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Effects of pressure on oxygen consumption in cottid fish from Lake Baikal

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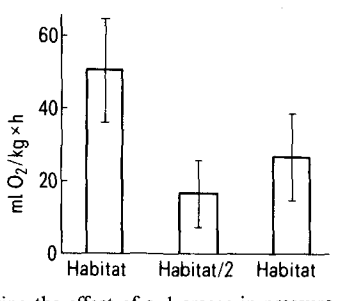
Summary. Rates of oxygen consumption in shallow dwelling cottid fish from Lake Baikal were unaffected by a change in pressure from 11 to 51 ata. The metabolic rate of deep cottids decreased by 72% when the pressure was decreased by 50% from that corresponding to habitat depth. Recovery from decreased pressures was incomplete in deep fish, suggesting that measurements of $\dot{V}O_2$ in deep dwelling fish in the literature may underestimate actual rates.

It has been proposed that adaptation to the deep-sea environment entails a lowering of the metabolic rate in fish compared to shallow water species[2–7]. The low metabolic rates in deep-sea fish are associated with lower caloric densities[8] and reduced activities of glycolytic[9] and tricarboxylic acid cycle[10,11] enzymes. Most studies on the rate of oxygen consumption ($\dot{V}O_2$) of deep-sea fish, however, have been performed at atmospheric pressure (1 ata) rather than at habitat pressures[2–5]. Notable exceptions are 2 studies done in situ at 1230–3650 m[6,7]. In only 2 studies, on *Anoplogaster*[3] and *Melanostigma*[12], were the effects of changes in pressure on $\dot{V}O_2$ investigated; these revealed no significant pressure effects.

The purpose of the present study was to determine the effects of pressure changes on the rates of oxygen consumption in shallow and deep dwelling cottid fish from Lake Baikal, Central Siberia, USSR; and to ascertain whether differences in metabolic rate obtain in closely related species as a function of depth of occurrence as suggested by Childress et al.[2,5].

Materials and methods. Specimens of the 3 deep species of cottid fish, *Batrachocottus nikolskii, Cottinella boulengeri* and *Abyssocottus korotneffi* were collected in the southern and central basins of Lake Baikal from the Soviet research vessel Titov in a 3-m beam trawl from depths of 660–1120 m. The fish were removed from the cod end to a pan of fresh lake water and only undamaged individuals which responded to touch with vigorous swimming were employed. Fish were used immediately after capture. Shallow water cottids *(Cottus kessleri)* were caught by hand from among rocks by the shore. Fish were placed individually in a stainless steel cylindrical pressure vessel equipped with a conical plexiglass window at each end and filled with fresh, aerated lake water. The chamber had a volume of 1250 ml and was connected on one end to the outflow from a pressure amplifier capable of delivering 50 ml of



Histogram showing the effect of a decrease in pressure and subsequent return to habitat pressure on $\dot{V}O_2$ of deep water cottid fish. All groups are significantly different from one another by the Mann-Whitney U-test.

Routine metabolic rates for deep and shallow cottid fish and deep marine fish at habitat and stress pressures

Animal species (source)	Capture depth (m)	Weight (g)	$\dot{V}O_2$ (ml O_2/kg · h)		Δ %
Deep cottids			Habitat	Habitat/2	
1	660	20.0	35.0	2.6	−92.5
2	1000	26.0	60.2	22.9	−62.0
3	1000	39.8	55.5	–	–
4	1120	19.8	49.9	18.6	−62.8
5	900	18.2	69.0	21.2	−69.3
6	1120	14.2	32.6	–	–
Average ± SD			50.4 ± 14.3†**	16.3 ± 9.3	−71.7 ± 14.3
Shallow species			Habitat	51 ata	Δ %
7	20	15.2	102.6	116.9	+14.0
8	< 1	30.5	82.6	50.5	−38.9
9	< 1	23.5	51.0	97.9	+92.0
Average ± SD			78.7 ± 26.0†	88.4 ± 34.2	+22.4 ± 65.8
Other studies			Habitat	1 ata	
10 (6)	1230	1800	2.4		
11 (6)	1230	100	2.2		
12 (11)	650–900	–	14.8		
13 (5)	2753, 3650	500–1200	3.2		
14 (2)	600	10–60	12.7		
15 (3)	550–900	39	–	32.5	

** Values for deep animals at habitat vs half of habitat pressure are significantly different at the level $p < 0.01$ by the Mann-Whitney U-test. † Values for shallow vs deep animals at habitat pressures are significantly different at the level $p < 0.05$ by the Mann-Whitney U-test. Species of animals: 1,2,3 *Batrachocottus nikolskii;* 4,5 *Cottinella boulengeri;* 6 *Abyssocottus korotneffi;* 7,8,9 *Cottus kessleri;* 10 *Coryphaenoides acrolepis;* 11 *Eptatretus deani;* 12 *Melanostigma pammelas;* 13 *Coryphaenoides armatus;* 14,15 *Anoplogaster cornuta.*

fresh, aerated lake water at a regulated pressure. The other end of the chamber was equipped with a metering valve connected to a 50 ml polyethylene syringe into which was inserted a Clark-type oxygen electrode hooked to a YSI oxygen meter. An O-ring around the electrode provided a seal with the inner wall of the syringe. Sampling was effected by opening the metering valve, allowing water in the syringe to be displaced by the action of the pressure amplifier. Water passed out of the syringe through a bleed-hole which was uncovered when the O-ring and the oxygen electrode were moved back slightly. The reading on the meter was recorded immediately after the 50 ml flowed through the syringe. The rate of oxygen uptake with no animal present was used as a blank and subtracted from all experimental values. The electrode was calibrated using fully aerated lake water, and the O_2 tension was never allowed to go below 5.3 ppm during the course of an experiment. The chamber and electrode were kept in an insulated water bath which was kept at habitat temperatures (6 °C for shallow and 3 °C for deep dwelling fish). 'Habitát' pressures were 11 ata for shallow fish and those corresponding to the trawl depth for deep fish. Shallow fish weighed 23.3 ± 4.4 g, deep fish weighed 23.0 ± 3.7 g (mean ± SEM). Because of the similarity in average size, no compensation for weight differences was made.

Results and discussion. The effect of a change in pressure of approximately 50 atm was quite different in the deep and shallow cottid species. Increasing the pressure from 11 to 51 ata did not affect the rate of oxygen consumption in the shallow water fish. However, a decrease in pressure from 61 to 31 ata or from 101 to 51 ata resulted in a marked and significant decrease in $\dot{V}O_2$ in the deep cottids (table). These changes in $\dot{V}O_2$ in the deep fish were not associated with overt changes in activity, as was the case for a deep dwelling mysid crustacean in which $\dot{V}O_2$ displayed an increase with increasing pressure[13]. Both the shallow and deep species of cottids are quite sedentary if not disturbed and were observed to remain stationary on the bottom of the vessel during experiments at these pressures.

The metabolic rates for animals measured at habitat pressures appeared to demonstrate a decrease with increasing depth of occurrence; $\dot{V}O_2$ of shallow cottids was significantly higher than that for deep species (table). This would tend to give support to the hypothesis of decreasing $\dot{V}O_2$ with increasing capture depth promulgated by Childress and co-workers[2–5]. It is possible, however, that even the values for $\dot{V}O_2$ measured in the deep cottids at habitat pressure are lower than they would be in situ and, thus, that the differences in $\dot{V}O_2$ between deep and shallow species may be overestimated.

We employed an A · B · A experimental design in the $\dot{V}O_2$ measurements for the deep cottids; measurements were made again at habitat pressure following the reduction by half of the pressure. Upon return to habitat pressure there was a significant increase in $\dot{V}O_2$ ($p < 0.05$ by the Mann-Whitney U-test, see fig.). The metabolic rate, however, was still significantly lower than that measured at the beginning of the experiment at the same pressure ($p < 0.01$). This implies either that recovery from decompression is incomplete or that recovery takes longer than the 1–1.5 h during which $\dot{V}O_2$ was measured. Since the deep cottids were brought to the surface without pressure protection, it is quite likely that a similar decrease in $\dot{V}O_2$ and subsequent incomplete recovery was in effect during our initial measurements of metabolic rate at habitat pressure. Thus, had we been able to measure them in situ or in animals brought to the surface in high pressure retrieval devices, there might have been little or no difference between the metabolic rates of the deep and shallow species.

Two points can be made as a result of these studies. First, there is clearly a difference between the reactions to pressure changes in the deep and shallow water cottids reflecting the long-term adaptation or acclimation of deep species to their high pressure environment. These differences are evident whether or not there exists a difference between the in situ metabolic rates of the deep and shallow fish. Second, the reduction in $\dot{V}O_2$ of deep fish upon decompression and the delay or absence of recovery upon recompression raises questions with regard to previous studies of metabolic rates in deep-sea fish. It is possible that measurements of $\dot{V}O_2$ in deep fish made at 1 ata are severe underestimates of actual rates at depth. Moreover, even measurements of metabolic rates performed under pressure could be erroneous if the animals experienced decompression during capture. These results underscore the need for pressure protective retrieval devices for the recovery of fish for physiologic studies.

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A new observation on *Halobacterium halobium;* light-induced volume flow through the whole organism

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Summary. A new observation on the *H. halobium* cells is reported. It has been observed that when the cells are exposed to light a volume flow is observed through them. The magnitude of the light-induced volume flow depends on the intensity and wavelength of the exciting light and is also influenced by temperature. The phenomenon appears to be relevant to the physiology of the organism.

The extreme halophile *Halobacterium halobium* has attracted attention in recent years, from the points of view of solar energy conversion and of bioenergetics[2,3]. In the present communication we report a new observation on *H. halobium* cells. It has been shown that when the cells are exposed to light, a volume flow is observed through them. Since under natural conditions the organism occurs in situations where it is invariably exposed to bright sunshine, the light-induced volume flow through the cells observed here may have a role to play in the physiology of the organism. The light-induced volume flow was found to increase with increase in the intensity of light and was found to depend on temperature and also on the wavelength of the incident light.

The experimental set-up designed to demonstrate the phenomenon of light-induced volume flow is depicted in figure 1, which has been labelled to make it self-explanatory. The U-tube (fig. 1) was partly filled with a 3% agar-agar (BDH) solution, prepared in a 4M aqueous sodium chloride (BDH analytical reagent) solution, containing 5 ml of an actively growing culture of *H. halobium* (cell mass 8.5×10^{-3} g on dry weight basis). After a few hours, when the solution in the U-tube had solidified, the rest of the glass cell (fig. 1) was filled with a 4M sodium chloride solution. At the beginning of the experiment the condition of no net pressure difference, $\Delta P = O$, was imposed on the system by adjusting the pressure head (fig. 1) – the pressure head was so adjusted that the liquid meniscus in the capillary L_1 L_2 remained stationary. The bulb B was then switched on and the consequent movement of the liquid meniscus in the capillary L_1 L_2 was noted with time using a cathetometer reading upto 0.001 cm and a stop watch reading upto 0.1 sec.

Table 1. Variation of the light-induced volume flow Jv with temperature

	Temperature (°C)		
	30	35	40
Jv × 10^{11} ($m^3 sec^{-1}$) (H. halobium cells entrapped in Agar-agar)	50.130	26.040	Not observable
Jv × 10^{11} ($m^3 sec^{-1}$)	4.098	2.306	Not observable

During the measurement of light-induced volume flow a constant and stabilised voltage of 220 V from A.C. mains was fed to the bulb B and the distance between the transport cell and the bulb B was kept fixed. To study the variation of the light-induced volume flow with intensity of the incident light, various voltages were fed to the bulb B to alter the intensity of the light. All measurements were made at constant temperature

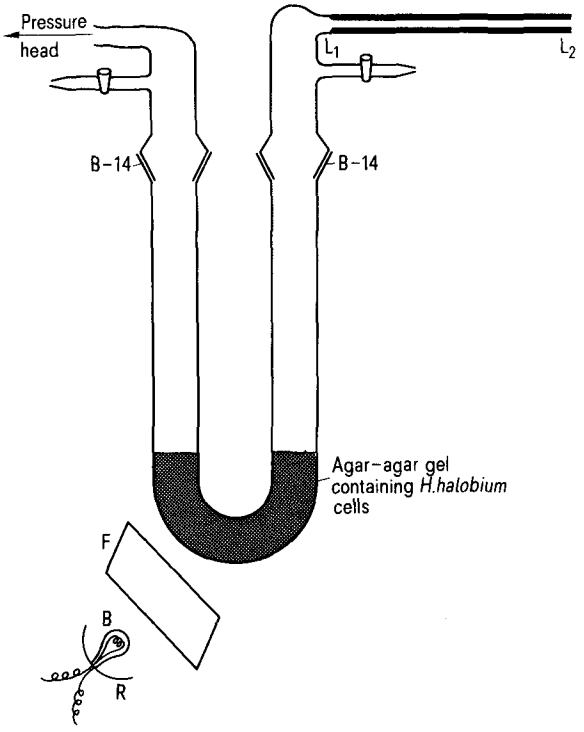

Figure 1. Experimental set-up for measurements of light-induced volume flow through *H. halobium* cells entrapped in agar-agar gel: R, reflector; B, 100 W bulb; F, Filter.