THE UNIVERSITY OF MANITOBA

MECHANISMS OF BUOYANCY ADJUSTMENT AND EFFECTS OF

WATER VELOCITY AND TEMPERATURE ON ABILITY TO MAINTAIN

BUOYANCY IN FATHEAD MINNOWS, PIMEPHALES PROMELAS, RAFINESQUE

BY

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

Physostomous fathead minnows (<u>Pimephales</u> <u>promelas</u>) were observed to determine (1) the relative importance of gas secretion/absorption and gulping/spitting as mechanisms of buoyancy adjustment, and (2) the effects of water velocity and temperature on ability to maintain a minimum buoyancy in current for an extended time.

Buoyancy is increased quickly by gulping air and slowly by secreting O_2 and CO_2. Small fish can increase buoyancy faster than large fish (0.048 cf. 0.028 $mL \cdot g^{-1} \cdot h^{-1}$). Relative contributions of gulping and secretion to buoyancy increases depend on the environmental conditions, and gulping appears to account for about 70% of any increase. Buoyancy is reduced quickly by spitting gas or slowly by resorbing O_2 and CO_2. Spitting is a fright response; resorption is the mechanism used to reduce swimbladder volume in response to an increase in water velocity. Small fish can reduce buoyancy by resorption faster than large fish (0.034 cf. 0.023 $mL \cdot g^{-1} \cdot h^{-1}$).

Except at $5^{o}C$ and following the initial period of adjustment, buoyancy is maintained in current for at least 42 days. Buoyancy level is determined by water temperature and velocity. Effects of high and increasing temperature and velocity on buoyancy are antagonistic; the buoyancy

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response to current being lost at high temperatures.
At extreme temperatures and velocities, internal pressure
is used to alter swimbladder volume, assisting in buoyancy
regulation.   Internal pressure may be a good indicator of
stress.

This thesis is dedicated to

my parents whose love and

encouragement have made my

work so much easier.

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INTRODUCTION

The ability of fishes to adjust buoyancy by altering
swimbladder volume is a common response to changes in
water velocity (Gee et al., 1974; Gee and Gee, 1976).
Plasticity in buoyancy permits efficient movement in
waters where velocity and turbulence change in time and
space.   In current, a negative buoyancy and a benthic
position help to prevent displacement downstream as the
fish can counteract hydrodynamic lift and at the same time
increase the frictional forces between the body and the
substrate (Alexander, 1966; Gee, 1968).   With a mid-water
position in current, a reduced buoyancy appears to counteract
hydrodynamic lift from the shape of the body, maintaining
vertical position and enabling locomotion with a minimum
expenditure of energy (Berezay and Gee, 1978).   In still
water, a neutral buoyancy is advantageous for efficient
maintenance of position and locomotion.

Buoyancy is affected by changes in photoperiod, water
temperature and velocity, hydrostatic pressure, size and age
of fish, degree of sexual development, and condition (Saunders,
1965; Neave et al., 1966; Gee, 1968, 1972, 1977; Pinder and
Eales, 1969; Berezay and Gee, 1978; Luoma and Gee, 1980).
Many of these factors interact.

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Little is known of the mechanisms of buoyancy adjustment in physostomes. Like physoclists, some alter their swimbladder volume by resorbing or secreting gas. Unlike physoclists they possess a pneumatic duct enabling them to exchange gas directly with the atmosphere by gulping air and passing it into the swimbladder or by forcing gas out of the swimbladder. Changes in internal pressure of the swimbladder gases may also be used to make minor corrections to buoyancy.

The ability to capitalize on buoyancy regulation depends upon the rate and extent of adjustment and the duration of maintenance of the appropriate level of buoyancy. Buoyancy changes in most stream fish are extensive, involving a 30-60% reduction in swimbladder volume when current is encountered, and are rapid requiring 12-96 h for completion (Gee et al., 1974; Gee and Gee, 1976). However, little is known of the ability to maintain a minimum buoyancy in current for extended periods of time. Objectives of this study were to determine (1) the relative importance of secretion and absorption of gas versus gulping and spitting of gas as mechanisms of buoyancy adjustment, and (2) the effects of water velocity and temperature on the ability to maintain a minimum buoyancy. The physostomous fathead minnow (Pimephales promelas) was chosen as the subject for the study. It is common throughout most of central North

America, inhabiting headwater streams and lakes (Scott
and Crossman, 1973).   In nature they are subject to
wide variations in water temperature and velocity,
especially during spring run-off (1-4 weeks).   Their
abundance and hardiness have made them a popular bioassay
fish.

MATERIALS AND METHODS

Fathead minnows were collected from the Pembina River, Manitoba as required. They were held in large fiberglass tanks (170 L) at either 5 or $11^{\circ}C$ under a 12L:12D photoperiod and fed #3 trout starter once a day. Prior to testing, fish were acclimated to the experimental temperature at a rate of no more than $1^{\circ}C \cdot d^{-1}$. Fish were not fed within 24 h of analysis.

To measure buoyancy, fish were captured by dipnet and anaesthetized in a solution of MS222 (ethyl m-aminobenzoate methanesulphonate). Swimbladder volume ($\pm$ 0.001 mL), weight of the gas-free fish in water ($\pm$ 0.001 g; Sartorius balance model 2255), and the volume of gases released at atmospheric pressure ($\pm$ 0.001 mL) were measured following the procedure of Gee (1970). Buoyancy was determined by dividing the swimbladder volume by the weight of the gas-free fish in water (1.0 $mL \cdot g^{-1}$ = neutral buoyancy). Internal pressure of swimbladder gases was measured by dividing the volume of gases released at atmospheric pressure (Pa; Appendix 1) by the swimbladder volume. Standard volume was determined by dividing the volume of gas released from the swimbladder at atmospheric pressure by the weight of the gas-free fish in water ($mL \cdot g^{-1}$). It gave a relative measure of the amount of gas in the swimbladder, facilitating comparisons between temperatures (Appendix 1).

Unless otherwise noted, fish were acclimated and tested in aquaria (90 x 45 x 45 cm) in either still water or current. Water velocities were created in stream tanks, based on the design of Gee and Bartnik (1969), where fish were held in an area 60 x 40 x 20 cm with stainless steel screens on the sides (2.3 meshes$\cdot$cm^{-1}) and plexiglass on the bottom. Water depth in the holding area varied between 7 and 10 cm. Water velocity could be provided up to 35 cm$\cdot$s^{-1}, and its measurement was determined from the average of six measurements taken 3 cm from the bottom with an Ott current meter (type Cl). Temperatures were regulated either by varying the inlet temperature (5-26 $\pm$ 0.5°C) or using a thermostat (26-35 $\pm$ 0.1°C). Illumination was provided by 60 W light bulbs on time clocks.

Mechanism of buoyancy alteration

To determine if gas spitting and gulping are used to alter swimbladder volume, fish must be observed during the period of buoyancy adjustment. Since it is not possible to measure buoyancy without disturbing fish and perhaps altering their spitting or gulping behaviour, some other

technique for assessing buoyancy must be used. Berezay and Gee (1978) found a strong relationship between swimming angle of attack and buoyancy in creek chub (Semotilus atromaculatus). If such a relationship exists in fathead minnows, then the period of buoyancy adjustment could be predicted by observing the swimming angle of attack without disturbing gas regulation.

To facilitate observations of gas spitting and gulping and swimming angles, a bubble-free current tank with a current of 20 cm·s^{-1} was constructed (Fig. 1). It consisted of a circular tank where fish, enclosed in a portion of the tank, were exposed to current generated by submersible pumps. Observations were made from behind a blind.

Prediction of buoyancy from swimming angles. Fish were exposed to either current or still water in the bubble-free current tank, and measures of swimming angles and buoyancy were recorded to determine if buoyancy could be predicted from swimming angle. Swimming angle of attack was considered to be horizontal if a horizontal line passing through the fish's eye crossed some part of its caudal fin. If the caudal fin was below the line it was swimming 'heads-up', and vice versa. Swimming angles were observed while fish swam forward or held position in an area of current where

OUTLET HOSES
FROM PUMPS

SWIMMING CHANNEL

INLET HOSES
TO PUMPS

FUNNEL

DRAIN

70 cm

BAFFLE SUPPORT

BAFFLE

SCREEN

WATER INFLOW

DRAIN

INLET HOSES TO PUMPS

BAFFLES

OUTLET HOSES
FROM PUMPS
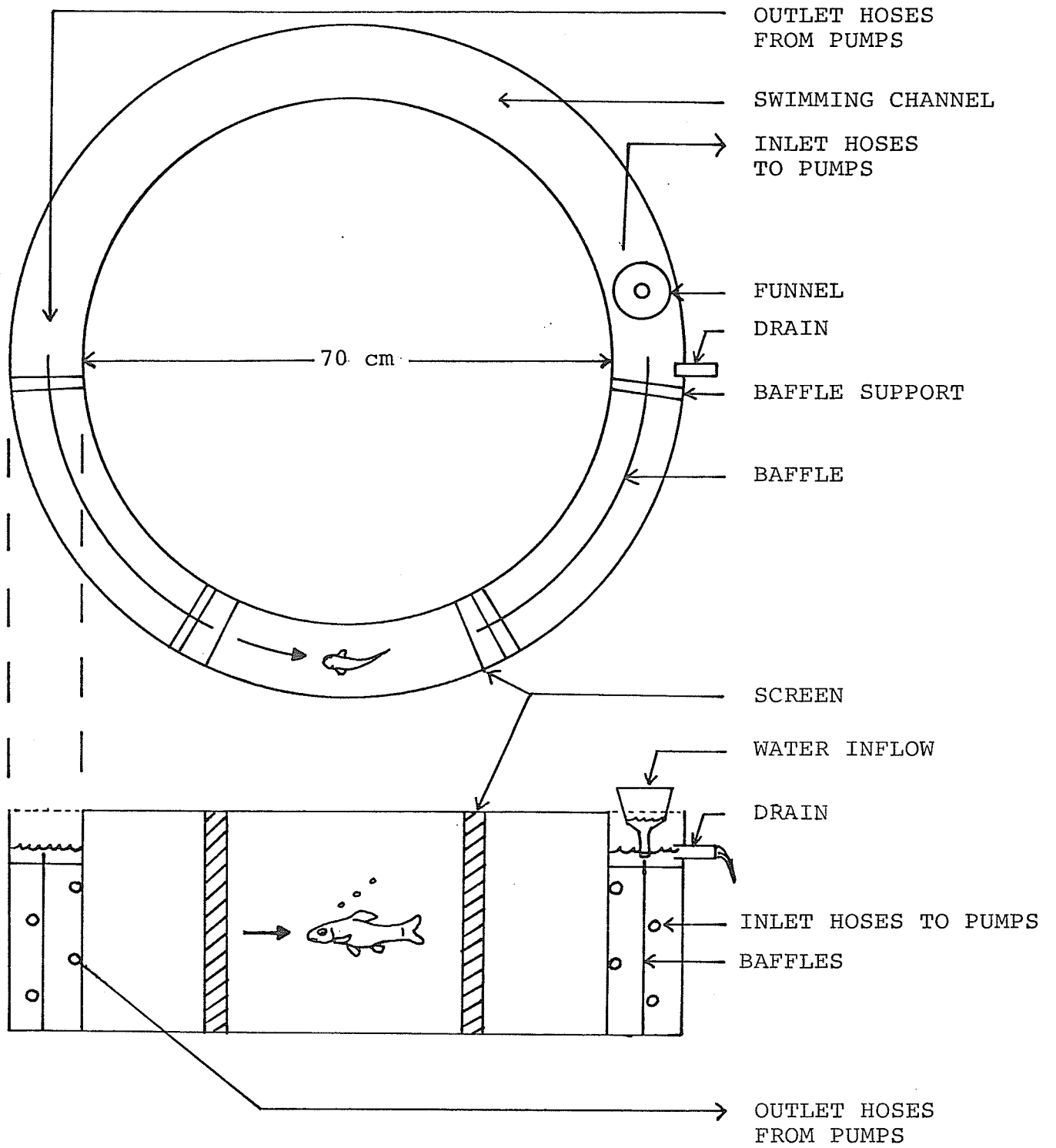
flow was as uniform as possible or in still water.
Fathead minnows (40-62 mm fork length) were acclimated
to 19°C and a 14L:10D photoperiod.   Light onset (800 h)
and offset were gradual over 15 minutes.   Groups of 42
fish were transferred to the test tank, allowed 2-4 d
for acclimation, and then exposed to current.   Water
velocity was increased gradually to 20 cm·s^{-1} over 8 min
beginning at 1000 h.   Measurements of buoyancy, internal
pressure and standard volume (2 fish) and observations on
swimming angle (5 fish every 5 min/for 40 min) were made
after 0, 1, 2, 5, 8, 11, and 23 h in current.   Then water
velocity was reduced to 0 over 8 min and the above procedure
was repeated.   These measurements were repeated four times
using four different groups of fish for a total of 8 buoyancy
measurements and 160 swimming angle observations at each
observation time.

Buoyancy adjustment by spitting or gulping gas.   To
determine whether gas spitting or gulping was used to alter
buoyancy, fish were observed while altering swimbladder
volume in response to current or still water.   Two groups
of 16 fish each were acclimated and exposed to still water
or current as described above.   Swimming angles were used
to predict the interval of buoyancy change and the frequency
of spitting or gulping was noted.   Gas spitting was indicated
by a release of bubbles from the mouth or opercular openings.

Gas gulping was indicated when a fish broke the meniscus and then descended, releasing bubbles from either the mouth or opercular openings. Spitting did not include gas bubbles released immediately following gulping.

Buoyancy adjustment by resorption and secretion

of gas. To determine whether gas resorption or secretion was used to alter buoyancy, swimbladder gas composition and buoyancy were measured following exposure to current and still water. Fifty fathead minnows (63-84 mm) were acclimated to $19^{\circ}C$ and a 12L:12D photoperiod in still water. Beginning 2 h before light and current onset, 4 fish were sampled from still water. Current was then increased to 30 cm·s^{-1}, and groups of 4 fish were sampled after 10 and 58 h in current. Current was shut off after 60 h and 4 fish were sampled in still water after 10 and 12 h. This procedure was repeated with a second group of 50 fish. Fish sampled after 10 h in still water had access to the surface but those sampled after 12 h had none. Fish measured after 10 or 12 h in still water were held in 0.5 L containers placed in the test aquarium. Prior to withdrawing gas, the pneumatic duct and the connection between the swimbladder lobes were tied off. One sample was taken from each lobe.

The composition of gases in the swimbladder was measured using a Carle model 8700 gas chromatograph (GC) equipped for syringe injection and respiratory gas separation (one Poropack QST 50/80 mesh and one molecular sieve 5A separation column).   Samples of gas (25-100 μL) withdrawn from the swimbladder were injected into the GC which separated them into CO_2, O_2 (includes Ar), and N_2 fractions. As each fraction passed the thermal conductivity detector in the GC it produced a peak on a recording chart whose area was proportional to the amount of the gas present. It was measured using the formula:

AREA = PEAK HEIGHT x WIDTH AT HALF PEAK HEIGHT

(McNair and Bonelli, 1969).   The instrument was calibrated using air and a known gas mixture of 9.59% by volume CO_2, 50.98% O_2 and Ar (inseparable), and 39.4% N_2.

Relative contribution of gas gulping and secretion
to buoyancy increases.   To determine if either gas gulping or gas secretion predominate as the method of buoyancy increase, the gulping behaviour and rate of buoyancy increase were observed in fish whose buoyancy had been lowered to about 0.5 mL$\cdot$g^{-1}.   Eight fish (49-83 mm), acclimated to 19°C and a 12L:12D photoperiod in still water, were individually subjected to ambient pressures of about 50 kPa (1 atm = 101.3 kPa).   Pressure

reduction was gradual and the number of gas bubbles released by the fish was noted. Once the fish was neutrally buoyant at this reduced pressure, pressure was returned to atmospheric and the negatively buoyant fish was transferred to an aquarium balance system (Gee and Graham, 1978) where its weight in water was recorded at regular intervals and the time of gulping was noted until no further changes occurred. The fish was then killed and its weight in water without the swimbladder was measured, making it possible to relate changes in the weight in water to actual increases in swimbladder volume and to identify the relative contribution of gulping and spitting to this increase.

Ability to Maintain a Minimum Buoyancy

Short term buoyancy maintenance. To determine if a diel rhythm in buoyancy fluctuation existed, buoyancy measurements were made every 4 h for 48 h in still water and in current. Fish (41-66 mm) collected in October, 1978 were held at $11^{o}C$ on a 12L:12D photoperiod for 30 days. During November and December, they were acclimated to $21^{o}C$, one group of 125 fish in still water and another in 20 $cm \cdot s^{-1}$ current. This

photoperiod was maintained in a quiet room until sampling
in late January.   Groups of 8 fish were examined from
both still water and current after 50 days of acclimation
to their test conditions.   Red lamps (40W) were used for
illumination during night sampling with no obvious effect
on fish behaviour.   The fish were starved during the
48 h period.

Long term effects of water velocity.   To determine
the effect of water velocity on ability to maintain a minimum
buoyancy in current fish (40-68 mm) collected in November,
1977 were divided into two batches, acclimated to either
21 or 30°C, and exposed to water velocities of either 10,
20, or 30 cm·s^{-1}.   Velocities in stream tanks were gradually
increased to the desired level over 6 h following introduction
of fish.   Testing occurred between February and May, 1978,
when 8 fish were examined from each treatment after 0, 2, 4,
7, 10, 18, 26, 34, and 42 d in current.   Fish were fed
following sampling.

Long term effects of water temperature.   The effect
of water temperature was measured at a velocity of 20 cm·s^{-1}
where fish were exposed to temperatures of 5, 11, 21, 26, and
30°C.   Sampling was conducted as above.

Effects of stress from temperature and velocity.

To determine the effects of stress from high water temperature and velocity on the ability to maintain a minimum buoyancy in current, fish were exposed to four treatments:

1) constant temperature of 25°C and water velocity of 12.5 $cm \cdot s^{-1}$,

2) increase in temperature from 25 to 31°C at $1°C \cdot d^{-1}$ and then from 31 to 34.5°C at $0.5°C \cdot d^{-1}$ with a constant water velocity of 12.5 $cm \cdot s^{-1}$,

3) increase in water velocity from 12.5 to 27.5 $cm \cdot s^{-1} \cdot 48 \ h^{-1}$ and then from 27.5 to 35 $cm \cdot s^{-1} \cdot 48 \ h^{-1}$ with temperature held constant at 25°C,

4) increase in temperature and velocity as described above, except temperature was increased to 35°C.

Four groups of fish (39-66 mm), collected in October 1978, were acclimated to 25°C in stream tanks and tested between 26 November and 10 December 1978. Treatments began following a 10 day acclimation to a 12.5 $cm \cdot s^{-1}$ water current and 8 fish per treatment were sampled daily. Temperatures and water velocities were adjusted and fish were fed following the daily sampling period.

Statistical Analysis

Regression analyses, lack of fit tests on the regressions, and two-way analyses of variance tests were done on an IBM/370 computer, using APL statistical programme 5796-PHW. All fish were chosen randomly for measurement and observation. Unless otherwise noted, analyses were done on individual measurements and not on the mean values for each treatment. All differences were considered significant if the probability of error was less than 5% ($P < 0.05$). Significance in the appendices is denoted by an asterisk (*) and the vertical lines from the means, on some of the graphs, represent the 95% confidence interval (CI). Note: In the appendices, F values calculated from the mean sum of squares (mss) often do not agree with the F values listed. This occurs because each of the mss values was rounded off from 16 digits before placement in the ANOVA tables. F values in the tables were calculated using all 16 digits of the mss and are correct.

RESULTS

Mechanism of Buoyancy Alteration.

Prediction of buoyancy from swimming angle.   When transferred from still water to current, fish decreased their buoyancy from 0.720 to 0.596 $mL \cdot g^{-1}$ over 12 h (Fig. 2).   On initial contact with current most fish adopted a heads-down angle of attack which gradually shifted to horizontal as buoyancy adjustment was completed. On exposure to still water buoyancy increased from 0.653 to 0.852 $mL \cdot g^{-1}$ over 12 h (Fig. 2).   When still water was encountered most fish initially adopted a heads-up swimming angle of attack which gradually became horizontal as buoyancy adjustment was completed.   The regressions of mean buoyancy on percent swimming horizontally during buoyancy adjustment downwards (in current) and upwards (in still water) were significant (Fig. 3, Appendix 2), indicating that swimming angle is a good predictor of buoyancy.

Buoyancy adjustment by spitting and gulping gas.
Fish seldom spit gas during the period of buoyancy adjustment. Spitting bubbles occurred in still water and in current just after light onset and during the first hour of exposure to

Figure 2.   Mean buoyancy (solid circles; n=8) and the

percent of fish swimming horizontally (open

circles) plotted for the first 12 h of

adjustment to either current or still water.
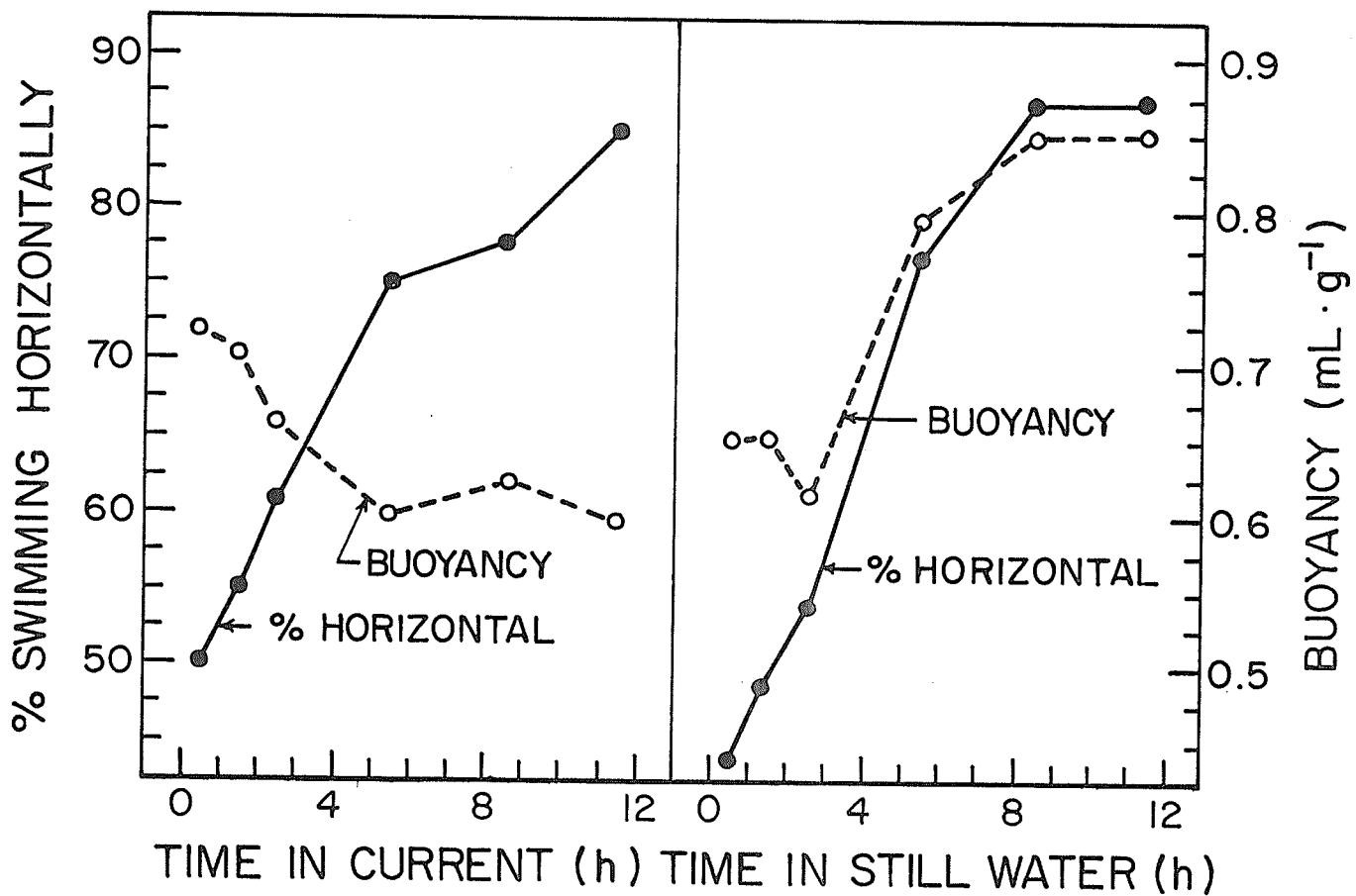
Figure 3.   Regression analysis of mean buoyancy (n=8)

on percent of the fish swimming horizontally

during the first 12 h of adjustment to

current (solid circles) and still water

(open circles).

STILL WATER
$y = 0.38 + 0.0053x$
$r^2 = 0.92$

CURRENT
$y = 0.90 - 0.0037x$
$r^2 = 0.96$
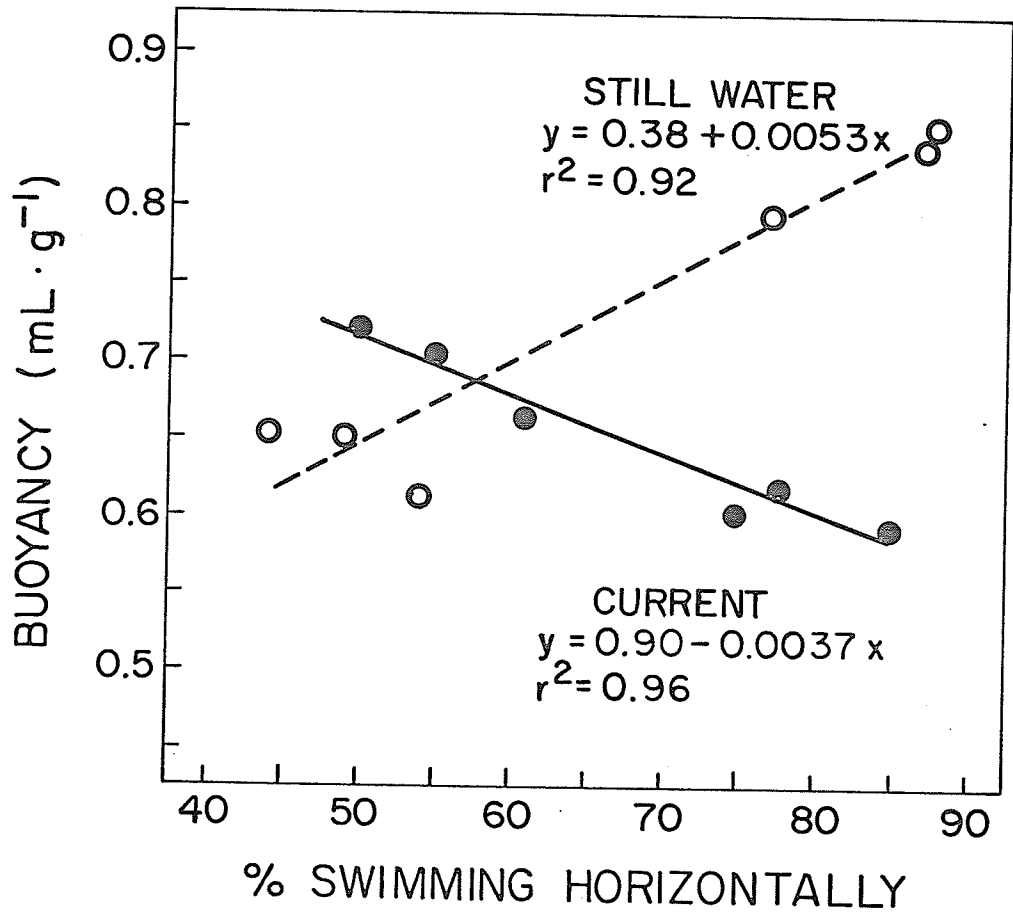
current (Fig. 4). Fish gulped air at the surface in both current and still water but the frequency was greatest during the initial 6 h of exposure to still water (Fig. 4). Apart from the hour following light onset, there was no spitting or gulping of gas when buoyancy was not being adjusted (i.e., when 95% of the fish were swimming horizontally).

Buoyancy adjustment by resorption and secretion of gas. When fathead minnows exposed to 30 $cm \cdot s^{-1}$ current reduced their buoyancy, they altered the composition of gases in their swimbladders by decreasing the amounts of CO_2 and O_2 relative to the amount of N_2 (Fig. 5). This was apparent in current after 12 h and the composition remained unchanged after 60 h. When they increased buoyancy after the current stopped, the reverse occurred. Fish with access to the surface returned their O_2 and N_2 levels to near the initial still water values but their CO_2 concentration was higher. Fish without access had similar CO_2 levels to those with access but O_2 levels were higher and N_2 levels were lower. The gas composition in anterior and posterior lobes of the swimbladder was similar.

Figure 4.　　Frequency of spitting bubbles and surface

gulping by fish when exposed to current for

24 h (buoyancy reduced) and then still water

for 24 h (buoyancy increased).　Buoyancy

adjustment was complete when 95% of the fish

swam horizontally.　Spitting and gulping

occurring outside the period of buoyancy

adjustment are indicated by solid bars.
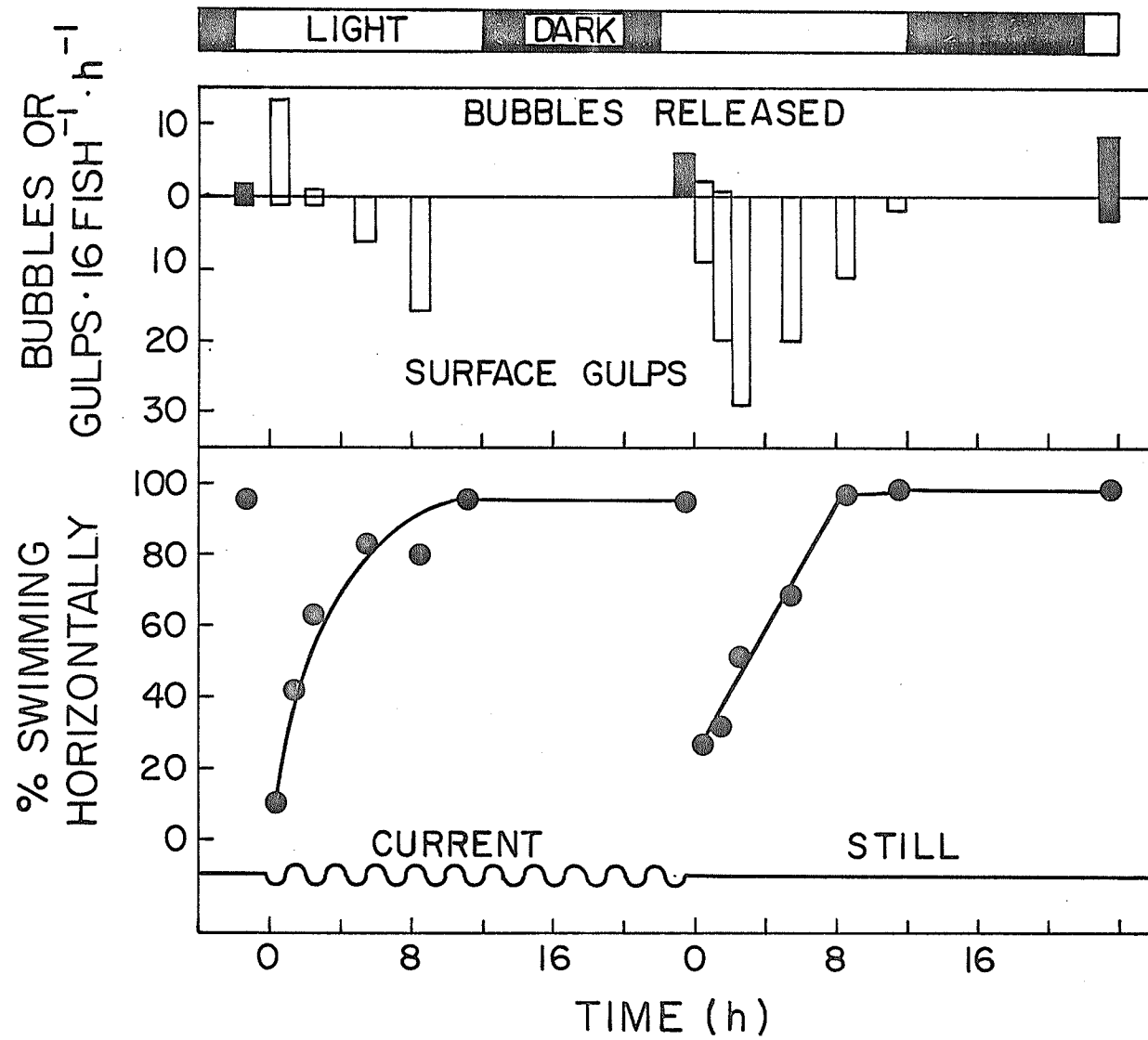
Figure 5.   Percent of the swimbladder gas volume

consisting of N_2, O_2, and CO_2 and buoyancy

in current and still water, with access to

the surface (solid circles) and without access

(squares).   Vertical lines are 95%

confidence intervals for the means.

BUOYANCY (mL·g^{-1})

PERCENT COMPOSITION

TIME (h)

CO_2

O_2

N_2
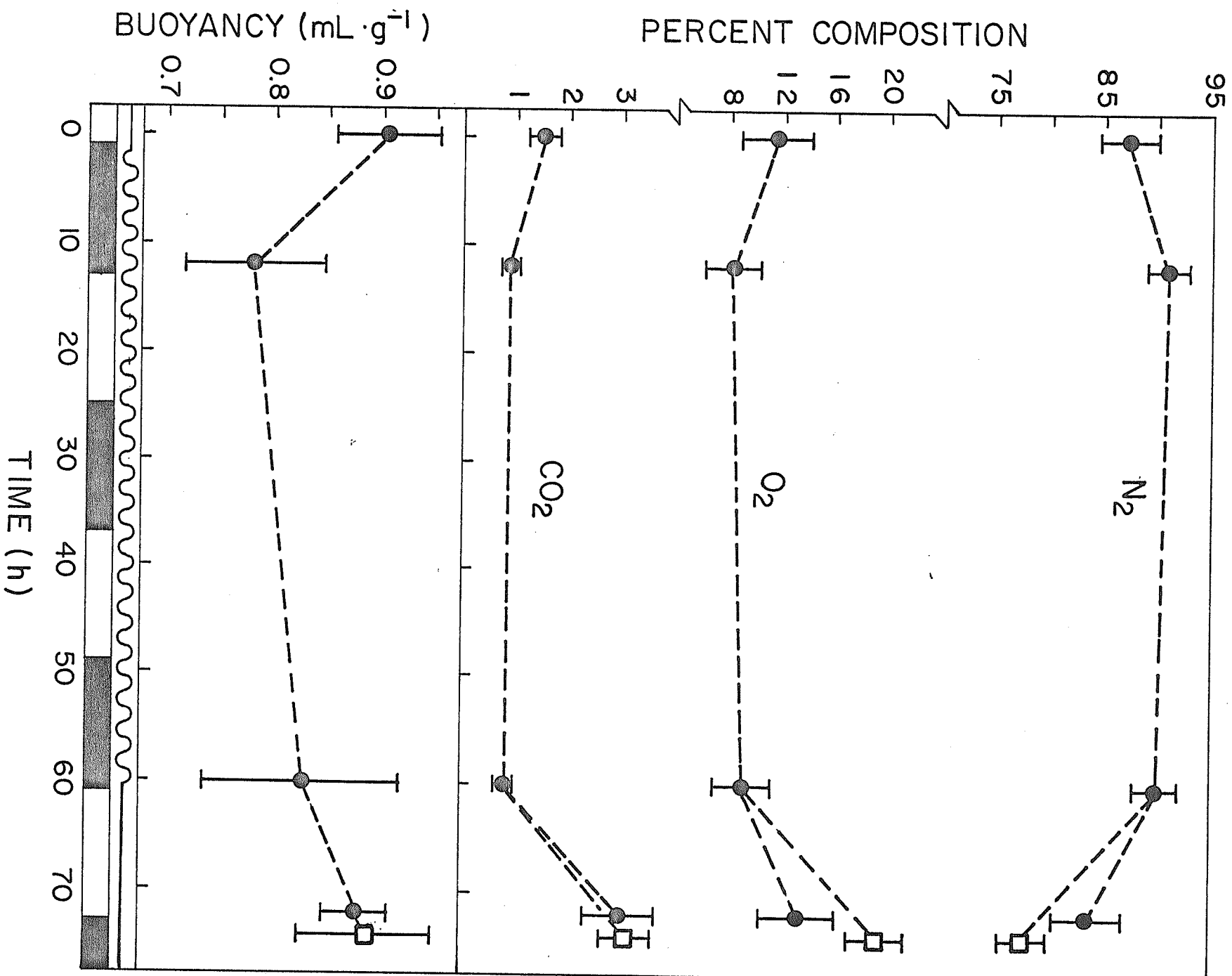
Relative contributions of gas gulping and secretion

to buoyancy increases.   Fish which had their buoyancy

reduced by about 50% were variable in the rate of return

to neutral buoyancy (Table 1).   The quickest did so mainly

by gulping air into the swimbladder, while slowly adjusting

fish relied more on gas secretion (Fig. 6).   Small fish

were able to increase their buoyancy by secretion significantly

faster than large fish, but the proportion of the overall

adjustment due to secretion was independent of size.   The

rate of buoyancy adjustment by gas secretion was related

to the length of the fish by the equation, $y = 0.0700 -$

$0.0005x$ ($r^2 = 0.76$; Appendix 3).   The volume of air forced

into the swimbladder during an average gulp was significantly

larger in large fish (72-83 mm; $\bar{x} = 20$ μL) than in small

fish (49-67 mm; $\bar{x} = 11$ μL) but the volume of gas released

by spitting gas bubbles during buoyancy decrease was

independent of length.

Ability to Maintain Buoyancy

Short term buoyancy maintenance in still water and

current.   Buoyancy was maintained over a narrower

range in still water than in current during the 48 h test

period (Fig. 7).   In still water, buoyancy was significantly

Table 1. Contributions of gas gulping and secretion to buoyancy increases during the return to neutral buoyancy in still water.

Length (mm)	Buoyancy ($mL \cdot g^{-1}$) Initial	Final	Adjustment Time (h)	Gulping $mL \cdot g^{-1} \cdot h^{-1}$	%	Secretion** $mL \cdot g^{-1} \cdot h^{-1}$	%	Mean Volume μL Gulp	Spit
83	0.35	0.98	7.0	0.062	69	0.028	31	17	4
81	0.36	0.98	5.0	0.091	77	0.029	23	17	7
78	0.50	0.98	4.5	0.084	78	0.023	22	20	2
72	0.54	0.99	1.0	0.418	93	0.032	7	26	3
67	0.75	0.99	2.0	0.077	64	0.043	36	9	1
62	0.44	0.94	10.5	0.011	23	0.037	77	5	3
49	0.53	0.95	3.5	0.076	63	0.044	37	3	1.5
49	0.66	1.00	1.5	0.187	81	0.043	19	4	1

** Mean secretion rates were: 49-67 mm = 0.042 $mL \cdot g^{-1} \cdot h^{-1}$

72-83 mm = 0.028 $mL \cdot g^{-1} \cdot h^{-1}$

Figure 6.   Progression of buoyancy alteration in

a fathead minnow using air gulping

(indicated by an arrow) and gas secretion

to increase buoyancy.   Solid lines indicate

continuous observations, dotted lines

interpolations between half-hourly

observations.
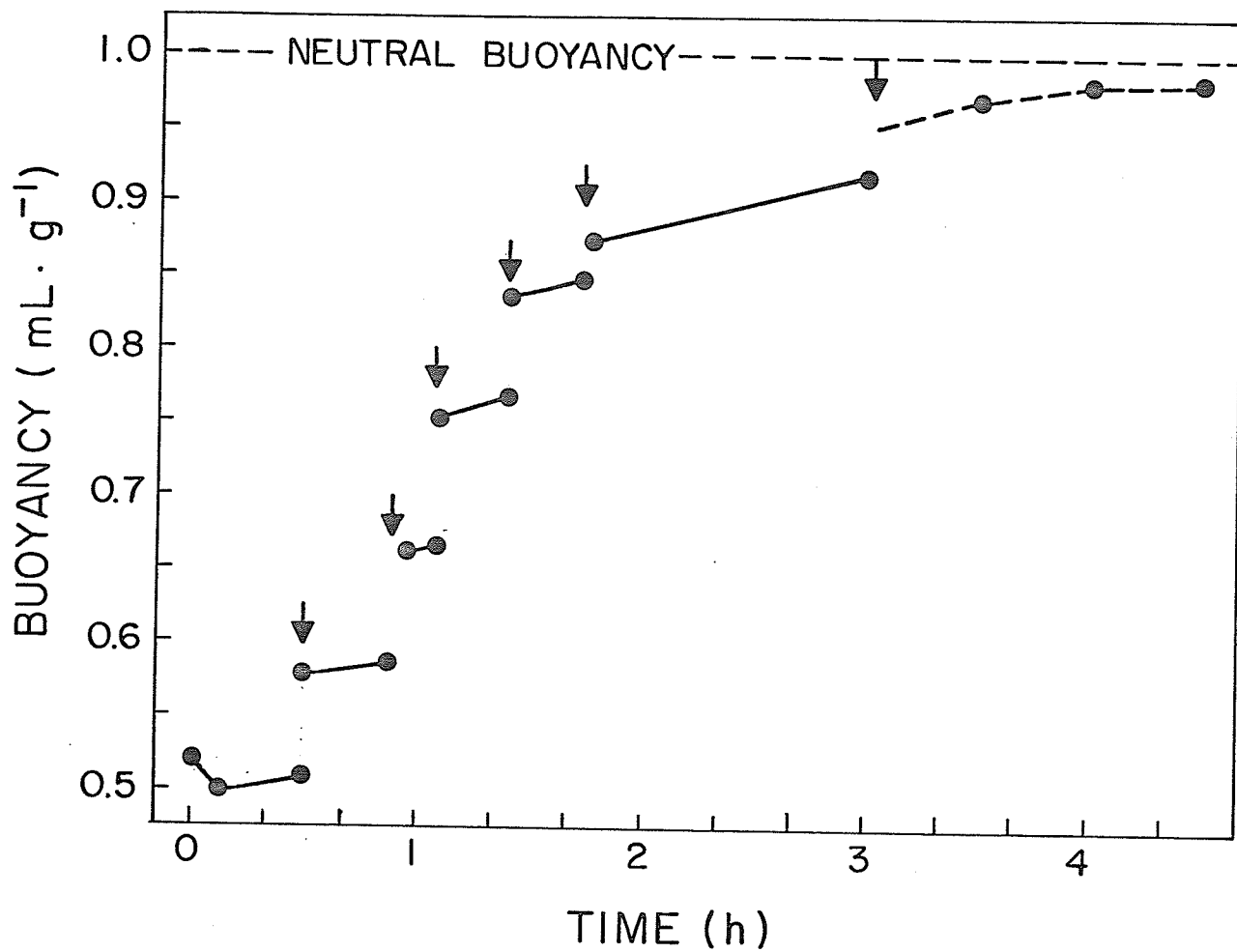
Figure 7. Buoyancy (solid circles), standard volume

(open circles), and internal pressure

(crosses) measured over 48 h in still water

and current. Points are means of 8 fish.
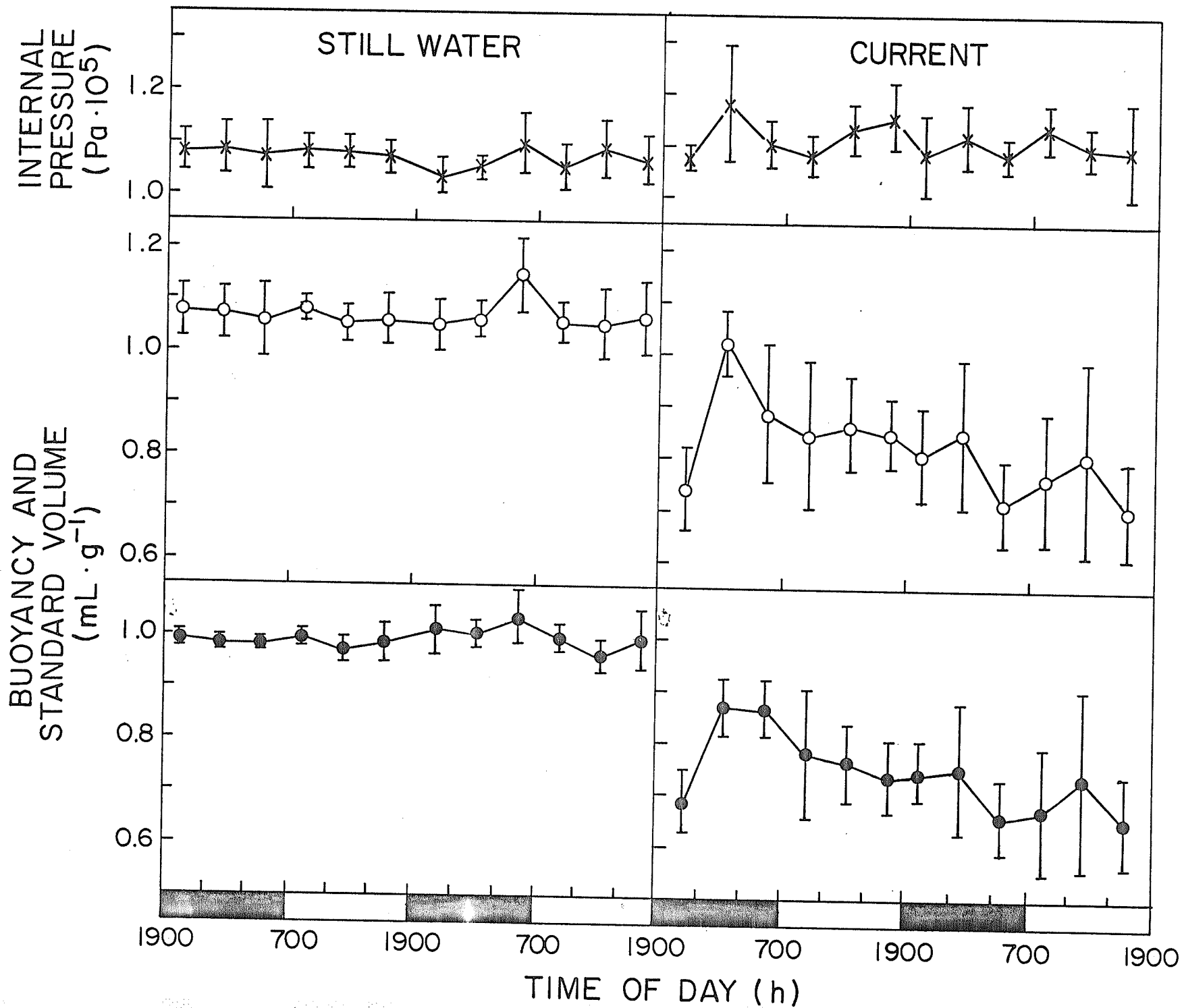
higher during the second dark period than during the rest
of the experiment while internal pressure and standard volume
remained constant (Appendix 4).   In current, buoyancy and
standard volume were greater on day 1 than on day 2 while
internal pressure remained constant.   No diel rhythm was
apparent in buoyancy, eliminating the need to sample at a
set time.

Long term effects of water temperature and velocity on

buoyancy maintenance.   At 21°C, a two-way analysis
of variance on effects of water velocity on buoyancy over
time in current (2-42 d) showed that the main effect of
velocity was not significant, that significant differences
occurred over time, and that there was no interaction between
the effects of water velocity and time in current (Appendix 5).
Following the initial decline between day 0 and 2, buoyancy
decreased slowly until day 18 after which there was little
change (Fig. 8).   Fish maintained similar buoyancies
at all velocities.

At 30°C, effects of water velocity were significant but
buoyancy did not vary significantly over time (day 2-48).
The interaction between these factors was significant
(Appendix 5).   Within each water velocity there was
considerable variation in buoyancy which did not stabilize
until after day 10, after which fish in faster velocities
maintained lower buoyancies (Figs. 8 and 9).

Figure 8.   Mean buoyancies (n=8) between 0 and 42

days in 10, 20 and 30 cm·s^{-1} current at

either 21°C or 30°C.

EXPERIMENTAL TEMPERATURE 21°C

EXPERIMENTAL TEMPERATURE 30°C

BUOYANCY (mL·g⁻¹)
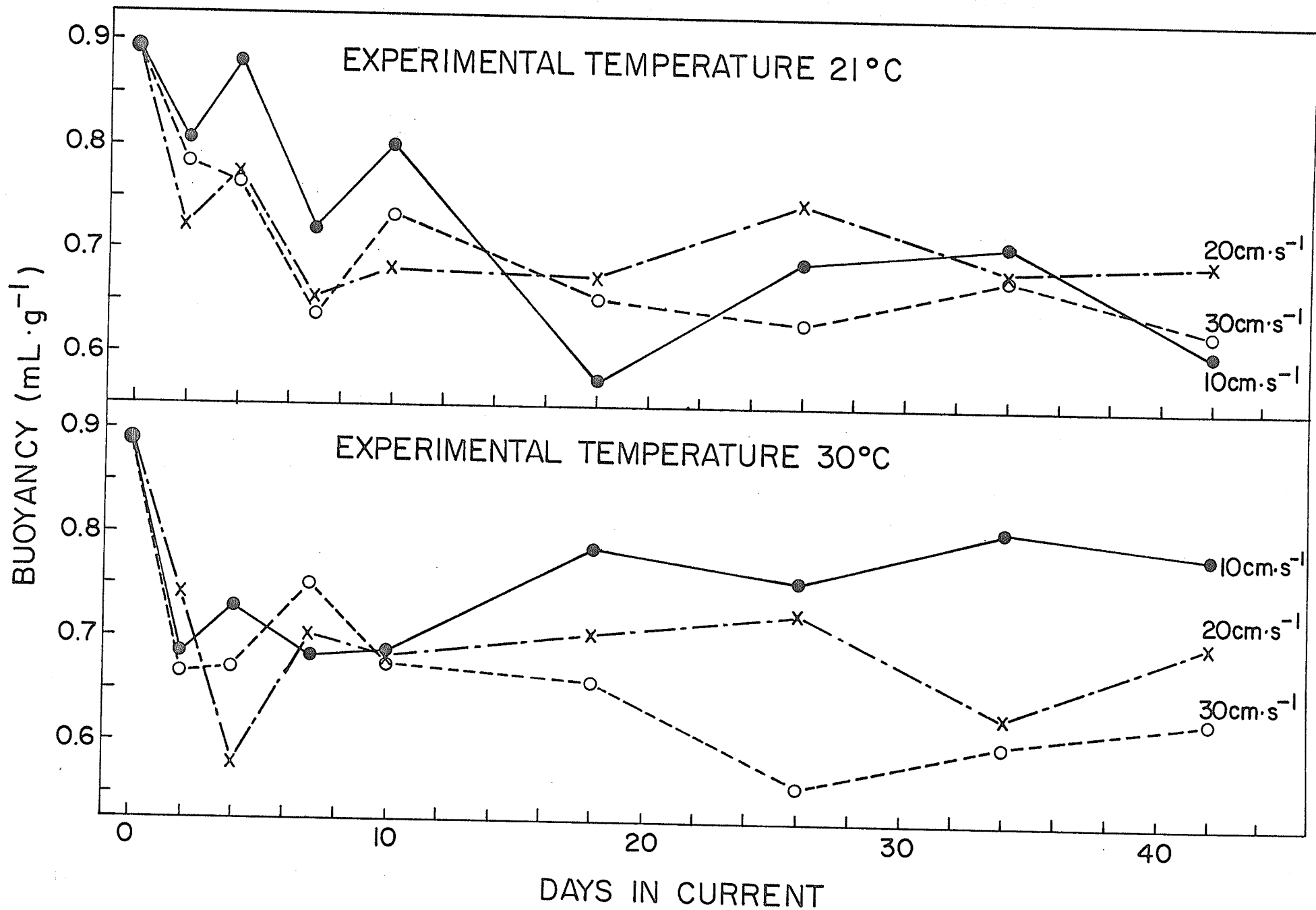
DAYS IN CURRENT

Figure 9.   Effects of water velocity on mean (n=40)

buoyancy (solid circles), standard volume

(open circles) and internal pressure

(crosses) at $30^{\circ}C$, between 10 and 42 days

in current.   Solid lines are significant.
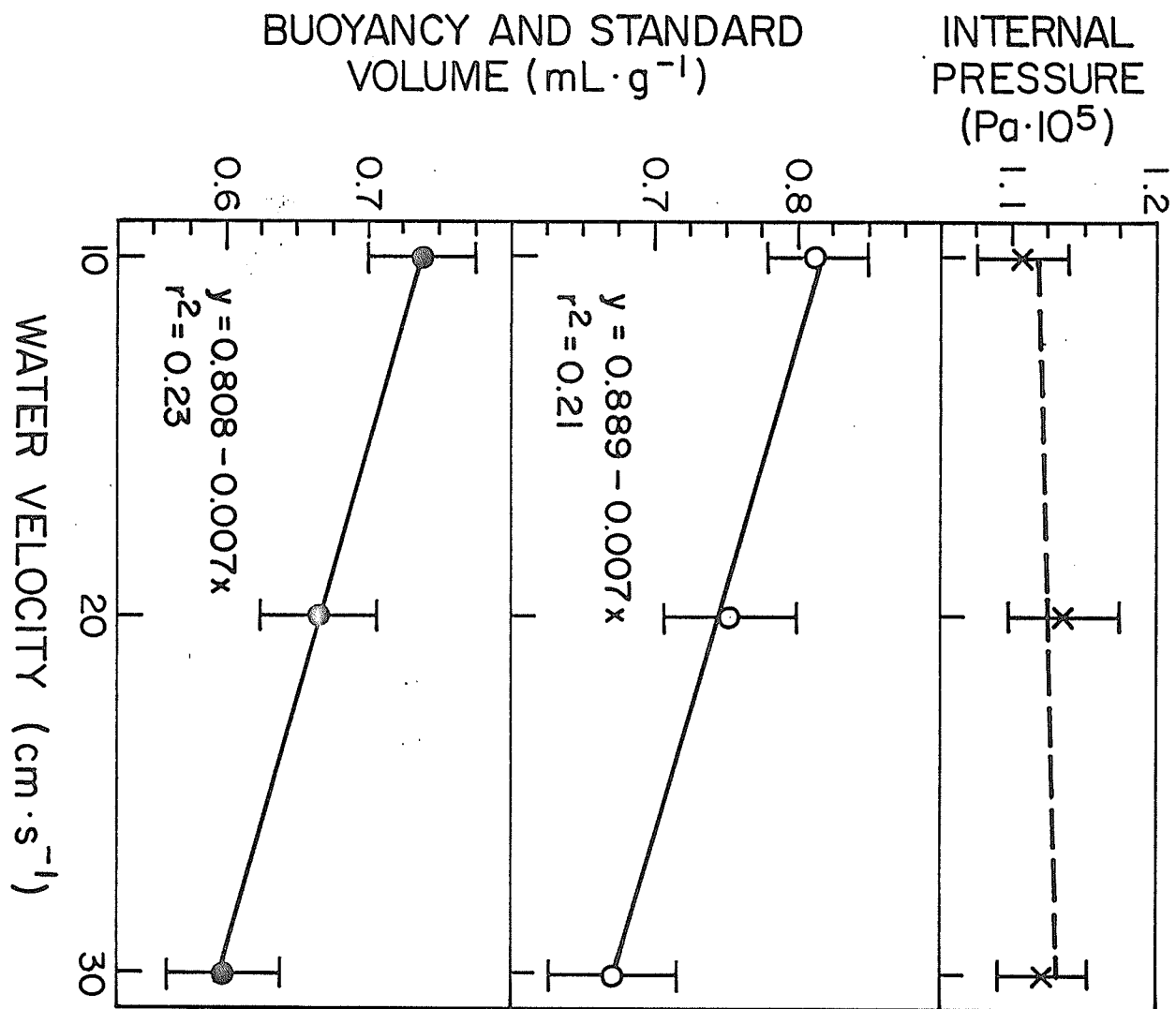
Buoyancy changes at each temperature resulted primarily from changes in standard volume of the swimbladder (Appendix 5, Fig. 9). Because there were interactions between effects of water velocity and time, neither buoyancy, internal pressure, nor standard volume could be lumped for overall regression analyses.

In 20 $cm \cdot s^{-1}$ current, the main effects of both water temperature and time in current on buoyancy were significant (Appendix 5). These effects were complicated by a just significant $(0.04 < P < 0.05)$ time-temperature interaction which, for the sake of comparison, was ignored. Following the initial decline between day 0 and day 2, buoyancy remained variable but was maintained at a relatively constant level for each temperature (Fig. 10). Buoyancy changes over time and at each temperature were determined by internal pressure and standard volume (Figs. 11 and 12).

In temperature and velocity experiments, internal pressure was generally highest and most variable during the first 10 days in current (Appendix 5).

Effects of stress from temperature and water velocity

on buoyancy maintenance. Control fish held at 25°C in 12.5 $cm \cdot s^{-1}$ current gradually reduced both buoyancy and standard volume significantly but did not change their internal pressure during the experiment (regression analyses: Fig. 13, Appendix 6). Lack of fit tests on the regressions for buoyancy and standard volume were significant.

Figure 10.   Mean buoyancies (n=8) between 0 and 42 days

in 20 cm·s^{-1} current at 5°C (solid circles),

11°C (solid triangles), 21°C (crosses), 26°C

(open triangles), and 30°C (open circles).

30
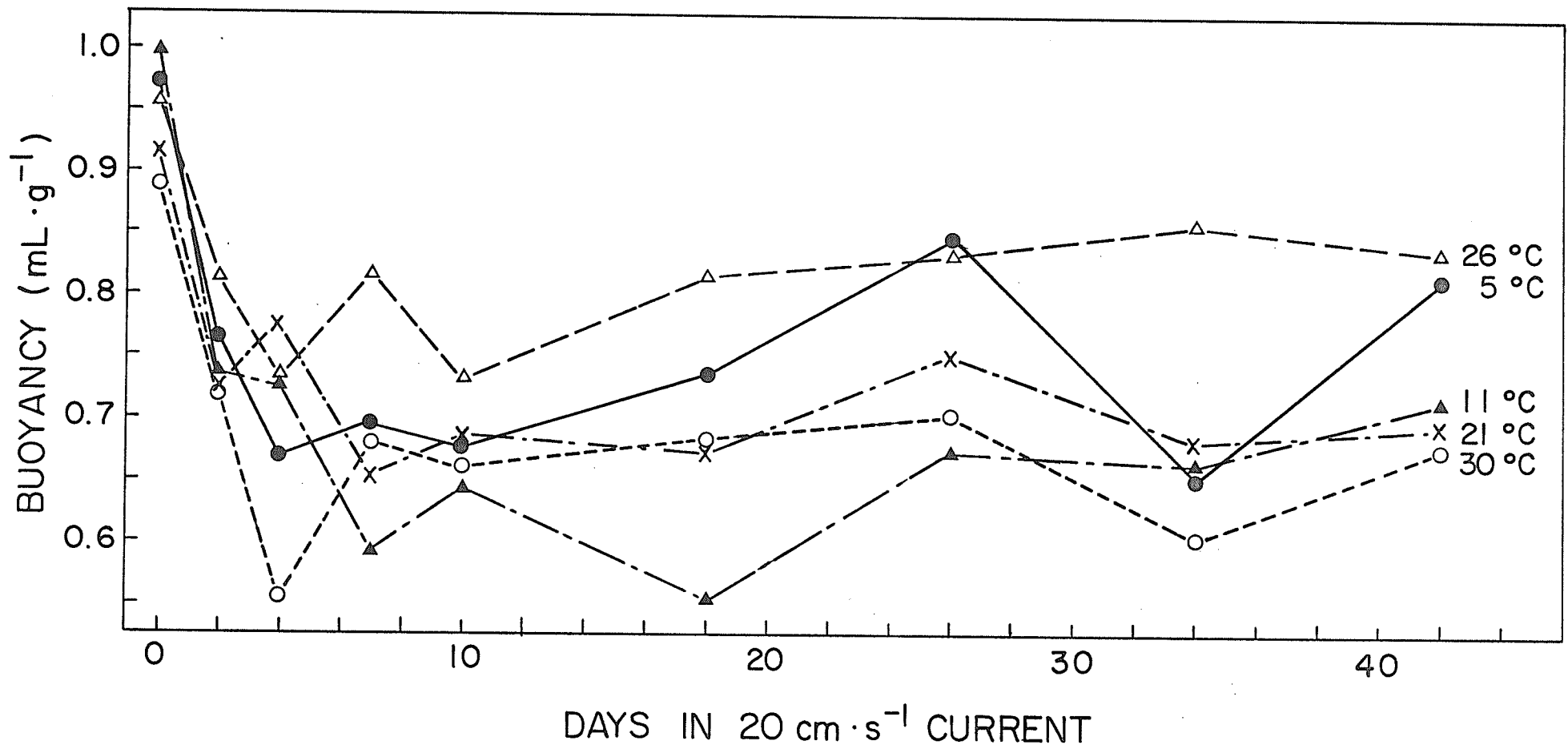
Figure 11.   Effect of time in current on mean (n=40)

buoyancy (solid circles), standard volume

(open circles), and internal pressure

(crosses).   Measurements are lumped for all

temperatures.
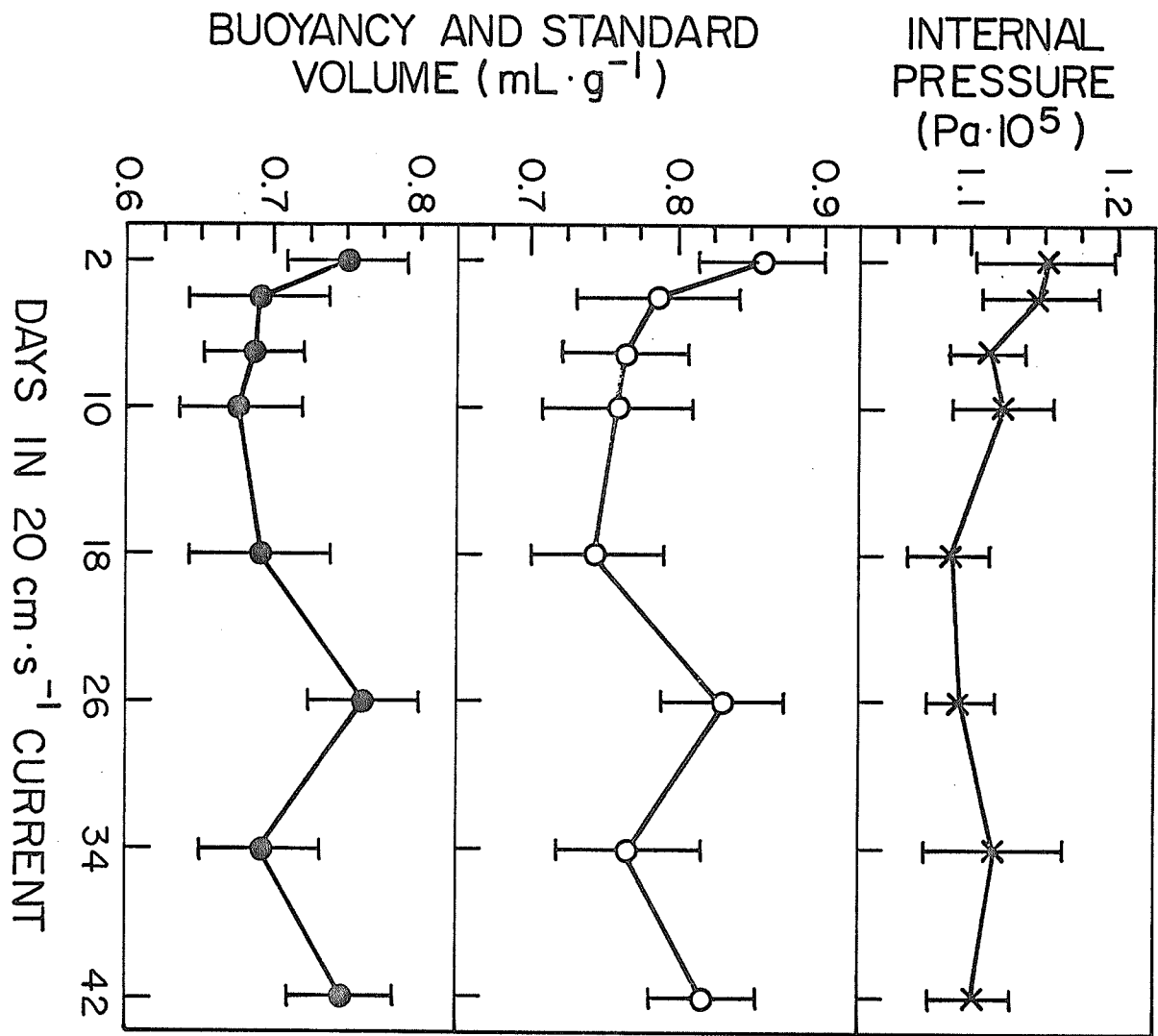
Figure 12. Effects of temperature on mean (n=64)

buoyancy (solid circles), standard volume

(open circles), and internal pressure

(crosses). Measurements are lumped

over time (2-42 d).
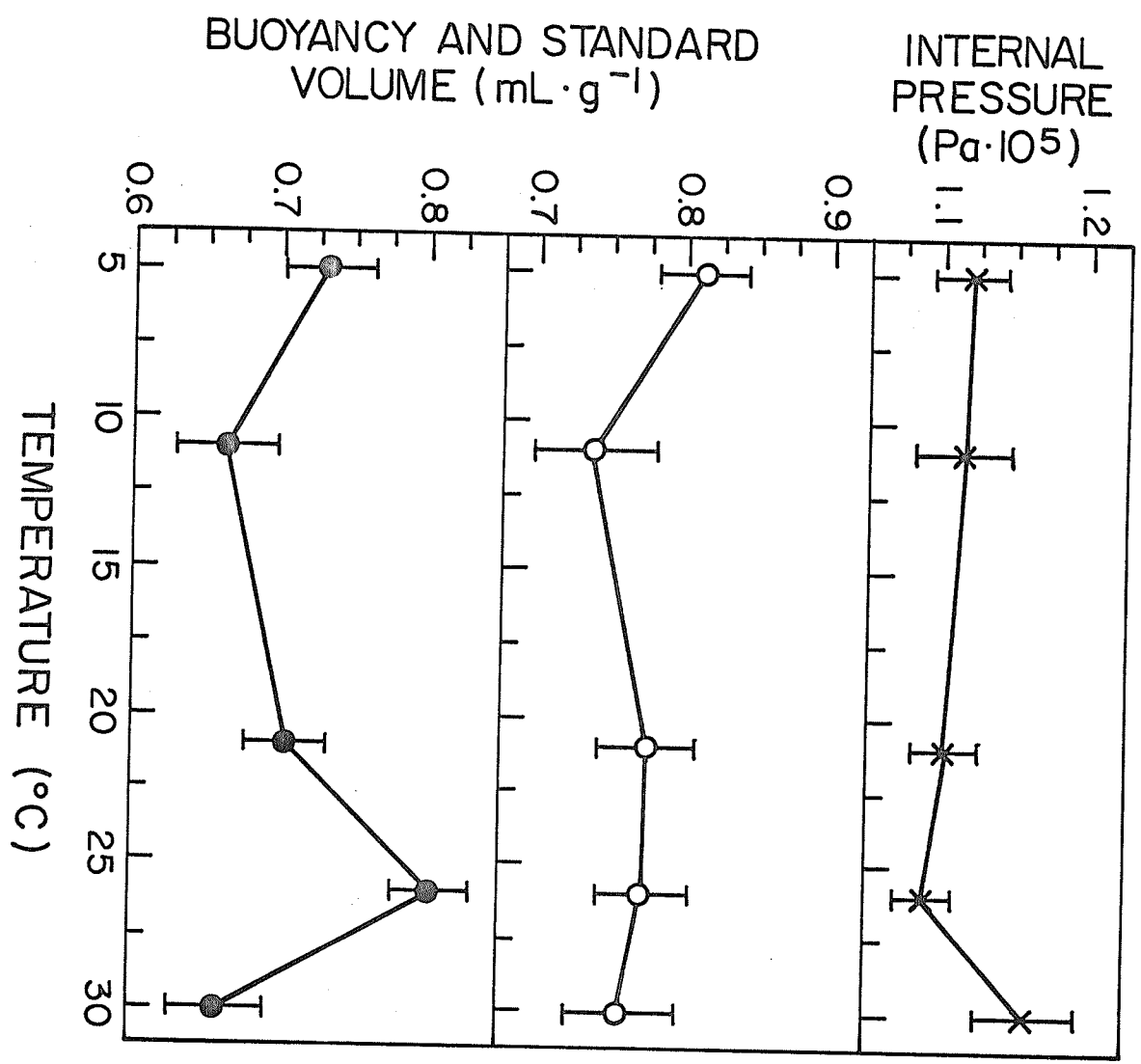
Figure 13.   Regressions against time for buoyancy

(solid circles), standard volume (open

circles), and internal pressure (crosses)

when water temperature and velocity are

held constant.   Solid lines are significant

and points are included for the means (n=8).

BUOYANCY AND STANDARD VOLUME (mL·g⁻¹)

INTERNAL PRESSURE (Pa·10⁵)

DAY OF EXPERIMENT

y = 0.827 − 0.006x, r² = 0.10

y = 0.887 − 0.006x, r² = 0.07
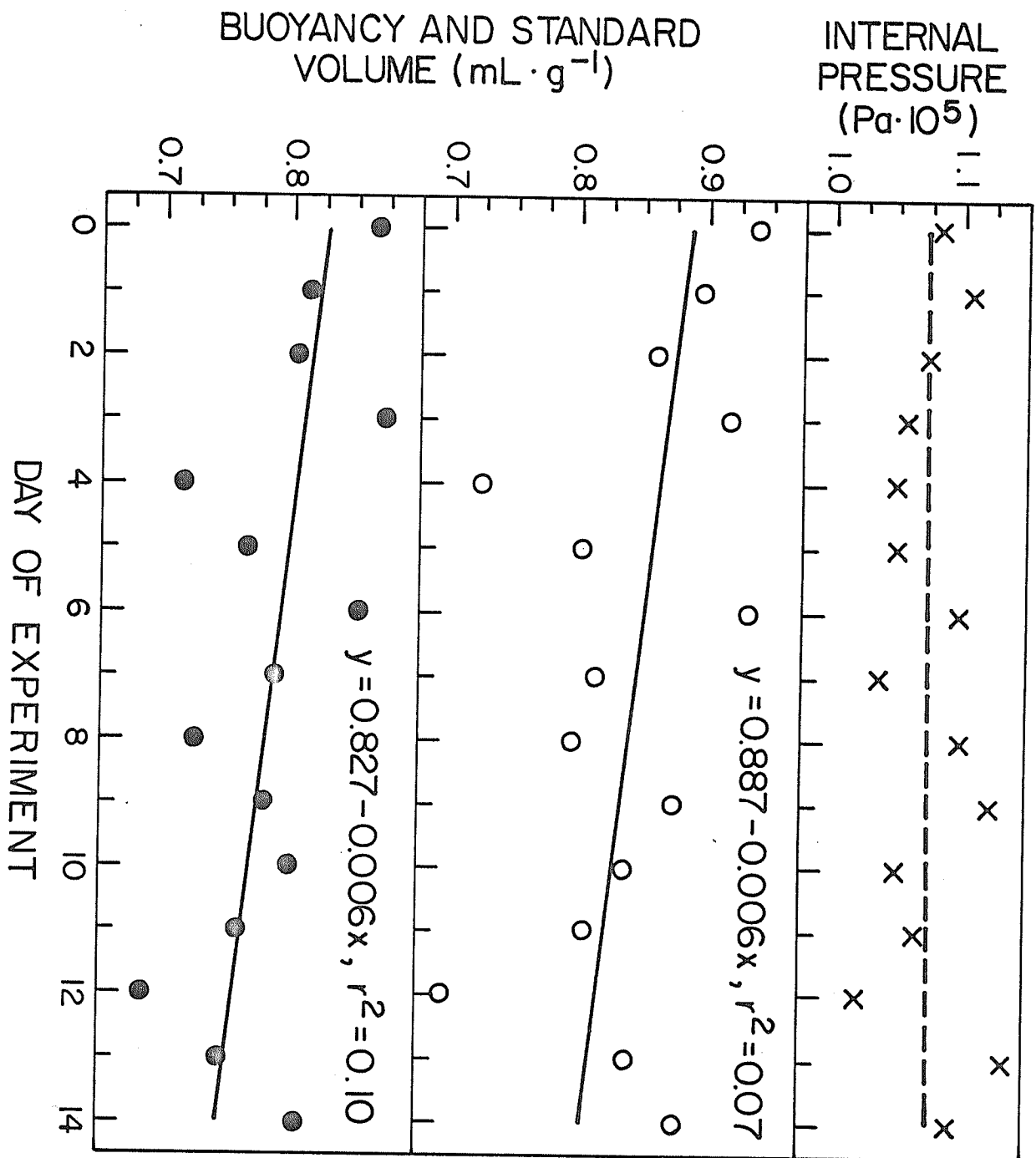
In 12.5 cm·s^{-1} current, increasing the water temperature resulted in a significant linear increase in the internal pressure of the swimbladder over time (regression analyses: Fig. 14, Appendix 6).

At 25°C, increasing the water velocity prompted significant decreases in buoyancy and standard volume and a significant increase in the internal pressure over time (regression analyses: Fig. 15, Appendix 6). Lack of fit tests on the buoyancy and internal pressure regressions were significant.

Increasing temperature and velocity prompted both internal pressure and standard volume to increase significantly (Fig. 16, Appendix 6). Both regressions had significant lack of fit tests.

Significant lack of fit tests suggested that many of the relationships were non-linear and might perhaps follow some more complex trend. However, in no case could a more complex trend be found to fit and the linear relationships remain for illustration. Poor fits of the regression lines to the data resulted from a high degree of variability in the measurements. Both buoyancy and standard volume means had high but constant variabilities. The variance of internal pressure measurements was not constant in conditions of increasing water temperature and/or velocity. When internal pressure was high so was variance (Fig. 17, Appendix 6). Logarithmic transformations were performed on the internal pressures to correct for this increasing variance. Because they had little effect on the significance, r^2, and fit of the lines, untransformed internal pressures were used.

Figure 14.   Regressions against time for buoyancy

(solid circles), standard volume (open

circles), and internal pressure (crosses)

when water temperature is increasing and

velocity is constant.   Solid lines are

significant and points are included for the

means (n=8).

$y = 1.067 + 0.0045\,x$, $r^2 = 0.05$

INTERNAL PRESSURE (Pa·10^5)

BUOYANCY AND STANDARD VOLUME (mL·g^{-1})

DAY OF EXPERIMENT

TEMPERATURE (°C)	25	26	27	28	29	30	31	31.5	32	32.5	33	33.5	34	34.5

Figure 15.   Regressions against time for buoyancy

(solid circles), standard volume (open

circles), and internal pressure (crosses)

when water temperature is constant and

velocity is increasing.   Solid lines are

significant and points are included for

the means (n=8).

INTERNAL PRESSURE (Pa·10⁵)

$y = 1.051 - 0.008x$, $r^2 = 0.07$

BUOYANCY AND STANDARD VOLUME (mL·g⁻¹)

$y = 0.884 - 0.024x$, $r^2 = 0.37$

$y = 0.835 - 0.026x$, $r^2 = 0.43$

DAY OF EXPERIMENT

H₂O VELOCITY cm·s⁻¹	12.5	17.5	17.5	22.5	22.5	27.5	27.5	30	30	32.5	32.5	35	35	35

Figure 16. Regressions against time for buoyancy

(solid circles), standard volume (open

circles), and internal pressure (crosses),

when both water temperature and velocity

are increasing.   Solid lines are significant

and points are included for the means (n=8).
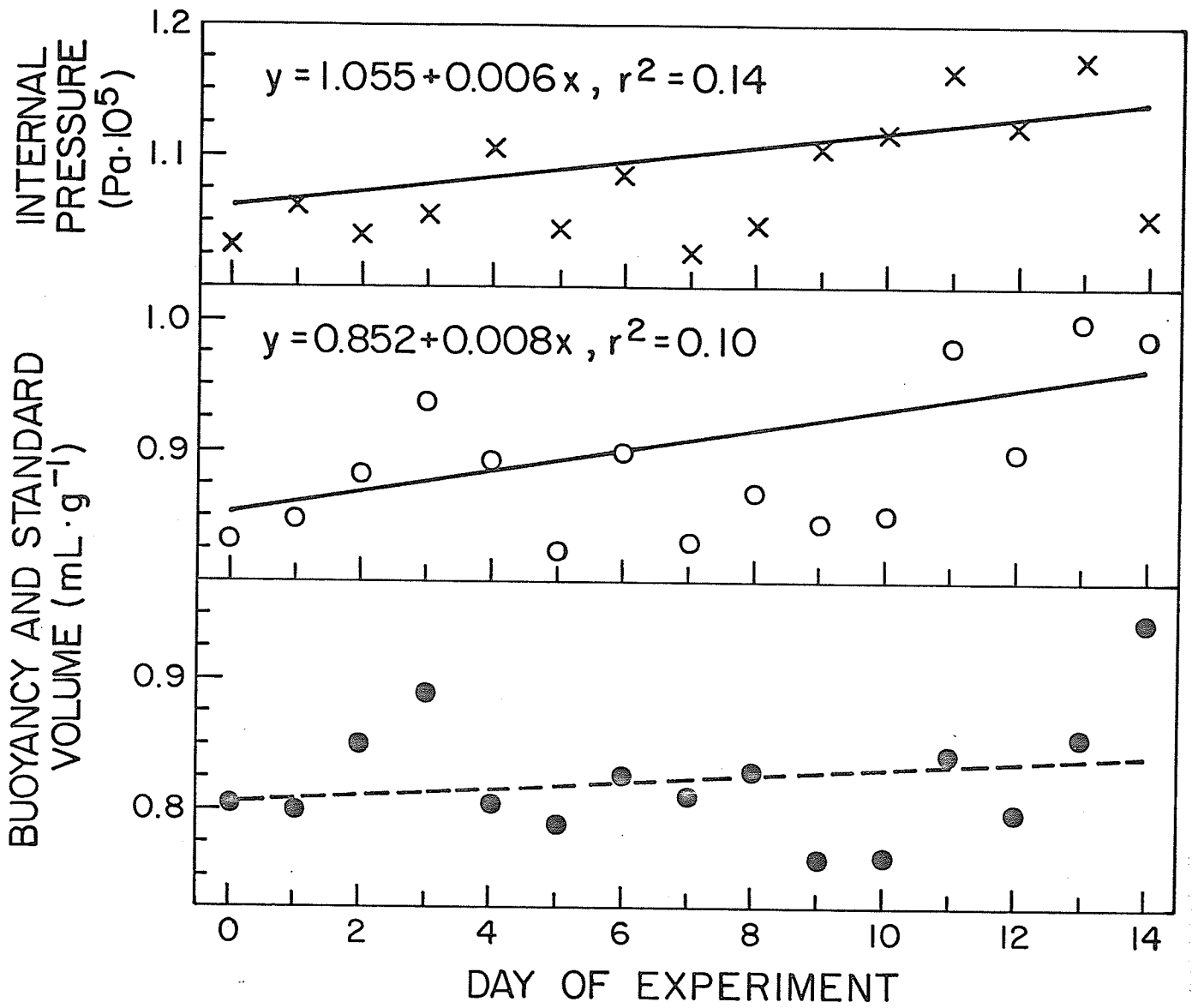
TEMPERATURE (°C)	25	26	27	28	29	30	31	31.5	32	32.5	33	33.5	34	34.5	35
H_2O VELOCITY (cm·s^{-1})	12.5	17.5	17.5	22.5	22.5	27.5	27.5	30	30	32.5	32.5	35	35	35	35

Figure 17. Variance of internal pressure measurements

vs. mean internal pressure (n=8) for:

I. constant water temperature and velocity,

II. increasing temperature,

III. increasing velocity, and

IV. increasing water temperature and velocity.

Solid lines are significant.

I. CONTROL

II. INCREASING TEMPERATURE
$y = ^-0.172 + 0.163x$
$r^2 = 0.88$

III. INCREASING VELOCITY
$y = ^-0.173 + 0.167x$
$r^2 = 0.79$

IV. INCREASING TEMPERATURE AND VELOCITY
$y = ^-0.046 + 0.046x$
$r^2 = 0.60$

VARIANCE

MEAN INTERNAL PRESSURE $(Pa \cdot 10^{-5})$
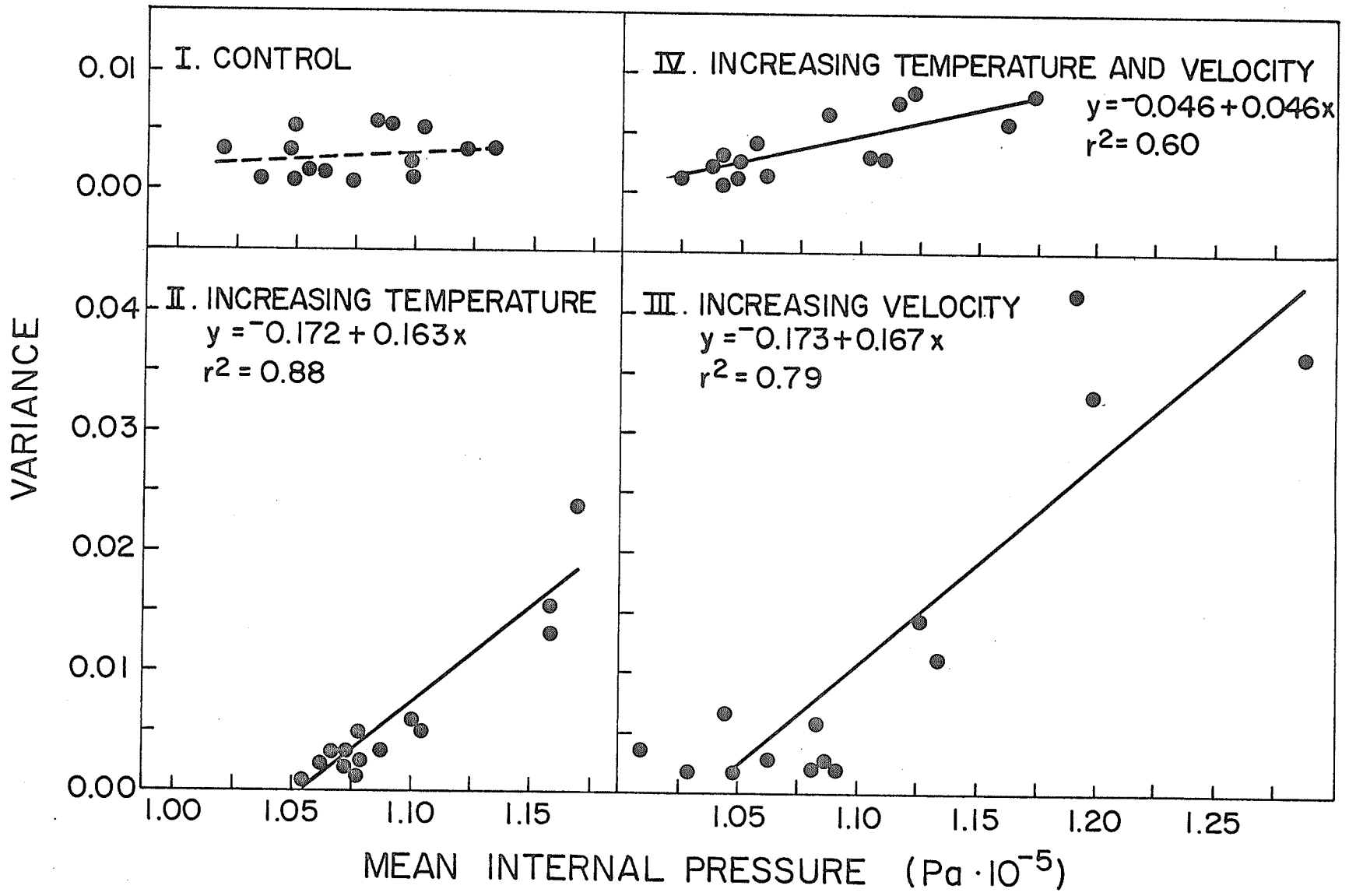
DISCUSSION

In nature, fish are exposed to fluctuations in water velocity that necessitate alteration of swimbladder volume and buoyancy. If a fish cannot respond to attain the appropriate buoyancy, its swimming effort increases, it may lose position, and its chances of survival and reproduction are diminished. Most buoyancy adjustment is accomplished by gas secretion or absorption and by gulping and spitting gas. Fathead minnows can make extensive adjustments at a rapid rate and maintain the optimal negative buoyancy indefinitely in current.

Mechanisms of Buoyancy Adjustment

Fathead minnows are able to increase buoyancy by gas secretion from their rete mirabile and by gulping air directly into the swimbladder through the pneumatic duct. They can decrease buoyancy by resorbing gas from the swimbladder and by forcing gas out of the swimbladder through the pneumatic duct. The extent to which each of these mechanisms is used varies between fish. Under extreme conditions, internal pressure may also be altered to help adjust buoyancy.

When fathead minnows are at less than optimal buoyancy, they increase swimbladder volume quickly by gulping air at the surface and slowly by secreting gas into the swimbladder. Evidence that they secrete gas includes the changing swimbladder gas composition during buoyancy increases and the ability to increase buoyancy without being able to gulp air. Gulping and secretion are used simultaneously to increase buoyancy with observations showing that the former normally accounts for about 70% of the increase.

If fathead minnows used either mechanism exclusively, and assuming that the increases were linear, they could fill a completely emptied swimbladder in 2.5 h by gulping (0.42 $mL \cdot g^{-1} \cdot h^{-1}$), 24 h by secretion for small fish (49-67 mm, 0.042 $mL \cdot g^{-1} \cdot h^{-1}$), and 36 h by secretion for large fish (72-83 mm, 0.028 $mL \cdot g^{-1} \cdot h^{-1}$). Gee's (1977) data give similar results, showing that small fathead minnows (47-63 mm, 0.040 $mL \cdot g^{-1} \cdot h^{-1}$) can fill their swimbladders completely in 25 h and juveniles (20-30 mm, 0.057 $mL \cdot g^{-1} \cdot h^{-1}$) in 17.5 h. Size related rate differences may reflect the greater relative metabolic and gas uptake rates of smaller fish (Ultsch, 1973; Jones and Randall, 1978).

These secretion rates are higher than those reported for other physostomes. Mudminnows (Umbra limi) take at least 24 days to completely fill an emptied swimbladder by secretion (Gee, in prep.). Rainbow and brown trout (Salmo gairdneri and S. trutta) take at least 13 days (Wittenberg,

1958), goldfish (Carassius auratus) between 5 and 7 days
(Evans and Damant, 1928; Wittenberg, 1958), and longnose
dace (Rhinichthys cataractae) between 3 and 4 days to
completely fill an emptied swimbladder by secretion (Gee,
1968).   When practical, slower secreting fish like
mudminnows and rainbow trout fill their swimbladders by
gulping air within 20 min (Gee, pers. comm.).   The rete
mirabile is generally less well developed than that of
physoclists, which are able to fill their swimbladders by
gas secretion in 2 to 24 h (Fange, 1966).   Lowering the
water temperature may reduce the fathead minnow's rate of
buoyancy increase (Gee, 1977), but it does not necessarily
mean that secretion rates are altered.   As temperature
changes, changes in the rate of O_2 secretion are offset
by changes in the rate of CO_2 secretion (McNabb and Mecham,
1971).   This may mean that fish are not able to gulp air
as effectively at low temperatures.

Little is known about the biochemistry of gas secretion
in the physostome swimbladder.   Indeed, in many species,
researchers have been unable to confirm the existence of
gas gland cells (Fange, 1976).   Microscopic examination of
the fathead minnow swimbladder reveals a well developed system
of counter current capillaries which, like those in the eel
(Anguilla vulgaris) (Steen, 1963), probably account for the
high rate of gas secretion. As in physoclists, most of the
newly-secreted gas consists of O_2 and CO_2, suggesting that

the secretory mechanisms may also be similar (Wittenberg, 1958; Fange, 1976). Nitrogen concentration in the swimbladder is low following secretion but it slowly rises to the normal still water level by diffusion and secondary O_2 resorption (Wittenberg, 1958; Alexander, 1966; Enns et al., 1967; Abernethy, 1972). Carbon dioxide diffuses out of the swimbladder following secretion. Still water gas composition (1.5% CO_2, 11.4% O_2, 87.1% N_2) closely resembles that reported for two other physostomes, the shallowwater cisco (Leucichthys artedi: 1.6% CO_2, 10.7% O_2, 87.7% N_2) and the American smelt (Osmerus mordax: 1.0% CO_2, 11.2% O_2, 87.9% N_2) (Saunders, 1953).

When fathead minnows are too buoyant, they can decrease swimbladder volume quickly by spitting gas bubbles or slowly by resorbing gas from the swimbladder. The former is used in response to fright; the latter is the main mechanism used to reduce swimbladder volume in response to an increase in water velocity. This is evidenced by the lack of spitting when current is increased and the change in composition of swimbladder gases. This change is opposite that which occurs during secretion. High N_2 and low O_2 and CO_2 concentrations following buoyancy reduction suggest that gas is removed from the swimbladder by diffusion. This is supported by the fact that CO_2 diffuses out of the swimbladder faster than O_2 which diffuses faster than N_2 (Piiper et al., 1962; Kutchai and

Steen, 1971; Denton et al., 1972). Thus, N_2 concentration rises as O_2 and CO_2 are preferentially resorbed. During exposure to current, fish were observed occasionally to gulp air at the surface. If it was taken into the swimbladder, buoyancy should have increased. It did not and this may mean that air was taken into the swimbladder where the O_2 and CO_2 were removed by resorption. Since O_2 consumption in current is one of the limiting factors to swimming performance, having the ability to supplement O_2 uptake during strenuous swimming, especially at non-optimal temperatures, would be a great survival advantage (Brett, 1964, 1972). The rate of gas resorption appears to be independent of temperature (Gee, 1977). Small fathead minnows (47-67 mm) can halve the volume of gas in their swimbladders using mainly resorption in 15 to 22 h ($0.034-0.023$ mL$\cdot$g$^{-1}\cdot$h^{-1}). If they are forced to spit gas they can halve their swimbladder volume without injury in less than 10 min.

Under extreme conditions, fathead minnows appear to contract or expand swimbladder volume by muscular activity, decreasing or increasing buoyancy. This is evidenced by changes in the internal pressure and has been observed to occur in several other species of physostomes and in some physoclists (McCutcheon, 1962; Gee, 1970; Gee et al., 1974). This method can only be used to alter buoyancy over a small range. It is used when fish are first exposed to current

and when water temperature and/or velocity are high and increasing. During the initial stages of buoyancy decrease, altering the internal pressure may prevent unnecessary gas resorption where buoyancy need only be altered for a short period. Under stressful conditions, internal pressure alterations, though slight, may be critical to the adjustment of swimbladder volume and buoyancy. The use of internal pressure may be limited to short term buoyancy adjustments by the energy cost of maintaining muscular tonus. Such short term adjustments may improve the precision of buoyancy control (McCutcheon, 1962).

Fathead minnows can alter swimbladder volume to adjust buoyancy by resorption/secretion, spitting/gulping and also by altering internal pressure of swimbladder gas. The former two mechanisms are used for major changes, the latter is used sparingly - usually for minor adjustments. There are advantages and disadvantages to each of these methods. Spitting and gulping have the advantage of changing buoyancy rapidly. They have the disadvantage of imprecision and gulping also requires time and energy to go to the surface, it exposes the fish to predators, and it is difficult when ice is present. Gulping air at atmospheric pressure also makes it difficult to obtain sufficient gas to maintain neutral buoyancy at depths where pressures are much greater than atmospheric. Secretion and resorption while slower have none of these disadvantages. Internal pressure alterations have

the advantage that they do not require gas exchange and the disadvantages of being energetically expensive and causing only limited swimbladder volume and buoyancy changes.

The mechanisms used to inflate or deflate the swimbladder may be influenced by several environmental factors. For example, predator disturbance may cause buoyancy reduction by spitting; it is fast, allowing fish to quickly seek cover on the bottom or at the surface and precision is not important (Jones, 1951, 1957; Verheijen, 1962; McCutcheon, 1966; Sullivan and Atchison, 1978). Fish entering current from still water resorb gas, tailoring buoyancy precisely to the level where swimming is most efficient. Ice cover may necessitate secretion under some conditions and fish far below the surface may secrete rather than swim to the surface to gulp. Following predator exposure and buoyancy reduction, it might be advantageous for fish to quickly return to neutral buoyancy by gulping air at the surface. In this event, adaptations such as group surfacing might be used to reduce exposure to predators (Gee, in press). Reducing buoyancy for short periods by increasing internal pressure might be advantageous for fish swimming from one pool to another up a riffle.

They would swim efficiently in the current of the riffle without having to gulp or secrete gas when they reached the upper pool.   Having a choice of mechanisms available may in itself be advantageous as the fish can use that strategy which is safest and most efficient under the prevailing environmental conditions.

Ability to Maintain Buoyancy

Fathead minnows can maintain a buoyancy level appropriate to the hydraulic demands of their environment for at least 42 days.   Two of the factors determining the level attained are water temperature and velocity.   In near lethal combinations, or when temperature is low, they may cause buoyancy maintenance to fail (Berezay and Gee, 1978).

Fish make buoyancy alterations to counteract the changing forces of lift on their bodies, thereby reducing swimming effort required to maintain position.   Because lift forces do not change unless the hydraulic forces on the fish's body change, to maximize swimming efficiency in constant conditions fish should maintain a constant buoyancy.   Lift forces (F_L) are determined by the density of the fish (ρ), the maximum projected surface area (S_h), swimming speed (U), and the coefficient of lift (C_L); where C_L equals a constant for a given hydrofoil shape (k) multiplied by the sine of the

swimming angle of attack (α). This results in the equation:

$$F_L = \tfrac{1}{2}\rho S_h U^2 C_L \qquad \text{(Webb, 1975)}.$$

Based on this information, lift and therefore buoyancy should change quadratically as the inverse of the water velocity when all other factors are constant. In practice, there is only a simple inverse linear relationship between buoyancy and water velocity. It holds over the long term at $30^{\circ}C$ between velocities of 10 and 30 $cm \cdot s^{-1}$ and for increasing water velocities when temperature is a constant $25^{\circ}C$. Gee (1977) working with fathead minnows at $21^{\circ}C$ and Neave et al. (1966) working with salmon parr also found that buoyancy was inversely related to water velocity with a linear relationship existing. Why buoyancy changes are linear and not quadrat as predicted by Webb's (1975) model remains unclear.

The lack of relationship between buoyancy and velocity observed at $21^{\circ}C$ is puzzling. It is contrary to other observations and suggests that some other factor influences buoyancy at that temperature. Since $21^{\circ}C$ is the optimal temperature for swimming performance and reproduction in the fathead minnow (Brungs, 1971; Gee, 1977), it may be that fish expend less energy swimming and consequently the energy saved by fine buoyancy adjustments is unimportant.

The relationship between temperature and buoyancy level is not a simple one. Average buoyancies were higher at 5 and $26^{\circ}C$ than at 11, 21 and $30^{\circ}C$. Gee (1977) obtained

similar results except that buoyancy remained low at 24 and 27°C after 3 days in current. Since increasing water temperature from 25 to 34.5^{o}C while holding velocity constant also had no effect on buoyancy level, other temperature dependent factors may have been operating at 26°C. Neither season of the year, degree of gonad development, condition, nor photoperiod which were found to affect buoyancy (Luoma, 1979) can explain why buoyancy should be higher than expected at 26°C. The inability to reduce buoyancy to the same extent at 5°C as at higher temperatures and to maintain it within narrow limits over a long period, may mean that only at low temperatures are fathead minnows unable to respond to lift created by increasing water currents. Why this should be so has yet to be explained. Since low temperatures encountered in nature are often coincident with spring run-off, fish unable to reduce their buoyancy at low temperatures risk being swept downstream.

In current, the initial buoyancy reduction takes less than 24 h but it appears to be approximate. Adjustments continue for between 4 and 18 days until a buoyancy level appropriate to the water temperature and velocity is reached. Following adjustment, buoyancy is usually maintained at a constant low level. During maintenance, buoyancy fluctuations occur both within and between sampling days. These follow

no set pattern and probably reflect minor changes in the
test conditions and/or in the groups of fish tested.
Most buoyancy differences between temperatures and
velocities result from differences in the amount
(standard volume) of gas in the swimbladder and not in
the internal pressure.   However, during the initial
buoyancy decrease and at high temperatures, internal
pressure may also be altered.

Stress, as defined by Brett (1958), is a state
produced by any environmental or other factor which extends
the adaptive responses of an animal beyond the normal range
or which disturbs the normal functioning to the extent
that, in either case, the chances of survival are
significantly reduced.   Two environmental stressors are
high water temperature and velocity.

When fish are stressed by increasing water temperature
and/or velocity, to near lethal levels, internal pressure
plays a much greater role in determining buoyancy levels.
It increases when temperature or both temperature and
velocity are increased, offsetting increases in standard
volume and keeping buoyancy level constant.   When velocity
alone increases, internal pressure rises as standard volume
falls, increasing the extent of buoyancy adjustments.
Internal pressure may be a good indicator of sublethal
temperature and velocity stress.   Initially, variability

is uniformly low as all of the fish can regulate their internal pressure.   Then, as temperature reaches 32.5°C or velocity 30 cm·s⁻¹, variability increases as some fish lose their ability to control internal pressure.   Finally, at extreme temperatures and velocities (34.5°C, 35 cm·s⁻¹), all of the fish lose control of their internal pressure and variability is again low.   When control is lost, buoyancy often rises abruptly.   It would be interesting to determine whether sublethal chemical stressors have similar effects.

It is interesting that effects of stress from increasing water temperature and velocity simultaneously are not cumulative.   Fish either no longer can or no longer need to reduce buoyancy in response to increasing current when temperature is also increasing.   Several observations suggest that the latter is the correct explanation. Internal pressure variability was generally low, fish did not appear to be labouring while swimming in current, and there were no mortalities.   Perhaps the combination of high temperature and velocity cause a slight change in the fish's body shape, thereby reducing lift and the need to compensate by reducing buoyancy.   Fish swimming in current also appear to withstand higher temperatures than those in still water.   On one occasion temperature was raised to 37°C overnight and fish in current all survived with no obvious ill affects.   The still water lethal level is

is uniformly low as all of the fish can regulate their internal pressure.   Then, as temperature reaches 32.5°C or velocity 30 $cm \cdot s^{-1}$, variability increases as some fish lose their ability to control internal pressure.   Finally, at extreme temperatures and velocities (34.5°C, 35 $cm \cdot s^{-1}$), all of the fish lose control of their internal pressure and variability is again low.   When control is lost, buoyancy often rises abruptly.   It would be interesting to determine whether sublethal chemical stressors have similar effects.

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reported to be 34°C (Brett, 1944; Hart, 1947; Brungs, 1971).
These differences may be partially due to the greater oxygen
availability in current than in still water at high
temperatures.   The effects of increasing water temperature
and velocity simultaneously, then, appear to be antagonistic
(Sprague, 1970).

Fathead minnows can maintain buoyancy in current over
long periods.   This ability enables them to swim efficiently
in current, facilitating migrations upstream to more desirable
habitats and preventing them from being swept downstream to
less desirable habitats where they would be more susceptible
to predation and extinction.   North temperate streams are
characterized by rapid changes in time and space of water
velocity and many have a prolonged interval of spring run-off.
The fathead minnow is adapted to such an environment partially
because it can adjust buoyancy rapidly and maintain a negative
buoyancy for an extended period.

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A P P E N D I C E S

APPENDIX 1

Gas volume corrections and sample calculations.

Gases released from the swimbladder during collection
were subject to changes in pressure, temperature, and volume.
To determine the effects of thermal expansion-contraction,
gas diffusion into the water bath, and water column height
in the collecting pipette on gas released from the swimbladder,
a test experiment was performed.

In the test experiment, conditions were exactly similar
to those of actual swimbladder gas volume measurements
except that known gas volumes were released.   Fifty and
100 μL ($\pm$ 1 percent) air samples were released under water
at 5, 10, 15, 20, 25, 30, and 35°C and collected in an
inverted funnel.   They were then measured at 22.2°C in
a 200 μL ($\pm$ 8 percent) pipette attached to the funnel.
Eight measurements of 100 μL samples were made at each
temperature using 2 different pipettes, A and B, and similar
measurements of 50 μL samples were made with pipette A.
Pipette A was used for all previous experimentation.

Gas volumes released differed from those collected both
with temperature and between pipettes (Table Al).   There
were no measureable gas volume changes arising from
solubility, diffusion or from expansion due to water column
pressure in the pipette.   Volumes of 50 and 100 μL changed
the same relative amount with temperature and while pipette A
and B showed similar slopes with temperature, their y-intercepts
differed markedly.

Table A1.　Regression equations testing for gas sampling
errors.　Asterisk (*) indicates statistical
significance (P< 0.05).

Pipette and Gas Volume Released	y-intercept	Slope (μL$\cdot^\circ$C^{-1})	F. Calc.	F. Crit.	R^2
pipette A- 100 μL	116.3 μL	-0.3964	1302.7*	4.06	0.960
pipette B- 100 μL	108.4 μL	-0.3054	612.3*	4.06	0.919
pipette A- 50 μL$\cdot$2	115.3 μL	-0.3732	538.2*	4.06	0.909
regression for pipette A based on error 100 μL	115.6 μL	-0.3514			

Table A2.　Correction factors for gas volumes released from
the swimbladder.

Temperature of Fish ($^\circ$C)	Correction Factor	Temperature of Fish ($^\circ$C)	Correction Factor
5	1.143	31	1.040
11	1.119	31.5	1.038
19	1.088	32	1.036
21	1.080	32.5	1.034
25	1.064	33	1.032
26	1.060	33.5	1.030
27	1.056	34	1.028
28	1.052	34.5	1.026
29	1.048	35	1.024
30	1.044		

The results suggested that volumetric errors in the gas measurement were due to volumetric errors in the pipettes and thermal expansion or contraction of the gas. For pipette A, the collected volume of a 100 μL sample, held and measured at 22.2°C, was 107.5 μL (i.e., y=116.3 μL− 0.3964 μL·°C^{-1}·22.2°C). This positive 7.5 percent error in the pipette, coupled with thermal contraction (1.0 μL = (273 K + 22.2K)÷(273K + 35K) = 0.9584 μL) or expansion (1.0 μL=(273K + 22.2K)÷(273 K + 5.0 K) = 1.0619 μL), yielded a regression equation within 1 percent of the observed regression for pipette A, confirming the sources of gas measurement error (Figure Al, Table Al). Both sources of measurement error and the daily barometric pressures were corrected for in the internal pressure and standard volume measurements. For example:

Fish C: - held at 25°C

   - body weight 1.848 g

   - weight in water + swimbladder 0.023 g

   - weight in water - swimbladder 0.103 g

   - gas volume released from swimbladder 0.092 mL

   - swimbladder displacement volume 0.080 mL

Buoyancy = $\dfrac{\text{Swimbladder Volume}}{\substack{\text{Weight in Water} \\ \text{Without Swimbladder}}}$ = $\dfrac{0.080 \text{ mL}}{0.103 \text{ g}}$ = 0.777 mL·g^{-1}

   - measures the flotation per gram weight of fish tissue in water, independent of temperature and pressure.

Figure Al. Measured and predicted error in gas sample
volume measurements arising from gas contraction
and expansion with temperature changes and from
errors in the pipette volume.
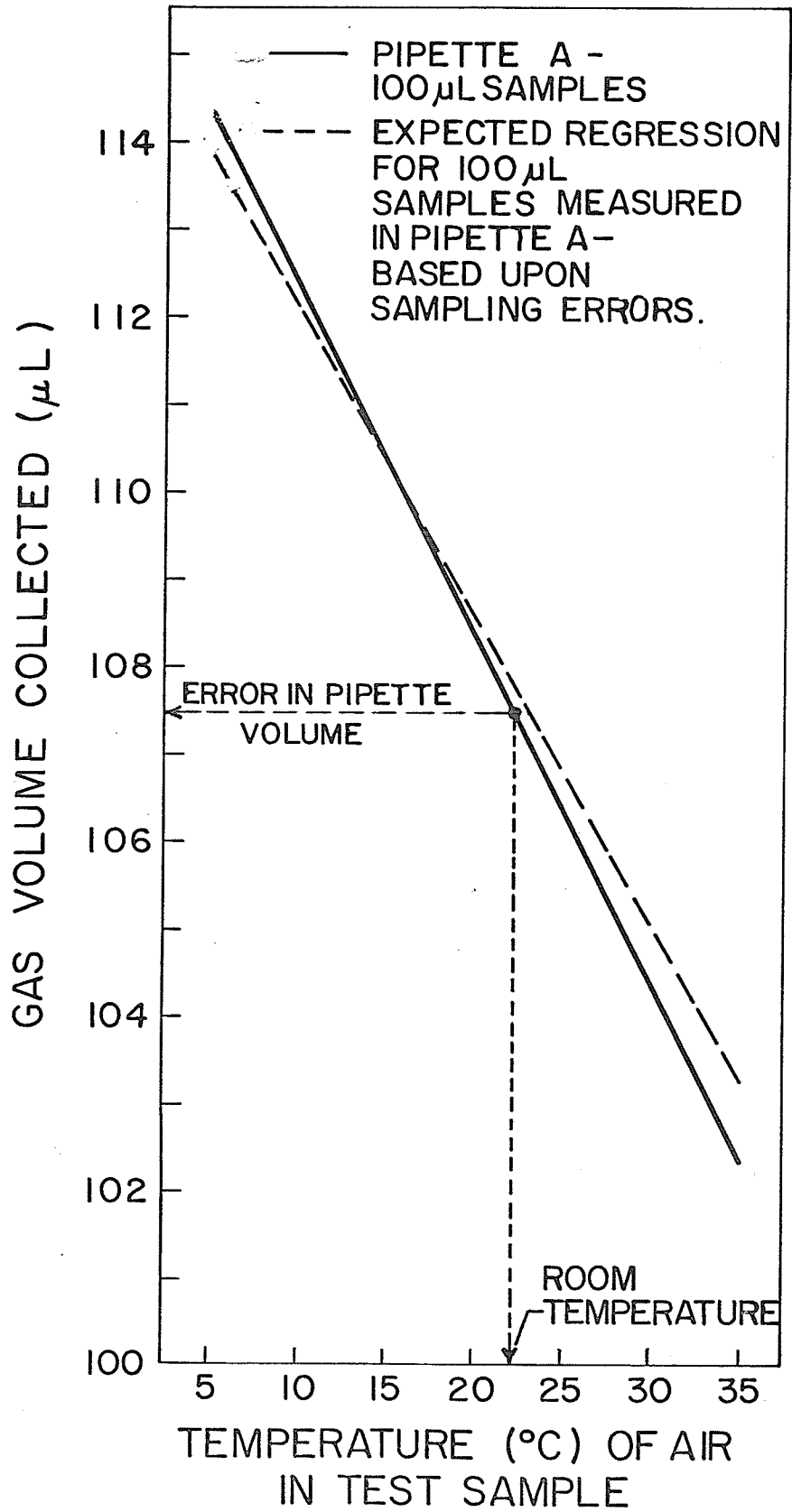
Internal Pressure = $\dfrac{\text{Gas Volume Released}}{\text{Swimbladder Volume}}$

$= \dfrac{0.092 \text{ mL}}{0.080 \text{ mL}} = 1.150$ Atmospheres

- the gas volume released was subject to measurement
  error from thermal contraction of gas, from
  pipette volumetric error, and from atmospheric
  pressure fluctuations.   Since gas volume measured
  was 1.064 times greater than the actual volume
  released, the internal pressure (atm) was divided
  by 1.064.   This corrected for thermal and pipette
  volumetric errors.   Daily pressure fluctuations
  also affected gas measurements as each 'Atmosphere'
  was at the altitude of Winnipeg, and subject to
  daily variation.   On 26/11/78, the day of
  measurement, the pressure was 993.2 millibars
  or for ease of comparison 0.993 $Pa \cdot 10^5$
  (Environment Canada, Winnipeg Airport, Table A2).
  Multiplying the internal pressure (atm) by pressure
  $(Pa \cdot 10^5 \cdot atm^{-1})$ gave the actual pressure of the
  gas inside the fish's swimbladder.   In this case:

Internal Pressure = $\dfrac{1.150}{1.064}$ atm . $\quad 0.993 \; Pa \cdot 10^5 \cdot atm^{-1}$

$= 1.073 \; Pa \cdot 10^5$

Standard volume = $\dfrac{\text{Gas Volume Released}}{\substack{\text{Weight in } H_2O \; - \\ \text{Swimbladder}}} = \dfrac{0.993 \; Pa \cdot 10^5}{1.064}$

$= \quad 0.834 \; mL \cdot g^{-1}$

- measures the gas volume released (at 25°C and
  standard pressure) per gram weight in water.
  It allows comparison of the relative amount
  of gas in the swimbladder between temperatures,
  not of the moles of gas in the swimbladder.

APPENDIX 2

Percentage of Fish Swimming Horizontally, Mean Buoyancy (mL·g^{-1}, n=8) and 95% Confidence Intervals (CI) During Adjustment to Still Water and Current at 19°C.

	Time (h)	Percent Swimming Horizontally	Mean Buoyancy (mL·g^{-1})	Standard Deviation	95% CI For Mean
Current	0.5	50.0	0.720	0.138	0.604 - 0.835
	1.5	55.2	0.705	0.123	0.602 - 0.808
	2.5	61.2	0.657	0.120	0.557 - 0.757
	5.5	75.0	0.600	0.096	0.520 - 0.681
	8.5	77.5	0.619	0.126	0.514 - 0.725
	11.5	85.0	0.596	0.134	0.484 - 0.708
Still Water	0.5	44.2	0.653	0.120	0.553 - 0.753
	1.5	48.5	0.653	0.145	0.532 - 0.775
	2.5	54.0	0.612	0.154	0.484 - 0.741
	5.5	76.8	0.794	0.127	0.687 - 0.900
	8.5	87.0	0.849	0.153	0.722 - 0.977
	11.5	87.5	0.852	0.114	0.757 - 0.947

Regression Analyses Tables: (See Note, page 14)

Percent of Fish Swimming Horizontally vs. Mean Buoyancy (mL·g^{-1}) During Adjustment to 20 cm·s^{-1} Current.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	5	0.0144	0.0029		
Regression	1	0.0133	0.0133	52.02*	7.71
Residual	4	0.0010	0.0003	**	

Regression Equation: y = 0.9004 - 0.0037 x, r^2 = 0.964

Percent of Fish Swimming Horizontally vs. Mean Buoyancy (mL·g^{-1}) During Adjustment to Still Water Following Exposure to 20 cm·s^{-1} Current.

Source	df	ss	mss	F. Calc.	F. Crit.
Total	5	0.0587	0.0118		
Regression	1	0.0539	0.0539	44.73*	7.71
Residual	4	0.0048	0.0012	**	

Regression Equation: y = 0.3834 + 0.0053 x, r^2 = 0.918

** No repeats lack of fit tests not possible.

APPENDIX 3

Regression Analyses of Fish Length(mm) vs. Rate of Buoyancy Increase
by Gas Secretion $(mL \cdot g^{-1} \cdot h^{-1})$.   (See Note, page 14)

Source	df	ss	mss	F.Calc.	F.Crit.
Total	7	0.00045	0.00006		
Regression	1	0.00034	0.00034	18.82*	5.99
Residual	6	0.00011	0.00002		
Lack of Fit	5	0.00011	0.00002	43.40	230.0
Pure Error	1	0.00000	0.00000		

Regression Equation: $y = 0.0700 - 0.0005 \, x$, $r^2 = 0.76$

A one-way ANOVA comparing the volumes of gas
bubbles forced into the swimbladder (gulps)
found that large fish (72-83 mm) gulped
significantly greater amounts of gas on the
average than did small fish (49-67 mm)
$(F_{Calc.} = 39.5^*, \; F_{0.05,1,6} = 5.99)$.

APPENDIX 4

Two-Way Analyses of Variance: Buoyancies ($mL \cdot g^{-1}$) of Fish Held in Still Water. (See Note, page 14)

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0068	0.0068	4.10	0.96*
Time Intervals	5	0.0167	0.0033	2.03	0.92
Interaction	5	0.0106	0.0021	1.28	0.72
Error	84	0.1386	0.0017		
Total	95	0.1727			

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0068	0.0068	4.08	0.96*
Light and Dark	1	0.0068	0.0068	4.10	0.96*
Interaction	1	0.0065	0.0065	3.92	0.95
Error	92	0.1526	0.0017		
Total	95	0.1727			

Buoyancies ($mL \cdot g^{-1}$) of Fish Held in 20 $cm \cdot s^{-1}$ Current.

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.1166	0.1166	7.63	0.99*
Time Intervals	5	0.1400	0.0280	1.83	0.86
Interaction	5	0.1100	0.0220	1.44	0.78
Error	84	1.2837	0.0153		
Total	95	1.6503			

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.1166	0.1166	7.11	0.99*
Light and Dark	1	0.0240	0.0240	1.46	0.77
Interaction	1	0.0005	0.0005	0.03	0.16
Error	92	1.5092	0.0164		
Total	95	1.6503			

Standard Volumes ($mL \cdot g^{-1}$) of Fish Held in Still Water.

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0009	0.0009	0.20	0.34
Time Intervals	5	0.0213	0.0043	1.01	0.58
Interaction	5	0.0373	0.0075	1.76	0.87
Error	84	0.3558	0.0042		
Total	95	0.4153			

APPENDIX 4 cont'd

Standard Volumes $(mL \cdot g^{-1})$ of Fish Held in Still Water.

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0009	0.0009	0.19	0.34
Light and Dark	1	0.0058	0.0058	1.32	0.75
Interaction	1	0.0032	0.0032	0.74	0.60
Error	92	0.4054	0.0044		
Total	95	0.4153			

Standard Volumes $(mL \cdot g^{-1})$ of Fish Held in 20 $cm \cdot s^{-1}$ Current.

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.2158	0.2158	11.56	1.00*
Time Intervals	5	0.2916	0.0583	3.12	0.99*
Interaction	5	0.1716	0.0343	1.84	0.89
Error	84	1.5675	0.0187		
Total	95	2.2466			

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.2158	0.2158	9.89	1.00*
Light and Dark	1	0.0238	0.0238	1.09	0.70
Interaction	1	0.0001	0.0001	0.005	0.10
Error	92	2.0069	0.0218		
Total	95	2.2466			

Internal Pressures $(Pa \cdot 10^5)$ of Fish Held in Still Water.

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0034	0.0034	1.13	0.71
Time Intervals	5	0.0089	0.0018	0.60	0.30
Interaction	5	0.0170	0.0034	1.14	0.66
Error	84	0.2500	0.0030		
Total	95	0.2793			

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0034	0.0034	1.12	0.71
Light and Dark	1	0.0002	0.0002	0.07	0.22
Interaction	1	0.0009	0.0009	0.29	0.44
Error	92	0.2749	0.0030		
Total	95	0.2793			

APPENDIX 4 cont'd

Internal Pressure (Pa·10^5) of Fish Held in 20 cm·s^{-1} Current.

Source	df	ss	mss	F. Calc.	Prob. of F.
Days	1	0.0103	0.0103	1.78	0.82
Time Intervals	5	0.0489	0.0098	1.70	0.86
Interaction	5	0.0430	0.0086	1.49	0.80
Error	84	0.4842	0.0058		
Total	95	0.5864			

Source	df	ss	mss	F.Calc.	Prob. of F.
Days	1	0.0103	0.0103	1.65	0.80
Light and Dark	1	0.0020	0.0020	0.33	0.42
Interaction	1	0.0005	0.0005	0.09	0.24
Error	92	0.5735	0.0062		
Total	95	0.5864			

APPENDIX 5

Two-Way Analyses of Variance: (See Note, page 14)

Effects on Buoyancy ($mL \cdot g^{-1}$) of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $21^{\circ}C$.

Source	df	ss	mss	F.Calc.	F.Crit.
Water Velocity	2	0.0378	0.0189	1.56	3.01
Time	7	0.6245	0.0892	7.36*	2.01
Interaction	14	0.2781	0.0199	1.64	1.69
Error	168	2.0378	0.0121		
Total	191	2.9782			

Effects on Standard Volume ($mL \cdot g^{-1}$) of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $21^{\circ}C$.

Source	df	ss	mss	F.Calc.	F.Crit.
Water Velocity	2	0.1009	0.0505	3.01	3.01
Time	7	0.7638	0.1091	6.52*	2.01
Interaction	14	0.5030	0.0359	2.15*	1.69
Error	168	2.8123	0.0167		
Total	191	4.1801			

Effects on Internal Pressure ($Pa \cdot 10^{5}$) of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $21^{\circ}C$.

Source	df	ss	mss	F.Calc.	F.Crit.
Water Velocity	2	0.0229	0.0155	1.50	3.01
Time	7	0.0501	0.0072	0.94	2.01
Interaction	14	0.2569	0.0184	2.41*	1.69
Error	168	1.2810	0.0076		
Total	191	1.6110			

Effects on Buoyancy ($mL \cdot g^{-1}$) of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $30^{\circ}C$.

Source	df	ss	mss	F.Calc.	F.Crit.
Water Velocity	2	0.2543	0.1271	10.56*	3.01
Time	7	0.0641	0.0092	0.76	2.01
Interaction	14	0.4421	0.0316	2.62*	1.69
Error	168	2.0225	0.0120		
Total	191	2.7830			

APPENDIX 5 cont'd

Effects on Standard Volume $(mL \cdot g^{-1})$ of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $30^{O}C$.

Source	df	ss	mss	F.Calc.	F. Crit.
Water Velocity	2	0.2615	0.1308	10.10*	3.01
Time	7	0.0437	0.0062	0.48	2.01
Interaction	14	0.4893	0.0350	2.70*	1.69
Error	168	2.1756	0.0129		
Total	191	2.9701			

Effects on Internal Pressure $(Pa \cdot 10^{5})$ of Long Term Exposure to 10, 20, and 30 $cm \cdot s^{-1}$ Current at $30^{O}C$.

Source	df	ss	mss	F.Calc.	F. Crit.
Water Velocity	2	0.0265	0.0133	1.38	3.01
Time	7	0.1736	0.0248	2.58*	2.01
Interaction	14	0.1218	0.0087	0.91	1.69
Error	168	1.6118	0.0096		
Total	191	1.9337			

Effects on Buoyancy $(mL \cdot g^{-1})$ of Long Term Exposure to 20 $cm \cdot s^{-1}$ Current at 5, 11, 21, 26, and $30^{O}C$.

Source	df	ss	mss	F.Calc.	F. Crit.
Temperature	4	0.8893	0.2223	16.46*	2.37
Time	7	0.3344	0.0478	3.54*	2.01
Interaction	28	0.5884	0.0210	1.56*	1.48
Error	280	3.7824	0.0135		
Total	319	5.5946			

Effects on Standard Volume $(mL \cdot g^{-1})$ of Long Term Exposure to 20 $cm \cdot s^{-1}$ Current at 5, 11, 21, 26, and $30^{O}C$.

Source	df	ss	mss	F.Calc.	F. Crit.
Temperature	4	0.7095	0.1774	10.11*	2.37
Time	7	0.4481	0.0640	3.65*	2.01
Interaction	28	0.9206	0.0329	1.87*	1.48
Error	280	4.9109	0.0175		
Total	319	6.9891			

APPENDIX 5 cont'd

Effects on Internal Pressure ($Pa \cdot 10^5$) of Long Term Exposure to
20 $cm \cdot s^{-1}$ Current at 5, 11, 21, 26, and 30°C.

Source	df	ss	mss	F.Calc.	F. Crit.
Temperature	4	0.1823	0.0456	4.35*	2.37
Time	7	0.1584	0.0226	2.16*	2.01
Interaction	28	0.7897	0.0282	2.69*	1.48
Error	280	2.9324	0.0105		
Total	319	4.0628			

Effects on Buoyancy ($mL \cdot g^{-1}$) of Long Term Exposure, Between 10 and 42
days, to 10, 20 and 30 $cm \cdot s^{-1}$ Current at 30°C.

Source	df	ss	mss	F.Calc.	F. Crit.
Water Velocity	2	0.3873	0.1936	18.57*	3.09
Time	4	0.0282	0.0071	0.68	2.46
Interaction	8	0.1695	0.0212	2.03	2.03
Error	105	1.0952	0.0104		
Total	119	1.6802			

Effects on Internal Pressure ($Pa \cdot 10^5$) of Long Term Exposure Between
10 and 42 days to 10, 20, and 30 $cm \cdot s^{-1}$ Current at 30°C.

Source	df	ss	mss	F.Calc.	F. Crit.
Water Velocity	2	0.0160	0.0080	0.96	3.09
Days	4	0.0284	0.0071	0.85	2.46
Interaction	8	0.0862	0.0108	1.29	2.03
Error	105	0.8752	0.0083		
Total	119	1.0058			

Effects on Standard Volume ($mL \cdot g^{-1}$) of Long Term Exposure Between 10
and 42 Days to 10, 20, and 30 $cm \cdot s^{-1}$ Current at 30°C.

Source	df	ss	mss	F.Calc.	F. Crit.
Water Velocity	2	0.4070	0.2035	16.70*	3.09
Days	4	0.0391	0.0098	0.80	2.46
Interaction	8	0.2307	0.0288	2.37*	2.03
Error	105	1.2791	0.0122		
Total	119	1.9559			

APPENDIX 5 cont'd

Regression Analyses: (See Note, page 14)

Buoyancy $(mL \cdot g^{-1})$ vs. Water Velocity $(cm \cdot s^{-1})$ at $30^{\circ}C$ Between 10 and 42 days in Current.

Source	df	ss	mss	F. Calc.	F. Crit.
Total	119	1.6802	0.0141		
Regression	1	0.3868	0.3868	35.29*	3.92
Residual	118	1.2934	0.0110		
Lack of Fit	1	0.0005	0.0005	0.045	3.92
Pure Error	117	1.2929	0.0111		

Regression Equation: $y = 0.8075 - 0.0070 x$, $r^2 = 0.230$

Standard Volume $(ml \cdot g^{-1})$ vs. Water Velocity $(cm \cdot s^{-1})$ at $30^{\circ}C$ Between 10 and 42 Days in Current.

Source	df	ss	mss	F. Calc.	F. Crit.
Total	119	1.9559	0.0164		
Regression	1	0.4042	0.4042	30.74*	3.92
Residual	118	1.5517	0.0132		
Lack of Fit	1	0.0028	0.0028	0.211	3.92
Pure Error	117	1.5489	0.0132		

Regression Equation: $y = 0.8889 - 0.0071 x$, $r^2 = 0.207$

Internal Pressure $(Pa \cdot 10^5)$ vs. Water Velocity $(cm \cdot s^{-1})$ at $30^{\circ}C$ Between 10 and 42 Days in Current.

Source	df	ss	mss	F.Calc.	F.Crit
Total	119	1.0058	0.0084		
Regression	1	0.0039	0.0039	0.45	3.92
Residual	118	1.0020	0.0085		
Lack of Fit	1	0.0121	0.0121	1.43	3.92
Pure Error	117	0.9898	0.0085		

Regression Equation: $y = 1.1079 + 0.0007 x$, $r^2 = 0.004$

APPENDIX 5   cont'd.

Comparisons of the Mean Internal Pressure and Variance Measurements Between the First Four Measurements in Current (2, 4, 7, 10 d) and the Last Four Measurements (18, 26, 34, 42 d).

Days in Current	Temperature (°C) Velocity cm·s⁻¹	21			30			5	11	21	Overall Means
		10	20	30	10	20	30	20	20	20	
Internal Pressure ($Pa \cdot 10^5$)											
2 – 10		1.144[a]	1.083	1.093	1.123	1.167	1.132	1.139	1.151	1.128	1.129
18 – 42		1.103[b]	1.122	1.104	1.114	1.126	1.126	1.097	1.078	1.047	1.102
Variance											
2 – 10		0.0154[c]	0.0082	0.0049	0.0084	0.0138	0.0088	0.0123	0.0166	0.0060	0.0105
18 – 42		0.0043[d]	0.0073	0.0056	0.0086	0.0104	0.0074	0.0076	0.0060	0.0028	0.0067

[a] mean (n=32) of internal pressure measurements taken after 2, 4, 7, and 10 days in 10 cm·s⁻¹ current at 21°C.

[b] as above except 18, 26, 34, and 42 days.

[c] mean (n=4) variance of the mean internal pressure (n=8) after 2, 4, 7, and 10 days in 10 cm·s⁻¹ current at 21°C.

[d] as above except 18, 26, 34, and 42 days.

APPENDIX 6

Buoyancy, Standard Volume, and Internal Pressure Response to Constant or Increasing Water Velocity and Constant or Increasing Water Temperature.

Water Temp. ($^{\circ}$C)	Water Velocity (cm·s^{-1})	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
	Buoyancy (mL·g^{-1})				
25°C	12.5	0	0.866	0.055	0.820 − 0.912
		1	0.813	0.112	0.719 − 0.906
		2	0.802	0.069	0.744 − 0.860
		3	0.871	0.039	0.838 − 0.904
		4	0.713	0.085	0.642 − 0.784
		5	0.764	0.032	0.738 − 0.790
		6	0.851	0.044	0.813 − 0.888
		7	0.786	0.095	0.706 − 0.865
		8	0.724	0.038	0.692 − 0.756
		9	0.779	0.079	0.713 − 0.846
		10	0.779	0.058	0.750 − 0.847
		11	0.758	0.065	0.704 − 0.812
		12	0.684	0.085	0.613 − 0.755
		13	0.744	0.064	0.690 − 0.778
		14	0.805	0.052	0.762 − 0.848
25°C	12.5	0	0.863	0.052	0.819 − 0.906
26		1	0.810	0.035	0.781 − 0.840
27		2	0.872	0.069	0.814 − 0.930
28		3	0.892	0.054	0.846 − 0.937
29		4	0.820	0.073	0.759 − 0.880
30		5	0.837	0.039	0.805 − 0.870
31		6	0.807	0.064	0.753 − 0.860
31.5		7	0.825	0.063	0.772 − 0.878
32		8	0.873	0.052	0.829 − 0.916
32.5		9	0.839	0.092	0.762 − 0.916
33		10	0.847	0.090	0.772 − 0.923
33.5		11	0.781	0.077	0.717 − 0.846
34		12	0.863	0.068	0.806 − 0.920
34.5		13	0.861	0.050	0.819 − 0.903
25	12.5	0	0.877	0.043	0.840 − 0.913
	17.5	1	0.732	0.070	0.674 − 0.791
	17.5	2	0.782	0.157	0.651 − 0.912
	22.5	3	0.781	0.065	0.727 − 0.836
	22.5	4	0.660	0.163	0.523 − 0.796
	27.5	5	0.802	0.073	0.741 − 0.863
	27.5	6	0.750	0.102	0.665 − 0.835
	30	7	0.637	0.138	0.522 − 0.753
	30	8	0.603	0.149	0.478 − 0.727

APPENDIX 6 cont'd

Water Temp. ($^{\circ}$C)	Water Velocity (cm·s^{-1})	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
25	32.5	9	0.511	0.130	0.403 - 0.620
	32.5	10	0.569	0.115	0.473 - 0.666
	35	11	0.566	0.124	0.463 - 0.670
	35	12	0.468	0.105	0.380 - 0.556
	35	13	0.552	0.106	0.464 - 0.640
25	12.5	0	0.804	0.086	0.732 - 0.876
26	17.5	1	0.800	0.094	0.716 - 0.883
27	17.5	2	0.849	0.069	0.791 - 0.907
28	22.5	3	0.889	0.044	0.852 - 0.926
29	22.5	4	0.805	0.117	0.768 - 0.903
30	27.5	5	0.788	0.080	0.721 - 0.855
31	27.5	6	0.826	0.036	0.796 - 0.856
31.5	30	7	0.809	0.082	0.741 - 0.877
32	30	8	0.829	0.069	0.771 - 0.887
32.5	32.5	9	0.762	0.076	0.669 - 0.826
33	32.5	10	0.764	0.090	0.689 - 0.840
33.5	35	11	0.841	0.061	0.790 - 0.893
34	35	12	0.798	0.091	0.723 - 0.874
34.5	35	13	0.855	0.088	0.781 - 0.930
35	35	14	0.941	0.109	0.851 - 1.033

Standard Volume (mL·g^{-1})

Water Temp. ($^{\circ}$C)	Water Velocity (cm·s^{-1})	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
25	25	0	0.940	0.086	0.868 - 1.013
		1	0.896	0.094	0.818 - 0.974
		2	0.860	0.061	0.808 - 0.911
		3	0.918	0.017	0.904 - 0.932
		4	0.723	0.130	0.644 - 0.861
		5	0.802	0.045	0.764 - 0.839
		6	0.933	0.049	0.892 - 0.975
		7	0.812	0.096	0.732 - 0.892
		8	0.795	0.051	0.752 - 0.837
		9	0.875	0.112	0.781 - 0.968
		10	0.836	0.087	0.763 - 0.909
		11	0.805	0.074	0.744 - 0.867
		12	0.694	0.114	0.603 - 0.794
		13	0.837	0.084	0.766 - 0.907
		14	0.877	0.078	0.812 - 0.943
25	25	0	0.925	0.074	0.862 - 0.987
26		1	0.872	0.054	0.826 - 0.917
27		2	0.946	0.063	0.894 - 0.999
28		3	0.957	0.062	0.906 - 1.009
29		4	0.865	0.082	0.796 - 0.933
30		5	0.900	0.044	0.864 - 0.937
31		6	0.887	0.064	0.834 - 0.941
31.5		7	0.912	0.079	0.846 - 0.978

cont'd

Water Temp. (^{o}C)	Water Velocity (cm·s^{-1})	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
32		8	0.932	0.083	0.862 – 1.002
32.5		9	0.982	0.169	0.841 – 1.123
33		10	0.901	0.110	0.809 – 0.993
33.5		11	0.901	0.090	0.826 – 0.976
34		12	0.998	0.113	0.903 – 1.092
34.5		13	0.924	0.062	0.873 – 0.976
25	12.5	0	0.919	0.055	0.873 – 0.964
	17.5	1	0.778	0.080	0.712 – 0.845
	17.5	2	0.848	0.183	0.695 – 1.002
	22.5	3	0.848	0.066	0.793 – 0.903
	22.5	4	0.737	0.144	0.617 – 0.857
	27.5	5	0.825	0.080	0.759 – 0.892
	27.5	6	0.820	0.127	0.715 – 0.926
	30	7	0.690	0.149	0.565 – 0.815
	30	8	0.606	0.150	0.480 – 0.732
	32.5	9	0.596	0.139	0.480 – 0.712
	32.5	10	0.674	0.145	0.554 – 0.795
	35	11	0.633	0.139	0.517 – 0.749
	35	12	0.598	0.130	0.490 – 0.707
	35	13	0.574	0.105	0.485 – 0.662
25	12.5	0	0.832	0.105	0.744 – 0.920
26	17.5	1	0.848	0.108	0.757 – 0.939
27	17.5	2	0.881	0.068	0.825 – 0.938
28	22.5	3	0.937	0.056	0.890 – 0.984
29	22.5	4	0.891	0.132	0.781 – 1.001
30	27.5	5	0.822	0.085	0.751 – 0.893
31	27.5	6	0.897	0.079	0.831 – 0.963
31.5	30	7	0.830	0.095	0.751 – 0.909
32	30	8	0.867	0.071	0.807 – 0.967
32.5	32.5	9	0.844	0.102	0.759 – 0.930
33	32.5	10	0.850	0.091	0.774 – 0.926
33.5	35	11	0.979	0.097	0.897 – 1.060
34	35	12	0.898	0.130	0.789 – 1.007
34.5	35	13	0.999	0.094	0.921 – 1.078
35	35	14	0.985	0.084	0.915 – 1.056

Internal Pressure (Pa·10^{5})

25	12.5	0	1.086	0.076	1.022 – 1.150
		1	1.109	0.073	1.048 – 1.170
		2	1.073	0.026	1.052 – 1.095
		3	1.056	0.039	1.023 – 1.088
		4	1.049	0.072	0.989 – 1.109
		5	1.049	0.026	1.028 – 1.071
		6	1.098	0.031	1.071 – 1.124

APPENDIX 6   cont'd

Water Temp. (°C)	Water Velocity (cm·s⁻¹)	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
25	12.5	7	1.035	0.033	1.007 – 1.062
		8	1.097	0.048	1.057 – 1.138
		9	1.121	0.056	1.074 – 1.168
		10	1.046	0.056	0.999 – 1.093
		11	1.063	0.039	1.030 – 1.095
		12	1.018	0.058	0.970 – 1.066
		13	1.134	0.183	0.981 – 1.288
		14	1.090	0.074	1.029 – 1.152
25	12.5	0	1.072	0.055	1.026 – 1.118
26		1	1.076	0.056	1.034 – 1.117
27		2	1.087	0.058	1.039 – 1.135
28		3	1.074	0.034	1.046 – 1.103
29		4	1.055	0.022	1.037 – 1.073
30		5	1.077	0.070	1.019 – 1.136
31		6	1.104	0.075	1.041 – 1.166
31.5		7	1.107	0.071	1.047 – 1.116
32		8	1.067	0.056	1.020 – 1.114
32.5		9	1.170	0.154	1.041 – 1.299
33		10	1.063	0.051	1.021 – 1.106
33.5		11	1.158	0.115	1.062 – 1.254
34		12	1.158	0.125	1.053 – 1.263
34.5		13	1.074	0.047	1.035 – 1.114
25	12.5	0	1.049	0.042	1.014 – 1.083
	17.5	1	1.063	0.053	1.019 – 1.107
	17.5	2	1.083	0.076	1.020 – 1.147
	22.5	3	1.087	0.050	1.045 – 1.129
	22.5	4	1.133	0.106	1.044 – 1.222
	27.5	5	1.029	0.043	0.993 – 1.065
	27.5	6	1.091	0.046	1.053 – 1.130
	30	7	1.082	0.048	1.042 – 1.122
	30	8	1.008	0.058	0.959 – 1.057
	32.5	9	1.191	0.024	1.021 – 1.361
	32.5	10	1.199	0.182	1.047 – 1.351
	35	11	1.126	0.120	1.025 – 1.226
	35	12	1.288	0.191	1.128 – 1.447
	35	13	1.043	0.082	0.975 – 1.112
25	12.5	0	1.034	0.050	0.992 – 1.075
26	17.5	1	1.061	0.038	1.029 – 1.093
27	17.5	2	1.040	0.050	0.998 – 1.082
28	22.5	3	1.055	0.064	1.002 – 1.108
29	22.5	4	1.108	0.054	1.063 – 1.153
30	27.5	5	1.043	0.027	1.020 – 1.066
31	27.5	6	1.086	0.083	1.016 – 1.156
31.5	30	7	1.025	0.036	0.995 – 1.056
32	30	8	1.046	0.031	1.020 – 1.073
32.5	32.5	9	1.106	0.055	1.061 – 1.152

APPENDIX 6 cont'd

Water Temp. (^{O}C)	Water Velocity ($cm \cdot s^{-1}$)	Day of Test	Mean (n=8)	Standard Deviation	95% Confidence Interval For Mean
33	32.5	10	1.116	0.087	1.043 – 1.188
33.5	35	11	1.163	0.075	1.101 – 1.226
34	35	12	1.123	0.094	1.044 – 1.201
34.5	35	13	1.172	0.092	1.095 – 1.249
35	35	14	1.050	0.051	1.008 – 1.093

APPENDIX 6 cont'd

REGRESSION ANALYSES TABLES: (See Note, page 14)

Time (d) in a Constant Water Temperature and Velocity vs. Buoyancy $(mL \cdot g^{-1})$.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	0.8269	0.0070		
Regression	1	0.0845	0.0845	13.42*	3.92
Residual	118	0.7424	0.0063		
Lack of Fit	13	0.2485	0.0191	4.07*	1.84
Pure Error	105	0.4937	0.0047		

Regression Equation: $y = 0.8269 - 0.0061 \, x$, $r^2 = 0.102$

Time (d) in a Constant Water Temperature and Velocity vs. Standard Volume $(mL \cdot g^{-1})$.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	1.2777	0.0105		
Regression	1	0.0923	0.0923	9.42*	3.92
Residual	118	1.1554	0.0098		
Lack of Fit	13	0.4205	0.0324	4.62*	1.84
Pure Error	105	0.7350	0.0070		

Regression Equation: $y = 0.8874 - 0.0064 \, x$, $r^2 = 0.074$

Time (d) in a Constant Water Temperature and Velocity vs. Internal Pressure $(Pa \cdot 10^5)$.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	0.6431	0.0054		
Regression	1	0.0001	0.0001	0.02	3.92
Residual	118	0.6430	0.0054		
Lack of Fit	13	0.1260	0.0097	1.97*	1.84
Pure Error	105	0.5170	0.0049		

Regression Equation: $y = 1.0733 + 0.0002 \, x$, $r^2 = 0.0002$

Time (d) in Increasing Water Temperature and Constant Velocity vs. Buoyancy (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	0.5127	0.0046		
Regression	1	0.0011	0.0011	0.23	3.94
Residual	110	0.5116	0.0046		
Lack of Fit	12	0.0980	0.0082	1.93	2.30
Pure Error	98	0.4136	0.0042		

Regression Equation: $y = 0.8470 - 0.0008 x$, $r^2 = 0.002$

Time (d) in Increasing Water Temperature and Constant Velocity vs. Standard Volume (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	0.9095	0.0082		
Regression	1	0.0145	0.0145	1.78	3.94
Residual	110	0.8950	0.0081		
Lack of Fit	12	0.1435	0.0120	1.56	2.30
Pure Error	98	0.7515	0.0077		

Regression Equation: $y = 0.9033 + 0.0028$, $r^2 = 0.016$

Time (d) in Increasing Water Temperature and Constant Velocity vs. Internal Pressure (Pa·10^5).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	0.7614	0.0069		
Regression	1	0.0368	0.0368	5.59*	3.94
Residual	110	0.7246	0.0066		
Lack of Fit	12	0.1178	0.0098	1.59	2.30
Pure Error	98	0.6068	0.0062		

Regression Equation: $y = 1.0666 + 0.0045 x$, $r^2 = 0.048$

APPENDIX 6 cont'd

Time (d) in a Constant Water Temperature and Increasing Velocity vs. Buoyancy (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	2.9190	0.0263		
Regression	1	1.2665	1.2665	84.30*	3.94
Residual	110	1.6526	0.0150		
Lack of Fit	12	0.3434	0.0286	2.14	2.30
Pure Error	98	1.3091	0.0134		

Regression Equation: $y = 0.8351 - 0.0264\ x$, $r^2 = 0.434$

Time (d) in a Constant Water Temperature and Increasing Velocity vs. Standard Volume (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	2.9373	0.0265		
Regression	1	1.0873	1.0873	64.65*	3.94
Residual	110	1.8500	0.0168		
Lack of Fit	12	0.9206	0.0242	1.52	2.30
Pure Error	98	1.5594	0.0159		

Regression Equation: $y = 0.8837 - 0.0244\ x$, $r^2 = 0.370$

Time (d) in a Constant Water Temperature and Increasing Velocity vs. Internal Pressure (Pa·10^5).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	111	1.7716	0.0160		
Regression	1	0.1256	0.1256	8.39*	3.94
Residual	110	1.6460	0.0150		
Lack of Fit	12	0.4849	0.0404	3.41*	2.30
Pure Error	98	1.1611	0.0118		

Regression Equation: $y = 1.0512 - 0.0082\ x$, $r^2 = 0.071$

Time (d) in Increasing Water Temperature and Velocity vs.
Buoyancy (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	0.9478	0.0080		
Regression	1	0.0137	0.0137	1.73	3.92
Residual	118	0.9341	0.0079		
Lack of Fit	13	0.2339	0.0180	2.70**	1.84
Pure Error	105	0.7002	0.0067		

Regression Equation: $y = 0.8067 + 0.0025\ x$, $r^2 = 0.014$

Time (d) in Increasing Water Temperature and Velocity vs.
Standard Volume (mL·g^{-1}).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	1.3662	0.0115		
Regression	1	0.1298	0.1298	12.39*	3.92
Residual	118	1.2364	0.0105		
Lack of Fit	13	0.2519	0.0194	2.07*	1.84
Pure Error	105	0.9845	0.0094		

Regression Equation: $y = 0.8516 + 0.0076\ x$, $r^2 = 0.095$

Time (d) in Increasing Water Temperature and Velocity vs.
Internal Pressure (Pa·10^5).

Source	df	ss	mss	F.Calc.	F.Crit.
Total	119	0.6528	0.0055		
Regression	1	0.0878	0.0878	18.35*	3.92
Residual	118	0.5649	0.0048		
Lack of Fit	13	0.1390	0.0107	2.63*	1.84
Pure Error	105	0.4260	0.0041		

Regression Equation: $y = 1.0553 + 0.0063\ x$, $r^2 = 0.135$

Mean Internal Pressure (Ip; Pa·10^5) vs. Variance in Ip with
Water Temperature and Velocity Constant.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	14	5 x 10^{-5}	0		
Regression	1	0	0	0.76	4.68
Residual	13	5 x 10^{-5}	0	**	

Regression Equation: y= -0.0111 + 0.013 x   , r^2 = 0.056

Mean Internal Pressure (Pa·10^5) vs. Variance in Ip with Increasing
Water Temperature at a Constant Water Velocity.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	13	0.0006	4 x 10^{-5}		
Regression	1	0.0005	0.0005	89.44*	4.75
Residual	12	0.0001	1 x 10^{-5}		
Lack of Fit	10	0.0001	1 x 10^{-5}	3.87	1.94
Pure Error	2	0.0001	0		

Regression Equation: y= -0.1723 + 0.1629 x   , r^2 = 0.882

Mean Internal Pressure (Pa·10^5) vs. Variance in Ip with Increasing
Water Velocity at a Constant Temperature.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	14	0.0027	0.0002		
Regression	1	0.0022	0.0022	48.26*	4.68
Residual	13	0.0006	4 x 10^{-5}	**	

Regression Equation: y= -0.1726 + 0.1667 x   , r^2 = 0.788

Mean Internal Pressure (Pa·10^5) vs. Variance in Ip with Increasing
Temperature and Water Velocity.

Source	df	ss	mss	F.Calc.	F.Crit.
Total	14	0.0001	1 x 10^{-5}		
Regression	1	0.0001	7 x 10^{-5}	19.76*	4.68
Residual	13	4 x 10^{-5}	0	**	

Regression Equation: y= -0.0456 + 0.0458 x   , r^2 = 0.603
** No repeats lack of fit tests not possible.