

RESPONSES TO LOW OXYGEN IN TWO SPECIES
OF THE MUD SNAIL NASSARIUS

A Thesis

Presented to

The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

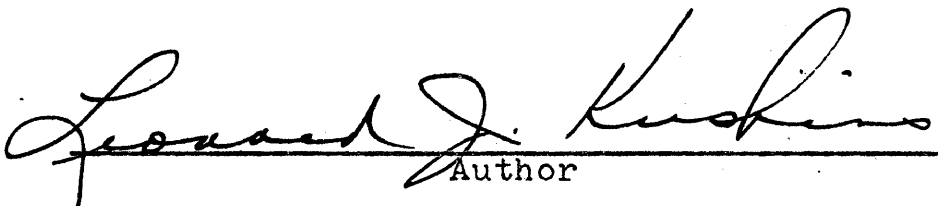
By

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1970

APPROVAL SHEET

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Master of Arts

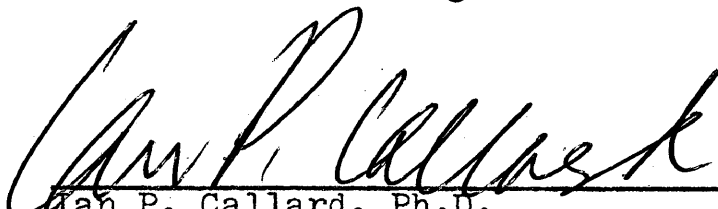


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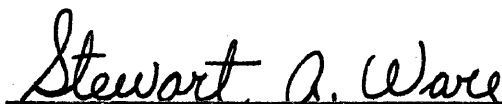
Approved, September 1970



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ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. Charlotte P. Mangum for her inspiration and guidance throughout this study. I am also indebted to Dr. Ian P. Callard and Dr. Stewart A. Ware for their critical review of and suggestions pertaining to the manuscript.

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ABSTRACT

1. Responses to low oxygen were investigated in two sympatric species of mud snail Nassarius obsoletus and N. trivittatus.

2. Both species survive anoxic conditions for more than nine days.

3. Both species show a significant increase in oxygen consumption after exposure to low oxygen conditions. Oxygen consumption is increased at each concentration in N. obsoletus but only at 2 and 3 ml O₂/l in N. trivittatus.

4. The relation of oxygen consumption to oxygen levels shows partial independence although after exposure to low oxygen conditions there is a marked tendency toward conformity.

5. In many cases, oxygen uptake ceases when oxygen is still available to the animal. Residual partial pressure decreases after exposure to low oxygen.

6. Gas bubbles are frequently found in N. obsoletus. Partial pressure of oxygen found in bubbles carried in the mantle cavity is significantly different from that of the external environment.

RESPONSES TO LOW OXYGEN IN TWO SPECIES
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INTRODUCTION

Despite an extensive literature on development and ecology, little is known about respiratory mechanisms and responses to low oxygen conditions in the mud snail Nassarius. Our knowledge of the metabolic response to low oxygen in other molluscs is also sparse. Collip (1921) showed that Mya arenaria survives low oxygen conditions for 8 days, after which there is an increased oxygen uptake. Van Dam (1935) demonstrated that, after exposure to low oxygen conditions, oxygen utilization in M. arenaria rises from 3 - 10% to 35%. The pulmonate gastropod, Ancylus fluviatilis, which lives in running streams, survives anoxic conditions only three to four days. However, Acroloxus lacustris, which lives in often hypoxic stagnant waters survives longer than 11 days (Berg, 1952).

The relationship of oxygen consumption to oxygen level is better known. In reviewing the literature, Hyman (1929) states that the several species of aquatic molluscs studied maintain their normal rate of oxygen uptake down to a certain critical level. The pulmonate Australorbis glabratus is a regulator from 100% air saturation down to very low levels where the rate falls off rapidly (von Brand et al., 1948). Ancylus fluviatilis is a regulator from 6 to 3 ml O₂/l, but the rate declines rapidly below this point.

Acroloxus lacustris, on the other hand, is a conformer (Berg, 1952).

Nassarius obsoletus (Say) and N. trivittatus (Say) tend to burrow in the mud flats and inhabit for some time a very low oxygen environment (Pearse et al., 1942). Their notable success in such environments must entail adaptation to low oxygen conditions. The casual observation that they release gas bubbles during several days of confinement in a closed container aroused our interest in these adaptations.

MATERIALS AND METHODS

The mud snails Nassarius obsoletus and N. trivittatus used in all but one aspect of this study were collected from various depths during August 1969 at Indian Field Creek, a tributary of the York River estuary in Virginia. Gas bubble analyses were performed on N. obsoletus collected during June and July, 1970 in the Little River, a tributary of Waquoit Bay, Massachusetts.

The animals were returned to the laboratory, separated by species and placed in aquaria. Animals were kept in aerated, recirculating York River estuary water (22.5°C, 17 - 22 o/oo salinity) or the running sea water system of the Marine Biological Laboratory (18 - 23°C, 31 - 32 o/oo).

Ecological distribution

Data on vertical distribution were collected during late June and July, 1969 at Indian Field Creek. Air and water temperatures ranged from 28 to 33 and 29 to 34°C respectively during the study period. Three transects, 10 meters apart, were extended from above the high water line to low water, and 21 to 44 continuous quadrats (0.25 meter square) were taken down each transect line. A total of 107 quadrats and 2000

animals were counted. A distinction was made between animals above the sediment and those which could not be seen protruding. No animals were counted below the low water level. The upper limit of the barnacle line was used as a biological reference point. These data were analyzed by Chi square.

Survival under anoxic conditions

Three animals were placed in each of 48 tightly stoppered 500 ml flasks. Sixty additional animals were kept individually in 125 or 250 ml flasks. The oxygen level, monitored with a Yellow Springs Instrument Co. Model 5420 self-stirring polarographic probe, was reduced to 0.07 - 0.35 ml O₂/l by bubbling nitrogen through the water. The flasks were stoppered and stored in the dark at room temperature (21 - 23°C). After one day exposure the oxygen concentration in the flasks had decreased to zero. The pH of each flask was measured before and after the experiment. At the termination of each experiment (1 - 10 days), animals in which the foot had become translucent, indicating the onset of decomposition, were inspected for parasites. When death was not obvious, animals were placed in an aerated aquarium for at least 48 hours, after which they were checked for response to tactile stimuli and then inspected for parasites. In all cases, recovery was 100%.

Metabolic response to low oxygen

Snails were kept at 22.5°C for 60 days. During this period, they were not fed but they browsed on other individuals' shells. We have kept unfed Nassarius in the laboratory for as long as ten months without observing large mortality. At least 5 days before the snails were used, the shell aperture was sealed with "Sira" adhesive wax (British Drug Houses, Ltd.) and the snail was dipped in melted paraffin wax momentarily and then dropped in sea water at 5°C to cool the animal quickly. The adhesive wax was then removed from the aperture and the animals returned to the 22.5°C aquaria. Of several hundred animals, only one died after this treatment, which was necessary to eliminate extraneous oxygen uptake. When the animal is removed from the shell large amounts of oxygen are used by the shell itself, despite careful millipore filtering of the sea water. Brushing the shell, scrubbing with alcohol, even treating the shell with 10^{-4} M sodium cyanide solution did not effectively eliminate oxygen uptake. When the shell was covered with paraffin wax, however, the oxygen uptake (probably due to algae that grow into the porous eroded shell) was negligible.

Measurements of oxygen depletion were made with the oxygen probe in a closed darkened container. The same animal was measured twice: once immediately prior to and once immediately after exposure to low oxygen conditions for 24 hours. In all cases, the water was millipore filtered and the experiments performed at 22.5°C (\pm 0.02 C). A continuous

record was made of oxygen depletion from air saturated sea water to a level where the rate equalled zero. The animal was then placed in the original experimental chamber containing air saturated sea water of the same salinity and temperature, and the measurement repeated. After removal from its shell, the animal was inspected for parasites, weighed, dried at 70°C for 24 hours and weighed again.

Results were compared by computing at each integral value of oxygen concentration the per cent difference between the pre-anoxic and post-anoxic measurement and placing 95% confidence intervals around the means. The first fifteen minute interval was not included in the calculations.

Changes in the oxygen level at which aerobic respiration can no longer be detected (residual partial pressure) were studied initially in animals kept under aerated conditions. The animal was allowed to deplete the oxygen in a closed container until the rate of depletion reached zero. The water was immediately replaced with air saturated water and the measurement immediately repeated. Changes in residual partial pressure after exposure to low oxygen were also measured. In this case, the animals were placed directly from the aerated aquaria into deoxygenated water and stored for 24 hours in darkened stoppered flasks at room temperature. The rate of oxygen depletion was then measured twice.

Gas bubble analysis

Bubbles were collected from the mantle cavity of

N. obsoletus. Of primary interest are bubbles found in contact with the ctenidium, but bubbles found in other areas of the cavity were also analyzed (Fig. 1). Bubbles were not used if they were surrounded by mucus. Oxygen determinations were made on bubbles from animals equilibrated to known pO_2 's, prepared by flushing water with nitrogen and measuring the oxygen level with a polarographic probe. Animals were equilibrated to air saturated conditions in an aerated container. Equilibration to lower pO_2 's was allowed to proceed for 3 hours by placing pairs of animals in one liter reagent bottles. The water was stirred constantly by a magnetic stirrer. During the exposure period, the use of oxygen in metabolic activities of the animal changed the equilibrium level by no more than 10%. To test the efficacy of a 3 hour equilibration period, equilibration to 100% and 0% saturation was allowed to proceed for 24 hours.

Before the animals were removed from the equilibration medium, they were tapped to force them to retract into their shells. The animals were quickly removed from the medium and their shells were cracked in a vise. The air bubble was removed from the mantle cavity with a gas syringe filled with alkaline citrate. It was then transferred to a Scholander microgas analyzer and analyzed according to Scholander et al. (1955). Control measurements on atmospheric air were performed to determine the volume of gas dissolved in the solutions.

The difference between pO_2 of the bubbles and pO_2 of the environment was tested according to Student's t.

RESULTS

Ecological distribution

Figure 2 is a distribution profile of the two mud snails Nassarius obsoletus and N. trivittatus at Indian Field Creek, which is surrounded by extensive mud flats that are virtually covered by these animals from spring to fall. The data indicate that the vertical ranges of the two species are identical at these depths. N. obsoletus occurs in significantly greater numbers from 0-75 cm below the barnacle line but N. trivittatus occurs in significantly greater numbers from 75 - 160 cm below the barnacle line ($p < .01$). Of the total number of animals counted, 59% of N. obsoletus were burrowed and 58.6% of N. trivittatus were burrowed. Seventy-eight percent of the N. trivittatus and 67% of the N. obsoletus were burrowed above 75 cm. The burrowing habits of the mud snails indicate that they must endure periods of low oxygen.

Survival under anoxic conditions

LD₅₀ is reached during the ninth day in both species (Tables 1 and 2). LD₁₀₀ is reached during the tenth day in N. obsoletus but not after 10 days in N. trivittatus. Changes in pH, which ranged from 0.05 to 1.6, show no correlation with

survival time or with the number of snails per flask. The number of animals with the characteristic larval trematode infection (Vernberg et al., 1969) was small, and there is no correlation with survival. An examination of the medium failed to reveal additional parasites.

Metabolic response to low oxygen

Nassarius is a partial oxygen regulator (Fig. 3); both species are capable of imperfectly regulating their oxygen consumption at higher pO_2 's while the rate falls rapidly at lower pO_2 's. If the data are subjected to all four possible forms of regression analysis (linear, logarithmic and the two forms of semi-logarithmic) the highest coefficients of determination (r^2) are obtained with either a semi-logarithmic ($\log X$) or logarithmic plot of oxygen consumption as a function of oxygen concentration (Fig. 3, Table 3). No sharp change that is comparable to the critical pO_2 of a more perfect oxygen regulation occurs in the curves (Prosser & Brown, 1961). After exposure to low oxygen conditions, the dependence of oxygen consumption on oxygen levels is markedly increased, approaching conformity (Fig. 3).

Regression analysis of the curve describing the relationship of oxygen consumption and oxygen concentration in each individual before and after the 24 hour anoxic period showed three tendencies (Fig. 4): 1) An upward translation of the regression line after exposure to anoxic conditions indicates an increased rate of oxygen consumption

TABLE 1
 SURVIVAL OF Nassarius obsoletus UNDER LOW OXYGEN CONDITIONS

Duration (hr)	No. animals per flask	Total no. animals	Mortality (%)
24	3	6	0
48	3	6	0
72	3	6	0
96	3	6	0
120	3	6	0
144	3	6	0
168	3	12	25
192	3	12	59.3
216	3	12	67.7
192	1	10	20
216	1	10	80
240	1	10	100

TABLE 2

SURVIVAL OF Nassarius trivittatus UNDER LOW OXYGEN CONDITIONS

Duration (hr)	No. animals per flask	Total no. animals	Mortality (%)
24	3	6	0
48	3	6	0
72	3	6	0
96	3	6	0
120	3	6	0
144	3	6	0
168	3	12	8.33
192	3	12	75
216	3	12	100
192	1	10	40
216	1	10	60
240	1	10	80

remaining constantly different over the complete range of oxygen concentrations. This change was the most common result for both species (45%). 2) Rotation of the curve in which the rate of post-anoxic oxygen consumption was initially greater at higher oxygen levels but eventually became equal to or lower than the pre-anoxic values was also noted in approximately 35% of animals. Van Dam (1935) reported a return to pre-anoxic rates of oxygen consumption in Mya arenaria. 3) In both species rotation in the opposite direction was apparent in twenty percent of the animals tested.

Immediately after the anoxic period, the rate of oxygen consumption was lower at high pO_2 's than before exposure. In 3 of 4 cases, the rate eventually became higher than the control. Apparently, there is a time lag before the animal begins to pay off oxygen debt.

In N. obsoletus (Fig. 5) the 95% confidence interval around the means of the percent difference between an initial rate of depletion and a succeeding measurement after 24 hour exposure to low oxygen levels does not include zero at any integral concentration. However, in N. trivittatus the confidence interval includes zero at 1, 4, and 5 ml O_2/l . At 2 and 3 ml O_2/l the percent difference does not include zero. Moreover, data pooled from all oxygen concentrations show significant differences for pre-anoxic and post-anoxic N. trivittatus (Table 6).

If the volume of water in the closed containers was relatively small (30 ml), and presumably the animals encountered low oxygen conditions rapidly, then they exhausted their

TABLE 3
 COMPARISON OF THE COEFFICIENT OF DETERMINATION (r^2) FOR
 THE FOUR TYPES OF REGRESSION ANALYSIS IN N. obsoletus

TYPE OF ANALYSIS	Means of the coefficient of determination	
	PRE-ANOXIC	POST-ANOXIC
Linear ($Y = a+bX$)	0.503 \pm 0.096	0.882 \pm 0.0396
Logarithmic ($\text{Log } Y = a+b \text{ Log } X$)	0.745 \pm 0.055	0.899 \pm 0.017
Semi-logarithmic ($Y = a+b \text{ Log } X$)	0.624 \pm 0.039	0.851 \pm 0.039
Semi-logarithmic ($\text{Log } Y = a+bX$)	0.461 \pm 0.095	0.633 \pm 0.081

supply of oxygen. If the volume was relatively large (60 ml) and thus the animals gradually encountered low oxygen conditions, oxygen depletion ceased while oxygen was still available to the animal (residual partial pressure of oxygen). This phenomenon has been shown in other animals as well (Mangum, 1970). Immediately upon replacing the oxygen depleted water with fresh air saturated water, 90% of the N. obsoletus tested consumed oxygen before ceasing to a lower residual level than in the first measurement (Table 4). This response, also detected previously (Mangum, 1970), is believed to be a compensatory adaptation to the preceding brief exposure to low oxygen conditions. After exposure to anoxic conditions for 24 hours, however, the animals removed more oxygen in the first measurement than in an immediately succeeding measurement (Table 5). This is the reverse of the pattern in animals which had been exposed to low oxygen conditions for only 3 hours.

After exposure of N. obsoletus to anoxic conditions for 24 hours, the residual pO_2 is significantly lower ($p < .05$) than before prolonged exposure (Table 6). A somewhat comparable phenomenon is shown by Shepard (1955), in the speckled trout, Salvelinus fontinalis. Trout acclimated to low oxygen levels have lower asphyxial levels than animals acclimated to higher levels. Unlike fish, however, snails survive at the residual pO_2 and lower levels for many days (Table 1).

Gas bubble analysis

TABLE 4
 CHANGES IN RESIDUAL pO_2 IN Nassarius obsoletus BEFORE
 EXPOSURE TO LOW OXYGEN CONDITIONS

Experiment No.	Residual pO_2 after first measurement (mm Hg)	Residual pO_2 after resaturation (mm Hg)	Net change in residual pO_2 (mm Hg)
1	41.0	12.3	28.7
2	26.4	16.6	9.8
3	20.6	0	20.6
4	16.6	47.6	-31.0
5	47.6	41.4	6.2
6	65.6	16.5	49.1
7	8.2	0	8.2
8	8.2	4.2	4.0
9	4.2	2.1	2.1
10	12.5	9.3	3.2

TABLE 5

CHANGES IN RESIDUAL pO_2 IN Nassarius trivittatus BEFORE
EXPOSURE TO LOW OXYGEN CONDITIONS

Experiment No.	Residual pO_2 after first measurement (mm Hg)	Residual pO_2 after resaturation (mm Hg)	Net change in residual pO_2 (mm Hg)
1	5.1	12.5	- 7.4
2	17.0	11.0	6.0
3	9.8	13.0	- 3.2
4	42.3	0	42.3
5	2.3	2.6	- 0.3
6	3.7	15.5	-11.8
7	8.4	10.4	- 2.0
8	0.6	1.6	- 1.0
9	0	1.3	- 1.3
10	0	0.6	- 0.6

TABLE 6
SUMMARY OF OXYGEN CONSUMPTION DATA

Experiment	Mean of the pre-anoxic measurements	Mean of the post-anoxic measurements	P	N
Total O ₂ in <u>N. trivirgatus</u> (paired data) μl/hr/g dry wt.	387.64 ± 72.15	984.02 ± 169.02	<.01	11
Total O ₂ in <u>N. obsoletus</u> (paired data) μl/hr/g dry wt.	414.52 ± 64.82	733.99 ± 74.85	<.01	10
Residual pressure in <u>N. obsoletus</u> (mm Hg) (non-paired)	40.5 ± 5.3	21.2 ± 5.5	<.05	24

Mud snails that had been submerged in aquaria for several days and then transferred under water to closed and darkened flasks released gas bubbles for at least three days. The origin of the bubble is unknown, but it seems reasonable to suppose that gas enters the mantle cavity through the siphon at low tide. We have no evidence to bear upon the question of oxygen secretion. The size of the bubble ranges from 0.3 to 3.6 μ l. Although only bubbles found in the mantle cavity were analyzed, bubbles were also observed in the esophagus, gut, anus and siphon.

About 50% of the animals opened contained gas bubbles in the mantle cavity. These animals were not subjected to sharp changes in temperature. On the single occasion when animals were not collected personally but instead by the Marine Biological Laboratory Supply Department, we were unable to locate bubbles in either the digestive tract or mantle cavity. We cannot explain their absence.

In Fig. 7, which gives the results of gas bubble analyses, the line connecting the means for bubble pO_2 crosses the equilibrium line between the data points at 40 and 80 mm Hg. At environmental pO_2 's above the data point at 80 mm Hg, the oxygen gradient is in the direction of the bubble, but the opposite gradient occurs at environmental levels below 40 mm Hg O_2 . Mean values are significantly different from one another and from the equilibrium line at all environmental pO_2 's. There is no difference between values obtained after 3 hour equilibration and those obtained after 24 hours. Ideally, the gas content of the bubble should be understood in relation

to the fluid in the mantle cavity but no measurements on mantle fluid were successful due to the small volume collected.

DISCUSSION

It is well known that Nassarius obsoletus is an intertidal animal (Sheltema, 1964). In her studies at Cold Spring Harbor, Long Island, Dimon (1903) reported that N. trivittatus is not exposed at low tides. However, an abundance is clearly exposed at low tide in the York River estuary.

The massive aggregation into schools (Jenner, 1956, 1957, 1958, 1959) may explain the bimodal distribution profile of N. obsoletus (Fig. 2). Crisp (1969) has demonstrated that schooling behavior is mediated largely by contact between snails. The effect of aggregation on vertical distribution at Indian Field Creek is confused by our lack of understanding of N. trivittatus. The possible influence of interspecific contact has not been considered. In addition, Sassaman & Mangum (1970) show a similar bimodality in two actinian populations with the trough in distribution at approximately the same depth at Indian Field Creek. It would be interesting to know whether the phenomenon is biological or physical in origin.

N. obsoletus and N. trivittatus both actively burrow into the black mud flats at Indian Field Creek. The percentage of animals burrowed may depend on the environmental conditions at the time of sampling. For example, heavy rains caused exposed animals to burrow, and therefore

animals were not counted on these days. Escaping into the mud suggests that the animals are better adapted to low oxygen than low salinity conditions. N. obsoletus contains bubbles with much greater frequency than N. trivittatus. These findings may be correlated with the observation that N. obsoletus is exposed for longer periods of time at low tide because they are in greater numbers at higher depths.

It was expected that the parasitized animals would be more sensitive to low oxygen (W. Vernberg, pers. comm. and Olivier et al., 1953). The fact that parasitism does not seem to affect survival may be due to the low number of parasitized animals.

The influence of oxygen levels on oxygen consumption in animals that have not experienced low oxygen exposure is typical of aquatic snails (Hyman, 1929), in that it shows partial regulation at high oxygen levels. However, we have not detected perfect regulation, which was reported by earlier workers (Hyman, 1929; von Brand et al., 1948; Berg, 1952). The tendency towards dependence of oxygen consumption on oxygen levels after exposure to anoxic conditions is probably due to the increased rate of oxygen depletion, also shown by Amberson et al. (1924).

Since previous exposure to low oxygen results in a compensatory adaptation which is reminiscent of temperature acclimation, classification of the response would seem desirable. Patterns of the response (Fig. 5) do conform to schemes widely used in classifying responses to temperature change (Prosser & Brown, 1961). However, the metabolic

response of Nassarius to prolonged exposure to low oxygen conditions is not consistent.

There is a change in rate of oxygen consumption at different oxygen levels in N. obsoletus (Fig. 5). However, the change in N. trivittatus (Fig. 6) is masked by an extreme rotational response in both directions, thus causing the large spread of 95% confidence intervals in Fig. 6. The increased rate of oxygen consumption to pay off an oxygen debt (Table 6), which is consistent, may be a typical molluscan exposure to low oxygen conditions (van Dam 1935, 1938).

A compensatory adaptation is evident from the comparison of the residual partial pressure of oxygen before and after exposure to anoxic conditions in N. obsoletus. Animals exposed to anoxic conditions for 24 hours remove more oxygen than animals acclimated to air saturation levels and consequently have a lower residual partial pressure. The greater oxygen depletion in post-anoxic animals at high oxygen levels may be responsible for lower residual levels.

Short term exposure to low oxygen brings about a similar response (Tables 4 and 5). When animals that had ceased consuming oxygen after reducing the level in a closed container were immediately placed in saturated water, their residual oxygen level was also reduced. We believe that this response results from the previous brief exposure to low oxygen conditions. The opposite response in animals measured twice after exposure to anoxic conditions for prolonged (24 hour) periods may be attributed to the demands of a greater

oxygen debt after a much longer exposure to anoxic conditions. The animal, measured immediately after the 24 hour anoxic period, may remove oxygen to low levels to pay off an oxygen debt. However, in the second measurement after exposure to low oxygen, this debt having been paid off, the animal removes less oxygen.

Grainger and Newell (1965), who have analyzed gas bubbles released by barnacles upon immersion in water after exposure to air, refer to the presence in prosobranchs of gas bubbles which are expelled with feces. This casual mention is the only reference to gas bubbles in prosobranchs of which we are aware. Interestingly enough, the authors conclude from measurements of oxygen consumption in air that barnacles use gaseous oxygen at low tide.

As the mantle fluid loses oxygen to respiring tissue, oxygen must diffuse from the bubble to the fluid. The change in direction of the oxygen gradient between the bubble and the environment suggests that the flow of water over the gill may stop between 40 and 80 mm Hg O_2 (Fig. 7). If exchange between the mantle cavity and the external environment does indeed stop here, it should be reflected by the oxygen uptake measurements. In fact, oxygen uptake does cease at a pO_2 of 40.5 mm Hg (Table 6).

The gas bubble may be a highly adaptive mechanism, in that the bubble could function as a reservoir, releasing oxygen, as it is consumed, to the mantle cavity fluid. A single average size bubble could supply the animal with oxygen for about 15 minutes at oxygen consumption rates immediately

before oxygen uptake ceases. Since animals may have up to a dozen bubbles, the total period during which at least partially aerobic respiration could be sustained by the supply of oxygen in mantle cavity bubbles may be important during tidal exposure when ventilation must cease.

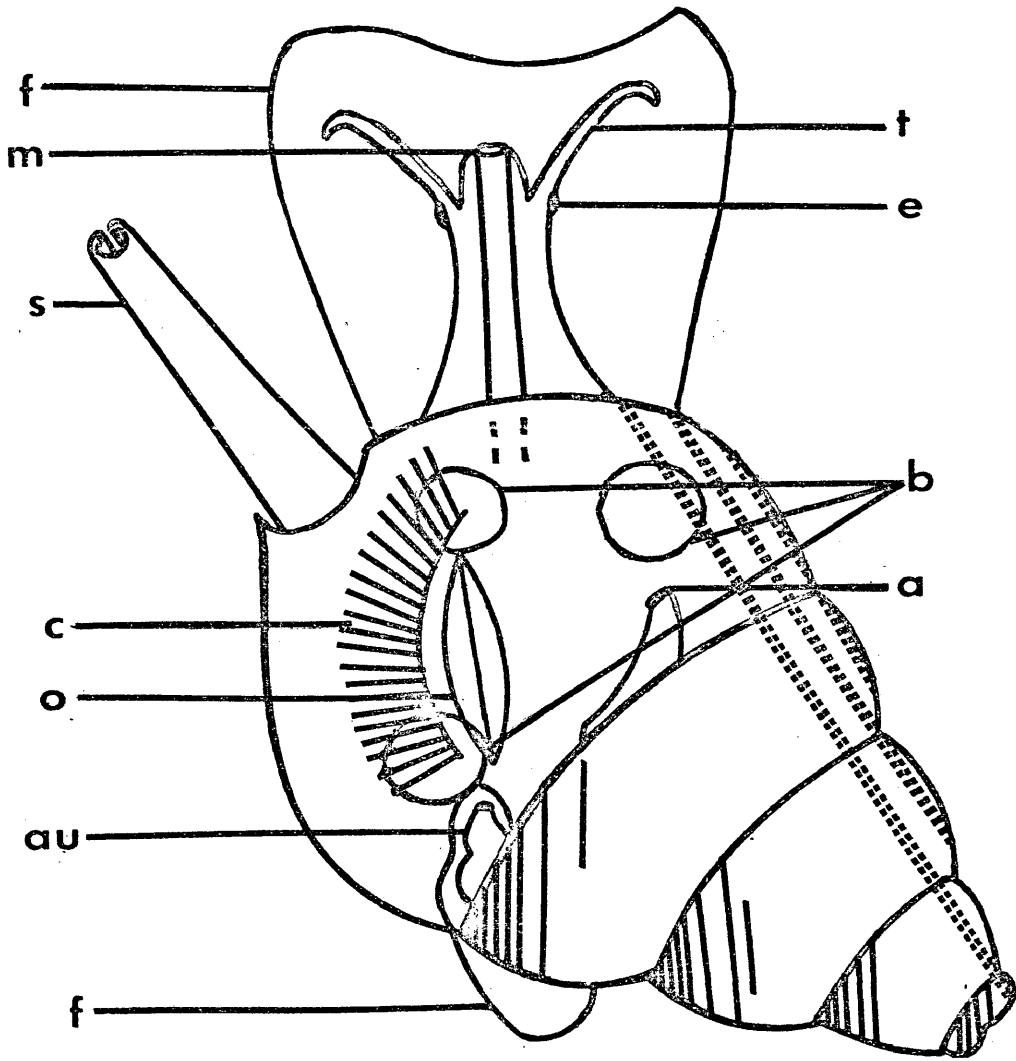
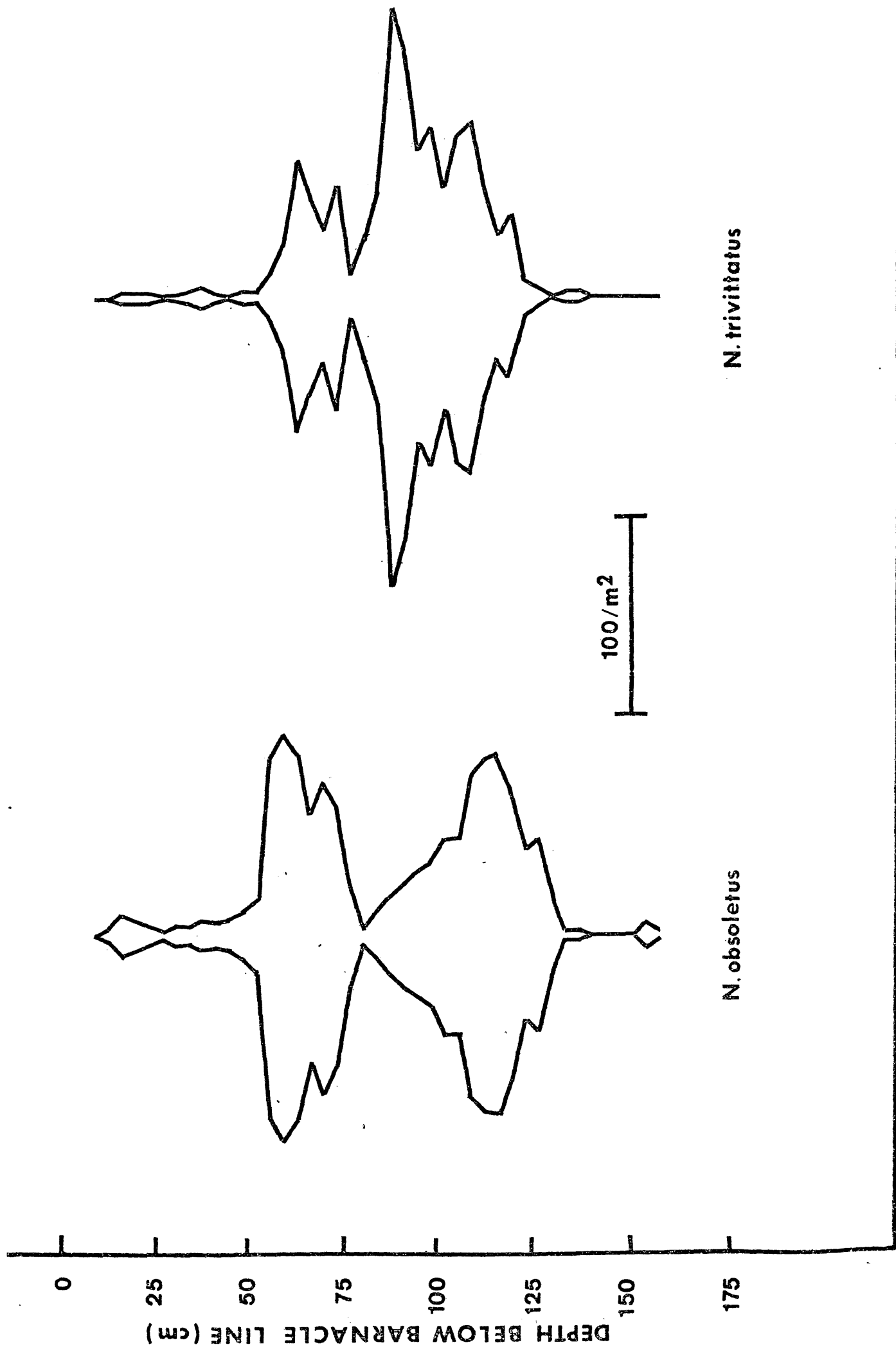


FIGURE 1: Diagram of *Nassarius obsoletus* showing locations of the gas bubble. a, anus; au, auricle; b, bubble; c, ctendium; e, eye; f, foot; m, mouth; o, osphradium; s, siphon; t, tentacle.



N. trivittatus

N. obsoletus

DENSITY
(animals / meter²)

FIGURE 2: Distribution of N. obsoletus and N. trivittatus at Indian Field Creek.

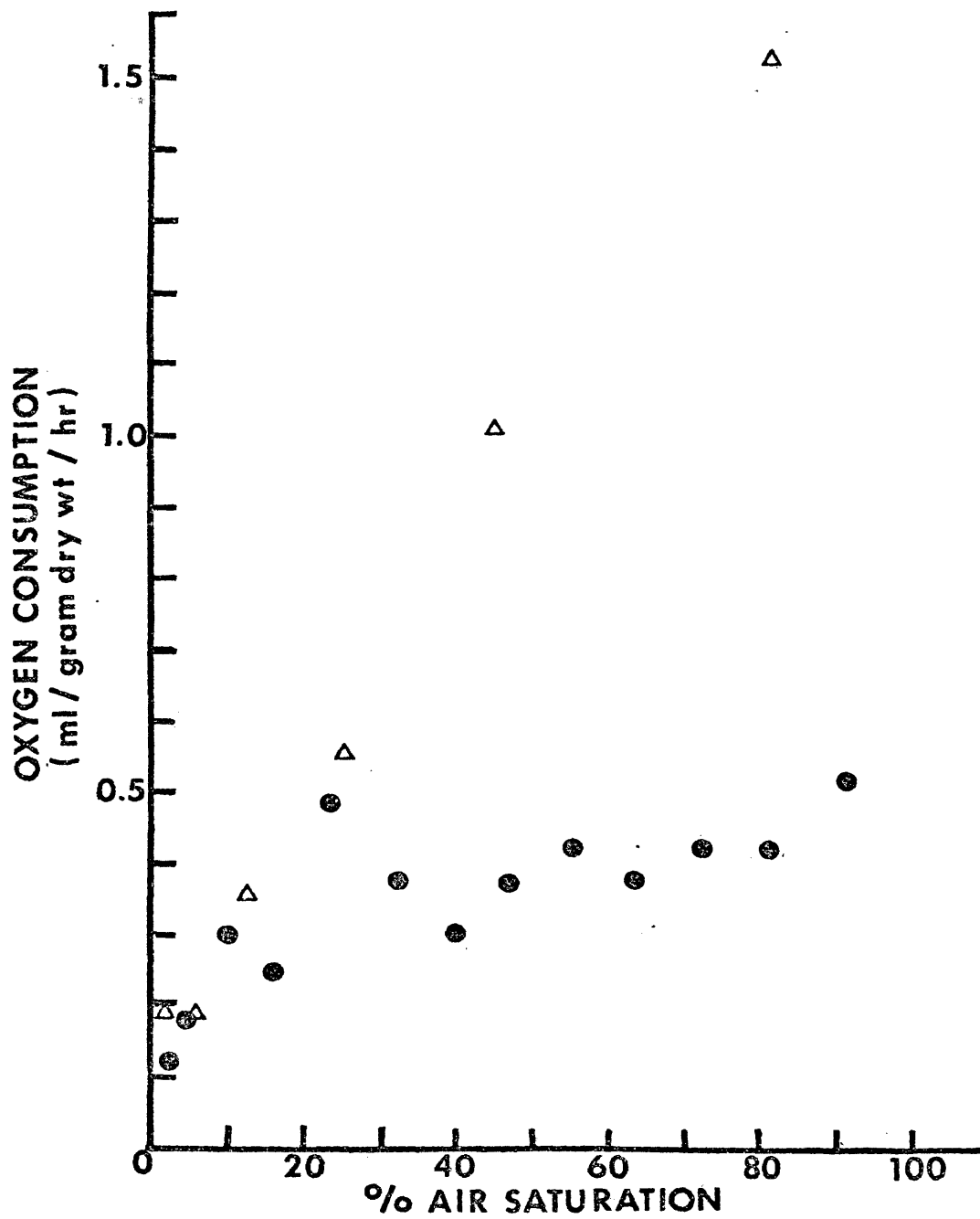


FIGURE 3: Relation of oxygen consumption of N. obsoletus to oxygen saturation of the medium. Circles are for pre-anoxic animals, triangles are for post-anoxic animals.

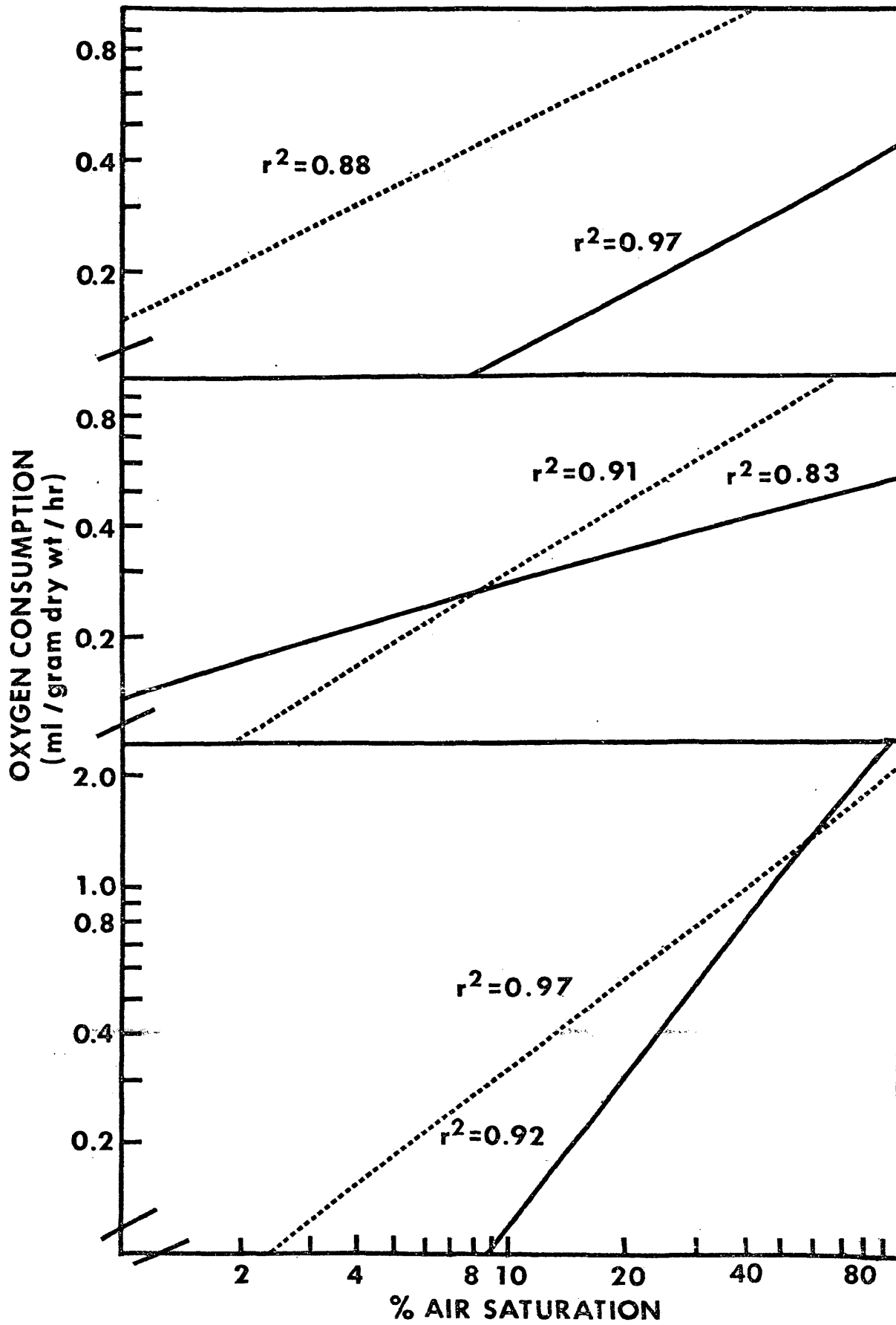


FIGURE 4: Three modes of response to low oxygen in paired measurements of N. obsoletus. Solid lines are pre-anoxic measurements, dashed lines are post-anoxic measurements.

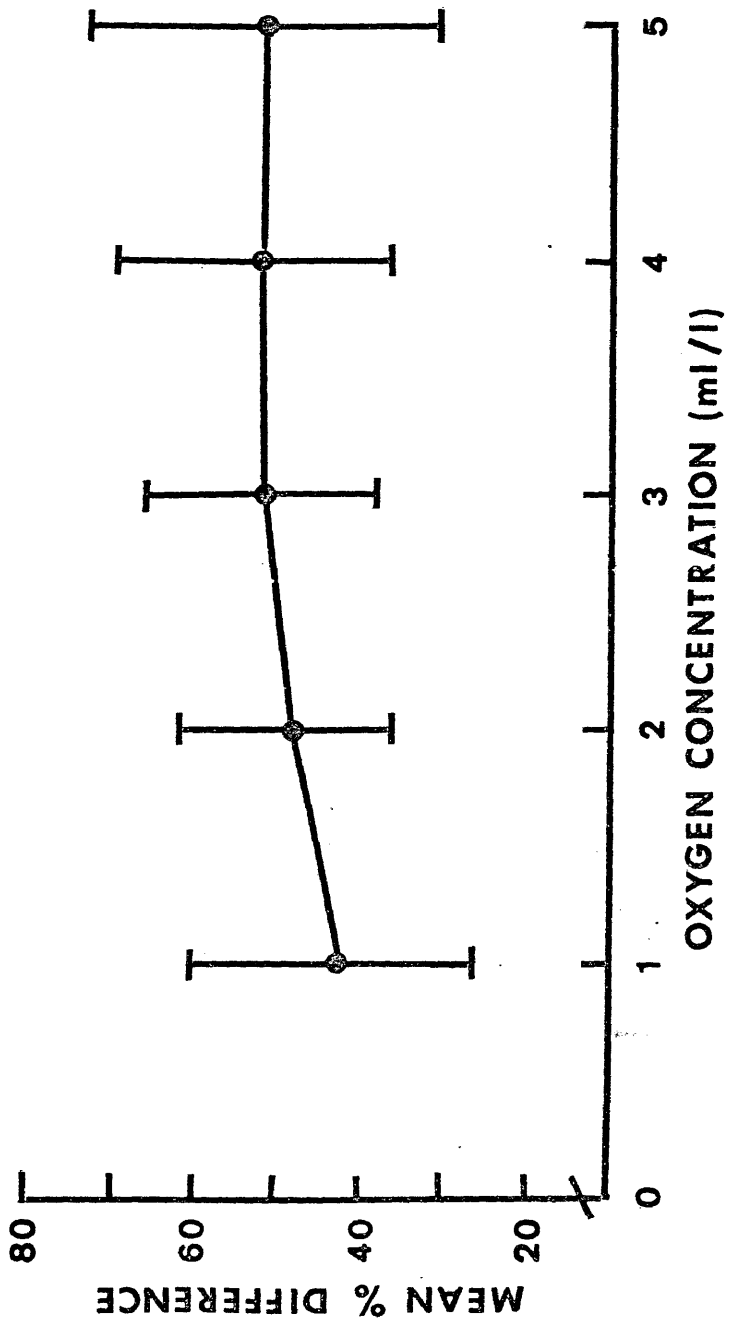


FIGURE 5: Mean percent differences of oxygen consumption at integral values of oxygen concentration before and after 24 hr exposure to anoxia in Nassarius obsoletus. The vertical lines are 95% confidence intervals.

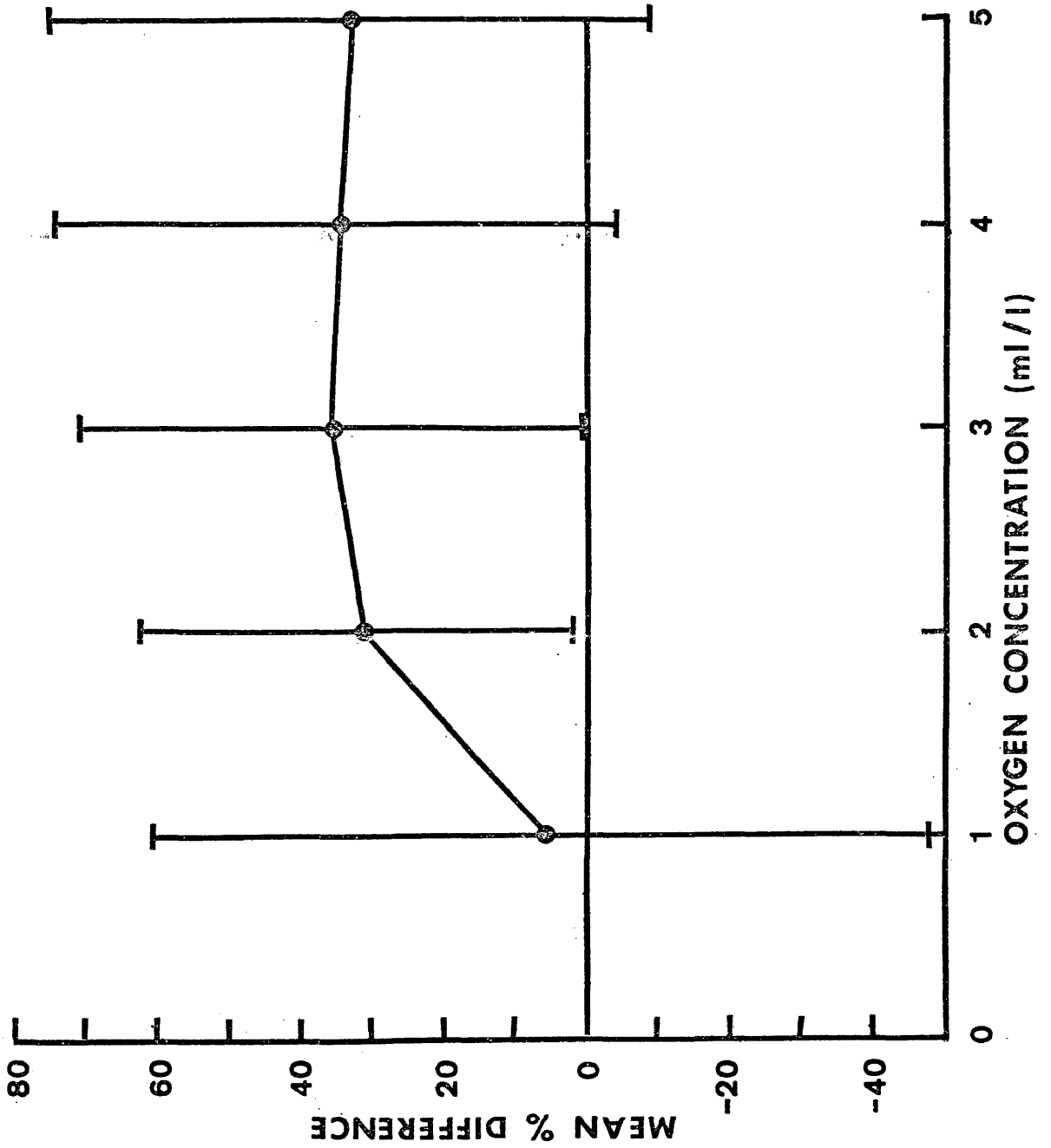


FIGURE 6: Mean percent differences of oxygen consumption at integral values of oxygen concentration before and after 24 hr exposure to anoxia in Nassarius trivittatus. The vertical lines are 95% confidence intervals.

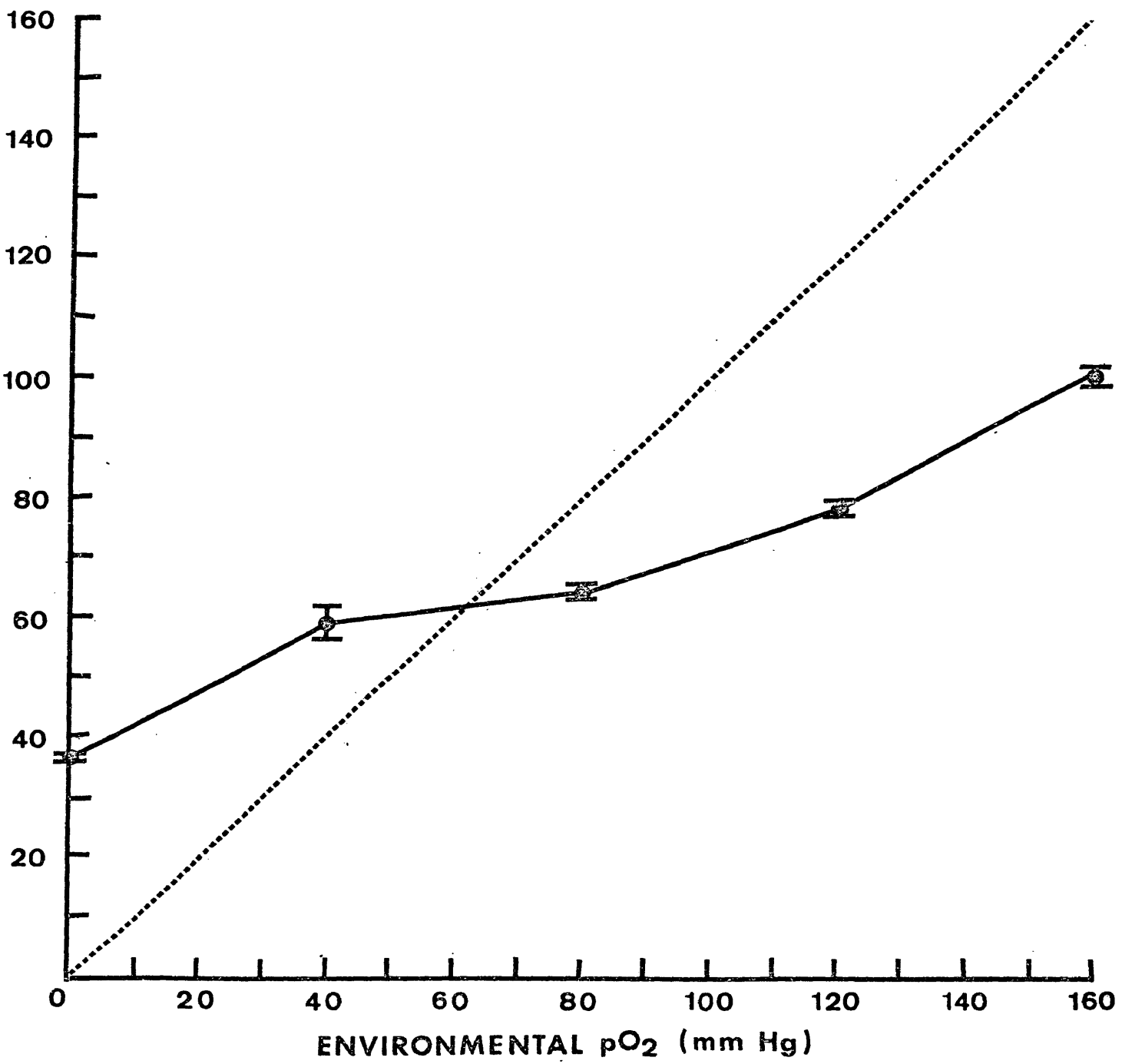


FIGURE 7: Partial pressure of oxygen of the gas bubble as a function of the partial pressure of oxygen of the environment. The vertical lines are 95% confidence intervals about the mean. The dashed line represents equilibrium.

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