THE UNIVERSITY OF MANITOBA

ADAPTIVE RESPONSES TO HYPOXIA IN THE FATHEAD MINNOW *PIMEPHALES PROMELAS*: BUOYANCY CONTROL AND UPTAKE OF AQUATIC AND AERIAL O_2

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

April, 1980

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ABSTRACT

Fathead minnows (Pimephales promelas) were exposed to hypoxia to determine changes in swimbladder gas composition, buoyancy, standard volume and internal pressure. Percentages of CO_2, O_2 and N_2 in the swimbladder did not change over 24 h in normoxic water. Evidence for gulping of air into the swimbladder, combined with uptake of O_2 during the first 12 h of exposure to hypoxia was indicated by: 1) increases in buoyancy and standard volume at the dissolved O_2 level of 0.5 ppm and 2) uptake of 31% of metabolic O_2 requirement directly from air (respirometer experiment). Declines in buoyancy, standard volume and percent swimbladder O_2 after 12 h of exposure to hypoxic water (0.5 ppm) suggested that gulping of air could no longer be sustained at previous levels. Uptake of aerial O_2 appeared to be a temporary and limited means of supplementing aquatic respiration during hypoxia. A model depicting the sequence of hypothesized and observed events leading to uptake of aerial O_2 and subsequent decline with prolonged exposure to hypoxia ($\leq$ 1.0 ppm) was constructed.

ACKNOWLEDGEMENTS

Sincere thanks to my supervisor Dr. Gee, for his patience and advise during the course of this study. To my committee members: Dr. Dandy, Dr. Rauch and Dr. Halvorson, my gratitude for their comments and suggestions in preparing this manuscript. This work was financially supported by the Natural Sciences and Engineering Research Council of Canada.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (continued)

INTRODUCTION

The ability to exploit residual and alternative sources of O_2 in hypoxic water is of critical survival value to fishes inhabiting shallow ponds and streams. Many species possess morphological, physiological and behavioural adaptations for reducing O_2 demand and utilizing low levels of dissolved O_2 in a water column. When this source of O_2 becomes unavailable, fishes may either move to the surface and breathe air or utilize dissolved O_2 found in the upper 1-2 mm of the surface water. Considerable literature is available on the air-breathing habits of fishes but information is scant on aquatic surface respiration. Although described by Carter and Beadle (1931) it was found only recently to occur commonly amongst tropical and temperate species (Lewis 1970; Gee et al. 1978; Graham et al. 1978; Kramer and Meghan 1980).

Fishes adopting this mode of respiration rise to the surface and irrigate their gills with the relatively O_2 rich surface water. Activity is reduced to minimize metabolic O_2 demand and precise hydrostatic control is required to reduce metabolic costs associated with surface skimming (Gee et al. 1978). However, a reduction in swimbladder O_2 during asphyxia has been noted in a number of species (Black 1946; Jones and Marshall 1953), which could disrupt buoyancy

control during aquatic surface respiration if not replaced by other gases. The ability to resolve such a conflict by gulping air is possible in physostome fishes. Graham et al. (1978) suggested that such a response was an important step in the sequence of events leading to the evolution of aerial respiration, although nothing is known of the changes in buoyancy and composition of swimbladder gases that occur during the initial stages of hypoxia when fish commence aquatic surface respiration. The purpose of this research is to analyze such changes in the fathead minnow, Pimephales promelas.

The fathead minnow is a physostome found in weedy ponds and streams in central North America. Depending on the amount and state of vegetation, hypoxia may be encountered on a nocturnal or extended basis. Aquatic surface respiration has been demonstrated in the fathead minnow, although it possesses no apparent morphological adaptations to do so (Gee et al. 1978). The ability to use its swimbladder as an accessory respiratory organ is unknown. However, fathead minnows have been shown to secrete, reabsorb and gulp in response to changes in the velocity of normoxic water (Stewart 1980). Secretion and gulping were employed to increase buoyancy in response to decreased water velocity, while reabsorption led to a reduction in buoyancy when water velocity increased.

The specific objectives of this study were to describe changes in buoyancy and swimbladder gas composition that occurred in the fathead minnow during hypoxia and to assess the swimbladder for a possible respiratory function during low levels of dissolved O_2. Three separate lines of investigation were undertaken:

1) proportions of gas present in the swimbladder were monitored over 24 h in fish held in normoxic water,

2) changes in swimbladder gas composition, buoyancy, standard volume and internal pressure were measured during exposure to hypoxia and

3) uptake of aquatic and aerial O_2 were measured during self induced hypoxia in a respirometer study.

MATERIALS AND METHODS

Fathead minnows were collected from the Red River drainage basin, Manitoba, in September 1978 and October 1979. Fish were held at 10 ± 2°C under the 12L:12D photoperiod and fed daily. Prior to each experiment fish were acclimated for at least 7 d to 20 ± 2°C. Daily increments of 1°C were employed to reach the desired temperature.

Changes in swimbladder gas composition over 24 h

To determine if changes in the gas composition of the swimbladder over 24 h would offer an advantage to fish entering hypoxia during a particular period of the day, 48 fish (total length 4.0 - 7.0 cm) were sampled at random every 30 min from a larger group held in a (45 x 90 x 45 cm) aquarium. Light period extended from 0600 h to 1800 h and dark period from 1800 h to 0600 h. At night fish were sampled under red light illumination.

To sample swimbladder gases, fish were anesthetized in MS222, dissected to expose the swimbladder, the ductus communicans and pneumatic duct immediately tied off, and two gas samples were withdrawn (25 - 100 µL): one from each of the anterior and posterior lobes using 100 µL gas tight Hamilton syringes (Hamilton, Inc., Reno, Nevado). The needle

of the first syringe was inserted into a septum cap to prevent leakage, while the second sample was analyzed using a Carle Basic Gas Chromatograph 8700 (Carle, California), modified for respiratory gases (one Poropack QST 50/80 mesh and one molecular seive 5A separation column). Processing of the first sample immediately followed the second. Peaks for CO_2, O_2 (actually O_2 plus Ar) and N_2 were resolved on a strip chart recorder as each fraction passed the thermal conductivity detector in the gas chromatograph. Peak areas were calculated using the formula: peak area = maximum peak height · width of the peak at half the peak height. The instrument was calibrated using a known gas mixture of 9.59% CO_2, 50.98% O_2 and Ar (inseparable) and 39.4% N_2.

Reaction to hypoxia

As it was impossible to measure gas composition, buoyancy and buoyancy dependent variables on one fish, two experiments were completed to describe the reaction of fathead minnows to hypoxia. In the first, changes in swimbladder gas composition and buoyancy were described whereas in the second changes in buoyancy, standard volume and internal pressures of swimbladder gases were analyzed. In all experiments the number of opercular beats · min^{-1} was counted as an index of the stress induced by hypoxia. In hypoxia experiments 5 fish were measured for opercular beat frequency at each sampling time,

whereas 4 fish were measured for opercular beat frequency in the uptake of aerial and aquatic O_2 experiment at each sampling time. This was measured by taking the reciprocal of elapsed seconds per 60 beats.

Changes in swimbladder gas composition and buoyancy.

Fish with and without access to the surface were examined to determine if changes in swimbladder gas composition and buoyancy occurred during exposure to hypoxia. Fish (total length 4.0 - 7.0 cm) were placed individually in plastic cups 24 h in advance of the experiment. Cups providing access to the surface were positioned with tops 1 cm above the surface, cups denying access to the surface were screened at the top and suspended 5 cm below the surface. Batches of 8 fish each were sampled from both treatments at 0 h (in normoxic water) and 12, 36 and 60 h after hypoxia was reached (0.5 ppm). Cups were 66 mm deep, bottoms 90 mm in diameter, tops 55 mm in diameter, covered with a screen (non-access only) and two screened openings 20 mm in diameter on opposite sides. A series of moveable glass rod supports placed across the tank regulated the height of the cups, determining access or non-access to the surface.

Hypoxia was generated and maintained by regulating the flow rate of N_2 bubbled into the water through an airstone. Although N_2 effectively reduced dissolved O_2 concentrations, identical and consistent profiles were difficult to achieve.

Dissolved 0_2 did not fluctuate during the 60 h of exposure to hypoxia beyond the values observed at each successive sampling time of 12, 36 and 60 h. Variations were within the limits of ascertaining responses made by fathead minnows during hypoxia. In view of the objectives a more elaborate method of stripping oxygen from the water was not adopted. Dissolved 0_2 was determined using a YSI oxygen meter (Model 57). A continuous record of dissolved 0_2 was obtained by means of a strip chart recorder connected to the YSI oxygen meter.

Gas samples were withdrawn and analyzed as above. Buoyancy was determined after Gee (1970) (Appendix 1) by dividing the volume of the swimbladder by the weight of the gas free fish in water. All weights were recorded to $\pm$ 0.001 g.

Volume of the swimbladder was determined by the following formula:

$$\frac{\text{wt fish in water} - \text{wt gas free fish in water}}{1.000 \text{ g} \cdot \text{mL}^{-1} \text{ (water density } 4^{\circ}\text{C)}}$$

(Appendix 1)

Changes in buoyancy, standard volume and internal pressure.

To determine if changes in buoyancy, standard volume and internal pressure occurred following exposure to hypoxic water, fish were examined at three levels of hypoxia with

access to the surface: 1.5, 1.0 and 0.5 ppm and at one level of hypoxia without access to the surface: 1.5 ppm. Cups, holding time before the experiment, sampling times, number of fish, size of fish and buoyancy measurements were as above. Volume of free swimbladder gas at atmospheric pressure and 20°C was determined by releasing swimbladder gas under water into a collecting funnel, suspended from an under-the-balance hook. Volume of gas released was calculated by the following formula:

$$\frac{\text{wt funnel without gas - wt funnel with gas}}{0.9982 \text{ g} \cdot \text{mL}^{-1} \text{ (density of water } 20^{\circ}\text{C)}}$$

(Appendix 1)

All weights were measured to $\pm$ 0.001 g. To determine standard volume of swimbladder gas, volume of gas released from the swimbladder was corrected to 101.3 kPa and divided by the weight of the gas free fish in water. Internal pressure was determined by dividing the volume of free swimbladder gas at atmospheric pressure by the volume of the intact swimbladder gas at atmospheric pressure, after substituting a water density of 1.000 g $\cdot$ mL^{-1} in the above formula. The theory and principles behind these measurements are presented in Appendix 1.

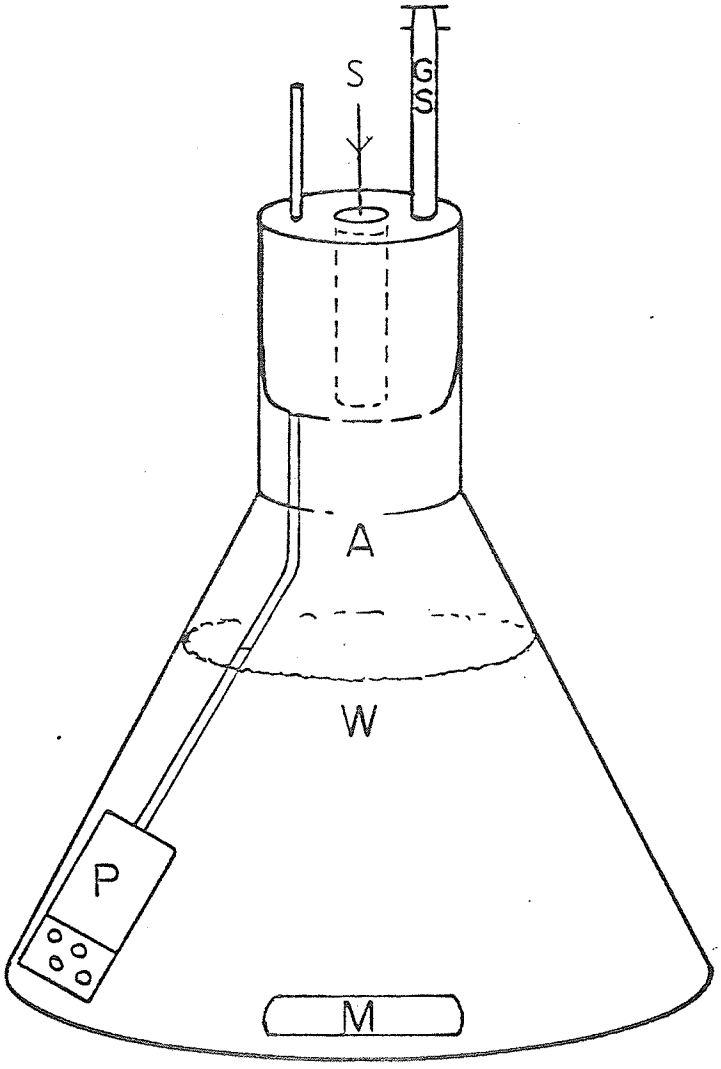
O_2 uptake from air and water

To determine if fathead minnows were able to take up O_2 directly from the air using the swimbladder as an accessory

respiratory organ, four fish were placed in a closed respirometer and decreases in aerial and aquatic 0_2 were monitored during self induced hypoxia. Utilization of aerial 0_2 would be demonstrated if aerial 0_2 declined faster than its diffusion rate into the water.

A 500 mL narrow mouth flask was used as a respirometer chamber. Fish were placed into the flask 24 h prior to the start of the experiment with a magnetic stirring bar in operation and an overflow of fresh water (20 $\pm$ 1^{o}C). During this period the flask with fish was placed in a 20 $\pm$ 1^{o}C water bath. The cable of the oxygen probe was cemented into a rubber stopper and the rubber stopper was fitted with a septum cap through which gas samples were withdrawn using a gas tight Hamilton syringe. The needle of a 1.0 cc glass syringe was driven through the rubber stopper to permit compensation for pressure differences upon inserting the rubber stopper into the flask and upon withdrawal of gas samples from the flask during the experiment. After 24 h the flask was submerged just below the water surface in a large aquarium (water, 20 $\pm$ 1^{o}C) and inverted, to retain 150 $\pm$ 0.5 mL of injected atmospheric air. The oxygen probe was then placed in the flask and the rubber stopper was inserted into the mouth of the flask and slowly tightened. The assembled apparatus was righted (Fig. 1), removed from the tank and the glass syringe filled with atmospheric air was attached to the needle.

Figure 1. Respirometer flask depicting: A, air phase; M, magnetic stirring bar; P, oxygen probe; S, septum; GS, glass syringe and W, water phase.


S
GS
A
W
P
M

The apparatus was placed back into the 20 $\pm$ 1°C water bath in which the water level reached the top of the rubber stopper but did not flood the septum cap area, thereby preventing contamination of gas samples with water. The magnetic stirrer was engaged and O_2 decrease in the air was determined using gas chromatograph samples drawn periodically through the septum, while O_2 decrease in the water was monitored using the YSI oxygen meter and strip chart recorder.

To determine milligrams of O_2 absorbed from the air within the flask it was assumed that the volume of N_2 in the air phase remained constant during the experiment. Since a known volume of air was injected, the volume of N_2 could be determined and the following equality applied:

$$\frac{\% \ O_2}{\% \ N_2} = \frac{\text{volume } O_2}{\text{volume } N_2 \text{ (constant term)}}$$

solving for volume O_2

$$\text{volume } O_2 = \frac{\text{volume } N_2 \cdot \% \ O_2}{\% \ N_2}$$

Milliliters were converted to milligrams by solving for n (number of moles) in the equation PV = nRT (gas equation) for 20°C and atmospheric pressure.

O_2 utilized by the probe was determined by measuring O_2 uptake from a sealed vessel of water without air and under constant circulation.

Diffision rate of 0_2 from atmospheric air into hypoxic water (0.9 ppm) was determined by using the same volume of water and air as found in the respiration experiment but without fish. The level of dissolved 0_2 was lowered to a concentration of 0.3 ppm by bubbling N_2 into the water. The air phase in the flask was then continually flushed with atmospheric air, while the water was under circulation by the magnetic stirrer to allow for diffusion of excessive N_2 out of the water phase. When dissolved 0_2 increased to 0.9 ppm, the vessel was sealed the magnetic stirrer operated at the same speed as in the first experiment and 0_2 uptake into the water measured by using the YSI oxygen meter and strip chart recorder.

Two estimates of metabolic rate were obtained: the first based on 0_2 loss from the water in the first 10 min of sealing the flask (this will be an underestimation due to diffusion from air into water) and a second more accurate estimate based on 0_2 loss from the gas phase and water phase after a hypoxia level of less than 1.0 ppm was reached.

STATISTICAL ANALYSIS

All means were constructed with 95% confidence limits. Differences between means were judged to be biologically significant if a clear separation (non-overlap) of 95% confidence limits was observed. Regression analysis (linear) was used to determine the various rates of oxygen uptake in the respirometer experiment and was evaluated at the alpha level of 0.05.

RESULTS

Changes in swimbladder gas composition over 24 h

Three gases, CO_2, O_2 and N_2 accounted for 98 to 100% of the sampled volumes. Mean concentrations over 24 h adequately predicted swimbladder gas composition for an intermediate time interval, no cycles were observed, nor were differences found between swimbladder lobes. Mean percentages(n = 45) and 95% confidence limits were as follows:

Gas	CO_2	O_2	N_2
Anterior lobe	1.49 (± 0.10)	10.03 (± 0.96)	89.37 (± 0.98)
Posterior lobe	1.56 (± 0.20)	10.37 (± 1.04)	89.11 (± 1.03)

Compared to atmospheric air CO_2 and N_2 values were above and O_2 values below percent concentrations.

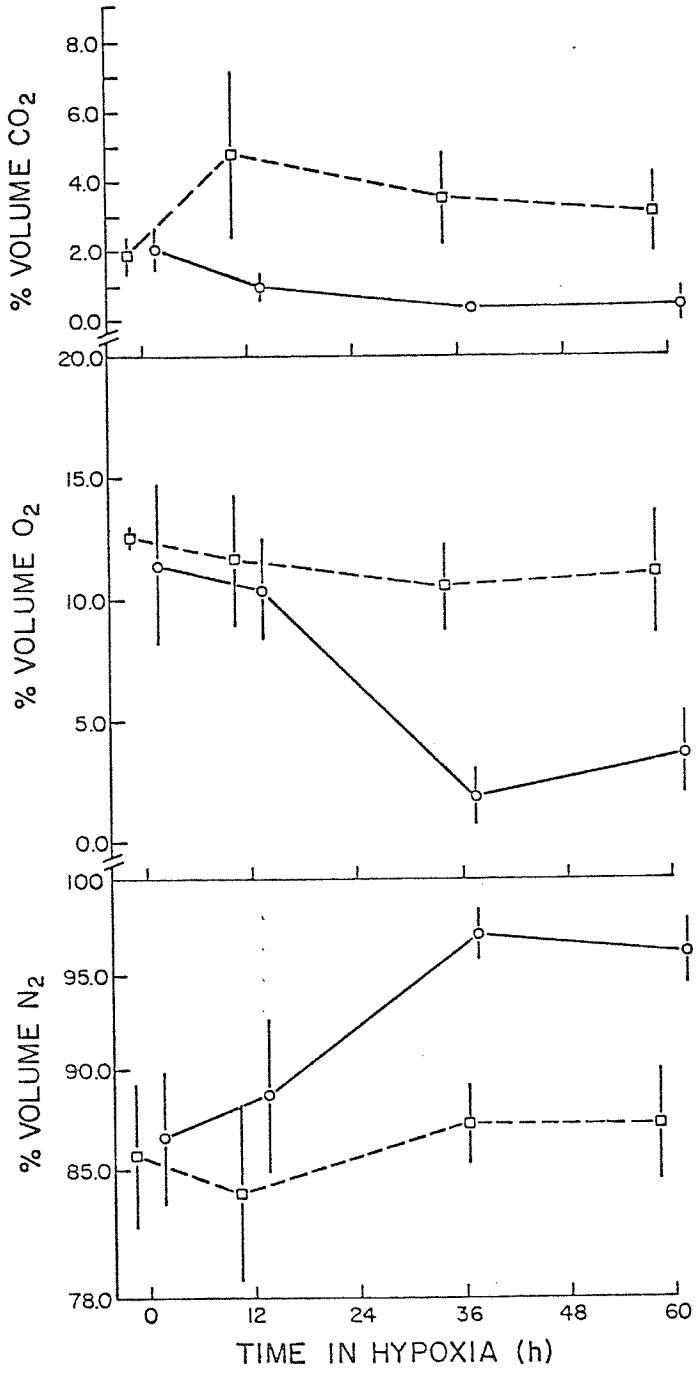
Reaction to hypoxia

Fathead minnows remained in the lower half of the cups until dissolved O_2 declined to 1.5 ppm, fish then rose to the surface or screens but immediately returned to deeper water. This behavior persisted in access fish throughout exposure to the hypoxia level of 1.5 ppm, whereas non-access fish remained pressed against the screens after 12 h at this same concentration of dissolved O_2.

At 1.0 ppm and lower fish with access remained just below the surface and swam around the perimeter of the cups. Non-access fish remained at the screens. Air bubbles were occasionally taken into the buccal cavity by access fish for approximately 10 seconds but this behavior was infrequent. Bubble retention appeared to be associated with accidental intake of existing bubbles at the surface. Otherwise fish did not demonstrate a gulping behavior at the surface that could be clearly sequenced and identified. However spitting of gas was observed in fish considered not to have taken an air bubble into the buccal cavity at the water surface.

Changes in swimbladder gas composition and buoyancy. In normoxic and hypoxic water, anterior and posterior lobes of the swimbladder were described by a common store of CO_2,O_2 and N_2 and were combined within each treatment (Fig. 2). The percentages of CO_2 and O_2 declined in fish with access to the surface (hypoxia level 0.5 ppm) and were complemented by an increase in N_2 (Fig. 2). Fish without access to the surface (hypoxia level 0.5 ppm) demonstrated no change in swimbladder gas composition during the 60 h of exposure to hypoxic water but compared to access fish after 12 h, CO_2 and O_2 were higher and N_2 was lower (Fig. 2).

Figure 2. Mean percent CO_2, O_2 and N_2 in the swimbladder of fish with access to the surface (solid line) and without access to the surface (dashed line) during exposure to hypoxic water at the level of 0.5 ppm. Vertical bars represent 95% confidence limits on the means.


% VOLUME CO_2
8.0
6.0
4.0
2.0
0.0
20.0
% VOLUME O_2
15.0
10.0
5.0
0.0
100
% VOLUME N_2
95.0
90.0
85.0
78.0
0
12
24
36
48
60
TIME IN HYPOXIA (h)

In fish with access to the surface buoyancy increased at 12 h but not to a degree considered significant in this study; however, a large increase in variance was observed at 12 h (Fig. 3). Fish without access to the surface did not demonstrate a change in buoyancy during the 60 h of exposure to hypoxic water; nor did variance increase at 12 h as observed in fish with access to the surface.

Opercular beats $\cdot$ min $^{-1}$ responded predictably with dissolved O_2 concentrations (Fig. 4). Access fish maintained an elevated frequency of opercular beats during consistently low concentrations of dissolved O_2 and non-accessfish decreased the frequency of opercular beats with increasing concentrations of dissolved O_2.

Changes in buoyancy, standard volume and internal pressure. The frequency of opercular beats (Fig. 5 B-D) was observed as above. Fish with access to the surface at the hypoxia level of 1.5 ppm (Fig. 5 A) did not follow this pattern and were able to decrease the frequency of opercular beats without the associated increases in dissolved O_2.

Fish without and with access to the surface at the hypoxia level of 1.5 ppm demonstrated no change in

Figure 3. Effects of exposure to hypoxic water (hypoxia level 0.5 ppm) on buoyancy in fish with and without access to the surface. Vertical bars represent 95% confidence limits on the means.

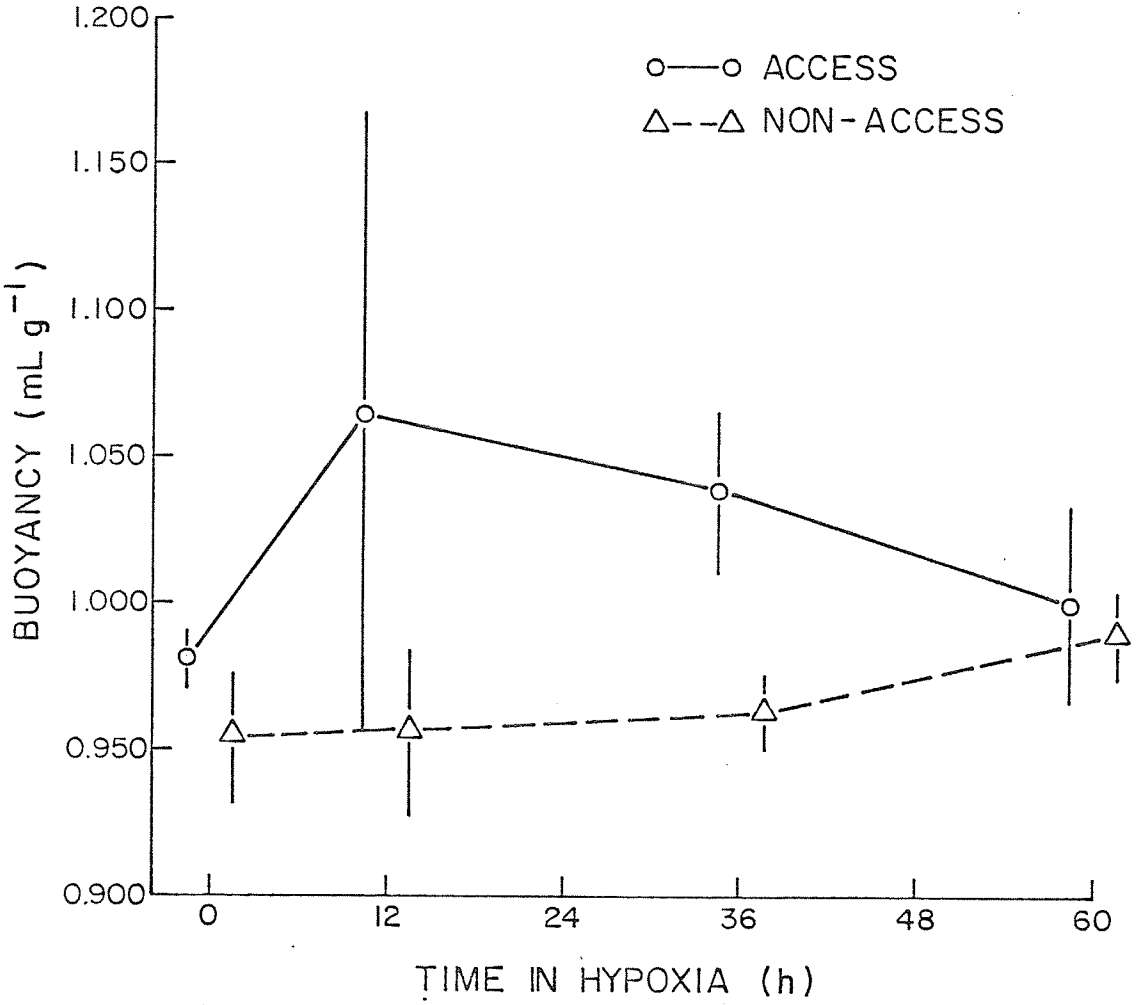

1.200
1.150
1.100
1.050
1.000
0.950
0.900
BUOYANCY (mL g⁻¹)
o—o ACCESS
Δ--Δ NON-ACCESS
0
12
24
36
48
60
TIME IN HYPOXIA (h)

Figure 4. Frequency of opercular beats (upper curves) and dissolved O_2 (lower curves) for fish with access to the surface (solid lines) and without access to the surface (dashed lines) at the hypoxia level of 0.5 ppm. Vertical bars represent 95% confidence limits on the means. Limits less than 5 beats $\cdot$ min^{-1} were not represented.

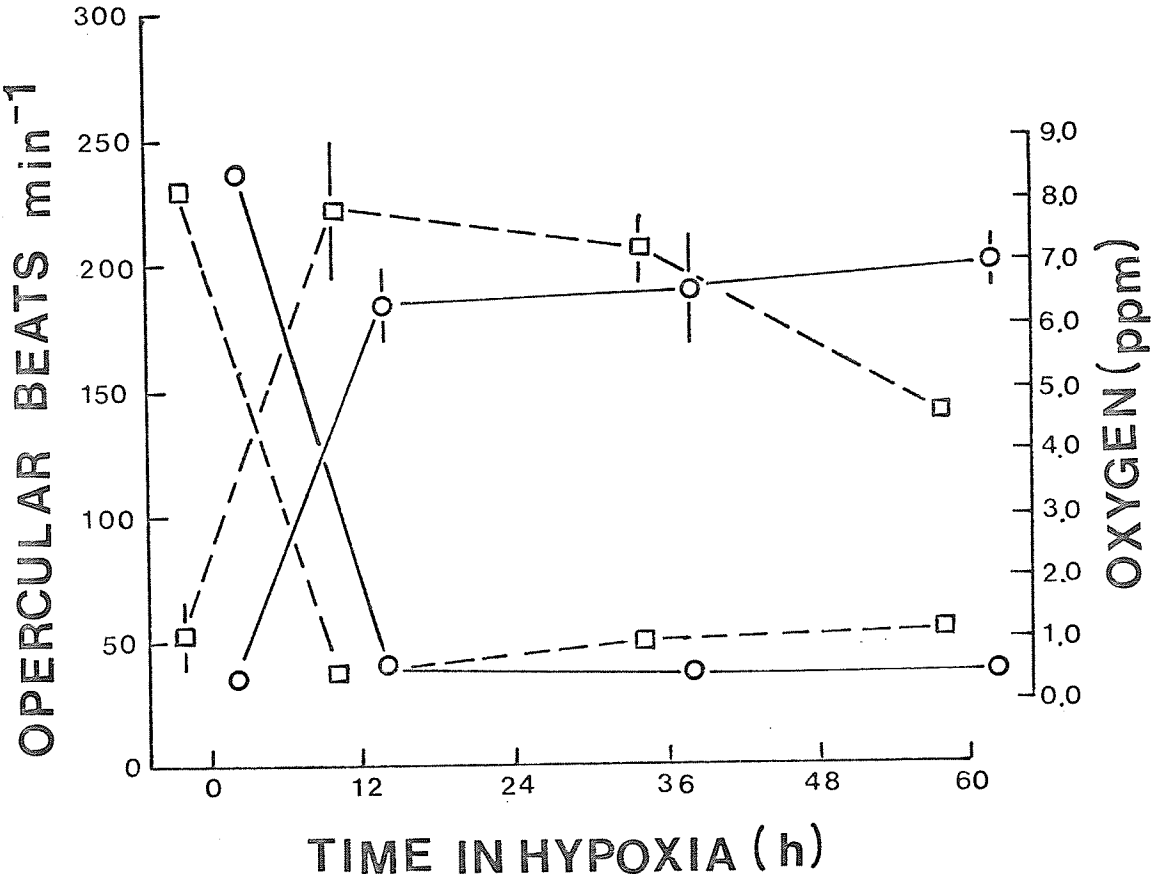
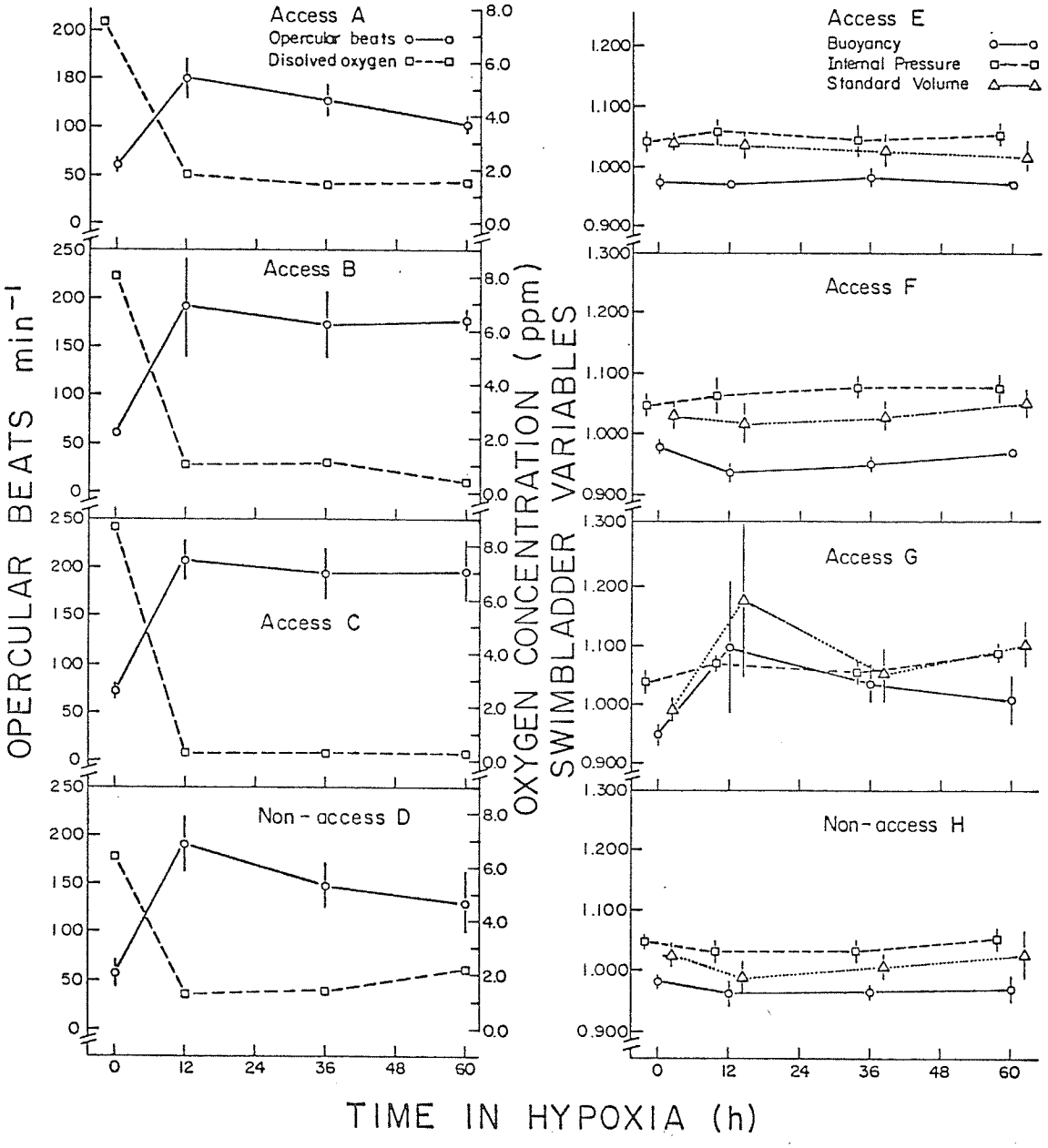

OPERCULAR BEATS min⁻¹
300
250
200
150
100
50
0
OXYGEN (ppm)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
12
24
36
48
60
TIME IN HYPOXIA (h)

Figure 5. Relationships between dissolved O_2 and frequency of opercular beats on exposure to three levels of hypoxic water with access (A, B, C) and one level of hypoxic water without access (D). Changes in buoyancy ($mL \cdot g^{-1}$), internal pressure (kPa) and standard volume ($mL \cdot g^{-1}$) appear for each treatment (E, F, G, H). Vertical bars represent 95% confidence limits on the means.

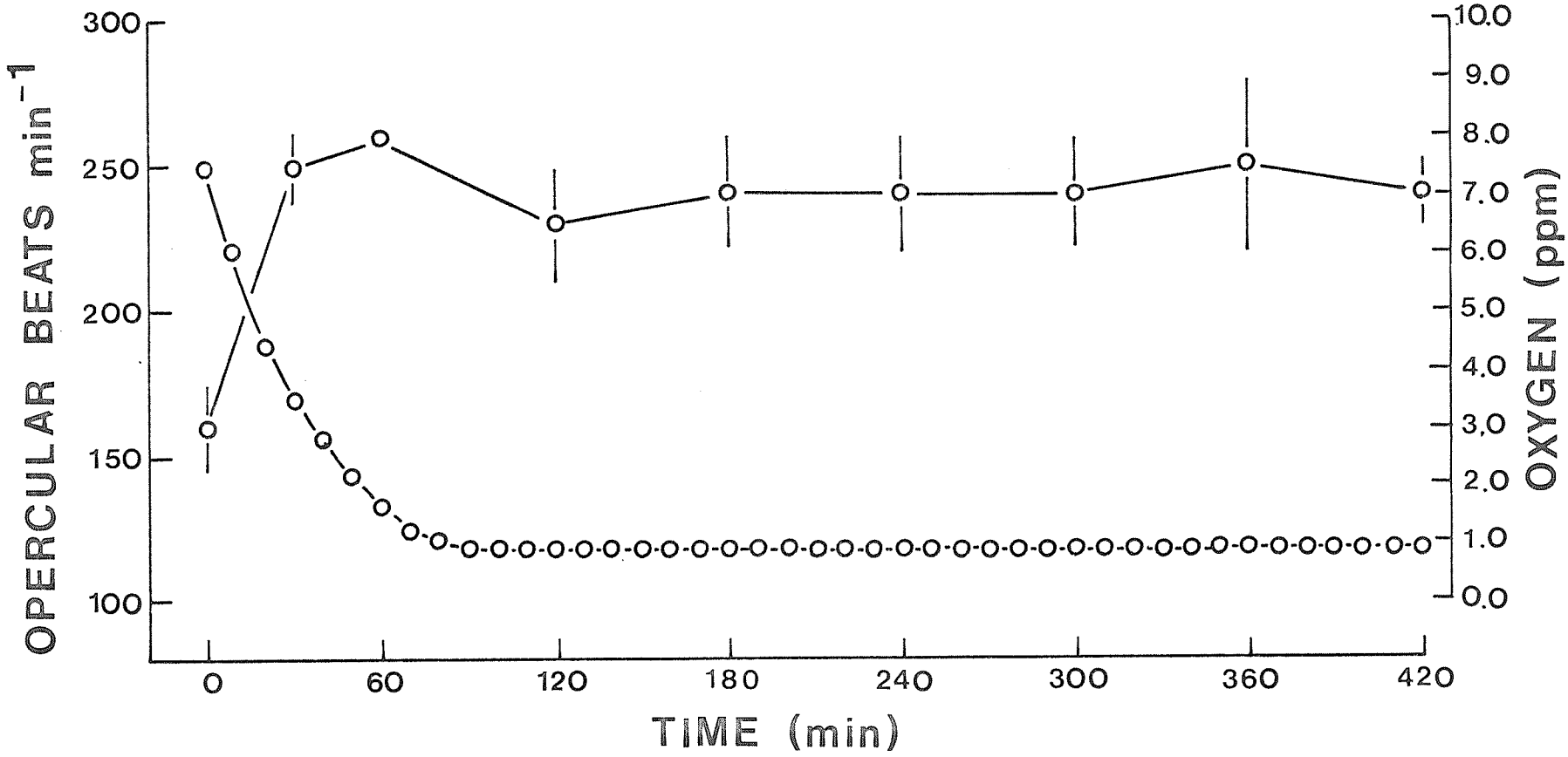

Access A
Opercular beats
Disolved oxygen
Access B
Access C
Non-access D
Access E
Buoyancy
Internal Pressure
Standard Volume
Access F
Access G
Non-access H
OPERCULAR BEATS min⁻¹
OXYGEN CONCENTRATION (ppm)
SWIMBLADDER VARIABLES
TIME IN HYPOXIA (h)

swimbladder variables during 60 h of exposure to hypoxic water (Fig. 5 E,H). At the hypoxia level of 1.0 ppm with access to the surface, buoyancy decreased at 12 h due to a combined but individually minor increase in internal pressure and decrease in standard volume (Fig. 5 F). An increase in buoyancy was apparent within the following 48 h of exposure to hypoxic water to values below normoxic conditions (0 h). Increases in buoyancy and standard volume occured in fish with access to the surface at the hypoxia level of 0.5 ppm (Fig. 5 G). The associated variance component for buoyancy and standard volume increased at 12 h to well above the observed variance at 0 h. Variances decreased 24 h after the peak increases observed at 12 h (Fig. 5 G). A similar pattern of buoyancy and variance increase at 12 h was observed in fish with access to the surface, sampled for swimbladder gas composition at the hypoxia level of 0.5 ppm.

O_2 uptake from air and water

The frequency of opercular beats increased to a maximum within 1 h of sealing the flask and remained elevated for the duration of the experiment (Fig. 6). Aquatic O_2 decreased exponentially, becoming asymptotic at $8.98 \cdot 10^{-4}$ $mg \cdot mL^{-1}$ (0.90 ppm) after 80 min (Fig. 6).

Figure 6. Frequency of opercular beats with declining levels of dissolved O_2 (respirometer study). Vertical bars represent 95% confidence limits on the means.


OPERCULAR BEATS min^{-1}
300
250
200
150
100
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
OXYGEN (ppm)
0
60
120
180
240
300
360
420
TIME (min)

The three regression equations obtained in this experiment were: 1) mg O_2 used by the oxygen probe = 0.05 mg · min^{-1} · time (min) + 0.009 mg O_2, n = 10, R^2 = 0.970; 2) mg O_2 diffused into hypoxic water (0.90 ppm) = 0.027 mg · min^{-1} · time (min) + 0.009 mg O_2, n = 10, R^2 = 0.988 and 3) mg O_2 loss from air in the presence of fish = 0.037 mg · min^{-1} · time (min) + 1.320 mg O_2, n = 8, R^2 = 0.996. The independent variable time provided significant information in predicting the dependent variable O_2 uptake ($P(F) \leq 0.95$, t test, on the coefficient of regression, R^2) in all regression equations. Corrected for utilization of O_2 by the oxygen probe, 31% of the O_2 used by fathead minnows during hypoxia (0.90 ppm) was obtained directly from the atmosphere.

Metabolic rate as calculated from O_2 uptake from the atmosphere and corrected for O_2 uptake by the probe during hypoxia (0.90 ppm) was 178 mg · kg^{-1} · h^{-1}. The estimate based on the first 10 min of O_2 loss from the water (normoxic conditions) was 618 mg · kg^{-1} · h^{-1}, more than 3 times the observed rate when fish were exposed to less than 1.0 ppm O_2.

DISCUSSION

In normoxic water swimbladder gas composition was independent of time of day. When fathead minnows were exposed to hypoxic water, percent swimbladder O_2 declined; without access to the surface no change in percent swimbladder O_2 was observed (hypoxia level 0.5 ppm). Effects of hypoxia on buoyancy, standard volume and internal pressure were dependent on: 1) levels of dissolved O_2, 2) times of exposure and 3) access to the surface in the presence of an air phase. A possible respiratory function for the swimbladder during the initial stages of hypoxia was suggested by: 1) increases in buoyancy and standard volume (12 h, hypoxia level 0.5 ppm) and 2) uptake of O_2 from the air in the respirometer experiment (1 - 5 h, 0.90 ppm).

Changes in swimbladder gas composition over 24 h

Endogenous daily rhythms in O_2 consumption have been observed in 8 species of salt-water fishes (Livingstone 1971) and daily fluctuations in 4 species of fresh-water fishes (Clausen 1936). It is not known if these patterns in O_2 consumption affect swimbladder O_2 stores. If such a pattern exists in the fathead minnow over 24 h, the proportions of swimbladder O_2 remain unchanged. Volume of swimbladder O_2 may also remain constant as Stewart (1980)

found no change in buoyancy over 24 h in the fathead minnow. Changes in buoyancy and percent swimbladder O_2 in the fathead minnow during hypoxia therefore reflect the effects of the treatments without the additive or subtractive components of an endogenous daily rhythm in buoyancy or percent swimbladder O_2.

Reaction to hypoxia

Changes in swimbladder gas composition and buoyancy. In fathead minnows with access to the surface, percent swimbladder O_2 declined between 12-36 h after fish were exposed to hypoxic water (hypoxia level 0.5 ppm). Non-access fish under the similar conditions did not change percent swimbladder O_2.

It is unlikely that swimbladder O_2 stores in the fathead minnow would go undepleted during exposure to hypoxic water unless fish gulped air or shunted blood away from the swimbladder to prevent loss of O_2. Klinger (1978) has indicated that the fathead minnow does not have prolonged capacity for anaerobic metabolism and Gee et al. (1978) have classified the species as morphologically unadapted for aquatic surface respiration. Many species also rapidly depleted swimbladder O_2 when asphyxiated (Jones and Marshall 1953).

The fathead minnow appears to be unusual in its ability to retain constant proportions of swimbladder O_2 during hypoxia without access to the surface. Buoyancy measurements also indicated that no net losses or gains in swimbladder gases were apparent. Both responses can be attributed to a non-reabsorption of swimbladder O_2 or equal rates of secretion and reabsorption of O_2. The latter would be unlikely if the O_2 content of the blood were to decline with progressive and prolonged hypoxia. Non-reabsorption could be achieved by shunting of blood away from the swimbladder, to the gills; such a response occurs in the primitive air-breather Amia calva when the gills become the primary site for uptake of O_2 (Johansen et al. 1970).

Fish with access to the surface decreased percent O_2 between 12-36 h of exposure to hypoxic water (hypoxia 0.5 ppm). Percent CO_2 declined from 0-36 h. Increases in buoyancy at 12 h were evidence of either gas secretion or gulping prior to this time. Graham et al. (1978) have observed buoyancy increases in Piabucina festae upon exposure to hypoxic water for 10 min and interpreted such increases as a means of increasing swimbladder volume to supply more aerial O_2. Secretion appears unlikely as high percentages of CO_2 are usually present in

the first phase of gas secretion (Fange 1966), this was not observed in the fathead minnow with access to the surface. The observed reduction in percent swimbladder CO_2 could be explained if fish gulped air. Such a response would: 1) increase supply of O_2 to the swimbladder, 2) balance uptake of O_2 from the swimbladder into the blood and 3) increase buoyancy and aid aquatic surface respiration. Based on buoyancy and swimbladder gas composition it is hypothesized that: 1) fathead minnows use the swimbladder as a site for uptake of O_2 during hypoxia when given access to the surface and 2) additional O_2 is obtained from the atmosphere by gulping.

However it was not apparent why swimbladder O_2 and buoyancy declined after 12 h of exposure to hypoxic water. A continued decline in CO_2 suggested that gulping and exchange of swimbladder gas with the atmosphere was less frequent or the volume exchanged was reduced.

Changes in buoyancy, standard volume and internal pressure. Fathead minnows at the hypoxia level of 1.5 ppm demonstrated no change in swimbladder variables during 60 h of exposure to hypoxic water. At this level of hypoxia access or non-access to the surface did not influence results. Fish with access to the surface at the hypoxia level of 1.0 ppm had a reduced buoyancy at

12 h compared to 0 h. A progressive increase in buoyancy was observed within the following 48 h. Fish with access to the surface at 0.5 ppm (hypoxia level) had increased buoyancy and standard volume at 12 h, both of which slowly declined within the following 48 h. This response in buoyancy was the same as that observed in access fish at the hypoxia level of 0.5 ppm in the previous experiment.

Analysis of swimbladder gas composition suggested that fathead minnows may have been absorbing swimbladder O_2 for the first 12 h of hypoxia but were also gulping air. Loss of optimal buoyancy due to reabsorption of swimbladder O_2 is hypothesized to have occured prior to gulping air and that the latter was in response to the former. The initial stages of such a response may have been evident in the observed decreased buoyancy at 12 h followed by a progressive increase at the hypoxia level of 1.0 ppm. Fathead minnows do respond to a less than optimal buoyancy (created by changes in atmospheric pressure) by gulping air at the surface (Stewart 1980) and such responses are common amongst air-breathing physostomes (Gee and Graham 1978; Farrel and Randall 1978; Johansen 1970; Gee in preparation).

At high levels of hypoxia (1.0 ppm) gulping of air may be in response to declining buoyancy which interfers with aquatic surface respiration. At lower levels of dissolved O_2 (0.5 ppm) gulping may serve two requirements:

1) the attainment of positive buoyancy to assist aquatic surface respiration and 2) the increase supply of O_2 to meet metabolic O_2 demands. The large standard volume observed at the hypoxia level of 0.5 ppm was more than that required to achieve positive buoyancy; additional gas could have been used to increase the supply of O_2 available to the blood.

Intake of atmospheric air into the swimbladder of the fathead minnow may involve a buccal force pump. In inspiration, air is taken into the mouth and is forced through the pneumatic duct into the swimbladder with the opercular and mouth closed (Fange 1976). Expiration is due to release of excess gas by relaxation of the pneumatic sphincter. The timing of expiration relative to inspiration was not observed. The cost of operating such a pump is not known but it may well interfer with efficient and essential aquatic surface respiration in fish that absorb swimbladder O_2 at slow rates. The costs would include time lost in aquatic respiration due to closing the opercula and the mouth and the possibility that blood might be shunted away from the gills to absorb O_2 at the swimbladder site. Prolonged exposure to hypoxia could eventually result in an oxygen debt or muscle fatigue due to the increased activity required to operate the inspiration-expiration pump. The anticipated response would

be to cut back on the frequency of gulping but to continue use of swimbladder O_2 and aquatic surface respiration. The fathead minnow may encounter this limit to the efficiency of aerial respiration after 12 h of exposure to severe hypoxia (0.5 ppm or less).

The potential utilization of swimbladder O_2 combined with repeated gulping to maintain buoyancy suggests a significant respiratory function for the swimbladder in the fathead minnow. The increased buoyancy to positive levels and increased standard volume of the swimbladder at 12 h increases the supply of aerial O_2 and simultaneously favors efficient aquatic surface respiration. However the efficiency of aerial respiration appears to be limited to 12 h of exposure in severe hypoxia.

O_2 uptake from air and water.

Fathead minnows obtained 31% of their metabolic O_2 requirement directly from atmospheric air during the first 5 h of exposure to hypoxic water (0.9 ppm). Here uptake of O_2 was linear and at 178 $mg \cdot kg^{-1} \cdot h^{-1}$ was less than the standard metabolic rate of 221 $mg \cdot kg^{-1} \cdot h^{-1}$ for 20°C extrapolated from Klinger's (1978) data on the fathead minnow.

The initial estimate of metabolic rate during the first 10 min of the experiment, 618 mg· kg^{-1}· h^{-1}, was well above the standard metabolic rate obtained from Klinger's data and the initial frequency of opercular beats at 0 h, 159 beats · min^{-1} was well above the normoxic rates 50-55 beats · min^{-1} at 0 h, observed in the previous experiments. Initial rates above standard and normoxic conditions indicated the degree of stress imposed by the presence of a water current in the respiration flask.

The frequency of opercular beats did not decline during the course of the experiment. Declining metabolic rate could be attributed to a decrease in the ability to take up O_2 with rising levels of aquatic CO_2 in the flask (Basu 1959), or the inability to extract enough aerial O_2 using the swimbladder, at a rate sufficient to meet previous metabolic O_2 demands.

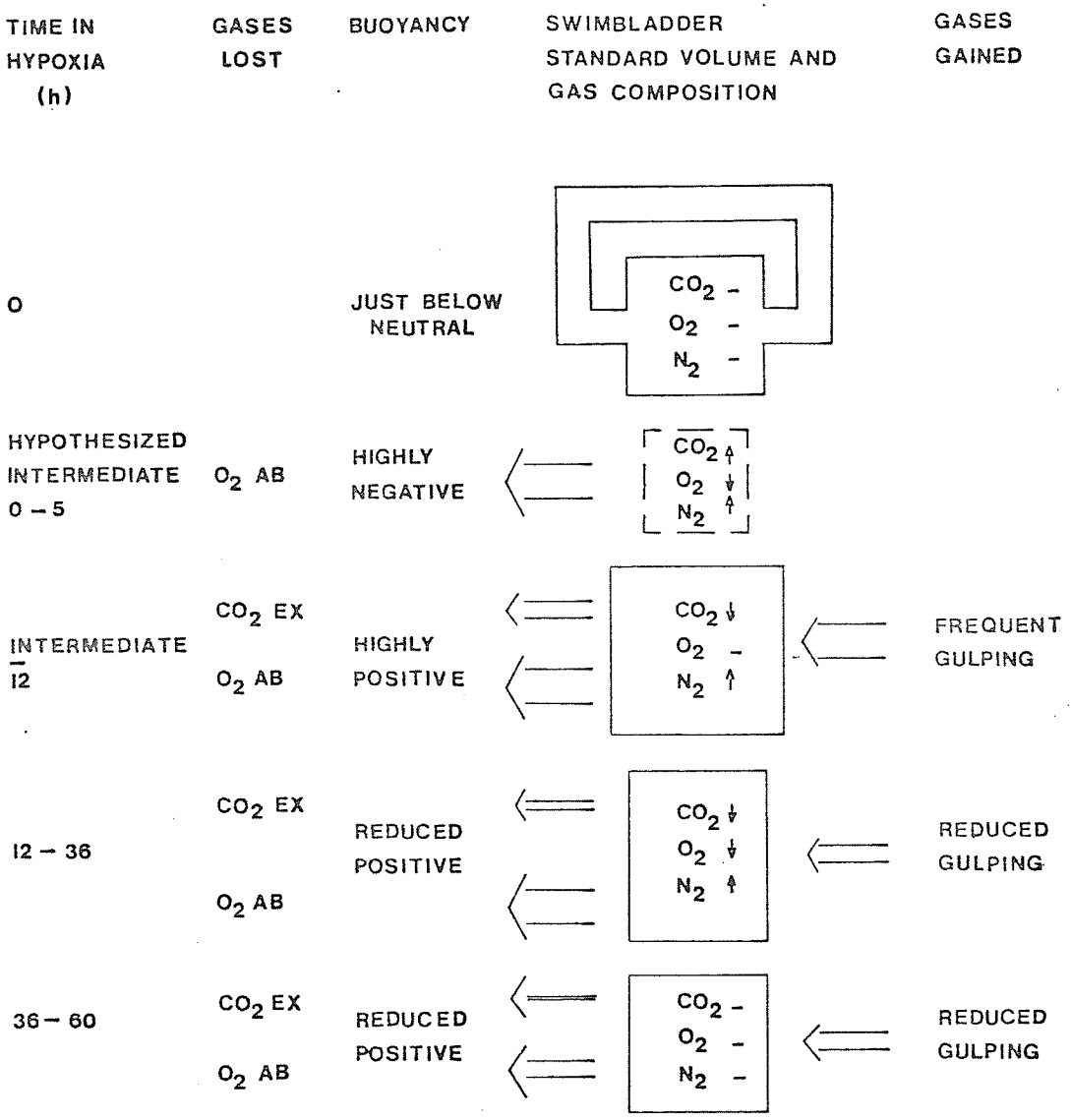
Bubble retention in the buccal cavity may represent an alternative explanation for uptake of aerial O_2. Although viewed as sporadic in this study, Klinger (1978) has also reported the response. An increase in dissolved O_2 could be achieved by passing hypoxic water over the bubble prior to irrigating the gills. Johansen (1970) has reviewed structural derivatives found in the mouth

of fish used as accessory breathing organs. No structural derivatives of this type have been observed in the fathead minnow and calculation of gas diffusion from the bubble into water passing over the gills would require further data. However it appears unlikely that the fathead minnow could obtain 31% of its metabolic O_2 requirement using this method as the behavior was infrequent and bubble retention was no longer than 10 seconds at a time.

It was therefore apparent that fathead minnows do exchange swimbladder gases with the atmosphere during the initial stages of hypoxia, 1-5 h. An extension of this mode of respiration to 12 h would agree with the lines of evidence given in the previous experiments in support of aerial respiration. They included: 1) concentrations of swimbladder O_2 that did not change with exposure to hypoxia at 12 h from those observed at 0 h and 2) increased standard volume at 12 h.

To explain the sequence of events encountered when fathead minnows were exposed to hypoxic water a model (Fig. 7) is proposed. The model is based on the hypothesis that the swimbladder is used as a respiratory organ during the initial stages of hypoxia. Gulping of atmospheric air is in response to a decline in buoyancy due to absorption of swimbladder O_2 during hypoxia. Repeated gulping of atmospheric air to ventilate the swimbladder during

Figure 7. Model depicting sequence of hypothesized and observed events during exposure to hypoxic water ($\leq$ 1.0 ppm) in the fathead minnow. Arrows represent percent increases $\uparrow$ and decreases $\downarrow$; AB, absorbed swimbladder gas and EX, expired swimbladder gas.


TIME IN HYPOXIA (h)
GASES LOST
BUOYANCY
SWIMBLADDER STANDARD VOLUME AND GAS COMPOSITION
GASES GAINED
0
JUST BELOW NEUTRAL
CO_2 –
O_2 –
N_2 –
HYPOTHESIZED INTERMEDIATE
0 – 5
O_2 AB
HIGHLY NEGATIVE
CO_2 ↑
O_2 ↓
N_2 ↑
INTERMEDIATE
12
CO_2 EX
O_2 AB
HIGHLY POSITIVE
CO_2 ↓
O_2 –
N_2 ↑
FREQUENT GULPING
12 – 36
CO_2 EX
O_2 AB
REDUCED POSITIVE
CO_2 ↓
O_2 ↓
N_2 ↑
REDUCED GULPING
36 – 60
CO_2 EX
O_2 AB
REDUCED POSITIVE
CO_2 –
O_2 –
N_2 –
REDUCED GULPING

hypoxia: 1) maintains an elevated level of percent O_2 in the swimbladder, 2) supplies a percentage of metabolic O_2 requirements, 3) decreases percent CO_2 in the swimbladder through expiration of CO_2 and 4) assists efficient aquatic surface respiration by maintaining positive buoyancy. However the fathead minnow maintains a high frequency of opercular beats throughout exposure to hypoxia and apparently decreases the frequency of aerial respiration after 12 h. Neither aquatic nor aerial respiration is employed to the exclusion of the other during hypoxia. This enables the fathead minnow to survive for extended periods of time by rising to the surface when waters become depleted of dissolved O_2.

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APPENDIX 1.

The calculation of buoyancy, standard volume and internal pressure in this study depend on the application of Archimedes' principle. This well known physical law states that an object in water displaces a weight of fluid proportional to its volume. This displacement results in a buoyant force opposite in direction to the gravitational constant. The weight of an object in any fluid can be mathematically expressed as

$$wt_{(fluid)} = wt_{(air)}\downarrow - \rho V\uparrow_{(object)} \qquad (1)$$

were: wt = weight of object; arrows, direction of force; ρ , density of fluid and V, volume of the object. Since the gravitational constant (9.8 $m \cdot sec^{-2}$) applies to all weights, force can be expressed as weight, rather than $N \cdot m \cdot sec^{-2}$. By providing a wire basket of weight greater than the maximum possible lift of the fish (so the basket and fish do not float at the surface), weight of the fish in water with an intact swimbladder (F + S), can be determined. To avoid false results the gills and buccal cavity are freed of residual air bubbles before weights are taken. Applying equation (1):

$$wt_{(F + S)} = wt_{air\ (F + S)} - \rho V_{(F + S)} \qquad (2)$$

for a fish in water with swimbladder removed (F - S)

$$wt_{(F - S)} = wt_{air\ (F - S)} - \rho V_{(F - S)} \qquad (3)$$

Subtracting equation (3) from (2) and simplifying

$$wt_{(F - S)} - wt_{(F + S)} = \rho(V_{(F + S)} - V_{(F - S)}) \qquad (4)$$

$$\frac{wt_{(F - S)} - wt_{(F + S)}}{\rho} = \text{volume of swimbladder} \qquad (5)$$

It is known that $wt_{air\ (F + S)} - wt_{air\ (F - S)}$ is less than 0.001 g in the fathead minnow and therefore assumed to be zero in equation (4).

Gee (1970) assumed a density of 1.000 $g \cdot mL^{-1}$ at all temperatures in calculating buoyancy as swimbladder volume (lefthand side of equation (4)) divided by the weight of the gas free fish in water. Uncorrected for density Gee (1970) has measured the buoyant force of the intact swimbladder volume divided by the weight of the gas free fish in water. To make data comparable to Gee (1970) a density of 1.000 $g \cdot mL^{-1}$ was used to calculate buoyancy in this study.

To calculate volume of swimbladder gas released at atmospheric pressure, swimbladder gas was collected in an immersed glass funnel, suspended from an under-the-balance hook. The weight of the funnel in water without swimbladder gas (Fl - G)

$$wt_{(Fl - G)} = W_{air\ (Fl - G)} - \rho V_{(Fl - G)} \quad (6)$$

with gas (Fl + G)

$$wt_{(Fl + G)} = wt_{air\ (Fl + G)} - \rho V_{(Fl + G)} \quad (7)$$

subtracting equations 7 from 6 and simplifying

$$wt_{(Fl - G)} - wt_{(Fl + G)} = \rho(V_{(Fl + G)} - V_{(F - G)})$$

$$\frac{wt_{(Fl - G)} - wt_{(Fl + G)}}{\rho} = \text{volume of free gas} \quad (8)$$

To calculate standard volume, volume of free gas is corrected to 101.3 kPa and then divided by the weight of the gas free fish in water. Internal pressure (IP) is calculated by dividing equation (8) by equation (5) and simplifying to yield

$$IP = \frac{wt_{(Fl - G)} - wt_{(Fl + G)}}{wt_{(F - G)} - wt_{(F + G)}}$$