

## THE VENTILATION, EXTRACTION AND UPTAKE OF OXYGEN IN UNDISTURBED FLOUNDERS, *PLATICHTHYS FLESUS*: RESPONSES TO HYPOXIA ACCLIMATION

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### SUMMARY

1. Gill ventilation ( $\dot{V}_g$ ), extraction of  $O_2$  from the ventilatory current and  $O_2$ -uptake ( $\dot{V}_{O_2}$ ) were measured in three groups of undisturbed flounders naturally buried in sand. Two groups were acclimated to normoxic water ( $P_{i,O_2} > 120$  mmHg). One was studied in normoxic water, the other during acute exposure to hypoxic water ( $P_{i,O_2} = 30$  mmHg). A third group was acclimated to and studied in hypoxic water.

2. Gill ventilation was measured directly using an electromagnetic flow-meter. The flowprobe, fitted to a plastic funnel, was placed over the fish's mouth without making physical contact with the fish. Exhaled water for calculation of  $O_2$ -extraction was siphoned from another plastic funnel placed over the upper operculum of the flounder.

3. Acute hypoxia exposure of normoxic fish caused nearly a doubling in ventilation volume. In flounders acclimated to chronic hypoxia and studied in hypoxic water the ventilation volume reached a value approximately 3.5 times that of normoxic fish.

4.  $O_2$ -extraction averaged 76% in normoxic flounders. During acute hypoxia the value declined to 52%, the same  $O_2$ -extraction was obtained in the chronically hypoxic fish.

5.  $\dot{V}_{O_2}$  for the normoxia acclimated fish in normoxic water was 0.45 ml  $O_2 \cdot kg^{-1} \cdot min^{-1}$  declining to 0.12 ml  $O_2 \cdot kg^{-1} \cdot min^{-1}$  during acute exposure to hypoxia.  $\dot{V}_{O_2}$  of hypoxia acclimated fish in hypoxic water was 0.24 ml  $O_2 \cdot kg^{-1} \cdot min^{-1}$ .

6. When compared in hypoxic water, the hypoxia acclimated fish show an unchanged  $O_2$ -extraction of 52% in spite of a nearly two-fold increase in ventilation relative to that of the normoxia acclimated fish. Possible causes for this may be a left shift of the  $O_2$ -Hb dissociation curves and an increase in the transfer factor for  $O_2$  over the gills in the hypoxia acclimated fish.

### INTRODUCTION

The flounder (*Platichthys flesus*) has a geographical distribution from the White Sea, in the north, to the Mediterranean and the Black Sea, in the south. It is a benthic, night-active, species showing activity peaks at dusk and dawn. During part of its

life it normally inhabits shallow coastal waters with a soft or sandy bottom and spends most of the day buried in the sediment. The species is notably tolerant of variable ambient conditions such as variations in temperature, salinity and  $O_2$ -availability (Muus, 1967). This author reported that the  $O_2$ -tension in quite shallow waters inhabited by small specimens of *P. flesus* could decrease to a value of about 20 mmHg at sunrise. Normally, however, the  $O_2$ -tension of these brackish waters did not fall to such low levels.

The objective of the present study has been to evaluate the influence of acclimation to different levels of oxygen availability on oxygen uptake and its principal determinants, the ventilation (gill water flow) and the  $O_2$ -extraction from the ventilatory current.

#### MATERIAL AND METHODS

Flounders (150–550 g) were caught by trawl from the Bay of Aarhus. In the laboratory the fish were divided into two groups and kept in filtered seawater (19 ‰ salinity) at 8–10 °C. In one tank the water was constantly aerated resulting in a high  $P_{O_2}$ , which never fell below 120 mmHg. In the other tank the  $P_{O_2}$  was regulated at a level of about 30 mmHg and monitored by a Radiometer  $O_2$ -electrode and PHM 71 acid-base analyser. The fish were gradually acclimated to the low oxygen tension over a period of 3 days, since sudden exposure to water of an oxygen tension of 30 mmHg for 24 h caused death of some fish, although it was tolerated for shorter periods of about 3 h (see below). Hypoxia induced changes in respiratory properties of blood in eel, plaice and carp are known to take place within 1–3 weeks (Wood & Johansen, 1972; Wood, Johansen & Weber, 1975; Weber & Lykkeboe, 1978). Consequently, an acclimation period of minimally 3 weeks was chosen. The fish were not fed during the acclimation and experimental periods.

Measurements were performed in an experimental aquarium with a 5 cm layer of sand covering the bottom. A fish was transferred to the experimental tank in the late afternoon and allowed to adjust overnight. During this period a flow of water of the same  $O_2$ -tension and temperature as that of the acclimation tank was directed through the experimental aquarium. Measurements were done during the daytime, which is the inactive period in the diurnal activity rhythm of the flounder.

Three types of experiments were performed:

1. Normoxia acclimated fish were exposed to normoxic water,  $P_{O_2} > 120$  mmHg (number of fish,  $n = 7$ ).
2. Normoxia acclimated fish were acutely exposed to hypoxic water,  $P_{O_2} = 30$  mm Hg ( $n = 7$ ).
3. Hypoxia acclimated fish were exposed to hypoxic water,  $P_{O_2} = 30$  mmHg ( $n = 5$ ).

Lowering the  $O_2$ -tension in the experimental aquarium from 120 to 30 mmHg was achieved by directing the water supply to the experimental aquarium through a nitrogen gas exchange column. The transition lasted 20 min. The oxygen tension in the experimental aquarium was continuously monitored by a Radiometer  $O_2$ -electrode.

Direct measurement of ventilation was achieved by placing a small plastic funnel equipped with an electromagnetic flow-probe (diameter 5 mm, length 25 mm) over

the mouth of the fish (Fig. 1). In order to avoid leaks, care was taken that the edge of the funnel was buried in sand. Drops of a concentrated dye (Evan's blue) were placed on the surface of the sand immediately outside the funnel to check that water did not penetrate around the edge. If any trace of dye was seen to penetrate into the funnel, more sand was pushed against the outer wall of the funnel until no leaks were present.

Expired water was collected via a second funnel placed over the upper opercular opening. A small fraction of the expired water was siphoned past an  $O_2$ -electrode placed in a thermostatted cuvette (Radiometer type E-5046) placed next to the experimental aquarium below the water surface. Water was siphoned from the experimental aquarium through the water jacket of the cuvette.

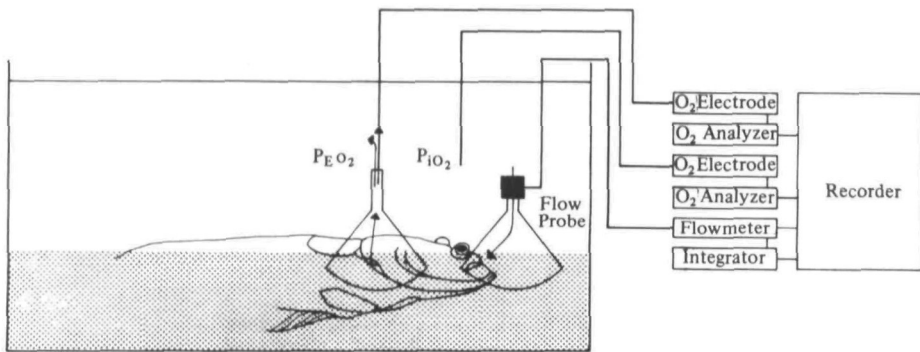


Fig. 1. Schematic illustration of a flounder in the experimental aquarium showing arrangement used for measurement of inspired ( $P_i, O_2$ ) and expired ( $P_e, O_2$ ) water  $O_2$ -tensions as well as inspired water flow.

Pressure pulses in the funnels caused by the ventilation of the fish could not be detected with a sensitive blood pressure transducer (Statham P 23 BB) and could therefore only have been a fraction of a millimeter of water at the most. This is indicative of a low resistance to flow of the funnels and flowprobe. The flow was recorded by a Micron Instruments electromagnetic flowmeter and integrated by a Gould-Brush integrator. Both flowmeter and integrator were calibrated volumetrically using a peristaltic pump (Harvard Apparatus) adjusted to known outputs of water per minute. The flowmeter was operated at a frequency response of 0.1 Hz. The use of this low frequency response was necessitated by a rather high level of electrical noise in the salt water system. Consequently the amplitude of the direct flow signal was somewhat damped. The magnitude of the integrated flow signal was, however, not affected, attesting to a true recording of mean flow (ventilation).

The flow signal, integrated flow signal, inspired and expired  $O_2$ -tensions were recorded on a Hewlett Packard 7404A 4-channel oscillograph.

A stable pattern of breathing was attained within half an hour after placing the funnels over the mouth and opercular openings of the flounder. In the case of the two groups of fish tested at the conditions of acclimation recordings were continued for 5-6 h. In the normoxia acclimated fish acutely exposed to hypoxia recordings were carried out for a period of about 3 hours. Within  $\frac{1}{2}$ -1 h of exposure to acute hypoxia a stable breathing pattern was established in these fish continuing for 1-2 h. After this

period of exposure to acute hypoxia the fish became restless and started to move about thus interrupting the recording. All fish survived acute exposure to hypoxia for this length of time and recovered upon being returned to normoxic water.

Average values of breathing frequency, stroke volume, ventilatory water flow and  $O_2$ -extraction were calculated for each fish based on the periods of stable breathing. Oxygen uptake was calculated for each fish from mean values of ventilation and  $O_2$ -extraction.

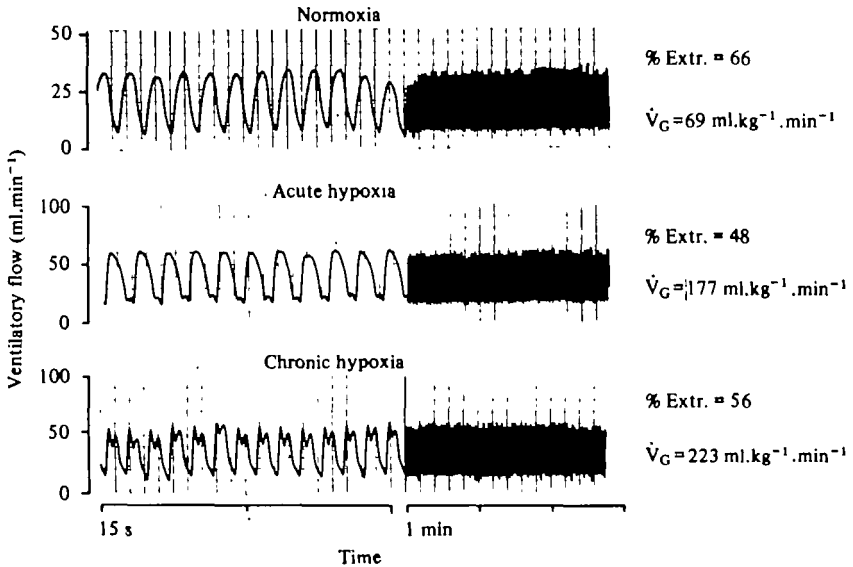


Fig. 2. Sample tracings of pulsatile ventilatory water flow in a specimen from each group of flounders studied. Note different scales on ordinates. The amplitude is damped as explained in the text.

## RESULTS

Fig. 2 shows typical recordings of ventilatory flow from a normoxic, an acutely hypoxic and a chronically hypoxic flounder. Although the amplitude of the flow pulses is damped, a difference in flow pattern between the three groups of fish is apparent. The shape of the signal is altered from a single peak per breath, in the normoxic fish, towards two peaks per breath in the acutely hypoxic fish and to a pronounced 'two-peak' signal per breath in the chronically hypoxic fish. This must be due to a change in the rhythm of the buccal force pump and the opercular suction pump which maintain the respiratory current (Hughes, 1960).

Breathing frequency was almost the same for the normoxic and the acutely hypoxic fish (21 and 22 beats.min<sup>-1</sup>) whereas the chronically hypoxic flounders showed a higher rate of breathing (29 beats.min<sup>-1</sup>) (Fig. 3). Ventilatory stroke volume was increased to 7.1 and 10.9 ml.kg<sup>-1</sup> in the acutely hypoxic and the chronically hypoxic fish compared to 4.3 ml.kg<sup>-1</sup> in the normoxic group (Fig. 3).

The near doubling of ventilation in the normoxia acclimated fish from 88.6 to 152.5 ml.kg<sup>-1</sup>.min<sup>-1</sup> when subjected to acute hypoxia was the result of an increase in stroke volume. In the flounders acclimated to chronic hypoxia the ventilation averaged

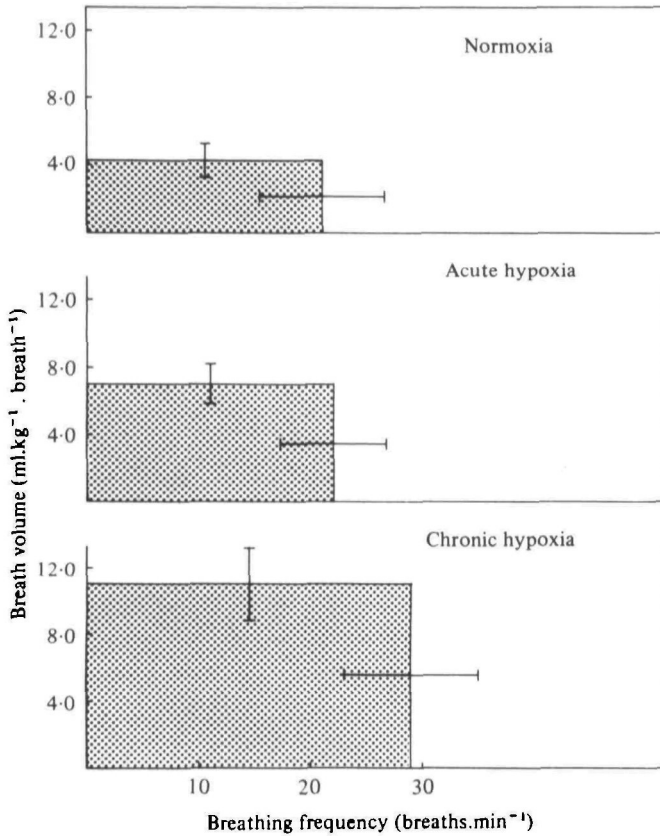


Fig. 3. Ventilation expressed as a product of breath volume and frequency in the three experimental groups. The values are averages for each group. Bars express  $\pm 1$  s.d. Student's *t*-test shows a significant difference between breath volume in normoxic vs. acutely hypoxic ( $P < 0.001$ ), normoxic vs. chronically hypoxic ( $P < 0.001$ ) and acutely hypoxic vs. chronically hypoxic ( $P < 0.01$ ) fish and a significant difference between breathing frequency in normoxic vs. chronically hypoxic ( $P < 0.05$ ) and acutely hypoxic vs. chronically hypoxic ( $P < 0.05$ ) fish. Number of fish in each group: normoxia:  $n = 7$ , acute hypoxia:  $n = 7$ , chronic hypoxia:  $n = 5$ .

308.0 ml.kg<sup>-1</sup>.min<sup>-1</sup>, a value approximately 3.5 times that of the normoxic fish owing to an increase in stroke volume as well as in the rate of breathing (Figs. 3 and 4).

Fig. 4 also shows per cent O<sub>2</sub>-extraction in relation to ventilation for the three groups of fish and average results for each individual specimen studied are given in Fig. 5. The normoxia acclimated flounders when studied in well aerated water had a very high O<sub>2</sub>-extraction averaging 76% with a maximum value of 85%. During acute exposure to hypoxia the extraction in normoxia acclimated fish fell to an average value of 52% with large individual variations. Notably, however, the flounders acclimated to chronic hypoxia had an average O<sub>2</sub>-extraction closely similar to the value for the acutely exposed fish in spite of the higher ventilation.

Fig. 6 illustrates the average O<sub>2</sub>-uptake for the three groups of flounders. Upon exposure to acute hypoxia the normoxia acclimated fish suffered a decline in O<sub>2</sub>-uptake from 0.45 ml.kg<sup>-1</sup>.min<sup>-1</sup> in normoxic water to 0.12 ml.kg<sup>-1</sup>.min<sup>-1</sup> in hypoxic

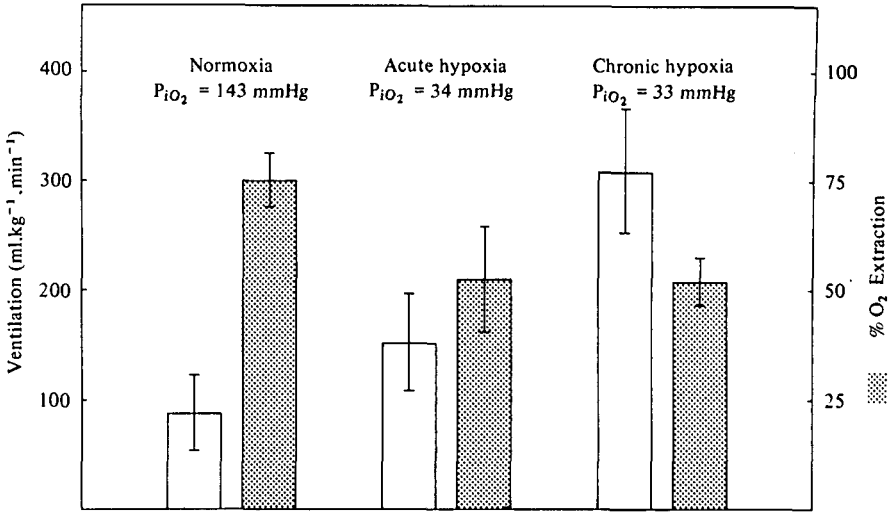


Fig. 4. Ventilation volume and % O<sub>2</sub>-extraction in the three experimental groups. The columns are averages for each group. Bars express  $\pm 1$  s.d. Student's *t*-test shows a significant difference between ventilation in normoxic vs. acutely hypoxic ( $P < 0.01$ ), normoxic vs. chronically hypoxic ( $P < 0.001$ ) and acutely vs. chronically hypoxic ( $P < 0.001$ ) flounders, and a significant difference in O<sub>2</sub>-extraction between normoxic and acutely hypoxic ( $P < 0.001$ ) as well as normoxic vs. chronically hypoxic ( $P < 0.001$ ) fish. Number of fish in each group: normoxia:  $n = 7$ , acute hypoxia,  $n = 7$ , chronic hypoxia,  $n = 5$ .

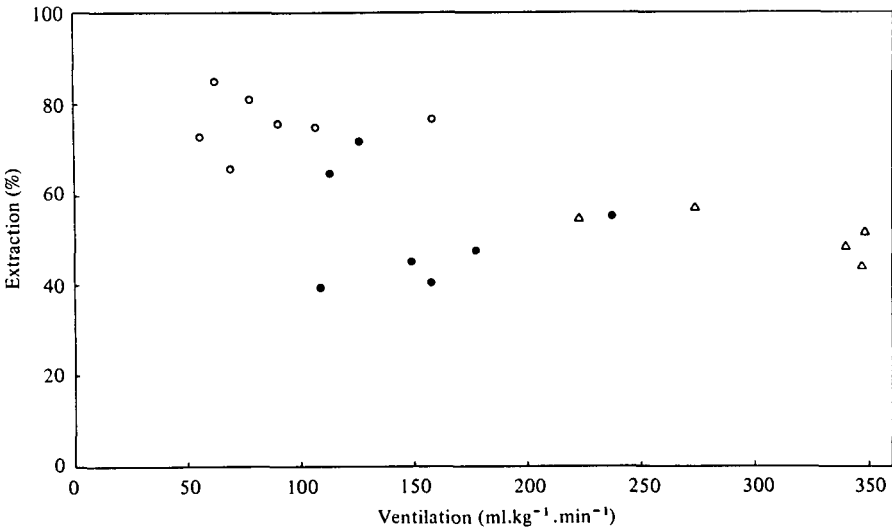


Fig. 5. Percent extraction of oxygen from the ventilatory water current in normoxic (open circles), acutely hypoxic (filled circles) and chronically hypoxic flounders (triangles) as a function of ventilation volume. The points are average values for the stable periods of measurements for each flounder.

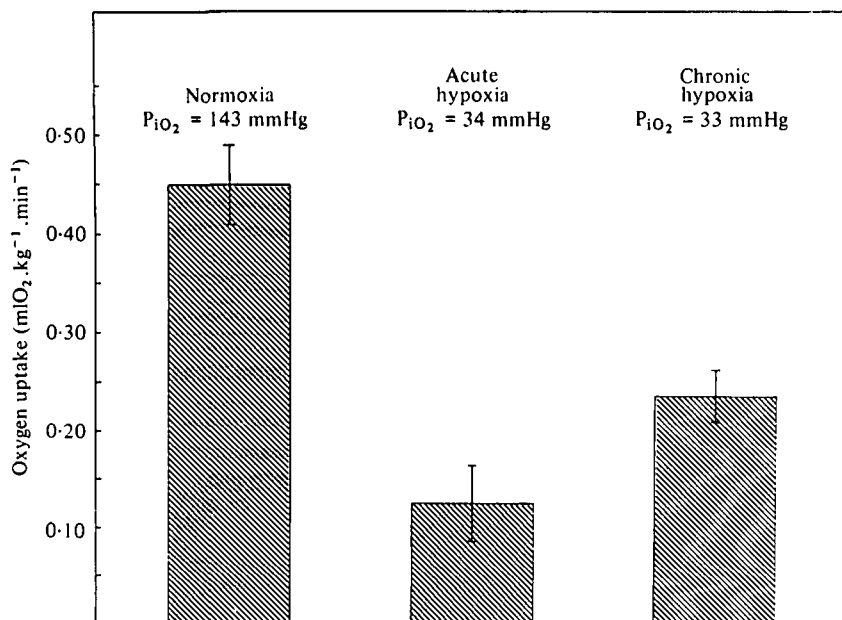


Fig. 6. Oxygen uptake for the three groups of flounders. The columns are average values for each group. Bars express  $\pm 1$  S.D. Student's *t*-test shows a significant difference between oxygen uptake in normoxic vs. acutely hypoxic ( $P < 0.001$ ), normoxic vs. chronically hypoxic ( $P < 0.02$ ) and acutely vs. chronically hypoxic ( $P < 0.001$ ) fish. Number of fish in each group: normoxia:  $n = 7$ , acute hypoxia:  $n = 7$ , chronic hypoxia:  $n = 5$ .

water. The chronically hypoxic fish were able to maintain an  $O_2$ -uptake of  $0.24$  ml.  $kg^{-1}$ .  $min^{-1}$  in hypoxic water, or twice the value seen in the acutely hypoxia exposed fish.

#### DISCUSSION

Quantification of gill ventilation in fish is technically difficult without disturbing the normal breathing rhythm or altering the physical conditions for a normal propagation of the respiratory water current. Accordingly most previous studies on gill ventilation have been based on either indirect estimates or measurements of ventilation on restrained fish. A few investigators have been working with unrestrained fish (Hughes & Umezawa, 1968; Watters & Smith, 1973; Piiper *et al.* 1977; Lomholt & Johansen, 1979), but even in these studies the fish have had rubber tubes glued or rubber membranes sewed around the gill openings or have been equipped with rubber masks. Hughes & Knights (1968) showed that the  $O_2$ -consumption of the dragonet (*Callionymus lyra*), at rest, was increased when the opercular and buccal cavities were cannulated or the fish had rubber tubes attached around the opercular openings.

Piiper *et al.* (1977) and Lomholt & Johansen (1979) have stressed the importance of achieving truly mixed samples of the exhaled water for correct estimation of  $O_2$ -extraction. Both groups of investigators found higher  $O_2$ -extraction values for dogfish and carp, respectively, than previously reported in studies of the same species when opercular sampling catheters were used. The experimental arrangement presently

used to study the flounder has the advantages of the mask or similar devices (direct recording of flow and collection of mixed exhaled water) and at the same time does not require any physical contact with the fish, thus leaving the fish in a completely normal and undisturbed situation.

Already Meyer (1935) employed a basically similar technique for respiratory studies on a burrowing species, the stargazer (*Uranoscopus scaber*). The system, however, included directing the water current through rather long glass tubes and did not allow continuous recording of pulsatile flow and exhaled  $O_2$ -tension.

During periods of elevated breathing, flatfish are known to expel some of the exhaled water through the lower operculum (Yazdani & Alexander, 1967; Watters & Smith, 1973). This phenomenon was regularly observed during the present study. It has not affected the measurements of ventilation which were done on the inflow side, but it has necessitated the assumption that the  $O_2$ -extraction of the gills is bilaterally similar. The fact that the gill surface area of flatfish is identical on the two sides and the opercula are almost symmetrical (Hughes, 1960) lends support to this assumption. Furthermore, even during quiet breathing when the entire water current is expelled from the upper operculum both gills are irrigated. This is possible because the opercular cavities communicate ventrally (Yazdani & Alexander, 1967).

The credibility of the direct measurement of ventilation and  $O_2$ -extraction for the calculation of  $O_2$ -uptake in our study is supported by the nearly similar value of  $0.39 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  obtained by Jørgensen (1978) on normoxic *P. flesus* at  $10^\circ \text{C}$  using conventional respirometry. Cech, Rowell & Glasgow (1977) also report an  $O_2$ -uptake of  $0.39 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the closely related *Pseudopleuronectes americanus*.

Only one study of ventilation and gas exchange in flatfish is available for comparison with our data on *Platichthys flesus*. Watters & Smith (1973) studied the respiratory dynamics of the starry flounder, *Platichthys stellatus*, in response to low oxygen and high temperature. Their fish had rubber membranes sewed around both gill openings for collection of the gill ventilation volume and sampling of expired water. The ventilation in normoxic water at  $9\text{--}12^\circ \text{C}$  and 30‰ salinity averaged  $141.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for specimens weighing between 1175 and 1475 g. The  $O_2$ -extraction averaged about 55%. Comparison of these results with our data shows a markedly smaller ventilation in *P. flesus*, averaging  $88.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and a much higher  $O_2$ -extraction of 76%. This difference is probably due to the unrestrained condition of our experimental animals. The high values of  $O_2$ -extraction result in a low ventilatory requirement ( $\dot{V}_g/\dot{V}_{O_2}$ ) being  $197 \text{ ml} \cdot \text{ml} \cdot \text{O}_2^{-1}$  for *P. flesus* compared to  $314 \text{ ml} \cdot \text{ml} \cdot \text{O}_2^{-1}$  in *P. stellatus*. A low ventilatory requirement is both energetically less costly and affords a large potential for compensatory increase in ventilation in hypoxic water or during or following physical activity. The  $O_2$ -uptake recorded by Watters & Smith (1973) for *P. stellatus* at normoxic conditions are in accord with the present data for *P. flesus* averaging  $0.404 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and  $0.45 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively.

Typically, teleost fishes respond to acute hypoxia by an increase in ventilation. This has been shown for trout (Holeton & Randall, 1967); juvenile catfish (Gerald & Cech, 1970); striped mullet (Cech & Wohlschlag, 1973); stargazer (Meyer, 1935); white sucker, brown bullhead, carp (Saunders, 1962); dragonet (Hughes & Umezawa, 1968); starry flounder (Watters & Smith, 1973); and carp (Lomholt & Johansen, 1979). The ventilation increase is mainly caused by larger stroke volumes and, to a lesser



extent, by a rise in frequency. Normally the  $O_2$ -extraction decreases during compensatory increase in ventilation. The data on *P. flesus* conform to this response pattern. During acute exposure to hypoxia the ventilation was doubled as a result of an increase in ventilatory stroke volume. The  $O_2$ -extraction decreased from 76 to 52%.

Acute exposure of starry flounders, *P. stellatus*, to an  $O_2$ -tension of about 60 mmHg, caused more than a doubling in ventilation and a modest reduction in  $O_2$ -extraction from 55 to 45%. This resulted in an  $O_2$ -consumption of  $0.35 \text{ ml } O_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  which is almost 80% of the oxygen uptake in well-aerated water (Watters & Smith, 1973). These data suggest that flounders may show respiratory independence down to an oxygen tension of about 60 mmHg at 10 °C. This suggestion has recently been verified also on *P. flesus* which showed a maintained  $O_2$ -uptake at 10 °C down to a water  $P_{O_2}$  of 50 mmHg (Jørgensen, 1978). At a water  $P_{O_2}$  of 30 mmHg, such as used in the present study, a clear respiratory dependence prevails with an oxygen uptake of only 28% of that during normoxia.

Few data on respiratory and metabolic responses of fish to hypoxic acclimation are available. Laboratory acclimation of goldfish to low oxygen content resulted in a reduction in standard oxygen consumption at high  $P_{O_2}$  (from an average of  $3.98$  to  $2.72 \text{ ml } O_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), while the critical oxygen tension fell from approximately 79 to 38 mmHg. At low  $P_{O_2}$  the  $O_2$ -uptake was higher in hypoxia acclimated than in normoxia acclimated goldfish (Prosser *et al.* 1957).

Beamish (1964) examined  $O_2$ -uptake rates in brook trout, carp and goldfish acclimated to normoxic and hypoxic conditions at high and low temperatures. Down to an oxygen partial pressure of about 80 mmHg the standard  $O_2$ -uptake remained almost constant and was the same for the two acclimation groups. Below this independent range the oxygen consumption first increased to a maximum value but then decreased as a result of a further reduction in  $O_2$  partial pressure. This increase below the independent range, explained to reflect the increased cost of breathing, was smaller in hypoxia acclimated than in normoxia acclimated fish.

Lomholt & Johansen (1979) studied hypoxia acclimation in carp. During exposure to water  $O_2$ -tensions between 10 and 40 mmHg in a flow-through respirometer, carp acclimated to an  $O_2$ -tension of 30 mmHg showed 30–40% higher average  $O_2$ -uptake values than acutely exposed normoxia acclimated carp. In well aerated water the  $O_2$ -extraction was nearly the same for the two groups but as the  $O_2$ -tension declined the normoxia acclimated fish showed a reduction in extraction while conversely the hypoxia acclimated carp showed a small increase in extraction. Concomitantly during normoxic conditions ventilation was the same in the two groups of fish whereas exposure to hypoxia resulted in a tendency towards a higher ventilation in normoxia acclimated compared to hypoxia acclimated fish.

The present data on *P. flesus* are partially in agreement with earlier studies. The finding of  $O_2$ -uptakes of the hypoxia acclimated flounders in hypoxic water of twice the value obtained for the flounders acutely exposed to hypoxia, is similar to that for goldfish (Prosser *et al.* 1957) and carp (Lomholt & Johansen, 1979). The compensatory increase in  $O_2$ -uptake of hypoxia acclimated *P. flesus* in hypoxic water resulted from an increase in ventilation, while the  $O_2$ -extraction was maintained. This is in contrast to the results for carp which lower the ventilation and increase the  $O_2$ -extraction under similar conditions.

The lowering of the  $O_2$ -tension in inspired water to about 30 mmHg will reduce the  $P_{O_2}$ -gradient from water to blood and thus lower the  $O_2$ -uptake by the blood. A compensation for the smaller diffusion gradients across the gills would be to shift the  $O_2$ -dissociation curve to the left augmenting the  $P_{O_2}$ -gradient across the gills and the  $O_2$ -saturation of the arterial blood. An increased  $O_2$ -affinity caused by a reduction in the red cell organic phosphate concentration during hypoxia has been demonstrated in eel (Wood & Johansen, 1972), plaice (Wood *et al.* 1975), and carp (Weber & Lykkeboe, 1978) under hypoxic conditions. In the eel (Wood & Johansen, 1972) the hypoxia acclimation also caused an increase in hemoglobin concentration and thus in the  $O_2$ -capacity of the blood, but this response was seen neither in plaice (Wood *et al.* 1975) nor in carp (Weber & Lykkeboe, 1978).

Another compensation against reduced  $O_2$ -gradients across the gill surface could be an increased transfer factor (diffusion capacity) for  $O_2$  across the gills. This might be caused by an increased functional area of the gills or a reduction in the diffusion distance between blood and water by changing the pattern of blood or water flow over the respiratory surfaces. Also the reduction in thickness of the water boundary layer at the respiratory surfaces, caused by the increased ventilation volume, may add to the transfer factor.

*P. flesus* as demonstrated presently exhibits a clear increase in  $O_2$ -uptake during hypoxia after a period of acclimation to low  $O_2$ -availability. The relative importance of the compensatory measures discussed above in bringing about this response must await information about blood respiratory properties and *in vivo* blood gas tensions and pH.

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