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Opercular differential pressure as a predictor of metabolic oxygen demand in the starry flounder

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The feasibility of using a differential pressure sensor connected to an acoustic telemetry device to monitor opercular activity as a correlate of oxygen consumption was investigated. Four starry flounders *Platichthys stellatus* were fitted with a miniature differential pressure sensor mounted close to the operculum. A cannula was connected to the sensor and inserted under the operculum, inside the branchial cavity. Measurements of oxygen consumption and opercular activity were carried out over a broad range of metabolic activity, from the post-surgery stress (high metabolic rate) to routine metabolic rate the following day. Relationships between differential pressure changes (rate and amplitude) were highly correlated with oxygen consumption ($r^2 = 0{\cdot}74$ and $0{\cdot}60$ respectively). The results indicate that monitoring opercular activity offers an alternative method for measuring aerobic metabolism in free-swimming fishes in nature.

Key words: flounder; O_2 consumption; opercular activity; pressure sensor; telemetry.

INTRODUCTION

Animals select a habitat based in part on environmental conditions that minimize bioenergetic costs. Because of current inability to accurately assess the energy expenditure of free-swimming fishes, however, the exact nature of the three-way interaction between environment, physiology and behaviour remains difficult to establish. This inability to measure the energy dissipated as activity metabolism is largely responsible for the poor understanding of fish production systems (Kerr, 1982; Boisclair & Leggett, 1989; Boisclair & Sirois, 1993; Ney, 1993; Krohn & Boisclair, 1994). Quite obviously what is needed is a reliable, accurate method for the direct measure of fish activity metabolism at the spatial and temporal scales of natural field conditions.

Laboratory determination of energy budget and allocation and their subsequent extrapolation to the field are highly error prone since, within the confines of the laboratory, behaviour and activity patterns differ from those under natural conditions (Kerr, 1982). In recent years, different tools have been tested

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to improve the ability to predict fish energy demand under natural conditions. The most promising techniques are based on the telemetry of physiological correlates of activity. For instance, many studies have measured electrocardiograms because a correlation between heart rate and oxygen consumption is readily observable in many species (Priede & Young, 1977; Priede, 1983; Lucas *et al*., 1991, 1993; Lefrançois *et al*., 1998; Webber *et al*., 1998; Mercier *et al*., 2000). These correlations, however, are often not as strong as would be desirable for direct translation into metabolic terms (Thorarensen *et al*., 1996).

Building upon research initiated on squid *Illex illecebrosus* L. by Webber & O'Dor (1986), Webber *et al*. (2001*a*, *b*) tested a novel approach to estimate locomotor-related metabolic expenditure in free-ranging fishes. Using an acoustic telemetry device, these authors investigated the feasibility of using a miniature differential pressure sensor to measure swimming speed in free-swimming Atlantic cod *Gadus morhua* L. and European sea bass *Dicentrarchus labrax* (L.). The sensor was mounted on the caudal peduncle and relationships between pressure changes (range and amplitude), tailbeat frequency, swimming speed and rate of oxygen consumption were determined, providing robust tools for analysing fish locomotor activity.

Although the cost of locomotion is the largest component in the energy budget of a fish, its quantification alone does not permit a full understanding of the impact of the environment on energy requirements. When investigating internal energy allocation strategies in energy demanding situations, a method allowing a direct and complete evaluation of metabolic rate is desirable. A number of studies have shown that a strong relationship exists between ventilation rate and oxygen consumption (Kiceniuk & Jones, 1977). Only a few studies, however, have investigated the possibility of using opercular rate as a correlate of activity in free-swimming fishes. Oswald (1978) examined feeding and ventilation activity in brown trout *Salmo trutta* L. by detecting the electromyogram of the *adductor mandibulea* and using it as the input signal for an ultrasonic transmitter. Rogers & Weatherley (1983) monitored the electromyogram signals emanating from the *levator arcus palatini*, a small muscle involved in the operation of the operculum in rainbow trout *Oncorhynchus mykiss* (Walbaum). In line with these early studies, the objective of the current study was to apply the differential pressure sensor employed by Webber *et al*. (2001*a*, *b*) to monitor ventilatory activity as a means of monitoring oxygen consumption in fishes.

MATERIAL AND METHODS

FISH

Starry flounders *Platichthys stellatus* (Pallas) ($n = 4$, mean $\pm$ S.E. mass $= 0{\cdot}68 \pm 0{\cdot}1$ kg) were collected by otter trawling a region of sandy substratum in East Sound of Orcas Island and West of San Juan Island, Washington State, U.S.A. in June 2002. Fish were held at Friday Harbor Laboratory, (University of Washington) in large outdoor tanks in 12° C sea water for 1 week before experiments began.

EXPERIMENTAL SETUP

The resting respirometer comprised a plastic box (11·5 l) hermetically sealed and submerged in a thermoregulated water-bath (Fig. 1). A submersible pump supplied the respirometer with fully aerated water at a flow rate of 1–2 l min^{-1}. A second pump prevented water stratification in the respirometer. In flow-through mode, the water oxygen level in the respirometer ranged from 70 to 90% saturation.

SURGERY

Fish were anaesthetized (MS-222, 100 mg l^{-1}), weighed and placed eyed side up on the operation table. While on the surgery table, fish were artificially ventilated with water containing 50 mg l^{-1} of anaesthetic. They were first fitted with a miniature differential pressure sensor. The sensor was secured using two stitches. A cannula (PE 100) was inserted under the operculum, 5 mm inside the branchial cavity. The cannula (3 cm) was attached to the skin *via* two stitches. The cannula was then filled with sea water and connected to the positive port of the pressure sensor. The four-conductor wire connecting the sensor to the acoustic transmitter was sutured to the surface of the body. Finally, the tag was firmly secured on the fish using two sutures. The positions of the sensor and transmitter are shown in Fig. 2. Surgery was completed within 15 min. A full description of the pressure sensor used in this study is given in Webber *et al*. (2001*b*).

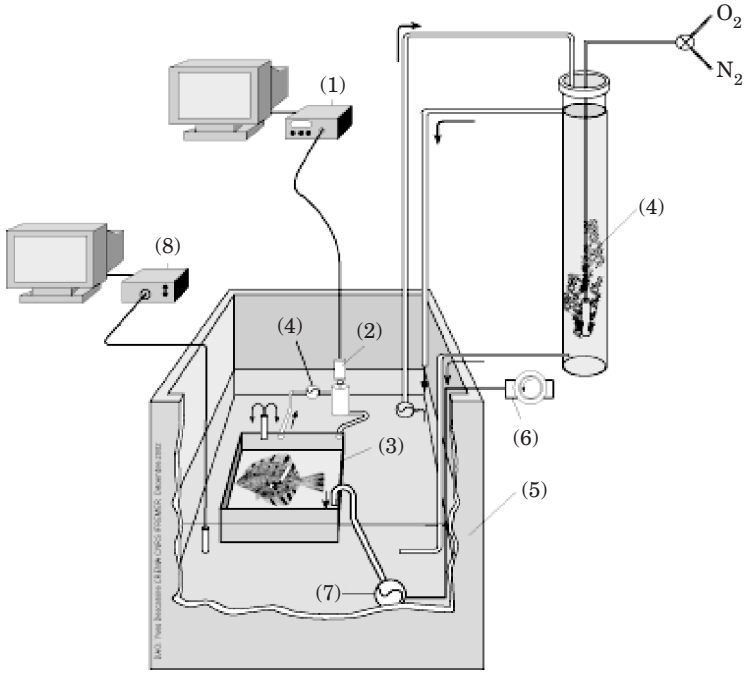

FIG. 1. The experimental setup. The respirometer chamber was made from a plastic box (3). A lid hermetically separated the measuring chamber (11·5 l) from a thermoregulation compartment (5). The measuring chamber was supplied with water from the outer compartment (1–2 l min^{-1}) *via* a pump (7) connected to a timer (6). The outer bath was bubbled with either air, in control condition, or nitrogen to induce hypoxia. A second pump prevented water stratification in the respirometer (4). Oxygen concentration in the respirometer chamber was measured using an Orion 083 060 oxygen probe (2) plugged to an Orion 1230 oxygen measuring system (1) connected to a computer. The acoustic pulses emitted by the transmitter were recovered *via* an hydrophone and a receiver (Vemco VR-20) interfaced with a computer using an RS-232 serial cable (8).

TELEMETRY

A V16TB transmitter (1·6 cm diameter, 8·0 cm length, 16 g mass in water) was provided by VEMCO (Shad Bay, Halifax County, Nova Scotia, Canada). The transmitter characteristics and mode of operation are described by Webber *et al.* (2001*b*). Briefly, V16TB transmitters are designed to operate in one of four modes. Mode 1 outputs a synchronization pulse followed by acoustic pulses representing the integrated pressure (*IP*) and pressure difference (*DP*). Modes 2 and 3 output a continuous stream of acoustic pulses proportional to 'raw' difference pressure. Mode 4 is sleep mode. In the present study, the transmitter was set in Mode 1. In this configuration, a digital algorithm estimates the baseline of the positive and negative electrical signal from the pressure sensor. The total area of raw pressure above and below the baseline is calculated,

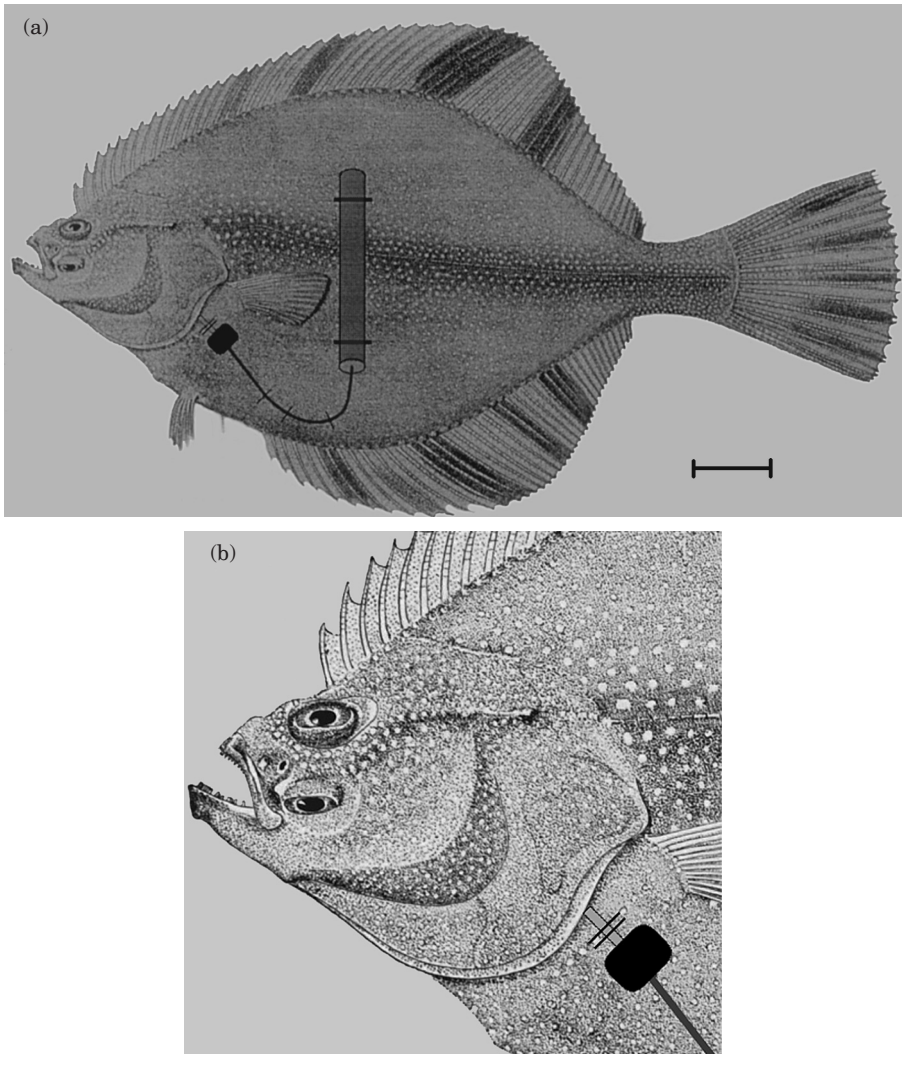

FIG. 2. Schematic presentation of the positioning of the (a) pressure sensor, cannula and acoustic transmitter. (b) Close-up. The original drawing of the fish is from H.L. Todd, U.S. National Museum, www.photolib.noaa.gov.

averaged and termed the 'integrated pressure' (*IP*). The 'pressure difference' (*DP*) is the difference between the lowest and the highest pressure measured. The time period from the synchronization pulse to the first pulse codes for *IP*. The time period between the first pulse and the second pulse gives the *DP*. Depending on the opercular activity level *IP* and *DP* were calculated every 1·75–3·3 s. The acoustic pulses were detected by a hydrophone (Vemco VH65) connected to a receiver (Vemco VR20) interfaced with a computer using an RS-232 serial cable.

EXPERIMENTAL PROTOCOL

Following tagging, the fish was revived in anaesthetic-free water. As soon as ventilation resumed (5 min), the fish was then transferred to the respirometer chamber and the monitoring of ventilatory activity started.

To measure oxygen consumption, the pump controlling the water supply to the respirometer was shut off and the water oxygen concentration continuously recorded for the next 30 min. During this period, oxygen in the respirometer decreased at a rate proportional to fish aerobic metabolism. The measuring chamber was then flushed for 30 min and a new determination of oxygen consumption was made. The control of the water supply to the respirometer was either manual or *via* a timer. In the later case, oxygen consumption (MO_2) was determined at hourly intervals. A full range of metabolic activities was measured, from the high metabolic rate of post-surgery stress to the low routine metabolic rate the next morning. Some measurements were also made during experimentally generated hypoxic episodes. In that case, the water in the outer compartment was bubbled with nitrogen before being pumped into the respirometer.

Linear regressions of water oxygen concentration *v.* time were performed and the slopes of the regression lines used to calculate oxygen consumption according to the equation:

$$MO_2 = b.V_{\text{resp}}.M^{-1}$$

where MO_2 (mg $O_2\,kg^{-1}\,h^{-1}$) = oxygen consumption, b (mg $O_2\,h^{-1}$) = dC_wO_2/dt (dC_wO_2 is the decrease in water oxygen concentration), V_{resp} (l) = volume of the respirometer minus the volume of the fish and M(kg) = mass of fish in the measuring chamber.

The MO_2 values resulting from a regression with $r^2 < 0{\cdot}9$ were excluded from the analysis. Oxygen consumption data were standardized ($MO_{2\,\text{stand}}$) to a 1 kg standard fish (M_{stand}) by:

$$MO_{2\,\text{stand}} = MO_2(M\ M_{stand}^{-1})^{0{\cdot}21}.$$

Acoustic pulse intervals were converted to pressure (pa) using calibration formulae provided by the tag manufacturer: $I.P._{\cdot\text{pa}} = (I.P._{\cdot 0\,\text{msec}\cdot} - I.P._{\cdot\text{mea msec}\cdot}) \times 0{\cdot}01156_{\text{cm msec}^{-1}} \times 1013{\cdot}25_{\text{pa}}$ and $D.P._{\cdot\text{pa}} = (P.D._{\cdot 0\,\text{msec}\cdot} - P.D._{\cdot\text{mea msec}\cdot}) \times 0{\cdot}0231_{\text{cm msec}^{-1}} \times 1013{\cdot}25_{\text{pa}}$. These calibration equations were checked according to the manufacturer recommendation and procedure (Vemco, V16TB Tail Beat Transmitter, Operation Manual).

RESULTS

Linear relationships between $\log(MO_2)$ and both *IP* and *DP* were established for four fishes (Fig. 3). The r^2 values ranged from 0·57 to 0·94 for *IP* and from 0·37 to 0·92 for *DP* (Table I). The regression lines showed no significant difference in slope ($P < 0{\cdot}001$). Intercepts for both *IP* and *DP*, however, were significantly different.

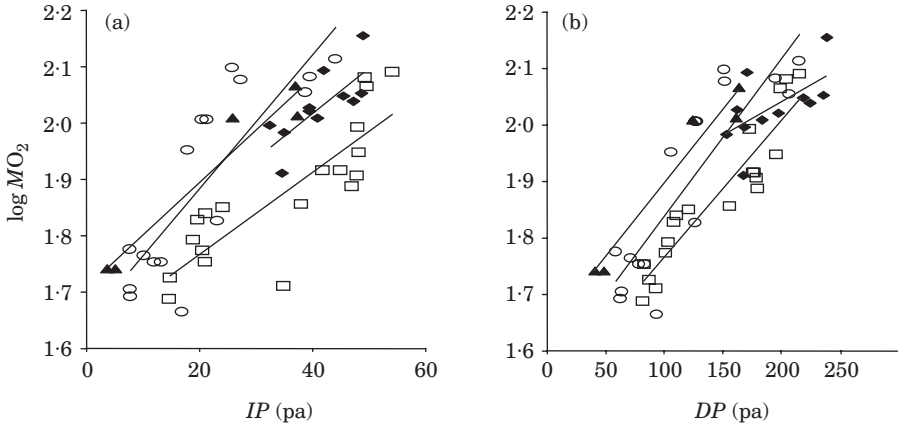

FIG. 3. The relationships between oxygen consumption and (a) integrated pressure and (b) pressure difference. Each symbol represents an individual fish.

DISCUSSION

The results indicate that the monitoring of opercular activity offers an alternative method for estimating aerobic metabolism of fishes in their natural environment. The use of differential pressure permits the experimenter to treat pressure as an alternating signal, reducing any error due to zero drift of the pressure sensor. In addition, any change in depth pressure as a result of vertical movement has no effect on the pressure output, making this sensor suitable for field applications.

In a large number of studies involving teleosts, a positive relationship has been observed between ventilatory activity and MO_2 (Smart, 1978; Perry & McDonald, 1993; van Rooij & Videler, 1996; Adams *et al.*, 2001). Since the measure of differential pressure integrates both the frequency and the amplitude of the opercular movement, it is likely to be a better predictor of MO_2 than frequency alone. This is particularly true at low levels of activity where ventilation incorporates both amplitude and frequency modulation.

TABLE I. Statistical analysis of linear relationships between oxygen consumption (log MO_2; mg kg^{-1} h^{-1}) and both integrated pressure (*IP*; pa) and differential pressure (*DP*; pa)

		Slope	Intercept	r^2	*P*
DP	Fish 1	0·001195	1·791	0·37	<0·05
	Fish 2	0·002414	1·527	0·87	<0·001
	Fish 3	0·002145	1·685	0·92	<0·01
	Fish 4	0·002784	1·562	0·77	<0·001
IP	Fish 1	0·008096	1·695	0·57	<0·05
	Fish 2	0·007274	1·624	0·71	<0·001
	Fish 3	0·009350	1·710	0·94	<0·01
	Fish 4	0·001188	1·648	0·70	<0·001

The telemetry of opercular pressure is a valid alternative to a number of approaches that have been used to investigate metabolism in free-ranging fishes. Several attempts have been made to assess energy dissipation using heart rate as a correlate to metabolic demand (Priede & Young, 1977; Priede, 1983; Armstrong, 1986; Lucas *et al.*, 1991, 1993; Claireaux *et al.*, 1995*a*, *b*; Mercier *et al.*, 2000). Since it is only one response in the multivariate cardio-respiratory system, however, it has been argued that heart rate is a poor correlate of integrated cardiac function and, therefore, of metabolic rate (Thorarensen *et al.*, 1996). The transmission of the electromyogram (EMG) signal of contracting muscles involved in opercular movement has also been proposed to monitor ventilation in fishes (Oswald, 1978; Rogers & Weatherley, 1983). This technique involves implanting electrodes and transmitting pulses whose period is a measure of the time it takes for the EMG voltage to sum to a preset threshold. One obvious disadvantage of this technique is that it gives reliable information about ventilation rate but not about ventilation amplitude.

The present experimental results suggest that both differential pressure and integrated pressure at the operculum are appropriate measures of ventilatory activity. Moreover, the correlations established between $M\mathrm{O}_2$ and both *IP* and *DP* suggest that opercular pressure can be directly translated into metabolic energy demand. Interindividual differences in the regression coefficients were observed in the value of the intercept. These differences are probably attributable to differences in the position of the cannula in the branchial cavity, although great care was taken to standardize the procedure. Interindividual variability may also be a result of differences in morphology. Gill arches were not specifically examined but differences in the condition of the edge of the opercula were observed. A third point is worth mentioning here. The present approach is based on the assumption that ventilation rate is directly determined by the rate of oxygen consumption. This is not always true. It has been shown that, in teleosts, ventilation rate is reflexly regulated *via* two populations of oxygen sensitive chemoreceptors (Smatresk, 1990; Burleson *et al.*, 1992). One group is situated on the gills and buccal cavity and monitors water oxygen partial pressure. The second group is situated within the vascular system and monitors blood oxygenation. The inconsistency in the intercept values, though limited, may result from small differences in water or blood oxygenation status, differences that were not reflected in the $M\mathrm{O}_2$ measurements. Fishes are known for being able to regulate oxygen uptake by mechanisms other than increased ventilatory work, *i.e.* increased lamellar recruitment or improved tissues perfusion (Randall, 1982). Finally, skin oxygen uptake is another factor that can influence the relationship between ventilation activity and oxygen uptake, particularly in flatfishes (Steffensen *et al.*, 1981). These points will obviously have to be examined carefully in future research.

There are particular situations where a fish's ventilatory activity does not mirror $M\mathrm{O}_2$. The most obvious of these situations is hypoxia, where ventilation rate can increase while $M\mathrm{O}_2$ remains unchanged (Randall, 1982). The present data do not depart from that rule, as illustrated in Fig. 4. This is a limitation that must be considered in future field applications. Another situation that leads to an uncoupling between ventilation and oxygen consumption rates is when fish ram ventilate (Steffensen & Lomholt, 1982).

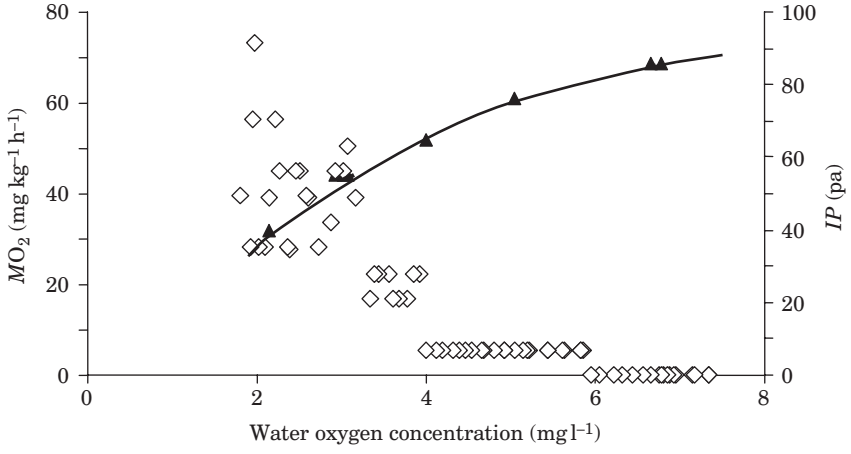

FIG. 4. An example of the simultaneous changes in oxygen consumption (▲) and integrated pressure (◇) during an hypoxic episode.

Despite these limitations, an overall model relating opercular activity to MO_2 was established. To do so, the slope and intercept values were averaged (Fig. 5). Both *IP* and *DP* showed strong linear relationships with MO_2 ($P < 0{\cdot}001$). The range of metabolic activity measured here, however, was relatively narrow (50–150 mg O_2 kg^{-1} h^{-1}) and further testing with a boarder range of oxygen consumptions is desirable. The increment in metabolic rate observed, *i.e.* three times the standard metabolic rate, is relatively small but is within the range of values reported in other flatfishes acclimated to similar temperature conditions

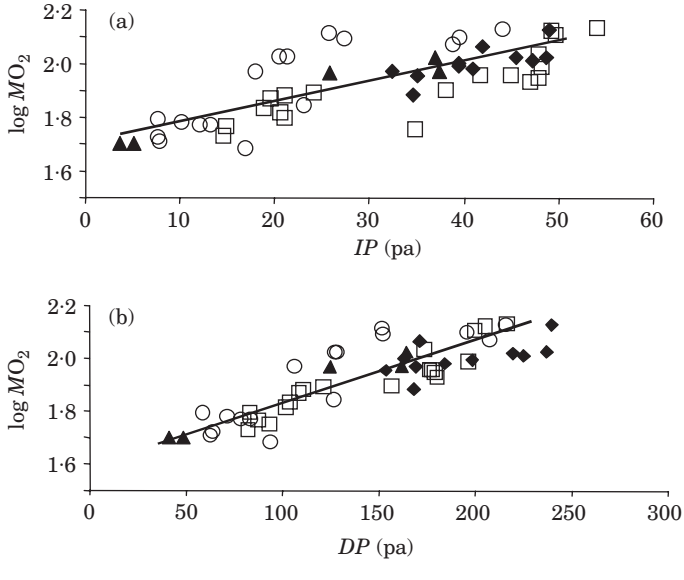

FIG. 5. The relationship between oxygen consumption and (a) integrated pressure ($r^2 = 0{\cdot}61$, $P < 0{\cdot}001$) and (b) differential pressure ($r^2 = 0{\cdot}74$ $P < 0{\cdot}001$). Each symbol represents an individual fish.

(Duthie, 1982; Van den Thillart *et al.*, 1994; Lefrançois, 2001; Mallekh & Lagardère, 2002). Although not specifically tested, the telemetry technique should also allow the detecting and monitoring of feeding events and cycles (Oswald, 1978).

In conclusion, the use of a pressure sensor to monitor opercular activity as a correlate of oxygen demand is a promising tool to investigate bioenergetics in free-ranging fishes. The reliability of the technique, however, may be limited under specific environmental conditions, particularly hypoxia. The proposed approach still needs to be tested on greater numbers of individuals from species representing a broad variety of physiology and habitat. Because of the size of the tag, application in the field is restricted to large animals (> 1 kg). Battery life span, external attachment and possibly fouling also restrain the duration of monitoring sequences to 3–4 weeks.

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