

Effects of Water Flow and Hypoxia on Respiration of the Frogfishes, *Histrio histrio* and *Phrynelox tridens*

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Abstract Effects of water flow rate and lowering the dissolved oxygen in ambient water on the respiration of the frogfishes, *Histrio histrio* and *Phrynelox tridens*, were investigated using continuous flow respirometry. Resting \dot{V}_{O_2} of isolated fish of both species did not increase with increase in flow rate through the respirometer tube, whereas the percentage of oxygen removed from that water decreased. With increasing flow rate the ventilatory frequency rose in *P. tridens*, but not in *H. histrio*. These differences are probably related to differences in the habitat of each species. Both the percentage of oxygen removal and ventilatory frequency in each species increased with decrease in the oxygen content in ambient water. \dot{V}_{O_2} did not change in *H. histrio* but increased with a decrease in water oxygen content in *P. tridens*. It is suggested that lowering of oxygen in the inspired water leads to compensatory responses in which the oxygen consumption of the fish during hypoxia is maintained in *H. histrio* or increased in *P. tridens* as a result of a rise in proportion of oxygen removed and elevated ventilatory frequency.

Ventilation of fish gills is produced by muscular activities which pump water through a resistance provided by the gills. The ventilation mechanism essentially involves a double pump, a buccal pressure pump which is mainly concerned with forcing water through the gills and two opercular suction pumps which mainly produce flow of water by reduction in pressure within the opercular cavities (Hughes, 1961)—the relative balance in the role of these two pumps and consequent variations in pattern of ventilation are related to the ecological conditions under which the fish live.

Either swimming speed or the rate of water flow have been considered important factors controlling ventilation in addition to the oxygen levels in the water. Thus when some fishes swim above a given speed they do not make normal ventilatory movement but rely to a greater or lesser extent upon the current entering the mouth as a result of their forward movement (Hughes, 1960 a, b; von Wahlert, 1964; Brown and Muir, 1970; Roberts, 1975; Hughes and Nyholm, 1979). However, in some species there is scarcely any change in ventilatory frequency when the rate at which water flows over a fish is changed experimentally, e.g. dogfish (Hughes and Umezawa, 1968 a) and killifish (Umezawa and Watanabe, 1973), but even so

there must be some increase in ventilation as they face upstream and depend on the buccal pressure pump.

Moreover, other fish which usually spend much of their life on the sea bottom and are subjected to a wide range of environmental oxygen tensions depend to a far greater extent on the suction pump mechanism, one feature of which is that the mouth usually remains closed for longer periods. Such teleosts as the dragonet, plaice, sole and other flatfishes are notable teleost examples (Hughes, 1963). Among the many other bottom-living forms there are two types of frogfish (Antennarioidei), which seem to show great dependence on their opercular suction pumps.

The present experiments were carried out in order to measure the oxygen consumption and ventilatory frequency of these sluggish fishes in relation to changing rates of water flow across the fish and its oxygen content. Water was passed continuously over two frogfishes, *Histrio histrio* (Linnaeus) (= *Pterophryne histrio*) and *Phrynelox tridens* (Temminck et Schlegel) (= *Antennarius tridens*) which have somewhat different life habits although they are related genera. *H. histrio* exhibits a tendency to live near the surface on floating seaweeds whereas *P. tridens* normally rests at the

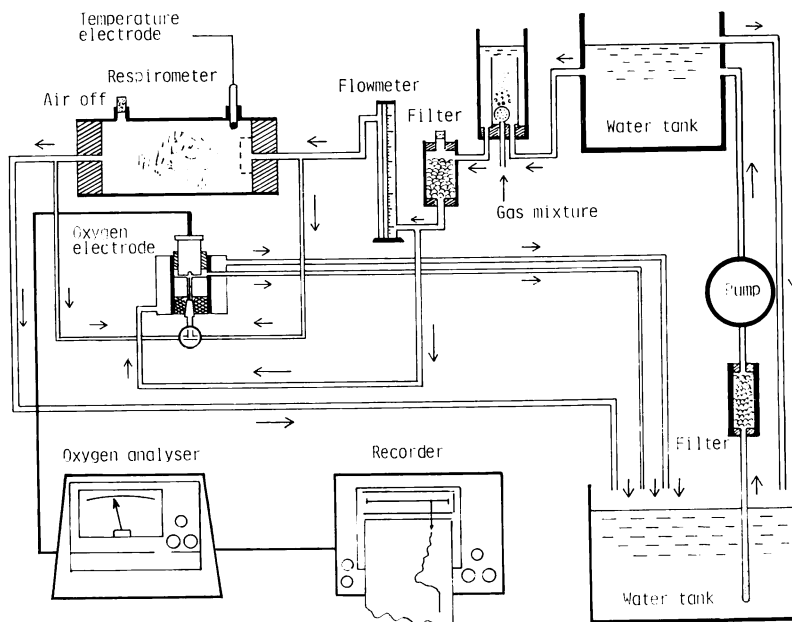


Fig. 1. Diagram of the experimental arrangements used to determine oxygen consumption under constant conditions of flow rate and water temperature in the frogfishes. For further explanations, see text.

bottom of the seaweed belt in the infra- or sublittoral zones during the summer season. Neither fish shows any tendency to utilize ramjet ventilation when swimming.

Materials and methods

The two species of frogfish used were: a) *Histrio histrio* (21~87 g) collected and kept in the seawater circulation at the Usa Marine Biological Station*, Kochi, Japan; the experiments were performed at a constant temperature in the range 23~25°C during May and June 1974; b) *Phrynelox tridens*, which spend their whole lives on the sea bottom; specimens (25~101 g) were collected from the seaweed belt of the infra- and sublittoral zone in the summer season and kept in similar aquaria at the Marine Station. Experiments on these fish were carried out at a constant temperature (25~27°C) in April, May, September and October 1974, and January 1975. In both *H. histrio* and *P. tridens* there is a thin tubular opening from each opercular cavity which is directed ventrally in the vicinity of the pectoral fin.

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Determinations of oxygen consumption and other features of the respiratory mechanism were made on individual fish kept in respirometer tubes of 7 and 10 cm diameter, having total volumes of about 600 cm³ and 750 cm³ for *H. histrio* and *P. tridens*, respectively. Water was passed continuously through the respirometer as the fish rested within it; the rate of flow could be varied as could its oxygen tension by bubbling nitrogen through a gas-exchange column included in the water supply line (Fig. 1). Oxygen consumption was determined using continuous flow respirometry involving the sampling of water in the inlet and outlet tubes of the respirometer (Hughes, 1978; Umezawa et al., 1981). It was found that all fish remained quiet and breathed regularly for prolonged periods and sometimes faced downstream to the current rather than the more expected upstream orientation. The amount of dissolved oxygen (O₂) before and after passing the fish in the respirometer tube was measured with an oxygen electrode (Oxygen Analyser, Field-Lab type, Beckman-Toshiba). The flow rate of water through the respirometer was kept constant and determined with a flowmeter (No. 71-5758, Nihon

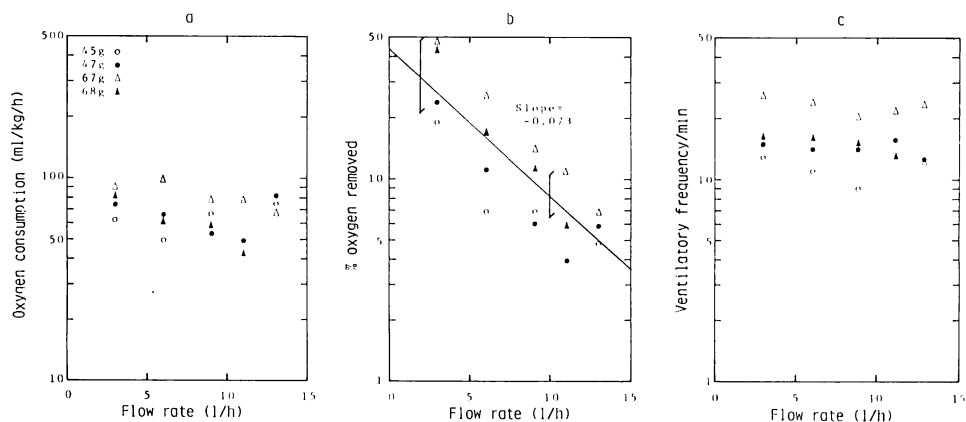


Fig. 2. Semi-logarithmic plots of (a) resting oxygen consumption, (b) the percentage of oxygen removed by fish, and (c) the ventilatory frequency of *Histrio histrio*, against flow rate of water over the fish in the respirometer tube, for the same 4 specimens, weighing between 45 and 68 g. Each point is the mean of four or five determinations. Bars indicating 95% confidence limits for the regression line in (b) are shown at flow rates of 2 and 10 l/h. No relationship between the two variables in (a) and (c) are represented. Temp.: $25 \pm 1^\circ\text{C}$.

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The results were analysed statistically by means of test of correlation coefficient and of regression coefficient according to Snedecor and Cochran (1967) and Ishii (1975).

Results

The species used in this investigation have several advantages, notably the way they rest quietly in the respirometer tube throughout almost the whole of the experimental period and the frequency of their ventilatory movements is relatively low (10~20 per min). As with other fishes (*Torpedo*, carp, etc.) high initial rates of both oxygen uptake and ventilatory frequency were found soon after the fish were placed in the respirometer. These values gradually subsided during the next 1~3 hours. In spite of this fairly rapid recovery, no measurements were made until at least 3 hours following the initial handling of the specimens.

Effects of changing the flow rate of water over the fish on respiration in normoxic conditions. In continuous flow respirometry a more rapid flow of water through the respirometer is preferred so long as the flow rate does not disturb the fish and that there is a sufficiently large difference between the inlet and outlet oxygen tensions. As a preliminary experiment in such studies it is important to establish the most

convenient flow rate which is then used during most of the other experimental procedures. During such preliminary experiments it is important to observe whether there are any significant effects of the flow rate itself on the fishes respiration. In these investigations measurements were made of oxygen consumption in relation to water flow, and from the differences between inlet and outlet oxygen levels the percentage of the oxygen removed from the water during its passage through the respirometer is calculated. The frequency of the ventilatory movements was also counted and is shown in Fig. 2 for *Histrio histrio* and Fig. 3 for *Phrynelox tridens*. These plots indicate that the percentage of oxygen removed from the water during its passage over the fish decreases as the water flow rate is raised, although the oxygen consumption does not change markedly. A statistically significant regression line of the percentage of oxygen removed on flow rate is drawn ($P < 0.001$ in *H. histrio* and $P < 0.01$ and 0.01 in *P. tridens*). It was also shown that there are no statistically significant correlations between ventilatory frequency and flow rate in *H. histrio* ($P > 0.5$) and that the regression lines for *P. tridens* do show a statistical significance ($P < 0.025$ and 0.025) so that frequency increases with increase in flow rate in *P. tridens*. It was found, however, that at the lower flow rate in *P.*

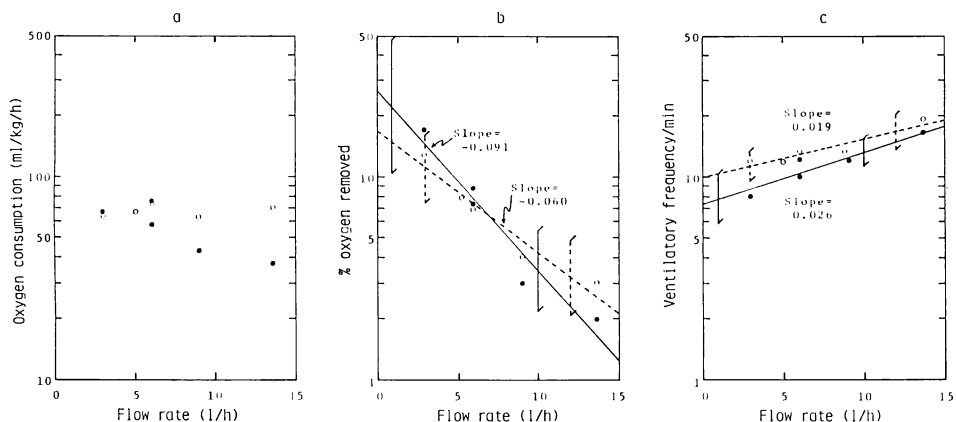


Fig. 3. Semi-logarithmic plots of (a) resting oxygen consumption, (b) the percentage of oxygen removed by fish, and (c) the ventilatory frequency of *Phrynelox tridens*, against flow rate of water over the fish in the respirometer tube, for the same 2 specimens. Filled circles and solid line refer to a 38 g fish at 22°C; open circles and dotted line refer to a 27 g fish at 27°C. Each point is the mean of four or five determinations. Bars indicating 95% confidence limits for each of the regression lines (solid and dotted lines) are given in (b) and (c) at flow rates of 1 and 10 l/h, and 3 and 12 l/h respectively. No relationship between the two variables in (a) is represented.

tridens the ventilatory frequency was not only reduced but showed variations. It is suggested, therefore, that an increase in ventilatory stroke volume may occur which depends on the fall in flow rate.

These results, excluding the rise in percentage of oxygen removed with decreasing flow rate, differ from those obtained by Hughes and Umezawa (1968a) for the dogfish and by Umezawa and Watanabe (1973) for the killifish.

Relationship between body mass and respiration under normoxic conditions. Comparisons have been made between the respiration of *Histrio histrio* and *Phrynelox tridens* on the basis of the relationships to body mass for each species. Results obtained are shown in Fig. 4, where resting oxygen consumption (\dot{V}_{O_2}), \dot{V}_{O_2} /body weight, and ventilatory frequency were plotted for each fish against body weight. These plots indicate that the mean \dot{V}_{O_2} is higher in larger specimens of *H. histrio* and *P. tridens* and that there are statistically significant regression lines ($P < 0.025$ and 0.001 , respectively). When these values for oxygen consumption were related to body weight, moreover, it was found that the weight specific oxygen consumption of smaller fish is greater than that in larger specimens for *H. histrio* and *P. tridens*, and there are statistically significant regression lines

($P < 0.05$ and 0.025 , respectively).

The mean values for ventilatory frequency of smaller specimens are scarcely greater than those of larger specimens of *H. histrio* and there is no statistically significant correlation ($P > 0.1$). However, the ventilatory frequency of small *P. tridens* is significantly greater than those of larger individuals ($P < 0.05$).

Thus, it can be suggested that the increase in weight specific oxygen consumption of smaller *Histrio histrio* is related to an increase in stroke volume as ventilatory frequency shows little change with body mass in this species. On the other hand, smaller specimens of *Phrynelox tridens* have higher ventilatory frequency which is not necessarily accompanied by an increase in stroke volume since the weight specific oxygen consumption increases with a decrease in body mass. These differences may be related to differences in the life habits of these species.

The effect of reducing inlet P_{O_2} . Measurements were carried out when the P_{O_2} of the water passed over the fish was reduced and the flow rate remained constant. No observations were made on the effect of varying the rate at which the oxygen tension was reduced, normally this took place at a rate of about 30 mmHg/min. The relationship between inlet oxygen content and \dot{V}_{O_2} , the proportion of the oxygen removed

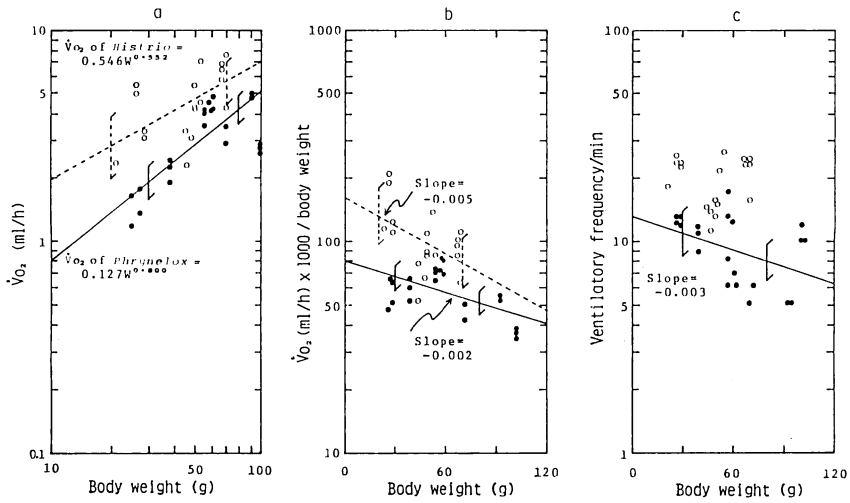


Fig. 4. Bi-logarithmic plots of (a) resting oxygen consumption (\dot{V}_{O_2}), and semi-logarithmic plots of (b) \dot{V}_{O_2} /body weight and (c) the ventilatory frequency of *Histrio histrio* for the same 18 specimens (○) at a temperature of about 25°C and *Phrynelox tridens* for the same 21 specimens (●) at temperatures ranging between 17 and 22°C, against body mass. Each of the points is the mean of four or five determinations. Bars indicating 95% confidence limits for the regression line in (a) and (b) are shown for body weights of 20 and 70 g in *H. histrio* and (a), (b) and (c) for 30 and 80 g in *P. tridens*. No relationship between the two variables in (c) in *H. histrio* is represented. Flow rate, 6 l/h.

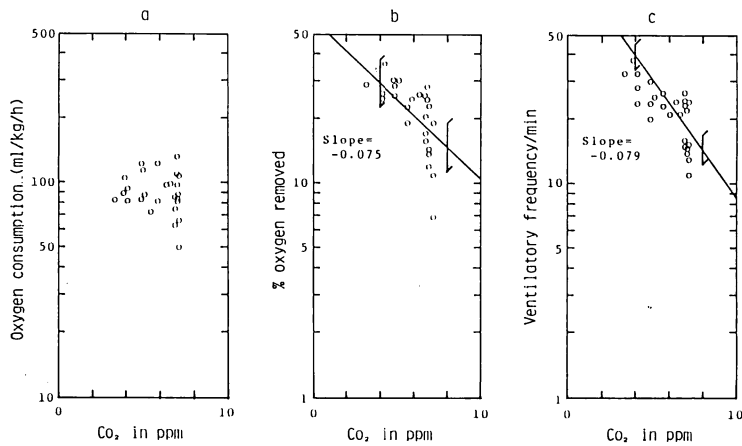


Fig. 5. Semi-logarithmic plots of (a) oxygen consumption, (b) the percentage of oxygen removed by fish, and (c) the ventilatory frequency of *Histrio histrio*, against oxygen content of inlet water to the respirometer tube, for the same 26 specimens, weighing between 50 and 70 g. Each point is the mean of four or five determinations. Bars indicating 95% confidence limits for the regression line in (b) and (c) are shown at oxygen contents of 4 and 8 ppm. No relationship between the two variables in (a) is represented. Temp.: 25 ± 1°C; flow rate, 6 l/h.

from water during its passage through respirometer, and ventilatory frequency are shown in Figs. 5 and 6. These plots indicate that both percentage of oxygen removal by the fish

$$\left(\frac{Co_2 \text{ inlet} - Co_2 \text{ outlet}}{Co_2 \text{ inlet}} \times 100 \right)$$

and ventilatory frequency increase as the oxygen content of inlet water is reduced in both species.

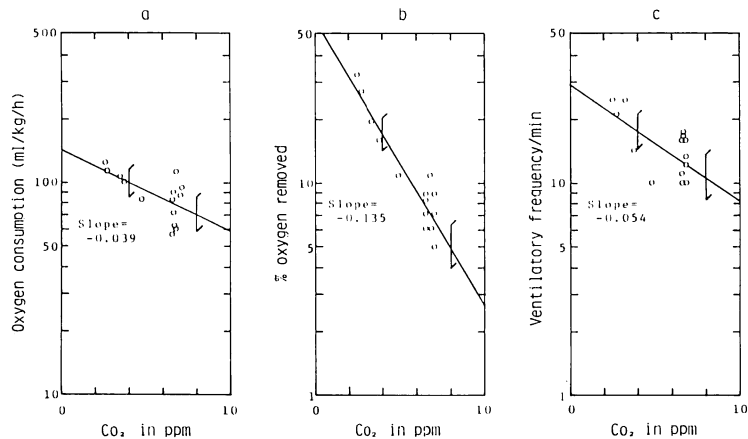


Fig. 6. Semi-logarithmic plots of (a) oxygen consumption, (b) percentage of oxygen removed, and (c) the ventilatory frequency of *Phrynelox tridens*, against oxygen content of inlet water to the respirometer tube, for the same 14 specimens, weighing between 26 and 27 g. Each point is the mean of four or five determinations. Bars indicating 95% confidence limits for the regression line in (a), (b) and (c) are shown at oxygen contents of 4 and 8 ppm. Temp.: 27~28°C; flow rate, 6 l/h.

Regression lines for a percentage of oxygen removed and ventilatory frequency plotted against inlet oxygen content show statistically significant changes ($P < 0.005$ and 0.001 , respectively for *Histrio histrio* and $P < 0.001$ and 0.01 , respectively for *Phrynelox tridens*). These results differ from those obtained in similar experiments carried out with the dragonet (Hughes and Umezawa, 1968b) and killifish (Umezawa and Watanabe, 1973). The increase in ventilatory frequency during hypoxia also agrees fairly well with observations on rainbow trout (Nakanishi and Itazawa, 1974; Hughes and Saunders, 1970) and carp (Itazawa and Takeda, 1978; Hughes et al., in preparation).

The present results (Figs. 5 and 6) also indicate that oxygen consumption does not change in relation to the fall in water oxygen content in *Histrio histrio*, whereas it increases with a decrease in oxygen content in *Phrynelox tridens*; the regression of oxygen consumption on Co_2 being statistically significant ($P < 0.025$). These relationships do not suggest the presence of a largely 'dependent' type of respiration similar to that found in some other fishes but are more typical of results obtained for species showing 'independent' respiration. Thus it may be surmised that lowering of ambient oxygen leads to compensatory responses which result in the oxygen consumption of a fish being maintained

during hypoxia in *H. histrio* or even increased in *P. tridens* as a result of the heightened ventilation frequencies and a rise in the proportion of the oxygen removed from the water flowing past the fish.

Discussion

Previous results using dogfish (Hughes and Umezawa, 1968a) and killifish (Umezawa and Watanabe, 1973) showed that as the flow rate of water was increased over a fish in a respirometer tube oxygen consumption rose but there was no change in the percentage of the oxygen removed from the water; ventilatory frequency remained unchanged, in both cases the fish face upstream and there must be a significant 'ram' component to their ventilation. Results in the present experiments with rather different fish have given different results which are probably related to differences in responsiveness to flowing water. *Histrio histrio* generally lives among surface-floating seaweed and *Phrynelox tridens* normally rests at the bottom of a seaweed belt in the infra- or sublittoral zones during summer. Neither of these fish is very active and when they swim they do not show ram ventilation. The lack of increase in oxygen consumption in frogfishes with increasing water flow rate is probably associated with the absence of any change in ventilation volume though the

ventilatory frequency increased in *P. tridens* but remained fairly constant in *H. histrio*.

Experiments in which the fish were kept in ambient water of low oxygen content and constant water flow rate showed consistent changes in oxygen consumption with a fall in oxygen content. A marked rise in oxygen consumption together with increases in both ventilatory frequency and percentage of oxygen removed from the water were observed when inspired oxygen levels were reduced in *Phrynelox tridens*. A similar relationship has been found by Itazawa and Takeda (1978) for the carp which contrasts with that for the rainbow trout (Hughes and Saunders, 1970) and for the dogfish and dragonet (Hughes and Umezawa, 1968a, b). A similar relationship was also found by Nakanishi and Itazawa (1974) for rainbow trout and Japanese eel. However, no correlation was obtained in *Histrio histrio* for the relationship between oxygen consumption and oxygen content of the ambient water whereas as the oxygen level fell there was a rise both in ventilatory frequency and percentage of oxygen removal. This seems to indicate that ventilation volume does not increase simultaneously with the increase in ventilation frequency.

Unfortunately, no direct determinations of ventilation volume, and consequently stroke volume, in relation to body mass were made in this study because it proved rather more difficult to attach tubes to the exhalant opercular openings than had been expected. In fact one of the reasons for beginning this study was in anticipation that such a procedure would be possible. However, it is possible that the raised oxygen consumption of smaller specimens of *Histrio histrio* was achieved because of an increase in ventilation volume independent of ventilatory frequency. For *Phrynelox tridens* the increase in oxygen consumption of smaller fish was associated with an increase in ventilatory frequency independent of ventilation volume.

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ハナオコゼとイザリウオの呼吸に及ぼす流速と低酸素の影響

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イザリウオ科のハナオコゼ及びイザリウオの呼吸に

及ぼす呼吸水の流速と低溶存酸素量の影響について流水式呼吸室を用いて調べた。酸素消費量はポーラログラフ酸素電極により測定された魚の呼吸前後の水の溶存酸素量の差から、また呼吸頻度は肉眼により呼吸運動に伴う鰓蓋の開閉数から求めた。流速が増加した場合、両種はともに酸素消費量の増加を示さなかったが、呼吸水からの酸素除去率は減少した。また、呼吸頻度はイザリウオでは増加し、ハナオコゼでは増加しなかった。これらの相違はハナオコゼが流れ藻に、イザリウオが藻場の底部に生活する習性の相違に基づくものと思われる。次に呼吸水の溶存酸素量が低下した場合、両種の酸素除去率及び呼吸頻度はともに増加したが、酸素消費量はイザリウオでは増加したが、ハナオコゼでは変化しなかった。これらは低酸素状態においてハナオコゼでは酸素消費量が一定に保たれ、イザリウオでは酸素除去率の増加と呼吸頻度の上昇により酸素消費量が増加するという補償作用の存在を示唆するものと思われる。

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