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Responses of the Fish *Blennius pholis* to Fluctuating Salinities

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ABSTRACT: *Blennius pholis* (L.) were exposed to fluctuating salinity regimes of near tidal periodicity and their blood osmolality, oxygen consumption, heart rate and opercular beat monitored. Despite salinity fluctuations from 34–0–34 ‰ S, the blood osmolality remained constant. Significant increases in oxygen consumption were observed at low salinity levels, but these may simply reflect changes in physical activity and the greater availability of oxygen at low salinities. Salinity effects upon heart rate/opercular beat were weak or negligible.

INTRODUCTION

The blenny *Blennius pholis* (L.) is a common inhabitant of rocky shores around Anglesey, U.K., particularly on the west coast of the island. It lives in rocky pools beneath stones, buried in muddy gravel and in rock crevices from just above the low water springtide level to the upper shore. Although it is a non-gregarious fish (Gibson, 1967a, 1968), its defended home ranges are small, and thus local abundances may be quite high. Blennies are particularly numerous in narrow rock gullies running at right angles to the coast, and these are often fed by freshwater streams or seepage. Consequently the fish are exposed to considerable salinity fluctuations ranging from 33 to 34 ‰ S at high tide, to freshwater at low tide. The individuals living highest on the shore may be exposed to freshwater influence for several hours.

The information available on the effects of salinity upon marine organisms in terms of their tolerance and physiology has been reviewed extensively in 'Marine Ecology', Volume I (Kinne, 1971a) and has received detailed attention notably from Krogh (1939), Potts and Parry (1964) and Kinne (1971b). However, almost all of the studies conducted thus far have involved laboratory experiments at constant salinities.

Steady-state salinity experiments are open to criticism on several grounds. Primarily they tend to give a false impression of the possible limits to distribution imposed upon animals by the salinity of their environment. A number of invertebrates can effectively counteract salinity stress during periods of each tidal cycle in littoral and estuarine areas, and thereby avoid or reduce damage by low environmental salinity levels. Such compensations have been considered by Kinne (1967, p. 526) under the headings of escape, reduction of contact, regulation and acclimation. In several cirripede, bivalve and polychaete species, compensation for salinity stress has been studied by Davenport (1976a), Davenport (1977) and Shumway and Davenport (1977). As suggested by Kinne (1971b, p. 905) and Spaargaren (1974), invertebrates which cannot reduce contact with adverse salinities tend to exhibit internal osmolarity changes which are damped by comparison with those of the medium, and thus allow the animals to survive brief exposures to salinity levels which would kill them if sustained for periods longer than those prevailing in their natural environment. Such damping has been confirmed by Tucker (1970) for the prosobranch *Scutus breviculus,* and for echinoderms and bivalves by Stickle and Ahokas (1974) and Shumway (1977). Data on fluctuating salinity effects upon organisms have been obtained with the aid of an apparatus which can mimic environmental salinity fluctuations: the most convenient and accurate apparatus is that described by Davenport et al. (1975).

Most of the information available thus far has been obtained in invertebrates, largely for osmoconformers rather than osmoregulators. In teleost fishes the situation is different. They have a low blood concentration maintained by an efficient osmoregulatory mechanism

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(e.g. Gilles, 1975) which, in euryhaline species, is responsible for the extrusion of salts at high salinity levels and for taking up salts at low seawater concentrations. The blenny appeared to be suitable for a first investigation with the fluctuating salinity apparatus, since this teleost is relatively small and its behaviour causes it to remain at positions on the shore which expose it to local tidal salinity fluctuations.

In the study reported here, blennies were exposed to fluctuating salinity regimes of near-tidal periodicity. During this exposure their plasma osmolarity, oxygen consumption, heart rate and opercular beat frequencies were monitored.

MATERIALS AND METHODS

Collection and Maintenance

Blennies *Blennius pholis* (L.) were mainly collected from the intertidal zone of the rocky west coast of Anglesey (U.K.) between Aberffraw and Rhosneigr. A few individuals were obtained from the shore of the Menai Strait beneath the northern end of Telford Suspension Bridge. The fish were collected from pools, crevices and muddy gravel beneath stones. Fish in pools were caught by the use of the anaesthetic quinaldine (Gibson 1967b). The blennies ranged in wet weight from 7.4–42.6 g. To obtain arrythmic fish, the blennies were held in the laboratory seawater system at 10 °C and under constant illumination (Gibson 1967a). They were fed flesh of *Mytilus edulis* regularly, but starved for 2 days before use in respirometry experiments. All experiments were performed in constant light at 10 °C.

Salinity Regimes

The blennies were exposed to salinity fluctuations produced by the apparatus described by Davenport et al. (1975). To determine the influence of these fluctuations upon heart/opercular beat frequencies and plasma osmolarity the fish were placed in 500 ml plastic boxes supplied with water at 250–300 ml min^{-1}. The contents of the boxes were aerated by diffuser stones connected to the compressed air supply. In these experiments only one type of salinity regime was used, namely an abruptly fluctuating cycle in which the test fish received alternate 6 h exposures to full seawater (33.5 ‰ S) and fresh water. No more gradual regimes than this were employed because the monitored physiological changes induced by the abrupt salinity fluctuations were either slight or negligible.

Two types of salinity regime were used in the respirometric experiments described below, an abrupt regime as described already, and a sinusoidal regime of 12 hour wavelength fluctuating between full seawater and fresh water.

Heart/Opercular Beat Frequency Measurements

Heart and opercular beat frequencies were both measured simultaneously in individual fish by the impedance pneumograph technique (Trueman, 1967). Each fish was anaesthetised with MS 222 and a silver-wire electrode sewn beneath the skin overlying each operculum. The two electrodes were connected, via fine polythene sheated copper wire to a Scientific Instruments* pneumograph coupler, itself connected to a twin channel George Washington 400 MD/2 chart recorder. Another pair of silver electrodes were inserted through the body wall (either side of the heart); they were anchored in place by sewing to the bases of the pelvic fins. These electrodes were also connected to a pneumograph coupler and the twin channel recorder. The fish was then allowed to recover for several hours before use in experiments. To conserve chart paper during long experiments the power supply of the chart recorder was controlled by a time clock which switched the recorder on for 2 min every hour. Results were obtained for 3 fish (19.0–27.5 g) exposed for 24 h to the abrupt salinity regime.

Plasma Osmolality Determinations

Blood samples were obtained by sacrificing fish at appropriate intervals during the first 12 h of an abrupt salinity regime. The tail of each fish was cut off and blood allowed to drip into a 1.5 ml Eppendorf plastic tube. After centrifuging the blood to remove cells, a 50 mm^3 sample of plasma was pipetted into the test vessel of Knauer Semi-micro osmometer and its osmolality established. Enough blood could be obtained from a single large fish for such a determination, but on several occasions the blood of 2–3 smaller fish had to be pooled.

Respirometry

Oxygen consumption measurements were carried out by the use of the apparatus shown in Figure 1. Air-saturated water was drawn by a peristaltic pump from a column supplied with water of fluctuating salinity by the apparatus described by Davenport et al. (1975). It

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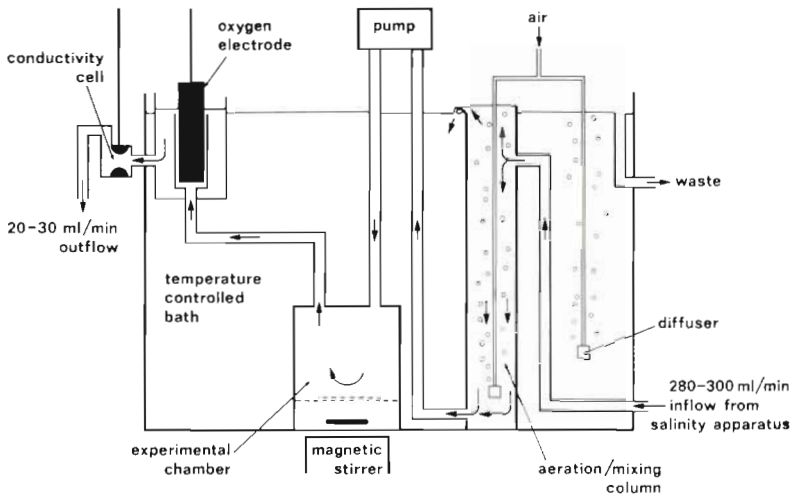

Fig. 1. Experimental set-up for measurement of oxygen consumption in various salinity regimes

was then delivered to a 250 ml glass experimental chamber fitted with a screw top plastic-coated metal lid, and magnetically stirred. From the experimental chamber the water flowed past a Radiometer E5046 oxygen electrode, connected via a Radiometer PHM 71 Mk II pH meter to a Smiths Servoscribe chart recorder adjusted to 100 mV. The water then flowed through a platinum conductivity cell, connected via a Carwyn Instruments* Salinity Monitor to the same chart recorder as the oxygen electrode. Thus a nearly simultaneous trace of the salinity and oxygen tension of the water leaving the experimental chamber was available.

To carry out an experiment the following experimental protocol was adhered to:

(1) Air-saturated full seawater (33.5 ‰ S) was allowed to flow through the apparatus for several hours, and measurements were made to confirm that temperatures remained the same in the temperature controlled bath, aeration column and outflow.

(2) The oxygen electrode was zeroed and adjusted so that 100 % seawater air saturation was equivalent to near full-scale deflection on the chart record (for details of calibration procedure see Davenport, 1976b).

(3) A fish was placed in the experimental chamber and allowed to settle down. The flow rate through the experimental chamber was adjusted, if necessary, so that the resting fish removed 10–15 % of the oxygen in the water passing over it.

(4) When the fish had become quiet and had shown no systematic decrease in respiration rate for several hours, the programmer of the salinity apparatus was set to deliver an abrupt or sinusoidal regime over the ensuing 24 h period.

(5) The fish was removed from the respirometer and weighed.

To avoid potential inaccuracies caused by oxygen-electrode response drifts, the electrode was regularly moved from its position in the experimental-chamber outflow and placed for a few minutes in the surrounding temperature-controlled bath. Since this bath is continually aerated, and at the same temperature as the experimental chamber and aeration column, this procedure allows regular reassessment of the chart deflection corresponding to full air saturation. When the flow rate of water through the respirometer is known, the oxygen uptake by the fish can be calculated from the difference in oxygen saturation between inflowing and outflowing water from the experimental chamber: the oxygen uptake was calculated for every hour of each of the 24 h periods, taking into account that the oxygen concentration of 100 % air-saturated water varies with salinity.

For each hourly interval of the salinity regimes the regression of the logarithm of the weight-specific oxygen uptake on the logarithm of wet weight was calculated. Thus a number of regressions were obtained, each relating oxygen consumption at a particular salinity to the size of the fish. These data were further treated by pooled regression analysis and analysis of variance (ANOVA). 7 fish were exposed to abruptly fluctuating salinity regimes and 14 to sinusoidal cycles.

RESULTS

Heart/Opercular Beat Frequency

In the abrupt salinity regime the fish were exposed to alternate periods of full seawater strength (34 ‰)

* Carwyn Instruments, Pentraeth Road, Menai Bridge, Gwynedd, U.K.

and fresh water (0 ‰). Each period lasted for 6 h, so that each fish was exposed to 12 h of full strength seawater and 12 h of fresh water. It appears that when tested as a group the heart beat frequencies differed significantly between individual fish and between salinities. There is probably no interaction ($0.10 > P > 0.05$); this indicates that the individuals are affected equally by salinity. All three fish showed signs of slight bradycardia in fresh water. However, a significantly lower heart beat frequency could only be demonstrated for one of the blennies (Table 1).

Table 1. *Blennius pholis*. Differences in heart beat frequencies in full salinity (34 ‰) and in fresh water. 12 observations in all cases. – not significant, +, + + and + + + significant at 2, 5 and 1 % levels, respectively

Fish No.	Wet weight (g)	Salinity (‰)	Heart beats min^{-1} $\bar{x}$	s	t-value	Level of significance
1	19.0	34	61.50	1.88	1.217	-
		0	60.42	2.43		
2	21.5	34	71.00	3.30	1.189	-
		0	65.83	5.39		
3	27.5	34	72.25	2.99	4.280	+ + +
		0	67.58	2.31		

Table 2. *Blennius pholis*. Opercular beat frequencies

Fish No.	Wet weight (g)	Opercular beats min^{-1} $\bar{x}$	s	Number of observations
1	19.0	36	43	24
2	21.5	66	21	23
3	27.5	48	43	24

Whereas the heart beats with a frequency of about 60–70 beats min^{-1} (Table 1), the opercular beat frequency seems slower (Table 2). ANOVA reveals that in none of the fish did the variations in opercular beat frequency follow the variations in heart beat frequency. Visual observations and traces from the chart recorder showed, however, that on occasion the opercular movements stopped completely. This explains the large standard deviations shown in Table 2. These large variations might have masked a possible covariation of heart and opercular beat frequencies.

Plasma Osmolarity

In an abrupt salinity regime *Blennius pholis* showed great constancy of blood osmoconcentration. Although the medium changed from full-strength seawater to fresh water, there occurred no significant ($0.50 > P > 0.25$) changes in the osmolarity of the plasma (Fig. 2).

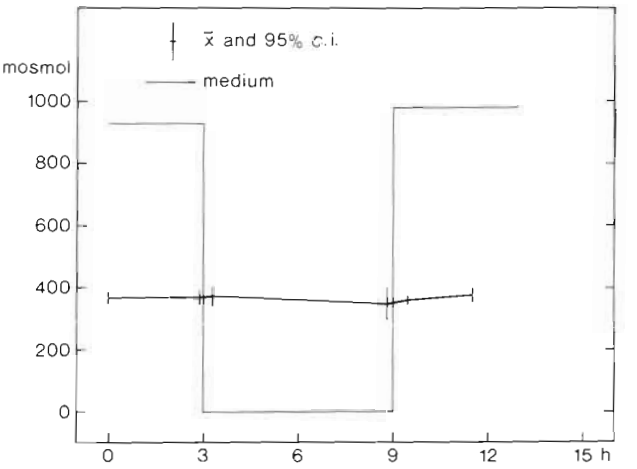

Fig. 2. *Blennius pholis*. Changes in the osmolarity of blood plasma during abrupt changes in salinity from full seawater to fresh water c.i.: confidence interval

Respirometry

In an abrupt salinity regime, pooled regression analysis of the total mass of data of oxygen consumption by the blenny (Fig. 3) indicates significant differences between regression coefficients (slopes) and between constants (intercepts). The regressions were therefore sorted into groups according to the part of the regime they were obtained from. It then appears that there were no significant differences between coefficients and constants, except in the second period at full salinity (9–15 h after start of the experiment). Therefore, all the regressions for the weight-specific oxygen consumption on weight in fresh water may be pooled to give a common regression. Similarly, the regressions for full salinity may be pooled for the experimental periods 0–3 and 21–24 h after the start of the experiment. Between these two pooled regressions there is a significant difference in coefficients, indicating that large and small fish react differently to fresh water. The regressions for the second period at full salinity (8–15 h after start of the experiment) cannot be pooled because of the significant differences among them. Since this implies that the number of degrees of freedom is not increased, the confidence limits (Fig. 3) are wider here than for those periods where the regressions could be pooled, a procedure which increases the number of degrees of freedom.

In the sinusoidal regime (Fig. 4) the blennies were exposed to the same salinities four times throughout the 24 h cycle. Two of these were experienced in a decreasing part of the regime and two in an increasing part. The pairs of regressions for each salinity in the decreasing part of the regime were compared, as were the pairs in the increasing part. It appears that a

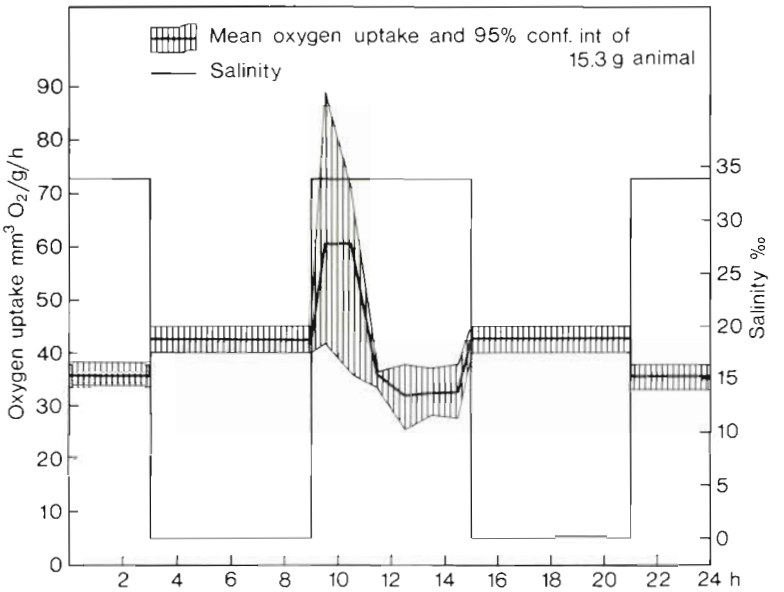

Fig. 3. *Blennius pholis*. Oxygen consumption of a 15.3 g individual during abrupt changes in salinity

slightly significant difference ($0.05 > P > 0.025$) within these pairs could only be demonstrated in one case, viz. in decreasing salinity at 12.6 ‰. As this difference occurs in one out of twelve regressions, the probability for this happening by chance is $(1/12) \times 100 = 8.3$ %. Therefore, it is probably justified to pool all pairs of regressions, thus obtaining 6 pairs in all. Each member of a pair obtained in the two decreasing parts of the regime and in the two increasing parts of the regime, respectively. Pooled regression analysis showed that, depending on whether the blenny was exposed to increasing or decreasing salinity, there were significant differences in the levels of oxygen consumption at salinities of 29.0, 21.4 and 12.6 ‰. Based on these pooled regressions, the changes in oxygen consumption throughout a 24 h sinusoidal salinity regime may be calculated for any size of blenny. This is illustrated for a 15.3 g individual in Figure 4. This weight was chosen since it was the mean weight of blennies used in a later study by Vahl and Davenport (1979).

CONCLUSIONS

The results obtained demonstrate that *Blennius pholis* is capable of maintaining a remarkable physiological stability when exposed to the most severe salinity fluctuations likely to occur in its intertidal habitat.

The constancy of blood osmoconcentration shown in

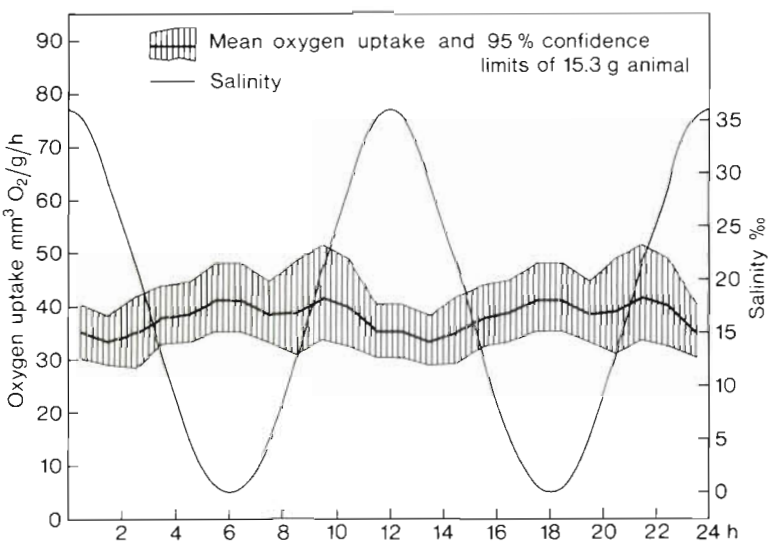

Fig. 4. *Blennius pholis*. Oxygen consumption of a 15.3 g individual exposed to a sinusoidal salinity regime

abrupt salinity regimes, where fish encounter fresh water for 6 h periods was somewhat unexpected. House (1963) showed that blennies acclimated to 10 ‰ seawater for 48 h had blood sodium and chloride concentrations some 70 % less than fish acclimated to full seawater, and it appears that *Blennius pholis* cannot maintain its blood concentration at the level held in full seawater in media below the isosmotic concentration of about 40 ‰ s.w. However, House was also able to demonstrate that the blenny differed from most other euryhaline teleosts e.g. *Anguilla anguilla* (Keys 1933) and *Salmo gairdnerii* (Houston, 1957) in that it exhibits a very rapid physiological response to salinity changes, switching in less than 5 min from pumping salts outwards across the gills in hyperosmotic media, to actively taking up salts from hypo-osmotic media (< 40 ‰ s.w.). This rapidity of response, which operates in both directions, means that the passive loss of salts caused by exposure to fresh water will be partially offset by active salt uptake, so that the blood osmoconcentration will decline relatively slowly. In the experiments reported here, it is clear that a 6 h exposure to fresh water is insufficient to produce a statistically significant decline in blood osmoconcentration – a situation which is analogous to the blood osmoconcentration damping response previously shown for invertebrates. It also remains possible that control of drinking rates, urine concentration and urine production rates may contribute to hemostasis, but no data to confirm this is available.

The variations in oxygen consumption are somewhat difficult to interpret. In the sinusoidal salinity regime, consumption is elevated at low and rising salinities but drops back to 'routine' level (Fry, 1957) at high and falling salinities. It is possible that the elevation at low salinity levels is associated with the cost of osmoregulation: the increased oxygen consumption as the salinity rises is less easy to explain, but may well reflect increased physical activity. Such an increase in physical activity is to be expected in a fish which is inactive at low tide and feeds at high tide. Possibly, rising salinity acts as a cue for searching activity to be resumed, whereas falling salinities precipitate inactivity. It should be noted that the fluctuations in mean oxygen consumption are relatively small, the maximum increase in mean rate above routine being about 19 %.

Kinne (1964) and Holliday (1971) have stressed that the oxygen content of water depends upon salinity and Holliday has stated that, since the oxygen uptake of fish is, to a large extent, determined by the oxygen concentration, it is difficult to assess whether a change in respiration rate is related to salinity or oxygen concentration. In our experiments the oxygen content of the air-saturated water supplied to the blennies fluctuated sinusoidally from 6.4 ml O_2 l^{-1} at high salinity to 8.0 ml O_2 l^{-1} at the lowest salinity level. This change in concentration of about 25 % is comparable in size to the change in respiration rates observed. However, it should be pointed out that Holliday's hypothesis is rather dubious, since oxygen tensions rather than oxygen concentrations control oxygen exchange. Moreover, the oxygen consumption data presented are assymetric in form; if the changes in uptake were solely influenced by oxygen concentration, it would be expected that consumption would vary sinusoidally and uptake maxima would coincide with salinity minima. These comments also apply generally to the results derived from the abrupt salinity regime experiments. Here, however, is an additional problem in that the fish appeared to show a large increase in oxygen uptake when they were returned to seawater after the first fresh water exposure, although the data were very variable. This response could not be demonstrated after the second fresh water exposure and may reflect an increase in physical activity of the fish. Overall it would appear, therefore, that blennies take up more oxygen when exposed to fluctuating salinity regimes than they do in full seawater, but the precise reasons for this remain obscure. The importance of the extra energetic cost of living in a fluctuating salinity environment will be explored further in the following paper dealing with apparent specific dynamic action and feeding strategies (Vahl and Davenport, 1979).

Since the oxygen consumption changes observed were small it is not surprising that the effects of salinity fluctuations upon heart and opercular beat should be weak. In any case, changes in blood perfusion rate of the gills may alter oxygen uptake without disturbance of the heart or opercular beat.

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