EFFECTS OF WATER ACIDITY ON SWIMBLADDER FUNCTION AND SWIMMING BEHAVIOR IN THE FATHEAD MINNOW, PIMEPHALES PROMELAS, RAFINESQUE

by

Wolfgang A. Jansen

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Department of Zoology, University of Manitoba

Winnipeg, Manitoba

ISBN 0-315-33924-1

EFFECTS OF WATER ACIDITY ON SWIMBLADDER FUNCTION AND SWIMMING BEHAVIOR IN THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*, RAFINESQUE

BY

WOLFGANG A. JANSEN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Wolfgang A. Jansen

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Wolfgang A. Jansen

The University of Manitoba requires the signatures of all persons using or photocopying this thesis. Please sign below, and give address and date.

To my grandparents and
the memory of my mother

ABSTRACT

Swimbladder function, buoyancy-related behavior and swimming performance were examined in fathead minnows (Pimephales promelas) following chronic (≥4 d) exposure to acidic water (pH 5.3). Fish were tested in water current with access to air and still water with and without access to air. Both treated and control (pH 7.7) fish in still water increased the proportions of CO_2 and O_2 inside the swimbladder when surface access was denied. Under the same conditions, treated fish attained significantly higher percentages in these gases and failed to increase buoyancy and standard volume over "access to air" levels, as did the controls. After 48 h in current, the extent of buoyancy adjustment was significantly reduced in treated fish compared to controls. While control fish increased the internal pressure of their swimbladder gases in response to a need for reduced buoyancy, treated fish were severely limited in their ability to do so. Treated fish were also unable to maintain the minimum buoyancy over 32 d in current. Upon transfer from current to still water without access to air, both treated and control fish reached almost neutral buoyancy within 48 h, and swimbladder gas composition (maximal 5% CO_2 and 45% O_2) remained unaffected by pH. Under otherwise identical conditions, but with surface access, fish of both groups filled

their swimbladders within 6-12 h followuing removal from current, using both air gulping and gas secretion. Based on the higher swimbladder CO_2 and O_2 proportions of the controls and direct observations on gulping frequencies, treated fish relied more on air gulping to increase swimbladder volume (buoyancy). When lift was increasing in still water, the frequency of pectoral fin beats (PFBF) was negatively correlated with buoyancy. Treated fish generally had a higher PFBF than controls in comparable experiments. The magnitude of this difference, however, varied between experimental conditions. Swimming performance, measured over 10 h, was unaffected by water pH, but treated fish lost more weight and were in poorer condition than controls. The possible mechanisms of acid-induced impairment of swimbladder function and some of its ecological consequences are discussed.

ACKNOWLEDGEMENTS

I express my sincere gratitude to my supervisor Dr. J.H. Gee for the opportunity to work on this project and the support and guidance he provided. I thank my committee members Dr. R.A. McArthur and Mr. J. Flannagan for their constructive criticism on the manuscript. Dr. B. Johnston provided valuable counsel and assistance on statistical analysis, which was greately appreciated. Also, I thank Raymond Ratynski and fellow graduate students of the Department of Zoology for many helpful discussions and Wolfgang Heck for helping with the illustrations. I am indebted to Ms. Maureen Sullivan for her patience and diligence while typing the appendices. Finally, I would like to acknowledge the assistance, encouragement and love of my wife, Pearl, especially during the final phase of this project.

This research was supported by grants from the National Research Council of Canada and by a World University Service of Canada Award to the author.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Over the past decade, acid precipitation has emerged as a major environmental issue in the industrialized nations, attracting considerable attention from both the general public and the scientific community (see review by Cowling 1982). Although acid rain may occasionally cause direct mortality in fish (Jensen & Snekvik 1972, Leivestad & Muniz 1976, Grahn 1980), its sublethal effects appear to be of greater ecological significance. Acid rain provides a reasonable explanation for the increasing number of fishless aquatic environments over large areas in Scandinavia and North America (Beamish & Harvey 1972, Almer et al. 1974, Schofield 1976, Rosseland et al. 1980).

Considerable research is underway, examining effects of such stress on lake-dwelling organisms but relatively little is directed towards fish in rivers and streams. Many fishes, however, spend part or all their lives in running water, where they must resist the downstream movement of current and hold position in order to survive and reproduce. Volume of discharge and water velocities are high during spring run-off, when input of acids from snowmelt is greatest, producing the lowest annual pH level (Jeffries et al. 1979).

When exposed to increased water velocity, fish swim faster, holding position and generating hydrodynamic lift from the camber of their bodies and paired fins (Webb 1975, Magnuson 1978). As a result, total lift (hydrodynamic and static) may exceed that required to offset weight. To counter this, fish can swim at a negative angle of attack (Berezay & Gee 1978), reducing lift, but at the expense of additional drag and higher energetic costs (Alexander 1972). Alternatively, fish can reduce total lift by decreasing static lift, or buoyancy (Saunders 1965, Neave et al. 1966, Gee et al. 1974) with reduced energetic costs (Alexander 1972). Similarly, when fish encounter lower velocities or still water, hydrodynamic forces are reduced, and fish must generate lift by increasing swimbladder volume or hydrodynamic forces (swimming) to maintain position in the water column. Shifting the primary source of lift rapidly by altering swimbladder volume is adaptive in terms of reducing drag because swimbladder lift is more efficient when swimming slowly in still water, and hydrodynamic lift is most efficient at fast swimming speeds when holding position in current (Gee 1983).

Physostomes can change swimbladder volume by gulping or secreting gas (inflation) or spitting and reabsorbing gas (deflation). Minor volume changes are also possible by altering the internal pressure of swimbladder gases (McCutcheon 1962). Both secreted and reabsorbed gases are of

characteristic composition (Steen 1970). Consequently, changes in swimbladder gas composition are indicative of the mechanism(s) employed in adjusting buoyancy. When access to air is denied (e.g. ice cover) or is undesirable (e.g. risk of predation), increases in swimbladder volume are restricted to gas secretion.

Three measurable components of buoyancy adjustment are of ecological significance. First the extent of adjustment (minimum buoyancy in current or maximum buoyancy in still water after exposure to current) ultimately affects the efficiency of locomotion and holding position. Secondly, the rate of increase or decrease in buoyancy is also important for the same reason. Finally, the ability to maintain the minimum buoyancy over an extended time (weeks) is critical for those species living in rivers where spring run-off takes place over a long period. These components of buoyancy adjustment are affected by individual characteristics (size, age etc.) and environmental factors (temperature, photoperiod etc.) (Saunders 1965; Neave et al. 1966; Pinder & Eales 1969; Gee 1972, 1977; Machniak & Gee 1975; Berezay & Gee 1978; Luoma & Gee 1980; Stewart & Gee 1981). Data pertaining to the effects of environmental pollutants, specifically water acidity, are not available. However, a number of studies have demonstrated severe physiological (e.g. haematological) disturbances encountered by fish exposed to water at reduced pH (see review by Wood & McDonald 1982). Con-

sidering the intimate link between the mechanisms responsible for gas reabsorption and secretion in the swimbladder, and the chemical, as well as physical properties of fish blood (see review by Steen 1970), the potential for impaired function of these processes becomes apparent.

Objectives of this research were to analyse effects of a reduced pH on buoyancy and related swimbladder parameters, and behavior. Particular attention was directed to effects on (1) extent and rate of buoyancy adjustment, (2) behavioral compensation of insufficient buoyancy, (3) the ability to maintain minimum buoyancy over extended time, and (4) the swimming performance in current.

The subject of the study was the fathead minnow (Pimephales promelas Rafinesque), a physostomous cyprinid, common to lakes and rivers in central North America where also environmental acidification occurrs (Scott & Crossman 1973). This species is sensitive to reduced water pH. Its reproduction may be affected at values as high as pH 6.0, while lethal levels seem to lie almost 2 pH units lower (Mount 1973). Also, fathead minnows can alter swimbladder volume extensively in response to changes in water velocity (Gee 1977).

MATERIALS AND METHODS

Fathead minnows, Pimephales promelas, were collected from the Brokenhead R., Manitoba, in September 1982 and thereafter from City of Winnipeg water retention ponds as required. Fish were held in fiberglass tanks at 11 (± 1°C) under a 12L:12D photoperiod and fed Tetramin flakes daily. Prior to each set of experiments fish were acclimated to 19.5 (± 1.5°C) by daily increments of 1°C until the required temperature was attained. Only fish with a coefficient of condition (K) (Luoma & Gee 1980) of 1 (range 0.94 - 1.02) and a total length between 5.05 and 7.10 cm were used in experiments. $K=(W/L^3)x100$, where W is wet weight in air (± 0.01 g) and L is total length (± 0.05 cm).

A. Experimental facilities

At least 10 d prior to testing, fish were transferred to 80-L holding aquaria and acclimated to a 14L:10D photoperiod. Light phase (40 Watt bulbs) extended from 07:30 to 21:30. Experiments were conducted in either still water tanks with or without access to air or in running water stream tanks always with access to air. Still water condi-

tions were created in glass aquaria (55 x 40 x 43 cm) using a gently bubbling airstone. Access to the surface and air bubbles were denied by use of 3 mm mesh plastic screens (fish held only in still water) or by submersing smaller aquaria (49 x 25 x 28 cm), covered with a perforated plastic lid, within the still water tanks (fish held in still water after transfer from current). Great care was taken to eliminate air bubbles within the smaller aquaria. In stream tanks (85 x 45 x 57 cm), current was created using a modification of the design by Gee & Bartnik (1969), in which fish were held in a current chamber (48 x 33 cm) with plastic screens at both ends, and plexiglas sides and bottom. Water depth varied from 2.5 to 3.5 cm, depending on water velocity. Metal components did not come in contact with the test water. An adjustable plastic shield fastened along the front of the chamber was used to direct flow. Fish were observed from behind a blind. Current speed was determined by averaging 10 measurements from six different locations, each taken 2 cm from the bottom using an Ott current meter (Type C1). The locations represented equidistant points 3 cm behind the front screen and 3 cm ahead of the rear screen. Front measurements always indicated higher water velocities. The maximum gradient between any front and rear measurements, however, never exceeded 7 cm/s. Water velocities were given as mean and range.

Dechlorinated Winnipeg City water was used for all experiments. Fish were held and tested at nominal pH values of either 7.7 (control) or 5.3 (treated) and received at least 2 L of water per gram body weight each day. A pH of 5.3 was chosen because (a) it is sufficient to cause sublethal stress in fathead minnows but does not affect their survival (Mount 1973), and (b) more research at the upper end of the "pH range of ecological significance" (pH 4.0-6.0) is required (Wood & McDonald 1982). For controls, two still water and one current aquaria received their inflow directly from the tap. Water at pH of 5.3 was provided to three identical aquaria as part of a circulation system, supplied from a 450 L storage tank via a 8 L mixing chamber. A float valve controlled water inflow to the storage tank. From there water was pumped (March Magnetic Drive Pump,Model 2CP-MD) into the mixing chamber from which aquaria were fed by gravity. The aquaria discharged back into the storage tank where water received sufficient amounts of H_2SO_4 and NaOH to maintain a pH of 5.3. On initial filling, storage tank water was acidified to pH 4.0, air-stripped of excess CO_2 for 2 d and back-titrated to the test pH with NaOH. The acid or base was delivered from a source through an automatic controller monitor (Chemcadet Model 5652-00, Cole-Parmer Instrument Co.) and two peristaltic pumps (Masterflex). A pH combination electrode (Cole-Parmer K-5991-12) continuously measured pH in the mixing chamber. In addition pH in all aquaria was recorded twice daily and adjusted manually if required (Table 1).

Water in the storage tank was filtered and continously aerated to equilibrate with atmospheric CO_2 and maintained at 19.5 ($\pm$ 1°C) by a thermostat and a heater. Depending on the duration of the experiment, water was exchanged from the circulation system after 16 - 47 d . Water was tested from control and treated aquaria 10 d after the start of each set of experiments (Table 2) using standard analytical methods (Stainton et al. 1977).

TABLE 1

Variation in pH over all experiments

	Nominal pH	
	7.7	5.3
Still water aquaria		
Mean	7.68	5.31
Range	7.51-7.78	5.22-5.46
Stream tanks		
Mean	7.91	5.33
Range	7.70-8.24	5.20-5.48

TABLE 2

Chemical characteristics of the experimental water. Values are means (n=5) with ranges given in parentheses. n=1 for stream tanks.

		pH 7.7		pH 5.3	
Analysis	unit	still water	stream tank	still water	stream tank
NH_4-N	$\mu g \cdot L^{-1}$	37.5 (30.0-50.0)	30.0 -	1848 (760-2800)	1240 -
Na^+	$mg \cdot L^{-1}$	1.94 (1.80-2.18)	2.07 -	10.17 (2.37-38.40)	2.31 -
K^+	$mg \cdot L^{-1}$	1.37 (1.30-1.46)	1.42 -	2.13 (1.46-2.90)	1.62 -
Ca^{2+}	$mg \cdot L^{-1}$	20.5 (13.6-22.9)	18.7 -	26.8 (15.1-36.8)	25.0 -
Mg^{2+}	$mg \cdot L^{-1}$	6.57 (5.19-9.28)	8.16 -	7.96 (6.31-10.9)	6.14 -
Cl^-	$mg \cdot L^{-1}$	4.83 (3.50-7.50)	6.07 -	6.38 (0.60-15.0)	5.10 -
SO_4^{2-}	$mg \cdot L^{-1}$	5.35 (3.50-7.20)	3.60 -	101.8 (13.9-175.0)	83.5 -
DIC	$\mu M \cdot L^{-1}$	1238 (690-1600)	1380 -	36 (20-50)	50 -
Con-25C	$\mu S \cdot cm^{-1}$	170 (160-190)	180 -	298 (220-450)	210 -
pH		7.72 (7.62-7.76)	8.19 -	5.35 (5.22-5.46)	5.44 -

Treated fish were initially exposed to increased hydrogen ion concentrations by supplying storage tank water (via the mixing tank) to the experimental aquaria at a rate of 100 mL/min, depressing pH from 7.7 to 5.3 within 2-3 d. Fish were then acclimated to pH 5.3 for at least 4 d and were not fed for 24 h prior to examination, or for 12 h prior to transfer to the stream tanks. Fish were sampled randomly and those with well-developed secondary sexual characteristics were discarded. In the stream tanks an acclimation period of 12 h was allowed before initiation of water current. All experiments started at 08:30 each day, minimizing possible effects of diel activity rhythms which may have complicated the interpretation of behavioral observations. With the exception of the swimming performance experiment, water velocity was increased every 15 min for 1 h until the required value was reached.

Fish mortality was recorded daily in the still water aquaria and is presented as percent dead fish/week. Fish mortality (percent number of stocked fish) in the stream tanks was recorded three times daily.

B. Description of variables examined

Swimbladder variables determined were buoyancy, standard volume (STV) (Gee 1970), internal pressure of swimbladder gases (IP) and swimbladder gas composition (SGC). To measure these, groups of 3-5 fish were captured by dip net and transferred to a 1 L container filled with a 0.03% solution of the anesthetic MS 222 (triacaine methanosulphate). Fish were immediately denied surface access to prevent accidental air gulping. Within 3-4 min after transfer to the anesthetic, the immobilized fish were processed (8 min/fish). Temperatures of the anesthetic solution and the water bath in which measurements were taken were similar ($\pm$ 2°C) to those in the experimental tanks.

Buoyancy was calculated (Gee 1970), by dividing the swimbladder volume ($\pm$ 0.001 mL) by the weight of the gas-free fish in water ($\pm$ 1.0 mg), where 1.0 mL/g is equivalent to neutral buoyancy. Fish were made gas-free by removing the swimbladder and all extraneous bubbles under water. The volume of the swimbladder was the difference in weight between the intact fish in water and the gas-free fish in water, as water density was assumed to be 1.0 g/mL. The volume of the gas released from the swimbladder at atmospheric pressure (Pa) was measured by rupturing the swimbladder wall and capturing the gas under water with a watch glass suspended from an under-the-balance hook. The difference in weight (g) of the watch glass before and after the gas was

released was equivalent to the gas volume (mL), as in water 1 mL of gas will support a weight of 1 g. The released volume of gas, corrected to 101.3 kPa (standard pressure) was divided by the weight of the gas-free fish in water to provide a measure of the weight specific gas volume inside the swimbladder (STV). IP was calculated by dividing the volume of the free swimbladder gas at atmospheric pressure by the swimbladder volume.

To measure SGC, the anesthetized fish was dissected to expose the swimbladder and the tip of a 100 µL gas-tight Hamilton syringe was inserted into the free end of the posterior lobe. Whenever the ductus communicans was closed light pressure applied on the anterior lobe induced it to open, thus allowing gas to be sampled from both lobes. Gas sample volume varied between 15 and 100 µL . Immediately after sampling, the syringe was inserted into a rubber septum cap to prevent leakage, and gases were analysed within 30 min using a Carle model 8700 gas chromatograph equipped to measure respiratory gases (Column 1: one Porapak QST 50/80 mesh, column 2: one molecular sieve 5 A 42/60 mesh). As CO_2, O_2 (plus Ar) and N_2 passed the thermal conductivity detector in the gas chromatograph, it produced a peak for each fraction on a chart recorder (Fisher Recordall Series 5000). To relate the peak shape to the sample concentration, the peak area, representing the amount of gas present, was calculated using the following formula:

AREA = PEAK HEIGHT X WIDTH AT HALF PEAK HEIGHT

Before and after each group of measurements, two calibration samples were injected into the gas chromatograph using 50 uL of a known gas mixture (10.0 % CO_2, 50.2 % O_2 and Ar (inseperable) and 39.8 % N_2). For each gas, a mean (n=4) value for area per unit gas volume ($mm^2/\mu L$) was calculated. This figure was then used to compute the volume of each gas present in the swimbladder, thus enabling conversion into volume percentage composition.

Fish in still water and negatively-buoyant fish, transferred from current into still water, were observed to determine effects of water acidity on mechanisms of increasing lift, particularly the relative importance of hydrodynamic, compared to static lift.

Except when gulping air, most of the fish in still water hovered in midwater while adopting a horizontal or slightly inclined position with the caudal peduncle above the head. Fish then beat their pectoral fins and, to a lesser extent, used undulating movements of the lower lobe of the caudal fin to maintain position in the water column. The pectoral fins beat synchronously with minor apparent differences in amplitude and phase difference between individual fin rays. This motion is termed oscilliatory by Blake (1983). The effective stroke of the beat was downwards. After transfer from current, fish sometimes rested on the bottom of the aquarium for extended periods of time and even returned there after gulping air.

Behavioral records included number of air gulps and unsuccessful air gulp attempts (access to air) or surfacing attempts (no access to air) per fish, pectoral fin beat frequency (PFBF) and number of fish resting on the bottom of the aquarium. In addition, the frequency (beats/min) of opercular beats was randomly recorded from different fish approximately 20 times for 4-10 s during the first and last h of each time series experiment. This provided some indication of the rate of recovery from exercise and handling stress.

An air gulp was recorded when a fish broke the meniscus with its mouth. An air gulp attempt was recorded when a fish swam vertically, but stopped short of the surface. Only those observations in which the caudal fin broke the surface during the attempt were recorded. Taken together, these two parameters represented the number of surface exposures. The PFBF was measured by counting each paired stroke (down and up) over a time period varying from 5 - 60 s, while the fish was hovering. Possible variation in beat amplitude was not taken into account. Care was exercised that each fish in a group was represented with equal numbers of observations, however, in some cases counts could not be obtained from one or two individual fish during a particular time period. To minimize bias in those cases, recordings on all other fish in that group were restricted to two observations.

The number of fish resting was measured by counting each fish resting on the bottom of the aquarium every 5 min on the minute and was expressed as percent of the number of fish in the group times the number of 5 min intervals.

In still water, without access to air, recordings were described as above. Instead of the number of airgulps, however, the number of surfacing attempts was recorded, representing observations when fish touched the cover of the submersed aquarium or the floating screen.

As it was impossible to attribute each surface exposure or surfacing attempt to a known individual, the mean frequency per fish was calculated by dividing the total number of air gulps, air gulp attempts or surfacing attempts in a given time period by the number of fish in the group. Similarly, swimbladder parameters could not all be measured on the same fish. Therefore experiments requiring SGC measurements were repeated, the first (buoyancy experiment) providing results on buoyancy, STV and IP, the second (gas composition experiment) provided data on SGC and buoyancy.

C. Experimental procedures

1. Extent of buoyancy adjustment

To determine the extent to which water acidity affected buoyancy, STV, IP, SGC or behavior, groups of 6-21 control and treated fish were held in still water with and without access to air for 10-25 d. All behavioral observations were performed between 19:00 and 20:00 on the evening before the day of sampling.

Groups of 14-16 control and treated fish were also held in current for 48 h after acclimation to specific pH conditions in still water to determine if reduced ambient pH affected the extent of buoyancy and STV adjustment or IP and SGC. Water velocity was 34.1 ($\pm$ 3.2 cm/s) for the treated, and 35.3 ($\pm$ 3.5 cm/s) for the control fish.

2. Rate of short-term buoyancy adjustment

The rate of adjustment of the measured swimbladder parameters was determined in time series experiments. In these, groups of fish were sampled and measured after the termination of predetermined time intervals which started with the initiation of current or the transfer from current to still water.

a) Decrease in buoyancy.

To determine whether the rate of decrease in buoyancy and STV or IP are affected by water acidity, fish were examined in groups of 10-15 after 0 (still water),1,3,6,12,24 and 48 h in current. Water velocity was as above.

b) Increase in buoyancy.

Effects of acidity on rate of increase in buoyancy was determined by holding groups (n=11-17) of fish in control and treated conditions for 48 h in current and then transfering them to still water. One group had access, a second was denied access to air. Fish were examined after 1,3,6,12,24 and 48 h in still water (48 h only for "non-access" fish), and buoyancy, STV, IP and SGC were recorded. Behavior was also examined to assess mechanisms by which fish overcome a less than optimal total lift. Water velocities for "non-access" fish were 35.2 $\pm$ 3.4 cm/s (control) and 34.6 $\pm$ 3.2 cm/s (treated). As approximately 40% of the "non-access" fish in the buoyancy experiment died after 48 h in current (Table 3), resulting in small sample sizes, a water velocity of 25.8 $\pm$ 2.9 cm/s (control) and 26.4 $\pm$ 3.2 cm/s (treated) was used for "access" fish and all fish of the gas composition experiment. In agreement with results provided by Berezay and Gee (1978), test experiments indicated that the measured swimbladder parameter were not sig-

nificantly affected by this change in water velocity (App.1).

Behavioral observations in the buoyancy experiment of "non-access" fish were first made on the swimming angle of attack, which was found to be strongly correlated with buoyancy in studies by Berezay & Gee (1978) and Stewart & Gee (1981). This line of investigation, however, proved to be impractical as fathead minnows removed from current did not swim at a discernible angle of attack in still water. Therefore no behavioral data were obtained for this experiment. All subsequent behavioral observations were made continuously for the first hour (two 30 min intervals) in still water after transfer from current, and then for 30 min intervals commencing at 2,5,11,23 and 47 h, if applicable.

As only the last experiment (24 h in "access" fish or 48 h in "non-access" fish) of each time series provided behavioral data for all time periods, this recording procedure lead to declining sample sizes in the behavioral parameters within the later time periods. More importantly, the behavioral measurements did not accurately reflect the buoyancy response recorded at the end of the specific time period, because most of the measurements incorporated into the means were obtained at times when no direct buoyancy measurements could be made. In order to more accurately detect possible interdependencies between the number of air gulps or surfacing attempts, and the PFBF and buoyancy, analyses were also

made including only those behavioral observations recorded during the time period for which the corresponding mean buoyancy had been measured.

3. Ability to maintain a minimum buoyancy over time

To determine whether water acidity affected the ability to maintain a minimum buoyancy and STV, or a particular IP over an extended period of time, fish were examined in groups of 11-17 after 1,2,3,6,12,20 and 32 days in current. Water velocity was 25.8 $\pm$ 2.9 cm/s for the control, and 26.4 $\pm$ 3.2 cm/s for the treated fish. While in the current chamber, fish were fed three times daily, the last feeding 48 h before examination.

4. Swimming performance

If the hypothesis that optimal buoyancy regulation saves energy is correct (Gee in press), then differences in ability to modulate buoyancy between control and treated fish should be reflected in swimming performance and condition after exposure to current. To measure these, four batches (n=10) of fish for each pH treatment were sampled from the still water aquaria, anesthetized in MS 222, and their coef-

ficient of condition before the start of the experiment (KB) was determined. Sketches showing characteristics of individual fishes were made, enabeling later identification. Fish were then placed into the current chamber which had neglible water flow (2 cm/s) and were allowed to acclimate for 12-14 h.

At 08:15 on the day of testing a gentle current (10 cm/s) was initiated. Starting at 08:30, water velocity was increased every 2 h to 15.2 $\pm$ 2.5, 25.3 $\pm$ 3.5, 35.2 $\pm$ 3.5, 43.7 $\pm$ 4.6 and 57.5 $\pm$ 5.5 cm/s, respectively. Fish were observed almost continuously and individuals resting against the back screen for 60 s without reacting to a gentle prodding were removed. As fish were not able to tolerate the highest water velocity for the full 2 h, the experiment was terminated after the last fish had been taken out. The swimming time for each fish was recorded from 08:30 to the nearest 15 min of the time of exhaustion together with its weight, buoyancy and IP. For individual fish the new factor of condition after the termination of the experiment (K_A) was calculated, using the length measurements taken before the start, and the weight measurements taken after the termination of the experiment.

D. Statistical analyses

Statistical analyses, except tests of variance equality, were done on an Amdahl 5850 computer using the Statistical Analasis System (SAS Institute Inc. 1982). Differences between pH treatments and between time periods were tested by analasis of variance (ANOVA), Student's t-test or least square regression analysis. As observations on surfacing behavior and PFBF were made for each time period of a time series experiment (see B.3.), repeated set of results were obtained for the same experiment but with different fish, ranging from 4 (access) and 5 (no access) at 0.5 and 1 h to zero repeats at 24 h (access) and 48 h (no access). Therefore surfacing behavior and PFBF were also tested for 'repeat' effects. If the ANOVA test revealed that group means were significantly ($p<0.01$) different, orthogonal comparisons using F-tests as tests of significance were applied to distinguish differences between means for control and treated fish for each time period. Duncan's new multiple range test was used to test differences between time intervals within pH treatments. A probability level of ≤ 0.05 was considered significant.

To obtain information on the rate of change in given parameters, these were regressed against time with the independent variable being transformed to $\sqrt{X}$ and $\log(X+1)$. Adding one to the values allowed for time zero. Untransformed and transformed time variables and their second and

third degree powers were used to find the best fitting model. When applicable, slopes of regression models were tested for parallelism.

Homogeneity of group variances was tested applying Hartley's Fmax-test (Sokal & Rohlf 1981). As sample sizes were unequal, the lesser degrees of freedom of the two variances needed in computing the variance ratio were used (Sokal and Rohlf 1981). If variances were not homogeneous, data were log- or arcsin- transformed, which in some cases stabilized the variances. Analyses were then performed on the untransformed and transformed data. When the probability values of the test statistics were influenced by data transformation, the analysis on the transformed data was used. On a few occasions data transformation did not result in the stabilization of the variances. Then the size of the samples, their variances and the probability values were compared, and following criteria given by Glass et al. (1972), it was decided that this violation of normal theory ANOVA did not seriously affect the validity of the test statistics. For clarity of presentation, arithmetic means of the untransformed data with 95% confidence intervals have been used in all figures and tables.

RESULTS

A. Mortality

Of 1252 fish held in the still water aquaria at pH 5.3 (n=618) and pH 7.7 (n=634), 38 treated and 41 control fish died before being sampled or transferred to the stream tank. Weekly mortality rates varied between 1-5% for both groups, and did not vary with time (n=4-42 d). At pH 5.3, eight male fish showed the 'hunch-backed' deformity described by Mount (1973). The appearance of the disproportionate head was accompanied by an overall darkening of the body whereafter the fish died within 4 - 5 d . Mortality in current increased with water velocity and over time, but was independent of water acidity (Table 3).

B. Extent of buoyancy adjustment

The results for buoyancy, STV and IP of the repeated still water and 48 h current experiments generally showed that the response in these swimbladder parameters did not differ between replicated tests (App.2). ANOVA tests indicated such differences only in the buoyancies of control

(p=0.06) and treated (p=0.02) fish with access to air and in IP of treated fish in current (p=0.01). This variability could not be attributed to any known experimental factor (fish size, time spent in still water, photoperiod, temperature etc.), and for ease of comparison, overall means are be presented here.

1. Still water

With surface access, control and treated fish were similar in most swimbladder variables (Table 4). Buoyancy was slightly negative and IP was just above atmospheric in both groups. Proportions of CO_2 were similar but treated fish had significantly ($p<0.01$) higher levels of O_2 and lower levels of N_2 (Table 4, App.3). Fish beat their pectoral fins at a rate of approximately 1 beat/s, a frequency not significantly ($p=0.10$) affected by water pH (Table 4). Treated fish gulped air more frequently than controls (Table 4). Unfortunately, the nature of this observation precluded statistical analysis.

Without surface access, control fish were almost neutrally buoyant and STV ($p<0.001$) and buoyancy ($p<0.0001$) were significantly higher than for treated fish (Table 4, App.3). IP was similar in both groups. Proportions of CO_2 and O_2 were elevated ($p<0.0001$), while N_2 was lower ($p<0.0001$) in treated fish (Table 4, App.3). PFBF ($p<0.01$) and the number

of surfacing attempts (no statistical analysis possible) were higher for treated fish (Table 4).

The effects of eliminating access to air in control fish were to significantly increase buoyancy ($p<0.0001$), STV ($p<0.05$), PFBF ($p<0.05$) and % O_2 ($p<0.01$), while % N_2 decreased ($p<0.01$) (Table 4, App.3). In treated fish, PFBF ($p<0.001$) and the proportions of O_2 and CO_2 were elevated compared to controls without access ($p<0.0001$) (Table 4, App.3). Both control and treated fish without access to air swam to the water surface almost 4 and 5 times more often (no statistical analysis possible), respectively, than when access was allowed (Table 4).

2. 48 h in current

Fish reduced buoyancy in current to 48.6 % (treated) and 41.8 % (control) of still water values. Control fish were less buoyant than treated fish ($p<0.01$) (Table 5, App.4). This difference mainly resulted from a higher ($p<0.0001$) IP among control fish, as mean STV values for the both groups were similar (Table 5, App.3).

Gas compositions of control and treated fish were similar (Table 5, App.4). In current, O_2 and CO_2 percentages for both groups were less ($p<0.01$) than in still water, while those of N_2 were increased ($p=0.001$) (Tables 3-4, App.5).

TABLE 3

Mortality of fathead minnows during exposure to,flowing water at velocities of 35 and 26 $cm \cdot s^{-1}$.

Experiment	Exposure time (h)	Mortality (%) pH 7.7	pH 5.3
a) water velocity 35 $cm \cdot s^{-1}$			
Decrease in buoyancy	1	0	0
	3	9.1	0
	6	10.0	0
	12	0	16.7
	24	27.3	8.3
	48	25.0	33.3
Increase in buoyancy; buoyancy experiment -no access to air	48	42.6	43.2
b) water velocity 26 $cm \cdot s^{-1}$			
Increase in buoyancy; buoyancy experiment -access to air	48	12.3	10.9
gas composition experiment -no access to air	48	4.6	6.6
gas composition experiment -access to air	48	10.0	6.1
Ability to maintain a minimum buoyancy	24	0	0
	48	27.3	8.3
	72	21.4	0
	144	11.8	18.2
	288	33.3	30.7
	480	35.7	21.4
	768	37.5	31.2

NOTE: A total of 906 fish were observed, with mortality values being based on 10-75 observations.

TABLE 4

Mean buoyancy, STV, IP, SGC, PFBF and number of air gulps (AG) or surfacing attempts (SA) per fish for fathead minnows in still water.

	access to air		no access to air	
	pH 7.7	pH 5.3	pH 7.7	pH 5.3
Buoyancy ($mL \cdot g^{-1}$)	0.922 ±0.022 (n=51)	0.899 ±0.020 (n=52)	0.982[b] ±0.008 (n=26)	0.871[a] ±0.022 (n=25)
STV ($mL \cdot g^{-1}$)	0.864 ±0.062 (n=11)	0.882 ±0.029 (n=31)	0.947[b] ±0.016 (n=12)	0.860[a] ±0.035 (n=12)
IP (atm)	1.012 ±0.007 (n=11)	1.003 ±0.006 (n=31)	1.008 ±0.010 (n=12)	1.001 ±0.011 (n=12)
CO_2 (%)	2.86 ±0.32 (n=17)	2.52 ±0.40 (n=16)	3.10 ±0.54 (n=14)	4.88[a b] ±0.48 (n=13)
O_2 (%)	13.81 ±3.14 (n=17)	23.84[a] ±4.13 (n=16)	20.57[b] ±4.34 (n=14)	45.51[a b] ±5.36 (n=13)
N_2 (%)	83.33 ±3.16 (n=17)	73.63[a] ±4.35 (n=16)	76.32[b] ±4.73 (n=14)	49.60[a b] ±5.60 (n=13)
PFBF ($beats \cdot min^{-1}$)	58.88 ±5.45 (n=25;n_1=68)	66.34 ±7.50 (n=24;n_1=50)	68.84[b] ±7.16 (n=12;n_1=32)	91.74[a b] ±15.34 (n=12;n_1=27)
AG or SA ($\cdot fish^{-1} \cdot h^{-1}$)	0.31 – (n=13;n_1=4)	1.14 – (n=14;n_1=16)	1.08 – (n=12;n_1=13)	5.08 – (n=12;n_1=61)

NOTE: Means ± 95% CI; n= number of fish tested, n_1= number of observations with repeated measurements.

[a] significantly ($p<0.05$) different from pH 7.7

[b] significantly ($p<0.05$) different from access to air at the same pH

TABLE 5

Mean buoyancy, STV, IP, and SGC for fathead minnows after 48 h in current.

	pH 7.7	pH 5.3
Buoyancy (mL g^{-1})	0.385 +0.024 (n=42)	0.437 +0.028 (n=50)
STV (mL g^{-1})	0.407 +0.033 (n=30)	0.431 +0.039 (n=35)
IP (atm)	1.054 +0.014 (n=30)	1.001 +0.016 (n=35)
CO_2 (%)	1.19 +0.20 (n=11)	1.24 +0.17 (n=13)
O_2 (%)	8.02 +2.27 (n=11)	9.53 +1.75 (n=11)
N_2 (%)	90.81 +2.25 (n=11)	89.23 +1.67 (n=11)

NOTE: Means + 95% CI; n= number of fish tested.

significantly ($p<0.01$) different from pH 7.7

C. Rate of short-term buoyancy adjustment

1. Decrease in buoyancy

When exposed to current for 48 h, treated and control fish reduced buoyancy from 0.905 to 0.452 mL/g, and from 0.876 to 0.380 mL/g, respectively (Fig.1A, App.5), with parallel changes recorded in STV (Fig.1B, App.5). Both time spent in current and water pH affected buoyancy ($p<0.0001$), with the result that, commencing at 6h, control fish had a consistantly lower buoyancy ($p<0.05$) than treated fish (Fig.1A, App.6). Similar results were found for STV, although differences between groups were only significant at 6,12 (borderline) and 24 h (Fig.1B, App.6).

Although curvilinear models (App.7) best described the regression of buoyancy and STV against time, a linear model, which explained 4% (buoyancy) or 5% (STV) less of the variability of the data than the curvilinear model, was used to compare slopes. Control fish reduced buoyancy ($p<0.05$) and STV ($p<0.09$) faster than treated fish (App.8), as indicated by the following regression equations (h=time in hours):

Buoyancy (Y):

Control $Y = 0.816 - 0.299 \log h$ ($r^2=0.74$)

Treated $Y = 0.830 - 0.240 \log h$ ($r^2=0.68$)

STV (Y):

Control $Y = 0.789 - 0.278 \log h$ ($r^2=0.70$)

Treated $Y = 0.810 - 0.228 \log h$ ($r^2=0.63$)

IP fluctuated considerably over time in current (0-48 h) and followed similar patterns in control and treated fish (Fig.1C). A two-way ANOVA indicated that IP was significantly affected by time ($p<0.0001$) and the interaction of time and water pH ($p<0.05$) (App.6).

2. Increase in buoyancy

After return to still water conditions with access to air, control and treated fish reached maximum buoyancy and STV after 6-12 h (Fig.1D,E; App.9). Water pH did not significantly affect these two swimbladder parameters over 1-24 h, but the interaction of time and pH was significant ($p<0.001$) (App.10). At 6h, treated fish were more ($p<0.01$) buoyant, at 24 h they were less ($p=0.06$) buoyant than controls. However, this pattern was not confirmed by the results of the gas composition experiment. In this, time and pH did not interact in affecting buoyancy and treated fish were less buoyant than controls at 6h ($p<0.05$), but equally buoyant at 24 h (App.10). Once maxima were attained, buoyancy of fish of both groups in the two experiments did not change significantly during subsequent hours of observation (Fig.1D, App.9). The adjustment in buoyancy and STV over 0-24 h was best described by polynomial models, which explained over 90% of the variability of the data (App.11).

Figure 1. Temporal changes in buoyancy (A,D,G), STV (B,F,H) and IP (C,F,J) in fathead minnows exposed to pH 5.3 (solid symbols) and pH 7.7 (open symbols) in current and in still water after current. Means ($\pm$ 95% CI) of 8-14 (except where indicated) fish are plotted. Means significantly different from control values are indicated with an asterisk. Time is given on a log scale but actual values (h) are shown.

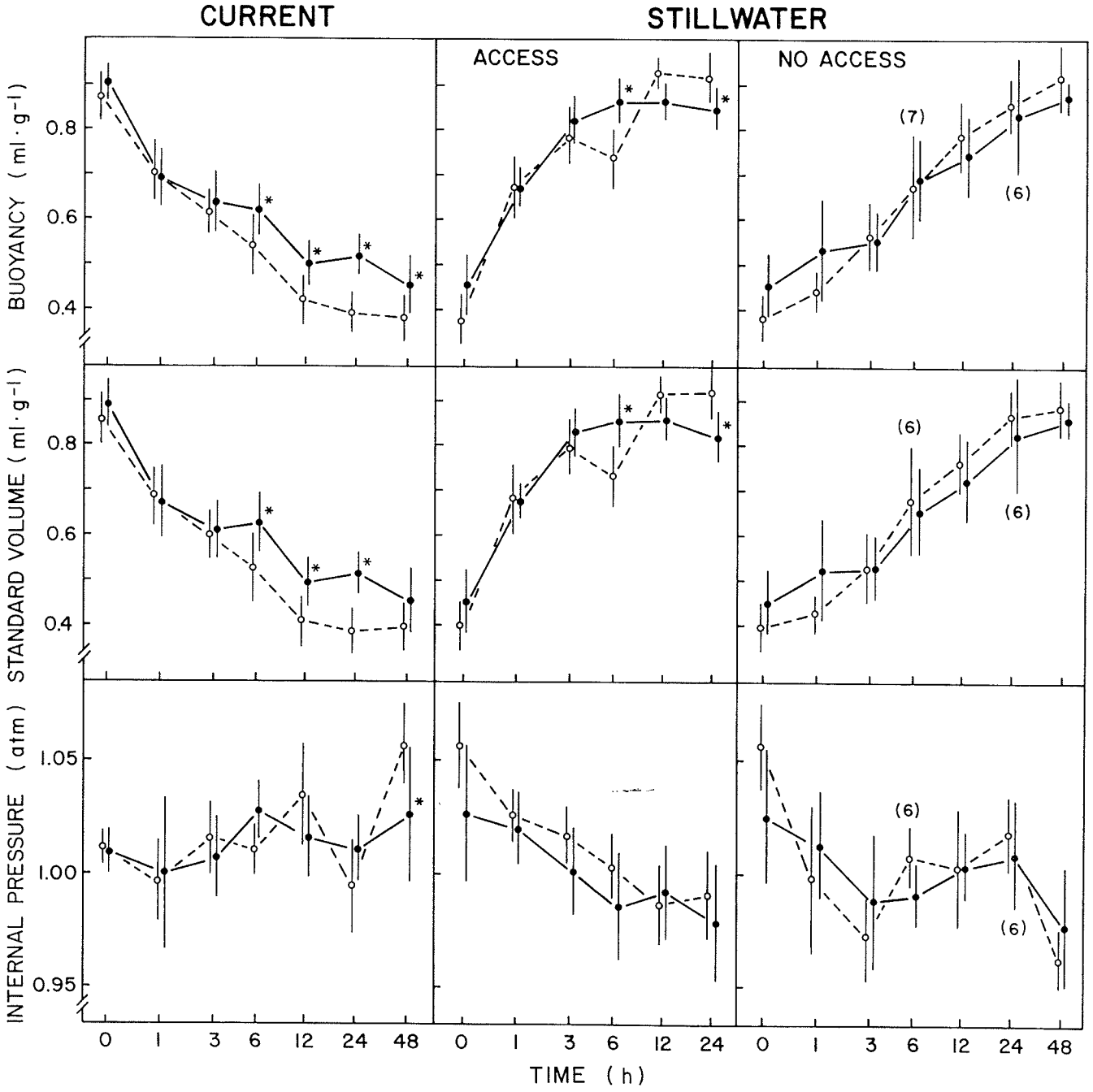
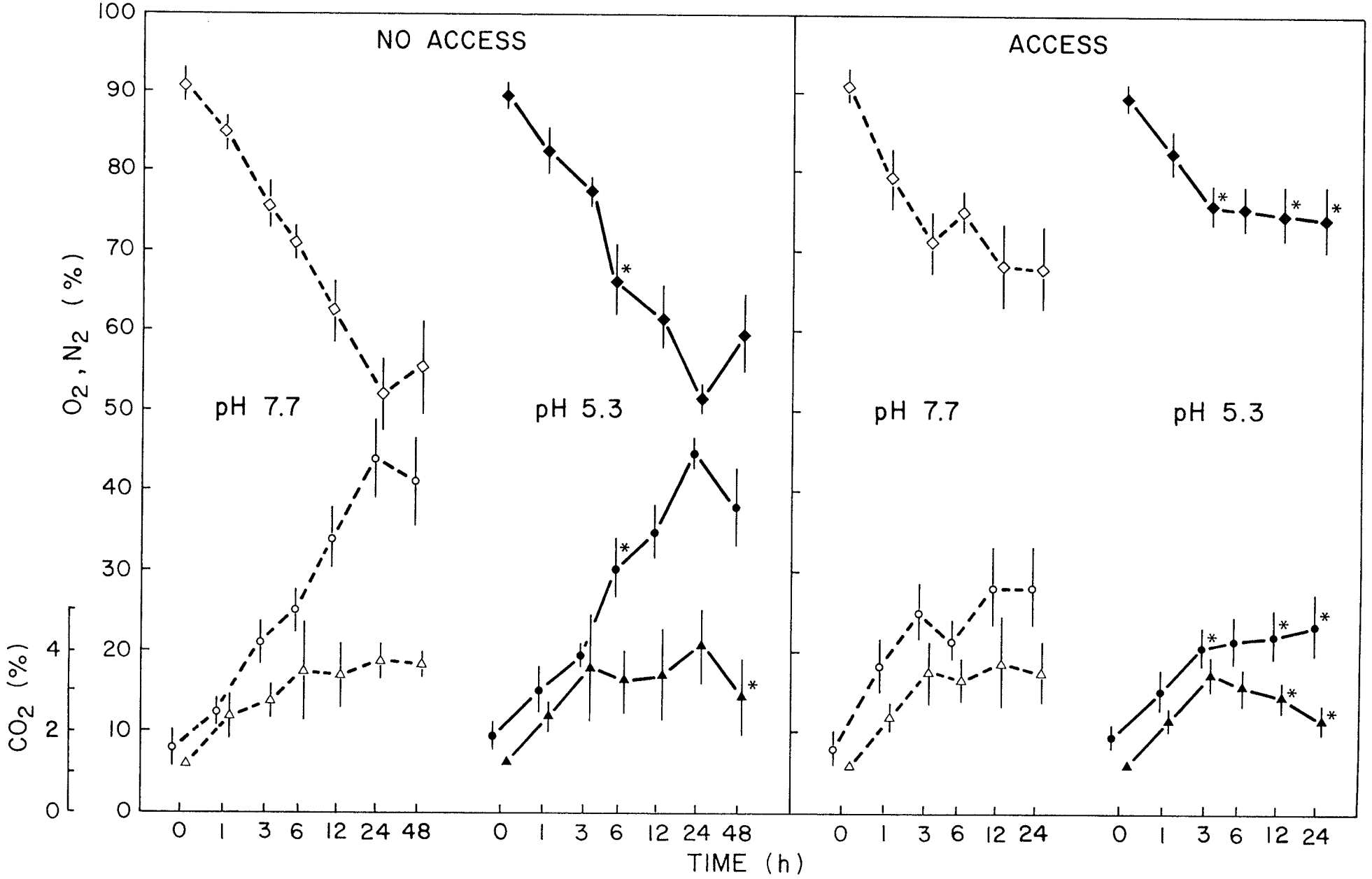

CURRENT
STILLWATER
ACCESS
NO ACCESS
BUOYANCY (ml · g⁻¹)
STANDARD VOLUME (ml · g⁻¹)
INTERNAL PRESSURE (atm)
0.8
0.6
0.4
1.05
1.00
0.95
(7)
(6)
0 1 3 6 12 24 48
TIME (h)

Figure 2. Temporal changes in the percent volume of swimbladder gases of control (open symbols) and treated (closed symbols) fathead minnows during the return to neutral buoyancy in still water. Points represent means ($\pm$ 95% CI) of 10-14 fish for CO_2 (triangles), O_2 (circles) and N_2 (diamonds). CI < 0.2 are not shown. Means significantly different from control values are indicated with an asterisk. Time is given on a log scale but actual values (h) are shown.


NO ACCESS
ACCESS
pH 7.7
pH 5.3
pH 7.7
pH 5.3
O_2, N_2 (%)
CO_2 (%)
TIME (h)
0 1 3 6 12 24 48
0 1 3 6 12 24
0 1 3 6 12 24 48
0 1 3 6 12 24
0 10 20 30 40 50 60 70 80 90 100
0 2 4
*

IP in treated and control fish decreased linearly with the logarithm of time (0-24 h; regression analysis, App.12) to less than atmospheric values (Fig.1F). The slopes of the regression lines were similar (App.12).

When fish had access to air, water pH affected changes in CO_2 ($p<0.05$), O_2 ($p<0.01$) and N_2 ($p<0.001$) percentages (Fig.2, App.10). The increase in the proportion of CO_2 and O_2 for treated and control fish during the first hour accounted for approximately 50% of the total increase of the respective gases. In both groups of fish, maximum CO_2 and O_2 percentages were reached after 3 or 12 h, respectively. With the exception of the % CO_2 of treated fish, which declined over 6-24 h (regression analysis, $p<0.0001$), these maxima were maintained over the remaining time periods.

On return to still water without access to air, control fish increased buoyancy from 0.380 to 0.917 mL/g, and STV from 0.394 to 0.880 mL/g (Fig.1G,H; App.13). The corresponding values for treated fish were 0.452 to 0.876 mL/g (buoyancy) and 0.453 to 0.853 mL/g (STV). For fish of both groups, buoyancy and STV increased linearly with the logarithm of time (App.14), as indicated by the following regression equations (h=time in hours):

Buoyancy (Y):

Control $Y = 0.370 - 0.341 \log h$ ($r^2=0.81$)

Treated $Y = 0.443 - 0.261 \log h$ ($r^2=0.64$)

STV (Y):

Control $Y = 0.370 - 0.325 \log h$ $(r^2=0.80)$

Treated $Y = 0.437 - 0.249 \log h$ $(r^2=0.61)$

The slopes of the regression equations were significantly different (App.8), as control fish increased both buoyancy and STV more rapidly then treated fish. Similar results to those given above for the buoyancy experiment were obtained from the gas composition experiment, and probability values for comparisons of the slopes of the regressions indicated the same significance levels (App.8).

Again, IP fluctuated (0.963 to 1.019 atm) considerably over time (1-48 h, Fig.1 J) with no significant difference between control and treated fish (ANOVA, App.15). Its response could be best described by a third degree polynomial model (App.14). For both groups IP reached similar minimum values after 48 h in still water, which were less than atmospheric and significantly reduced compared to those after 48 h in current. However, these minima did not differ between control and treated fish (App.15).

Concomittant with the elevation in buoyancy and STV, swimbladder O_2 and CO_2 percentages increased sharply over time (0-24 h) after transfer into still water without access to air (Fig.2, App.13). There was no indication of an overall pH effect (ANOVA, App.16). Peak concentrations of these gases were reached after 24 h, representing a more than

threefold increase in the CO_2 and an approximately fivefold increase in the O_2 percentages, compared to the minimum values at 48 h in current. The subsequent decline in the proportions of CO_2 and O_2 at 48 h was apparent for both pH treatments, and was significant ($p<0.05$) for treated fish.

During the first hour after transfer from current to still water, fish beat their pectoral fins at a rate of approximately 270 beats/min (Table 6), independent of water pH or surface access. The freqency then declined and reached minimum values during the last observation period (Table 6). For all data sets (access, pH combinations), the decline in PFBF was best described by curvilinear models, which accounted for 64 and 82 % of the observed variability ($p<0.0001$) (App.17). ANOVA tests for fish without access to air, indicated that water pH did not significantly ($p=0.12$) influence mean PFBF, however, at 6 and 12 h the frequency of treated fish was higher ($p\leq0.05$) than of controls (App.18). PFBF differed between times ($p<0.0001$) and repeats ($p<0.05$) and there was a just significant ($0.04<p<0.05$) interaction between time and repeat (App.18).

For fish with access to air, buoyancy and gas composition experiments showed different results in terms of PFBF response (Table 6). For the buoyancy experiments, ANOVA demonstrated an effect ($p<0.001$) of water pH on PFBF, for which, commencing at 12 h, treated fish showed a significantly higher frequency (App.18). No such effect on the

overall mean was apparent for fish in the gas composition experiment, however, the PFBF of treated fish at 6 h was significantly ($p<0.0001$) higher than of controls (App.18). The interactions of all three main effects (time, pH and repeat) were significant ($p<0.0001$) in the buoyancy experiment, while in the gas composition experiment pH significantly interacted with time ($p<0.0001$) and repeat ($p<0.05$) (App.18).

Upon transfer to still water, fish typically sank to the bottom of the aquarium where they rested motionless. As suggested by the frequency of opercular beats which declined from 113 (control) and 131 (treated) to 85 beats/min (both groups) and stabilized at that level during the first 60 min upon transfer, recovery from exercising and handling stress proceeded fairly rapidly. At 24 or 48 h, fish in both groups had opercular frequencies of about 55 beats/min. After 4 to 8 min in still water, individual fish started to orientate towards the water surface, making short vertical swimming bursts, returning to the bottom of the aquarium within 3 s. After 60 min these individual surface exposures or surfacing attempts became less frequent. Instead fish tended to follow other individuals, swimming to the surface in groups of 3 to 5, staying there for 2 to 8 s, while gulping or attempting to gulp air.

Fish with access to air, regardless of pH treatment, made over 85% of their air gulps within the first 3 h after

transfer to still water (Fig.3). ANOVA tests indicated that in the gas composition experiment both the number of surface exposures and air gulps differed significantly with water pH, time and replication, while in the buoyancy experiment only the effect of time was significant ($p=0.001$, App.19). The number of air gulp attempts in the two experiments was significantly affected by time or repeat, but not by water pH ($p>0.10$, Fig.4, App.19). Independent of water pH, air gulp attempts contributed 18-32% (buoyancy experiment) and 4-21% (gas composition experiment) to the number of surface exposures.

Fish without access to air swam to the surface much more frequently than fish with access (Fig.3-4). Treated fish made 80% and contol fish 85% of their attempts to gulp air during the first 6 h after transfer to still water. The ANOVA on effects of water pH, time and repeats showed that all three main effects were significant ($p<0.05$), but they did not interact (App.19).

The percentage of fish with access resting on the bottom of the aquarium declined from initial values 29% (control) and 16% (treated) in the buoyancy experiment, and 6% (both groups) in the gas composition experiment, to less than 3% after 3 to 6 h (Fig.5). Thereafter, with the exception of treated fish in the buoyancy experiment, fish usually remained swimming in the water column. In contrast, the proportions of resting, "non-access" fish did not decrease but

remained rather stable near 20% during the first 2-3 h, increasing to over 30% by 12 h. The proportions then declined rapidly, leaving almost all fish swimming at 48 h (Fig.6).

When behavioral data for all pH and access conditions were pooled, mean PFBF accounted for 71% of the variance in mean buoyancy (Table 7, Fig.7). The linear regression equation was significant ($p<0.0001$), indicating that PFBF is a useful predictor of buoyancy. There was, however, some indication that its predictive power varied depending on the data subset used. PFBF was more closely correlated to buoyancy at pH 7.7 and when access to the water surface was denied. Consequently, it explained only 63% of the variability in treated fish with access but 94% of the variability in control fish without access to air (Table 7). The introduction of the variables time and air gulps or surfacing attempts ($p=0.07$) did not significantly improve the regression equation (Table 7). This can partly be explained by the significant ($p<0.05$) correlations among these variables (eg. between air gulps ($r= -0.46$) or surfacing attempts ($r= -0.54$) and PFBF), obviously detracting from the efficiency of their use for prediction. Even when used as a single predictor of buoyancy, the number of air gulps was not significant ($p>0.05$) while the number of surfacing attempts was ($p=0.01$, Table 7).

Figures 8 and 9 summarize observations on buoyancy, PFBF and air gulps or surfacing attempts for fish adjusting their

lift in still water with and without access to air. Only behavioral observations made during the time period of consequent buoyancy measurement were used.

TABLE 6

Temporal changes in mean PFBF (beats·min^{-1}) of fathead minnows during the return to neutral buoyancy in still water.

Time intervall (h)	Buoyancy experiment		Gas composition experiment			
	Access		Access		No access	
	pH 7.7	pH 5.3	pH 7.7	pH 5.3	pH 7.7	pH 5.3
0 - 1	270.2 ± 4.4	270.6 ± 4.1	277.7 ± 4.2	269.3 ± 4.5	272.5 ± 3.4	273.0 ± 3.0
2.0-2.5	194.3 ± 8.0	206.6[a] ± 5.0	210.6 ±15.0	199.5 ±12.1	259.9 ± 4.4	265.5 ± 4.8
5.0-5.5	174.8 ± 9.4	151.6[a] ±10.6	81.6 ±13.7	117.8[a] ±10.9	237.7 ± 7.7	256.0[a] ± 4.3
11.0-11.5	85.9 ±10.2	132.0[a] ±12.1	70.9 ±15.7	67.8 ± 7.5	208.8 ±12.2	219.6[a] ±10.5
23.0-23.5	63.8 ±12.7	114.6[a] ±12.7	63.4 ±20.7	65.8 ±12.4	112.0 ±17.9	116.8 ±20.7
47.0-47.5	-	-	-	-	76.7 ± 9.9	80.7 ±17.7

NOTE: Means ± 95% CI; the analysis was based on 2397 observations of 349 fish, with 16-163 observations included in the means.

[a] significantly ($p<0.05$) different from pH 7.7

TABLE 7

Linear multiple regression analysis of factors accounting for the variation in buoyancy (dependent variable) of fathead minnows increasing lift in still water.

Independent variables	Multiple correlation coefficient	Simple correlation coefficient
a) all fish (n=32)		
PFBF (beats·min^{-1})	0.8418[a]	-0.8418[b]
Time ($\sqrt{\text{min}}$)	0.8519	+0.6017[b]
b) control fish (n=16)		
PFBF (beats·min^{-1})	0.8738[a]	-0.8738[b]
Time (log·min)	0.8863	+0.6716[b]
c) treated fish (n=16)		
PFBF (beats·min^{-1})	0.8264[a]	-0.8264[b]
Time (min)	0.8461	+0.4737[b]
d) fish with access (n=20)		
PFBF (beats·min^{-1})	0.8453[a]	-0.8453[b]
Air gulps	0.8465	+0.2966[b]
Time (min)	0.8695	+0.5486[b]
e) fish without access (n=12)		
PFBF (beats·min^{-1})	0.9199[a]	-0.9199[b]
Surfacing attempts	0.9455	+0.7036[b]
Time (log min)	0.9732	+0.9529[b]
f) treated fish with access (n=10)		
PFBF (beats·min^{-1})	-	-0.7925[b]
g) control fish without access (n=6)		
PFBF (beats·min^{-1})	-	-0.9680[b]

[a] variable does not contribute significantly to the regression equation

[b] $p \leq 0.05$

Figure 3. Effect of water pH on the mean frequency of surface exposures (full bar), air gulps (patterned portion) and air gulp attempts (blank portion) in selected 30-min time periods for fathead minnows during return to neutral buoyancy in still water of pH 7.7 (light stippling) and pH 5.3 (heavy stippling) with access to air. The number of 30-min observation intervals (n) is given across the top of the graph, and if $n \leq 3$, the upper 95% confidence limit for the mean number of surface exposures is included. Time period (after transfer to still water)

1: 1- 30 min
2: 31- 61 min
3: 120- 150 min
4: 300- 330 min
5: 660- 690 min
6: 1380-1410 min

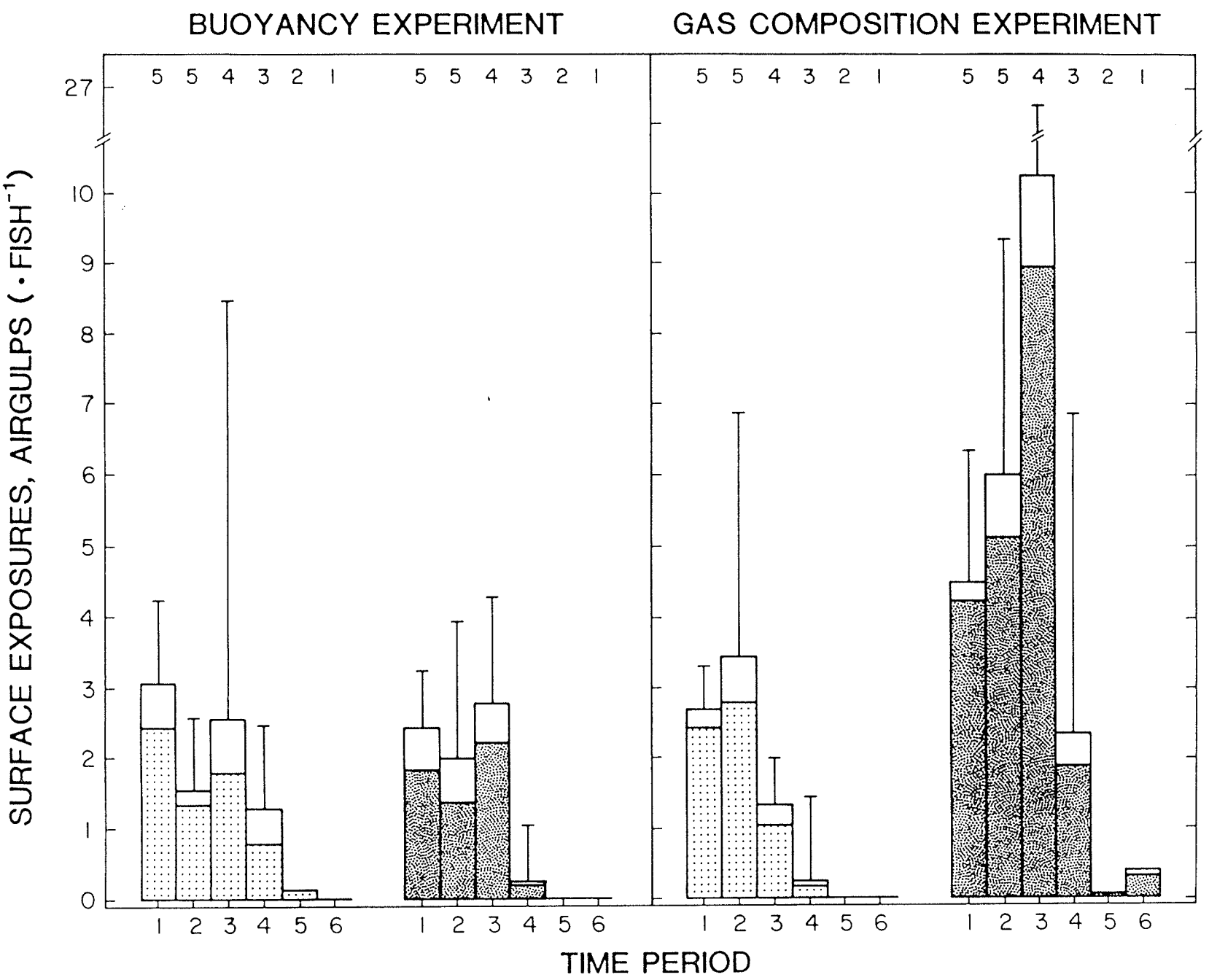

BUOYANCY EXPERIMENT
GAS COMPOSITION EXPERIMENT
SURFACE EXPOSURES, AIRGULPS (•FISH⁻¹)
TIME PERIOD
27
10
9
8
7
6
5
4
3
2
1
0
5 5 4 3 2 1
1 2 3 4 5 6

Figure 4. Effect of water pH on the mean frequency of surfacing attempts in selected 30-min time periods for fathead minnows during the return to neutral buoyancy in still water of pH 7.7 (light stippling) and pH 5.3 (heavy stippling) without access to air (gas composition experiment). The number of 30-min observation intervals is given across the top of the graph, and if $n \leq 3$, the upper 95% confidence limit for the mean is included.

Time period (after transfers to still water)

1: 1- 30 min
2: 31- 61 min
3: 120- 150 min
4: 300- 330 min
5: 660- 690 min
6: 1380-1410 min
7: 2820-2850 min

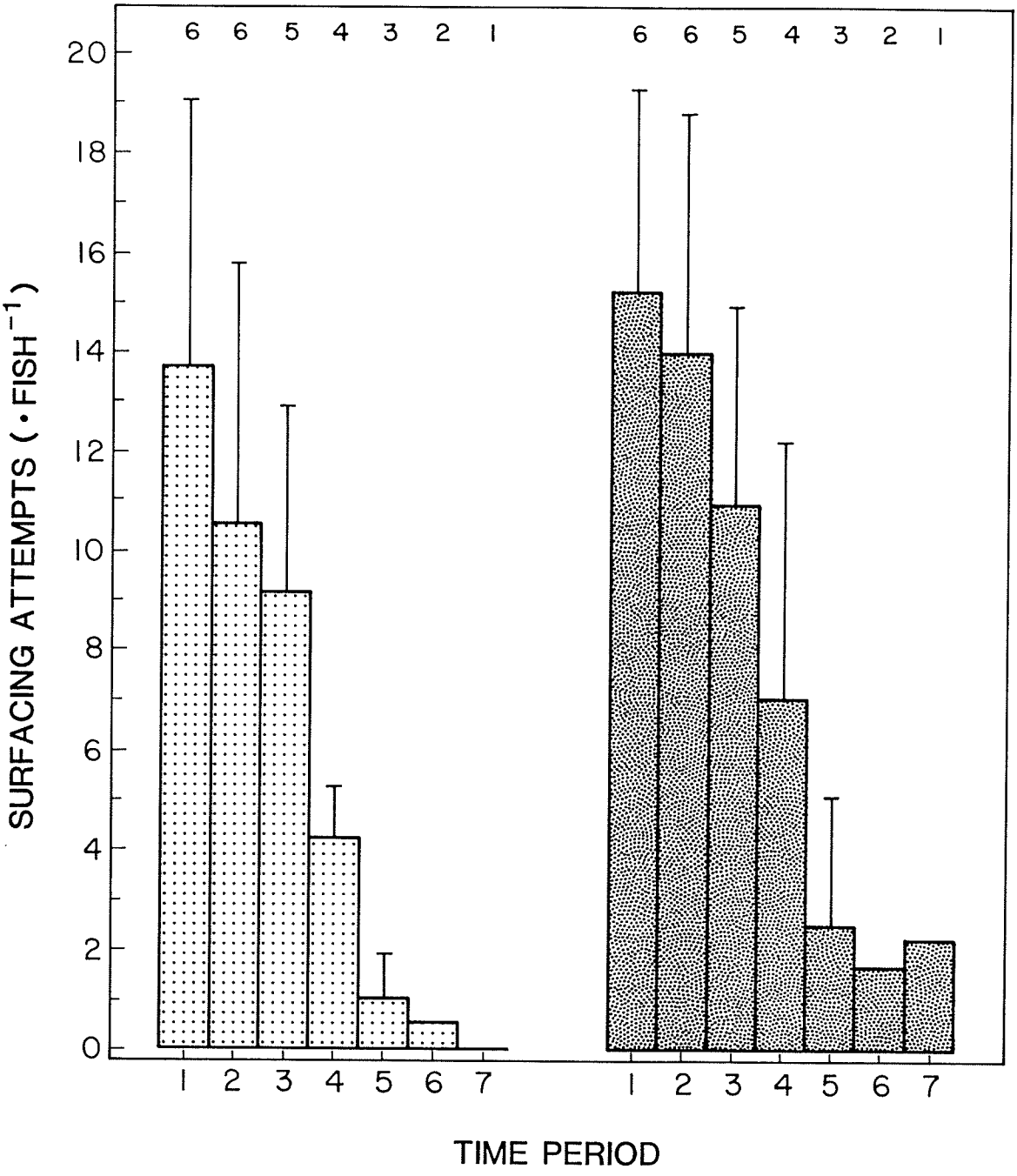

6 6 5 4 3 2 1
6 6 5 4 3 2 1
20
18
16
14
12
10
8
6
4
2
0
SURFACING ATTEMPTS (•FISH^{-1})
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TIME PERIOD

Figure 5. Effect of water pH on the percentage of fathead minnows resting on the bottom of the aquarium in selected 30-min time periods during the return to neutral buoyancy in still water of pH 7.7 (open triangles) and pH 5.3 (solid triangles) with access to air.

A - gas composition experiment

B - buoyancy experiment

The number of 30-min observation intervals sampled is given across the top of the graphs.

Time period (after transfer to still water)

1: 1- 30 min
2: 31- 61 min
3: 120- 150 min
4: 300- 330 min
5: 660- 690 min
6: 1380-1410 min

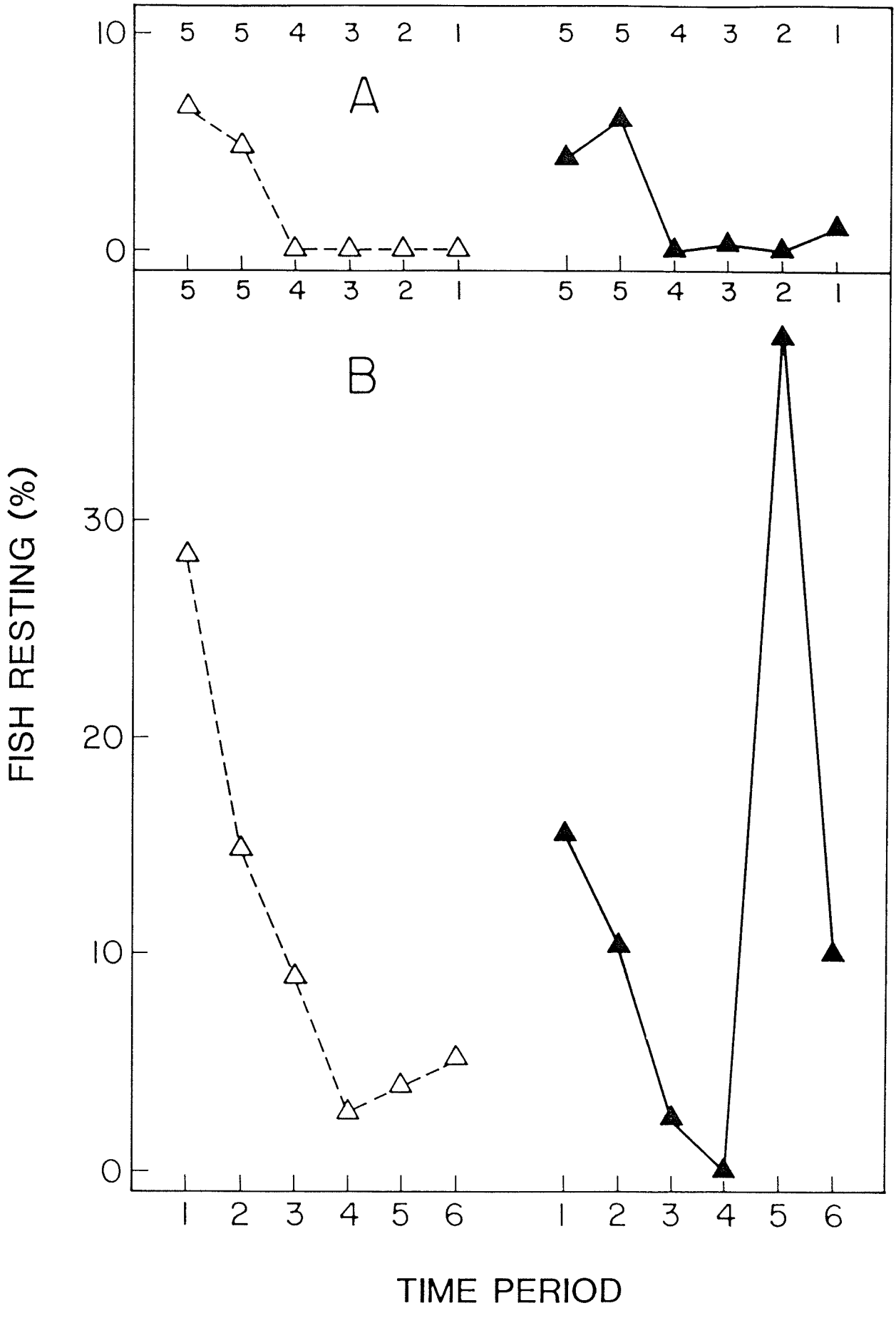

A
B
10
0
30
20
10
0
5 5 4 3 2 1
5 5 4 3 2 1
1 2 3 4 5 6
1 2 3 4 5 6
FISH RESTING (%)
TIME PERIOD

Figure 6. Effect of water pH on the percentage of fathead minnows resting on the bottom of the aquarium in selected 30-min time periods during the return to neutral buoyancy in still water of pH 7.7 (open triangles) and pH 5.3 (solid triangles) without access to air. The number of 30-min observation intervals sampled is given across the top of the graph.

Time period (after transfer to still water)

1: 1- 30 min
2: 31- 61 min
3: 120- 150 min
4: 300- 330 min
5: 660- 690 min
6: 1380-1410 min
7: 2820-2850 min

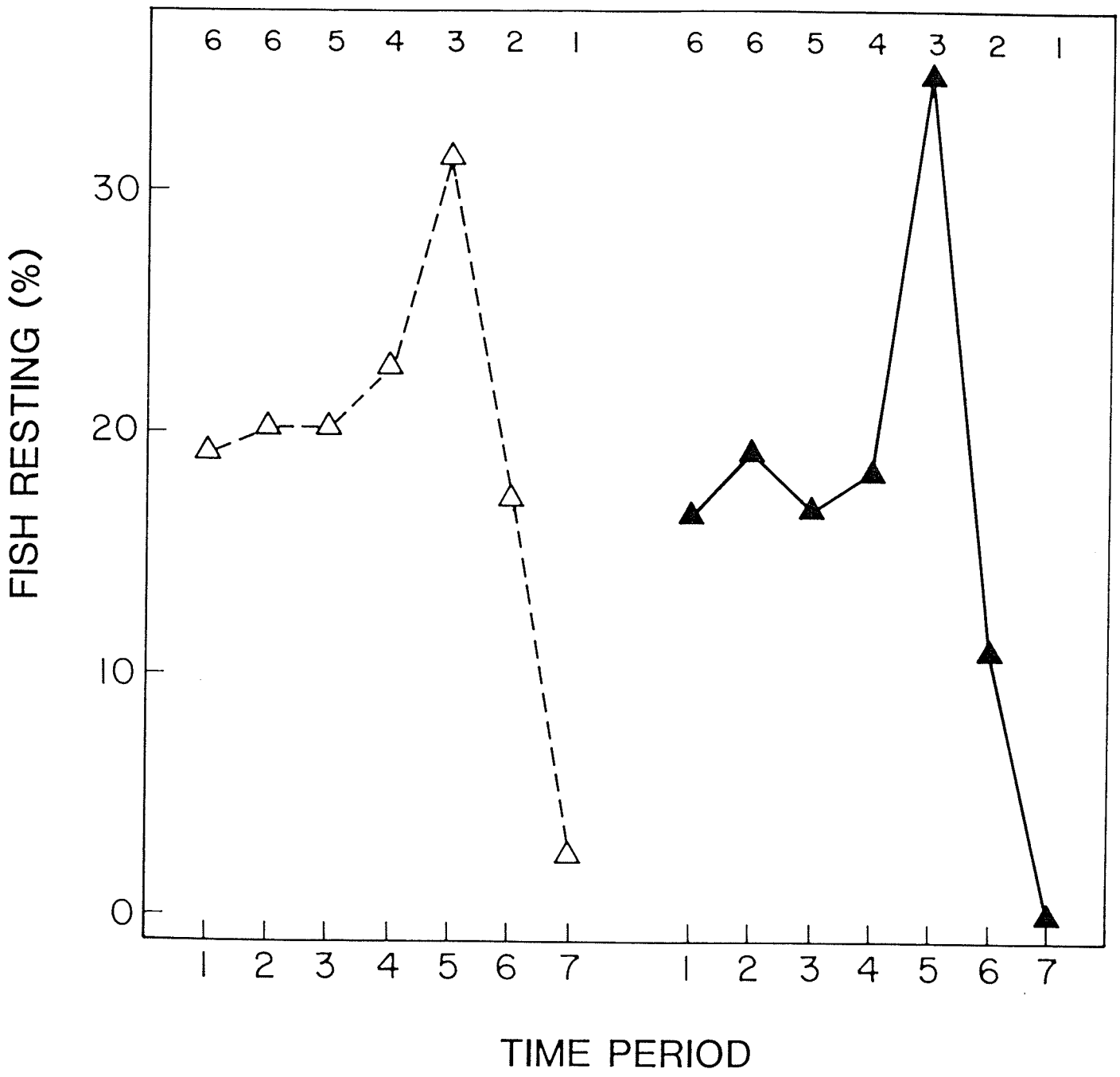

6 6 5 4 3 2 1
6 6 5 4 3 2 1
30
20
10
0
FISH RESTING (%)
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TIME PERIOD

Figure 7. Relation between mean PFBF and mean buoyancy for fathead minnows increasing lift in still water with access to air. Solid symbols are for treated fish, open symbols are for control fish.

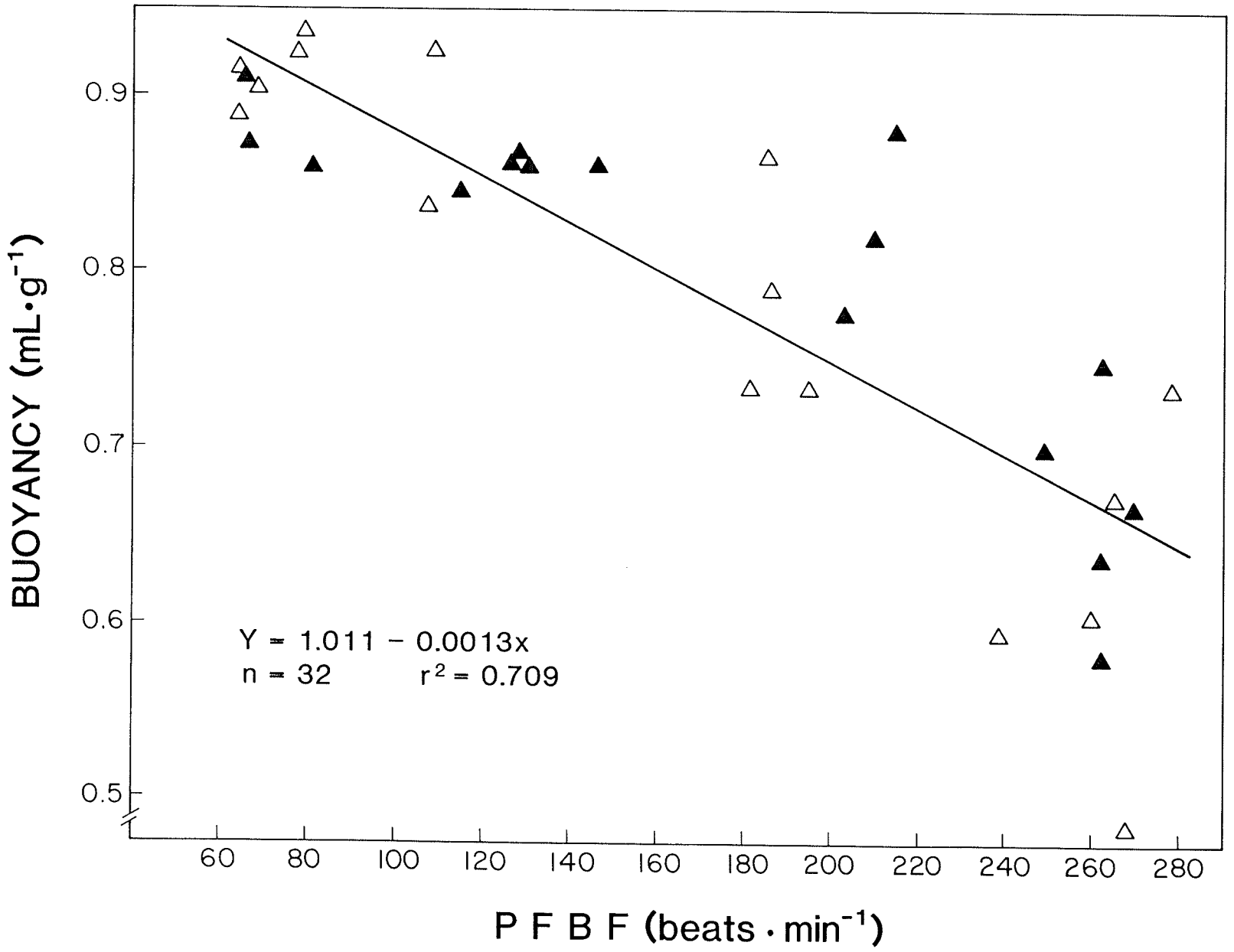
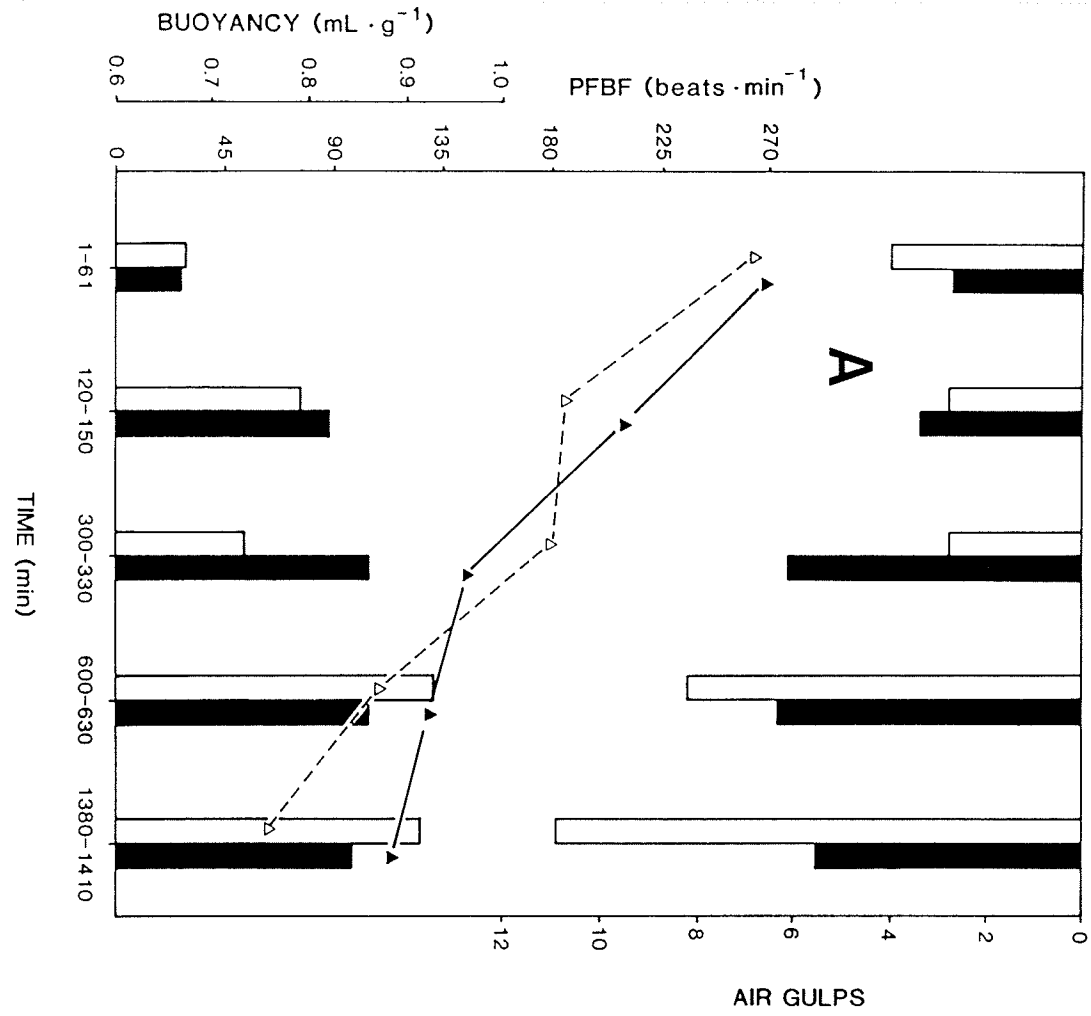

BUOYANCY (mL•g⁻¹)
0.9
0.8
0.7
0.6
0.5
Y = 1.011 − 0.0013x
n = 32
r² = 0.709
60
80
100
120
140
160
180
200
220
240
260
280
P F B F (beats • min⁻¹)

Figure 8. Mean buoyancy (bottom bars), accumulated total number of air gulps per fish (top bars) and PFBF (triangles) for fathead minnows exposed to current and then still water of pH 7.7 (open symbols) and pH 5.3 (solid symbols) with access to air. Time given represents min after removal from current.

A - buoyancy experiment

B - gas composition experiment


BUOYANCY (mL · g⁻¹)
0.6
0.7
0.8
0.9
1.0
PFBF (beats · min⁻¹)
0
45
90
135
180
225
270
TIME (min)
1-61
120-150
300-330
600-630
1380-1410
A
12
10
8
6
4
2
0
AIR GULPS

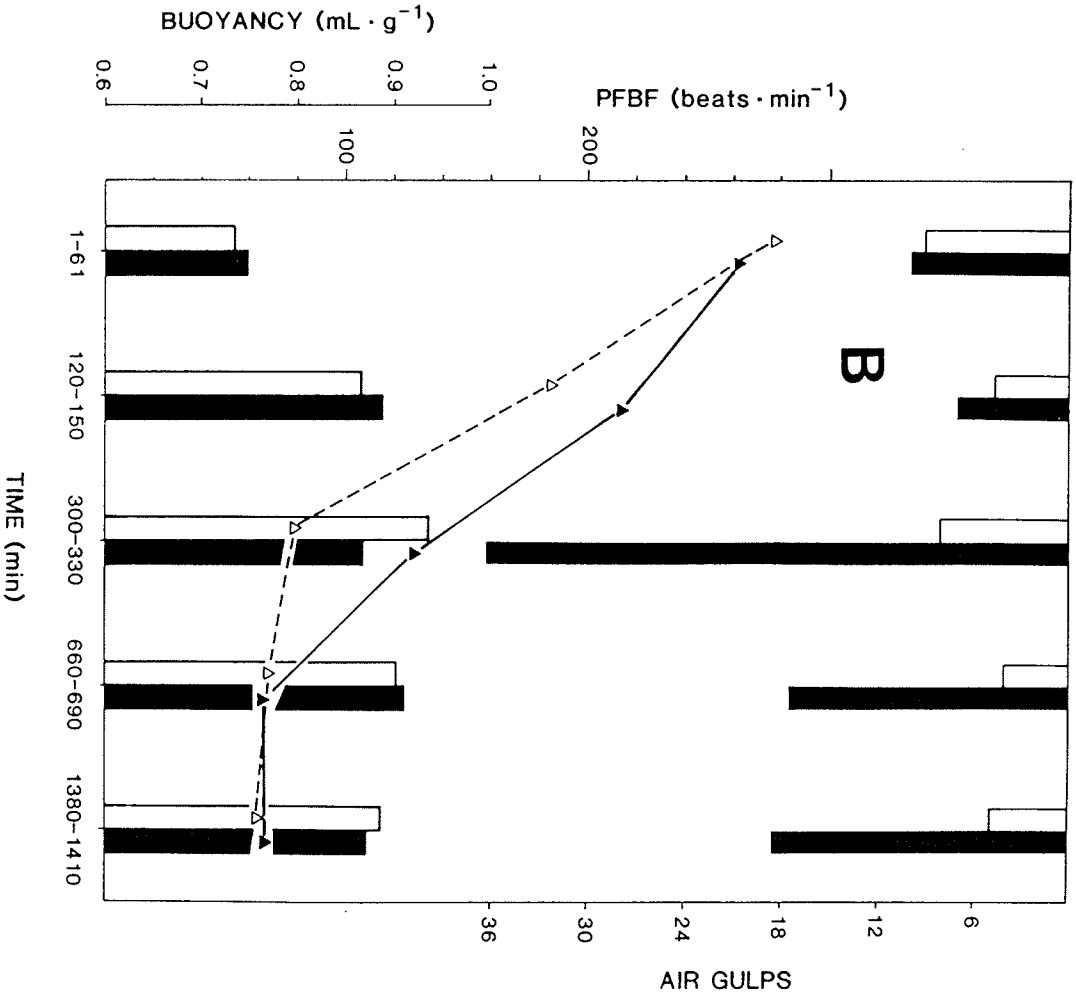
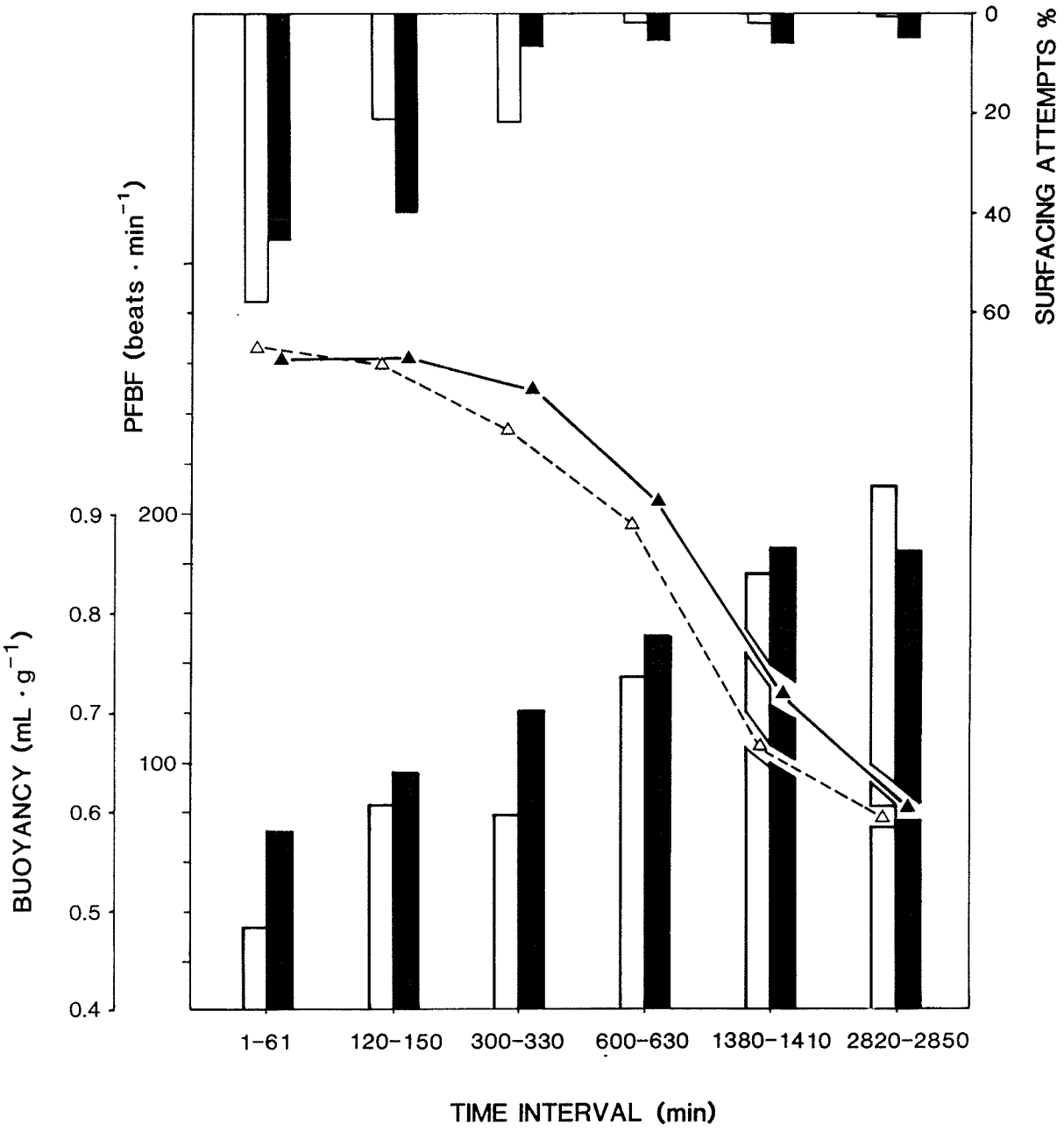

BUOYANCY (mL · g⁻¹)
0.6
0.7
0.8
0.9
1.0
PFBF (beats · min⁻¹)
100
200
TIME (min)
1-61
120-150
300-330
660-690
1380-1410
B
36
30
24
18
12
6
AIR GULPS

Figure 9. Mean buoyancy (bottom bars), number of surfacing attempts as percentage of the total number over all time periods* (top bars) and mean PFBF (triangles) for fathead minnows exposed to current and then still water of pH 7.7 (open symbols) without access to air. Time given represents min after removal from current.

*at 1-61 min the percentage of attempts between 31 and 61 min was used

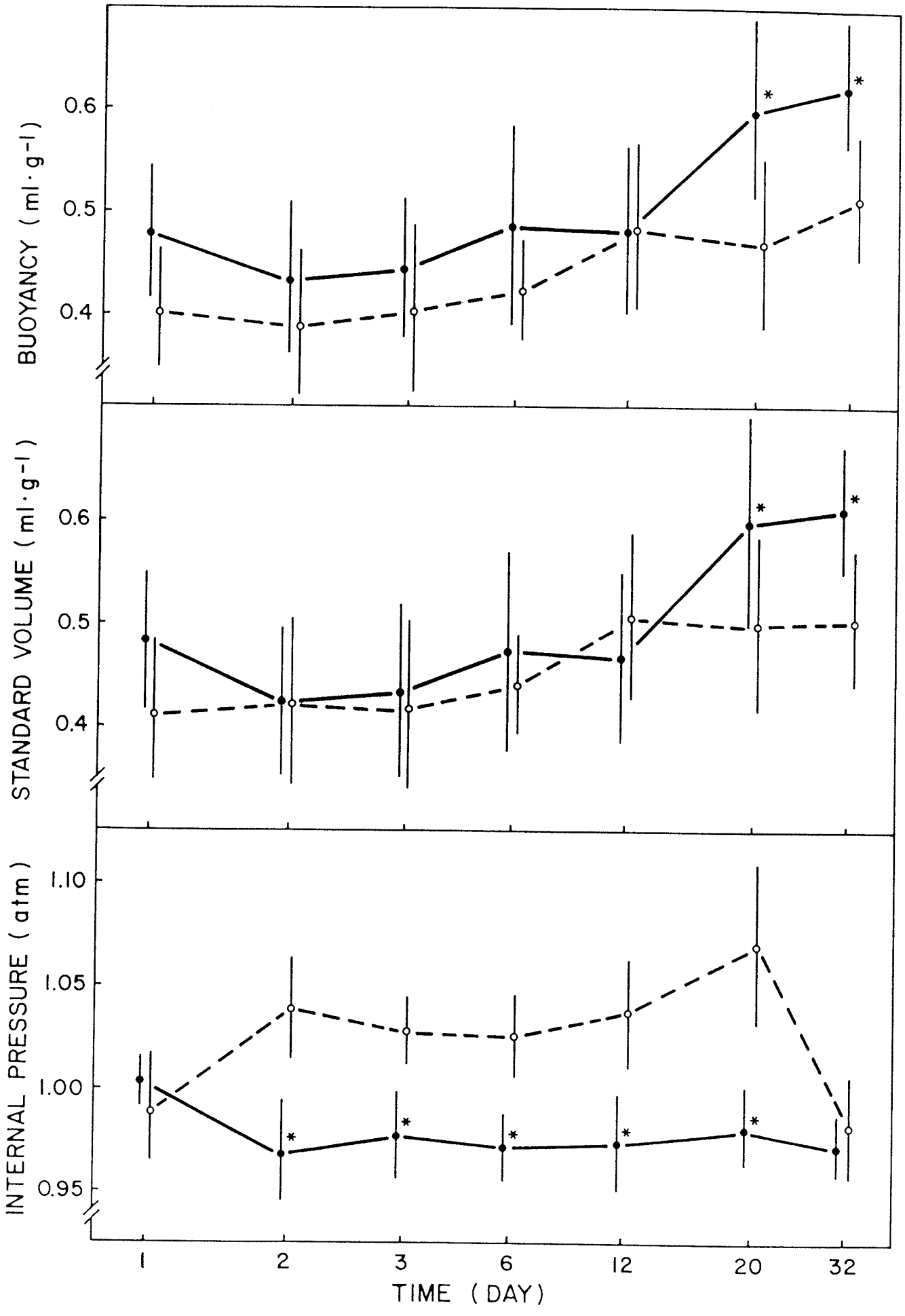

PFBF (beats · min⁻¹)
SURFACING ATTEMPTS %
BUOYANCY (mL · g⁻¹)
0.9
0.8
0.7
0.6
0.5
0.4
200
100
0
20
40
60
1-61
120-150
300-330
600-630
1380-1410
2820-2850
TIME INTERVAL (min)

D. Ability to maintain a minimum buoyancy over time

When exposed to water current over an extended period of time, both treated ($p<0.0001$) and control ($p<0.01$) fish significantly increased buoyancy and STV from day 2 (minimum values) to day 32 (Fig.10; regression analysis, App.20). This increase was not uniform for both groups, and as shown by ANOVA tests, buoyancy was significantly affected by water pH ($p=0.001$), while for STV the effect of pH was not quite significant ($p=0.07$) (App.21). Also, ANOVA showed that in control fish, only buoyancy at day 32 was significantly ($p<0.05$) higher than that at day 2. For both buoyancy and STV, treated fish showed higher ($p\leq0.05$) values than control fish at day 20 and 32 (Fig.10, App.21).

IP was significantly ($p<0.001$) affected by water pH, time and their interaction. IP of treated fish was consistantly lower ($p\leq0.001$) between day 2 and 20 than that of controls. Regression analysis showed that control fish increased ($p<0.01$) IP over this time period (App.20). In treated fish IP remained at similar values over day 2 to 32. IP of control fish declined dramatically between day 20 and 32, attaining similar values to treated fish (Fig.10).

Figure 10. Buoyancy, STV, and IP after 1 to 32 days in 26 cm/s current at pH 5.3 (solid symbols) or pH 7.7 (open symbols). Points represent means (± 95% CI) of 7-13 fish. Means significantly different from control values are indicated with an asterisk. Time is given on a log scale but actual values (d) are shown.


BUOYANCY (ml·g-1)
0.6
0.5
0.4
STANDARD VOLUME (ml·g-1)
0.6
0.5
0.4
INTERNAL PRESSURE (atm)
1.10
1.05
1.00
0.95
1
2
3
6
12
20
32
TIME (DAY)

E. Swimming performance

The data for all 4 batches of fish were pooled for analysis (App.22). When subjected to a stepwise increase in water velocity, no significant ($p>0.10$) difference could be found in the mean swimming times of treated (6.91 h) and control (7.05 h) fish (Table 8). This trend was confirmed when numbers of resting fish for individual velocity stages of each replicated test were compared. All fish of both groups resisted water velocities of 15 and 25 cm/s, 3-4 fish tired at 35 cm/s and 44 cm/s, respectively, and none of the fish swam longer than 60 min in 57 cm/s current.

The approximately 7 h of forced swimming resulted in an average weight loss of 9.2 and 5.5% body weight in the treated and control fish, respectively. This difference was manifested in a significant ($p<0.001$) reduction in the K of treated fish. Before the start of the experiment fish from both pH treatments were of similar K (Table 8). Similar to results of the experiment in which buoyancy was reduced in the short term (C1), treated fish did not reduce their buoyancy to the same extent as controls ($p<0.001$). Unlike the previous experiment, no differences between treated and control fish could be detected for IP ($p>0.10$) (Table 8).

The relationship of swimming time and KA to the other measured parameter was investigated by means of multiple linear regression. IP varied positively ($p<0.05$) with swim-

ming time of treated fish, accounting for 16% in the variability in the latter variable (Table 9). The addition of KB, as the second strongest predictor, into the model accounted for an additional 9% of the variance in swimming time, however, the contribution of KB was only marginally ($p=0.075$). No other variable contributed significantly to the regression equation. For control fish no significant relationship between swimming time and any measured parameter was observed, though here again, IP was the strongest predictor of swimming time, accounting for 10 % of its variance ($p=0.07$) (Table 9). KA was not significantly correlated to either buoyancy, IP or swimming time.

TABLE 8

Swimming time (SWT), buoyancy IP, and K and weight before the start (B) and after the termination (A) of the experiment for fish subjected to a stepwise increase in water velocity.

Variable	pH 7.7	pH 5.3
SWT (h)	6.91 ±0.51	7.05 ±0.51
Buoyancy ($mL \cdot g^{-1}$)	0.439 ±0.027	0.520[a] ±0.027
IP (atm)	1.006 ±0.010	0.992 ±0.016
KB	0.986 ±0.008	0.988 ±0.013
KA	0.932 ±0.010	0.897[a] ±0.018
WeightB (g)	2.74 ±0.10	2.80 ±0.10
WeightA (g)	2.60 ±0.10	2.55 ±0.10

NOTE: Means ± 95% CI; n=33-40, representing the number of fish tested.

[a] significantly ($p<0.001$) different from pH 7.7

TABLE 9

Linear multiple-regression analysis of factors accounting for the variation in swimming time (dependent variable) of fish subjected to a stepwise increase in water velocity.

Independent variable	Multiple correlation coefficient	Simple correlation coefficient
	a) all fish (n=68)	
IP (atm)	0.3557 [a]	+0.3557 [b]
KB	0.4120 [a]	-0.1806
	b) control fish (n=35)	
IP (atm)	0.3098 [a]	+0.3098
KB	0.3300 [a]	-0.1378
	c) treated fish (n=33)	
IP (atm)	0.3992 [a]	+0.3992 [b]
KB	0.4950 [a]	-0.2121

[a] variable does not contribute significantly ($p<0.05$) to the regression equation

[b] $p \leq 0.05$

DISCUSSION

Exposure of fathead minnows to a mean pH of 5.3 resulted in frequent disturbances in swimbladder parameters and buoyancy related behaviors. These effects were less pronounced under "normal" conditions, i.e. still water with access to air, but were magnified when fish experienced further stressors, i.e. water current or denial of surface access.

Although this investigation was not designed as a lethality study, it is apparent that the mortality of adult fathead minnows was not noticeably affected by exposure to ambient pH of 5.3 over 2-42 d. Mount (1973) obtained similar results in still water for pH values between 4.5 and 6.6 in a long-term (13 month) laboratory study. Zischke et al. (1983), however, working with caged fathead minnows in a simulated stream environment acidified to pH 5.0, reported a 50% lower survivorship of treated fish compared to controls (pH 7.8-8.5) after 4 month of exposure. Fluctuating pH levels (range: 4.4-6.9) and interacting effects of heavy metal mobilisation might help to explain these discrepencies.

A. Swimbladder parameters and behavior in still water

In still water with access to air, control and treated fish were not quite neutrally buoyant and had similar STV and PFBF. Under the same conditions, however, treated fish gulped air almost 4 times more frequently, had slightly less IP ($p=0.06$), and increased swimbladder O_2 concentrations by 73% . Similar buoyancy values were reported by Gee et al. (1974), Gee (1977), Stewart & Gee (1981) and Gee & Ratynski (unpubl. data), indicating that fathead minnows must generate some hydrodynamic lift to remain pelagic. McCutcheon (1966) has described a slightly negative buoyancy as the "normal" resting and emergency state. Despite metabolic costs involved in maintaining elevated fin beat frequencies or hydrodynamical lift in general, negative buoyancy has several adaptive advantages. It aids in predator avoidance (Gee 1977, 1983; Stewart & Gee 1981), by allowing fish to sink to the bottom and remain there motionessly. It further permits fish to rest on vegetation or substrate without swimming activity and provides some insurance against a sudden buoyant ascent to the surface. Jones (1952), working on perch (Perca fluviatilis Lin.), suggested that once a certain expansion in swimbladder volume is exceeded, swimbladder volume control is lost. In subcarangiform fish, such as cyprinids (Lindsey 1978), compensatory hydrodynamic lift is thought to be derived from both the camber of the body and the paired fins during forward locomotion. However, instead

of continuously cruising around the tank, fish in this study often hovered in still water for hours at a time, compensating negative buoyancy with appropriate movements of their pectoral fins.

With 2.9% CO_2, 13.8% O_2 and 83.3% N_2, the gas composition of control fish is fairly typical for a swimbladder in long-term, dynamic equilibrium. Under steady state conditions, the relative contribution of the individual gases is maintained by the more or less continuous processes of gas reabsorption and secretion (Fange 1966). Cyprinids in general show relatively high CO_2 (2.0-5.8) and low O_2 (3.4-12.3) percentages (Hufner 1892, Hall 1924, Evans & Damant 1929, Jacobs 1934 , Krohn & Piiper 1962). According to my data, and those of Stewart & Gee (1981), Chiasson & Gee (1983) and Gee & Ratynski (unpubl. data), fathead minnows conform to this pattern. The O_2 percentages, which are substantially lower than atmospheric, suggest that under normal conditions (i.e. still water with access to air) and when at "optimal" buoyancy, fatheads do not rely extensively on atmospheric air to replace gas lost from the swimbladder. This is supported by direct observations on air gulping frequency, which was negligable. Similarly, this gas composition suggests that under normal conditions diffusional losses are minor. Otherwise these losses would have to be compensated for, if not by air gulping, by gas secretion. In most species, freshly secreted swimbladder gas consists mainly of O_2,

and to a lesser extent, of CO_2 and N_2 (Akita 1936, Scholander 1956, Steen 1963b, McNabb & Mecham 1971). Considering the high O_2 and CO_2 percentages attained after filling of a partially-emptied swimbladder by gas secretion (Fig.2, no access), fathead minnows seem to conform to the normal pattern. Any substantial gas secretion must therefore lead to elevated O_2 and CO_2 percentages inside the swimbladder compared to the composition of air. Such were not found in still water control fish A high impermeability of the swimbladder wall was reported by Moreau (1876), Evans & Damant (1929), Kutchai & Steen (1971), Denton et al. (1972), Ross (1979a) and Wittenberg et al. (1980). In this study, intact isolated swimbladders were left underwater for several hours and no appreciable (>0.002 mL) difference in volume could be detected. It seems that unstressed fathead minnows can maintain a constant swimbladder volume at a very low metabolic cost. Alexander (1972) has estimated this cost, in terms of O_2 consumption, at 2 $cm^3/kg/h$ for a 1 g fish at 100 m. This value becomes even less with decreasing water depth and increasing fish size (i.e. swimbladder volume).

Considering the probable composition of freshly secreted gas, and the fact that air transferred into the swimbladder cannot account for O_2 concentrations in excess of 21%, the swimbladder gas composition of treated fish strongly suggests that gas secretion is stimulated even when access to air is provided. Treated fish are able to maintain buoyancy

and STV at the required level under "optimal" conditions (i.e. still water with access to air), but can only do so at the expense of an increased rate of gas secretion supplemented by an elevated number of air gulps (Table 4). Denial of surface access apparently increases these regulatory problems of treated fish and also affects controls. Compared to treated and control fish with access to air, the corresponding "non-access" fish increased % O_2 and % CO_2 of swimbladder gas (Table 4). Similar results were obtained by Gee & Ratynski (unpubl. data) who found CO_2 and O_2 percentages of fish held at neutral pH without access of 3.3% and 27.5%, respectively. Chiasson & Gee (1983) reported only slightly elevated values of these gases. However, fish in their study were acclimated to the "non-access" conditions for only 24 h, which might have been too short a time for full development of compensatory gas secretion.

These changes in gas composition suggest that fathead minnows increase secretion of gas into the swimbladder when surface access is denied. In controls, this volume of gas seems to be relatively small and serves to increase STV and buoyancy, while in treated fish these two swimbladder parameters actually tended to decrease, though not significantly. This is surprising, because as inferred from the very high CO_2 (4.9%) and O_2 (45.5%) percentages, which equal those obtained after the filling of a partially emptied swimbladder by gas secretion (values at 24 h in Fig.2), a high rate of

gas secretion must have persisted over an extended period before measurements were taken. The reason for this discrepency between apparently intensive gas secretion and stable STV in fish, both with and without surface access, is not known. One possible explanation is an extensive gas loss out of the swimbladder, which must equal the rate of gas secretion in magnitude. Gas reabsorption occurs passively by diffusion of gases into the blood (Moreau 1876) and is, in many fishes, restricted to a vascular area known as the "oval", where the rate of gas loss can be regulated by the degree of closure of a relatively impermeable tissue layer and by constriction or dilation of the underlying capillaries (von Ledebur 1937). The degree of vasoconstriction and muscular contraction is controlled by autonomic reflexes and hormones (Fange 1973, Ross 1978). Whether physiological imbalances induced by acid exposure affect these control mechanisms or the gas permeability of the swimbladder wall is not known. In a recent review of acid toxicity, Wood & McDonald (1982) notes the paucity of information availible on endocrinological effects and the absence of neurophysiological studies.

Whatever the reason, treated fish in still water without access have obvious difficulties in maintaining buoyancy. They can only do so at the expense of extensive gas secretion and mechanical costs of increasing hydrodynamic lift via elevated PFBF (Table 4). The increased number of sur-

facing attempts of "non-access" fish (Table 4), suggesting insufficient swimbladder lift, further indicates their inability to regulate buoyancy efficiently under acid exposure and denial of surface access.

The almost neutral buoyancy (0.98 mL/g) of control fish is of possible adaptive advantage when access to air is denied, (eg. winter ice cover). Under those conditions, fish might utilize photosynthetic O_2 released by aquatic vegetation, and which is trapped under the ice, for respiration. Neutral buoyancy facilitates adoption of a position close to the water-ice interface and indirectly reduces O_2 demand otherwise resulting from locomotory activity for hydrodynamical compensation of insufficient lift. Klinger et al. (1982) report that fathead minnows survive anoxic conditions in winter by using trapped gas bubbles. Although O_2 was not a limiting factor in the experiments reported here, the denial of surface access alone, ultimately responsible for anoxic conditions in winter, might have stimulated a similar response.

Some of the above theoretical considerations are supported by casual behavioral observations. Apart from surfacing attempts, "non-access" control, but not treated fish, moved upwards in the water column as a group, frequenting the undersurface of the submersed screen for substantial periods of time. On three occasions small gas bubbles trapped beneath the screen were immediately sensed and engulfed whole or in fractions.

Despite the increase in buoyancy, PFBF of control fish did not decrease when access to air was denied, but instead increased from 59 to 69 beats/min. The exact measurement of PFBF, however, was hampered by an overall increase in swimming activity. Fish hovered rarely, and then, only for a few seconds. As PFBFs, recorded under these conditions, were generally higher than when fish hovered for longer times, the unsuspectedly high PFBF of "non-access" control fish is probably not directly comparable with the frequencies of "access" fish. Hoglund (1961) for salmonids in an hypoxic gradient and Klinger et al. (1982) for fish (i.e. fathead minnows) under simulated winterkill conditions also found an unexpected increase in locomotory activity and argued that this might be an adaption to finding local sources of O_2.

B. Swimbladder parameters on exposure to current

When fathead minnows experience surplus lift as a result of increased water velocity, they reduced buoyancy and STV, but minimum levels attained after 48 h could not be maintained over 32 d. As suggested by the significant difference in minimum buoyancy, but not in STV, between treated and control fish (Table 5), the extent of adjustment in STV remained unaffected by water pH, while the extent of buoyancy adjustment was larger in controls. These latter fish also decreased buoyancy and STV faster, with the changes in the former variable being somewhat greater (Fig. 1 A,B). This possible discrepancy between the response in buoyancy and STV may be explained in terms of differences in IP. It can be hypothesized that under "normal" conditions (i.e. pH 7.7) two mechanisms are operative in buoyancy reduction of fathead minnows : reabsorption of gases and increase in IP. In the early phase, reduction is achieved mainly by gas reabsorption, while muscular contraction, leading to increased IP (see below) contributes largely to the final rate of change. Some evidence of the possible temporal interaction of these two mechanism is provided by the response of control fish in still water between 24 and 48 h after transfer from current. During that time, the mean STV increased by 3%, but a 7% increase in IP may have been responsible for the further reduction in buoyancy of 3% (Fig.1 A-C, App.6). In treated fish IP remained stable over time and may thus not have contributed to buoyancy adjustment (Fig.1 C).

The possible important contribution of IP in slight, but critical buoyancy adjustment, is further supported by the results of fish attempting to maintain a minimum buoyancy over an extended time. Similar to results for the rate of adjustment, here the increase in buoyancy and STV over day 2-32 was affected by water pH, but this effect was only significant for buoyancy. Although control fish increased buoyancy between 2 and 32 days (regression analysis), the concomitant increase in IP dampened this response, and it was not until day 32, when control fish lost any excess IP, that buoyancy became significantly different from its value at day 2 (ANOVA). In treated fish, which over days 2-32 maintained IPs 2-3% below atmospheric, buoyancy intimately followed STV. When STV increased dramatically on day 20 and 32, the control of buoyancy may have failed.

Stewart & Gee (1981) did not find an increase in buoyancy in fathead minnows swimming at 20 or 30 cm/s for up to 42 d. This discrepency is possibly attributable to a difference in the velocity profiles, which in their experiment provided sheltered areas of lower current (Gee, pers. comm.), thus reducing the stress from fast swimming. Mortality rates found in present experiments further indicate the considerable stress imposed on fish by water velocity alone. Stress of increasing temperature and/or water velocity was shown to adversely affect control of buoyancy (Gee 1977, Berezay & Gee 1978, Stewart & Gee 1981) and IP (Stewart & Gee 1981).

If, in control fish, this effect is assumed to be critically manifested between day 20 and 32, then the introduction of the additional stressor, reduced pH, may influence both magnitude and timing of impairment in buoyancy control. This control is likely affected both by loss of IPs greater than ambient and, to a lesser degree, by an increase in STV.

There is ample evidence in the literature for excess IP in the order of 20-130 mm Hg (Evans & Damant 1929, Franz 1937, McCutcheon 1958, Alexander 1959, Gee et al. 1974, Gee & Gee 1976). Furthermore, Gee (1970), Gee et al. (1974) and Machniak & Gee (1975) reported an increase in IP as buoyancy decreased in current. McCutcheon (1958,1966) suggested that muscular compression of the swimbladder wall changes IP and thereby provides for precise swimbladder control. Mean IP in excess of 20 mm Hg above atmospheric was never found in treated fish. From these results it can be infered that acid-exposed fish are unable to utilize this mechanism of precise buoyancy adjustment, or are at least severely restricted in its use. If and to what extent the operation of the Weberian apparatus, which apparently relies on a certain tension of the swimbladder wall (Evans & Damant 1929, Alexander 1959), is affected in acid-exposed fish, remains unknown.

Compared to still water values, the swimbladder percentages of CO_2 and O_2 in current were reduced after 48 h, with no significant difference between treated and control fish

(Table 5). The proportions were about 1 and 9% respectively, very similar to results obtained by Stewart & Gee (1981) for fathead minnows after 12 and 60 h in current of close to neutral water pH.

Gas reabsorption occurs passively by diffusion (see above). Steen (1963a) demonstrated that the final proportion of O_2 in the swimbladder is dependent on the O_2 tension of the arterial blood supplied to the swimbladder. Once swimbladder and arterial CO_2 and O_2 tensions have equilibrated, gas reabsorption will cease. This ignores minor diffusion of N_2 for which at that moment exists a positive pressure gradient between swimbladder and blood. The final swimbladder O_2 content of fathead minnows indicates an arterial blood PO_2 of 0.09 atm, which must have limited any further O_2 absorption. The drastic change in swimbladder gas composition between fathead minnows at maximum and minimum buoyancy together with the observed lack of gas spitting while fish were in current, supports Stewart & Gee's (1981) suggestion that gas reabsorption is the principle mechanism used to reduce swimbladder volume when water velocities increase.

There is little information on gas reabsorption rates. Using data from Steen (1963a) on eel (Anguilla vulgaris), assuming a weight of 200-400 g, the rate of O_2 reabsorption is 4.0-8.0 mL/h/kg (body weight) at an initial swimbladder PO_2 of 0.2 atm and a 20% saturation of arterial blood. Tyt-

ler & Blaxter (1973) and McCutcheon (1958) obtained similar results for cod (Gadus morhua) and saithe (Polladius virens) (8 mL/h/kg) and pinfish (Lagodon rhomboides) (4 mL/h/kg), respectively, when the saturation of the arterial blood was not manipulated and must have been more than 20% saturated with O_2. From 279 weight measurements of fathead minnows in air and water, the following regression equation was obtained: weight in air (g) = 0.19 + 16.29 gas-free weight in water (r^2=0.90). Using this relationship to calculate gas reabsorption rates per kg body weight in air, rates were 5.3 mL/h/kg for the first 3 h, and 2.3 mL/h/kg for the first 12 h after stimulus. This assumes that the changes in STV precisely reflect the amounts of gas reabsorbed, thereby ignoring the possibility of concomitant gas secretion. The rates for the latter time period are somewhat lower than those from the literature. This, however, could be due to the much smaller absolute swimbladder volumes of fathead minnows. From this comparison there is no evidence for quantitative difference in reabsorption rates of physoclists and physostomes, which, except for the eel, supposedly do not possess special vascular adaptations for gas reabsorption (Fange 1976).

C. Swimbladder parameters after transfer to still water

When water velocity is altered from current to still, fathead minnows decrease IP. As for control fish in current, IP contributes to buoyancy adjustment under these conditions. When buoyancy is increased, however, IP decreases similarily in treated and control fish. This decrease in IP is to be expected, assuming that muscular action is involved in swimbladder control. However, less than atmospheric IPs were frequently recorded for fish increasing buoyancy with or without surface access (Fig.1 F,J). This requires not only a reduced muscular tonus of some swimbladder wall and abdominal muscles, but a contraction of other muscles with the effect of increasing the space of the abdominal cavity. A negative pressure of at least 30 mm Hg is required to account for the observed expansion of the swimbladder wall. This process not only has to be sustained against the relative inextensibility of the cyprinoid swimbladder wall (Alexander 1959, Jones 1951), but while the amount of gas molecules inside the swimbladder is constantly increasing. The occurrence of a "negative" swimbladder pressure (relative to the ambient) is supported by Sundnes & Gytre (1972). They measured transient swimbladder gas pressures as low as 0.93 atm. No causal mechanism was suggested by these authors. McCutcheon (1962) states that fish lack the "morphology of muscular control to increase swimbladder volume".

It is conceivable that the "negative" IPs recorded were artifacts. The volume of swimbladder gas and consequently IP, was measured by releasing the gas under water. Due to its high diffusability, CO_2 might have quickly diffused into the water, resulting in low volume readings. However, Stewart (1980) was unable to find gas volume changes arising from diffusion into the water bath. Furthermore, to affect the volume readings at a magnitude required to account for IPs of 0.92 atm, which were the lowest individual measurements recorded, the swimbladder of those fish must have contained up to 8% CO_2 diffusing completely into the water within 20 s (time allowed for the balance to settle). While such CO_2 values were occasionally measured for fish increasing buoyancy, they are more than 3 times the maximal values recorded for fish at minimal buoyancy. Efforts to exclude the possibility of underestimating IP due to gas volume changes arising from diffusion by releasing gas of a known composition under the watchglass and reanalysing it, failed. In any case it is difficult to explain why treated fish should create and maintain (as in experiment D) "negative" IPs of up to 30 mm Hg if the negative IP is counter-adaptive with regard to buoyancy adjustment (experiment D).

Regardless of pH treatment, fish increasing STV and buoyancy without access to air require at least 48 h to attain close to neutral buoyancy, but when access is provided only a fourth of this time or less is needed. Gee (1968) report-

ed similar results for the physostome Rhinichthys cataractae, while his experiments with fathead minnows were less conclusive (Gee 1977). In the latter study, however, the extent of buoyancy adjustment (0.58 to 0.93 mL/g) was considerably less than reported here (0.38 to 0.92 mL/g) and might help to explain the less distinctive differences in adjustment times between "access" and "non-access" fish.

In both buoyancy and gas composition experiments "non-access" fish had not yet attained long-term still water buoyancy levels after the last measurements (48 h, see Table 3). These differences in buoyancy were small, and it seems reasonable to assume that, independent of water pH, fatheads can fill a more than half-empty swimbladder by gas secretion within 48 h. Stewart & Gee (1981) calculated even shorter times. However, if changes in STV are taken as a direct measure of swimbladder gas secretion, thus ignoring possible diffusional losses during this short time span, the amount of gas secreted by control fish during that time was 22% larger than that of treated fish (Fig.1 H, App.13). Consequently, the gas secretion rate by control fish was higher than of treated fish. Changes in SGC during periods of buoyancy increase followed almost identical patterns for "non-access" control and treated fish (Fig. 2). SGC at minimum buoyancy also was similar for both groups of fish (see above). These similarities in swimbladder gas composition are not neccessarily in contradiction with the previously

established differences in gas secretion rates between treated and control fish. Treated fish secreted O_2 and CO_2 rich gas into a swimbladder of initially lower specific volume but similar gas composition. Consequentely, a smaller gas volume, compared to control fish, could have been sufficient to result in the nearly identical gas concentrations. It can therefore be assumed that the mechanisms of gas secretion and reabsorption are not affected qualitatively by acid exposure but rather quantitatively.

Physostomes are generally believed to be slow gas secretors (Fange 1976), and have been shown to require several days to a few weeks to fill their partially empty swimbladder when denied surface access (Evans & Damant 1929, Jacobs 1934, Wittenberg 1958, Krohn & Piiper 1962). Physoclists take only 4 to 24 h (Scholander et al. 1956, McCutcheon 1962, Fange 1976). Rates of gas secretion of physoclists calculated from rates of buoyancy or swimbladder volume adjustment given in the literature, range from 1.1-15.0 mL/kg/h (kg body weight in air), but values between 2.0 and 6.0 mL/kg/h are most common (Jacobs 1932, Akita 1936, Scholander et al. 1956, Wittenberg et al. 1964, McNabb & Mecham 1971, Tytler & Blaxter 1973). For Carassius auratus, a physostome, Overfield & Kylstra (1971) reported secretion rates of 0.2-0.6 mL/kg/h. In the study presented here, secretion rates (calculated from STV results) for control fish were 1.2-2.7 mL/kg/h, almost identical to rates obtained by Stew-

art & Gee (1981). These authors attribute the high rate of gas secretion in fatheads to the well-developed system of counter-current capillaries observed in these fish. Additional support for the extraordinary ability to secrete gas stems from the SGC of fathead minnows. O_2 percentages increased to over 43% after gas secretion was stimulated. These values are similar to those of physoclists (eg. Jacobs 1930, Akita 1936, Rostorfer 1942, McNabb & Mecham 1971) and considerably exceed those of most physostomes (Evans & Damant 1929, Jacobs 1934, Krohn & Piiper 1962). Although a direct correlation between buoyancy and air gulps could not be established, the rapid increase in STV and buoyancy of access fish can be mainly attributed to air gulping which commenced immediately after transfer to still water and almost ceased after 6 h (Fig.8,9). Contrary to "non-access", the STV and buoyancy of "access" fish show some anomalies, i.e. higher values at earlier than at later time periods (Fig.1, App.10). This can partly be explained by the fact that groups of fish could only be measured once at the end of their predetermined time period and therefore did not represent the "true" response of all other groups at this point in time, but it also reflects the crude nature of air gulping as a buoyancy adjustment mechanism. The decrease in buoyancy during the final time periods suggests that adjustment by air gulping leads to "overshooting" as the size of a bubble cannot be exactly controlled and too much air is transported into the swimbladder. The more precise mecha-

nisms of buoyancy control (muscular action, gas secretion/ reabsorption) are almost exclusively employed for the final adjustment. However, as indicated by the sharp increase in the proportions of CO_2 and O_2, which exceed atmospheric values (Fig.2), and the decrease in IP, gas secretion and changes in muscular tonus are also utilized in the early phase of increases in buoyancy, then supplementing air gulping. Stewart & Gee (1981) attributed 30% of the overall increase in buoyancy to gas secretion.

Assuming a STV of 0.400 mL/g after 48 h in current, control fish in the buoyancy experiment increased STV by 0.285 mL/g to 0.685 mL/g during the first hour upon exposure to still water while gulping air an approximately 4 times (Fig.8 A) From the results for "non-access" fish, an increase in STV due to gas secretion of more than 0.100 mL/g (i.e. 35%) seems to be highly unlikely during the first hour. Then the remaining increase of 0.185 mL/g is probably due to gulping. Considering the mean gas-free weight of a 1.9 g (59 mm) fathead minnow is 0.11 g, this results in a mean gulp volume of 5.1 µl. Corresponding calculations for treated fish arrive at a value of 5.3 µl. Stewart (1980) measured the gulp volume of a 62 mm fathead and found it to be 5 µL. Apparently acid-exposed fish are not limited in the amount of gas they can successfully pass into their swimbladder with a single air gulp. However, the relative contributions of air gulping and gas secretion to increases

in buoyancy are affected by environmental pH in as much that acid-exposed fish compensate for their possible inferior rate of gas secretion by relying more extensively on air gulping. Several sources of evidence support this theory. As buoyancy was similar in both groups of "access" fish over 1 to 24 h, the total adjustment by control fish must have been greater because they were at lower values when being transferred from current to still water. This larger increase in buoyancy was accomplished while gulping air as often as treated fish (buoyancy experiment) or less than treated fish (gas composition experiment). Secondly, the proportions of CO_2 and O_2 in the swimbladders of "access" control fish were higher (Fig.2). In these fish, air brought into the swimbladder by gulping will mix with freshly secreted gas, proportions of CO_2 and O_2 being higher as the contribution of gas secretion increases. The third line of evidence is more indirect and stems from the more frequent attempts to gulp air by treated than control fish when access is denied.

D. Behavioral compensation of insufficient buoyancy

Kopec (1927), Meesters & Nagel (1934), Jones (1952), McCutcheon (1958,1962) and Bishai (1961) have all shown that an increased body density incurred by a reduction in swimbladder volume can be compensated for by appropriate fin movements. Under those conditions, primarily the pectoral fins aided by the caudal fin will provide increased hydrodynamic lift. At lower body densities some percoid fish could resist a hydrostatic pressure reduction of about 30% (i.e. a 60% increase in swimbladder volume) by beating their pectoral fins upwards at a frequency of 150-200 beats/min (Jones 1952, Bishai 1961). Using these data, Alexander (1972) calculated the maximal upward force which could be counteracted with 0.25 N/kg body weight. In the present study, the percentage volume calculated according to Alexander (1966) as mL swimbladder gas/g body weight of fathead minnows after 48 h in current was approximately 2.5% . If the average density of a fish, excluding its swimbladder is taken as 1.076 (Taylor 1921, cited in Jones 1951), then this percentage volume reduces the vertical force to 4.9% of the fish's body weight or 0.48 N/kg. In hovering, fatheads must produce a downwardly-directed force from its pectoral fins that equals the weight of the fish in water. Inferred from the percentage of resting fish, which never exceed 30% during the first h (Fig.5,6), most fatheads tolerated their higher densities and remained at fixed positions in the water column for pro-

longed periods of time. There was, however, some indication that the time during which fish could hover in this manner was affected by its density. After transfer from current, "access" fish reached buoyancies of 0.8 mL/g or higher, after 3 h in still water, and only negligible numbers of fish rested beyond this time (Fig.5). Corresponding percentages of resting "non-access" fish were also observed after their buoyancy had reached about 0.8 mL/g, however, as they increased static lift much slower than those with access, this value was only reached between 24 and 48 h. Prior to this time, when buoyancy was much less, significant (20-30%) proportions of fish rested on the bottom. The increasing number of resting fish observed at time period 5 (i.e. 20:00-20:30) in "non-access" and treated fish with access (buoyancy experiment) was probably not a buoyancy-related phenomenon, but indicative of the diel activity pattern of the fish in this study, which generally found fatheads very inactive at this particular time. Kopec (1927) observed the cyprinid Phoxinus laevis to hover for short periods of time after the whole swimbladder had been surgically removed. Here the sustained vertical force must have been even greater. This somewhat greater tolerance to downward forces is plausible, as negative buoyancy is a resting state of definite survival value (McCutcheon 1966; see above). Alternatively, this trend might simply reflect differences in the maximal sustainable frequency of pectoral fin beats. Immediately upon transfer from current to still water, fathead

minnows had beat frequencies of 280-315 beats/min, substantially more than the maximal frequencies (150-200 beats/min) reported by Jones (1952) and Bishai (1961). Differences in beat amplitude and effective fin area, which could have had modifying effects, were not measured.

Generally, the frequencies of fin beats were higher in treated than in control fish, although not always significantly, as probabilities varied between 0.0001 and 0.12 . This inequality in beat frequencies could often be attributed to differences in buoyancies (eg. in still water) but was sometimes at variance with buoyancy changes (eg. Fig.9). As suggested by the linear relationship between increasing PFBF and decreasing buoyancy found in the present study and as supported by results of Jones (1952) and McCutcheon (1958), static and hydrodynamic lift are directly complementary in providing neutral total lift. Based on this relationship, cases in which treated fish displayed higher beat frequencies than controls of similar or lower buoyancy, are difficult to explain. Perhaps different fish vary in their ability to generate hydrodynamic lift per fin beat. Another explanation could be inaccuracies in counting beat frequencies above 240/min and the time delay between the measurements of beat frequencies and buoyancies. Furthermore, the less tight relationship (in terms of r) between PFBF and buoyancy in treated fish and for those with access to air (Table 7) indicates the presence of modifying factors which could not be assessed.

Regarding the ecological significance of these results, it has to be asked if, and to what extent, these laboratory observations pertain to the behavior of fathead minnows in their natural habitat. Lindsey (1978) cautions that "much of the repertoire of behavioral patterns characteristic of a species in the wild may be physically impossible under laboratory conditions." Fathead minnows feed on detritus, insect larvae and small crustaceans (Held & Peterka 1974, Tallman et al. 1984), food objects that are immobile or slow moving. This feeding behavior involves prolonged periods of slow swimming and intermittent hovering. As forward locomotion will not provide much lift under these conditions, extensive use of the pectoral fins therefore seems to be a very likely strategy in generating hydrodynamic lift.

E. Swimming performance

Both control and treated fish withstood the increasing current for about 7 h. In these trials, IP was also unaffected by water pH and remained close to atmospheric. However, treated fish had higher buoyancies and were in poorer condition than controls after they fatigued.

MacLeod & Smith (1966) measured the "maximum" swimming speed of adult fathead minnows at about 38.5 cm/s. However, as indicated by the previous experiments in which the majority of fish could sustain speeds of 35 cm/s for at least 48 h, maximum, prolonged and even sustained swimmming speeds seemed to be considerably higher (see Beamish 1978 for review on categories of swimming speed). To accomodate the above considerations, a stepwise progression in current speed with a 2 h interval was applied. Unfortunately at the high water velocities it became increasingly difficult to maintain an acceptable velocity profile and the magnitude of velocity increments were limited by the design of the stream tank. Obviously a smaller step increase to the highest velocity would have resulted in a more precise seperation in the swimming time of individual fishes. At 57.5 cm/s, fish swept against the downstream screen blocked water flow and resulted in a greatly increased velocity gradient. Generally, fish often reacted repeatedly to prodding by "bursting" to the upstream screen just to be swept back within 20 to 120 s before being removed. Occasionally, though, fish swam

for up to 60 min after a brief rest (<60s) at the downstream screen.

Considering these limitations, the similar swimming times of treated and control fish and the lack of relationship between buoyancy and swimming time do not preclude the possibility that acid-exposed fish fatigue more easily at a given water velocity or have lower prolonged swimming speeds. Both buoyancy and water pH have previously been shown to affect swimming performance. Berezay & Gee (1978) found that creek chub (Semotilus atronaculatus) at non-optimal buoyancy swam at a negative angle of attack in current, thereby increasing drag and fatigued more quickly than fish at optimal buoyancy. Graham & Wood (1981) reported a linear decline in critical swimming speed with acid-exposure between pH 4.6 and 3.0 in rainbow trout (Salmo gairdneri). These pH levels are sufficiently low (see chapter F, p.84) to attribute the reduced swimming performance directly to respiratory disturbances (Graham & Wood 1981) but elevated energetic costs for ionoregulation and increased swimming effort due to suboptimal buoyancy may have also contributed. It can be assumed that energy expenditure during locomotion is directly related to swimming effort. In the present study, the amount of energy expended during exercise was probably less affected by the experimental conditions and measuring techniques than the somewhat arbitrary measure of swimming time. Changes in condition provide some indirect information on energy expen-

diture over the course of the experiment. It is here where the experiment is probably more telling. Fish were not fed for 24-26 h before KB was measured. No specific data are available for fathead minnows, but according to Beamish (1978) it can be assumed that this time period is sufficiently long to assure the postabsorptive state. Consequently, energy requirements must have been met by metabolizing stored reserves. On a percentage basis, treated fish lost more weight than controls and were in poorer condition after swimming for similar times. The composition of this weight loss is not known. Sockeye salmon, Oncorhynchus nerka, increased percentage body water and decreased lipid, protein and caloric content per gram wet weight during sustained swimming (Brett 1973). It seems reasonable, therefore, to attribute the weight loss in fathead minnows to a reduction in caloric content of the fish.

Considering the inefficiency of hydrodynamic compensation (i.e. negative angle of attack) of excess lift (Alexander 1972), it is intriguing to speculate that the higher buoyancy of treated fish, which was of similar magnitude to that found in fish after 48 h in current of constant velocity, may have been responsible for the increased energy demands. Surprisingly the negative correlation found between buoyancy and KA was not significant ($p=0.10$). The reason for this is not known. Probably other factors than buoyancy contribute to increased energy demands. In treated fish the cost of

maintainance could have been increased due to elevated energy expenditure for ventilation and osmoregulation (see next chapter). Neville (1979a) and Janssen & Randall (1975) did not find a longterm ventilatory response of rested rainbow trout, Salmo gairdneri, at reduced pH . However, such was reported by Rosseland (1980) for brown trout, Salmo trutta. Furthermore, the higher breathing rate of treated (132 opercular beats/min) compared to control (114 beats/min) fish, measured within 15 min after transfer from current into still water in the time series experiments, indicates that under severe exercise, when oxygen consumption is maximal, ventilation can be affected. Apparently these demands were met in the short term (0-9 h) without a dramatic effect on swimming performance, but treated fish could only do so by increasing energy mobilisation. The response in IP of the control fish was somewhat unexpected. In contrast to fish in current of constant velocity (Fig.1 C), IP never exceeded atmospheric pressure significantly. A comparible response occurred in control fish after 32 d in 26 cm/s current (Fig.10). It can be speculated that physiological demand incurred by the stress of tolerating water current has both a time and magnitude component. Intolerable velocities immediately cause the control over IP to fail, a process which at lower velocities only occurs after extended periods of time.

The positive correlation between swimming time and IP again indicates the critical role of this precise mechanism

of buoyancy control. As mean IP for fish of both treatments was close to atmospheric, individual fish that were able to maintain excess IP most likely swam longer than those which did not.

F. Mechanisms of impaired swimbladder function

Effects of reduced environmental pH on the physiology of freshwater fish include disturbances in (a) branchial gas exchange (Packer & Dunson 1972, Packer 1979, Ultsch et al.1981), (b) plasma acid-base status (Packer & Dunson 1970, Packer 1979, Neville 1979b, McDonald et al. 1980, Ultsch et al. 1981, Hobe et al. 1983, McDonald & Wood 1981, Booth et al. 1982) and (c) haematological homeostasis (Dively et al. 1977, Neville 1979b, McWilliams 1982, Milligan & Wood 1982, Giles et al. 1984).

Of these factors, reduced O_2 uptake at the respiratory surface and consequently decreased blood PO_2, which via an elevated O_2 gradient between swimbladder and blood (see below), potentially results in increased gas reabsorption, appears to arise only at acutely lethal pH values (2.0-3.5). This was shown at least for salmonids (Packer & Dunson 1972, Packer 1979) and the carp (Cyprinus carpio) (Ultsch et al. 1981). It should therefore not be considered as a factor at the current pH levels, which are in terms of hydrogen ion concentration two order of magnitude higher. Similarly, a net acid uptake can be normally avoided at ambient pH at least as low as 5.1 (Ultsch et al. 1981), thus preventing significant changes in blood pH. However, fathead minnows are among the more acid-sensitive species (Rahel & Magnusson 1983, Mills 1982, Rahel 1984). If this intolerance reflects their greater physiological sensitivity to acid exposure,

then the possible development of a mild acidosis cannot be completely ignored even at a more moderate pH.

Contrary to the first two factors, net acid uptake and impaired O_2 transport, an elevated haematocrit has been reported for acid-exposed fish over a wider pH range (4.0-6.4), (McWilliams 1982, Lee et al. 1983, Giles et al. 1984). With regard to the O_2 carrying capacity of the blood, it is important to note that this increase in blood haematocrit is not linked to a parallel increase in blood heamoglobin content, but is partly attributable to a simple swelling (increase in volume) of the erythrocytes (Milligan & Wood 1982, Giles et al. 1984). In an extensive study on haematological disturbances, Milligan & Wood (1982) measured a 68-125% increase in blood viscosity, which was directly attributed to the rise in haematocrit. The authors suggested that the red cell swelling results from an "ionic dilution" of the plasma evoked by severe ionregulatory failure. They thereby drew an intimate linkage between ionic imbalance, elevated haematocrit and increased blood viscosity. If this interpretation is correct, then, the strong correlation between moderately depressed environmental pH (4.0-6.0) and ionoregulatory failure (Leivestad & Muniz 1976, McDonald et al. 1980, McDonald & Wood 1981, McWilliams 1982, Booth et al. 1982, Fraser & Harvey 1984, Giles et al. 1984), indicates that higher blood viscosity must be a common phenomenon at reduced ambient pH.

Both gas secretion and reabsorption are critically dependent on (a) the partial pressure gradient between the blood and gas phase, (b) the gas (particularly O_2)-combining capacity of the blood and (c) the blood flow (Steen 1963a,b; Kuhn and Kuhn 1961, Sund 1977). At a given partial pressure, the affinity of haemoglobin for O_2 and the O_2-carrying capacity of blood falls with declining blood pH (Bohr and Root effect). In the rete mirabile of the swimbladder, where a localized reduction in blood pH is achieved by the addition of lactic acid (Hall 1924, Steen 1963b), these effects are used to produce high partial gas pressures within the swimbladder. Arterial blood entering the rete at a reduced plasma pH and therefore lowered absolute O_2 content, should theoretically diminish the effectiveness of the gas secreting mechanisms. In the reabsorptive area of the swimbladder, a reduced O_2 combining capacity of the afferent blood equally results in a depressed gas reabsorption rate. From the Poiseuille equation (Poiseuille 1842, cited in Ross 1979b) it can be deduced that the blood flow rate inside a vessel is, under otherwise constant conditions, inversely proportional to the blood viscosity. Increases in heart rate and mean dorsal aortic pressure (Milligan & Wood 1982), provide some indication that acid-exposed salmonids do not compensate for the higher blood viscosity by reducing vascular resistance. If this is true for fathead minnows, then the efficiency of gas secretion and reabsorption will be further reduced by the acid-induced increase in blood viscosity.

G. Ecological implications

Compared to terrestrial organisms, fish do not require strong skeletons to support their weight or need to expend much energy to move vertically because the relatively high density of water buoys up their bodies (Lindsey 1978). Nevertheless, fish without a swimbladder are denser than water by 6-9% (Alexander 1966) and are comparatively ill-equipped to resist the resulting vertical forces by movements of their fins. At low to moderate (<70 cm/s) swimming speeds, a gas-filled swimbladder, which reduces density by increasing volume but not appreciably mass, is energetically the most economical way to compensate for the differences in densities, thus providing neutral buoyancy (Alexander 1972, Gee 1983). The vast majority of lotic or epipelagic freshwater fish species use this efficient method of generating lift. However, lift requirements are not constant. Water velocities in lotic environments can vary profoundly over time and space. Vertical migrations in the upper layers of the water column result in the relative greatest pressure, and therefore, swimbladder volume changes. Factors such as these necessitate rapid and precise adjustment of swimbladder volume if these locomotor costs and therefore the costs of total metabolic output are to be minimized. In an energy budget

$$C = (R + A + SDA) + (F + U) + (B + G)$$

where C=energy consumed by feeding, R=standard metabolism, A=costs of activity, SDA=specific dynamic action, F=egestion of indigestible waste, U=excretion of soluble waste, B=somatic growth and G=gonad growth, the total metabolic output comprises R,A and SDA (Kitchell 1983).

The results presented in this study indicate that fathead minnows in a moderately acidic environment are limited in their capacity to efficiently regulate buoyancy. Indirectly, suboptimal buoyancy may reduce the tolerance to maximal water velocities or the time to fatigue in current. If fish can not withstand the flow of the water, upstream dispersal will be limited and/or drift to unfavorable downstream habitats increased. These factors potentially affect the viablility of localized populations, spawning success, food availability etc. Prolonged periods of gas secretion, and hydrodynamical compensation or more frequent air gulping will increase standard metabolism, the cost of activity and therefore maintenance costs. Elevated costs of ion regulation and ventilation, at least during active metabolism, may also be incurred. Furthermore, increased activity may render fatheads more susceptible to predation.

Following the energy output hierarchy, maintenance costs must first be met before any surplus energy can be allocated to growth or reproductive tissue (Kitchell 1983). Consequently, in acid-exposed fish a lesser proportion of the available food can be funnelled into net energy gain. In

times of food shortage, body energy reserves more rapidly deplete as illustrated by the larger weight loss of unfed treated fish during sustained swimming (Table 8).

Almer et al. (1974), Hendry et al. (1976), Malley et al. (1981), Nero & Schindler (1982) who reported an acid-induced decline in invertebrate species constituting important elements of fish diets suggest that the intake of energy is potentially reduced at decreased environmental pH. These effects on species composition and abundance start to occur at pH values as high as pH 6.0 (Almer et al. 1974). Reduced growth rates (Beamish et al. 1975, Muniz & Leivestad 1979, cited in Overrein et al. 1980, Rosseland 1980, Ryan & Harvey 1980) or equal growth rates at increased food intake (Rosseland et al. 1980) have been reported from field and laboratory studies. Rosseland (1980) observed increased metabolism in brown trout (Salmo trutta) at pH 4.5 and attributed response in growth rates to the "higher costs of living in acid water" (Rosseland et al. 1980).

In summary, the results of this study confirm the high sensitivity of fathead minnows and cyprinids in general to even moderate acidification of their environment. Further information is provided on some of the possible physiological disturbances contributing to their disappearance from aquatic ecosystems at pH levels of 5.2-6.2 as reported by Almer (1974), Mills (1982), Rahel & Magnusson (1983) and Rahel (1984).

LITERATURE CITED

Akita,Y.K. 1936. Studies on the physiology of the swimbladder. J. Faculty Sci. Tokyo Univ. Sect.IV Zool. 4: 111-135.

Alexander, R.McN. 1959. The physical properties of the swimbladder in intact cypriniformes. J. exp. Biol. 36: 315-332.

Alexander, R.McN. 1966. Physical aspects of swimbladder function. Biol. Rev. 41: 141-176.

Alexander, R.McN. 1972. The energetics of vertical migration by fishes. Symp. Soc. Exp. Biol. 26: 273-294.

Almer, B, W. Dickson, C. Eckstrom, and E. Hornstrom. 1974. Effects of acidification on Swedish lakes. Ambio 3: 30-36.

Beamish, F.W.H. 1978. Swimming capacity. In: Fish Physiology, vol.7, pp. 101-187, edited by W.S. Hoar and D.J. Randall. Academic Press, New York and London.

Beamish, R.J. and H.H. Harvey. 1972. Acidification of the La Cloche Mountain lakes, Ontario, and resulting fish mortalities. J. Fish. Res. Board Can. 29: 1131-1143.

Beamish, R.J., W.L. Lockhart, J.C. Van Loon, H.H. Harvey. 1975. Long term acidification of a lake and resulting effects on fishes. Ambio 4: 98-102.

Berezay, G. and J.H. Gee. 1978. Buoyancy response to changes in water velocity and its function in creek chub (Semotilus atromaculatus). J. Fish. Res Board Can. 35: 295-299.

Bishai, H.M. 1961. The effect of pressure on the distribution of some Nile fish. J. exp. Zool. 147: 113-124.

Blake, R.W. 1983. Median and paired fin propulsion. In: Fish Biomechanics, pp. 214-247, edited by P.W. Webb and D. Weihs. Praeger Publishers, New York.

Booth, J.H., G.F. Jansz, and G.F. Holeton. 1982. Cl^-, K^+, and acid-base balance in rainbow trout during exposure to, and recovery from, sublethal environmental acidification. Can. J. Zool. 60: 1123-1130.

Brett, J.R. 1973. Energy expenditure of sockeye salmon, Oncorhynchus nerka, during sustained performance. J. Fish. Res. Board Can. 30: 1799-1809.

Chiasson, A.G. and J.H. Gee. 1983. Swimbladder gas composition and control of buoyancy by fathead minnow (Pimephales promelas) during exposure to hypoxia. Can. J. Zool. 61: 2213-2218.

Cowling, E.B. 1982. An historical perspective on acid precipitation. In: Acid Rain/Fisheries, pp.15-31. Proc. Int. Symp. on Acid Precipitation and Fishery Impacts in Northeastern North America. Cornell University, Ithaca, N.Y., August 2-5, 1981; publ. by the Amer. Fish. Soc., 1982.

Denton, E.J., J.D. Liddicoat, and D.W. Taylor. 1972. The permeability to gases of the swimbladder of the conger eel (Conger conger). J. mar. biol. Ass. U.K. 52: 727-746.

Dively, J.L., J.E. Mudge. W.H Neff, and A. Anthony. 1977. Blood PO_2, PCO_2, and pH changes in brook trout (Salvelinus fontinalis) exposed to sublethal levels of acidity. Comp. Biochem. Physiol. 57A: 347-351.

Evans, H.M. and G.C.C. Damant. 1929. Observations on the physiology of the swimbladder in the cyprinoid fishes. J. exper. Biol. 6: 42-55.

Fange, R. 1966. Physiology of the swimbladder. Physiol. Rev. 46: 299-322.

Fange, R. 1973. The physiology of the swimbladder. In: Comperative Physiology, pp. 135-159, edited by L. Bolis, K. Schmidt-Nilson, H.P. Madrell. North-Holland Publ. Co.

Fange, R. 1976. Gas exchange in the swimbladder. In: Respiration of amphibious vertebrates, pp. 189-211, edited by G.M.Hughes. Academic Press London.

Franz, G. 1937. Uber den Reflex des Gasspuckens bei Fischen und die Funktion des Weberschen Apparates. Z. vergl. Physiol. 25: 193-238.

Fraser, G.A. and H.H. Harvey. 1984. Effects of environmental pH on the ionic composition of the white sucker (Catostomus commersoni) and pumpkinseed (Lepomis gibbosus). Can. J. Zool. 62: 249-259.

Gee, J.H. 1968. Adjustment of buoyancy by longnose dace (Rhinichthys cataractae) in relation to velocity of water. J. Fish. Res. Board Can. 25: 1485-1496.

Gee, J.H. 1970. Adjustment of buoyancy in blacknose dace, Rhinichthys atratulus. J. Fish. Res. Board Can. 27: 1855-1859.

Gee, J.H. 1972. Adaptive variation in swimmbladder length and volume in dace genus Rhinichthys. J. Fish. Res. Board Can. 29: 119-127.

Gee, J.H. 1977. Effects of size of fish, water temperature and water velocity on buoyancy alteration by fathead minnows, Pimephales promelas. Comp. Biochem. Physiol. 56A: 503-508.

Gee, J.H. 1983. Ecological implications of buoyancy control in fish. In: Fish biomechanics, pp.140-176, edited by P.W. Webb and D. Weihs. Praeger Publishers, New York.

Gee, J.H. In press. Ecological and evolutionary implications of phenotypic plasticity of swimbladder volume and lift in stream environments. In: Evolutionary and community ecology of North American stream fishes, edited by D.C. Heinz and W.J. Matthews. University of Oklahoma Press.

Gee, J.H. and V.G. Bartnik. 1969. Simple stram tank simulating a rapids environment. J. Fish. Res. Board Can. 26: 2227-2230.

Gee, J.H. and P.A. Gee. 1976. Alteration of buoyancy by some Central American stream fishes, and a comparison with North American species. Can. J. Zool. 54: 386-391.

Gee, J.H., K. Machniak, and S.M. Chalanchuk. 1974. Adjustment of buoyancy and excess internal pressure of swimbladder gases in some North American freshwater fishes. J. Fish. Res. Board Can. 31: 1139-1141.

Giles, M.A, H.S. Majewski, and B. Hobden. 1984. Osmoregulatory and hematological responses of rainbow trout (Salmo gairdneri) to extended environmental acidification. Can. J. Fish. Aquat. Sci. 41: 1686-1694.

Glass, G.V., P.D. Peckham, and J.R. Sanders. 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. Rev. Educ. Res. 42: 237-288.

Graham, M.S. and C.M. Wood. 1981. Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type, and exercise. Can. J. Zool. 59: 1518-1526.

Grahn, O. 1980. Fish kills in two moderately acidic lakes due to high aluminum concentration. In: Proc. Internat. Conf. Ecol. Impact of Acid Precipitation, pp.310-311, SNSF Project Report, Oslo, Norway, March 1980.

Hall, F.G. 1924. The function of the swimbladder of fishes. Biol. Bulletin 47: 79-126.

Held, J.W. and J.J. Peterka. 1974. Age,growth and food habits of the fathead minnow, Pimephales promelas, in North Dakota saline lakes. Trans. Amer. Fish. Soc. 4: 743-756.

Hendry, G.R., K. Baalsrud, T.S. Traan, M. Laake, and G. Raddum. 1976. Acid precipitation: Some hydrobiological changes. Ambio 5: 224-227.

Hobe, H., P.R.H. Wilkes, R.L. Walker, C.M. Wood and B.R. McMahon. 1983. Acid-base balance,ionic status, and renal function in resting and acid-exposed white suckers (Catostomus commersoni). Can. J. Zool. 61: 2660-2668.

Hoglund, L.B. 1961. The reaction of fish in concentration gradients. Rep. 43. Inst. Freshwater Res.

Hufner, G. 1892. Zur phsikalischen Chemie der Schwimmblasengase. Arch. Anat. Physiol., Physiol. Abtl. pp. 54-80.

Jacobs, W. 1930. Untersuchungen zur Physiologie der Schwimmblase der Fische. I. Uber die Gassekretion in der Schwimmblase von Physoclisten. Z. Vergl. Physiol. 11: 565-629.

Jacobs, W. 1932. Untersuchungen zur Physiologie der Schwimmblase der Fische. II. Die Volumenregulation in der Schwimmblase des Flussbarsches. Z. Vergl. Physiol. 18: 125-156.

Jacobs, W. 1934. Untersuchungen zur Physiologie der Schwimmblase der Fische. III. Luftschlucken und Gassekretion bei Physostomen. Z. Vergl. Physiol. 20: 674-698.

Janssen, R.G. and D.J. Randall. 1975. The effects of changes in pH and PCO_2 in blood and water on breathing in rainbow trout, Salmo gairdneri. Respir. Physiol. 25: 235-245.

Jeffries, D.S., C.M. Cox and P.J. Dillon. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640-646.

Jensen, K.W and E. Snekvik. 1972. Low pH levels wipe out salmon and trout populations in southernmost Norway. Ambio 1: 223-225.

Jones H.F.R. 1951. The swimmbladder and the vertical movement of teleostean fishes. I. Physical factors. J. exp. Biol. 28: 553-566.

Jones H.F.R. 1952. The swimmbladder and the vertical movement of teleostean fishes. II. The restriction to rapid and slow movement. J. exp. Biol. 29: 94-109.

Kitchell, I.F. 1983. Energetics. In: Fish biomechanics, pp.313-338, edited by P.W. Webb and D. Weihs. Praeger Publishers, N.Y.

Klinger, S.A., J.J. Magnuson, and G.W. Gallepp. 1982. Survival mechanisms of the central mudminnow (Umbra limi), fathead minnow (Pimephales promelas) and brook stickleback (Culea incostans) for low oxygen in winter. Env. Biol. Fish. 7: 113-120.

Kopec, S. 1927. A contribution to the knowledge of the hydrostatic role of the airbladder during swimming. Biologia generalis 3: 253-258.

Krohn, H. and J. Piiper 1962. Gassekretion in die Schwimmblase der Schleie (Tinca tinca L.) in Wasser mit erniedrigtem N-Druck. Naturwissenschaften 49: 428-429.

Kuhn, W. and H.J. Kuhn. 1961. Multiplikation von Aussalz- und anderen Einzeleffekten fur die Bereitung hoher Gasdrucke in der Schwimmblase. Z. fur Elektrochemie 65: 426-439.

Kutchai, H. and J.B. Steen. 1971. The permeability of the swimbladder. Comp. Biochem. Physiol. 39A: 119-123.

Ledebur, J.von. 1937. Uber die Sekretion und Resorption von Gasen in der Fischschwimmblase. Biol. Rev. 12: 217-244.

Lee, R.M., S.D. Gerking, and B. Jezierska. 1983. Electrolyte balance and energy mobilisation in acid-stressed rainbow trout, Salmo gairderi, and their relation to reproductive success. Biol. Fish. 8: 115-123.

Leivestad, H. and I.P. Muniz. 1976. Fish kill at low pH in a Norwegian river. Nature 259: 391-392.

Lindsey, C.C. 1978. Form, function and locomotory habits in fish, 1-100. In: Fish Physiology, edited by W.S. Randall, vol.7, Academic Press, New York and London.

Luoma, M.E. and J.H. Gee. 1980. Seasonal factors affecting buoyancy attained in still water and current by fathead minnows, Pimephales promelas. Can. J. Fish. Aquat. Sci. 37: 670-678.

Machniak, K. and J.H. Gee. 1975. Adjustment of buoyancy by tadpole madtom, Noturus gyrinus, and black bullhead, Ictalurus melas, in response to a change in water velocity. J. Fish. Res. Board Can. 32: 303-307.

MacLeod, J.C. and L.L. Smith, Jr. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, Pimephales promelas. Trans. Am. Fish. Soc. 95: 72-84.

Magnuson, J.J. 1978. Locomotion by scombrid fishes: hydromechanics, morphology, and behavior, pp. 239-313. In: Fish Physiology, vol.7, pp. 239-313 edited by W.S. Hoar and D.J. Randall. Academic Press, New York and London.

Malley, D.F., D.L. Findley, and P.S.S. Chang. 1981. Ecological effects of acid precipitation on zooplankton. In: Acid precipitation: effects on ecological systems, pp.297-327, edited by F.M. D'Itri. Ann Arbor Science Publishers, Ann Arbor, MI.

McCutcheon, F.H. 1958. Swimbladder volume, buoyancy, and behavior in the pinfish, Lagodon rhomboides (Linn.) J. Cell. Comp. Physiol. 52: 453-479.

McCutcheon, F.H. 1962. Swimbladder volume control in the pinfish, Lagodon rhomboides(Linn.) J. Cell. Comp. Physiol. 59: 203-214.

McCutcheon, F.H. 1966. Pressure sensitivity, reflexes and buoyancy responses in teleosts. Anim. Behav. 14: 204-217.

McDonald, D.G. and C.M. Wood. 1981. Branchial and renal acid and ion fluxes in the rainbow trout, Salmo gairdneri, at low environmental pH. J. Exp. Biol. 93: 101-118.

McDonald, D.G., H. Hobe, and C.M. Wood. 1980. The influence of calcium on the physiological responses of the rainbow trout to low environmental pH. J. Exp. Biol. 88: 109-131.

McNabb, R.A. and J.A. Mecham. 1971. The effects of different acclimation temperatures on gas secretion in the swimbladder of the bluegill sunfish, Lepomis macrochirus. Comp. Biochem. Physiol. 40A: 609-616.

McWilliams, P.G. 1982. A comparison of physiological characteristics in normal and acid exposed populations of the brown trout, Salmo trutta. Comp. Biochem. Physiol. 72A: 515-522.

Meesters, A. and F.G.P. Nagel. 1934. Uber Sekretion und Resorption in der Schwimmblase des Flussbarsches. Z. Vergl. Physiol. 21: 646-657.

Milligan, C.L. and C.M. Wood. 1982. Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, Salmo gairdneri. J. Exp. Biol. 99: 397-415.

Mills, K.H. 1982. Fish population responses to experimental acidification of a small Ontario lake. Division of Environmental Chemistry, American Chemical Society. Las Vegas, NV., March 1982.

Moreau, A. 1876. Recheres experimentales sur le functions de la vessie natatoire. Annales Sci. Nat. Zool. Biol. Anim. Ser.b. 4: 1-85.

Mount, D.I. 1973. Chronic effect of low pH on fathead minnow survival, growth and reproduction. Water Research 7: 987-993.

Neave, N.M., C.L. Dilworth, J.G. Eales, and R.L. Saunders. 1966. Adjustment of buoyancy in Atlantic salmon parr in relation to changing water velocity. J. Fish. Res. Bd. Can. 23: 1617-1620.

Nero, R.W., and D.W. Schindler. 1983. Decline of Mysis relicta during the acidification of lake 223. Can. J. Fish. Aquat. Sci. 40: 1905-1911.

Neville, C.M. 1979a. Ventilatory response of rainbow trout to increase H^+-Ion concentration in blood and water. Comperative Biochem. Physiol. 63A: 373-376.

Neville, C.M. 1979b. Sublethal effects of environmental acidification on rainbow trout. J. Fish. Res. Can. 36: 84-87.

Overfield, E.M. and J.A. Kylstra. 1971. The volume and rate of volume change of the swimbladder of the goldfish. Respir. Physiol. 13: 283-291.

Overrein, L.N., H.M. Seip, and A. Tollan. 1980. Acid precipitation - effects on forest and fish. Final report of the SNSF-project 1972-1980. Oslo - As, April 1980.

Packer, R.K. 1979. Acid-base balance and gas exchange in brook trout (Salvelinus fontinalis) exposed to acidic environment. J. Exp. Biol. 79: 127-134.

Packer, R.K. and W.A. Dunson. 1970. Effects of low environmental pH on blood pH and sodium balance of brook trout. J. Exp. Zool. 174: 65-72.

Packer, R.K. and W.A. Dunson. 1972. Anoxia and sodium loss associated with the death of brook trout at low pH. Comp. Biochem. Physiol. 41A: 17-26.

Pinder, L.J. and J.G. Eales. 1969. Seasonal buoyancy changes in Atlantic salmon (Salmo salar) parr and smolt. J. Fish. Res. Board Can. 26: 2093-2100.

Rahel, F.J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology 65: 1276-1289.

Rahel, F.J. and J.J. Magnuson. 1983. Low pH and the absence of fish species in natural acidic Wisconsin lakes: Inferences for cultural acidification. Can. J. Fish. Aquat. Sci. 40: 3-9.

Ross, L.G. 1978. The innervation of the resorptive structures in the swimbladder of a physoclist fish Pollachius virens (L.) Comp. Biochem. Physiol. 61C: 385-388.

Ross, L.G. 1979a. The permeability to oxygen and the guanine content of the swimbladder of a physoclist fish, Pollachius virens. J. Mar. Biol. Ass. U.K. 59: 473-441.

Ross, L.G. 1979b. The haemodynamics of gas resorption from the physoclist swimbladder: the structure and morphometrics of the oval in Pollachius virens (L.) J. Fish. Biol. 14: 261-266.

Rosseland, B.O. 1980. Physiological responses to acid water in fish. 2. Effects of acid water on metabolism and gill ventilation in brown trout, Salmo trutta, and brook trout Salvelinus fontinalis. In: Proc. Internat. Conf. Ecol. Impact of Acid Precipitation, pp. 34-35. SNSF Project Report, Oslo, Norway, March 1980.

Rosseland, B.O. I. Sevaldrud, D. Svalastog and I.P. Muniz. 1980. Studies on freshwater fish populations - effects of acidification on reproduction, population structure, growth and food selection. In: Proc. Internat. Conf. Ecol. Impact of Acid Precipitation, pp.336-337, SNSF Project Report, Oslo, Norway, March 1980.

Rostorfer, H.H. 1942. The gas content of the swimbladder of the rock bass, Ambloplites rupestris, in relation to hydrostatic pressure. Biol. Bull. 82: 138-153.

Ryan, P.M. and H.H. Harvey. 1980. Growth response of yellow perch, Perca flavescens, to lake acidification in the La Cloche Mountain lakes of Ontario. Environ. Biol. Fish. 5: 97-108.

Saunders, R.L. 1965. Adjustment of buouyancy in young Atlantic salmon and brook trout by changes in swimbladder volume. J. Fish. Res. Board Can. 22: 335-351.

SAS. 1982. User's guide: Statistics. SAS Institute Inc. Cary, Noth Carolina.

Schofield, C.L. 1976. Acid precipitation: effects on fish. Ambio 5: 228-230.

Scholander, P.F. 1956. Observations on the gas gland in the living fish. J. Cell. Comp. Physiol. 48: 523-528.

Scott, W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Co., San Francisco.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analysis of fresh water. Fish. Res. Board Can. Misc. Spec. Publ. 25: 125 p.

Steen, J.B. 1963a. The physiology of the swimbladder of the eel, Anguilla vulgaris. II. The reabsorption of gases. Acta Physiol. Scand. 58: 138-149.

Steen, J.B. 1963b. The physiology of the swimbladder of the eel, Anguilla vulgaris. III. The mechanism of gas secretion. Acta Physiol. Scand. 59: 221:241.

Steen, J.B. 1970. The swim bladder as an hydrostatic organ. In: Fish Physiology, vol.4, pp. 413-443, edited by W.S. Hoar and D.J. Randall. Academic Press, New York and London.

Stewart, D.B. 1980. Mechanisms of buoyancy adjustment and effects of water velocity and temperature on the ability to maintain buoyancy in fathead minnow, Pimephales promelas, Rafinesque. M.Sc. Thesis, University of Manitoba, Winnipeg.

Stewart, B. and J.H. Gee 1981. Mechanisms of buoyancy adjustment and effects of water velocity and temperature on ability to maintain buoyancy in fathead minnow, Pimephales promelas, Rafinesque. Comp. Biochem. Physiol. 68A: 337-347.

Sund, T. 1977. A mathematical model for counter-current multiplication in the swim-bladder. J. Physiol. 267: 679-696.

Sundnes, G. and T. Gytre. 1972. Swimbladder gas pressure of cod in relation to hydrostatic pressure. I. Cons. int. Explor. Mer. 34: 529-532.

Tallman, R.F., K.H. Mills, and R.G. Rotter. 1984. The comperative ecology of pearl dace (Semotilus margarita) and fathead minnow (Pimephales promelas) in lake 114, the experimental lakes area, northwestern Ontario, with an appended key to the cyprinids of the experimental lakes area. Can. Manuscr. Rep. Fish. Aquat. Sci. 1756: iv + 27 p. .

Tytler, P. and J.H.S. Blaxter 1973. Adaptation by cod and saithe to pressure changes. Netherl. J. Sea Res. 7: 31-45.

Ultsch, G.R., M.E. Ott, and N. Heisler. 1981. Acid-base and electrolyte status in carp (Cyprinus carpio) exposed to low environmental pH. J. Exp. Biol. 93: 65-80.

Webb, P.W. 1975. Hydromechanics and energetics of fish propulsion. Bull. Fish. Res. Board Can. 190, 159 pp. .

Wittenberg, J.B. 1958. The secretion of inert gas into the swimbladder of fish. J. Gen. Physiol. 41: 783-804.

Wittenberg, J.B., M.J. Schwend and B.A. Wittenberg 1964. The secretion of oxygen into the swimbladder of fish, III. The role of carbon dioxide. J. Gen. Physiol. 48: 337-355.

Wittenberg, J.B., D.E. Copeland, R.L. Haedrich and J.S. Child 1980. The swimbladder of deep-sea fish: The swimbladder wall is a lipid-rich barrier to oxygen diffusion. J. Marine Biol. Assoc. U.K. 60: 236-276.

Wood, C.M and D.G. McDonald. 1982. Physilogical mechanisms of acid toxicity in fish. In: Acid Rain/Fisheries, Proc. Int. Symp. on Acid Precipitation and Fishery Impacts in Northeastern North America, pp. 197-226. Cornell University, Ithaca, N.Y., August 2-5, 1981; publ. by the Am. Fish. Soc., 1982.

Zischke, J.A., J.W. Arthur, K.J. Nordlie, R.O. Hermanutz, D.A. Standen, and T.P. Henry. 1983. Acidification effects on macroinvertebrates and fathead minnow (Pimephales promelas) in outdoor experimental channels. J. Water Res. 1: 47-63.

APPENDICES

APPENDIX 1

Buoyancy, internal pressure (IP) and standard volume (STV) of fish after 48 h in current of 26 cm/s to determine if a change in water velocity from 35 to 26 cm/s affected the given swimbladder parameter. Individual observations and the mean ($\bar{x}$ ± 95% CL) are given.

Buoyancy (mL/g)	IP (atm)	STV (mL/g)
	a) pH 7.7	
0.311	1.064	0.330
0.426	1.029	0.436
0.511	1.047	0.533
0.274	1.080	0.295
0.348	1.036	0.359
0.419	1.040	0.434
0.375	1.026	0.383
0.430	1.030	0.441
0.387 ± 0.064	1.044 ± 0.017	0.401 ± 0.064
	b) pH 5.3	
0.608	1.006	0.606
0.405	0.998	0.400
0.500	0.992	0.491
0.410	1.012	0.411
0.343	1.004	0.341
0.380	1.020	0.384
0.517	1.003	0.514
0.426	1.000	0.422
0.449 ± 0.073	1.004 ± 0.007	0.446 ± 0.071

APPENDIX 2

Results of still water with access to air and 48 h current experiments:

Buoyancy, standard volume (STV) and internal pressure (IP) of fathead minnows in still water.

pH 7.7			pH 5.3		
d	$\bar{x}$ ± 95% CL	n	d	$\bar{x}$ ± 95% CL	n
		a) Buoyancy (mL/g)			
14[a]	0.876 ± 0.054	13	13	0.905 ± 0.041	14
10	0.948 ± 0.031	21	18	0.878 ± 0.042	7
20	0.922 ± 0.075	6	25	0.865 ± 0.041	15
11	0.925 ± 0.038	11	15	0.899 ± 0.054	6
			18	0.956 ± 0.041	10
	0.922 ± 0.022	51		0.899 ± 0.020	52
		b) STV (mL/g)			
14	0.864 ± 0.062	11	13	0.892 ± 0.053	12
			18	0.897 ± 0.039	6
			25	0.865 ± 0.048	13
	0.864 ± 0.062	11		0.882 ± 0.028	31
		c) IP (atm)			
14	1.012 ± 0.007	11	13	1.010 ± 0.011	12
			18	0.993 ± 0.018	6
			25	1.001 ± 0.004	13
	1.012 ± 0.007	11		1.003 ± 0.006	31

[a] days in still water

APPENDIX 2 continued

Buoyancy, standard volume (STV) and internal pressure (IP) of fathead minnows after 48 h in current.

pH 7.7			pH 5.3		
d	$\bar{x}$ ± 95% CL	n	d	$\bar{x}$ ± 95% CL	n
	a) Buoyancy (mL/g)				
18[a]	0.380 ± 0.053	12	10	0.452 ± 0.067	14
25	0.391 ± 0.040	8	18	0.396 ± 0.062	11
8	0.392 ± 0.071	11	9	0.437 ± 0.074	11
12	0.379 ± 0.069	11	5	0.455 ± 0.054	14
	0.385 ± 0.024	42		0.437 ± 0.028	50
	b) STV (mL/g)				
18	0.394 ± 0.055	12	10	0.453 ± 0.074	13
25	0.408 ± 0.043	8	18	0.408 ± 0.071	11
8	0.424 ± 0.033	10	9	0.426 ± 0.071	11
	0.407 ± 0.033	30		0.431 ± 0.039	35
	c) IP (atm)				
18	1.057 ± 0.018	12	10	1.026 ± 0.031	13
25	1.067 ± 0.038	8	18	1.006 ± 0.025	11
8	1.039 ± 0.025	10	9	0.970 ± 0.025	11
	1.054 ± 0.014	30		1.001 ± 0.016	35

[a] days in still water before transfer to current

APPENDIX 2 continued

One-Way ANOVA and Duncan's New Multiple Range Test (means with the same letter are not significantly different):

Buoyancies (mL/g) of control fish held in still water with access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	3	0.0426	0.0142	2.69	0.0570
Error	47	0.2482	0.0052		
Total	50	0.2908			

Duncan Grouping		Mean	n
	A	0.948	21
B	A	0.925	11
B	A	0.922	6
B		0.876	13

Buoyancies (mL/g) of treated fish held in still water with access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	4	0.0533	0.0133	3.40	0.0160
Error	47	0.1842	0.0039		
Model	51	0.2375			

Duncan Grouping		Mean	n
	A	0.956	10
B	A	0.905	14
B	A	0.899	6
B		0.878	7
B		0.865	15

Internal pressures (atm) of treated fish in still water with access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0013	0.0006	3.03	0.0642
Error	28	0.0058	0.0002		
Total	30	0.0071			

Standard volumes (mL/g) of treated fish in still water with access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0061	0.0031	0.56	0.5792
Error	28	0.1540	0.0055		
Total	30	0.1601			

APPENDIX 2 continued

Buoyancies (mL/g) of control fish held in still water without access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	1	0.0007	0.0007	1.82	0.1895
Error	24	0.0084	0.0004		
Total	25	0.0101			

Buoyancies (mL/g) of treated fish held in still water without access to air.

Source	df	ss	ms	F value	Prob > F
Experiment	1	0.00007	0.00007	0.02	0.8849
Error	23	0.0785	0.0034		
Total	24	0.0786			

Buoyancies (mL/g) of control fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	3	0.0014	0.0005	0.07	0.9754
Error	38	0.2570	0.0068		
Total	41				

Internal pressures (atm) of control fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0036	0.0018	1.51	0.2391
Error	27	0.0326	0.0012		
Total	29				

Standard volumes (mL/g) of control fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0049	0.0025	0.29	0.7489
Error	27	0.2273	0.0084		
Total	29	0.2322			

Buoyancies (mL/g) of treated fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	3	0.0261	0.0087	0.83	0.4833
Error	46	0.4813	0.0105		
Total	49	0.5074			

APPENDIX 2 continued

Internal pressures (atm) of treated fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0189	0.0095	5.23	0.0108
Error	32	0.0579	0.0018		
Total	34	0.0768			

Duncan Grouping		Mean	n
	A	1.026	13
B	A	1.004	11
B		0.970	11

Standard volumes (mL/g) of treated fish held in current for 48 h.

Source	df	ss	ms	F value	Prob > F
Experiment	2	0.0125	0.0062	0.50	0.6139
Error	32	0.4029	0.0126		
Total	34	0.4154			

APPENDIX 3

t - Test: Means comparison for treated and control fish in still water with access to air.

a) Buoyancy (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.899	52	0.0095	equal	-1.61	101.0	0.1101
pH 7.7	0.922	51	0.0107				

b) Internal pressure (atm)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	1.003	31	0.0028	equal	-1.95	40.0	0.0586
pH 7.7	1.012	11	0.0033				

c) Standard volume (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.881	31	0.0131	equal	0.74	40.0	0.4624
pH 7.7	0.864	11	0.0289				

d) CO_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	2.52	16	0.192	equal	-1.41	31.0	0.1699
pH 7.7	2.86	17	0.152				

e) O_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	23.84	16	1.936	equal	4.15	31.0	0.0002
pH 7.7	13.81	17	1.482				

f) N_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	73.64	16	2.037	equal	-3.88	31.0	0.0005
pH 7.7	83.33	17	1.489				

g) Pectoral fin beat frequency (beats/min)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	66.34	50	3.732	equal	1.65	116.0	0.1013
pH 7.7	58.88	68	2.732				

APPENDIX 3 continued

Means comparison for treated and control fish in still water without access to air.

a) Buoyancy (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.871	25	0.0114	unequal	-9.11	29.7	0.0001
pH 7.7	0.982	26	0.0039				

b) Internal pressure (atm)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	1.001	12	0.0048	equal	-1.11	22.0	0.2783
pH 7.7	1.008	12	0.0045				

c) Standard volume (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.860	12	0.0159	unequal	-4.97	15.5	0.0002
pH 7.7	0.947	12	0.0073				

d) CO_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	4.88	13	0.219	equal	5.32	25.0	0.0001
pH 7.7	3.10	14	0.251				

e) O_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	45.51	13	2.462	equal	7.90	25.0	0.0001
pH 7.7	20.57	14	2.010				

f) N_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	49.60	13	2.576	equal	-7.94	25.0	0.0001
pH 7.7	76.32	14	2.192				

g) Pectoral fin beat frequency (beats/min)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	91.74	27	7.459	unequal	2.78	37.2	0.0085
pH 7.7	68.84	32	3.505				

APPENDIX 3 continued

Means comparison for control fish in still water with and without access to air.

a) Buoyancy (mL/g)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	0.922	51	0.0107	unequal	-5.25	62.3	0.0001
No Access	0.982	26	0.0039				

b) Internal pressure (atm)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	1.012	11	0.0033	equal	0.70	21.0	0.4932
No Access	1.008	12	0.0045				

c) Standard volume (mL/g)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	0.864	11	0.0289	unequal	-2.89	11.3	0.0145
No Access	0.947	12	0.0073				

d) CO_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	2.86	17	0.152	equal	-0.83	29.0	0.4147
No Access	3.10	14	0.251				

e) O_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	13.81	17	1.482	equal	-2.77	29.0	0.0098
No Access	20.57	14	2.010				

f) N_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	83.33	17	1.489	equal	2.72	29.0	0.0109
No Access	76.32	14	2.192				

g) Pectoral fin beat frequency (beats/min)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	58.88	68	2.732	equal	-2.14	98.0	0.0348
No Access	68.84	32	3.505				

APPENDIX 3 continued

Means comparison for treated fish in still water with and without access to air.

a) Buoyancy (mL/g)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	0.899	52	0.0095	equal	1.74	75.0	0.0860
No Access	0.871	25	0.0114				

b) Internal pressure (atm)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	1.003	31	0.0028	equal	0.27	41.0	0.7855
No Access	1.001	12	0.0048				

c) Standard volume (mL/g)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	0.881	31	0.0131	equal	0.92	41.0	0.3611
No Access	0.860	12	0.0159				

d) CO_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	2.52	16	0.1921	equal	-8.73	27.0	0.0001
No Access	4.88	13	0.2191				

e) O_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	23.84	16	1.936	equal	-7.02	27.0	0.0001
No Access	45.51	13	2.462				

f) N_2 (volume %)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	73.64	16	2.037	equal	7.42	27.0	0.0001
No Access	49.60	13	2.576				

g) Pectoral fin beat frequency (beats/min)

Air	Mean	N	Std Error	Variances	T	df	Prob > T
Access	66.34	50	26.392	unequal	-3.05	39.3	0.0041
No Access	91.74	27	38.769				

APPENDIX 4

t-Test: Means comparison for treated and control fish after 48 h in current.

a) Buoyancy (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.437	50	0.0144	equal	2.72	90.0	0.0078
pH 7.7	0.385	42	0.0123				

b) Internal pressure (atm)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	1.001	35	0.0080	equal	-5.01	63.0	0.0001
pH 7.7	1.054	30	0.0065				

c) Standard volume (mL/g)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	0.430	35	0.0187	equal	0.92	63.0	0.3635
pH 7.7	0.407	30	0.0163				

d) CO_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	1.24	13	0.0795	equal	0.37	22.0	0.7172
pH 7.7	1.19	11	0.0879				

e) O_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
pH 5.3	9.53	13	0.7645	equal	1.18	22.0	0.2495
pH 7.7	8.02	11	1.0081				

f) N_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob> T
pH 5.3	89.23	13	0.7645	equal	-1.26	22.0	0.2190
pH 7.7	90.81	11	1.0081				

APPENDIX 4 continued

Means comparison for control fish in still water with access to air and after 48 h in current.

a) CO_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	2.86	17	0.1516	unequal	-9.54	24.2	0.0001
current	1.19	11	0.0879				

b) O_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	13.81	17	1.4822	equal	-2.86	26.0	0.0083
current	8.02	11	1.0233				

c) N_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	83.33	17	1.4893	equal	3.68	26.0	0.0011
current	90.81	11	1.0081				

d) Internal pressure (atm)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	1.012	11	0.0326	unequal	5.74	38.4	0.0001
current	1.054	30	0.0646				

Means comparison for treated fish in still water with access to air and after 48 h in current.

a) CO_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	2.52	16	0.1921	unequal	6.18	19.9	0.0001
current	1.24	13	0.0795				

b) O_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	23.84	16	1.9355	unequal	6.83	19.9	0.0001
current	9.53	13	0.8046				

c) N_2 (volume %)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	73.64	16	2.0368	unequal	-7.17	19.1	0.0001
current	89.23	13	0.7645				

d) Internal pressure (atm)

Treatment	Mean	N	Std Error	Variances	T	df	Prob > T
still water	1.003	31	0.0028	unequal	-0.17	41.8	0.8667
current	1.001	35	0.0080				

APPENDIX 5

Temporal changes in mean buoyancy, standard volume and internal pressure of fish exposed to current at pH 7.7 and pH 5.3.

Time (h)	pH 7.7 Mean	n	95% CI for mean	pH 5.3 Mean	n	95% CI for mean
			a) Buoyancy (mL/g)			
0	0.876	13	0.821 - 0.931	0.905	14	0.864 - 0.946
1	0.711	12	0.645 - 0.777	0.695	11	0.630 - 0.760
3	0.611	14	0.557 - 0.665	0.638	10	0.570 - 0.706
6	0.536	12	0.457 - 0.615	0.621	10	0.562 - 0.680
12	0.415	10	0.361 - 0.469	0.502	10	0.452 - 0.552
24	0.391	13	0.345 - 0.437	0.520	15	0.475 - 0.565
48	0.380	12	0.327 - 0.433	0.452	14	0.385 - 0.519
			b) Standard volume (mL/g)			
0	0.864	11	0.802 - 0.926	0.892	12	0.839 - 0.945
1	0.683	12	0.619 - 0.747	0.674	9	0.593 - 0.755
3	0.600	14	0.544 - 0.656	0.611	9	0.546 - 0.676
6	0.525	12	0.446 - 0.604	0.628	9	0.559 - 0.697
12	0.405	8	0.346 - 0.464	0.493	9	0.438 - 0.548
24	0.384	13	0.332 - 0.436	0.517	15	0.468 - 0.566
48	0.394	12	0.339 - 0.449	0.453	13	0.379 - 0.527
			c) Internal pressure (atm)			
0	1.012	11	1.005 - 1.019	1.010	12	1.000 - 1.020
1	0.996	12	0.977 - 1.015	1.000	9	0.966 - 1.034
3	1.016	14	1.000 - 1.032	1.007	9	0.989 - 1.025
6	1.010	12	0.999 - 1.021	1.028	9	1.015 - 1.041
12	1.035	8	1.012 - 1.058	1.016	9	0.998 - 1.034
24	0.993	13	0.972 - 1.014	1.011	15	0.996 - 1.026
48	1.057	12	1.039 - 1.075	1.026	13	0.996 - 1.056

APPENDIX 6

Two-Way Analysis of Variance and Means Comparison:

Effects of water pH and time in current on buoyancy (mL/g).

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	0.876	0.905	0.4069
pH	1	0.0001	1	0.711	0.695	0.6763
Interaction	6	0.1125	3	0.611	0.638	0.4759
Error	156		6	0.536	0.621	0.0315
Total	169		12	0.415	0.502	0.0345
			24	0.391	0.520	0.0003
			48	0.380	0.452	0.0449

Effects of water pH and time in current on standard volume (mL/g).

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	0.864	0.892	0.4773
pH	1	0.0002	1	0.683	0.674	0.8194
Interaction	6	0.1126	3	0.600	0.611	0.7716
Error	144		6	0.525	0.628	0.0138
Total	157		12	0.405	0.493	0.0580
			24	0.384	0.517	0.0003
			48	0.394	0.453	0.1155

Effects of water pH and time in current on internal pressure (atm).

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	1.012	1.010	0.8270
pH	1	0.4949	1	0.996	1.000	0.7870
Interaction	6	0.0287	3	1.016	1.007	0.4616
Error	144		6	1.010	1.028	0.1497
Total	157		12	1.035	1.016	0.1652
			24	0.993	1.011	0.0946
			48	1.057	1.026	0.0074

APPENDIX 7

Regression Analyses:

Time (0-48 h) in current vs. buoyancy (mL/g).

a) pH 7.7

Source	df	Prob > F	r^2
Model	2	0.0001	0.784
Log time; x	1	0.0001	
(Log time)^2	1	0.0001	
Error	83		
Total	85		

Regression Equation: $Y = 0.876 - 0.552x + 0.150x^2$

b) pH 5.3

Source	df	Prob > F	r^2
Model	2	0.0001	0.723
Log time; x	1	0.0001	
(Log time)^2	1	0.0004	
Error	81		
Total	83		

Regression Equation: $Y = 0.878 - 0.464x + 0.132x^2$

Time (0-48 h) in current vs. standard volume (mL/g).

a) pH 7.7

Source	df	Prob > F	r^2
Model	2	0.0001	0.749
Log time; x	1	0.0001	
(Log time)^2	1	0.0001	
Error	79		
Total	81		

Regression Equation: $Y = 0.859 - 0.559x + 0.163x^2$

b) pH 5.3

Source	df	Prob > F	r^2
Model	2	0.0001	0.676
Log time; x	1	0.0001	
(Log time)^2	1	0.0013	
Error	73		
Total	75		

Regression Equation: $Y = 0.861 - 0.455x + 0.134x^2$

APPENDIX 7 continued

Time (0-48 h) in current vs. internal pressure (atm).

a) pH 7.7

Source	df	Prob > F	r^2
Model	4	0.0001	0.374
Time; x	1	0.0076	
$Time^2$	1	0.0013	
$Time^3$	1	0.0004	
$\sqrt{Time}$	1	0.0461	
Error	77		
Total	81		

Regression Equation: $Y = 1.012 + 0.018x - 0.0008x^2 + 0.00001x^3 - 0.029\ x$

b) pH 5.3

Source	df	Prob > F	r^2
Model	3	0.2069	0.061
Time	1	0.1231	
$Time^2$	1	0.1184	
$(Log\ time)^2$	1	0.1138	
Error	72		
Total	75		

APPENDIX 8

Test for equality of slopes of model: $Y = a - b \log h$

Still water - 48 h current.

a) Buoyancy (mL/g)

Source	df	Prob > F
pH	1	0.6018
Log time (h)	1	0.0001
Interaction	1	0.0302
Error	166	
Total	169	

b) Standard volume (mL/g)

Source	df	Prob > F
pH	1	0.4623
Log time (h)	1	0.0001
Interaction	1	0.0889
Error	154	
Total	157	

Test for Equality of Slopes of Model: $Y = a + b \log h$

48 h current - still water without access to air; buoyancy experiment.

a) Buoyancy (mL/g)

Source	df	Prob > F
pH	1	0.0187
Log time (h)	1	0.0001
Interaction	1	0.0131
Error	128	
Total	131	

b) Standard volume (mL/g)

Source	df	Prob > F
pH	1	0.0351
Log time (h)	1	0.0001
Interaction	1	0.0219
Error	125	
Total	128	

48 h current - still water without access to air; gas composition experiment.

a) Buoyancy (mL/g)

Source	df	Prob > F
pH	1	0.0010
Log time (h)	1	0.0010
Interaction	1	0.0211
Error	167	
Total	170	

APPENDIX 9

Temporal changes in mean buoyancy, standard volume and internal pressure of fish exposed to still water with access to air after 48 h in current at pH 7.7 and pH 5.3; buoyancy experiment.

Time(h)	pH 7.7 Mean	n	95% CI for mean	pH 5.3 Mean	n	95% CI for mean
			a) Buoyancy (mL/g)			
0	0.380	12	0.327 - 0.432	0.452	14	0.385 - 0.519
1	0.672	9	0.603 - 0.741	0.667	10	0.633 - 0.701
3	0.792	10	0.733 - 0.851	0.821	9	0.768 - 0.874
6	0.733	11	0.666 - 0.800	0.862	9	0.813 - 0.911
12	0.926	10	0.894 - 0.958	0.862	10	0.821 - 0.903
24	0.915	10	0.848 - 0.972	0.845	9	0.796 - 0.894
			b) Standard volume (mL/g)			
0	0.394	12	0.339 - 0.449	0.453	13	0.379 - 0.527
1	0.685	8	0.600 - 0.770	0.675	10	0.637 - 0.713
3	0.803	10	0.744 - 0.862	0.831	9	0.778 - 0.884
6	0.731	11	0.662 - 0.800	0.855	8	0.793 - 0.917
12	0.915	9	0.871 - 0.959	0.860	9	0.811 - 0.909
24	0.916	9	0.859 - 0.973	0.819	9	0.761 - 0.877
			c) Internal pressure (atm)			
0	1.057	12	1.039 - 1.075	1.026	13	0.996 - 1.056
1	1.026	8	1.012 - 1.038	1.020	10	1.004 - 1.036
3	1.017	10	1.004 - 1.030	1.001	9	0.981 - 1.021
6	1.003	11	0.988 - 1.018	0.985	8	0.961 - 1.009
12	0.986	9	0.968 - 1.004	0.992	9	0.971 - 1.013
24	0.990	9	0.971 - 1.009	0.978	9	0.952 - 1.004

APPENDIX 9 continued

Temporal changes in mean buoyancy and composition of swimbladder gases of fish exposed to still water with access to air after 48 h in current at pH 7.7 and pH 5.3; gas composition experiment.

Time(h)	pH 7.7 Mean	n	95% CI for mean	pH 5.3 Mean	n	95% CI for mean
			a) Buoyancy (mL/g)			
0	0.379	11	0.330 - 0.428	0.455	14	0.402 - 0.508
1	0.735	11	0.675 - 0.795	0.748	12	0.669 - 0.827
3	0.866	12	0.811 - 0.921	0.882	13	0.847 - 0.917
6	0.936	11	0.901 - 0.971	0.868	12	0.822 - 0.914
12	0.905	10	0.860 - 0.950	0.910	12	0.870 - 0.950
24	0.887	10	0.826 - 0.948	0.873	13	0.842 - 0.904
			b) CO_2 (Volume %)			
0	1.19	11	0.99 - 1.39	1.24	13	1.07 - 1.41
1	2.38	11	2.05 - 2.71	2.30	12	1.99 - 2.61
3	3.51	12	2.67 - 4.35	3.46	13	3.02 - 3.90
6	3.27	11	2.73 - 3.81	3.14	12	2.66 - 3.62
12	3.70	10	2.62 - 4.88	2.88	12	2.51 - 3.25
24	3.52	10	2.71 - 4.33	2.33	13	2.00 - 2.66
			c) O_2 (Volume %)			
0	8.01	11	5.73 - 10.29	9.53	13	7.78 - 11.28
1	18.47	11	14.72 - 22.22	15.33	12	12.56 - 18.10
3	25.33	12	21.46 - 29.20	20.79	13	18.35 - 23.23
6	21.57	11	19.09 - 24.05	21.63	12	18.95 - 24.31
12	28.30	10	23.24 - 33.34	22.33	12	19.03 - 25.63
24	28.35	10	23.22 - 33.48	23.53	13	19.56 - 27.50
			d) N_2 (Volume %)			
0	90.81	11	88.56 - 93.06	89.23	13	87.57 - 90.89
1	79.09	11	75.10 - 83.08	82.38	12	79.61 - 85.15
3	71.16	12	67.24 - 75.08	75.75	13	73.40 - 78.10
6	75.17	11	72.49 - 77.85	75.24	12	72.45 - 78.03
12	68.30	10	62.83 - 73.77	74.78	12	71.26 - 78.30
24	68.13	10	62.57 - 73.69	74.74	13	70.01 - 78.17

APPENDIX 10

Two-Way ANOVA and Means Comparison:

Effect of water pH and time (1-24 h) in still water with access to air on buoyancy and standard volume.

a) Buoyancy experiment - buoyancy (mL/g)

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	4	0.0001	1	0.672	0.667	0.8849
pH	1	0.7758	3	0.792	0.821	0.4244
Interaction	4	0.0002	6	0.733	0.862	0.0005
Error	87		12	0.926	0.862	0.0747
Total	96		24	0.915	0.845	0.0591

- standard volume (mL/g)

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	4	0.0001	1	0.685	0.675	0.7970
pH	1	0.8931	3	0.803	0.831	0.4730
Interaction	4	0.0005	6	0.731	0.855	0.0020
Error	82		12	0.915	0.860	0.1677
Total	91		24	0.916	0.819	0.0157

b) Gas composition experiment - buoyancy (mL/g)

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	4	0.0001	1	0.735	0.748	0.6951
pH	1	0.5226	3	0.866	0.882	0.6051
Interaction	4	0.3264	6	0.936	0.868	0.0397
Error	106		12	0.905	0.910	0.8713
Total	115		24	0.887	0.873	0.6661

APPENDIX 10 continued

Effect of water pH and time (0-24 h) in still water with access to air on the gas content (volume %) of the swimbladder.

a) CO_2 (log transformed)

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	5	0.0001	0	1.19	1.24	0.7954
pH	1	0.0158	1	2.38	2.30	0.7603
Interaction	5	0.0393	3	3.51	3.46	0.8615
Error	128		6	3.27	3.14	0.7015
Total	139		12	3.70	2.88	0.0512
			24	3.52	2.33	0.0004

b) O_2

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	5	0.0001	0	8.01	9.53	0.4731
pH	1	0.0016	1	18.47	15.33	0.7461
Interaction	5	0.0941	3	25.33	20.79	0.0299
Error	128		6	21.57	21.63	0.9761
Total	139		12	28.30	22.33	0.0078
			24	28.35	23.53	0.0280

c) N_2

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	5	0.0001	0	90.81	89.23	0.4749
pH	1	0.0008	1	79.09	82.38	0.1445
Interaction	5	0.0648	3	71.16	75.75	0.0345
Error	128		6	75.17	75.24	0.9756
Total	139		12	68.30	74.78	0.0055
			24	68.13	74.14	0.0088

APPENDIX 11

Least-squares regression analysis to determine the functional relationship between buoyancy or STV and time (x) for fish in still water with access to air. A = Buoyancy experiment; B = Gas composition experiment.

Experiment	pH	Independent variables in the best fitting model	Multiple correlation coefficient
		a) Buoyancy (mL/g)	
A	7.7	x x^2 x^3 $(\log x)^3$	0.927
	5.3	$\log x$ $(\log x)^2$	0.912
B	7.7	$\sqrt{x}$ $(\log x)^2$	0.927
	5.3	$\sqrt{x}$ $(\log x)^2$	0.894
		b) STV (mL/g)	
A	7.7	x x^2 x^3 $(\log x)^3$	0.917
	5.3	$\log x$ $(\log x)^2$	0.895

APPENDIX 12

Regression Analyses:

Time (0-24 h) in still water with access to air vs. internal pressure (atm).

a) pH 7.7

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.527
Error	57		
Total	58		

Regression Equation: Y = 1.049 - 0.050x

b) pH 5.3

Source	df	Prob > F	r^2
Log Time; x	1	0.0002	0.219
Error	56		
Total	57		

Regression Equation: Y = 1.026 - 0.035x

Time (3-24 h) in still water with access to air vs. CO_2 content (volume %, log transformed) of the swimbladder.

Source	df	Prob > F	r^2
CO_2; x	1	0.0001	0.312
Error	48		
Total	49		

Regression Equation: Y = 0.654 - 0.0058x

Test for equality of slopes of model: Y = a - b log time

48 h current - still water with access to air; internal pressure.

Source	df	Prob > F
pH	1	0.0097
Log time	1	0.0001
Interaction	1	0.1757
Error	113	
Total	116	

APPENDIX 13

Temporal changes in mean buoyancy, standard volume and internal pressure of fish exposed to still water without access to air after 48 h in current at pH 7.7 and pH 5.3; buoyancy experiment.

Time(h)	pH 7.7 Mean	n	95% CI for mean	pH 5.3 Mean	n	95% CI for mean
			a) Buoyancy (mL/g)			
0	0.380	12	0.327 - 0.433	0.452	14	0.385 - 0.519
1	0.439	9	0.393 - 0.485	0.530	9	0.417 - 0.643
3	0.567	10	0.481 - 0.653	0.550	10	0.484 - 0.616
6	0.673	7	0.555 - 0.791	0.685	10	0.592 - 0.778
12	0.784	11	0.706 - 0.862	0.737	9	0.647 - 0.827
24	0.856	8	0.801 - 0.911	0.828	6	0.699 - 0.957
48	0.917	9	0.848 - 0.986	0.876	8	0.833 - 0.919
			b) Standard volume (mL/g)			
0	0.394	12	0.339 - 0.449	0.453	13	0.379 - 0.527
1	0.426	9	0.382 - 0.470	0.524	9	0.417 - 0.637
3	0.530	10	0.451 - 0.609	0.530	10	0.458 - 0.602
6	0.677	6	0.559 - 0.795	0.652	9	0.555 - 0.649
12	0.761	11	0.690 - 0.832	0.718	9	0.628 - 0.808
24	0.860	8	0.801 - 0.919	0.823	6	0.692 - 0.954
48	0.880	9	0.815 - 0.945	0.853	8	0.813 - 0.893
			c) Internal pressure (atm)			
0	1.057	12	1.040 - 1.074	1.026	13	0.996 - 1.056
1	0.998	9	0.965 - 1.031	1.013	9	0.989 - 1.037
3	0.971	10	0.952 - 0.990	0.988	10	0.958 - 1.018
6	1.008	6	0.994 - 1.022	0.991	9	0.977 - 1.005
12	1.003	11	0.978 - 1.028	1.004	9	0.989 - 1.019
24	1.019	8	1.003 - 1.035	1.009	6	0.985 - 1.033
48	0.963	9	0.950 - 0.976	0.977	8	0.950 - 1.004

APPENDIX 13 continued

Temporal changes in mean buoyancy and composition of swimbladder gases of fish exposed to still water without access to air after 48 h in current at pH 7.7 and pH 5.3; gas composition experiment.

Time(h)	pH 7.7 Mean	n	95% CI for mean	pH 5.3 Mean	n	95% CI for mean
			a) Buoyancy (mL/g)			
0	0.379	11	0.330 - 0.468	0.455	14	0.402 - 0.508
1	0.484	10	0.409 - 0.559	0.580	12	0.488 - 0.672
3	0.605	12	0.519 - 0.691	0.638	14	0.575 - 0.701
6	0.595	11	0.517 - 0.683	0.700	12	0.641 - 0.759
12	0.734	12	0.637 - 0.831	0.776	14	0.726 - 0.826
24	0.837	12	0.760 - 0.914	0.863	13	0.793 - 0.933
48	0.925	11	0.889 - 0.961	0.860	13	0.803 - 0.917
			b) CO_2 (volume %)			
0	1.19	11	0.99 - 1.39	1.24	13	1.07 - 1.41
1	2.38	10	1.79 - 2.97	2.33	11	1.91 - 2.75
3	2.75	12	2.31 - 3.19	3.58	14	2.22 - 4.94
6	3.49	11	2.22 - 4.76	3.23	12	2.42 - 4.04
12	3.38	12	2.54 - 4.22	3.43	14	2.26 - 4.60
24	3.75	12	3.33 - 4.17	4.12	13	3.18 - 5.06
48	3.68	11	3.39 - 3.97	2.85	13	1.89 - 3.81
			c) O_2 (volume %)			
0	8.01	11	5.73 - 10.29	9.53	13	7.78 - 11.28
1	12.68	10	10.44 - 14.92	15.23	11	12.29 - 18.07
3	21.24	12	18.18 - 24.30	19.00	14	17.92 - 21.08
6	25.18	11	22.06 - 28.30	30.46	12	26.63 - 34.29
12	34.04	12	30.01 - 38.07	34.86	14	31.49 - 38.23
24	43.89	12	38.63 - 49.15	44.76	13	43.04 - 46.48
48	41.09	11	35.23 - 46.95	38.05	13	33.14 - 42.96
			d) N_2 (volume %)			
0	90.81	11	88.56 - 93.06	89.23	13	87.57 - 90.89
1	84.96	10	82.88 - 87.04	82.44	11	79.36 - 85.52
3	76.00	12	72.85 - 79.15	77.42	14	75.69 - 79.15
6	71.33	11	69.10 - 73.56	66.31	12	61.82 - 70.80
12	62.58	12	58.44 - 66.72	61.71	14	57.65 - 65.77
24	52.09	12	47.23 - 56.95	51.40	13	49.76 - 53.04
48	55.23	11	49.11 - 61.25	59.11	13	54.70 - 64.52

APPENDIX 14

Regression Analyses:

Time (0-48 h) in still water without access to air vs. buoyancy (mL/g); buoyancy experiment.

a) pH 7.7

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.810
Error	64		
Total	65		

Regression Equation: Y = 0.370 + 0.341x

b) pH 5.3

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.639
Error	64		
Total	65		

Regression Equation: Y = 0.443 + 0.261x

Time (0-48 h) in still water without access to air vs. standard volume (mL/g); buoyancy experiment.

a) pH 7.7

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.0802
Error	63		
Total	64		

Regression Equation: Y = 0.370 + 0.325x

b) pH 5.3

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.606
Error	62		
Total	63		

Regression Equation: Y = 0.437 + 0.249x

Time (0-48 h) in still water without access to air vs. internal pressure (atm); buoyancy experiment.

a) pH 7.7

Source	df	Prob > F	r^2
Model	3	0.0001	0.531
Log time; x	1	0.0001	
$(\text{Log time})^2$	1	0.0001	
$(\text{Log time})^3$	1	0.0001	
Error	61		
Total	64		

Regression Equation: $Y = 1.059 - 0.354x - 0.477x^2 - 0.178x^3$

b) pH 5.3

Source	df	Prob > F	r^2
Model	3	0.0093	0.173
$\sqrt{\text{Time}}$; x	1	0.0086	
$(\sqrt{\text{Time}})^2$	1	0.0205	
$(\sqrt{\text{Time}})^3$	1	0.0253	
Error	60		
Total	63		

Regression Equation: $Y = 1.028 - 0.038x + 0.013x^2 - 0.001x^3$

Time (0-48 h) in still water without access to air vs. buoyancy (mL/g); gas composition experiment.

a) pH 7.7

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.712
Error	77		
Total	78		

Regression Equation: $Y = 0.381 + 0.318x$

b) pH 5.3

Source	df	Prob > F	r^2
Log time; x	1	0.0001	0.636
Error	90		
Total	91		

Regression Equation: $Y = 0.485 + 0.248x$

APPENDIX 15

One-Way ANOVA and Means Comparisons (means with the same letter are not significantly different):

Internal pressures (atm) of fish held in still water without access to air after 48 h in current at pH 7.7 and pH 5.3.

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	1.057	1.026	0.0144
pH	1	0.6598	1	0.998	1.013	0.3465
Interaction	6	0.1189	3	0.971	0.988	0.2231
Error	115		6	1.008	0.991	0.3340
Total	128		12	1.003	1.004	0.9682
			24	1.019	1.009	0.5623
			48	0.963	0.977	0.3851

Duncan Grouping				Mean	n	Time(h)	pH
		A		1.057	12	0	7.7
		B		1.026	13	0	5.3
C		B		1.019	8	24	7.7
C		B		1.013	9	1	5.3
C		B	D	1.009	6	24	5.3
C		B	D	1.008	6	6	7.7
C	E	B	D	1.004	9	12	5.3
C	E	B	D	1.003	11	12	7.7
C	E	B	D	0.998	9	1	7.7
F C	E	B	D	0.991	9	6	5.3
F C	E		D	0.988	10	3	5.3
F	E		D	0.977	8	48	5.3
F	E			0.971	10	3	7.7
F				0.963	9	48	7.7

APPENDIX 16

Two-Way ANOVA and Means Comparison:

Effect of water pH and time in still water without access to air on the gas content (volume %) of the swimbladder.

a) CO_2 (arcsine transformed)

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	1.19	1.24	0.8831
pH	1	0.8100	1	2.38	2.33	0.8831
Interaction	6	0.4006	3	2.75	3.58	0.1920
Error	155		6	3.49	3.23	0.6632
Total	168		12	3.38	3.43	0.9159
			24	3.75	4.12	0.5760
			48	3.68	2.85	0.0465

b) O_2

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	8.01	9.53	0.5016
pH	1	0.3349	1	12.68	15.23	0.2905
Interaction	6	0.1660	3	21.24	19.00	0.3032
Error	155		6	25.18	30.46	0.0230
Total	168		12	34.04	34.86	0.7040
			24	43.89	44.76	0.6948
			48	41.09	38.05	0.1796

c) N_2

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time	6	0.0001	0	90.81	89.23	0.5054
pH	1	0.3884	1	84.96	82.44	0.3186
Interaction	6	0.2105	3	76.00	77.42	0.5343
Error	155		6	71.33	66.31	0.0384
Total	168		12	62.58	61.71	0.7008
			24	52.09	51.40	0.7660
			48	55.23	59.11	0.1029

APPENDIX 17

Results of least-squares curve-fitting procedures to determine the functional relationship between pectoral fin beat frequency and time (x) for fathead minnows during the return to neutral buoyancy in still water. A = Buoyancy experiment; B = Gas composition experiment.

Experiment	pH	Independent variables in the best fitting model		Multiple correlation coefficient
		a) Access to air		
A	7.7	x	x^3	0.880
	5.3	$(\log x)^2$	$(\log x)^3$	0.797
B	7.7	$(\log x)^2$	$(\log x)^3$	0.872
	5.3	$(\log x)^2$	$(\log x)^3$	0.904
		b) No access to air		
B	7.7	x	x^3	0.861
	5.3	x^2	x^3	0.837

APPENDIX 18

Three-Way ANOVA and Means Comparison:

Effects of water pH, time and experimental repeat on the frequency of pectoral fin beats of fish returning to neutral buoyancy in still water at pH 7.7 and pH 5.3.

a) Access to air; buoyancy experiment

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time(T)	4	0.0001	0-1.0	270.2	270.6	0.9279
pH	1	0.0003	2.0-2.5	194.3	206.4	0.0230
Repeat(R)	4	0.5566	5.0-5.5	174.8	151.6	0.0001
Interaction T x R	6	0.0001	11.0-11.5	85.9	132.0	0.0001
Interaction R x pH	4	0.0001	23.0-23.5	63.8	114.6	0.0001
Interaction T x pH	4	0.0001				
Error	701					
Total	724					

b) Access to air; gas composition experiment

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time(T)	4	0.0001	0-1.0	277.7	269.3	0.0796
pH	1	0.7135	2.0-2.5	210.6	199.5	0.1055
Repeat(R)	4	0.8191	5.0-5.5	81.6	117.8	0.0001
Interaction T x R	6	0.2404	11.0-11.5	70.9	67.8	0.7445
Interaction R x pH	4	0.0184	23.0-23.5	63.4	65.8	0.8671
Interaction T x pH	4	0.0001				
Error	695					
Total	718					

c) No access to air; gas composition experiment

Source	df	Prob > F	Time(h)	Contrast pH 7.7 vs. 5.3 Means		Prob > F
Time(T)	5	0.0001	0-1.0	272.5	273.0	0.9128
pH	1	0.1219	2.0-2.5	259.9	265.5	0.3275
Repeat(R)	5	0.0122	5.0-5.5	237.7	256.0	0.0024
Interaction T x R	10	0.0434	11.0-11.5	208.8	219.6	0.0528
Interaction R x pH	5	0.6068	23.0-23.5	112.0	116.8	0.5274
Interaction T x pH	5	0.5812	47.0-47.5	76.7	80.7	0.6846
Error	921					
Total	952					

APPENDIX 19

Three-Way ANOVA:

Effects of water pH, time and experiment repeat on the frequency of surface exposures, air gulps and air gulp attempts* of fish returning to neutral buoyancy in still water with access to air at pH 7.7 and pH 5.3. * (all variables log transformed)

1) Buoyancy experiment

a) surface exposures

Source	df	Prob > F
Time(T)	5	0.0010
pH	1	0.9185
Repeat(R)	4	0.0642
Interaction T x R	10	0.7784
Interaction R x pH	4	0.7394
Interaction T x pH	5	0.3228
Error	10	
Total	39	

b) air gulps

Source	df	Prob > F
Time(T)	5	0.0011
pH	1	0.9993
Repeat(R)	4	0.1050
Interaction T x R	10	0.8365
Interaction R x pH	4	0.5663
Interaction T x pH	5	0.4129
Error	10	
Total	39	

c) air gulp attempts

Source	df	Prob > F
Time(T)	5	0.0535
pH	1	0.8813
Repeat(R)	4	0.1607
Interaction T x R	10	0.7909
Interaction R x pH	4	0.8484
Interaction T x pH	5	0.5472
Error	10	
Total	39	

APPENDIX 19 continued

2) Gas composition experiment

a) surface exposures

Source	df	Prob > F
Time(T)	5	0.0002
pH	1	0.0123
Repeat(R)	4	0.0108
Interaction T x R	10	0.5847
Interaction R x pH	4	0.1905
Interaction T x pH	5	0.1100
Error	10	
Total	39	

b) air gulps

Source	df	Prob > F
Time(T)	5	0.0002
pH	1	0.0125
Repeat(R)	4	0.0185
Interaction T x R	10	0.6070
Interaction R x pH	4	0.2132
Interaction T x pH	5	0.1029
Error	10	
Total	39	

c) air gulp attempts

Source	df	Prob > F
Time(T)	5	0.0799
pH	1	0.3262
Repeat(R)	4	0.0241
Interaction T x R	10	0.6271
Interaction R x pH	4	0.3591
Interaction T x pH	5	0.6416
Error	10	
Total	39	

Effects of water pH, time and experimental repeat on the frequency of surfacing attempts (log transformed) of fish returning to neutral buoyancy in still water without access to air at pH 7.7 and pH 5.3.

Source	df	Prob > F
Time(T)	6	0.0001
pH	1	0.0021
Repeat(R)	5	0.0188
Interaction T x R	15	0.8833
Interaction R x pH	5	0.7240
Interaction T x pH	6	0.4827
Error	15	
Total	53	

APPENDIX 20

Regression Analyses:

Time (d) in current vs. buoyancy (mL/g).

a) pH 7.7; 2-32 d

Source	df	Prob > F	r^2
Log time; x	1	0.0008	0.180
Error	57		
Total	58		

Regression Equation: $Y = 0.358 + 0.101x$

b) pH 5.3; 2-32 d

Source	df	Prob > F	r^2
$(\text{Log time})^3$; x	1	0.0001	0.316
Error	60		
Total	61		

Regression Equation: $Y = 0.441 + 0.059x$

Time (d) in current vs. standard volume (mL/g).

a) pH 7.7; 2-32 d

Source	df	Prob > F	r^2
Log time; x	1	0.0082	0.120
Error	55		
Total	56		

Regression Equation: $Y = 0.392 + 0.082x$

b) pH 5.3; 2-32 d

Source	df	Prob > F	r^2
$(\text{Log time})^3$; x	1	0.0001	0.300
Error	57		
Total	58		

Regression Equation: $Y = 0.429 + 0.059x$

Time (d) in current vs. internal pressure (atm).

a) pH 7.7; 2-20 d

Source	df	Prob > F	r^2
Time^3; x	1	0.0046	0.165
Error	45		
Total	46		

Regression Equation: $Y = 1.030 + 0.000005x$

b) pH 5.3; 2-32 d

Source	df	Prob > F	r^2
Log time; x	1	0.7565	0.002
Error	57		
Total	58		

Regression Equation: $Y = 0.973 + 0.0025x$

APPENDIX 21

Two-Way ANOVA and Means Comparison:

Effects of water pH and time on buoyancy, standard volume and internal pressure of fish swimming in current at pH 7.7 and pH 5.3 for 2-32 d.

a) Buoyancy (mL/g)

Source	df	Prob > F	Time(d)	Contrast pH 7.7 vs. 5.3 Means		Prob> F
Time	5	0.0001	2	0.392	0.437	0.2976
pH	1	0.0011	3	0.407	0.446	0.4148
Interaction	5	0.3458	6	0.425	0.488	0.1691
Error	109		12	0.488	0.482	0.9009
Total	120		20	0.472	0.604	0.0048
			32	0.515	0.628	0.0144

b) Standard volume (mL/g)

Source	df	Prob> F	Time(d)	Contrast pH 7.7 vs. 5.3 Means		Prob> F
Time	5	0.0001	2	0.424	0.426	0.8661
pH	1	0.0668	3	0.421	0.434	0.7153
Interaction	5	0.3410	6	0.442	0.474	0.4906
Error	104		12	0.509	0.468	0.4994
Total	115		20	0.502	0.601	0.0535
			32	0.505	0.611	0.0310

c) Internal pressure (atm)

Source	df	Prob > F	Time(d)	Contrast pH 7.7 vs. 5.3 Means		Prob> F
Time	5	0.0006	2	1.039	0.970	0.0001
pH	1	0.0001	3	1.028	0.978	0.0013
Interaction	5	0.0066	6	1.026	0.972	0.0003
Error	104		12	1.037	0.974	0.0001
Total	115		20	1.071	0.982	0.0001
			32	0.981	0.972	0.5276

APPENDIX 22

Results of swimming performance experiment:

Fish of all 4 batches within each pH treatment responded similarly (Table A1) and showed no significant ($p > 0.10$) differences in most of the measured parameters. However ANOVA tests indicated such differences in the KA ($p = 0.005$) between the batches of experimental and in buoyancy ($p = 0.05$) between the batches of control fish. The difference in KA could mainly be attributed to one batch of fishes, which already showed a higher, though not significant, KB at the start of the experiment. To faciliate comparison between pH treatments, the just significant difference in buoyancy of control fish was ignored and results within each treatment were pooled.

Table A1. Mean (n = 10) swimming time (SWT), buoyancy, IP, weight (W) and coefficient of condition (K) before the start (B) and after the termination (A) of the experiment for 4 batches of fathead minnows subjected to a stepwise increase in water velocity ($\bar{x} \pm 95\%$ CL).

Batch	pH 7.7	pH 5.3
	a) SWT (h)	
1	7.05 ± 1.11	7.38 ± 1.33
2	7.05 ± 1.43	6.85 ± 0.79
3	6.58 ± 1.12	7.00 ± 1.15
4	6.98 ± 1.03	6.98 ± 0.92
	b) Buoyancy (mL/g)	
1	0.458 ± 0.047	0.529 ± 0.062
2	0.439 ± 0.058	0.504 ± 0.056
3	0.480 ± 0.077	0.517 ± 0.050
4	0.380 ± 0.040	0.530 ± 0.078
	c) IP (atm)	
1	1.000 ± 0.025	0.998 ± 0.041[a]
2	1.007 ± 0.015[a]	0.972 ± 0.031[b]
3	0.998 ± 0.035[c]	0.983 ± 0.045[c]
4	1.016 ± 0.027	1.020 ± 0.020

APPENDIX 22 (Table A1) continued

Batch	pH 7.7	pH 5.3
	d) KB	
1	0.994 ± 0.018	0.995 ± 0.015
2	0.974 ± 0.019	0.988 ± 0.015
3	0.992 ± 0.016	0.964 ± 0.047
4	0.985 ± 0.018	1.007 ± 0.024
	e) KA	
1	0.923 ± 0.022	0.890 ± 0.031
2	0.932 ± 0.019	0.884 ± 0.046
3	0.945 ± 0.022	0.868 ± 0.032
4	0.929 ± 0.025	0.947 ± 0.028
	f) WB (g)	
1	2.68 ± 0.22	2.84 ± 0.27
2	2.77 ± 0.19	2.86 ± 0.17
3	2.76 ± 0.25	2.72 ± 0.16
4	2.77 ± 0.21	2.81 ± 0.26
	g) WA (g)	
1	2.49 ± 0.20	2.54 ± 0.25
2	2.65 ± 0.18	2.56 ± 0.20
3	2.63 ± 0.25	2.45 ± 0.18
4	2.61 ± 0.21	2.64 ± 0.24

[a] n = 6; [b] n = 8; [c] n = 9

APPENDIX 22 continued

Table A2. One-Way ANOVA:

1) Parameters of control fish

Source	df	ss	ms	F value	Prob > F
		a) Swimming time(h)			
Batch	3	1.556	0.5188	0.19	0.9030
Error	36	98.637	2.7399		
Total	39	100.193			
		b) Buoyancy (mL/g)			
Batch	3	0.0553	0.0185	2.85	0.0511
Error	36	0.2332	0.0065		
Total	39	0.2886			
		c) IP (atm)			
Batch	3	0.0017	0.0005	0.58	0.6320
Error	31	0.0298	0.0010		
Total	34	0.0315			
		d) KB			
Batch	3	0.0026	0.0009	1.36	0.2697
Error	36	0.0225	0.0006		
Total	39	0.0251			
		e) KA			
Batch	3	0.0026	0.0009	0.91	0.4442
Error	36	0.0345	0.0010		
Total	39	0.0371			
		f) WB (g)			
Batch	3	5.371	1.7903	0.19	0.9043
Error	36	343.869	9.5519		
Total	39	349.240			
		g) WA (g)			
Batch	3	15.237	5.0789	0.56	0.6467
Error	36	328.183	9.1162		
Total	39	343.420			

APPENDIX 22 (Table A2 continued)

2) Parameters of treated fish

Source	df	ss	ms	F value	Prob > F
		a) Swimming time			
Batch	3	1.537	0.5125	0.23	0.8749
Error	36	80.238	2.2288		
Total	39	81.775			
		b) Buoyancy (mL/g)			
Batch	3	0.0045	0.0015	0.20	0.8980
Error	36	0.2766	0.0077		
Total	39	0.2811			
		c) IP (atm)			
Batch	3	0.0122	0.0041	2.34	0.0941
Error	29	0.0504	0.0017		
Total	32	0.0626			
		d) KB			
Batch	3	0.0098	0.0033	2.06	0.1230
Error	36	0.0571	0.0016		
Total	39	0.0669			
		e) KA			
Batch	3	0.0363	0.0121	5.12	0.0047
Error	36	0.0851	0.0024		
Total	39	0.1215			
		f) WB (g)			
Batch	3	11.228	3.2917	0.40	0.7521
Error	36	334.871	9.3020		
Total	39	346.010			
		g) WA (g)			
Batch	3	17.151	5.7169	0.60	0.6175
Error	36	341.457	9.4849		
Total	39	358.608			