

## Oxygen Minimum Layer: Vertical Distribution and Respiration of the Mysid *Gnathopausia ingens*

**Abstract.** The large mysid crustacean *Gnathopausia ingens* (between 35 and 125 millimeters long) lives at oxygen concentrations between 1.25 and 0.20 milliliter per liter along the coast of California. It lives aerobically at these low oxygen concentrations by regulating its rate of oxygen consumption in water having down to and below 0.26 milliliter of oxygen per liter.

Zones of minimum oxygen are found at intermediate depths in most of the world's oceans; and, although the dissolved oxygen in some of these "oxygen minimum layers" is considerably less than 0.5 ml/liter, populations of metazoans exist there (1). Minimum layers are generally thought to be formed by the consumption of oxygen by living organisms (2), but the relative roles of bacteria and metazoans in consuming the oxygen have never been determined. Several authors have suggested that the oxygen concentration may be too low for aerobic respiration by metazoans (3, 4). Longhurst (5) has suggested that "oxygen tensions as low as 0.2 ml/liter at depth may support either a resting stock of zooplankton for a long period or a population of vertically migrating animals during their daytime residence at depth." However, Teal and Carey (6) found that *Euphausia mucronata* from the eastern Pacific minimum layer consumed oxygen down to unmeasurable concentrations in a closed vessel.

Intermediate-sized individuals of the mysid *Gnathopausia ingens* live in the eastern Pacific minimum layer (Table 1). With an opening and closing Tucker trawl (7) 2 meters square at the mouth and towed at 2 knots, these animals were captured within 200 miles of the coast of California between 29° and 37°N on *Te Vega* cruises between March and December 1967. Table 1 includes data only for those specimens between 35 and 125 mm long from eye to telson tip; all animals smaller than this occurred at higher oxygen concentrations above the minimum layer, and larger animals have been reported below the minimum layer (8). The fact that all the intermediate specimens were taken in water containing less than 1.25 ml of oxygen per liter, and most of them from water with less than 0.5 ml/liter, suggests that these animals live continuously and grow at low oxygen tensions. This distribution is not a sampling artifact since about one-third of the trawls were taken in water with between 0.20 and 0.50 ml of oxygen

per liter, one-third in water with between 0.50 and 1.25 ml/liter, and one-third in water with between 1.25 and 6.00 ml/liter.

To obtain rates of oxygen consumption at different oxygen concentrations (Fig. 1), I sealed individual *G. ingens* in a chamber filled with seawater. The rate of change in the partial pressure of dissolved oxygen in the chamber was measured continuously with a Clark-type oxygen electrode (9), as the animal reduced the oxygen from partial pressures at air-saturation to unmeasurable partial pressures. The time required for this reduction ranged from 12 to 32 hours. The experiments were performed at normal environmental temperatures for these animals ( $5.5^\circ \pm 0.1^\circ\text{C}$ ), and the electrode was calibrated before and after each run with air-saturated seawater and with nitrogen-saturated seawater at the experimental temperature. Because *G. ingens* normally lives under very low illumination, the experimental chamber was kept in darkness during each experiment. To control pH, I used seawater buffered with 2.5 g of tris(hydroxymethyl)aminomethane per liter, adjusted to pH 8.0 with HCl, and diluted with distilled water to the osmolality of seawater. Streptomycin (6 mg/

liter) and aureomycin (20 mg/liter) were added to minimize microbial growth.

Rates of oxygen consumption were calculated (10), at the indicated oxygen concentrations, from the slopes of the curves of oxygen partial pressure as a function of time. At the end of each experiment, the animal was removed from the chamber, enough air-saturated seawater was added to replace the volume of the animal, and the rate of oxygen consumption was again measured for 3 to 24 hours. These rates, which were independent of oxygen concentration and always less than 5 percent of the total measured rate, were taken to be the respiration rates of microbial contaminants and were subtracted from the total rates to obtain the values in Fig. 1.

Figure 1 shows the regulation of oxygen uptake by *G. ingens* at three different degrees of activity. The intermediate curve (solid line) represents the mean of the curves from 23 runs with eight different individuals (0.3 to 15.0 g, wet weight), in which the animal was swimming slowly at the beginning and end of each run. The oxygen concentrations at which these animals failed to regulate (that is, to maintain their characteristic rates of oxygen consumption) ranged from 0.14 to 0.45 ml/liter with a mean of 0.26 ml/liter and a standard error of  $\pm 0.04$  ml/liter. The oxygen concentration at which an animal ceased to regulate was directly proportional to its respiration rate; the greater the rate, the higher the concentration at which regulation stopped. In four runs, animals increased their oxygen consumption

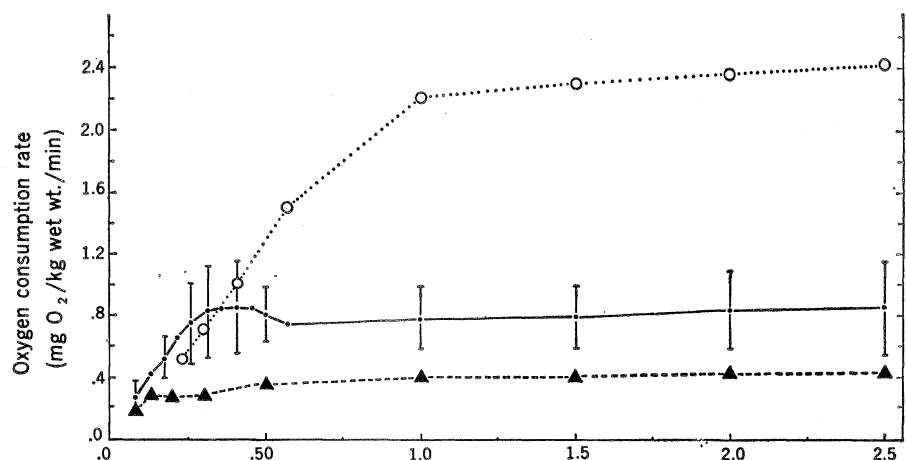


Fig. 1. Oxygen consumption rate of *G. ingens* as a function of oxygen concentration (in milliliters of oxygen per liter). ○, Single very active animal; ●, mean of 23 runs with eight individuals; ▲, single nonswimming animal. The vertical lines represent plus or minus one standard deviation.

Table 1. Numbers of *G. ingens* captured in different ranges of oxygen concentration during day and night, and total numbers of 1-hour net tows taken in each range.

Oxygen concentration (ml/liter)	Individuals captured (No.)		Tows (total No.)
	Day	Night	
0.20-0.50	21	8	49
0.50-0.75	2	3	19
0.75-1.00	0	6	14
1.00-1.25	0	1	10
1.25-6.00	0	0	49

rates 100 to 200 percent at concentrations between 0.5 and 0.3 ml of oxygen per liter, producing a slight but not significant increase in the average curve; in the remaining 19 runs the slopes at this concentration were the same as those at higher oxygen concentrations. Thus, *G. ingens* from the minimum layer can maintain a nearly constant rate of oxygen consumption in seawater having down to the lowest oxygen concentration found where they live (0.20 ml/liter). All of the animals continued to consume oxygen until none was detectable. The animals continued swimming for less than 30 minutes after they exhausted the oxygen in the chamber, although they could be revived for up to 6 hours after this time.

The upper and lower curves of Fig. 1 show the extremes in respiratory rates observed. The upper curve represents one animal which was forced, by the action of a magnetic stirring bar, to swim at the maximum rate which it could sustain for the duration of the experiment (12 hours). The lower curve represents one animal which was not swimming at either the beginning or end of the experiment.

The animals used in these experiments lived in the laboratory for up to 3 months, during which time they showed no detectable change in respiratory rate and readily ate pieces of fish.

The results indicate that *G. ingens* lives aerobically in the oxygen minimum and requires, for long-term survival, at least 0.14 to 0.26 ml of oxygen per liter in its environment. Its ability to remain active under anaerobic conditions is strictly limited, however; this may explain why *G. ingens* is absent from the regions of the eastern tropical Pacific where oxygen concentrations of less than 0.1 ml/liter prevail at the depths which the species occupies elsewhere (8, 11).

The ability of *G. ingens* to regulate its oxygen consumption at low oxygen concentrations is remarkable. Most

crustaceans and fishes previously investigated cannot regulate oxygen consumption at concentrations below 1.2 ml of oxygen per liter (12), and the best regulators previously examined regulate at concentrations only down to 0.6 ml/liter (13). It is quite possible that other animals found in the minimum layer are also able to live aerobically in this nearly anaerobic environment. If this is the case, these populations may make a substantial contribution to the biological consumption of oxygen which is believed responsible for the formation and maintenance of these layers.

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## Transverse Tubular System in Glycerol-Treated Skeletal Muscle

**Abstract.** *Horseradish peroxidase used as an extracellular marker fills 98.5 percent of the central triadic elements (tubules) in normal muscle and 97.2 percent in muscle soaked in Ringer solution to which 400-millimolar glycerol is added. A glycerol-soaked muscle rapidly returned to normal Ringer solution has a disrupted transverse tubular system and has only 3.2 percent of triads filled with peroxidase.*

Frog skeletal muscle has an extensive network of transverse tubules that originate as invaginations of the plasma membrane, invade the muscle fiber at the Z-disk, and branch so as to surround the myofibrils. It seems likely that flow of current down these tubules is an essential link between excitation (that is, an action potential at the plasma membrane) and contraction deep in the muscle fiber (1). Frog skeletal muscle also has a set of electrical properties quite distinct from those of nerve; they have often been attributed to the transverse tubular system. In particular it has been suggested that the membrane of the transverse tubules increases the capacitance of muscle fibers (2), that the lumen of the tubules forms a restricted diffusion space in which flow of current can produce slow changes in ion concentration and thus slowly change membrane potential (3), and that the potassium conductance system, which rectifies in the direction opposite from that of squid nerve, may be located in the tubular membrane (4).

The most direct method of proving that these properties do indeed require an intact transverse tubular system would be removal of the system selectively. A recently described procedure (5) is said to disrupt the transverse tubular system. Moreover many of the electrical properties of the muscle fibers treated in this manner are those to be expected from an isolated sarcolemma lacking tubular infoldings (6). In particular the specific capacitance of these treated muscle fibers is strikingly low—close to the value previously predicted for the sarcolemma (2). So that one may interpret with confidence the properties of these treated fibers, the morphology of the fibers and the number of tubules still connected to the extracellular space (and thus presumably functional) must be determined.

The procedure for disrupting the transverse tubular system has two stages: (i) the muscle is soaked for 1 hour at room temperature (17° to 21°C) in Ringer solution made hypertonic by addition of 400 mM glycerol;