically sharpened stainless steel electrodes which showed no photoelectric effect. Amplification was with a neutralized input capacity amplifier (Bioelectric Instruments, Inc., NF1), and the light source was a microscope illuminator (tungsten filament) to which was attached a shutter assembly. Illumination at the level of the eye was 5.3×10^4 erg (cm²) sec; stimulus duration was 500 msec. Mosquitoes were dark-adapted for a minimum of 30 minutes before recordings were made.
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Oxygen Consumption and Pumping Rates in the Hard Clam *Mercenaria mercenaria*: A Direct Method

Abstract. *Respiratory and pumping rates in the hard clam Mercenaria mercenaria were measured directly in running seawater; the quantity of oxygen used was calculated from the difference in concentrations in incurrent and excurrent water. A linear relationship between pumping and respiratory rates suggests regulation of water transport by oxygen requirement rather than by feeding.*

Reports of respiration in lamelli-branch mollusks emphasize the variability in measurements of ventilation rates, and the lack of control of ventilation rate by oxygen requirements because the gill is highly specialized for filter-feeding (1, 2). Feeding currents are thought to be greater than those required for respiration and "this flow is kept up for feeding purposes, and the respiratory function is incidental" (2).

Indirect measurements of water flow through bivalves, such as clearance rate of particles in suspension, are questionable because the animals may vary unpredictably the percentage of particle retention. Because measurements of oxygen uptake in flowing water systems are calculated on the basis of water flow through the bivalves, many of these are also questionable. Oxygen uptake has been measured in closed standing-water systems of low volume in which metabolites may accumulate (3, 4).

Our methods avoid these problems and are especially suited for the siphonate bivalves. The direct method of measurement of water pumpage (5) is combined with the polarographic method of measuring oxygen concentration in flowing water (6) (Fig. 1). *Mercenaria mercenaria* are kept in aquariums with bottoms covered by sand (15 to 20 cm deep), supplied with running seawater cooled or warmed as needed. Seawater, well aerated and dyed with green food coloring (1 ml/liter), is held in reservoirs at controlled temperature. The dye solution leaving the reservoir passes through a coil set in the aquarium for temperature adjustment, and through a flowmeter to a fine glass tube mounted within a wide tube which carries away the overflow from the aquarium as well as the excurrent stream from the clam, to a weir which maintains a constant level within the aquarium. An adjustable capillary tube is also mounted within the wide tube. The system of tubes is positioned with

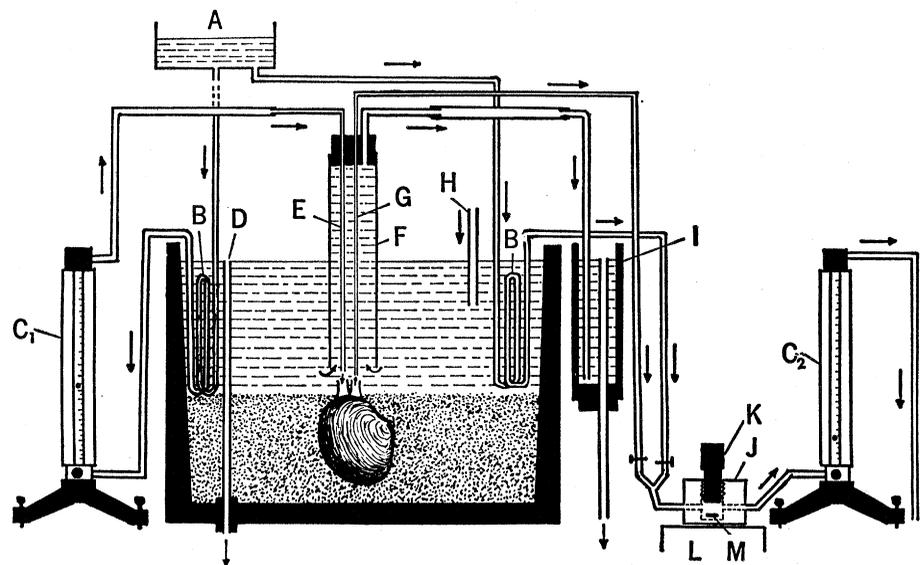


Fig. 1. Apparatus for measurement of pumping and respiration rates in *Mercenaria*. (A) Reservoir for dyed seawater, (B) coil for temperature adjustment, (C₁) flowmeter in incurrent water, (C₂) flowmeter for outflow from polarographic chamber, (D) overflow, (E) inflow of dyed seawater, (F) aquarium overflow, (G) capillary tube for sampling constant level in aquarium, (H) inflow of seawater to aquarium, (I) weir to maintain constant level in aquarium, (J) polarographic chamber, (K) Beckman oxygen electrode, (L) magnetic stirrer, and (M) stirring bar.

the fine glass tube about 0.5 cm from the incurrent siphon and the capillary tube at the center of the stream from the excurrent siphon, and frequently within the siphon.

To determine pumping rate, the flow of dyed seawater is increased gradually until any further increase results in an overflow from the siphon; at this point the dye solution is providing all the incurrent water and the flowmeter is read.

A water sample from the excurrent stream passes through the capillary tube and the polarographic chamber into a flowmeter that controls the speed of the flow. Care is taken to maintain this flow at not more than half of the pumping rate of the animal to avoid entrainment of aquarium water in the sample.

The polarographic chamber was constructed by drilling three channels in a plastic block, two for the flowing water and a third for a Beckman oxygen electrode. A stirring bar is inserted into the hole and the polarographic chamber is placed, during the measurements, on a magnetic stirrer. To determine oxygen concentration in incurrent water, the dye solution from the reservoir is shunted to the polarographic chamber through a coil submerged in the aquarium. Pumping rate and oxygen content of the excurrent stream are measured at 3-minute intervals and the period of observation on each clam extends from 18 to 60 minutes.

The quantity of oxygen consumed by each clam is determined by multiplying the reduction in oxygen content (in milliliters) from the incurrent to the excurrent streams by the pumping rate in liters per hour and is expressed as milliliters (at 0°C and 760 mm-Hg) consumed per hour per clam.

Pumping and respiratory rates for the hard clam may be studied with this method under a variety of combinations of temperature and salinity, oxygen tension, and so forth.

Pumping rates and oxygen consumption of 17 clams were measured at 24°C and a salinity of 20.5 to 21.3 per mille (Fig. 2). We calculated the following linear regression equation from these data by the method of least squares:

$$R = 1.118X + 0.836$$

where R is the pumping rate in liters per hour and X is the oxygen uptake in milliliters per hour per clam. The

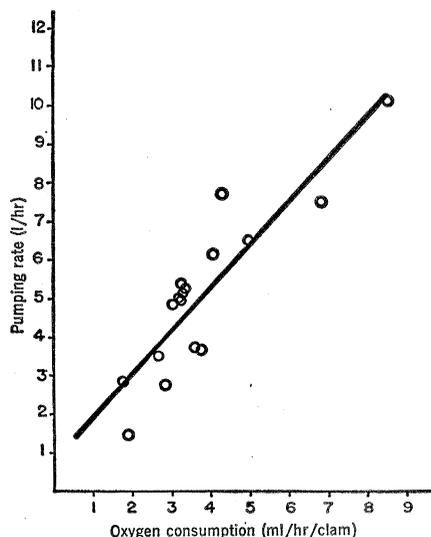


Fig. 2. Pumping rate of *M. mercenaria* plotted against respiration rate at 24°C and a salinity of 20.5 to 21.3 per mille, line fitted as $Y = 1.118X + 0.836$. Each point represents the mean of 6 to 20 values of pumping and respiration rates obtained for one clam.

correlation coefficient (r) was .88.

In similar determinations with 11 clams at 15°C and with salinity ranging from 19.7 to 20.2 per mille, the linear regression equation was

$$R = 1.262X + 0.230$$

The correlation coefficient (r) was .95.

The linear relationship between water transport and oxygen consumption indicates a constant efficiency in withdrawal of oxygen from the water. The ratio of oxygen reduction to oxygen content of the incurrent water at 15°C averaged 13 percent and at 24°C averaged 15 percent. All values were within ± 5 percent.

Calculations based on these results differ significantly from reported values in two additional respects. (i) The relation between water transport and oxygen consumption for several species of bivalves ranges from 1 to 40 liters of water per milliliter of oxygen removed (3). The range for *M. mercenaria* is 0.95 to 2.0 liters at 15°C, and 0.76 to 1.8 liters at 24°C lower than reported values for any other bivalve. (ii) Oxygen consumption for a variety of bivalves expressed as Q_{O_2} (weight specific respiration in micro-liters of oxygen per gram wet weight per hour) ranges from 4 to 71 (1, 4, 7). For *M. mercenaria* Q_{O_2} at 15°C ranges between 20 and 203, and at 24°C be-

tween 68 and 324. For *M. mercenaria* Q_{O_2} is generally higher than that reported for other bivalves. Furthermore, the Q_{O_2} is a function of pumping rate which is not necessarily dependent on size and may vary widely for a given animal at constant temperature and salinity.

Thus, oxygen removal is a consistent function of water transport in *M. mercenaria* and water transport for this species may be at least partially regulated by oxygen requirements. Similar suggestions were made by Koch and Hers (8) concerning regulation of water transport by the exhalant siphon in *Anodonta* in connection with respiratory needs ("les intermittences d'ouverture et de fermeture du siphon exhalant peuvent régler la ventilation branchiale en rapport avec les besoins respiratoires de l'anodonte") and by Bayne (9) for *Mytilus perna* ("these results suggested that *Mytilus perna* is able to regulate its oxygen consumption by increasing the volume of water passing through its mantle cavity per unit time").

This is in contrast to the generally accepted idea that respiration in bivalves is only incidental to water flow maintained for feeding (1, 2). This has been concluded by others because of the low oxygen demand and the low and inconsistent percentage of utilization. Our technique avoids the drawbacks of previous methods; pumping rate is determined directly, and the determinations are made in an open system closely approximating natural conditions.

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