

Respiration Studies With Fresh-Water Molluscs:

I. Oxygen Consumption in Relation to Oxygen Tension

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The following investigations were carried out with the purpose of determining, first, the rate of oxygen consumption of molluscs in relation to the oxygen tension of the surrounding water, and second, to what low tensions oxygen consumption could progress. Numerous trials were made, and the most significant are recorded in this paper.

Such determinations have been recorded by other investigators with other forms of animal life. In a previous paper, Hyman (1929) has given a summary of the results of other workers. For the sake of brevity, her summary and bibliography are omitted here with the exception of those applying to the Mollusca and certain other forms recently investigated. The aquatic molluscs investigated by others have been shown to maintain oxygen consumption uniformly at lowered tensions. Amberson et al. (1924) have shown that oxygen consumption of *Loligo pealii* is constant to 30% saturation of the water. Henze (1910) has reported oxygen consumption of *Limax agrestis*, a terrestrial slug, lowered at 5 to 15% oxygen. Thunberg (1905) reports oxygen consumption of the same form increased at 50 to 97% oxygen. Other molluscs able to respire uniformly at reduced tensions are *Anodonta* (Dakin and Dakin, 1925), *Buccinum*, *Aplysia*, *Fusus* (Moore et al., 1912), *Aplysia* and *Eleodone* (Henze, 1910).

The author (1931) working with aquatic insects, found that the nymphs of the dragon-fly, *Nasiaeschna penthacantha*, were able to respire at a fairly uniform rate to tensions of about one-sixth that of normal equilibrium of air and water. The same was true of the larvae of the caddis-fly, *Limnephilus rhombicus*. Increasing the percentage of dissolved oxygen in the water above normal equilibrium did not increase the respiration rate of this species.

Hall (1931) reports that oxygen consumption of the worm, *Urechis caupo*, is independent of oxygen pressures down to a value of 70 mm. of mercury. Lindemann (1932) reports a "straight-line" relationship between oxygen consumption and oxygen tension in the leech, *Hirudo*. Hiestand and Singer (1934), also using *Hirudo*, find oxygen consumption to be independent of oxygen tensions above normal air-water equilibrium but slightly dependent upon tensions below air-water equilibrium. They report cessation of oxygen consumption at about 0.8 mgm. oxygen per liter or approximately one-tenth that of normal water. More recently, Maloeuf (1936), using a closed respiration jar of rather small capacity, found that the crayfish, *Cambarus bartoni*, and the nymphs of the dragon-fly, *Anax junius*, have a respiratory rate that is more or less independent of the oxygen of the medium. He showed that both forms are capable of some anerobic metabolism when all of the oxygen has been consumed.

It may well be mentioned here that the conflicting results of various investigators of oxygen tension effects are to no small part *due to the size of the respiration chamber*. In other words, if the experimental animal be placed in a large volume of water, there will be *more total available oxygen* for its use than if it be placed in a small container. That this often erroneously influences the results obtained was demonstrated by Hiestand (1931). Thus when a crayfish is placed in a large volume of water, its respiration will show an independence of oxygen tension over a considerable range; but if it be placed in a jar too small, a "curved line" relationship will appear, since there is not enough available oxygen for a uniform consumption. In such a condition as the latter the interpretation is often that of a dependence of oxygen consumption upon oxygen tension, where as a matter of fact, the animal suffers from asphyxia and does not repay its oxygen debt until removed to media of higher oxygen content. This fact should be carefully considered in all experiments in which oxygen tension is a factor in oxygen consumption.

Material and Methods

Three species of fresh-water molluscs were used. With the exception of the Japanese snail, *Vivipara japonica*, which was obtained from a local dealer in aquarium supplies, the molluscs were obtained from the Wabash River in the vicinity of Lafayette, Indiana. The Wabash in this region is a muddy water, well supplied with branchiate molluscs. The pH of the river was found to be 7.25, or nearly neutral. The molluscs, after removal from the river, were washed carefully with a brush and cloth and placed in laboratory distilled water having a pH value of 5.4. They apparently suffered no harmful effects by being kept in distilled water. In order to determine the metabolic rates in relation to body weight, it became necessary to remove them from their shells, which was done at the conclusion of the experiments. The weights given, therefore, refer to the live weights of the animals exclusive of their shells.

The apparatus used consisted of a wide-mouth jar of 960 ml. capacity, stoppered with a rubber stopper bearing two holes. One hole received a capillary tube to allow inlet of mercury; the other hole admitted a thermometer. No air was entrapped in the bottle during any experiment. Suspended from a hook in the stopper by a thread was a wire screen cage holding the animals. At the side of the jar, midway from top to bottom, was a capillary stop-cock for the withdrawal of water samples for oxygen determination. Clean, washed mercury was admitted by a burette through the capillary tube to replace the water withdrawn for sampling. A more complete and illustrated description of the apparatus can be found in a previous paper by the writer (1931).

Samples of water were withdrawn into special 10 ml. glass-stoppered volumetric flasks for oxygen determination by the micro-Winkler method of Lund (1921). Sodium thiosulphate of N/400 strength was used. Using a 10 ml. sample, each ml. of thiosulphate is equivalent to 2 mgm. of oxygen per liter (2 parts per million), or 1.4 cc. of oxygen per liter. At each withdrawal of sampling water an additional amount (1 or 2 ml.)

of water was removed and allowed to flow over the top of the 10 ml. flask to remove surface water which had come into contact with air during the procedure. It is doubtful that this precaution was necessary because of the short interval of time elapsing during the withdrawal of the sample. Specially prepared micro-pipettes were made to deliver 0.1 ml. of the reagents used in the determination. The micro-Winkler method of oxygen determination has been thoroughly tested by the writer in previous work and found to be as accurate as the ordinary method using a 250 ml. sample of water.

Experiments With Diminishing Oxygen Tension

1. *Vivipara japonica* (Von Martens).—When individuals of this species were placed in the cage and allowed to decrease the oxygen tension by their own oxygen consumption, quite uniform results were obtained. The gastropods remained quiet during the entire test; so the respiratory conditions can be considered basal. Table I and Figure 1 show the results of one experiment. It will be noted that a lapse of 11 hours occurred during which no readings were made. At the end of this period (the following morning) the same procedure was followed until it became apparent that the low point had been reached. The experiment required 34 hours for completion. Had a smaller animal jar been used, a shorter time would have been necessary for the final oxygen tension to be reached. As can be seen from the graph (Fig. 1), oxygen consumption was quite regular until a low tension of 0.52 cc. per liter was reached. During the next hour oxygen consumption was greatly lessened. The experiment was stopped at this point to prevent injury to the snails. Thus the experiment demonstrates that oxygen consumption can continue to a tension less than one-tenth of that of normal saturation of oxygen in water.

TABLE I.

Vivipara japonica:
2 individuals.

Weight (without shells) 33.15 gm.
Temp. 23° C.

Hours.....	Water vol. ml.	Thios. ml.	O ₂ per L. mgm.	O ₂ per L. cc.
0.....	960	3.95	7.90	5.53
2.....	948	3.75	7.50	5.25
4.....	936	3.54	7.08	4.95
6.....	924	3.30	6.60	4.62
8.....	912	3.10	6.20	4.34
10.....	900	2.87	5.74	4.02
12.....	888	2.60	5.20	3.64
(lapse of 11 hours)				
23.....	876	1.64	3.28	2.29
25.....	864	1.45	2.90	2.03
27.....	852	1.23	2.46	1.72
29.....	840	0.97	1.94	1.35
31.....	828	0.70	1.40	0.98
32.....	816	0.55	1.10	0.77
33.....	804	0.37	0.74	0.52
34.....	792	0.35	0.70	0.49

2. *Utterbackia imbecillis* (Say).—For a comparison with the previous experiment, bivalves of the above species were used, two being placed in the container at the same time. Table II and Figure 1 show the data obtained. It will be seen from these data that oxygen consumption of *Utterbackia* is relatively uniform from normal tensions to approximately 0.70 cc. per liter.

TABLE II.

Utterbackia imbecillis:
2 individuals.

Weight (less shells) 48.43 gm.
Temp. 23° C.

Hours	Water vol. ml.	Thios. ml.	O ₂ per L. mgm.	O ₂ per L. cc.
0.....	960	4.10	8.20	5.74
2.....	949	3.52	7.04	4.93
4.....	938	3.08	6.16	4.31
6.....	927	2.74	5.48	3.84
8.....	916	2.14	4.28	2.99
10.....	905	1.76	3.52	2.46
12.....	894	1.25	2.50	1.75
14.....	883	0.82	1.64	1.15
15.....	872	0.64	1.28	0.89
16.....	861	0.56	1.12	0.78
17.....	850	0.52	1.04	0.73

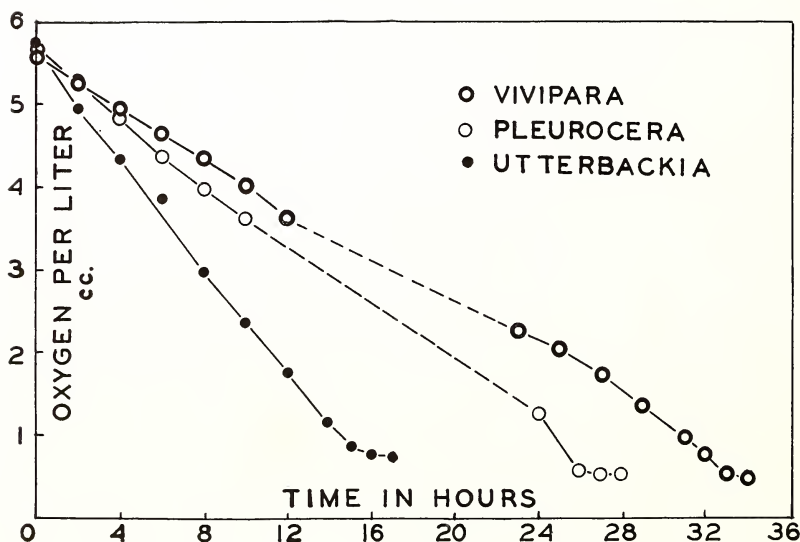


Fig. 1.

3. *Pleurocera undulatum canaliculatum* (Say).—Twenty-eight specimens of this species of *Gastropoda* were used simultaneously. The conditions were similar to those of Table I. During the course of the

experiment, which lasted 28 hours, a period of 13 hours elapsed over night. Here it can be seen that oxygen consumption continued quite regularly down to a concentration of oxygen of approximately 0.50 cc. per liter (about one-tenth that of the start of the experiment). Other experiments, the data of which are not included here, definitely proved that respiration of this species stopped at a tension of 0.50 cc. oxygen per liter.

TABLE III.

Pleurocera undulatum: Weight (less shells) 10.95 gm.
28 individuals. Temp. 23° C.

Hours	Water vol. ml.	Thios. ml.	O ₂ per L. mgm.	O ₂ per L. cc.
0.....	960	4.05	8.10	5.67
2.....	949	3.78	7.56	5.29
4.....	938	3.44	6.88	4.82
6.....	927	3.10	6.20	4.34
8.....	916	2.85	5.70	3.99
10.....	905	2.58	5.16	3.61
(lapse of 13 hours)				
24.....	894	0.88	1.76	1.23
26.....	883	0.42	0.84	0.58
27.....	872	0.38	0.76	0.53
28.....	861	0.38	0.76	0.53

Discussion

These investigations have shown that branchiate molluscs are able to remove oxygen from the surrounding water at a fairly uniform rate from a tension of normal oxygen saturation to levels of relatively low oxygen percentages. The writer has been unable to find evidence of any free-living animal able to withstand such low tensions as the molluscs, unless living under anoxybiotic conditions as *Corethra* and *Chironomus*, as demonstrated by Juday (1908). When we bear in mind that molluscs of these varieties live in streams, the water of which is exposed to atmospheric air, it appears a remarkable fact that they should be able to respire at a uniform rate over a range of oxygen pressures as considerable as these. The fact that oxygen consumption is not proportional to oxygen tension seems to indicate one of two possibilities. Either (1) *the animals use so little oxygen that all pressures above the minimum are greatly in excess of their needs*, or (2) *they are able to regulate their metabolic rate in such a way that they are able to consume proportionally more oxygen at lower tensions than at higher ones*. When one considers the slow rate of metabolism of molluscs as compared with that of other animals, the former explanation appears more plausible than the latter.

In relation to gram-body weight the rate of metabolism is greatest for the genus *Pleurocera*, less for the genus *Utterbackia*, and least for the genus *Vivipara*. Table IV has been compiled from the data of the

preceding experiments. It indicates the rate of metabolism of each species when reduced to the same standard conditions.

To this may be added the metabolic rate of another gastropod which has been recorded in other respiration studies, at same pH,—

TABLE IV.—Standard rate of respiration at pH 5.4

Pleurocera: .016 cc. O₂/gram body wt./hour.

Utterbackia: .008 cc. O₂/gram body wt./hour.

Vivipara: .004 cc. O₂/gram body wt./hour.

Campeloma crassulum (raf.) .009 cc. O₂/gram body wt./hr.

It can be seen from the metabolic rates that the smaller species of molluscs respire at a greater rate than the larger ones, which fact agrees with the comparative rates of other animals in general.

Summary

Each of the three species of molluscs used in this investigation consumed oxygen at a fairly uniform rate until quite low tensions were reached. For the Japanese snail *Vivipara japonica* (Von Martens), oxygen consumption ceased at a concentration of about 0.49 cc. per liter. For the bivalve *Utterbackia imbecillis* (Say), the low point reached was approximately 0.73 cc. per liter. For the gastropod *Pleurocera undulatum canaliculatum* (Say), the lowest tension was about 0.53 cc. per liter. Explanations of the ability of molluscs to respire at very low concentrations of oxygen have been suggested in this paper. The relationship of metabolic rate of four species of molluscs to size have been shown.

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