Comp. Biochem. Physiol., 1978, Vol. 59A, pp. 199 to 203. Pergamon Press. Printed in Great Britain

OXYGEN CONSUMPTION IN THE PROSOBRANCH SNAIL *VIVIPARUS CONTECTOIDES* (MOLLUSCA: GASTROPODA)—IV. EFFECTS OF DISSOLVED OXYGEN LEVEL, STARVATION, DENSITY, SYMBIOTIC ALGAE, SUBSTRATE COMPOSITION AND OSMOTIC PRESSURE

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(*Received* 13 *April* 1977)

Abstract—1. Oxygen consumption (OC) is directly related to dissolved oxygen levels.
2. While starvation for up to 12 days has no consistent long-term effect on OC of male snails, OC of females is depressed by lack of food.
3. OC is not related to density of snails either in single or mixed sex groups of up to eight individuals per 250 ml flask.
4. Symbiotic algae produce amounts of oxygen sufficient to significantly depress apparent OC of snails in a closed system.
5. OC over short periods is independent of substrate composition.
6. Rising osmotic pressure (OP) due to NaCl is associated with decreasing OC; increasing OP due to $CaCl_2$ is accompanied by rising OC; and, changing OP due to evaporation or dilution of natural water has no effect on OC.

INTRODUCTION

Maintenance metabolism in snails (measured as oxygen consumption), as in most animals is apparently the most important route of daily energy expenditure (see Studier *et al.*, 1975). Evaluation of various factors which might be expected to significantly effect oxygen consumption (OC) provides a measure of the importance of these intrinsic and extrinsic factors to the daily energy budget of the species. These findings also point out precautions which must be taken in future related studies of OC. Previous studies of the prosobranch snail, *Viviparus contectoides*, have reported the effects and relationships of OC to weight and activity (Fitch, 1975), to ambient temperature and pH (Buckingham & Freed, 1976) and natural and artificial light cycles (Fitch, 1976). This report concludes our studies of parameters which may effect OC in *V. contectoides* and evaluates the effects of dissolved oxygen level, starvation, density, symbiotic algae, substrate composition and osmotic pressure.

The ranges of these parameters in the natural habitats of *Viviparus contectoides* are only poorly known. Harman & Berg (1971) found this species (as *V. georgianus*) in waters ranging in O_2 concentration from less than 2 mg O_2/l (i.e. < 1.4 cm^3 O_2/l), while Horst (1971) collected this species from McGargo Lake which had a surface reading as low as 0.4 mg O_2/l (*ca.* 0.28 cm^3 O_2/l). In central New York, Harman (1974) has found these snails in water with O_2 concentrations as high as 8 mg O_2/l (*ca.* 5.6 cm^3 O_2/l).

Viviparus contectoides is frequently found at rather low densities burrowing in sandy substrates with seemingly little organic matter. However, densities as high as 250/m^2 (Van Cleave & Lederer, 1932) and 864/m^2 (Pace *et al.*, 1975) have been sampled from substrates rich in silt and fine organic detritus. Harman (1972) also found that these snails prefer silt and detritus substrates.

The physical association between *Viviparus contectoides* and the algal communities covering their shells has often been noted (e.g. Van Cleave & Lederer, 1932; Fitch, 1975). To our knowledge, however, no measures of its possible symbiotic significance or influence on apparent snail OC have ever been reported.

While no direct measurements of the range of osmolarity of the habitats of this species have been made, Harman (1974) listed these snails from habitats ranging in alkalinity (as $CaCO_3$) from 20 to 230 mg/l. The water from which our snails were collected had an alkalinity of about 200 mg/l and an osmolarity of 10 mOsm/l.

MATERIALS AND METHODS

Methods for the determination of OC have been described (Fitch, 1975). Initial oxygen levels were varied by slowly bubbling pure oxygen or nitrogen gas through the test water for varying periods of time. In addition to normal oxygen levels of approximately 5 cm^3/l (*ca.* 7.1 mg/l), lowered oxygen levels of about 1 and 4 cm^3/l (*ca.* 1.4 and 5.7 mg/l) and elevated oxygen levels of about 14 and 23 cm^3/l (*ca.* 20 and 33 mg/l) were developed and OC of snails determined at all five levels. The OC of eight individuals of each sex was measured in 250-ml Erlenmeyer flasks over a period of 2 hr at each oxygen concentration. To test the possible effects of starvation, OC of five snails of each sex was determined immediately after their removal from holding tanks and on the 3rd, 9th and 12th days thereafter. Between tests, the same snails were separately

maintained in labeled containers of natural water containing no food. To determine the effects of density on the OC of these snails, tests were performed on individuals and on groups of 2, 4, 6 or 8 snails. All OC-density determinations were performed on single-sex groups of both sexes and on mixed-sex groups in a 1:1 sex ratio of individuals. As stated previously, the shells of most *Viviparus contectoides* harbored a dense periphyton community whose most conspicuous members were *Oedogonium* sp. and *Cladophora* sp. This was scoured off with an abrasive cloth in all other tests. To determine if the algal growth produced sufficient oxygen during lighted periods to significantly effect apparent OC of the snails, OC determinations were performed on eight scoured and eight unscoured individuals of each sex. Substrate composition effects were examined by testing the OC of eight male and eight female snails in unmodified 250 ml Erlenmeyer flasks and in identical flasks to which had been added enough of the following to cover the bottom to a depth of about one-fourth the height of the flask: sterilized natural sediment, fine sand, coarse sand, or small (dia. *ca.* 2 mm) aquarium gravel. Some initial studies concerning the effects on OC of a range of osmotic pressures (OP) of 3.4, 6.8, 10 and 11.2 mOsm/l were performed. In one series natural water (10 mOsm/l) was evaporated to obtain the higher OP and distilled water was added to obtain the lower OP levels. In two other sets of experiments, these same OPs were achieved by adding either NaCl or $CaCl_2$ to distilled water.

All OC determinations except for effects of symbiotic algae were performed in minimal light for a period of 2 hr between 2000 and 2400, the approximate time of minimal activity (Fitch, 1975). All snails had been maintained in natural water (OP = 10 mOsm/l; pH = 8.1) with natural food-laden sediment in a light cycle of 12 hr light (lights on at 0800); 12 hr dark for at least 10 days prior to testing. Water temperature was maintained at 20–25°C. With the exception of individuals used in the starvation study, OC was never determined more than once per snail. Regression analyses were performed with Dual IBM 360/67 processes and statistical significance was defined as $P < 0.05$.

RESULTS

A direct, predictive ($r^2 = 0.714$) relationship exists between OC and dissolved oxygen level in *V. contectoides*. There is no difference in the relationship when comparing males to females. The regression coefficient is significant ($t = 14.05$; df = 80; $P < 0.0001$) while the intercept does not differ from zero ($t = 1.822$; df = 80; $P > 0.5$).

The relationship is expressed by the following equation:

$$\underset{}{OC} = \underset{(0.00038)}{0.00534\,OL} + \underset{(0.00478)}{0.00870},$$

where OC is cm^3g/hr and OL is oxygen level in cm^3/l ($n = 81$; values in parentheses are S.E.M.).

In male snails, starvation resulted in a significant rise in OC after 3 days ($t = 7.714$; df = 8; $P < 0.01$) followed by a return to pre-starved rate of OC at 9 and 12 days (Table 1). Starvation in female snails was accompanied by a slow drop in OC until day 9 when OC was significantly lower than the pre-starved level ($t = 6.335$; df = 8; $P < 0.01$). After 9 days of starvation, OC apparently remains constant until at least day 12 (Table 1).

Grouping of snails of the same sex or in equal ratio mixed sex groupings showed no significant relationships to OC of individual snails regardless of the total number in the test group.

Table 1. Weight-specific oxygen consumption (cm^3/g/hr) of *Viviparus contectoides* under fed conditions (day 0) and after 3, 9 and 12 days of starvation. Sample size is 5 in each case. Upper value is the mean; lower value is the S.E.M.

Day:	0	3	9	12
Females	0.03228	0.03026	0.02506	0.02590
	0.00095	0.00095	0.00020	0.00058
Males	0.07462	0.08916	0.07228	0.07396
	0.00037	0.00185	0.00071	0.00079

The filamentous algae naturally present on the shell significantly decreases the apparent OC of male ($t = 2.573$; df = 14; $P < 0.05$) and female ($t = 2.641$; df = 14; $P < 0.02$) *V. contectoides* when compared to the OC of snails from which the algal growth is removed (Table 2).

Surprisingly, there was no acute effect of substrate composition on OC in *V. contectoides*. Oxygen consumption of snails exposed to natural sediment substrate did not differ significantly for either sex from OC of snails studied in all glass vessels or in the presence of fine sand, coarse sand, or small aquarium gravel.

Analysis of preliminary data concerning acute effects on OC of varying OP using diluted and concentrated natural water indicate that no relationship exists. Increasing OP due to addition of NaCl to distilled water, however, shows that OC is inversely related to OP by the following equation ($n = 30$; $r^2 = 0.601$).

$$\underset{}{OC(cm^3/g/hr)} = \underset{(0.00119)}{-0.00775\,OP} + \underset{(0.011)}{0.234},$$

where the values in parentheses are S.E.M. Increasing OP due to the addition of $CaCl_2$ to distilled water is accompanied by rising OC as shown by the following equation ($n = 29$; $r^2 = 0.163$):

$$\underset{}{OC(cm^3/g/hr)} = \underset{(0.00039)}{0.00092\,OP} + \underset{(0.0052)}{0.0157},$$

where the values in parentheses are S.E.M.

DISCUSSION

The direct relationship between the OC of *Viviparus contectoides* and environmental oxygen availability is typical of the metabolic conformity characteristic of many aquatic ectotherms, especially benthic invertebrates and sedentary aquatic vertebrates (see Prosser, 1973, p. 198). In comparison, many mollusks, including other snails, show a complete range of re-

Table 2. Weight-specific oxygen consumption (cm^3/g/hr) under lighted conditions of *V. contectoides* with and without the presence of algal growth on the shell. Sample size is 8 in each case. Values given are the mean (upper) and S.E.M. (lower)

	With algae	Without algae
Males	0.0684	0.1274
	0.0200	0.0113
Females	0.0983	0.2314
	0.0139	0.0484

sponses of OC to ambient oxygen tension—"from total or partial dependence to complete independence" (Ghiretti, 1966a, p. 201; also see von Brand *et al.*, 1948; Berg & Ockelmann, 1959; Åkerlund, 1974). The observation that the ecological and phylogenetic distribution of oxyconformity and oxyregulation make little biological sense has been extensively reviewed (Mangum & Van Winkle, 1973; Van Winkle & Mangum, 1975). These authors have noted that the mode of response is primarily determined by the path of oxygen permeation into aerobic tissue. This is, of course, influenced by the area of surface available for gas transport; the area, volume and turnover rate of respiratory chambers (e.g. mantle cavity); the partial pressure differences between the environmental medium, the blood and tissues; and the respiratory pigment cooncentration, loading tension and storage capacity of the blood. Prosobranchs can easily restrict both the cutaneous and respiratory chamber gas transport by simply withdrawing into their shells and closing the operculum. Although Jones (1961) has shown that certain aquatic pulmonates can adjust cutaneous and pulmonary gas exchange rates so as to maintain OC constant over a wide range of pO_2, no similar experiments have been reported for freshwater prosobranchs. A further complication in analyzing this response is the fact that part of the feeding behavior of *Viviparus* involves ctenidial filtering as water currents are drawn through the mantle cavity (Cook, 1949). How this ciliary feeding is controlled and how it may influence OC is unknown. Fox (1955) showed that hemoglobin synthesis may vary with ambient oxygen tension in certain pulmonates. The concentration of hemocyanin in most mollusks, however, remains quite constant under normal physiological conditions (Ghiretti, 1966*b*). No specific studies on *Viviparus* species have been reported. Finally, one study of a European species, *Viviparus fasciatus*, reported that after 24 hr under elevated oxygen tension, the snails returned to original rates of OC (see Hyman, 1967, p. 389). This, in conjunction with some of the above questions, suggests some interesting possibilities for future investigation. From an ecological standpoint, however, the wide range of oxygen concentration in which *V. contectoides* has been found (see above) indicates that other factors are more likely to be limiting in many natural aquatic habitats.

Past studies on the effects of starvation on the OC of several species of snails vary both in duration and results. In an early study of four aquatic pulmonates, the OC of all four had dropped to about 50% of normal after 6 days and below 40% after 9 days of starvation (von Brand *et al.*, 1948). The OC of a freshwater pulmonate limpet was found to decrease to two-thirds of the initial rate after 3 days of starvation (Berg *et al.*, 1958). In another series of studies, Berg & Ockelmann (1959) showed that for four of five species of freshwater pulmonates starved for 10 hr and for two of three species of freshwater prosobranchs starved for 24 hr, very little or no decrease in OC occurred. In *Potomatopyrgus jenkinsi*, a prosobranch, several days of starvation caused a substantial reduction in OC (Lumbye & Lumbye, 1965). On the other hand, Åkerlund's (1969) study of the tropical prosobranch, *Marisa cornuarietis*, starvation resulted in no consistent effect on OC. Similarly, Newell & Pye (1971) detected no significant difference in the OC of the intertidal prosobranch, *Littorina littorea* after 3 weeks of starvation at 7°C. Although the low temperature maintained in the latter study undoubtedly reduced the effects, there are obviously substantial interspecific differences in the effects of starvation on OC. Therefore, possible starvation effects must be considered in all studies of molluscan OC. None of the previous studies included any species of *Viviparus*, nor distinguished between the effects of starvation on the OC of the two sexes. An explanation of the differences noted in this study between the sexes of *V. contectoides* requires some knowledge of the fat and carbohydrate metabolism of these snails. In 1932, Rosen found that 6 weeks of starvation were required to deplete fat reserves stored in the midgut gland of *Viviparus* (see Hyman, 1967, p. 397). Fat is evidently not mobilized as fast as carbohydrates. This has been demonstrated in oysters (Baker *et al.*, 1942). In studies of the polysaccharides of the Indian aquatic snails, *Viviparus* was one of those in which galactogen was found (Meenakshi, 1954). In the common European land snail, *Helix pomatia*, May and his associates (see Goddard & Martin, 1966) found that galactogen is present only in the albumen gland and in the eggs, while glycogen was found exclusively in the rest of the body. Studies of some operculate snails having separate sexes revealed that the mates contained only glycogen. May's studies of hermaphroditic *Helix*, revealed that glycogen reserves were used up after 10 days of starvation while galactogen levels remained constant. Thereafter, it took nearly 20 days for the galactogen reserves to be depleted. Goddard & Martin (1966) speculated that galactogen acts primarily as a reserve for reproduction and is protected from easy mobilization. We might speculate further that starved males, lacking any galactogen reserves, might soon be driven to increased food-finding activity resulting in temporarily higher OC rates. Females, on the other hand, may reduce the conversion of glycogen to galactogen until food becomes more readily available, possibly resulting in the noted gradual decline in OC. This extra source of stored energy could also account for the apparently higher survival rate of females during the difficult overwintering period (Pace *et al.*, unpublished data). While all of this is highly conjectural, it does suggest a number of interesting studies for future investigation.

With the knowledge that natural densities frequently reach several hundred per square meter (see above) it is not surprising that the densities tested here did not significantly effect OC in these snails. The activity and metabolism of these snails evidently accomodates early to the high frequency of intraspecific contacts.

The symbiotic relationship between *Viviparus* and its shell-borne periphyton community is certainly much less specific and intense than that of the marine bivalves, *Cardium* and *Tridacna*, and opisthobranchs, *Aeolidiella*, with their intracellular zooxanthellae (Yonge, 1966). We might speculate that as the snails plow up and down through the food-bearing substrate, algae are alternately brought into regions of relatively high light intensity (substrate surface) and high CO_2 and nutrient levels (substrate depths). Since the snails are relatively large, the chances of the

attached algae being washed away by wave action or streamflow is reduced. Attachment to moving animals decreases the chances of the algae being permanently covered by silt. If any advantage accrues to the snails it is probably in the form of increased oxygen and pH levels and decreased CO_2 tension. We might also speculate that another indirect advantage to the snails might result from $CaCO_3$ being precipitated by the algae as CO_2 is withdrawn from the water. The evidence presented here, at least demonstrates the potential of these algae for effecting the ambient oxygen levels of the snails. The other suggestions, of course, require further study.

While these snails are found on a variety of substrates, higher frequencies of occurrence (Harman, 1972) and higher densities (Van Cleave & Lederer, 1932; Pace *et al.*, 1975) in those rich in organic sediments show an undeniable preference. Therefore, the lack of effects of substrate type on OC over a 2-hr period is at first perplexing. Further consideration, however, suggests that these experiments actually parallel short-term starvation experiments. If this is the case, we are actually comparing fed snails (natural substrate) with starved snails (fine and coarse sand, small gravel). The above starvation experiment, however, has demonstrated that males require 3 days and females require 9 days of starvation to demonstrate OC effects. In all probability, the snail's distribution is more reflective of the food content of the substrate than on texture.

Several authors (Fredericq, 1904; Obuchowicz, 1958; Little, 1965*a*, *b*) have studied the osmotic properties of the blood and tissues of species of *Viviparus*. The OP of the blood of *Viviparus viviparus* has been reported as 50–60 mM/l NaCl (*ca.* 95–114 mOsm/l NaCl) by both Fredericq (1904) and Potts & Parry (1964). Little (1965*a*), however, reported a figure of 40.4 mM/l NaCl (*ca.* 76.9 mOsm/l NaCl). Assuming that the OPs of blood for *V. viviparus* and *V. contectoides* are similar, all our experimental OPs (3.4–11.2 mOsm/l) are substantially hypoosmotic to the blood of the snails. Little (1965*a*) showed that in concentrations from de-ionized water up through 15% sea water (0–85 mM/l NaCl) *V. viviparus* specimens maintained their blood significantly hyperosmotic to the external medium. In this same range, the difference between internal and external osmotic pressures decrease, however, as external OP increases. Our observation that OC decreases as external OP increases due to NaCl, may therefore reflect a reduction in active transport work required. It should be noted, however, that *Viviparus* is unknown from waters greater than 8.5% sea water (Baltic Sea) and Little (1965*a*) suggests that an increase of intracellular sodium for long periods may be limiting. The importance of calcium to the size and strength of the shell is obvious and well documented (see Wilbur, 1964). Therefore, the increase in OC as OP was increased by $CaCl_2$ addition might be explained as follows. Increased OC may reflect increased rates of shell formation stimulated by elevated Ca^{2+} and possibly elevated CO_2 levels in mantle tissue.

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