Chapter II.2 The Application of Polarographic Oxygen Sensors for Continuous Assessment of Gas Exchange in Aquatic Animals

J.P. Lomholt and K. Johansen[1]

1 Introduction

Most aquatic invertebrates, as well as fish, depend on active ventilation of water for irrigating respiratory surfaces. These may be specialized in the form of gills or ctenidia, or be unspecialized parts of the general body surface. Movement of water may occur by active muscular pumping or by ciliary action. Typically, the passage of water across respiratory surfaces occurs in discrete channels and most commonly unidirectionally.

For the purpose of analyzing respiratory gas exchange, it becomes important to confine the ventilatory current for measurement of both volume ventilated and gas composition of the expired water. This paper reports on the application of polarographic oxygen sensors (POS) for continuous measurement of O_2 tension in expired water from fishes and selected invertebrates. Electromagnetic flow-measuring techniques have been used in combination with the POS for evaluation of total external gas exchange. Traditionally such analysis has depended on sequential sampling of water for analysis of water O_2 content by the Winkler method (App. D), or measurement of p_{O_2} in water by injection of sampled water into cuvettes holding POS (Chap. II.10). Such sample measurements of expired water gas composition may often give a distorted picture of normal gas exchange, since natural breathing movements may be periodic or phasic, giving intermittent values of O_2 tension in expired water of indeterminable value unless related to ventilatory changes.

We will describe some experimental arrangements, which we have used to record continuously the inspired as well as expired oxygen tension and hence the extraction of oxygen from the ventilatory current in fishes and invertebrates like decapod crustaceans and polychaetes. Additionally, these arrangements have permitted simultaneous and direct recording of the magnitude of the ventilatory current.

The equipment consists of a standard Radiometer p_{O_2} sensor (E 5046) mounted in a thermostatted cuvette (D 616) and connected to a Radiometer PHM 71 or 72 acid-base analyzer. The sensor cuvette is placed alongside the experimental aquarium, a few centimeters below the water surface. Water to be analyzed for O_2 tension is passed to the sensor cuvette by gravity through polyethylene cannula (Clay Adams PE 100).

1 Department of Zoophysiology, University of Aarhus, DK–8000 Aarhus C., Denmark

Polarographic Oxygen Sensors (ed. by Gnaiger/Forstner)

This material is sufficiently impermeable to allow the passage of water of zero oxygen tension without any measurable rise in tension, even if the passage time extends to 1–2 min.

A further check on the validity of the p_{O_2} readings of water passed through polyethylene catheters was made by setting the POS at zero by injecting an O_2-free solution (sodium sulfite and borax solution) into the cuvette. Subsequently, water deoxygenated by N_2 equilibration was passed through the polyethylene catheter into the cuvette without any readable change in the zero setting. This checking procedure excluded the possibility that O_2 molecules could pass through the catheter walls and distort the true water p_{O_2} values at the water flow rates employed.

By way of a three-way stopcock and a catheter equal in length to the sampling catheter the cuvette holding the POS is connected to a beaker placed in the experimental aquarium and containing water of the experimental temperature. During calibration the stopcock is switched and air-saturated water passes the POS at the same flow rate as during sampling from the animal.

In experiments involving rapid changes of the inspired oxygen tension two sensors should be employed, one of which is used for continuous monitoring of ambient p_{O_2}. If measurements are carried out at conditions of stable inspired p_{O_2}, one sensor will suffice.

The ventilatory flow of water passing the respiratory exchange surfaces is recorded by a Statham SP 2202 electromagnetic flowmeter.

2 Applications on Fish

Obtaining samples of exhaled water from undisturbed and unrestrained fish presents various problems. If exhaled water is sampled via a single catheter in the branchial cavity, uncertainty may exist as to whether the sample represents mixed exhaled water [1, 2]. On the other hand, attaching rubber membranes to animals to confine the expired water current in order to obtain truly mixed expired water, may place considerable restraint on the animal.

In a study of ventilation and gas exchange in the carp (*Cyprinus carpio*), we have employed a respiration mask (Fig. 1) made from thin flexible rubber [6]. The mask is tailored to the fish on the basis of a cast of the fish head, made by rapid impression of the head in dental cement in the way described in detail by Glass et al. [3]. The mask can be equipped with electromagnetic flowmeter probes at the exit orifices extending from each of the opercular openings. Catheters for continuous water sampling were similarly placed well inside the outflow extensions from the opercular openings (Fig. 1). The success of this procedure depends on the special morphology of the fish head and the magnitude of breathing movements. In the carp the movements of the mouthparts involved in breathing are rather small, whereas in other species, like trout, breathing involves large movements of the jaws. In such cases a mask technique is difficult to apply because it will restrict normal breathing movements. When useful, the mask will permit continuous sampling of truly mixed expired water for monitoring of oxygen tension as described above.

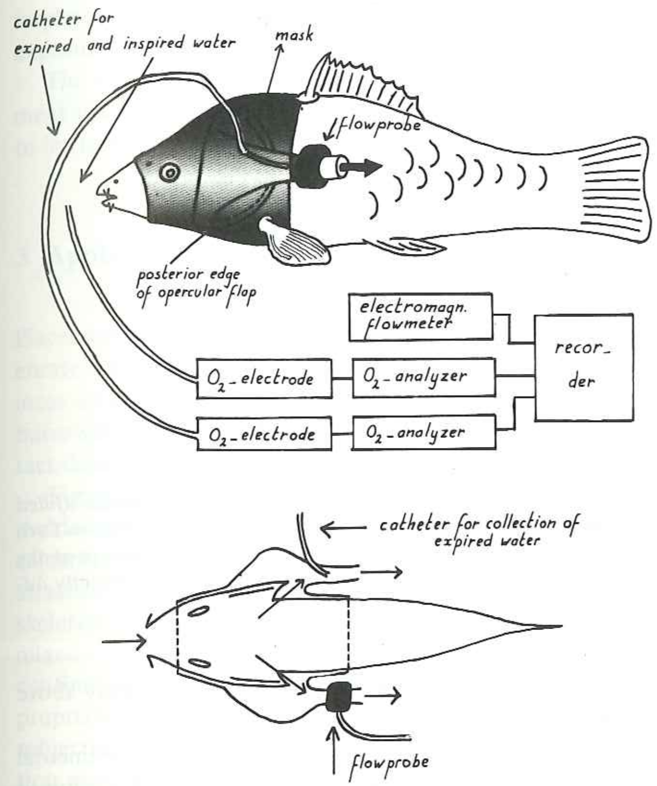

Fig. 1. Carp equipped with rubber mask permitting channeling of respiratory water current for measurement of ventilation by an electromagnetic flow probe and sampling of exhaled water [6]

This experimental arrangement was used on the carp to study how gas exchange parameters were affected by acclimation to hypoxic water [6]. It was demonstrated that not only were values for O_2 extraction from the respiratory water current higher than those hitherto reported for fish, but hypoxia acclimation resulted in a further elevation of O_2 extraction when compared with values from normoxic fish studied during acute exposure to hypoxic water (Fig. 2). These significant findings most certainly would have escaped notice if water O_2 tension had not been continuously monitored in combination with concurrent measurements of water ventilation.

A similar procedure was employed to study respiratory adjustments of the flounder (*Platichtys flesus*) to hypoxic water [5]. In this case advantage was taken of the fact that during undisturbed ventilation the fish rests buried in the sediment. Expired water was sampled continuously via a catheter attached inside the outflow orifice of a funnel placed in the sediment over the upper operculum (Fig. 3). Gill ventilation was recorded concurrently by attaching a flow probe to the neck of the collecting funnel. Alternatively, flow could be recorded from a separate funnel placed over the mout of the fish (Fig. 3). The latter procedure excludes the possibility that a portion of the water current may exit from the lower opercular opening. If care was taken that the edges of the funnels were buried in sediment, no water leaks around the edges could be detected.

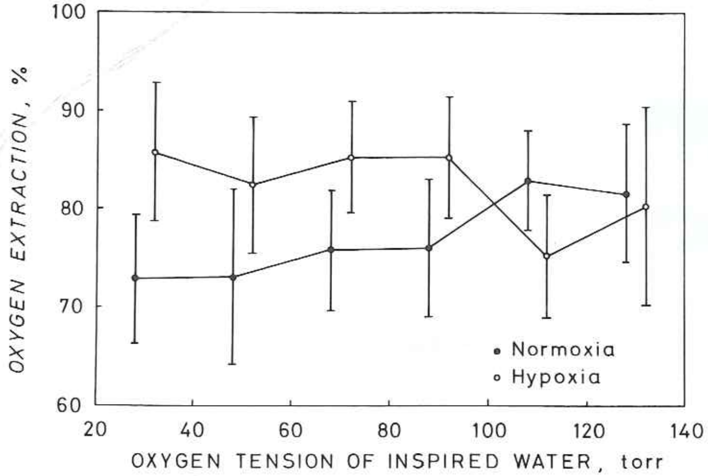

Fig. 2. Percentage extraction of oxygen from the ventilatory water current in normoxia- (*filled circles*) and hypoxia- (*open circles*) acclimated carp as a function of inspired oxygen tension. Each *point* is the average of 30–60 determinations in five different fish. *Bars* are 1 SD. Except at the highest level of oxygen tension average values for the two acclimation groups are significantly different (*t*-test, $P < 0.001$) [6]

This was checked by placing small amounts of a concentrated dye immediately above the sediment surface close to the edge of the funnel.

The main finding of the *Platichtys* study, directly dependent upon the experimental arrangement described above, was that hypoxia acclimation of the flounders allowed them to maintain an O_2 uptake rate in hypoxic water twice the value for flounders acutely exposed to hypoxia (cf. Chap. II.3). The higher level of O_2 uptake in the hypoxia-acclimated flounder compared to the acutely hypoxia-exposed fish resulted from an ability to maintain the same O_2-extraction in spite of a higher level of ventilation. The recording of these differences depended on the simultaneous monitoring of

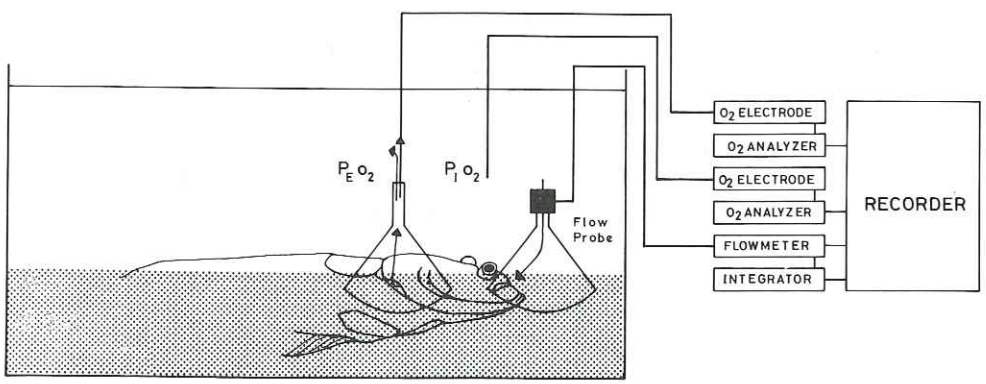

Fig. 3. Schematic illustration of a flounder in the experimental aquarium showing arrangement used for measurement of inspired ($p^I_{O_2}$) and expired ($p^e_{O_2}$) water O_2 tensions as well as inspired water flow [5]

inspired-expired O_2-tensions and concomittant ventilation changes during hypoxic exposure.

The funnel technique as applied to the flounder has the advantage that no equipment needs to be attached to the animal itself, but its application naturally is limited to burrowing animals.

3 Applications on Invertebrates

Placements of masks or other structures confining respiratory water currents in invertebrate animals are easily done if the species studied possesses an exoskeleton, such as most decapod crustaceans, shell-bearing mollusks, or tubiculous polychaetes. Similarly, burrowing forms, like many holothurians and polychaetes, can be studied without contact disturbance with the animal itself.

Figure 4 pictures the brachyuran crab, *Cancer magister,* equipped with a mask molded for an optimal fit after casting the area surrounding the mouth parts in dental cement. The mask can be glued to the exoskeleton by various fast-setting glues or be attached by anchoring bands (sutures, rubber bands) to protruding parts on the exoskeleton. A POS was placed with the sensing end inside the mask screening the well-mixed exhaled water before it exited through an electromagnetic flow probe giving a continuous record of the ventilated volume. Other catheters could be placed at appropriate sites for sampling of arterial and venous blood as indicated in Fig. 4. Crabs subjected to the procedure allowed samplings and continuous recordings of information needed for a complete gas exchange characterization of the animal [4]. The same approach has recently been applied on four other species of crabs including *Carcinus*

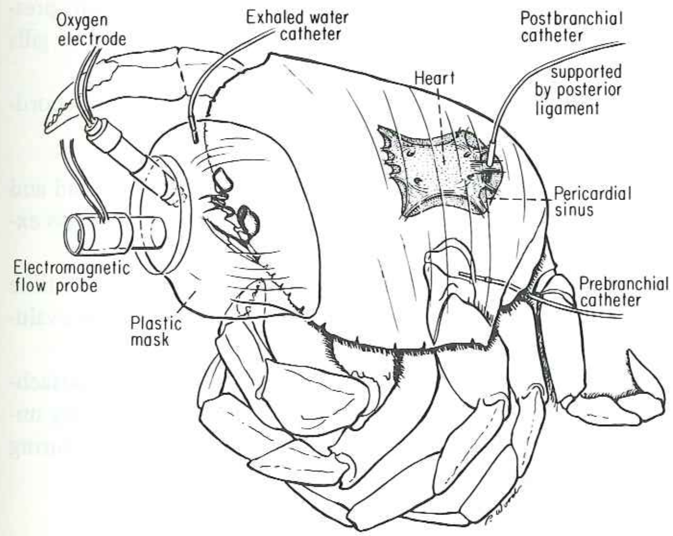

Fig. 4. Schematical representation showing placement of catheters for blood sampling and the mask containing the oxygen sensor and the electromagnetic flow probe [4]

maenas, weighing only about 20 g, thus demonstrating the applicability of the technique also to small species.

Among tubiculous polychaetes, species practicing unidirectional ventilation of a burrow are ideally suited for gas exchange analysis by the described method. Preliminary experiments on the polychaete *Nereis diversicolor,* weighing as little as 0.2 g, allowed simultaneous measurements of ventilation and O_2 extraction, yielding values for O_2 uptake corresponding well with those obtainable by closed respirometry.

For small species having a low ventilation, the direct passage of exhaled water from the expired current may not be possible, since a certain flow rate through the O_2-sensor cuvette is necessary for continued, stable sample flow. In such cases the application of a funnel for confinement of the expired water current in burrowing forms will act as a reservoir, giving average values of O_2-extraction when the funnel water is intermittently siphoned by gravity or aspirated by a pump through the O_2 cuvette.

Another important limitation of the technique relates to the dimensions of tubes and other flow channels interposed in the natural ventilatory current set up to connect the latter to the flow and p_{O_2} measuring devices. The propagation of ventilatory currents in all aquatic animals, whether they employ active muscular pumping or ciliary activity, is powered by very small pressure gradients, often less than 1 cm of H_2O (0.1 kPa). This fact implies that normal ventilatory currents are subject to very low values of resistance. If the arrangements of masks and tubes connecting the measuring equipment significantly alter the resistance to water flow, distorted values of ventilation may be recorded, leading in turn to nonrepresentative values of O_2-extraction. Because of this limitation, the largest possible flow probes and channeling devices should be used. However, since the flow probes are velocity sensors, the size of the probe diameter must be selected as a compromise between the requirement for a low resistance and a velocity high enough to detect pulsatile flow. In the described applications the added resistance imposed by connecting channeling devices in direct series with the natural ventilatory current have been small and negligible judged from pressure buildups less than 1 mm H_2O. By comparison the pressure gradient across the gills of fish is typically more than an order of magnitude greater.

In summary we see the following principal advantages of using continuous recordings of ventilatory flow and water O_2 tensions in aquatic animals:

a) The simultaneous and continuous monitoring of ventilatory flow and inspired and expired water p_{O_2} permits detection of phasic changes in O_2 extraction and gas exchange.

b) When ambient water p_{O_2} (inspired) varies as during hypoxic exposure, the time course of compensatory changes in ventilatory flow and O_2 extraction can be evaluated.

c) A minimum of physical disturbance of the experimental animal is required. Attachment of masks or connecting vessels imposes a minimum of restraint, allowing unhindered breathing movements and for many species data may be obtained during locomotory activity, like swimming.

d) For burrowing species no physical contact with the animal is required.

e) Calibration of POS, as well as flow probes, can be done easily and rapidly in situ.

References

1. Davis JC, Watters K (1970) Evaluation of opercular catherization as a method for sampling water expired by fish. J Fish Res Board Can 27:1627–1635
2. Garey WF (1967) Gas exchange, cardiac output and blood pressure in free-swimming carp (*Cyprinus carpio*). Ph D Dissertation, State Univ New York Buffalo
3. Glass ML, Wood SC, Johansen K (1978) The application of pneumotachography on small unrestrained animals. Comp Biochem Physiol 59A:425–427
4. Johansen K, Lenfant C, Mecklenburg TA (1970) Respiration in the crab, *Cancer magister.* Z Vergl Physiol 70:1–19
5. Kerstens A, Lomholt JP, Johansen K (1980) The ventilation, extraction and uptake of oxygen in undisturbed flounders, *Platichtys flesus.* (Responses to hypoxia acclimation). J Exp Biol 83: 169–179
6. Lomholt JP, Johansen K (1979) Hypoxia acclimation in carp – How it affects O_2-uptake, ventilation and O_2-extraction from water. Physiol Zool 52(1):38–49