Marine Biology 19, 133—148 (1973)

Respiratory Dynamics of the Starry Flounder *Platichthys stellatus* in Response to Low Oxygen and High Temperature

K. W. Watters, Jr. and L. S. Smith

Fisheries Research Institute, University of Washington; Seattle, Washington, USA

Abstract

Starry flounder (*Platichthys stellatus* Pallas) were fitted with masks for measurements of ventilation volume and post-gill oxygen tensions, and with arterial and venous cannulae. They were then subjected to periods of low oxygen levels and high temperature. Pre- and post-gill oxygen tension, blood oxygen tension and content, oxygen uptake, and ventilation volume were measured. Effectiveness of gas exchange across the gills, cardiac output, and transfer factor were calculated. A series of blood-oxygen dissociation curves were also constructed at two pH's and temperatures. The data suggest that flounder are able to regulate oxygen uptake down to an environmental oxygen tension of at least 50 mm Hg by increasing ventilation volume and transfer factor while not changing blood flow rate or pattern. Also, the flounder's response to temperature increase is similar to the expected response to exercise, with the exception of an increase in gill diffusion resistance.

Introduction

The effects of sub-optimal water quality on fish has been of concern for some time. Earlier studies evaluating the biological effects of pollution consisted of placing fish in polluted water and observing their reaction (Knight, 1901). As more was learned about using fish as experimental animals, more elaborate techniques were evolved. Metabolic measurements of oxygen uptake were made, and the effects of specific environmental factors such as dissolved gas and temperature were noted (Powers and Shipe, 1928; Belding, 1929).

However, it was not until van Dam's (1938) classic work that modern fish respiratory physiology was developed. Investigators since have examined many aspects of gas exchange in fishes. Fry's (1957) review article covered many considerations of respiration, including the effects of temperature and CO_2 on the blood-oxygen dissociation curves, and the effects of lowered oxygen tensions on metabolism of several fishes. Basu (1959) examined the effects of various concentrations of oxygen and carbon dioxide on oxygen uptake, and Hickman (1959) performed measurements of oxygen consumption on the starry flounder *Platichthys stellatus* Pallas as part of a study on the role of the thyroid gland. Randall *et al.* (1967) presented an analysis of the exchange of oxygen and carbon dioxide across the gills of the rainbow trout *Salmo gairdnerii*. *Piiper and Baumgarten-Schumann* (1968) did an analysis of the effectiveness of oxygen removal from the water and oxygen uptake by the blood in the dogfish *Scyliorhinus stellaris*, and Hanson and Johansen (1970) examined water and blood flow rates at the gills of the dogfish *Squalus suckleyi*. Davis and Cameron (1970) studied the relationship of water flow to gas exchange in the rainbow trout *S. gairdnerii*.

The role of blood in respiration in fishes has been studied by Black (1940), and Black *et al.* (1966a, b). More work has also been carried out on the effects of environmental oxygen concentration on breathing in fishes (Randall and Shelton, 1963; Holeton and Randall, 1967; Randall and Smith, 1967; Smith *et al.*, 1971), and the effects of temperature on respiration (Beamish and Mookherjii, 1964). Together with these investigations, more accurate methods for making physiological measurements have been developed (Tucker, 1967; Davis and Watters, 1970).

The bulk of the above work has been performed on pelagic fishes; comparatively little has been done with benthic animals. A flounder, which is physically adapted for living on a mud bottom, would probably encounter extremes of oxygen concentrations and temperatures unknown to pelagic forms. Although bottom temperatures in Eastsound, Washington, USA (a common collecting site for flounders) have been reported to range between 6° and 12 °C (Miller, 1969), young *Platichthys stellatus* have been found in shallow bays with water temperatures as high as 20 °C, and adults have been seined from silt in the bottom of a polluted estuary (personal observation).

It was the objective of this study to (1) develop surgical procedures for obtaining biological samples from a flounder; (2) to obtain comprehensive respiratory reactions to adverse environmental conditions in a flounder as a representative of a benthic species; (3) to compare these findings with similar results obtained on pelagic species.

Methods

Studies were carried out during 1970—1971 at the University of Washington's Friday Harbor Laboratories. Starry flounders (*Platichthys stellatus* Pallas) were collected periodically by bottom trawling in Eastsound, Orcas Island, and were kept in 8 ft (2.5 m) diameter concrete tanks without feeding until used. Weights of the experimental flounders ranged between 1175 and 1475 g. Fish to be tested were anesthetized in MS-222 at a concentration of 0.1 g/l seawater, and were placed on a net platform suspended over a tub containing anesthetic. A pump was used to continuously perfuse the fish's gills with the anesthetic solution. With the fish thus immobilized, the necessary surgical procedures involved in collecting samples of blood and water for oxygen measurements could be performed. A recovery period of 24 h was allowed before starting experimentation.

Measurements of Gill Water Flow

Gill water flow (Vg) was measured directly during all experiments. The technique consisted of cutting off the fingers of a disposable rubber glove, and forming a gasket on the glove with silicone rubber caulking which conformed to the fish's operculae. The glove was then sutured to the fish along the gasket, covering the gill openings on both the eyed and blind side, with the thumb serving as the outflow for both gills. Total flow of water past the gills was then collected and measured. Food-coloring dye was periodically injected into the glove via the fish's mouth, where an absence of dye leakage insured a perfect seal (Fig. 1).

When covering the operculae, care was taken to insure that opercular movement was not restricted. No restriction was apparent, as a wide range of ventilatory flows was measured.

Collection of Blood

The arterial and venous systems of several 1 to 1.5 kg *Platichthys stellatus* were injected with liquid silicone injection compound in order to make blood vessels easily visible to determine sampling sites. The ideal blood sampling sites for oxygen concentrations would have been (1) the dorsal aorta immediately efferent to the gills for arterial blood, and (2) the ventral aorta immediately afferent to the gills for mixed venous blood, as samples were to be used for analysis of oxygen transfer across the gills. Unfortunately, neither trial cannulations or dissections showed a method for inserting a chronic cannula directly into either of the above sites. Consequently, the caudal blood vessels were chosen as the most convenient site for cannulation. Subsequent dissection showed that tubing could be pushed almost to the gills via the caudal artery, and to the kidney via the caudal vein. The caudal vein thus provided the most representative collection of venous blood possible. Both Piiper and Baumgarten-Schumann (1968) and Itazawa (1970) obtained venous blood from both the downstream side of the heart and the caudal vein in the dogfish and in a variety of teleosts. Neither reported any significant differences between the two sampling sites.

PE-60 Polyethylene tubing filled with heparinized Cortland saline (Wolf, 1963) was inserted into the artery and vein through a lateral incision in the caudal peduncle. Each tube was pushed anteriorly until resistance was felt, then pulled back approximately 1 cm and finally checked for free flow. In this way, arterial samples were collected from the anterior dorsal aorta, and venous samples from the anterior end of the caudal vein. When there was any question of which vessel was being sampled, source of the samples was confirmed by visually comparing pressures in the cannulae; the artery would usually show a 6 to 10 cm blood pressure head over the vein.

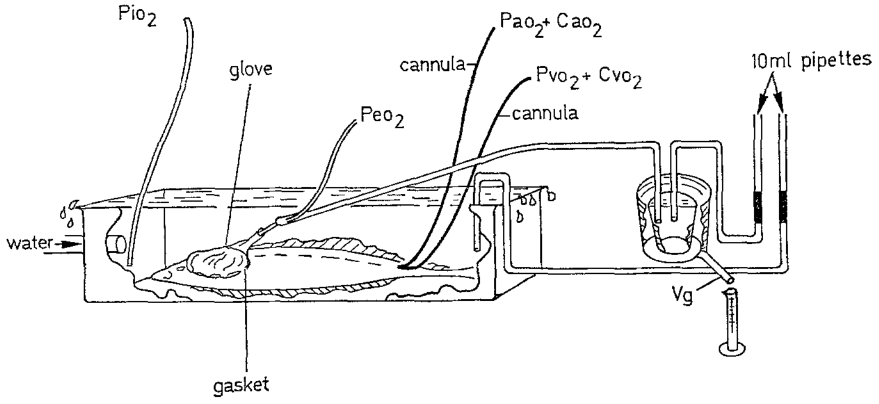

Fig. 1. Experimental design used in collection of samples from *Platichthys stellatus* (see Table 1 for symbols)

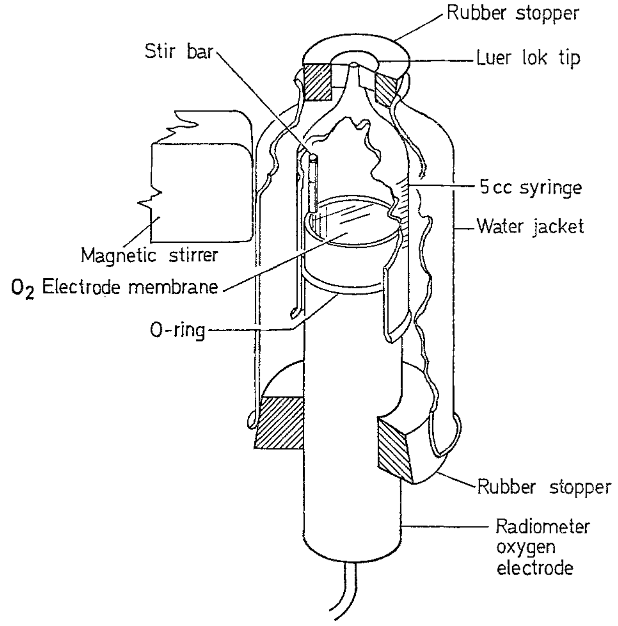

Fig. 2. Cuvette for determining blood-oxygen content

Experimental Procedure

All blood and water samples were analyzed for oxygen tension and content with a Radiometer acid-base analyzer type PHM71 and PO_2 module type PHA930. Blood oxygen contents were determined adapting a method described in Tucker (1967) and using a Radiometer model E 54046 oxygen electrode inserted into a cut-off 5 cm³ glass syringe (Fig. 2). This yielded a cuvette volume of approximately 1.98 ml. Blood sample volumes varied between 0.027 and 0.033 ml. The electrode was calibrated between samples with known gas mixtures.

Flounders were kept in a flat plexiglass box of about 20 l capacity during recovery from surgery and during the experimental period. The box was covered with black plastic sheeting to avoid any startled reactions, and blood and water samples were taken together at approximately 1 h intervals throughout the day, for periods up to 1 week, on each fish. Air and water temperatures were also noted at each sample period, and hemoglobin content was determined at least once a day by the cyanmethemoglobin method (Cannan, 1958). The blood oxygen capacity was calculated by multiplying hemoglobin content in g % by 1.34 (Torrance and Lenfant, 1969—1970). The hematocrit was also noted daily.

Partial pressures and contents of oxygen in water and blood were determined using the content cuvette as shown in Fig. 2. Samples of inspired and expired water were taken in 10 cm³ glass syringes, allowed to come to room temperature, and injected into the cuvette. Blood samples for oxygen content were taken at the same time by allowing blood to drip from the cannulae for a few seconds, and then filling a glass pipette of appropriate volume. Blood samples for oxygen tension were taken in a 2 cm³ syringe, injected into the cuvette, and then reinjected into the fish.

Oxygen tensions in the water were controlled by a stripping column. A pyrex tube 45 cm long and 6 cm in diameter was filled with glass marbles. Nitrogen was bubbled into the bottom of the column through an airstone, and water was pumped in at the top. In this fashion, a counter-current was established, and oxygen tension of the water collected at the bottom could be controlled over a wide range by varying nitrogen and water flows.

Water temperature was controlled by pumping water through a heat exchanger of glass tubing immersed in a thermostatically controlled water bath. A water flow of at least 2 l/min was established through the experimental chamber at all times.

Construction of Blood-Oxygen Dissociation Curves

The mixing technique described by Torrance and Lenfant (1969—1970) was used in producing blood-oxygen dissociation curves. In this technique, the amount of oxygen in a blood sample is estimated as percent saturation by mixing oxyhemoglobin and deoxyhemoglobin in known proportions. A simple tonometer was constructed using a small plexiglass box as a water jacket, with two 25 ml round-bottom flasks as equilibrators (Fig. 3). The tonometer was placed on an Eberbach rotating table, and nitrogen and air were bubbled through water into the flasks to obtain gases saturated with water vapor. This was necessary in order to prevent dehydration of blood samples. The water temperature in the jacket was monitored using a thermistor-type thermometer. Ten ml of blood were withdrawn from the fish and mixed with 0.5 ml 1000-unit heparin and 1.5 ml TRIS buffer. This was then divided into two 6 ml samples, one going into each 25 ml flask. The two samples were equilibrated, one with air and one with nitrogen, by passing humidi-

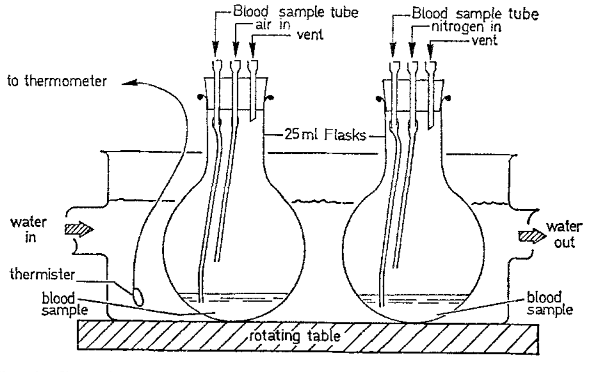

Fig. 3. Simple tonometer for equilibrating blood with air and nitrogen

fied gas through the flasks while shaking the tonometer on the rotating table. After approximately 2 h equilibration, a blood sample containing deoxygenated hemoglobin and a blood sample containing oxygenated hemoglobin was obtained. Oxygen tension and pH were then recorded on the two equilibrated samples that were 0 and 100% air saturated, and on three blood mixtures, 1:3, 1:1, and 3:1, corresponding to 25, 50, and 75% oxyhemoglobin. To make the mixtures, blood was taken from the flasks directly into a 1 cm^3 syringe in the desired proportions, and injected into the Radiometer PO_2 cuvette. After noting oxygen tension, pH was then taken on blood withdrawn from the cuvette. At the completion of a set of determinations, the temperature of the circulating water in the jacket was raised by 9° to 10 C°, and equilibration was continued for 1 h. Zero, 25, 50, 75, and 100% saturation points were again determined as above. The entire sequence was then repeated using fresh blood and TRIS buffer of a different pH. In this fashion, four curves were constructed, one for each of two temperatures and two pH's, and each experiment was replicated once. Plots were thus made of percent saturation versus partial pressure of oxygen to construct each dissociation curve.

Calculations

All oxygen tensions were temperature corrected, as sampling and measuring temperatures differed in most cases. By saturating blood and water samples with air at various temperatures and noting the oxygen tensions, it was found that the temperature correction for water applied to flounder blood as well.

To calculate the effectiveness of oxygen removal from water and uptake by blood, the equations of Piiper and Baumgarten-Schumann (1968) were used. Symbols used are shown in Table 1.

The equations are:

$$\frac{Veff}{Vg} = \frac{Ci - Ce}{Ci - Cveq} = Ew \text{ for water, and similarly,} \quad (1)$$

$$\frac{Qeff}{Qt} = \frac{Ca - Cv}{Cieq - Cv} = Eb \text{ for blood.} \quad (2)$$

Cardiac output, *Qt*, was calculated by the Fick principle, where:

$$Qt = \frac{Ci - Ce}{Ca - Cv} \cdot Vg. \quad (3)$$

Oxygen consumption, *Vo*, was calculated as follows:

$$Vo = (Ci - Ce) \cdot Vg. \quad (4)$$

All of the above operations were performed on a CDC 6400 series computer using BASIC language.

Ci and *Ce* were calculated from Pio_2 and Peo_2 by referring to an oxygen dissociation curve for water and to an oxygen solubility table, as was *Cveq*. *Cieq* was calculated by referring to the appropriate blood dissociation curve, and multiplying the percent saturation thus obtained by the oxygen capacity of the blood. $\Delta\ Pg$, the mean difference of oxygen tension across the gills, was calculated by the following formula:

$$\Delta\ Pg = \tfrac{1}{2}\,(Pio_2 + Peo_2) - \tfrac{1}{2}\,(Pao_2 + Pvo_2)\,. \quad (5)$$

Transfer factor, To_2, was defined by Randall *et al.* (1967) as:

$$To_2 = \frac{Vo}{\Delta\ Pg}\,. \quad (6)$$

Table 1. *Symbols used in calculations and text*

Symbol	Meaning	Units
Pio_2	Partial pressure of oxygen in inspired water	mm Hg
Peo_2	Partial pressure of oxygen in expired water	mm Hg
Ci	Content of oxygen in inspired water	ml O_2/100 ml H_2O
Ce	Content of oxygen in expired water	ml O_2/100 ml H_2O
Pao_2	Partial pressure of oxygen in arterial blood	mm Hg
Pvo_2	Partial pressure of oxygen in venous blood	mm Hg
Ca	Content of oxygen in arterial blood	ml O_2/100 ml blood
Cv	Content of oxygen in venous blood	ml O_2/100 ml blood
Cieq	Content of O_2 in blood in complete equilibrium with inspired water	ml O_2/100 ml blood
Cveq	Content of O_2 in water in complete equilibrium with venous blood	ml O_2/100 ml H_2O
Veff	Flow of water over gills in complete exchange with blood	ml/min/kg
Vg	Total flow of water over the gills	ml/min/kg
Qeff	Flow of blood through gills in complete exchange with water	ml/min/kg
Qt	Total flow of blood through gills	ml/min/kg
Ew	Effectiveness of removal of oxygen from water	
Eb	Effectiveness of uptake of oxygen by blood	
Vo	Total oxygen uptake of fish	ml O_2/min/kg
$\Delta\ Pg$	Mean partial pressure difference of oxygen across gills	mm Hg
To_2	Transfer factor	(ml/min)/(mm Hg·kg)
V/Q	Ventilation-perfusion ratio (ratio of water to blood flow in gills)	

All data for ventilation volume, cardiac output, and oxygen uptake were divided by weight for comparative purposes.

In making graphs, data was plotted as means ± one standard deviation. In all cases except Figs. 4, 6, 21, and 22, lines were drawn by regressions calculated through the means. In Figs. 4 and 21, the line was drawn arbitrarily.

Results

General

A total of 8 *Platichthys stellatus*, weighing an average of 1340.5 ± 120.5 g (mean ± standard deviation), were used in the study. Data used in making up the graphs that follow were obtained from averages of all 8 fish. Table 2 shows a summary of calculated data for each fish, broken down into 3 groups representing (1) low temperature and high oxygen tension; (2) low temperature and low oxygen tension; (3) high temperature and high oxygen tension.

Where temperature was plotted as an environmental factor, only low and high extremes were shown. Intermediate temperatures were not considered in order to present an adequate number of data points at the extremes, as the experimental objective was to measure respiratory effects of high temperature rather than changing temperature. Each high temperature mean represents between 12 and 13 observations.

Blood Factors

The hematocrit and hemoglobin content were measured daily in most fish. The hematocrit was found

Table 2. *Platichthys stellatus. Respiratory parameters for 8 starry flounders. (All values are means ± 1 standard deviation)*

No. of observations	Temperature (°C)	*Pio*$_2$ (mm Hg)	*Vg* (ml/min/kg)	*Qt* (ml/min/kg)	*V/Q*	*Vo* (ml O$_2$/min/kg)	*Ew*	*Eb*
	Fish # 1, 1425 g							
9	11	129 ± 8	173 ± 47	58 ± 29	3.50 ± 1.75	0.489 ± 0.312	0.792 ± 0.109	0.489 ± 0.201
8	11	65 ± 8	330 ± 37	35 ± 4	3.55 ± 1.47	0.338 ± 0.045	0.611 ± 0.062	0.577 ± 0.072
	Fish # 2, 1290 g							
5	9	126 ± 5	127 ± 10	33 ± 22	5.05 ± 2.37	0.277 ± 0.050	0.510 ± 0.039	0.776 ± 0.311
4	9	72 ± 12	173 ± 68	36 ± 17	6.19 ± 3.85	0.184 ± 0.045	0.516 ± 0.145	0.667 ± 0.436
5	16.6 ± 2.3	143 ± 7	224 ± 74	49 ± 15	4.66 ± 1.05	0.454 ± 0.163	0.464 ± 0.057	0.479 ± 0.089
	Fish # 3, 1268 g							
10	9.5	128 ± 13	110 ± 20	45 ± 14	2.72 ± 1.12	0.398 ± 0.074	0.946 ± 0.049	0.560 ± 0.270
6	9	60 ± 14	251 ± 43	42 ± 14	6.21 ± 1.56	0.376 ± 0.056	0.841 ± 0.059	0.347 ± 0.071
8	17.8 ± 2.7	153 ± 7	199 ± 34	56 ± 24	3.98 ± 1.34	0.741 ± 0.099	0.803 ± 0.081	0.735 ± 0.186
	Fish # 4, 1475 g							
8	10.2	133 ± 2	176 ± 47	44 ± 9	4.45 ± 2.80	0.529 ± 0.092	0.804 ± 0.156	0.655 ± 0.234
6	11.5	58 ± 15	423 ± 78	40 ± 4	10.77 ± 2.02	0.368 ± 0.057	0.610 ± 0.123	0.406 ± 0.143
	Fish # 5, 1175 g							
5	11.2	125 ± 5	162 ± 9	88 ± 44	2.08 ± 0.621	0.380 ± 0.022	0.721 ± 0.031	0.637 ± 0.310
5	11.5	61 ± 17	538 ± 212	50 ± 14	11.24 ± 5.69	0.354 ± 0.021	0.506 ± 0.141	0.602 ± 0.159
6	20 ± 1	155 ± 4	511 ± 73	103 ± 16	5.04 ± 0.98	0.979 ± 0.057	0.439 ± 0.053	0.560 ± 0.135
	Fish # 6, 1450 g							
5	11.8	125 ± 3	90 ± 19	17 ± 5	5.46 ± 1.08	0.195 ± 0.051	0.641 ± 0.125	0.367 ± 0.099
6	19.5 ± 0.5	143 ± 4	390 ± 78	98 ± 29	4.45 ± 2.14	1.062 ± 0.164	0.676 ± 0.053	0.502 ± 0.246
	Fish # 7, 1200 g							
7	11.4	124 ± 7	80 ± 35	27 ± 26	4.57 ± 2.94	0.244 ± 0.119	0.933 ± 0.101	0.504 ± 0.147
6	19.8	148 ± 6	366 ± 30	70 ± 20	5.66 ± 1.86	0.849 ± 0.053	0.632 ± 0.034	0.467 ± 0.115
	Fish # 8, 1440 g							
8	12.3	120 ± 5	214 ± 41	49 ± 16	4.74 ± 1.99	0.613 ± 0.112	0.927 ± 0.090	0.861 ± 0.156
4	13	58 ± 8	466 ± 39	47 ± 7	10.26 ± 2.35	0.516 ± 0.070	0.991 ± 0.018	0.838 ± 0.101
6	19.8	143 ± 6	394 ± 19	103 ± 17	3.91 ± 813	1.023 ± 0.067	0.673 ± 0.041	0.726 ± 0.130

to range between 6 and 22%, with a mean of 13.7%. and the hemoglobin content ranged between 2.4 and 5.7 g %, with a mean of 3.98 g %. From hemoglobin, blood oxygen capacity was calculated, and varied between 3.216 and 7.638 volume %, with a mean of 5.352 volume %. These values were at their highest at the onset of experimentation on a fish, and declined throughout the experimental period.

Exactly why the hematocrit and hemoglobin content of flounder blood declined during experimentation is unknown, although it is expected that it was a response to the stress of handling, operating, and blood sampling, even though very small quantities of blood were withdrawn. A flounder with only an arterial cannula, not subjected to stress, did not show such a decline upon periodic sampling. Changes in blood oxygen capacity with decreasing hemoglobin were calculated daily in all fish. Cameron and Davis (1970) showed that the only effect of decreasing blood oxygen capacity in the rainbow trout *Salmo gairdnerii* was an increased cardiac output.

Six samples of arterial blood and 5 samples of venous blood were taken at the end of the study to determine pH. Mean pH of arterial blood was found to be 7.685 ± 0.045, and mean pH of venous blood 7.544 ± 0.085. These samples were all taken at a temperature of about 12 °C, and pH was immediately noted at the same temperature.

Blood Oxygen Tensions and Saturations

Blood oxygen tensions were noted for a total of 82 observations in 6 fish. For each tension, an appropriate percent saturation was found using the blood-oxygen dissociation curve. The high pH curves were used for arterial blood, and the low pH curves were used for venous blood. Mean values are shown in Table 3, together with water temperatures and oxygen tensions.

Ventilation Volume (Vg)

Direct measurement of ventilation volumes were made on all fish. A reduction in oxygen tension produced a sharp rise in water flow over the gills (Fig. 4). Even though there was considerable variability between fish and in individual fish (Table 2), mean ventilation volume increased about 3-fold during lowered oxygen tension, from 150 to 450 ml/min/kg. An increase in temperature at a fixed oxygen tension also produced a 3-fold increase in ventilation (Fig. 5).

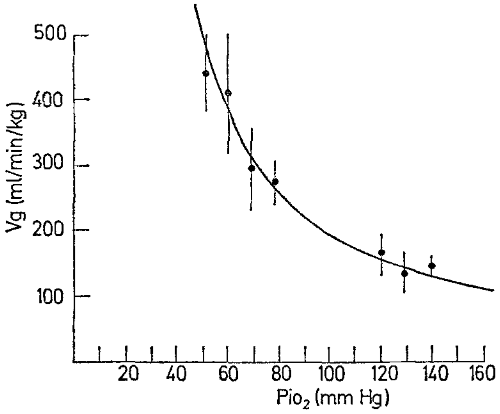

Fig. 4. *Platichthys stellatus.* Effect of decreasing environmental oxygen tension on ventilation volume. All points are shown ± 1 standard deviation

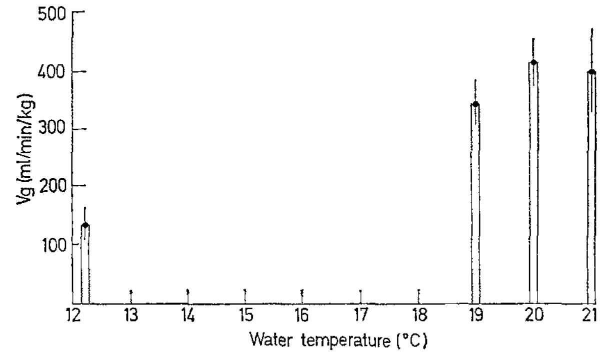

Fig. 5. *Platichthys stellatus.* Effect of increasing temperature on ventilation volume. All points are shown ± 1 standard deviation

Table 3. *Platichthys stellatus. Mean arterio-venous oxygen tensions, % saturation (S), and transfer factors. (All values ± 1 standard deviation)*

No. of observations	Water temperature (°C)	Pio_2 Peo_2 (mm Hg)	Pao_2 (mm Hg)	Arterial %S	Pvo_2 (mm Hg)	Venous %S	To_2
36	11.4 ± 1.1	126.3 ± 5.8 54.2 ± 14.5	75.5 ± 17.9	88.6 ± 4.6	42.9 ± 7.3	72.1 ± 5.2	0.0288 ± 0.0571
15	11.7 ± 1.1	59.0 ± 13.7 35.7 ± 7.1	41.3 ± 14.9	75.2 ± 9.6	23.9 ± 6.8	52.6 ± 10.8	0.0471 ± 0.0554
31	19.4 ± 1.5	148.5 ± 7.3 71.8 ± 7.8	62.3 ± 13.9	85.6 ± 5.7	29.8 ± 8.4	54.5 ± 10.0	0.0149 ± 0.0030

Gill water flow increased with increasing oxygen uptake. Considerable similarity was noted between the rate of increase in gill ventilation and the rate of increase of cardiac output with increasing oxygen uptake (Fig. 6). The disparity at low uptakes might be explained by the fact that at low oxygen tensions the fish's oxygen consumption was depressed as ventilation volume increased, whereas at high temperatures both consumption and ventilation increased.

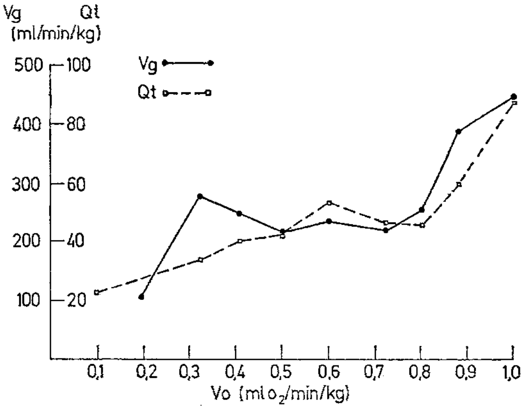

Fig. 6. *Platichthys stellatus*. Changes in ventilation volume and cardiac output with oxygen uptake

Platichthys stellatus has a functional opening between the upper and lower gill chamber, which is used to route all of the expired water out through the upper chamber during normal respiration (Yadzani and Alexander, 1967). This was confirmed in this study by injecting food-coloring dye into the buccal cavity of quiescent fish, and observing the lower opercle through a plexiglas sheet. It was also noted that water was expelled from the lower gill chamber during periods of high ventilatory flow, generally greater than 150 ml/min/kg.

Cardiac Output (Qt)

Table 2 shows a slight drop in cardiac output in individual fish with decreased oxygen tension, which has been masked by averaging the data for Fig. 7. This result is to be expected, as an increase in cardiac output takes place with increasing oxygen consumption (Fig. 6), and consumption is lowered with decreased oxygen tension (Fig. 12).

However, an increase in temperature appeared to produce an increase in cardiac output (Fig. 8). Blood flow was approximately doubled with a 10 C° rise in temperature. Cardiac output also rose with an increase in ventilation volume, although considerable variation was noted (Fig. 9). The variation was, in part, the result of decreased blood flow with increased ventilation during periods of lowered oxygen tension.

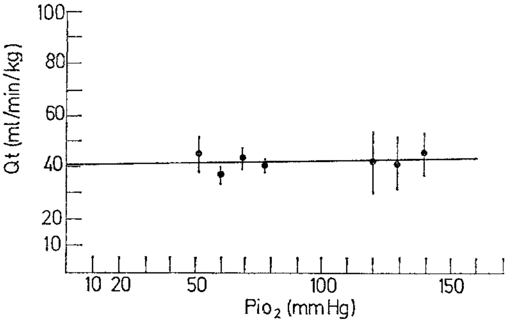

Fig. 7. *Platichthys stellatus*. Effect of decreasing environmental oxygen tension on cardiac output. All points are shown ± 1 standard deviation

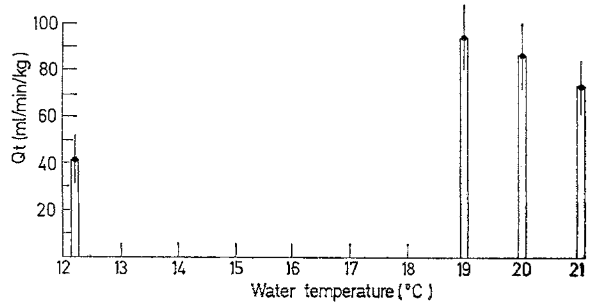

Fig. 8. *Platichthys stellatus*. Effect of increasing temperature on cardiac output. All points are shown ± 1 standard deviation

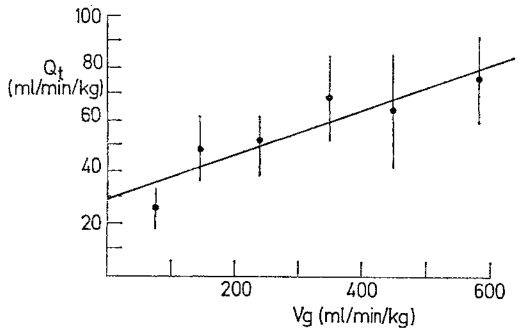

Fig. 9. *Platichthys stellatus*. Changes in cardiac output with ventilation volume. All points are shown ± 1 standard deviation

Ventilation-Perfusion Ratio (V/Q)

From the foregoing data, ventilation-perfusion ratios, or ratios of water to blood flow in the gills were calculated on all fish. The effects of lowered oxygen and increased temperature on V/Q were much as expected, with a linear increase in the ratio with lowered oxygen (Fig. 10), and virtually no effect in elevated temperature (Fig. 11).

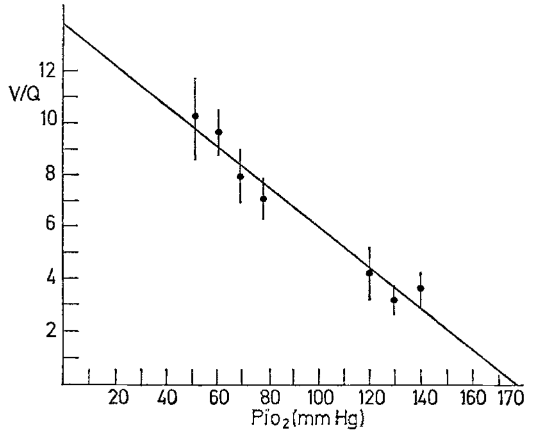

Fig. 10. *Platichthys stellatus.* Effect of decreasing oxygen tension on ventilation-perfusion ratio. All points are shown ± 1 standard deviation

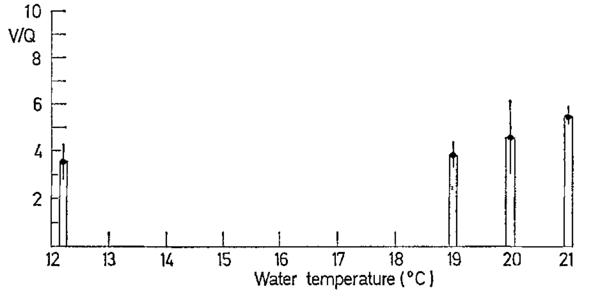

Fig. 11. *Platichthys stellatus.* Effect of increasing temperature on ventilation-perfusion ratio. All points are shown ± 1 standard deviation

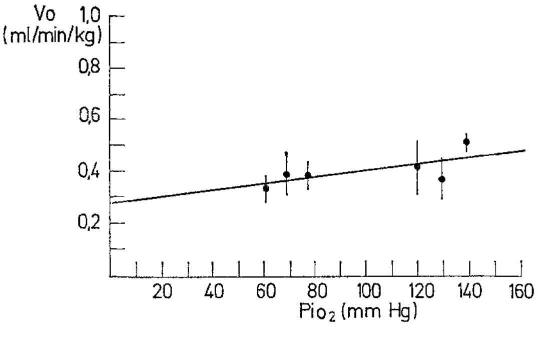

Fig. 12. *Platichthys stellatus.* Effect of reduced oxygen tension on oxygen uptake. All points are shown ± 1 standard deviation

Oxygen Uptake (VO)

A considerable range of oxygen consumptions was noted with varying oxygen tensions and temperatures. Even though gill ventilation increased markedly with lowered oxygen tension, consumption appeared to decrease slightly (Fig. 12). A two-fold increase in consumption was noted with an increase in temperature of from 12° to 21 °C (Fig. 13).

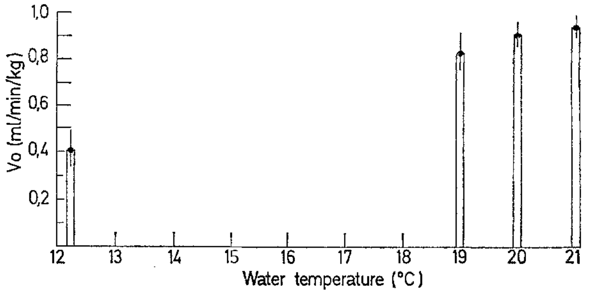

Fig. 13. *Platichthys stellatus.* Effect of increasing temperature on oxygen uptake. All points are shown ± 1 standard deviation

Effectiveness of Oxygen Removal from Water (Ew)

The effectiveness of removal of oxygen from water was calculated as the ratio of effective water flow past the gills (*Veff*) to total ventilation volume (*Vg*). The effectiveness declined with decreasing oxygen tension and increasing temperature (Figs. 14 and 15). The effectiveness also dropped with increasing ventilation volume (Fig. 16) and increasing ventilation-perfusion ratio (Fig. 17), since at higher *Vg*'s and hence at higher *V*/*Q*'s, relatively higher volumes of water were shunted past the gills.

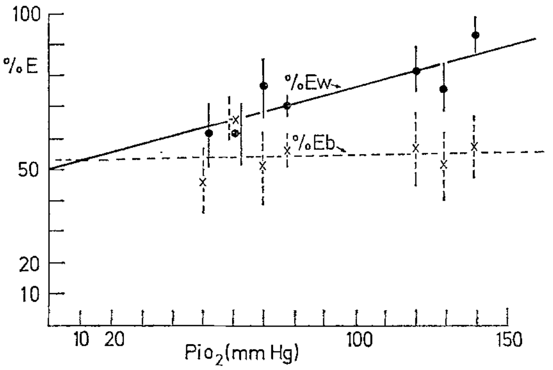

Fig. 14. *Platichthys stellatus.* Effect of decreasing oxygen tension on effectiveness of oxygen removal from water and oxygen uptake by blood. All points are shown ± 1 standard deviation

Effectiveness of Oxygen Uptake by Blood (Eb)

Similarly to the above, effectiveness of oxygen uptake by blood was calculated as the ratio of effective blood flow through the gills (*Qeff*) to cardiac output

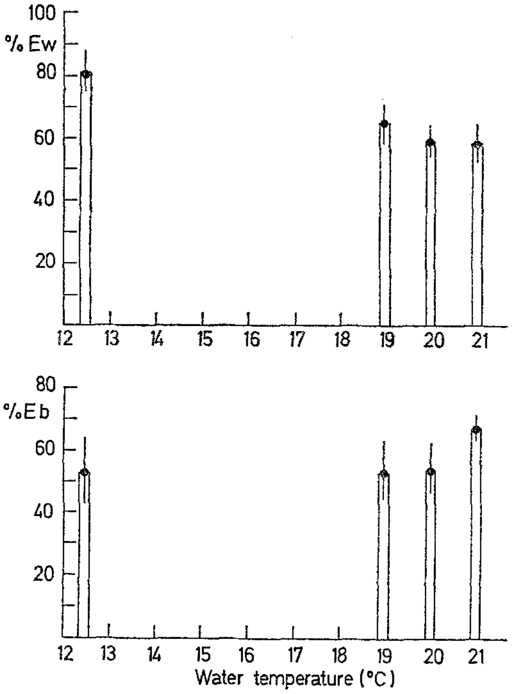

Fig. 15. *Platichthys stellatus.* Effect of increasing temperature on effectiveness of oxygen removal from water and uptake by blood. All points are shown ±1 standard deviation

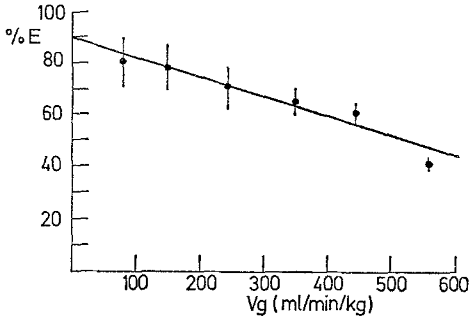

Fig. 16. *Platichthys stellatus.* Changes in effectiveness of oxygen removal from water with ventilation volume. All points are shown ±1 standard deviation

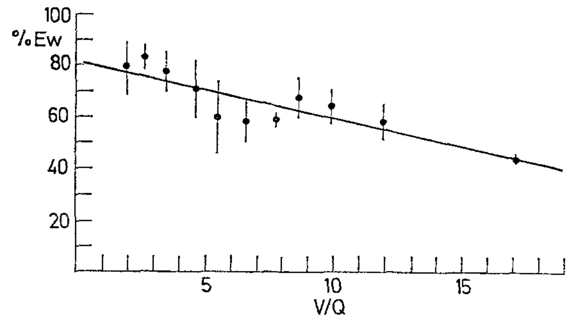

Fig. 17. *Platichthys stellatus.* Changes in effectiveness of oxygen removal from water with ventilation-perfusion ratio. All points are shown ± 1 standard deviation

(Qt). Although there was wide variation in values within individual fish as well as between fish, a remarkable constancy of Eb was noted in response to environmental changes (Figs. 14 and 15), cardiac output (Fig. 18), and V/Q (Fig. 19). Most means were between 0.5 and 0.6 regardless of oxygen tension, temperature, cardiac output, or V/Q.

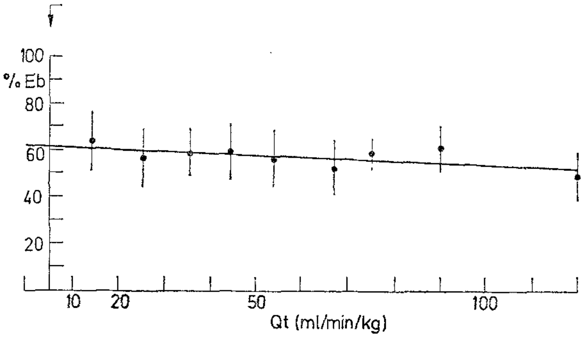

Fig. 18. *Platichthys stellatus.* Changes in effectiveness of oxygen uptake by blood with cardiac output. All points shown are ± 1 standard deviation

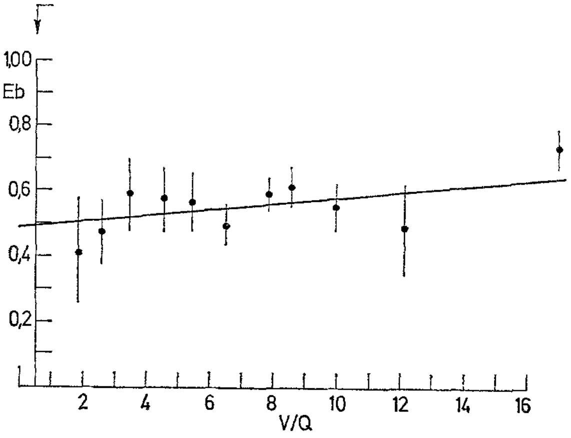

Fig. 19. *Platichthys stellatus.* Changes in effectiveness of oxygen uptake by blood with ventilation-perfusion ratio. All points are shown ± 1 standard deviation

Mean Oxygen Tension Difference between Blood and Water (Δ Pg)

Mean oxygen tension differences were calculated for 6 fish. Δ Pg declined with lowered oxygen tensions. Too few points were obtained to confidently draw a meaningful line, but the data were important in describing transfer factors. Mean difference rose with an increase in temperature as shown in Fig. 20.

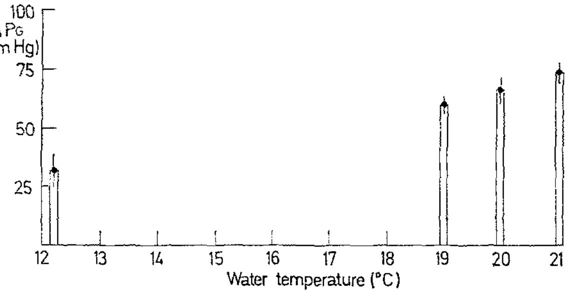

Fig. 20. *Platichthys stellatus*. Effect of increasing temperature on mean partial pressure difference of oxygen across gills. All points are shown ± 1 standard deviation

Transfer Factor (To_2)

The transfer factor, a measure of the relative ability of the respiratory surface to exchange gases, was calculated for 6 fish and averaged into three groups: (1) high oxygen, low temperature; (2) low oxygen, low temperature; (3) high oxygen, high temperature. As can be seen in Table 3, the highest transfer factor, or lowest gill diffusion resistance, was seen in low oxygen, and the lowest in high temperature.

Inspired versus Expired Water

A graph was made relating oxygen tensions of expired and inspired water in both *Salmo gairdnerii* (from Holeton and Randall, 1967) and *Platichthys stellatus* (Fig. 21). As environmental oxygen decreased, an initial increase was seen in expired tension, followed by a decrease. However, mean expired tensions in the flounder were in all cases below 60 mm Hg.

Arterial oxygen tensions were found to be considerably higher than those of expired water in periods of high and low oxygen tension and low temperature, indicating efficient exchange in a counter-current system (Steen, 1971). In elevated temperatures, arterial tension was lower than expired water tension (Table 3).

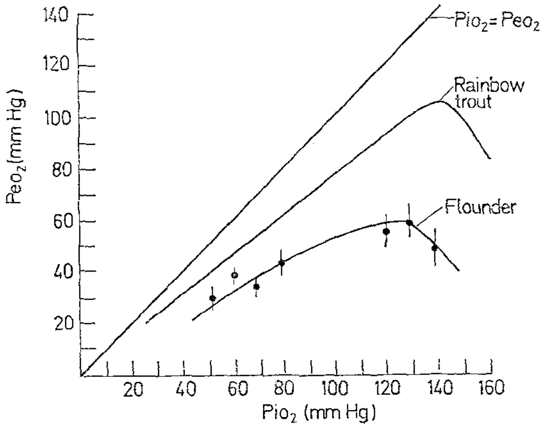

Fig. 21. Relationship of inspired and expired water in flounder *Platichthys stellatus* and rainbow trout *Salmo gairdnerii*. (Trout curve from Holeton and Randall, 1967)

Blood-Oxygen Dissociation Curve

Table 4 and Fig. 22 show a summary of values for 4 blood oxygen dissociation curves in *Platichthys stellatus*: (1) low temperature, high pH; (2) high temperature, high pH; (3) low temperature, low pH; (4) high temperature, low pH. These values illustrate both the temperature and Bohr shifts for oxygen loading of flounder blood. P_{50}'s were, respectively, (1) 15.3 mm, (2) 18.3 mm, (3) 20.1 mm, and (4) 24.6 mm. Two separate determinations were done in making each curve.

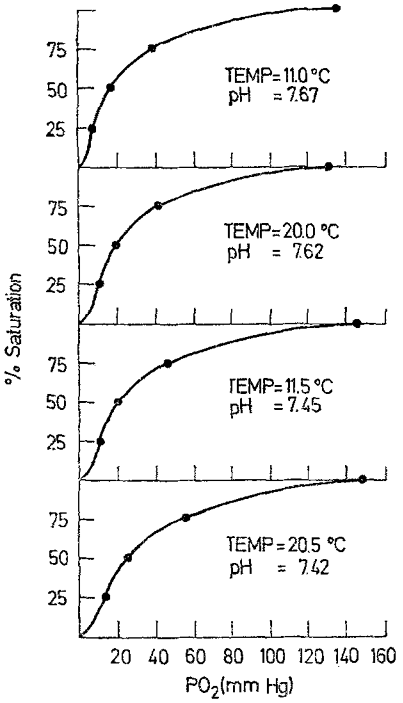

Fig. 22. *Platichthys stellatus*. Blood-oxygen dissociation curves at 2 temperatures and 2 pH's

Discussion

General

Flounders (*Platichthys stellatus*) were chosen as the experimental animals, as they present an obvious degree of adaptation to their environment and are often found in extreme temperatures and oxygen levels (personal observation). They also survive well in captivity and are able to tolerate long periods of starvation.

Table 4. *Platichthys stellatus. Values for blood oxygen dissociation curve. (mm Hg ± 1 standard deviation)* (*N* = 2)

Saturation (%)	Temperature = 11.0 ± 0 °C pH = 7.67 ± 0.034	Temperature = 20.0 ± 0 °C pH = 7.62 ± 0.047	Temperature = 11.5 ± 0.527 °C pH = 7.45 ± 0.040	Temperature = 20.5 ± 0.527 °C pH = 7.42 ± 0.042
100	132.8 ± 1.2	130.2 ± 6.6	144.6 ± 16.1	148.0 ± 7.9
75	37.0 ± 0.4	39.9 ± 2.7	46.0 ± 0.2	54.2 ± 4.3
50	15.3 ± 2.1	18.3 ± 0.9	20.0 ± 1.9	24.6 ± 0.6
25	7.5 ± 1.5	9.5 ± 1.3	10.2 ± 2.3	12.0 ± 0.5
0	0	0	0	0

Tucker's (1967) method of directly determining blood oxygen content permits more accurate and efficient determinations of cardiac output by the Fick principle than by using blood oxygen tensions. Both Holeton and Randall (1967) and Stevens and Randall (1967) made Fick principle estimates of cardiac output using partial pressures of blood oxygen. Holeton and Randall stated that their estimates of cardiac output were indicative of an order of magnitude rather than exact measurement. Stevens and Randall indicated that the accuracy of the values for cardiac output depended largely on estimates for percentage saturation of venous blood. Direct measurements of blood oxygen content in this study rather than tension eliminated many uncertainties in calculation of cardiac output.

A direct method of measuring gill-water ventilation volume was also used for purposes of accuracy. Davis and Watters (1970) pointed out that considerable variability of results is often encountered in the use of cannulae for obtaining post-gill water samples. Stevens and Randall (1967) showed a maximum ventilation volume calculated by the Fick principle of about 10 l/min/kg in exercising the rainbow trout *Salmo gairdnerii*, a figure that seems quite high. Accurate measurement of post-gill oxygen tensions and gill-water flows were central in the calculation of most parameters in this study.

Piiper and Baumgarten-Schumann's (1968) model of effectiveness of gas exchange in gills was deemed particularly suitable to this work, as it permitted a description of the ratio of effective to total fluid flow past the gills. They based their model on the premise that gas exchange is almost never at 100% effectiveness in gills, and proposed two reasons for this: (1) Either part of the respired water and blood flowing through the gills may not come into close enough contact to permit gas exchange, or (2) resistance to gas diffusion between blood and water in the gills is not infinitely low. To examine both possibilities in the flounder, transfer factor as well as effectiveness of gas exchange was calculated.

Responses to Hypoxia

A fish may deal with water of low oxygen content in several ways. If the prime objective is maintenance of arterial oxygen saturation and oxygen uptake, the fish may increase water flow over the gills or increase blood flow through the gills in an effort to obtain more oxygen. Alternatively, the fish may be able to increase transfer efficiency by presenting a greater surface area for gas exchange, or by lowering diffusion resistance.

In a low oxygen environment, oxygen uptake appeared to be the limiting factor in *Platichthys stellatus*. The fish may be able to increase uptake slightly by pumping more water over the gills, but at a greatly increased cost of energy. The cardiac pump appeared to be well governed; evidently the energy cost of increased cardiac output was too high. From Fig. 4 it can be seen that a small drop in oxygen tension below 70 to 80 mm Hg produced a large increase in ventilation and, therefore, an increase in cost. One would expect, therefore, that progressive anaerobic metabolism might occur down to some point below 50 mm Hg (Beamish, 1964), where metabolic collapse would take place due to the inability of the respiratory pump to supply oxygen for its increasing demands. Indeed, Holeton and Randall (1967) and Smith *et al.* (1971) showed clear evidence of anaerobiosis in salmonids in an hypoxic environment.

The data obtained on starry flounder seem to agree fairly well with work on other fish. Jones *et al.* (1970) predicted, on the basis of their model of oxygen transfer in fish, that oxygen consumption and arterial oxygen content could be maintained during a fall in environmental oxygen by an increase in gill water flow, a reduction in mean partial pressure difference across the gills, and a reduction in venous oxygen tension. Even though the flounders were not able to maintain arterial oxygen content at its highest level (Table 3), they performed as predicted in other respects.

Several other investigators noticed an increase in ventilation with low oxygen levels, followed by a decrease that may signify respiratory collapse. Holeton and Randall (1967) observed an increase in gill water flow in rainbow trout down to about 40 mm Hg O_2, followed by a decrease. Marvin and Heath (1968), working on catfish, trout, and bluegill, noted a rise in breathing rate with a decline in oxygen to about 50% environmental saturation, or 80 mm Hg O_2, followed by a progressive fall to apnea at much lower tensions. They reported complete respiratory collapse in trout at about 25% oxygen saturation, or 40 mm Hg O_2. It would seem that a reduction in ventilation rate at lowered oxygen levels in these fish signified an inability to sustain activity rather than an effort to adapt to lower oxygen. Although no decline in respiratory flow was seen in the flounder down to 50 mm Hg O_2, a sudden drop in ventilation would be expected at an oxygen tension not far below that level, as uptake did not increase to meet the rapidly increasing ventilatory expense. The cost of ventilation alone in an active fish was shown to be as high as 69% of the total metabolism (Shelton, 1970).

Both Holeton and Randall (1967) and Marvin and Heath (1968) noted a decreased heart rate (bradycardia) during hypoxia in fishes. However, Holeton and Randall found no significant change in cardiac output with the bradycardia, indicating an increased stroke volume. They hypothesized that a changing pattern of blood flow in the gills may augment gas exchange, permitting an increased residence time for blood. Bradycardia in hypoxia was shown to be due to increased vagal inhibition of the heart, and not to be directly the result of oxygen lack (Randall, 1966). Marvin and Heath (1968) estimated that the energy required by fish heart-muscle could account for 25% of the metabolic demand of the resting animal. Therefore, it is possible that, by slowing the heart and increasing stroke volume, the fish is attempting to utilize less energy to pump blood through the gills.

Oxygen uptake in *Platichthys stellatus* exposed to an hypoxic environment remained fairly steady down to an environmental tension of 50 mm Hg O_2. This is in contrast to the findings of Marvin and Heath (1968), who reported that oxygen uptake of trout fell off quite sharply below a tension of about 96 mm Hg O_2, oxygen consumption of bluegill declined at a tension of 120 mm Hg O_2, and uptake of catfish was depressed immediately upon lowering environmental oxygen. Clearly, then, the flounder was able to regulate its uptake to a considerable degree, and did not become respiratory dependent until an oxygen tension of below 50 mm Hg was reached (critical pressure), at which point consumption would be expected to fall steadily to zero (Basu, 1959; Prosser and Brown, 1961). The changeover from independent to dependent respiration may be mediated by the increasing ventilation volume. At the point where the respiratory pump uses all of the oxygen made available by its increased action, consumption will start to fall (Hughes 1964).

Fry (1957) stated that the major limitation producing respiratory dependence in fishes is the volume of water that can be pumped across the gills by the ventilatory apparatus, and not the affinity of hemoglobin for oxygen, as a decrease in uptake with lower oxygen levels occurs at a partial pressure of oxygen higher than that necessary to saturate the hemoglobin. This did not appear to be the case with the flounder, as the dissociation curve showed the hemoglobin to be 80% saturated at a PO_2 of 50 mm Hg, where uptake had not fallen off significantly. However, it would appear that the fish was rapidly approaching its limit of gill ventilation at that concentration of oxygen (Fig. 4), so possibly both ventilation limits and blood oxygen affinity play a part in determining the point of respiratory dependence in the flounder.

Comparison of average oxygen-uptake figures in the present study with those of Hickman (1959) for starry flounder show fair agreement. Hickman reported a consumption of about 0.07 mg O_2/g/h for fish weighing between 10 and 300 g, at a temperature of 15 °C and salinity of 25‰. This converts to 0.822 ml O_2/min/kg, compared with an average of 0.404 ml O_2/min/kg in the present study for fish weighing between 1200 and 1475 g at 12 °C and 30‰. Hickman also reported a depression of metabolic rate with an increase in size, which may partly account for the disparity.

As pointed out by Piiper and Baumgarten-Schumann (1968), calculations of effectiveness based on their model assume maximum possible gas transfer across the gills, which requires not only complete equilibration of blood and water, but also a perfect counter-current arrangement of blood and water in the gills. A comparison of expired water and arterial blood oxygen tensions (Table 3) leaves little doubt that a functional counter-current arrangement of some sort exists in the flounder, as mean arterial tensions were higher than mean expired water tensions in all cases except that of elevated temperature.

In looking at the data for effectiveness of oxygen removal from the water (*Ew*) and oxygen loading by blood (*Eb*), several facts stand out. *Ew*, although declining with lowering oxygen tensions, was generally higher than *Eb*. This is in contrast to the findings of Randall *et al.* (1967) who found *Eb* in the rainbow trout exposed to hypoxia to be generally higher than *Ew*. Similarly, Piiper and Baumgarten-Schumann (1968) and Hanson and Johansen (1970) found *Eb* to be always higher than *Ew* in the dogfish. This disparity may partly be due to the fact that the flounder is normally an inactive fish, in contrast to the trout and dogfish, and, therefore, could be expected to have developed a more efficient ventilatory pump. The flounder cannot depend on "ram-effect" to augment its respiratory flow and, therefore, must maintain a highly effective water flow past its gills.

Effectiveness of oxygen uptake by blood remained

Table 5. *Platichthys stellatus. Summary of reactions to oxygen and temperature stress*

Action	Reaction								
	Ventilation volume (Vg)	Cardiac output (Qt)	Ventilation-perfusion ratio (V/Q)	Oxygen uptake (Vo)	Effectiveness of O_2 removal (Ew)	Effectiveness of O_2 removal (Eb)	Transfer factor (To_2)	Arterial O_2 content (Ca)	Venous O_2 content (Cv)
Decrease Pio_2 126—62 mm	Sharp increase	Very slight decrease	Linear increase	Slight decrease	Linear decrease	No change	Increase	Decrease	Decrease
Increase temperature 11°—20 °C	Increase	Increase	Very slight increase	Linear increase	Decrease	No change	Decrease	Very slight decrease	Decrease
Increase Vo	Increase	Increase							
Increase Vg		Increase			Linear decrease				
Increase Qt						No change			
Increase V/Q					Decrease	Slight increase			

practically constant throughout the range of environmental oxygen tensions, as did cardiac output. This indicates that the flounder did not change the pattern of blood flow through its gills in hypoxia, even though the ability to regulate the amount of respiratory versus shunted blood flow in the gills has been demonstrated for several teleosts (Steen and Kruysse, 1964; Richards and Fromm, 1969). Indeed, Randall *et al.* (1967) found that *Eb* dropped markedly with decreasing oxygen tensions, even though cardiac output remained unchanged.

The fall in effectiveness of oxygen removal from water from about 90 to 60% with hypoxia was expected, as ventilatory volume rose sharply during this period, decreasing the residence time of water at the gills. Hanson and Johansen (1970) noted a fall in *Ew* in the dogfish *Squalus suckleyi* from 67 to 19% as ventilatory volume was raised from 89 to 867 ml/min/kg, and Davis and Cameron (1970) reported a fall from 45 to about 5% utilization with an increase in gill perfusion in the rainbow trout *Salmo gairdnerii*.

A summary of *Platichthys stellatus*' response to low oxygen is shown in Table 5. Evidently the fish was not entirely able to compensate for the environmental change, as both arterial oxygen content and oxygen uptake declined. As expected, ventilation volume rose, but cardiac output decreased slightly. The fish appeared to be able to increase transfer efficiency (Table 3), as evidenced by lowering the mean partial pressure difference across the gills. The greater difference between arterial and venous oxygen content may have contributed to this increase. However, more blood was not shunted to the gill surface, as the ratio of effective to total blood flow remained unchanged. Unloading of oxygen from the blood to the tissues was increased in hypoxia, as evidenced by lowered venous oxygen content. However, unloading was by no means complete, as venous blood remained more than half saturated.

Response to Temperature Increase

Jones *et al.* (1970) considered exercise in fishes as causing an increase in oxygen consumption at a constant level of environmental oxygen. An increase in consumption is also achieved by subjecting quiescent fish to an increase in environmental temperature. In fact, the responses of flounder to high temperature were similar to those of rainbow trout to exercise as reported by Stevens and Randall (1967). Not only was oxygen consumption elevated, but ventilation volume and cardiac output both increased. Arterio-venous oxygen content difference was also increased.

However, the diffusion resistance of the gills seemed to be increased by high temperature, whereas it was decreased in exercise. Transfer factor decreased in the flounder, as reflected by an increase in mean partial pressure difference across the gills (ΔPg), (Fig. 20), even though oxygen uptake was elevated. Jones *et al.* (1970), however, looking at the above data on trout, estimated that ΔPg should remain relatively unchanged during exercise, yielding an increase in transfer factor. In fact, Randall *et al.* (1967) noted an increase in transfer factor with exercise in trout. Of interest, however, is the fact that the partial pressure of oxygen in expired water of exercising trout did not increase despite an increase in ventilation volume. Consequently, *Ew* remained unchanged, as did ΔPg.

In the present study, however, oxygen tension of inspired water actually increased with temperature, as did that of expired water, even though the inspired-expired difference (% utilization) was relatively unchanged. Coupled with a slight decline in arterial tension, and a greater arterio-venous difference, mean partial pressure difference increased, yielding a lowered transfer factor.

Both Fry (1957) and Basu (1959) considered the effects of increasing temperature on fish in terms of "scope of activity", the difference between active and standard metabolism. In general, the higher the temperature, the lower the level of activity that can be maintained. One would expect, therefore, that the effect of an increase in temperature on the flounder, a relatively inactive fish, would be less severe than on a more active species, such as a trout or a salmon, as a flounder's normal metabolic level appears to be much closer to standard than to active.

From Table 5, then, it appeared that the main effect of elevated temperature on the flounder was simply an increase in oxygen uptake, or metabolic level, with concurrent increases in water and blood-flow rates. Fig. 11 shows that the ratio of water to blood flow remained fairly constant throughout the range of temperatures, allowing the fish to most efficiently oxygenate its blood (Hughes, 1964). The major difference between the effects of temperature and activity seemed to be an increase in diffusion resistance of the gills. It is probably due to this resistance that arterial oxygen tension had fallen below that of expired water, and not due to a failure of countercurrent flow.

Other Considerations

Although it seems that flounder respond generally in the same way to respiratory stress as do other fish, certain differences stand out. By comparison with rainbow trout and dogfish, it is obvious that the flounder was very effective at extracting oxygen from the water. On a comparison of inspired and expired water tensions (Fig. 21), the flounder clearly was able to expire water of a lower oxygen tension at all environmental PO_2's than was the trout. From a cursory examination of the data of Hanson and Johansen (1970), it would appear that the dogfish might be between the trout and flounder in effectiveness of oxygen removal. However, hypoxia was not a factor in that study, so no actual comparison could be made.

Another unusual feature of flounder respiratory response was the high venous oxygen reservoir. Table 3 shows that venous content in *Platichthys stellatus* dropped from 72 to 53% saturation in hypoxia and to 54% in temperature stress. This was in sharp contrast to trout, which lowered venous content from 67 to 73% saturation to a mere 3% in hypoxia (Holeton and Randall, 1967). The venous reservoir in flounder may either signify a high degree of resistance to hypoxia (Itazawa, 1970), or an inability to unload oxygen to the tissues below 50% saturation, accounting for the similarity of venous content in both oxygen and thermal stress.

Blood-Oxygen Dissociation Curves

The mixing technique has been well established as an accurate method for determining blood-oxygen dissocation curves (Edwards and Martin, 1966; Torrance and Lenfant, 1969—1970). The method is quite simple, and depends on equilibrating only two samples of blood to construct a single curve. However, complete saturation of the oxygenated sample is necessary, and complete removal of the oxygen from the reduced sample, while not absolutely necessary, is very helpful, as otherwise a correction must be applied for residual oxygen. Great care must be taken not to contaminate samples as they are being mixed.

The effects of CO_2 and temperature on fish blood-oxygen dissocation curves have been described by several authors (Fry, 1957; Black *et al.*, 1966a, b). CO_2 is generally present in venous blood in greater amounts than in arterial blood, hence the shape of arterial and venous dissociation curves differ. In the present study, where temperature was a factor, it was essential to assess the effects of temperature on the curves as well. As CO_2 was not available in the equilibrating gas mixtures, pH was used as an index of the Bohr shift, and two curves of different pH were determined at two temperatures (Table 4). Since atmospheric air and laboratory nitrogen were used as the equilibrating gases, PCO_2's of all samples were assumed to be zero. Two determinations were used for each curve to note variability. Mixed (pooled) blood samples were not used.

Results were much as expected, with a noticeable shift to the right (Bohr shift) with decreasing pH and increasing temperature. Of interest is the greater pH shift seen at higher temperature. This would presumably aid the fish in unloading oxygen to the tissues at high environmental temperatures. The pH's selected for the two curves were, at best, arbitrary, and may only indicate a general shape for arterial and venous bloods.

Summary

1. Oxygen tensions of water and blood afferent and efferent to the gills were measured, as well as oxygen contents of arterial and venous blood, in the starry flounder *Platichthys stellatus* Pallas subjected to low environmental oxygen and high temperature. Ventilation volume was measured directly by impounding expired water. A series of blood-oxygen dissociation curves were determined from mixtures of fully air-saturated and fully deoxygenated blood.

2. Fick principle cardiac output, ventilation-perfusion ratio, oxygen uptake, effectiveness of gas

exchange across the gills, and transfer factor of oxygen exchange were calculated during exposure to low environmental oxygen and high temperature.

3. The mean ventilation volume was found to be 141 ml/min/kg at a mean oxygen tension of 126 mm Hg and a mean temperature of 11 °C. This rose to a mean value of 363 ml/min/kg at a mean tension of 62 mm Hg at the same temperature. A mean ventilation volume of 347 ml/min/kg was noted at an average tension of 147 mm Hg and average temperature of 19 °C.

4. Mean cardiac output of 45 ml/min/kg was calculated at high oxygen tension and low temperature. A mean value of 42 ml/min/kg was noted at low tension and low temperature. An average output of 80 ml/min/kg was noted at high oxygen tension and high temperature.

5. The ratio of water to blood flow averaged 4.07 at high oxygen levels and low temperatures, and rose to 9.04 at low oxygen and low temperature. A mean ventilation-perfusion ratio of 4.62 was calculated at high oxygen tensions and high temperatures.

6. Mean oxygen uptake of 0.391 ml O_2/min/kg declined to an average of 0.356 ml O_2/min/kg in low oxygen, and rose to a mean of 0.851 ml O_2/min/kg at higher temperature.

7. Mean efficiency of oxygen removal from water was found to be 78.4% in high oxygen and low temperature, which declined to an average of 50.9% in low oxygen and to an average of 61.5% in high temperature.

8. Mean efficiency of oxygen uptake by blood was calculated to be 60.6% at high oxygen tension and low temperature. In low oxygen, mean efficiency was 57.3%, and in high temperature 57.8%.

9. Average transfer factor for exchange of oxygen across the gills was found to be 0.0288 (ml/min)/(mm Hg · kg) in high oxygen and low temperature conditions. This rose to a mean value of 0.0471 (ml/min)/(mm Hg · kg) in low oxygen and fell to a mean value of 0.0149 (ml/min)/(mm Hg · kg) in high temperature.

10. In a low oxygen environment, it appeared that *P. stellatus* attempted to maintain oxygen uptake (to oxygen regulate) by increasing ventilation volume while regulating cardiac output and decreasing gill diffusion resistance.

11. The starry flounder's response to elevated temperature was similar to that expected in exercise. Ventilation volume and cardiac output both increased in order to meet the increasing metabolic demand. However, diffusion resistance of the gills increased as well.

12. Flounder blood was found to be half saturated with oxygen at a partial pressure of 15.3 mm Hg when the blood temperature was 11 °C and blood pH was 7.67. The P_{50} increased to 18.3 mm Hg at a temperature of 20 °C. When blood pH was lowered to 7.45, P_{50} was 20.1 mm Hg at 11.5 °C and 24.6 mm Hg at 20.5 °C.

Acknowledgements. Many people were instrumental to the successful completion of this project, including Dr. R. L. Burgner, Director of the Fisheries Research Institute, Dr. R. L. Fernald, Director of the Friday Harbor Laboratories, Dr. R. T. Paine, Associate Professor of Zoology, Dr. K. Johansen, Professor of Zoology, Dr. J. Petersen, and Miss A. Henry, who did the illustrations.

Literature Cited

Basu, S. P.: Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Bd Can. **16**, 175—212 (1959).

Beamish, F. W. H.: Respiration of fish with special emphasis on standard oxygen consumption. III. Influence of oxygen. Can. J. Zool. **42**, 355—366 (1964).

— and P. S. Mookherjii: Respiration of fish with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, *Carassius auratus* L. Can. J. Zool. **42**, 161—175 (1964).

Belding, D. L.: The respiratory movements of fish as an indicator of a toxic environment. Trans. Am. Fish. Soc. **59**, 238—245 (1929).

Black, E. C.: The transport of oxygen by the blood of freshwater fish. Biol. Bull. mar. biol. Lab., Woods Hole **79**, 215—229 (1940).

—, D. Kirkpatrick and H. H. Tucker: Oxygen dissociation curves of the blood of brook trout (*S. fontinalis*) acclimated to summer and winter temperatures. J. Fish. Res. Bd Can. **23**, 1—13 (1966a).

—, H. H. Tucker and D. Kirkpatrick: Oxygen dissocation curves of the blood of Atlantic salmon (*S. salar*) acclimated to summer and winter temperatures. J. Fish. Res. Bd Can. **23**, 1187—1195 (1966b).

Cameron, J. N. and J. C. Davis: Gas exchange in rainbow trout with varying blood oxygen capacity. J. Fish. Res. Bd Can. **27**, 1069—1085 (1970).

Cannan, R. K.: Proposal for a certified standard for use in hemoglobinometry. Clin. Chem. **4**, 246—251 (1958).

Van Dam, L.: On the utilization and regulation of breathing in some aquatic animals. Ph. D. Dissertation, Groningen 1938.

Davis, J. C. and J. N. Cameron: Water flow and gas exchange at the gills of rainbow trout, *S. gairdnerii*. J. exp. Biol. **53**, 1—18 (1970).

— and K. W. Watters: Evaluation of opercular catheterization as a method for sampling water expired by fish. J. Fish. Res. Bd Can. **27**, 1627—1635 (1970).

Edwards, M. J. and R. J. Martin: Mixing technique for the oxygen-hemoglobin equilibrium and Bohr effect. J. appl. Physiol. **21**, 1898—1902 (1966).

Fry, F. E. J.: The aquatic respiration of fish. *In:* Physiology of fishes, pp 1—63. Ed. by M. E. Brown. New York: Academic Press 1957.

Hanson, D. and K. Johansen: Relationship of gill ventilation and perfusion in Pacific dogfish, *S. suckleyi*. J. Fish. Res. Bd Can. **27**, 551—564 (1970).

Hickman, C. P., Jr.: The osmoregulatory role of the thyroid gland in the starry flounder, *Platychthys stellatus*. Can. J. Zool. **37**, 997—1060 (1959).

Holeton, G. F. and D. J. Randall: The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. exp. Biol. **46**, 317—327 (1967).

Hughes, G. M.: Fish respiratory homeostasis. Symp. Soc. exp. Biol. **18**, 81—107 (1964).

Itazawa, Y.: Characteristics of respiration of fish considered from the arterio-venous difference of oxygen content. Bull. Jap. Soc. scient. Fish. **36**, 571—577 (1970).

Jones, D. R., D. J. Randall and G. M. Jarman: A graphical analysis of oxygen transfer in fish. Respiration Physiol. **10**, 285—298 (1970).

Knight, A. P.: The effects of polluted waters on fish life. Contr. Can. Biol. Fish. **22** A, 9—18 (1901).

Marvin, D. E. and A. G. Heath: Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of freshwater fish. Comp. Biochem. Physiol. **27**, 349—355 (1968).

Miller, B. S.: Life history observations on normal and tumor-bearing flathead sole in Eastsound, Orcas Island (Washington). Ph. D. Thesis, University of Washington, Seattle, Washington 1969.

Piiper, J. and D. Baumgarten-Schumann: Effectiveness of O_2 and CO_2 exchange in the gills of the dogfish, *Scyliorhinus stellaris*. Respiration Physiol. **5**, 338—349 (1968).

Powers, E. B. and L. M. Shipe: The rate of oxygen absorption by certain marine fishes as affected by oxygen content and carbon dioxide tension of the sea-water. Publs Puget Sound mar. biol. Stn **5**, 365—372 (1928).

Prosser, C. L. and F. A. Brown: Comparative animal physiology, 2nd ed. 688 pp. Philadelphia: W. B. Saunders Co. 1961.

Randall, D. J.: The nervous control of cardiac activity in the tench, *Tinca tinca*, and the goldfish, *Carassius auratus*. Physiol. Zoöl. **39**, 185—192 (1966).

—, G. F. Holeton and E. D. Stevens: The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. exp. Biol. **6**, 339—348 (1967).

— and G. Shelton: The effects of changes of environmental gas concentrations on the breathing and heart rate of a teleost fish. Comp. Biochem. Physiol. **7**, 169—174 (1963).

— and J. C. Smith: The regulation of cardiac activity in fish in an hypoxic environment. Physiol. Zoöl. **40**, 104—113 (1967).

Richards, B. D. and P. O. Fromm: Patterns of blood flow through filaments and lamellae of isolated-perfused rainbow trout gills. Comp. Biochem. Physiol. **29**, 1063—1070 (1969).

Shelton, G.: The regulation of breathing. *In:* Fish physiology, Vol. IV. Ed. by W. S. Hoar and D. J. Randall. London: Academic Press 1970.

Smith, L. S., J. B. Saddler, R. C. Cardwell, A. J. Mearns, H. M. Miles, T. W. Newcomb and K. W. Watters: Responses of teleost fish to environmental stress. Wat. Pollut. Control Res. Ser. 18050EBK02/71, Fed. Water Qual. Admin., Environ. Protection Agency, 114 pp. (1971).

Steen, J. B.: Comparative physiology of respiratory mechanisms, 182 pp. London: Academic Press 1971.

— and A. Kruysse: The respiratory function of teleostean gills. Comp. Biochem. Physiol. **12**, 127—142 (1964).

Stevens, E. D. and D. J. Randall: Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. J. exp. Biol. **46**, 329—337 (1967).

Torrance, J. D. and C. Lenfant: Methods for determination of oxygen dissociation curves, including Bohr effect. Respiration Physiol. **8**, 127—136 (1969—1970).

Tucker, V. A.: Method for oxygen content and dissociation curves on microliter blood samples. J. appl. Physiol. **23**, 410—414 (1967).

Wolf, K.: Physiological salines for freshwater teleosts. Progve Fish Cult. **25**, 135—140 (1963).

Yazdani, G. M. and R. M. Alexander: Respiratory currents of flatfish. Nature, Lond. **213**, 96—97 (1967).

First author's address: Dr. K. W. Watters, Jr.
Puerto Rico Nuclear Center
College Station, Mayaguez
Puerto Rico 00708

Date of final manuscript acceptance: October 27, 1972. Communicated by J. Bunt, Miami