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# INSTRUMENTS AND METHODS

## A pressure vessel for the simultaneous determination of oxygen consumption and swimming speed in zooplankton

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**Abstract**—A pressure vessel suitable for the simultaneous determination of oxygen consumption and swimming speed in zooplankton is described. The relationship between respiratory rate ($y$; $\mu$l $O_2$ mg $DW^{-1}h^{-1}$) and swimming speed ($x$; m $h^{-1}$) for the euphausiid *Thysanoessa spinifera* at 12°C is described by the equation $y = 0.015 \pm 0.003x + 0.620$. The data suggest that increased swimming speed results in a substantial increase in oxygen consumption rate, indicating that locomotion can be energetically costly for zooplankton.

### INTRODUCTION

THE total oxygen consumption rate of an aquatic organism is the sum of a basal, or standard, metabolism and additional consumption attributable to physiological processes other than subsistence (FRY, 1971; PROSSER, 1973; BRETT and GROVES, 1979). Among the latter, the demand for energy by locomotion is by far the most important (BRETT, 1972). Increased swimming activity has been shown to triple the metabolic rate of pelagic crustaceans (IVLEV, 1963; MICKEL and CHILDRESS, 1978; QUETIN, MICKEL and CHILDRESS, 1978) and to raise that of fishes by as much as an order of magnitude (FRY, 1971). Thus, in order effectively to assess the influence of an experimental variable on metabolic rate, activity must be considered.

The technical difficulty of evaluating activity vs oxygen consumption in small zooplankton has prevented much advance in this area. Five studies deal directly with the topic; two used experimental methods (FOULDS and ROFF, 1976; KLYASHTORIN and KUZ'MICHEVA, 1976) and three used hydrodynamic modeling (VLYMEN, 1970; KLYASHTORIN and YARZHOMBEK, 1973; SVETLICHNYI, ZAGORODNYAYA and STEPANOV, 1977) to evaluate the energetic cost of swimming. All five studies concluded that active metabolism was at most 160% of basal metabolism.

The data differ considerably from a large body of literature on larger swimming organisms (IVLEV, 1963; HALCROW and BOYD, 1967; FRY, 1971; MICKEL and CHILDRESS, 1978; BRETT and GROVES, 1979; QUETIN, 1979; QUETIN *et al.*, 1978), in which the energy cost of locomotion has been empirically determined to be far greater than that of basal metabolism.

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Given the importance of locomotory requirements in modeling the energy expended by zooplankton during vertical migration (McLAREN, 1974; ENRIGHT, 1977) and in oceanic ecosystems in general (STEELE, 1974), there is a clear need for further research in this area. The need is of particular concern if one considers the scant data base available for understanding energy flow in oceanic systems.

This paper describes an apparatus designed to simultaneously measure oxygen consumption and spontaneous activity in an individual zooplankter as a function of temperature and pressure. The relationship between respiratory rate and swimming speed for the euphausiid *Thysanoessa spinifera* at 12°C is described.

## MATERIALS AND METHODS

The device is a teflon-coated stainless annulus (1, Fig. 1) integrated with an impeller pump (2), an oxygen electrode (3) (CLARK, 1956) for the continuous determination of oxygen consumption, and a photoresistor (4) light emitting diode (5) (LED) system for monitoring activity. The annulus is machined in two halves (6 and 7) from two disks of 316 stainless steel. A groove 101.6 in outer diameter, 7.0 mm wide, and 6.0 mm deep is milled into the bottom disk. A complementary annular groove is milled into the top disk to a depth of 2.0 mm. Two O-ring grooves (8) form concentric circles inside and outside the annular groove. Both O-rings seal against the flat surface of the top disk when the halves are bolted together. Six 9.5-mm bolts (9) secure the periphery of the annulus, while three bolts (not shown) seal the center.

The seawater within the vessel is mixed by an impeller pump; the impeller is a 17.5-mm 'spin fin' magnetic stirring disk (10) that directs the medium tangentially into three outlets (11). This initiates a gentle flow of water from the pump to the annulus that returns through the three inlets (12).

The pump head is constructed in two halves from 316 stainless steel rod with the top half (13) bored to accept the stirring disk. The outlets enter the pump head tangentially at precisely the level of the fins on the spin fin magnet. Holes through the pump head for the inlets and the outlets accept 3.2-mm outer diameter 316 stainless steel tubing, which is welded at its point of exit from the pump. The bottom half of the pump (14) is simply a disk fitted with an O-ring groove and three bolts to secure it to the top.

The outlet tubes penetrate the annulus at three equally spaced points in the small area between the outer O-ring and the annular groove. Inlets are located precisely between the outlets within the inner O-ring and the annular groove. The offset position of the inlets and outlets minimizes turbulence within the annulus and the staggered yet equidistant placement of the inlets and outlets creates no directed current of water.

The 'X' type valves (15) (Whitey, Inc.) allow unrestricted flow from the pump to the annulus at all times. Seawater can be admitted through them when open, and when used together they allow for a flow-through system.

The oxygen electrode, photoresistor, and LED used to measure oxygen partial pressure and activity consist of electrical elements soldered to coaxial cable and cast in epoxy resin (Hysol HD 3561-R9-2039). A section of aluminum rod reinforces the low pressure end of each probe (A, Fig. 2) and is incorporated during the casting procedure.

Holes in the vessel allow each probe to penetrate until its tip is flush with the annulus (cf. Fig. 2). The outer end of each hole is threaded to accept a 316 stainless steel nipple. The probes are secured by a 316 stainless steel pipe cap (D, Fig. 2) that threads onto the nipple. An O-ring near the tip of each probe (E, Fig. 2) seals it to pressure.

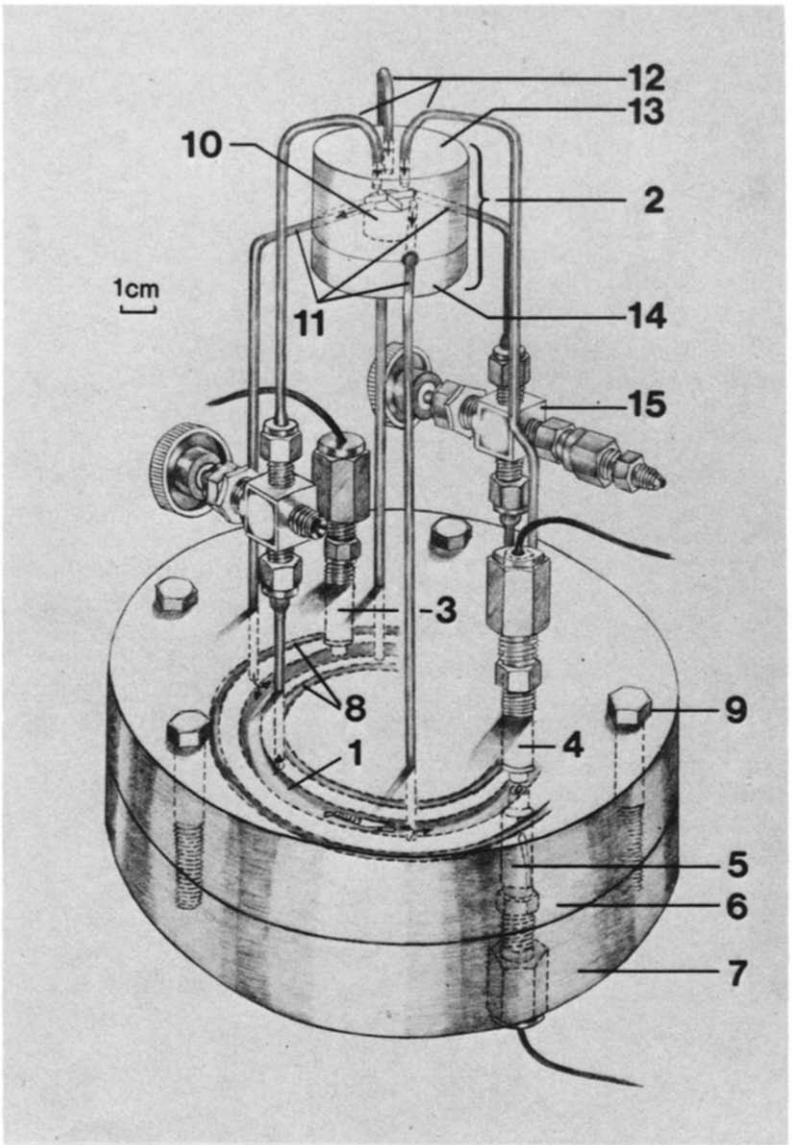


Fig. 1. Schematic representation of vessel. 1, annulus; 2, impeller pump; 3, oxygen electrode; 4, photoresistor; 5, light emitting diode; 6, top half of vessel; 7, bottom of vessel; 8, O-rings; 9, bolts for assembly; 10, impeller; 11, pump outlets; 12, pump inlets; 13, 14, top and bottom of pump; 15, X valves. Direction of water flow indicated by arrows. See text.

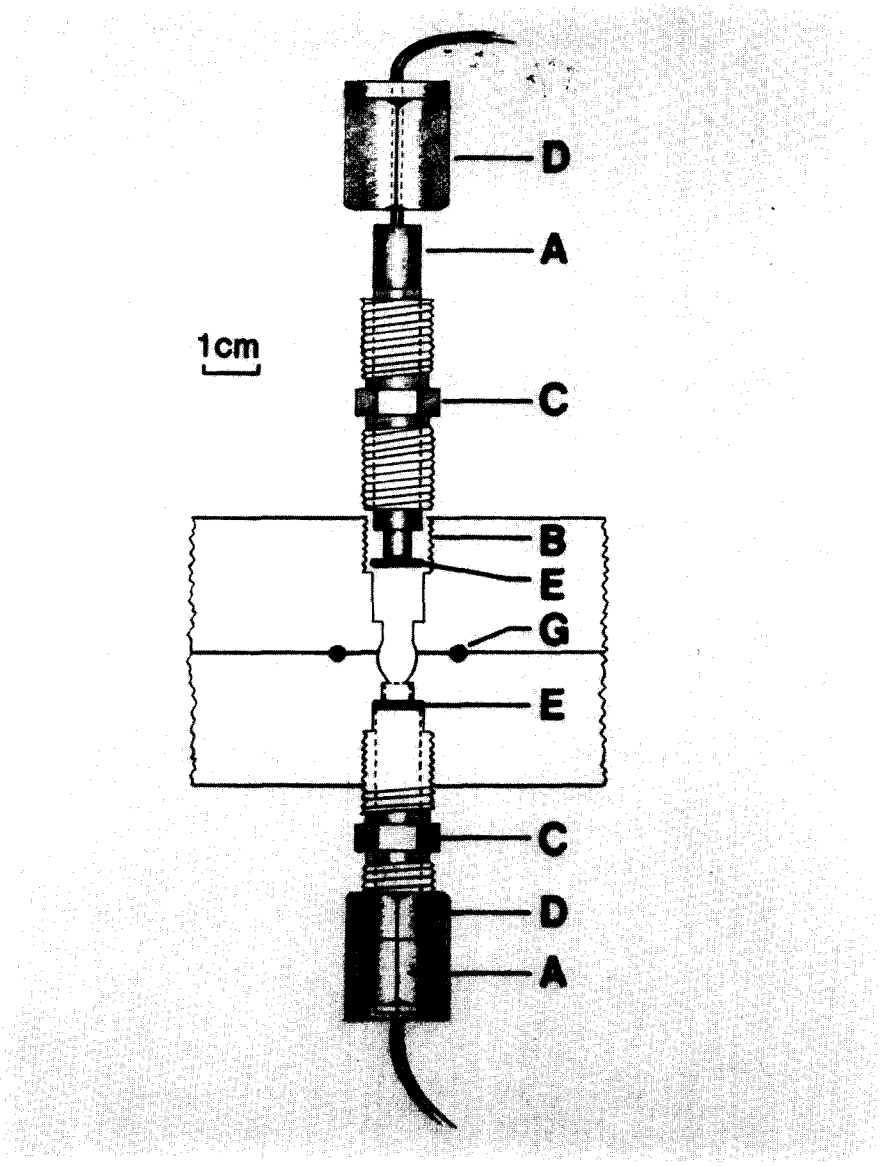


Fig. 2. Cross section of activity monitoring system. A, aluminum cap of probe; B, threaded section of corridor for probe; C, stainless steel nipple; D, pipe cap; E, O-ring for sealing probe into annulus; G, O-rings that isolate annulus. Each revolution of the zooplankter breaks the beam of the LED (bottom probe) resulting in a spike on a strip chart recorder.

Oxygen partial pressure is monitored by a microcathode Clark-type polarographic oxygen electrode (3, Fig. 1) (CLARK, 1956; MICKEL, QUETIN and CHILDRESS, in press). Opposite it is the activity monitor (4,5, Fig. 1; Fig. 2), a photoresistor in conjunction with an LED. The LED (Monsanto MV60) emits at 735 mm, outside the sensitivity of both euphausiid (BODEN and KAMPA, 1965) and human eyes. The photoresistor (Clairex CL 903) is wired as one arm of a balanced wheatstone bridge. Each time an animal crosses the light beam focused on the photoresistor the bridge is unbalanced, creating a pulse that is routed through a voltage follower circuit to a potentiometric strip chart recorder, where it appears as a spike.

The entire vessel, with the exception of the bore of the tubing used on the pump, is coated with a 0.08-mm layer of teflon (Crest Coatings, Buena Park, CA) that provides a low friction surface and prevents oxidation of the pressure vessel.

The entire vessel is placed in a circulating refrigerated water bath to control temperature. The large mass of steel relative to the 21.5-ml volume of the annulus is a good heat sink and quickly cools water admitted through the valves to the experimental temperature. Pressure flow-through can be achieved by using a pump and a back-pressure valve.

The apparatus was tested with the euphausiid *T. spinifera* at 12°C and 1 atm. Typically a neritic species, these animals are reported to live within the upper 100 m as non-migrators (BRINTON, 1962). However, they have been captured to a depth of 200 m, and there is some evidence that the species does a limited vertical migration (PEARCY and SMALL, 1968). Animals were captured in the Santa Barbara Basin (California) using a 1.5-m (2.3-$m^2$ mouth area) Tucker trawl in November and December of 1978. Once on deck *T. spinifera* were immediately sorted into 3.79-l polyethylene jars and transported on ice to the laboratory.

In the laboratory they were placed in 1.5-l cylindrical polyethylene dishes at a density of two individuals per container and maintained in a cycling temperature regime in the dark. Temperature was cycled from 8° during daylight hours to 12° at night; the time taken to shift from one temperature to the other was 1.5 h. The regime, designed to mimic the diel temperature change encountered by a strongly migrating species, corresponds to the temperatures encountered by *T. spinifera* in nature. Animals were fed every two to three days with the diatom *Coscinodiscus angstii* well in excess of saturation feeding (ROSS, 1979).

Experiments were begun after the bath had reached 12°C, or well into the night cycle of the temperature regime. The vessel was first cooled to 12°; it was then thoroughly flushed with filtered seawater (0.45 μm, Millipore®) containing streptomycin and neomycin (25 mg each $l^{-1}$). The oxygen electrode was first calibrated outside the vessel using the methods of CHILDRESS (1971). Just before flushing, the electrode was inserted; flushing continued until the electrode had stabilized for at least 30 min at a point equal to that determined outside the vessel. This required from 1.5 to 2.0 l of seawater.

Once the vessel was prepared, a single *T. spinifera* was gently pipetted through the photoresistor port into the annulus. The bomb was then sealed, and the magnetic stirrer was turned on. After each 12-h run the animal was removed, weighed wet, and then dried to a constant weight at 60°C.

The oxygen electrode was recalibrated after each experiment. Results were accepted only if the difference between the pre- and post-run calibrations was less than 5%.

Possible contributions by bacterial action and oxidation of the vessel to the total oxygen consumption were evaluated through post- and pre-run control measurements; no measurable contribution to total oxygen consumption occurred.

The data were reduced by dividing each experiment into consecutive increments of 1 h. The

activity for each hour was determined by counting the number of revolutions recorded as spikes on the strip chart for each period. Oxygen consumption rate was calculated from the simultaneous record of oxygen concentration on the chart recorder.

## RESULTS

Directly after transfer to the vessel animals showed erratic activity, usually short bursts of high activity or no activity at all. After 2 h they generally settled into a period of intermittent swimming, subsiding into quiescence at lower oxygen concentrations. The oxygen consumption rates and total activity were determined for each 1-h increment of the intermediate period of each run (Fig. 3).

The relationship between oxygen consumption ($y$, $\mu l\ O_2\ mg\ DW^{-1}H^{-1}$) and swimming speed ($x$, m h ) for five *T. spinifera* is described by the equation $y = 0.015 \pm 0.003\ x + 0.620$ ($r = 0.83$) (Fig. 3). The standard rate, by definition (FRY, 1971), is the $y$ intercept, or $0.620\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$. Oxygen consumption for the highest spontaneous activity recorded ($60\ m\ h^{-1}$) was $1.67\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$, or about three times the standard rate, which corresponds to a swimming speed of $1.7\ cm\ s^{-1}$ or about one body length per second. This may not be the active rate (FRY, 1971), because the maximum sustained swimming speed of *T. spinifera* is unlikely to be spontaneous. Other euphausiids are known to swim at sustained speeds of $150\ m\ h^{-1}$ ($4.2\ cm\ s^{-1}$) or more, both in the laboratory (TORRES, 1980) and during vertical migration (KAMPA and BODEN, 1954).

## DISCUSSION

The routine metabolic rate of a species is defined as the average oxygen consumption rate associated with spontaneous activity (BRETT, 1972). All previous measurements of euphausiid respiration may be defined as routine rates, because the term 'routine' is generally applied to any measurement of oxygen consumption rate in which activity is not monitored, such as Winkler bottle and Gilson respirometer determinations. Measurements of routine oxygen consumption using these techniques fall at or above the maximum activity levels shown in Fig. 3. SMALL, HEBARD and MCINTYRE (1966), using a Gilson respirometer, reported a rate of $1.28 \pm 0.07\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$ for *T. spinifera* at 10°C. If a $Q_{10}$ of 2.0 is assumed, the rate becomes $1.54\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$ at 12°C, which corresponds to a swimming speed of $61.3\ m\ h^{-1}$. Similarly, PEARCY and SMALL (1968) obtained a value of $2.21 \pm 0.69\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$ with Winkler bottle respirometers ($2.65\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$ at 12°C), which corresponds to a swimming speed of $135.3\ m\ h^{-1}$ using the relationship shown in Fig. 3. Both sets of results suggest a high level of spontaneous activity or nervous excitement during the course of the measurements resulting in very high routine metabolic rates. An approximation for the routine rate of the five animals used to produce Fig. 3 is the average oxygen consumption rate (as defined above) or $0.99 \pm 0.35\ \mu l\ O_2\ mg\ DW^{-1}h^{-1}$. That rate corresponds to the average swimming speed of $25.3 \pm 19.6\ m\ h^{-1}$; a speed much lower than that calculated from the data of SMALL *et al.* (1966) and PEARCY and SMALL (1968). The disparity may reflect differences in the techniques used for measuring oxygen consumption. Both Gilson (SMALL *et al.*, 1966) and Winkler bottle respirometers (PEARCY and SMALL, 1968) confine small pelagic animals, which may cause elevated metabolic rates. The annular respirometer described here, despite its small volume, allows for swimming.

The relationship shown in Fig. 3 indicates that swimming activity has a marked effect on

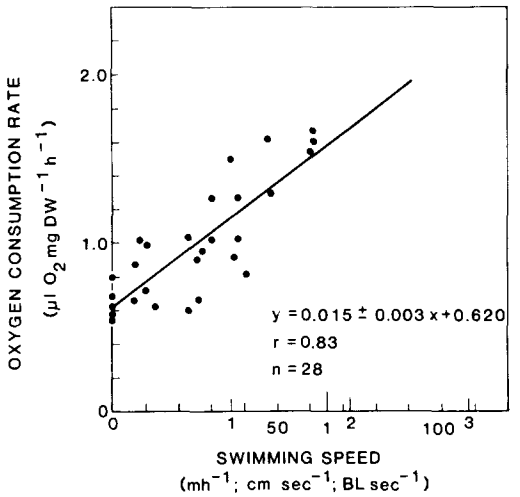


Fig. 3. Relationship between swimming speed and oxygen consumption rate of five *T. spinifera* at 12°C. (DW = 8.7 to 9.4 mg). $y = 0.015 \pm 0.003x + 0.620$ ($y = \mu l\ O_2\ mg\ DW^{-1}\ h^{-1}$; $x = m\ h^{-1}$); $n = 28$ $r = 0.83$. Swimming speed is expressed in $m\ h^{-1}$ (small hash marks), $cm\ s^{-1}$ (medium hash marks), and body lengths $s^{-1}$ (large hash marks).

the oxygen consumption rate of *T. spinifera*. While this conclusion is apparently in disagreement with prevailing views (cf. VLYMEN, 1970; KLYASHTORIN and YARZHOMBEK, 1973; FOULDS and ROFF, 1976; KLYASHTORIN and KUZ'MICHEVA, 1976; SVETLICHNYI *et al.*, 1977), differences may be reconciled by a consideration of those authors' assumptions and by carefully examining the difference between standard and routine metabolism.

VLYMEN (1970), KLYASHTORIN and YARZHOMBEK (1973), and SVETLICHNYI *et al.* (1977) all calculated the energy expended by swimming copepods in overcoming frictional drag using well established hydrodynamic techniques. VLYMEN (1970) and SVETLICHNYI *et al.* (1977) assumed that the organisms were capable of 100% metabolic and hydrodynamic propulsive efficiency. That assumption is reflected in their results, which indicate that normal swimming would account for only a 0.3% increase in metabolism over the routine rate. A generally accepted figure for the efficiency of mechanical work by muscle (i.e., metabolic efficiency) is between 10 and 20%, depending on the speed of contraction (ALEXANDER, 1970), and the hydrodynamic efficiency of paddle propulsion has been calculated to be 10 to 30% (NACHTIGALL, 1977). KLYASHTORIN and YARZHOMBEK (1973) considered both factors in their calculations and arrived at a more reasonable figure of 1.4 to 1.6 times the routine rate for energy expended in 'normal' swimming. This is the same as our figure of 1.4 to 1.6 times the routine rate at swimming speeds between 1 and 2 $cm\ s^{-1}$. Thus, the major discrepancy between the mathematically derived costs of swimming and our measurements has been in a lack of correction for the inefficiency of the biological machine. The total chemical energy required for swimming greatly exceeds that used to overcome hydrodynamic resistance.

A second source of discrepancy is in a failure by the hydrodynamicists to recognize the difference between standard and routine metabolism. Routine metabolism by definition is elevated well above standard or resting metabolism, because it incorporates normal spontaneous activity into the measured metabolic rate. In our study, routine metabolism was nearly twice the standard rate. The figures quoted by VLYMEN (1970), KLYASHTORIN and YARZHOMBEK (1973), and SVETLICHNYI *et al.* (1977) as standard rates are in fact routine rates.

The difference has resulted in their reporting very small increases in energy consumption with increased swimming speed, when in fact the increases are nearly three times that of resting metabolism (Fig. 3). Similarly, FOULDS and ROFF (1976), using a swimming tunnel respirometer, found that groups of *Mysis relicta* showed no significant increase in respiration over the routine rate when swimming at a rate of 0.7 cm $s^{-1}$ (normal speed). The same relationship was found in *T. spinifera*; however, the conclusion is a function of the elevated metabolic values characteristic of the routine rate and does not suggest cost-free activity.

Highly excitable pelagic animals such as copepods, euphausiids, and mysids do not deal well with interfaces, largely because they live in a relatively interface-free environment. Situations of close confinement such as those found in Warburg, Gilson, or Winkler bottle respirometers may affect routine metabolic measurements considerably, because an animal may be intermittently bumping into the wall of the respirometer. The vessel described above, though also confining, does not present a perpendicular interface for the animal to encounter. In addition, it allows for the determination of metabolic rate as a function of swimming speed. The increased resolution this type of system yields should resolve much of the existing confusion regarding the energetics of zooplankton swimming.

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