THE UNIVERSITY OF MANITOBA

SEASONAL FACTORS AFFECTING BUOYANCY ATTAINED IN STILL WATER AND IN CURRENT BY FATHEAD MINNOWS, *PIMEPHALES PROMELAS*

BY

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ABSTRACT

This study investigated the effects and biological significance of various seasonal factors on maximum (in still water) and minimum (in current) buoyancy attained by fathead minnows, *Pimephales promelas*.

Fish were always less buoyant in current than in still water. Maximum and especially minimum buoyancy varied seasonally depending upon the effects of various seasonal factors, such as maximum tolerable water velocity, water temperature, photoperiod and condition of the fish. Buoyancy also varied seasonally independent of variation in water velocity and temperature. There was no significant effect of time of year on maximum or minimum buoyancy attained by fish held under constant environmental conditions.

To further explain these seasonal differences, the effects of photoperiod, sex, sexual development, fat content and condition of the fish on buoyancy were determined. Small and large fish showed different buoyancy responses in current at various spring photoperiods after simulated winter conditions and small fish were always less able to reduce buoyancy than large fish at 15°C. Also, length of exposure (1 or 7 days) to a particular spring photoperiod had no significant effect on buoyancy. Long-term (3 weeks) exposure to various constant photoperiods and changing photoperiod, as well as direction of change affected buoyancy in still water and in current at 15°C. No consistent trend in buoyancy response was observed when the effect of photoperiod was tested in isolation from other factors.

There was no significant difference in buoyancy attained between female and male fish in either still water or current. However, buoyancy decreased significantly especially in current as sexual development increased.

Buoyancy increased significantly in still water and in current with days of starvation. Also, as coefficient of condition decreased in starved fish, buoyancy increased significantly in current but only slightly in still water. Fat content, determined by a densitometric method, had no significant effect on buoyancy.

Seasonal changes in buoyancy were related to water velocity, water temperature, photoperiod, size, age, sexual development and condition of the fish. These factors interact to influence buoyancy.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

INTRODUCTION

The ability to reduce swimbladder volume and thus buoyancy as water velocity increases and to increase swimbladder volume to attain near neutral buoyancy when still water is encountered is a common adaptation amongst North American stream-dwelling fish (Gee et al. 1974). A variable buoyancy enables fish to successfully occupy a lotic environment, where water velocities vary considerably in time and space. The appropriate buoyancy, with respect to water velocity, permits efficient movement in still water and maintenance of position in faster waters with minimum expenditure of energy (Saunders 1965; Gee et al. 1974; Gee and Gee 1976; Berezay and Gee 1978).

Buoyancy is affected by the size of fish (Gee 1968, 1972, 1977; Machniak and Gee 1975; Berezay and Gee 1978), water velocity (Neave et al. 1966; Gee 1977), water temperature (Pinder and Eales 1969; Gee 1977) and by an interaction between water velocity and temperature (Berezay and Gee 1978). These factors interact to influence buoyancy. Other factors affecting buoyancy, especially those related to season, remain largely unknown. Neave et al. (1966) and Pinder and Eales (1969) found seasonal variations in buoyancy in still water and in current, which were related to size of fish and water temperature, in juvenile Atlantic salmon (Salmo salar).

The greatest demand on temperate stream fish to hold position is during spring runoff, when velocities are greatest and waters are cold. Yet, the extent of reduction in buoyancy in current by fathead minnows (Pimephales promelas) is minimal at cold temperatures (Gee 1977). Gee (1977) suggested that other variables related to seasonal change, such as photoperiod or increasing water temperature could stimulate a greater decrease in buoyancy at cold temperature ranges.

The purpose of this study was to assess effects of season and its dependent variables on maximum and minimum buoyancy attained by fathead minnows. Primary objectives were to determine if buoyancy varies (1) seasonally with water velocity, water temperature, photoperiod and possibly other related factors, (2) seasonally but independent of variation in water velocity and temperature and (3) seasonally but independent of variation in direct environmental cues. Secondary objectives were to determine effects of photoperiod, sex, sexual development, fat content and condition of the fish on buoyancy, to further explain any seasonal differences found.

MATERIALS AND METHODS

Fathead minnows were collected periodically from Crystal Creek, an intermittent stream in Manitoba. Environmental variables including photoperiod, air temperature, water temperature, salinity, conductivity, oxygen, pH and Secchi disc transparency were measured for each time of collection (Appendix 1). Fish were transported to the laboratory in Crystal Creek water, in styrofoam coolers with plexiglass windows in the top, to maintain field photoperiod and water temperature. Fish were fed Tetramin flakes or Trout Starter (No. 3) once a day, except 24 h prior to buoyancy measurements.

To determine maximum and minimum buoyancy, fish were held in either still water or current, respectively, for approximately 24 h (Gee 1977). Current was created in an aquarium (90 x 44 x 44 cm) using the design of Gee and Bartnik (1969). Water depth was about 6 cm and no substrate was present. Maximum water velocity was set such that all fish could hold position without resting against the back of the stream tank. The mean velocity in any vertical velocity curve occurs at about six-tenths of the depth (Grover and Harrington 1966). Therefore, water velocity was recorded by averaging six measurements from different locations taken 2.5 cm from the bottom with an Ott current meter (Type C1). Still water conditions were created in an aquarium (90 x 44 x 44 cm) using only a gently bubbling airstone. Water dechlorinated by the charcoal method was continuously exchanged in all aquaria to prevent build-up of wastes.

To measure buoyancy, fish were dip-netted from either still water or current, anesthetized with MS-222 (ethyl m-aminobenzoate methanesulfonate) and swimbladder volume and weight of gas-free fish in water were measured using the procedure of Gee (1970). Buoyancy was expressed by dividing the swimbladder volume ($\pm$ 0.001 mL) by the weight ($\pm$ 0.001 g) of the gas-free fish in water, where 1.0 $mL \cdot g^{-1}$ is neutral buoyancy. The difference between the weight (g) of the fish in water with its swimbladder inflated and the weight (g) of the gas-free fish in water equals swimbladder volume (mL), because at a given depth 1 mL voume of gas supports 1 g of fish tissue, assuming that the specific gravity of water equals 1.0. No correction to swimbladder volume was made for depth of capture because the hydrostatic pressures resulting from depths in the aquarium (maximum 40 cm) were negligible in buoyancy measurements (Gee et al. 1974). The temperatures of the anesthetic solution and water bath in which fish were weighed were similar to the one in which fish were held and tested. Buoyancy measurements were made during mid-day in all experiments.

Seasonal factors

To determine if maximum (in still water) and minimum (in current) buoyancy varies (A) seasonally and (B) seasonally but independent of variation in water velocity and temperature, fish were collected from the field, divided into two groups and each group was held and tested under the appropriate conditions (Table 1). Fish were collected approximately once a month from June 1976 to May 1978 when possible, since during

Table 1. Plan of experiment to determine if maximum and minimum buoyancy varies (A) seasonally, (B) seasonally but independent of variation in water velocity and temperature and (C) seasonally but independent of variation in direct environmental cues.

Group	A	B	C
Source of fish	Collected from Crystal Creek, once a month when possible from June 1976 to May 1978.		Collected from Crystal Creek in May 1976.
Laboratory holding conditions	Held at field photoperiod and water temperature for 6 days prior to testing.	Held at field photoperiod and acclimated to 15^{o}C for 7 days prior to testing.	Held at a photoperiod of 12 h and 5^{o}C. Fish were acclimated to 15^{o}C for 8 days prior to testing.
Testing conditions	Tested at field photoperiod and water temperature. One batch in still water and one in maximum water velocity tolerated by all fish (20-53 cm·s^{-1} for 24 h.	Tested at field photoperiod and 15^{o}C. One batch in still water and one in a velocity of 35 ($\pm$ 2) cm·s^{-1} for 24 h.	Tested at a photoperiod of 12 h and 15^{o}C. One batch in still water and one in a velocity of 35 ($\pm$ 2) cm·s^{-1} for 24 h.
Duration of experiment (months)	24	24	16

winter fish populations were subjected to partial winter kill. Group A fish were held in the laboratory for 6 days before testing buoyancy, as this was the minimum time found for fish to recover from the stress of capture and transportation (Appendix 2). The lowest water temperature which could be obtained in the laboratory was $5^{\circ}C$ and therefore, fish collected from temperatures less than this were held and tested at $5^{\circ}C$. Fish were acclimated to desired temperatures by allowing at least 1 day for every $1.5^{\circ}C$ change. Temperatures were controlled to within $\pm\ 0.5^{\circ}C$. Photoperiod was regulated by 60 watt incandescent bulbs on 24 h time clocks. To determine if maximum and minimum buoyancy varies (C) seasonally but independent of variation in direct environmental cues, fish were collected in May 1976 and acclimated to laboratory conditions for 2 months before the start of this experiment (Table 1). Group C fish were held at a temperature of $5^{\circ}C$ to slow down growth of the fish and then acclimated to $15^{\circ}C$ for 8 days before testing buoyancy in order to compare results with group B. Batches of fish from group C were tested at times of the year similar to those of groups A and B. In each group, buoyancy measurements were made on eight fish from still water and eight fish from current. Fish tested were of similar size (4.3 - 5.7 cm, fork length) and both sexes were used at random.

In addition to buoyancy measurements, weight in air, fork length, sex and the coefficient of condition (K) were determined. K was calculated according to the method of Hile (1936), where K = weight (g) $\div$ length^3 (cm) x 100.

Variation in either maximum or minimum buoyancy by group A fish would reflect combined effects of various seasonal factors such as water velocity, water temperature, photoperiod and possibly others. Variation by group B fish would reflect effects of field photoperiod and possibly other related factors. Group C fish would reflect effect of time of year, independent of variation in direct environmental cues, suggesting an endogenous influence on buoyancy.

Various seasonal factors, such as photoperiod, sexual development, fat content and condition of the fish were then further studied to determine their influence on seasonal variations in buoyancy found in groups A and B. In all following experiments photoperiod was regulated by 40 watt incandescent bulbs on 24 h time clocks.

Photoperiod

Size of fish and length of exposure to various spring photoperiods. To determine if the ability to reduce buoyancy in current following spring break-up was affected by photoperiod, fish collected in June 1976 were held in simulated winter conditions of darkness at $5^{\circ}C$ for 5 months from December 1976 to April 1977. Two size groups of fish (3.3 - 4.5 and 5.5 - 6.8 cm) were then acclimated to $15^{\circ}C$ for 7 days and exposed to photoperiods of either 8.5, 10.5, 12.5 or 14.5 h for either 1 or 7 days of the acclimation period. In each treatment, buoyancy was measured on eight fish after 24 h in current (35 ± 2 $cm \cdot s^{-1}$) at $15^{\circ}C$.

For the rest of the experiments, fathead minnows were collected in October or November 1977 and held at 5 or 11°C and at a 12 h photoperiod.

Long-term exposure to various photoperiods. To determine effects of long-term exposure to various photoperiods on maximum and minimum buoyancy, groups of fish (4.5 - 5.7 cm) were acclimated in December 1977 to 15°C and photoperiods of either 9, 10.5, 12, 13.5, 15 or 16.5 h for 3 weeks. At each photoperiod eight fish were held in still water and eight fish in current (35 ± 2 $cm \cdot s^{-1}$) at 15°C for 24 h before measuring buoyancy.

Decreasing and increasing length of photoperiod. To determine effects of decreasing and increasing photoperiods on maximum and minimum buoyancy, fish (4.5 - 6.0 cm) were divided into four groups and exposed from February to April 1978 to either simulated summer, autumn, winter or spring photoperiod regimes. Photoperiod was decreased or increased at a rate of 30 min per week, similar to nature. Each group was acclimated to 15°C and to the photoperiod regime which is encountered in nature prior to the photoperiod regime tested, for 3 weeks. Photoperiod was then either decreased or increased over 8 weeks and buoyancy was measured in each group every 2 weeks (Table 2). At each photoperiod tested, eight fish were held in still water and eight fish in current (35 ± 2 $cm \cdot s^{-1}$) at 15°C for 24 h before measuring buoyancy.

Table 2. Plan of experiment to determine if maximum and minimum buoyancy varies with decreasing and increasing photoperiods. Photoperiod used during acclimation and testing period and photoperiods at which buoyancy was measured for each group are given.

	Photoperiod (h)		
Group	Acclimation period (3 wk)	Testing period (8 wk)	Buoyancy measured biweekly at:
I (summer)	Constant at 16.5[a]	Decreased from 16.5 to 12.5	16.5, 15.5, 14.5, 13.5, 12.5
II (autumn)	Decreased from 14 to 12.5	Decreased from 12.5 to 8.5	12.5, 11.5, 10.5, 9.5, 8.5
III (winter)	Constant at 8.5[a]	Increased from 8.5 to 12.5	8.5, 9.5, 10.5, 11.5, 12.5
IV (spring)	Increased from 11 to 12.5	Increased from 12.5 to 16.5	12.5, 13.5, 14.5, 15.5, 16.5

[a] Groups were acclimated to constant photoperiods because during these times in nature photoperiod remains relatively constant for about 3 weeks.

Sex and sexual development

To determine effects of sex and sexual development on maximum and minimum buoyancy, adult fish (5.0 - 7.0 cm) were set up in four aquaria (90 x 44 x 44 cm) at a density of about 45 fish per tank and gradually acclimated to 21°C and to a photoperiod of 16 h over 5 weeks. Each aquarium contained a gently bubbling airstone, an opaque cover to decrease light intensity from a 40 watt overhead light source and spawning tiles of PVC piping and broken clay flower pots. Approximately once a month for 4 months, from March to June 1978, a batch of about 10 female and 10 male fish were held in still water and another batch in current (40 ± 2 cm$\cdot$s^{-1}) at 21°C for 24 h before measuring buoyancy. Fish were then drip-dried for 1 h to obtain total body weight and then gonads were removed and weighed. The weight of the gonads divided by the total body weight was used as an index of gonad development. The largest ratio of gonad:body-weight for each sex was assumed to be 100% sexual development. Sexual development was then calculated for each sex by the formula:

$$\%\ \text{sexual development} = \frac{\text{gonad:body-weight ratio}}{\text{largest ratio of gonad:body-weight}} \times 100$$

Condition and fat content

The effect of condition on maximum and minimum buoyancy was examined in fish (5.2 - 6.7 cm) acclimated to 15°C, a photoperiod of 12 h and fed daily for 4 weeks. On day 0 feeding ceased and all debris was removed from the aquarium. Buoyancy was measured after 0, 4, 8, 12, 16, 20, 24, 36 and 60 days of starvation during May and June 1978. Two batches of eight fish were taken at the above times and one was held in still water and the other in current (35 ± 2 cm$\cdot$s^{-1}) at 15^{o}C for 24 h before measuring buoyancy. The coefficient of condition (K), used to describe the degree of starvation, was determined and related to variation in buoyancy.

The effect of fat content on maximum and minimum buoyancy was determined in these starved fish, using the indirect method of Horak (1966) to find fat content.

$$\% \text{ fat} = 100 \times \left[\frac{Df}{Dff - Df}\right] \times \left[\frac{Dff}{\text{Specific Gravity}} - 1\right]$$ where Df is the density of body fat (0.9348), Dff is the density of the fat-free body (1.1000) and specific gravity is determined by $\frac{Wa \times K}{Wa - Ww}$ where Wa is the body weight of the fish in air, Ww is the weight in water and K is the density of water containing the fish at 15^{o}C (0.99913, from a density table).

Statistical analysis

Statistical analysis of data was done on an IBM/370 computer using APL statistical library program 5796-PHW.

RESULTS

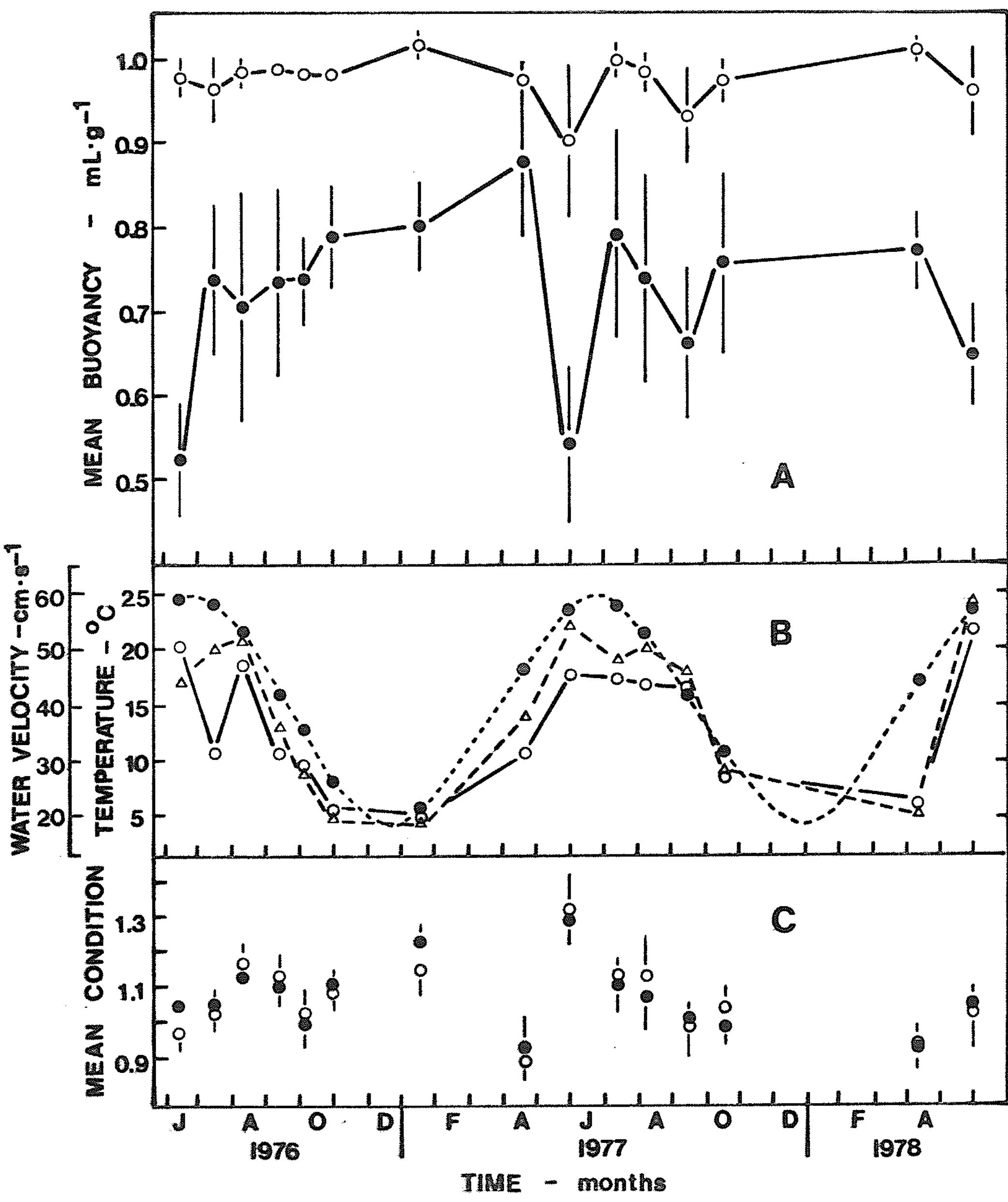
Seasonal factors

Group A fish. Both maximum (in still water) and minimum (in current) buoyancy varied significantly ($p < 0.05$, one-way analyses of variance; Appendix 3) with time of year (Fig. 1A). The pattern of variation also differed between the 2 years of the study. Fish were close to neutral buoyancy in still water, varying from a mean of 0.900 to 1.015 $mL \cdot g^{-1}$, with the lowest buoyancy values occurring in breeding fish and the highest values in the coldest waters. In current, fish were negatively buoyant, varying from a mean of 0.523 to 0.877 $mL \cdot g^{-1}$, with the lowest buoyancy values in May or June of each year. This coincides with the potential for high tolerable water velocity and high water temperature, a long photoperiod and the start of the breeding season. Fish tested in April of each year during spring run-off showed a minimal ability to reduce buoyancy in current, especially in 1977, when fish were parasitized (Fig. 1A).

The maximum tolerable water velocity, water temperature and photoperiod in which fish were tested varied throughout the year (Fig. 1B) (Appendix 4). The coefficient of condition varied seasonally with the lowest values occurring in the spring and the highest value in May 1977 in breeding fish (Fig. 1C).

The single linear regressions of buoyancy attained in still water on water temperature, photoperiod and coefficient of conditon were significant ($p < 0.05$, single linear

Figure 1. (A) Mean buoyancy ($n = 8$) attained by group A fish over 2 years of study in still water (open circles) and in current (closed circles). Vertical lines represent 95% confidence limits on the mean (those < 0.014 are not shown). (B) Maximum tolerable water velocity (o——o), water temperature (△---△) and photoperiod (●----●) at which fish were tested. (C) Mean coefficient of condition ($n = 8$) for fish tested in still water (open circles) and in current (closed circles). Only one side of the 95% confidence limits on the mean is shown (those < 0.030 are not shown).


MEAN BUOYANCY - mL·g⁻¹
1.0
0.9
0.8
0.7
0.6
0.5
A
WATER VELOCITY -cm·s⁻¹
60
50
40
30
20
TEMPERATURE - °C
25
20
15
10
5
B
PHOTOPERIOD - h
17
15
13
11
9
MEAN CONDITION
1.3
1.1
0.9
C
J
A
O
D
F
A
J
A
O
D
F
A
1976
1977
1978
TIME - months

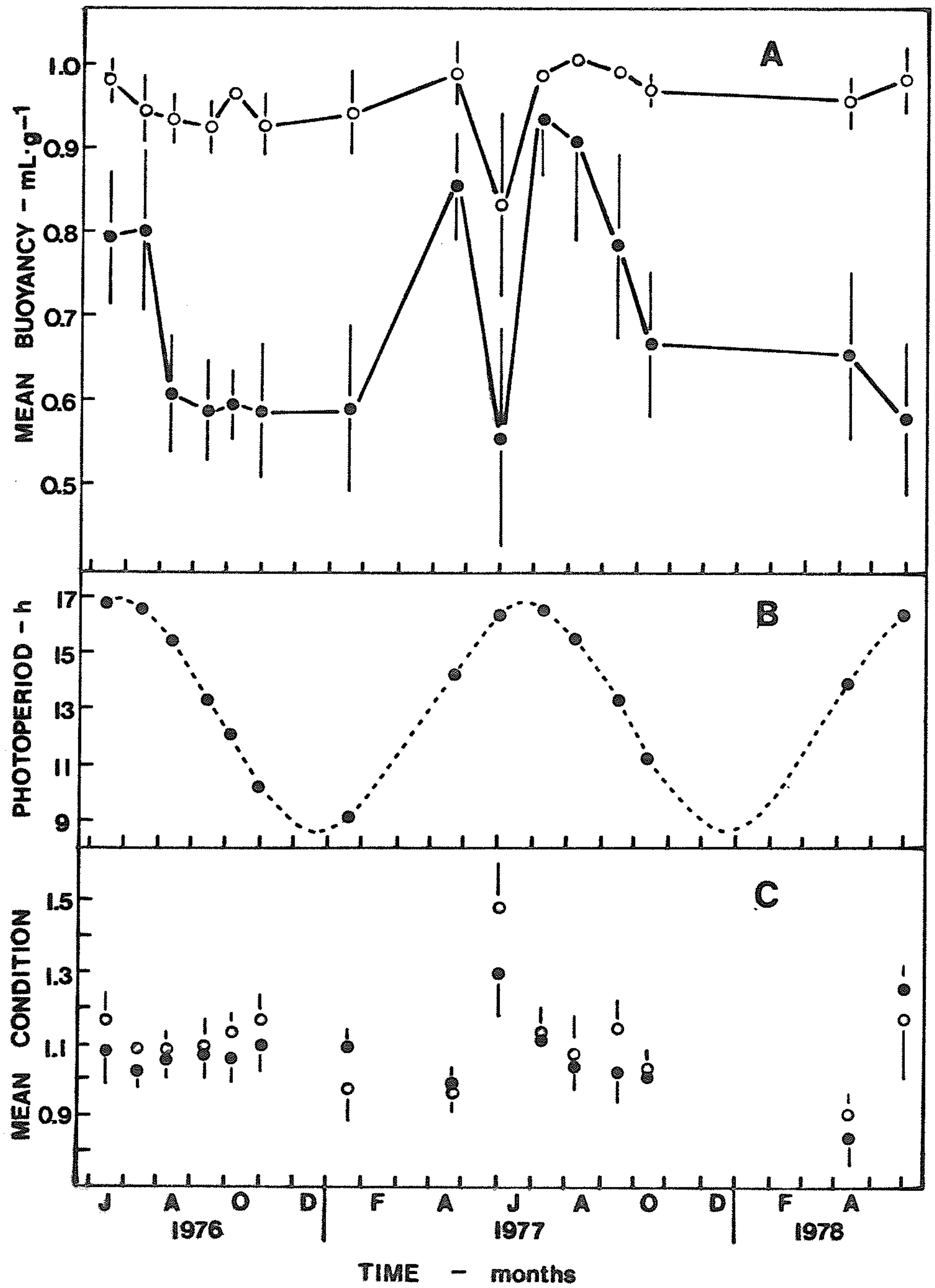
regression analyses; Appendix 4) in group A fish. In current, the regressions of buoyancy on water velocity, water temperature, photoperiod and coefficient of condition were also significant ($p < 0.05$, single linear regression analyses; Appendix 4). The slope of the regression line and the percentage of the total variation that is explained by the regression (r^2) for each factor are as follows:

	Still water		Current	
Factor	Slope	r^2 (%)	Slope	r^2 (%)
Water velocity	-	-	-0.0057	20.0
Water temperature	-0.0025	10.3	-0.0076	12.2
Photoperiod	-0.0041	4.2	-0.0187	10.9
Condition	-0.0787	4.5	-0.2470	4.2

Group B fish. Independent of variation in water velocity and temperature, both maximum (in still water) and minimum (in current) buoyancy varied significantly ($p < 0.05$, one-way analyses of variance; Appendix 5) with time of year (Fig. 2A). In still water, fish were close to neutral buoyancy varying from a mean of 0.833 to 1.006 $mL \cdot g^{-1}$ and in current, negative buoyancy varied from a mean of 0.556 to 0.933 $mL \cdot g^{-1}$. The ability to reduce buoyancy in current was minimal in midsummer of each year and when fish were parasitized in April 1977 (Fig. 2A). Photoperiod and coefficient of condition were the factors varying over time for group B fish (Fig. 2B and C). The pattern of variation also differed between group A and B fish (Fig. 1A and 2A).

The single linear regression of buoyancy attained in

Figure 2. (A) Mean buoyancy ($n = 8$) attained by group B fish over 2 years of study in still water and in current ($35\ cm \cdot s^{-1}$) at $15^{o}C$. (B) Photoperiod at which fish were tested. (C) Mean coefficient of condition for fish in still water and in current. Notation as in Fig. 1.

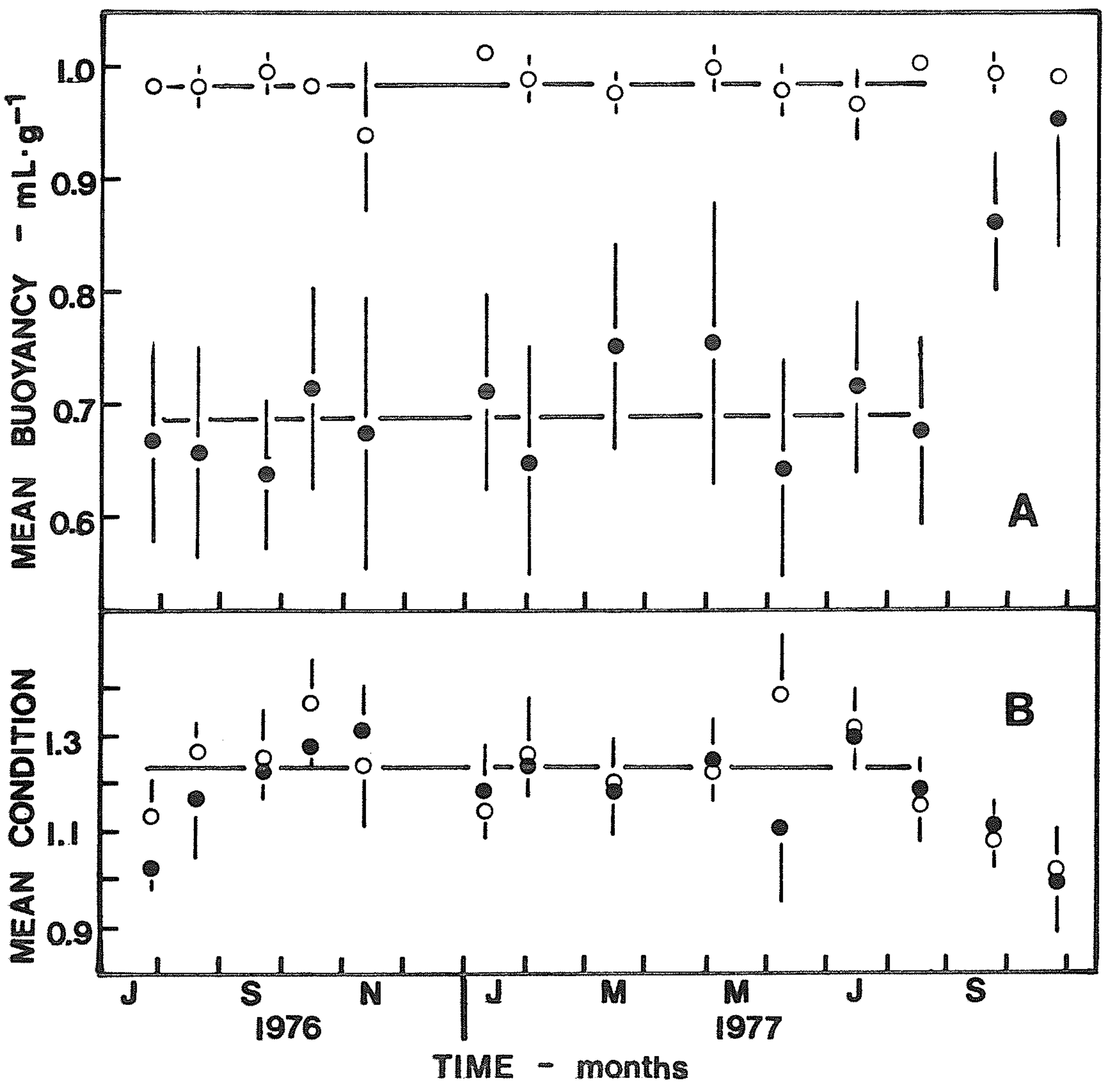

A
MEAN BUOYANCY - mL·g⁻¹
1.0
0.9
0.8
0.7
0.6
0.5
B
PHOTOPERIOD - h
17
15
13
11
9
C
MEAN CONDITION
1.5
1.3
1.1
0.9
J
A
O
D
F
A
J
A
O
D
F
A
1976
1977
1978
TIME - months

still water on coefficient of condition was significant ($p < 0.05$, single linear regression analysis; Appendix 6), but photoperiod was not significant ($p > 0.05$, single linear regression analysis; Appendix 6) in group B fish. In current, the regressions of buoyancy on photoperiod and coefficient of condition were significant ($p < 0.05$, single linear regression analyses; Appendix 6). The slope of the regression line and the percentage of the total variation that is explained by the regression (r^2) for each factor are as follows:

	Still water		Current	
Factor	Slope	r^2 (%)	Slope	r^2 (%)
Photoperiod	Not significant		0.0225	11.2
Condition	-0.1099	8.3	-0.3619	8.2

<u>Group C fish</u>. For fish held under constant laboratory conditions of a 12 h photoperiod and 15°C, the effect of time of year on maximum (in still water) and minimum (in current) buoyancy was not significant ($p > 0.05$, two-way analysis of variance; Appendix 7) over the first 14 months (Fig. 3A). There was a significant difference ($p < 0.05$, two-way analysis of variance; Appendix 7) between buoyancy in still water and in current. In still water, fish were close to neutral buoyancy with an overall mean of 0.981 $mL \cdot g^{-1}$ and in current the overall mean negative buoyancy was 0.686 $mL \cdot g^{-1}$ (Fig. 3A). Interaction between water velocity and time was not significant ($p > 0.05$, two-way analysis of variance; Appendix 7) and thus extent of buoyancy adjustment was similar at all times over the first 14 months. The extent of buoyancy

Figure 3. Effect of constant environmental conditions on mean buoyancy (n = 8) attained by group C fish in still water and in current ($35\ cm \cdot s^{-1}$) at $15^{o}C$. Horizontal lines represent mean buoyancies from July 1976 to August 1977 in still water and in current. (B) Mean coefficient of condition for fish in still water and in current. Horizontal line represents mean condition for all fish from July 1976 to August 1977. Notation as in Fig. 1.



1.0
0.9
0.8
0.7
0.6
MEAN BUOYANCY - mL·g⁻¹
A
B
1.3
1.1
0.9
MEAN CONDITION
J
S
N
J
M
M
J
S
1976
1977
TIME - months

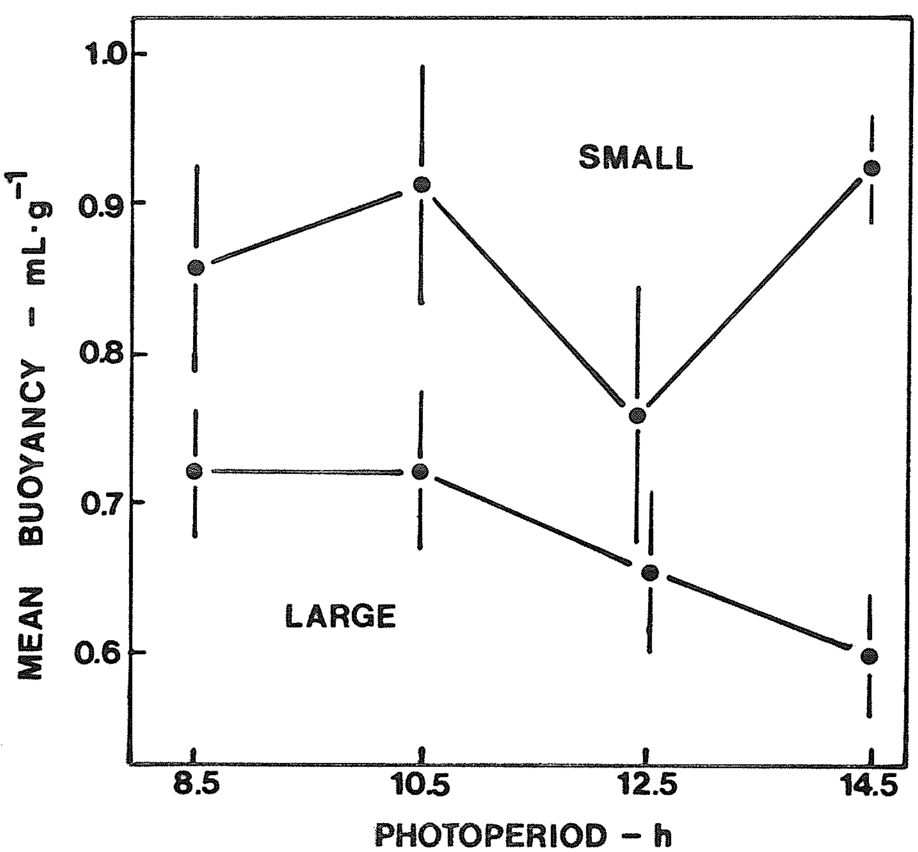
adjustment is the difference in mean buoyancy between still water and current.

The coefficient of condition increased several months after the fish were brought into the laboratory and then remained relatively constant over the first 14 months, with an overall mean of 1.229 (Fig. 3B). Fish tested in September and October 1977 showed a decrease in coefficient of condition and mean buoyancy attained in current increased to almost neutral (Fig. 3A). Group C fish gradually died from September to November 1977.

Photoperiod

Size of fish and length of exposure to various spring photoperiods. The effect of length of exposure to photoperiod (1 or 7 days), after simulated winter darkness on buoyancy attained in current was not significant ($p > 0.05$, two-way analyses of variance; Appendix 8) in either small or large fish. Data for 1 and 7 day exposures were then grouped in both small and large fish and re-analyzed. Size of fish and photoperiod each had a significant effect ($p < 0.05$, two-way analysis of variance; Appendix 8) on buoyancy attained in current (Fig. 4). Interaction between the effects of size and photoperiod was significant ($p < 0.05$, two-way analysis of variance; Appendix 8), indicating that they are dependent effects. Large fish attained the lowest mean buoyancy (0.601 $mL \cdot g^{-1}$) in current at a photoperiod of 14.5 h and small fish attained it (0.760 $mL \cdot g^{-1}$) at a photoperiod of 12.5 h (Fig. 4).

Figure 4. Effect of various spring photoperiods on mean buoyancy (n = 16; except for small fish at 8.5 h n = 12, 10.5 h n = 13, large fish at 10.5 h n = 14) in current (35 $cm \cdot s^{-1}$) at 15°C by small and large fish, with 95% confidence limits on the mean.



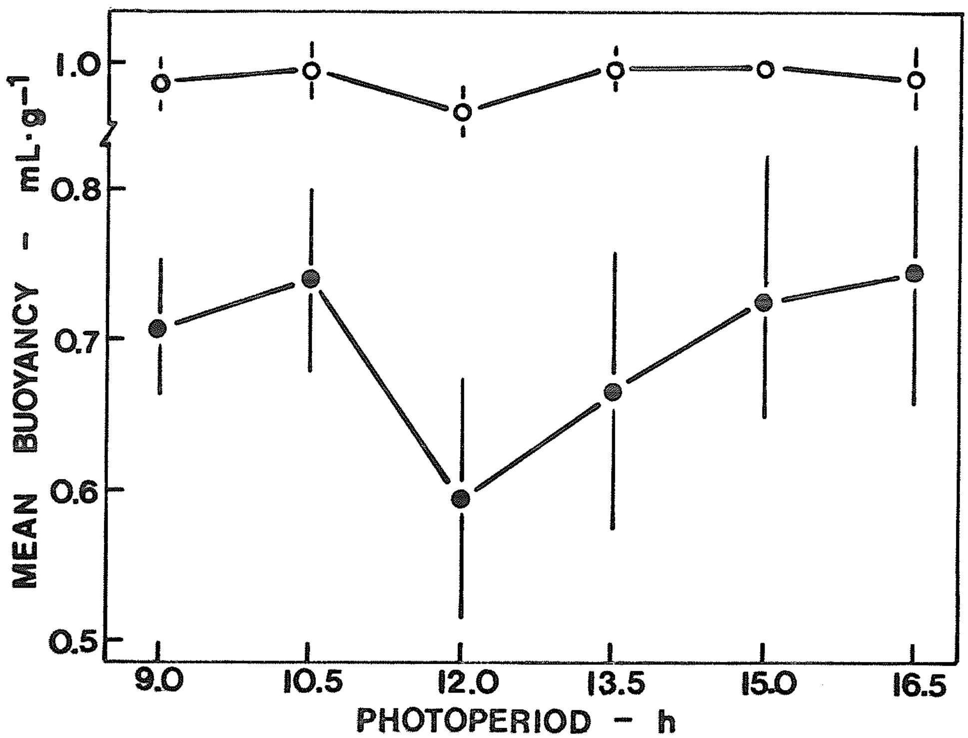
MEAN BUOYANCY - mL·g^-1
1.0
0.9
0.8
0.7
0.6
SMALL
LARGE
8.5
10.5
12.5
14.5
PHOTOPERIOD - h

Long-term exposure to various photoperiods. The effects of water velocity (still or current) and long-term exposure to photoperiod on buoyancy attained by medium sized fish were significant ($p < 0.05$, two-way analysis of variance; Appendix 9). The lowest mean buoyancy in still water ($0.968\ mL \cdot g^{-1}$) and in current ($0.596\ mL \cdot g^{-1}$) was attained at a photoperiod of 12 h (Fig. 5). Interaction between the effects of water velocity and photoperiod was not significant ($p > 0.05$, two-way analysis of variance; Appendix 9) and thus extent of buoyancy adjustment was similar at all photoperiods.

Decreasing and increasing length of photoperiod. The effects of water velocity (still or current), decreasing photoperiod and their interaction on buoyancy were significant ($p < 0.05$, two-way analysis of variance; Appendix 10) (Fig. 6 I and II). The significant interaction indicated that water velocity and decreasing photoperiod were dependent effects and thus extent of buoyancy adjustment varied with photoperiod. A very low buoyancy in current occurred with a decreasing photoperiod at 13.5 and 9.5 h (Fig. 6 I and II).

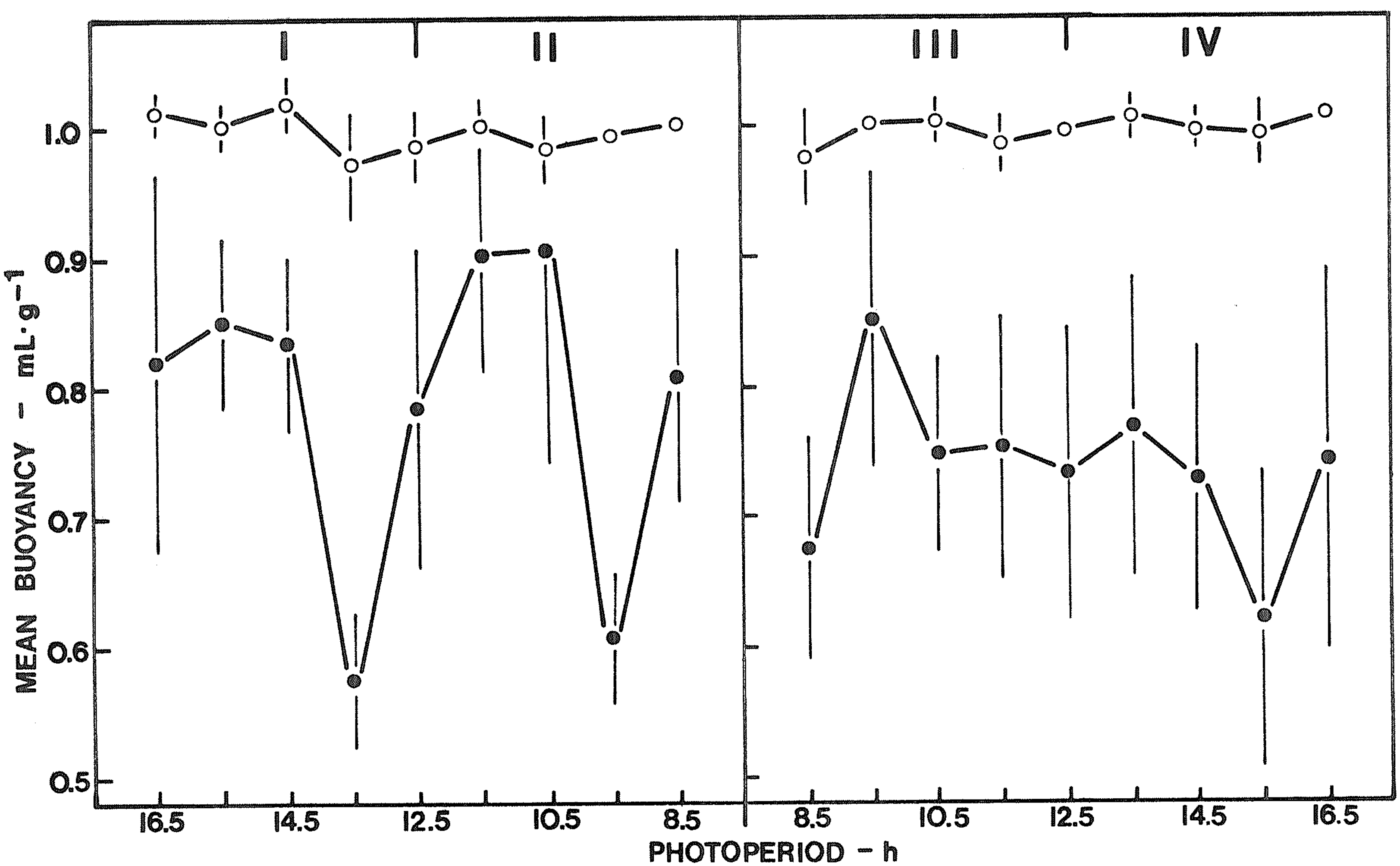
The effects of water velocity (still or current) and increasing photoperiod on buoyancy were significant ($p < 0.05$, two-way analysis of variance; Appendix 10) (Fig. 6 III and IV). Interaction between the effects of water velocity and photoperiod was not significant ($p > 0.05$, two-way analysis of variance; Appendix 10) and thus extent of buoyancy adjustment was similar at all increasing photoperiods (Fig. 6 III and IV). The direction of change in photoperiod also affected buoyancy

Figure 5. Effect of long-term exposure to photoperiod on mean buoyancy ($n = 8$) attained in still water (open circles) and in current (35 $cm \cdot s^{-1}$) (closed circles) at 15°C, with 95% confidence limits on the mean. Confidence limits < 0.014 are not shown.



1.0
0.8
0.7
0.6
0.5
MEAN BUOYANCY - mL·g⁻¹
9.0
10.5
12.0
13.5
15.0
16.5
PHOTOPERIOD - h

Figure 6. Effects of decreasing (I and II) and increasing (III and IV) photoperiods on mean buoyancy ($n = 8$; except at increasing 16.5 h in current $n = 6$) attained in still water (open circles) and in current (35 $cm \cdot s^{-1}$) (closed circles) at $15^{o}C$, with 95% confidence limits on the mean. Confidence limits < 0.014 are not shown. Mean buoyancies of fish tested at a decreasing (in group I and II) or increasing (in group III and IV) photoperiod of 12.5 h were similar. Therefore, eight fish were randomly chosen from the two batches in order to join group I to II and III to IV in still water and in current.



I
II
III
IV
MEAN BUOYANCY - mL·g-1
1.0
0.9
0.8
0.7
0.6
0.5
16.5
14.5
12.5
10.5
8.5
8.5
10.5
12.5
14.5
16.5
PHOTOPERIOD - h

attained in still water and in current (Fig. 6).

Fish tested at decreasing photoperiods of 13.5 and 9.5 h and increasing photoperiods of 15.5 and 16.5 h were observed to be in breeding condition and this may have affected the results.

Sex and sexual development

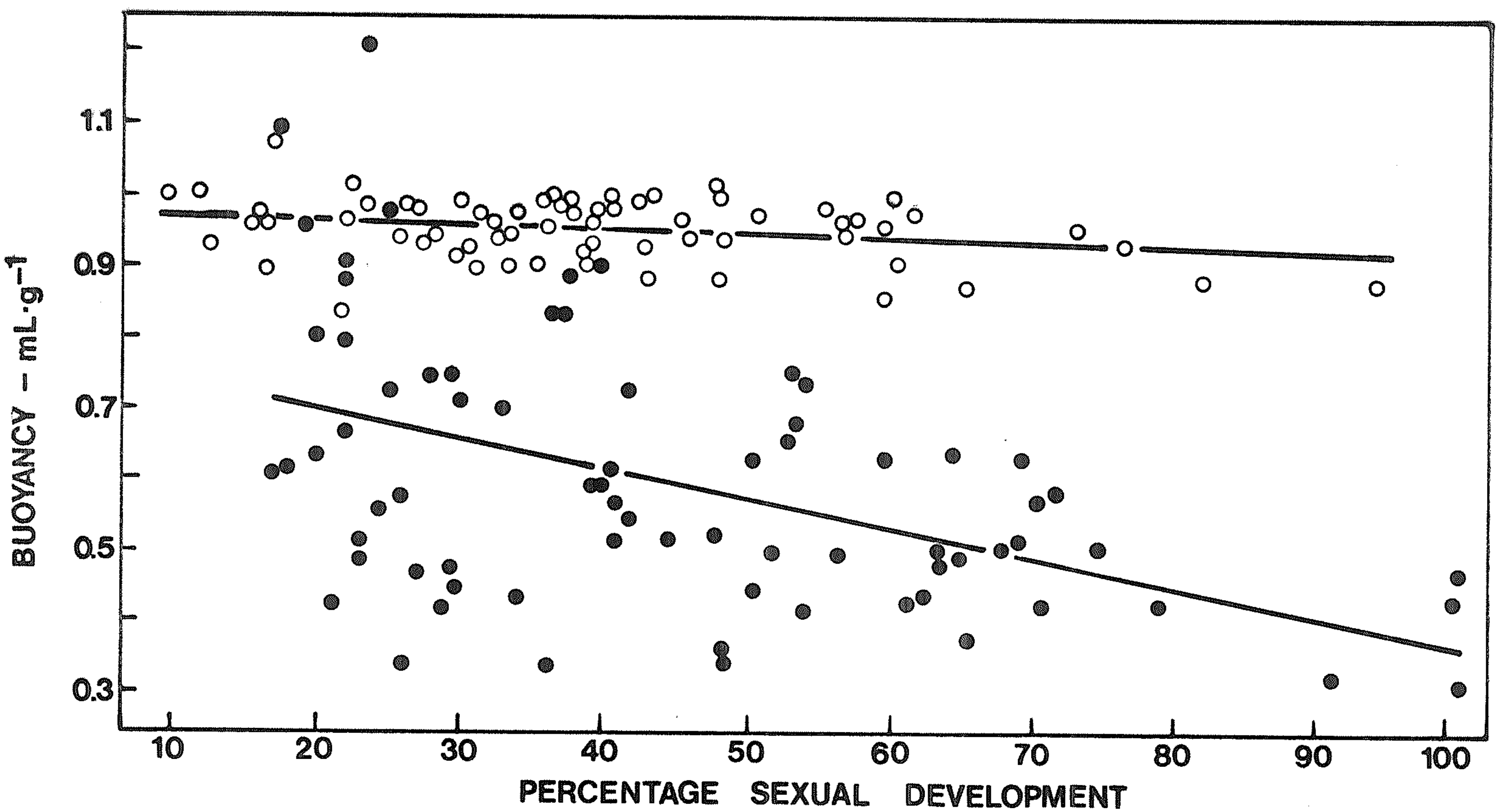
Slopes of regression lines for female and male fish were not significantly different ($p > 0.05$, analyses of covariance; Appendix 11), in either still water or current. There was also no significant difference between treatments (sex) ($p > 0.05$, analyses of covariance; Appendix 11), in either still water or current. Therefore, single regression lines of $Y = 0.979 - 0.0007\ X$ for still water and $Y = 0.784 - 0.0042\ X$ for current suffice for combined female and male data (Fig. 7).

Buoyancy attained in still water and in current decreased significantly ($p < 0.05$, analyses of covariance; Appendix 11) with increasing sexual development, with a greater decline occurring in current (Fig. 7). Thus extent of buoyancy adjustment increased with increasing sexual development. Variation in the buoyancy attained in current was greatest amongst sexually undeveloped fish.

Condition and fat content

Mean buoyancy of starved fish increased significantly ($p < 0.05$, single linear regression and lack of fit analyses; Appendix 12) with increasing time of starvation in both still

Figure 7. Effect of sexual development on buoyancy attained in still water (open circles) and in current ($40\ cm \cdot s^{-1}$) (closed circles) at $21^{o}C$. Lines represent significant regression lines for combined female and male fish in still water and in current.


BUOYANCY – mL·g⁻¹
1.1
0.9
0.7
0.5
0.3
10
20
30
40
50
60
70
80
90
100
PERCENTAGE SEXUAL DEVELOPMENT

water and in current (Fig. 8). Regression lines are $Y = 0.985 + 0.0004\ X$ for still water and $Y = 0.771 + 0.0021\ X$ for current.

Mean coefficient of condition decreased over 60 days of starvation (Appendix 12), but the relationship between coefficient of condition and buoyancy attained in still water was not significant ($p > 0.05$, single linear regression analysis; Appendix 13). Although non-significant, there was a slight decreasing trend in buoyancy in still water with increasing coefficient of condition (Fig. 9). Buoyancy attained in current by starving fish decreased significantly ($p < 0.05$, single linear regression analysis; Appendix 13) with increasing coefficient of condition (Fig. 9). The regression line is $Y = 1.240 - 0.4348\ X$. Thus extent of buoyancy adjustment increased with increasing coefficient of condition (Fig. 9).

Fat content in starved fish varied from 16.2 to 28.1%, but there was a non-significant ($p > 0.05$, single linear regression analyses; Appendix 14) effect of fat content on buoyancy attained in either still water or in current.

Figure 8. Effect of starvation on mean buoyancy ($n = 8$) attained in still water (open circles) and in current ($35\ cm \cdot s^{-1}$) (closed circles) at $15^{o}C$, with 95% confidence limits on the mean. Confidence limits < 0.014 are not shown. Lines represent significant regression lines. Mean indicated by $\triangle$ was omitted from analysis.

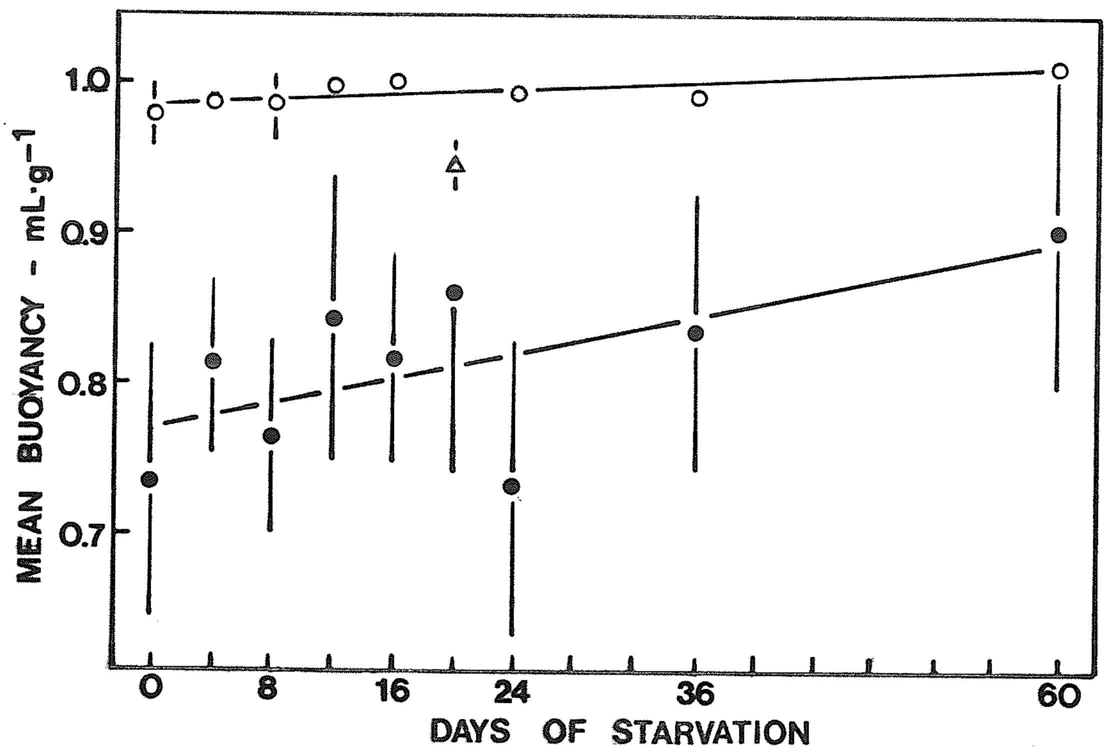
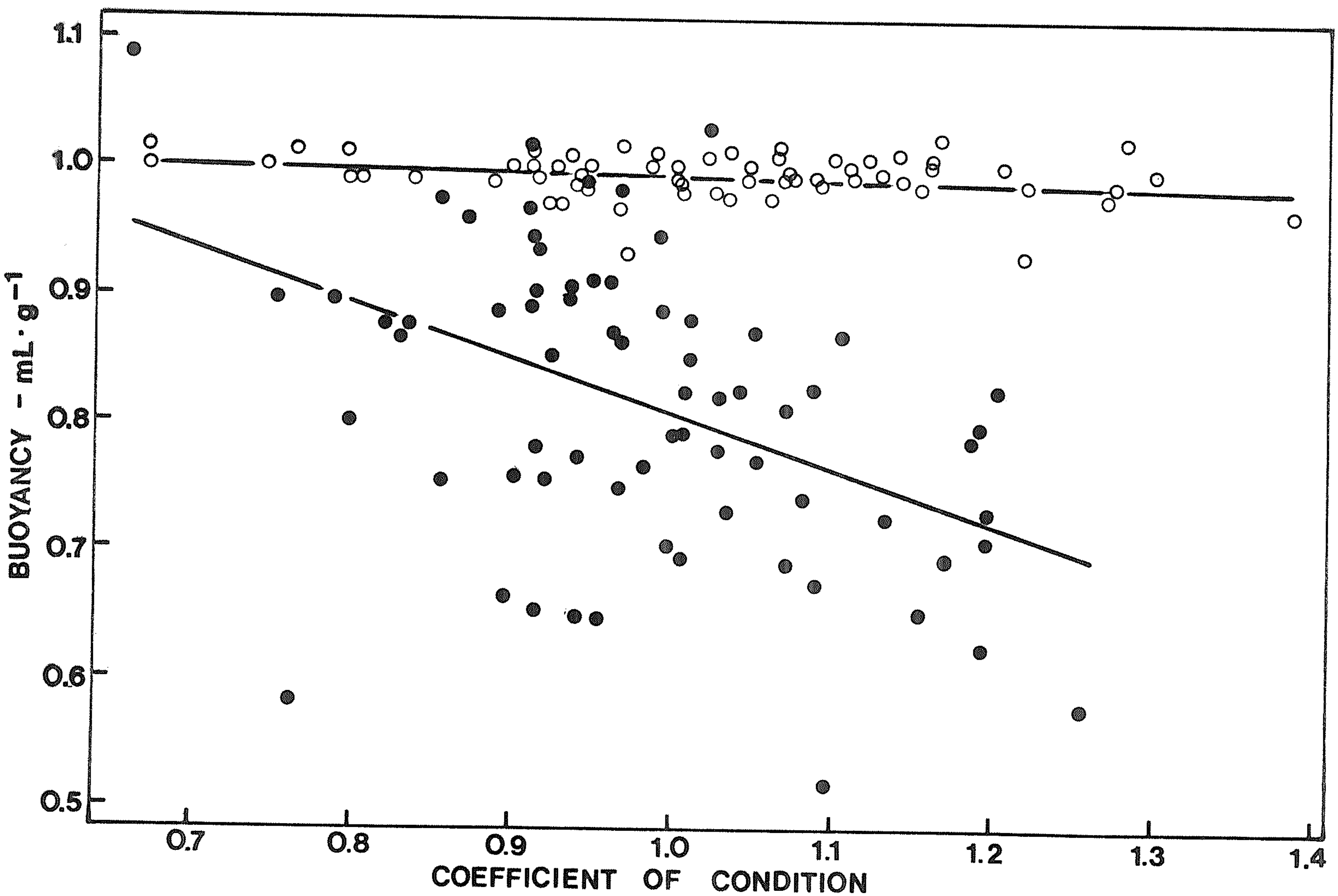

MEAN BUOYANCY - mL·g-1
1.0
0.9
0.8
0.7
0
8
16
24
36
60
DAYS OF STARVATION

Figure 9. Effect of coefficient of condition on buoyancy attained by starved fish in still water (open circles) and in current (35 $cm \cdot s^{-1}$) (closed circles) at 15°C. Lines represent significant regression line for current and non-significant for still water.


BUOYANCY - mL · g⁻¹
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
COEFFICIENT OF CONDITION

DISCUSSION

Fathead minnows attained a near neutral buoyancy in still water and reduced buoyancy when exposed to current. There was seasonal variation in maximum (in still water) and minimum (in current) buoyancy (group A fish) depending upon the effects of various seasonal factors, such as maximum tolerable water velocity, water temperature, photoperiod and condition of the fish. The pattern of variation also differed between the 2 years of the study. Independent of variation in water velocity and temperature, maximum and minimum buoyancy still varied seasonally (group B fish), suggesting that photoperiod, sexual development and condition of the fish may affect buoyancy. The seasonal pattern of variation between group A and B fish differed, indicating that water velocity or temperature or both affect buoyancy. Maximum and minimum buoyancy did not vary with time of year under constant environmental conditions (group C fish) showing that there was no endogenous influence on buoyancy. Buoyancy appears to be affected by external factors so that it can be adjusted rapidly to sudden and unexpected changes in the environment. This type of behavioural plasticity is common in North American stream fish (Gee et al. 1974).

Water temperature and velocity

In still water, as temperature decreased buoyancy increased from slightly negative to positive (group A fish) (negative slope of regression line). Similar effects of temperature were obtained by Gee (1977) in fathead minnows. Such a positive buoyancy at very low temperatures could be adaptive. Magnuson and Karlen (1970) showed that in an ice-covered lake oxygen depletion started at the bottom and proceeded upward in the water column. Fathead minnows were observed to move up in the water column as oxygen became depleted at lower levels (Mills 1972). They have also been observed to ventilate their gills with water drawn over the surface of gas bubbles containing oxygen, found at the ice-water interface or to take a bubble into their buccal cavity and pass water over it (Klinger 1978). Therefore, a positive buoyancy at cold temperatures would allow fish to remain at the ice-water interface with minimum energy expenditure. During winter sampling, fathead minnows were observed to be positively buoyant, remaining at the water surface in holes recently augered through the ice.

In current, as water velocity and temperature increased, buoyancy decreased (group A fish) (negative slope of regression lines). Water velocity, water temperature and an interaction of these factors are known to affect buoyancy (Neave et al. 1966; Pinder and Eales 1969; Gee 1977; Berezay and Gee 1978).

During the major part of spring runoff, which lasted for about a week in April 1978 in Crystal Creek, the water temperature increased from 1 to 4°C. Greater than a 5°C difference in temperature is required before a significant difference in buoyancy in current occurs (Gee 1977). Therefore, it is unlikely that an increasing water temperature during spring runoff could stimulate a greater decrease in buoyancy as suggested by Gee (1977).

To maintain position in current, fishes may either swim against current, seek nearby areas of reduced velocity (Allen 1969), reduce buoyancy by decreasing swimbladder volume or all of these (Gee and Gee 1976). In April, group A fish showed a minimal ability to reduce buoyancy in current in the laboratory and the maximum tolerable water velocity was 20 $cm \cdot s^{-1}$. Water velocity in Crystal Creek during spring runoff in April 1978 was observed to be as fast as 48 $cm \cdot s^{-1}$. To maintain position fathead minnows must then seek areas of reduced water velocity (in association with an irregular substrate, stream bank or in back waters) or be displaced downstream. Fathead minnows were observed to drift head-downstream (water velocity approximately 50 $cm \cdot s^{-1}$, water temperature was 12^{o}C) in the Whitemouth River, Manitoba in April 1977 (H. Smart, personal communication). This seems to indicate that fathead minnows do not reduce buoyancy enough to hold position in the fast water velocities encountered during spring runoff. However, downstream displacement may not be a disadvantage.

Stream invertebrates are known to drift downstream (Waters 1972). Drift can serve as a mechanism of dispersal, thereby reducing intra- and interspecific competition (Bishop and Hynes 1969). It can have the effect of transporting invertebrates to areas where the conditions for survival are more advantageous, it can be related to partner-searching behavior as in water beetles, water mites and amphipods (Müller 1974) and it can distribute adults to all areas of the stream suitable for reproduction (Waters 1972). Drift can also be ecologically important in the recolonization of downstream areas in which populations have been seriously reduced by pollution, flooding (Bishop and Hynes 1969) or by winter conditions such as anchor ice (subsurface) (Maciolek and Needham 1951; Waters 1972). The level of drift in invertebrates is controlled by water velocity, water temperature, photoperiod and density (Elliott 1967; Bishop and Hynes 1969; Waters 1969; Chaston 1972).

Winter conditions in streams in the great plains of North America often cause at least partial winterkill. Therefore, the minimal ability to reduce buoyancy in current and subsequent drifting downstream observed in fathead minnows during spring runoff may be a mechanism to disperse, as in invertebrates and to repopulate all of the suitable areas in the stream prior to the breeding season. Fathead minnows are characteristic of the headwaters of many streams in the great plains, hence a downstream displacement could be advantageous.

Photoperiod

Buoyancy in small and large fish was affected differently by various spring photoperiods and small fish were less able to reduce buoyancy in current than large fish. Size dependent differences in buoyancy have been shown, regardless of photoperiod in various species by Gee (1968, 1972, 1977), Machniak and Gee (1975) and Berezay and Gee (1978). Fish were able to respond to a particular photoperiod within 1 day, as length of exposure (1 or 7 days) to a particular photoperiod produced a similar buoyancy response. Long-term (3 weeks) exposure to various constant photoperiods and changing photoperiod as well as direction of change affected maximum and minimum buoyancy at a constant temperature. The photoperiod experiments were conducted in order to assess the independent effect and significance of photoperiod on buoyancy. However, it is not possible from the results of these experiments to comment on the biological significance of photoperiod on buoyancy alone.

Ivlev (1964) stated that light as an ecological and physiological factor is almost as important as temperature to fish and numerous vital processes are known to take place under the direct or indirect effects of light. Girsa (1972) also stated that the combined influence of photoperiod and temperature is of great biological significance and is the basis of seasonal variations in the physiological state of organisms. A change in photoperiod is usually accompanied by a change in temperature in nature which can strengthen or weaken the effect of photoperiod, as was found in the photoreaction response of various fish (Girsa 1972). Northcote (1958) showed that the water current response of young rainbow trout (Salmo gairdneri) was related to day length and temperature. The interaction of photoperiod and temperature regulated the frequency of upstream movement of common shiners (Notropis cornutus) (Dodson and Young 1977). Clarke et al. (1978) showed that temperature controlled the rate of response to photoperiod, so that changes in growth rate of sockeye salmon fry (Oncorhynchus nerka) caused by photoperiod treatments were apparent sooner at higher temperatures than at lower ones. It was also found that the sensitivity to photoperiod varied seasonally and that direction of change and rate of change of day length were the most important cues related to photoperiod. Pinder and Eales (1969) concluded that photoperiod plays a negligible role in the development of smolt buoyancy in current in Atlantic salmon parr.

Group A fish showed that as photoperiod increased, maximum and especially minimum buoyancy decreased (negative slope of regression line). However, in group B fish, tested under constant water velocity and temperature, minimum buoyancy increased as photoperiod increased (positive slope) and there was no significant change in maximum buoyancy. It appears that a different response in buoyancy to seasonal photoperiod was attained between fish tested under various seasonal water temperatures and ones tested at a constant temperature. Thus photoperiod, in isolation from other factors, has a meaningless effect on buoyancy but in combination with water temperature, the effect on buoyancy could be biologically significant.

Sexual development

There was no difference in maximum or minimum buoyancy attained between female and male fish. Buoyancy decreased as sexual development increased, with a greater decline occurring in current than in still water. The lowest buoyancy in still water and in current in group A and B fish was also attained when the fish were observed to be in breeding condition. This could be an adaptive feature for the spawning fish. Fathead minnows have a very wide geographic range and are found in both lotic and lentic environments (Scott and Crossman 1973).

In such environments where water velocity can vary in time and space, the ability to reduce buoyancy to a minimum should there be a sudden increase in water velocity would be an important adaptation during spawning. A reduced buoyancy in current would enable fish to effectively hold position in order to facilitate maintenance of territorial position and thus enhance reproductive success. It would also be advantageous to have neutral buoyancy in still water as was found in breeding fish, since a greater speed and maneuverability are required, especially in males to facilitate nest building and spawning which occurs beneath objects (McMillan and Smith 1974).

Variation in buoyancy in current was greatest amongst sexually undeveloped fish. This variation may be due to differences between sexually immature and spent fish since differences were not determined during the experiment.

Several fish tested during the decreasing and increasing photoperiod experiment were noted to be in breeding condition and therefore, may have influenced the buoyancy response to photoperiod. The breeding condition observed during decreasing photoperiods is not unusual since fathead minnows are known to spawn throughout the summer until September (Scott and Crossman 1973; McMillan and Smith 1974).

Condition

The coefficient of condition is a numerical representation of heaviness or robustness. Condition varied seasonally in fathead minnows (group A and B fish), with the lowest values occurring in the spring and highest in breeding fish as would be expected. Condition can be affected by sex, length, age, maturity and environmental factors such as food supply, temperature and parasitization (Hoar 1939; LeCren 1951). There are also individual variations among fish and therefore, Hoar (1939) and LeCren (1951) suggested that small variations in condition are of little significance.

Buoyancy increased in still water and especially in current with days of starvation. MacLeod and Smith (1966) observed that over 4 days active metabolism decreased in unfed fathead minnows. It appears that starvation decreases active metabolism and the capacity for work (MacLeod 1967). Buoyancy increased significantly in current but only slightly in still water as condition decreased in starved fish. Group A and B fish also showed similar results with significant buoyancy increases in still water as well as in current as condition decreased. In group C fish, condition remained relatively constant due to the constant feeding and laboratory conditions, as did maximum and minimum buoyancy. This indicates that the coefficient of condition can be used to describe the condition of the fish and that the more robust a fish, the greater its ability is to reduce buoyancy in current.

Group C fish tested at the end of the experiment were about 26 to 29 months old and fathead minnows are rarely known to live beyond age 2 in nature (Scott and Crossman 1973). It appears that old age greatly reduces the ability to decrease buoyancy in current, either directly, or indirectly due to the poor condition (low K values) of these fish. Fish which were parasitized also showed a poor ability to reduce buoyancy in current. However these fish (group A and B in April 1977) were also influenced by other seasonal factors, so that it is not certain whether parasitism can affect buoyancy.

Fat content

Fat content had no significant effect on maximum or minimum buoyancy. The formula used to determine fat content was initially developed for rainbow trout. Even though the values may not represent actual body-fat concentrations in fathead minnows, differences in fat concentration would be apparent between individuals of the same population (Horak 1966). Variations in fat content have also shown little influence on density differences of fish tissues and therefore, swimbladder volume in various Cyprinidae (Taylor 1922; Alexander 1959, 1967) and in juvenile Atlantic salmon (Pinder and Eales 1969). However, maintenance of neutral buoyancy in some marine fish may be aided by the presence of large quantities of lipid in various body organs (Brawn 1969; Butler and Pearcy 1972; Bone 1973; Lee et al. 1975; DeVries and Eastman 1978).

By studying monofactorial experiments, as in this study, it was possible to provide insight into some of the more important factors influencing buoyancy in the natural environment. However, the natural environment is more complicated and therefore, the introduction of multifactorial experiments holds greater promise for revealing the respective roles of environmental factors (Brett 1969) and this appears to be true for buoyancy studies.

Water velocity, water temperature, photoperiod, size, age, sexual development and condition of the fish affect the buoyancy response in fathead minnows. It is likely that many if not all of these factors interact to influence buoyancy.

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Appendix 1.

Environmental variables measured for Crystal Creek, Manitoba from May 1976 to May 1978.

Date	Photoperiod (h)	Temperature (°C)		Salinity (o/oo)	Conductivity (μmhos·cm^{-1})	Oxygen (ppm)	pH	Secchi disc transparency (cm)
		Air	Water					
31 May	16.5	30	22	2.0	1000	9.1	7.6	51
15 Jun	16.8	26	17	1.0	900	10.4-11.2[f]	7.8	36
16 Jul	16.6	23	20	0.5	910	8.8	8.4	28
10 Aug	15.5	27	21	0.7	1000	6.5-9.0[f]	8.5	20
13 Sep	13.3	11	13	0.8	920	9.1	8.7	28
4 Oct	12.1	10	9	0.8	880	10.8	8.8	10
1 Nov	10.2	11	5	1.9	720	12.5	7.9	10
17 Jan	9.1	-26	0	6.0	1900	1.0	5.5	8/46/61[e]
1 Mar[a]	11.2	1	1	5.0	2700-2600[f]	0.3-1.3[f]	6.7	46/107/30[e]
21 Apr[b]	14.3	15	14	0.5	470	12.4	7.5	33
30 May	16.4	27	22	3.5	910-950[f]	2.5-5.3[f]	7.2	28
4 Jul[c]	16.7	28	23-26[f]	0.7	1150-1210[f]	8.4-10.2[f]	7.6	20
13 Jul	16.5	18	19	0.7	1050	7.5-8.2[f]	7.5	-
8 Aug	15.5	25	20	0.8	1110-1150[f]	9.2-11.2[f]	8.1	25
15 Sep	13.3	26	18	0.6	920	9.8	7.9	25
17 Oct	11.2	15	9	0.8	720	-	7.7	42
16 Nov[d]	9.6	7	3	0.9	730	-	7.6	46
12 Dec[d]	8.7	-8	2	1.9	1090-1000[f]	1.3	7.5	3/51/66[e]
24 Jan[a]	9.3	-8	2-0[f]	6.0	1250	1.0-2.0[f]	7.2	50/80/70[e]
10 Apr	13.9	5	2	0.2	135	6.5	8.1	13
29 May	16.4	30	22-24[f]	0.1	260	4.0	7.7	6

a no fish found

b sampling site (Crystal Cr.-Pembing R. junction)

c fish developed fungus infection - not tested

d fish too small - not tested

e depth of snow/ice/water

f bottom - surface readings

Appendix 2.

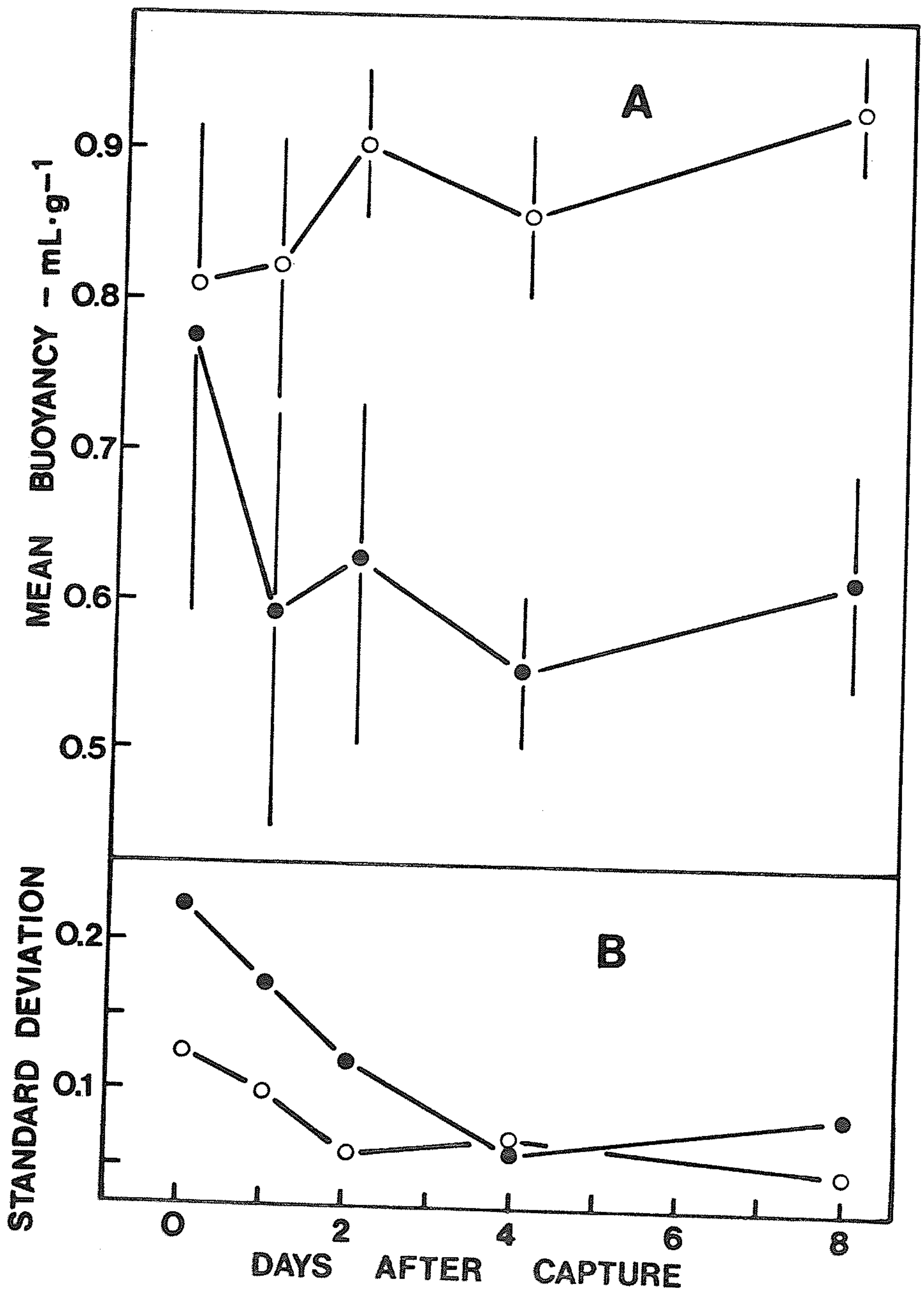
Effect of capture and transportation to the laboratory (Day 0) on buoyancy. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (51 $cm \cdot s^{-1}$) at 22°C.

	Still		Current	
Days after capture	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
0	0.810 ± 0.104	0.125	0.777 ± 0.186	0.222
1	0.822 ± 0.084	0.100	0.592 ± 0.143	0.171
2	0.905 ± 0.051	0.060	0.630 ± 0.100	0.119
4	0.858 ± 0.056	0.067	0.554 ± 0.050	0.060
8	0.929 ± 0.041	0.049	0.617 ± 0.073	0.087

To determine effect of capture and transportation on maximum (in still water) and minimum (in current) buoyancy, fish collected in May 1976 were tested 0, 1, 2, 4 and 8 days after capture and transportation to the laboratory. On each day eight fish were held in still water and eight fish in current (51 ± 2 $cm \cdot s^{-1}$) at 22°C for 24 h before measuring buoyancy.

Buoyancy was affected by capture and transportation. After 4 days mean buoyancy and standard deviation stabilized in current. For fish in still water, standard deviation stabilized after 2 days but mean buoyancy continued to fluctuate (Appendix Fig. 1A and B). Therefore, 6 days was chosen as a conservative minimal time for fish to return to

Appendix Figure 1. (A) Mean buoyancy ($n = 8$) over time after capture and transportation (Day 0) in still water (open circles) and in current ($51\ cm \cdot s^{-1}$) (closed circles) at $22^{o}C$, with 95% confidence limits on the mean. (B) Standard deviation of mean buoyancy in still water and in current.


A
B
MEAN BUOYANCY – mL·g⁻¹
0.9
0.8
0.7
0.6
0.5
STANDARD DEVIATION
0.2
0.1
0
2
4
6
8
DAYS AFTER CAPTURE

a stable buoyancy level in still water and current.

Hattingh (1976) found that it takes about 4 days after capture and transportation for swimbladder gas composition of various freshwater fish to return to a stable level, but that fractional volumes (buoyancy) in still water were not affected.

Appendix 3.

Seasonal changes in buoyancy in group A fish over 2 years. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (maximum tolerable water velocity) at field water temperature and photoperiod.

Date	Still: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Still: SD	Current: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Current: SD
15 Jun 1976	0.976 ± 0.021	0.026	0.523 ± 0.066	0.078
16 Jul	0.964 ± 0.038	0.045	0.738 ± 0.091	0.109
10 Aug	0.983 ± 0.014	0.017	0.704 ± 0.136	0.163
13 Sep	0.986 ± 0.009	0.012	0.734 ± 0.112	0.134
4 Oct	0.980 ± 0.012	0.015	0.737 ± 0.052	0.062
1 Nov	0.979 ± 0.013	0.016	0.787 ± 0.060	0.072
17 Jan 1977	1.015 ± 0.014	0.016	0.799 ± 0.049	0.059
21 Apr	0.974 ± 0.019	0.023	0.877 ± 0.089	0.106
30 May	0.900 ± 0.091	0.109	0.539 ± 0.094	0.112
13 Jul	0.996 ± 0.022	0.027	0.790 ± 0.123	0.148
8 Aug	0.983 ± 0.024	0.029	0.738 ± 0.124	0.148
15 Sep	0.931 ± 0.056	0.068	0.662 ± 0.090	0.108
17 Oct	0.973 ± 0.025	0.030	0.757 ± 0.106	0.127
10 Apr 1978	1.011 ± 0.014	0.017	0.771 ± 0.048	0.057
29 May	0.959 ± 0.053	0.063	0.647 ± 0.061	0.072

One-Way Analysis of Variance for Fish in Still Water

Source	df	SS	MS	F
Time	14	0.0919	0.0066	3.590*
Error	105	0.1920	0.0018	
Total	119	0.2839		

* Tested and found significantly different ($p < 0.05$).

One-Way Analysis of Variance for Fish in Current

Source	df	SS	MS	F
Time	14	1.0089	0.0721	6.050*
Error	105	1.2506	0.0119	
Total	119	2.2595		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test) in still water and in current, however the analyses of variance are robust due to equal sample sizes (Glass et al. 1972). A robust statistical test preserves the validity of the probability statements applied to it, even though the assumptions upon which it is based are violated.

Appendix 4.

Maximum tolerable water velocity, water temperature and photoperiod used for testing group A fish.

Date	Water velocity $(cm \cdot s^{-1})$	Water temperature (°C)	Photoperiod (h)
15 Jun 1976[a]	50	17	16.8
16 Jul	31	20	16.6
10 Aug	47	21	15.5
13 Sep	31	13	13.3
4 Oct	29	9	12.1
1 Nov	21	5	10.2
17 Jan 1977	20	5	9.1
21 Apr[b]	31	14	14.3
30 May[a]	45	22	16.4
13 Jul	44	19	16.5
8 Aug	43	20	15.5
15 Sep	42	18	13.3
17 Oct	27	9	11.2
10 Apr 1978	22	5	13.9
29 May[a]	53	24	16.4

[a] Fish in breeding condition
[b] Fish were parasitized

Single Linear Regressions of Buoyancy in Still Water on Various Factors

Water Temperature

Source	df	SS	MS	F	r^2
Regression	1	0.0292	0.0292	13.537*	10.3%
Residual	118	0.2547	0.0022		
Total	119	0.2839			

Regression line is Y = 1.010 - 0.0025 X.

Photoperiod

Source	df	SS	MS	F	r^2
Regression	1	0.0120	0.0120	5.212*	4.2%
Residual	118	0.2720	0.0023		
Total	119	0.2840	0.0480		

Regression line is Y = 1.032 - 0.0041 X.

Coefficient of Condition

Source	df	SS	MS	F	r^2
Regression	1	0.0128	0.0128	5.566*	4.5%
Residual	118	0.2712	0.0023		
Total	119	0.2840			

* Tested and found significantly different ($p < 0.05$).
Regression line is Y = 1.058 - 0.0787 X.

Single Linear Regressions of Buoyancy in Current on Various Factors

Water Velocity

Source	df	SS	MS	F	r^2
Regression	1	0.4525	0.4525	29.546*	20.0%
Residual	118	1.8070	0.0153		
Total	119	2.2595			

Regression line is Y = 0.925 - 0.0057 X.

Water Temperature

Source	df	SS	MS	F	r^2
Regression	1	0.2765	0.2765	16.456*	12.2%
Residual	118	1.9830	0.0168		
Total	119	2.2595	0.1296		

Regression line is Y = 0.832 - 0.0076 X.

Photoperiod

Source	df	SS	MS	F	r^2
Regression	1	0.2462	0.2462	14.431*	10.9%
Residual	118	2.0133	0.0171		
Total	119	2.2595			

Regression line is Y = 0.984 - 0.0187 X.

Coefficient of Condition

Source	df	SS	MS	F	r^2
Regression	1	0.0959	0.0959	5.229*	4.2%
Residual	118	2.1636	0.0183		
Total	119	2.2595			

* Tested and found significantly different ($p < 0.05$).
Regression line is Y = 0.983 - 0.2470 X.

Appendix 5.

Seasonal changes in buoyancy, independent of water velocity and temperature in group B fish over 2 years. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

	Still		Current	
Date	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
15 Jun 1976	0.979 ± 0.026	0.031	0.794 ± 0.080	0.096
16 Jul	0.947 ± 0.039	0.047	0.804 ± 0.096	0.115
10 Aug	0.937 ± 0.030	0.035	0.605 ± 0.069	0.082
13 Sep	0.927 ± 0.033	0.039	0.587 ± 0.059	0.070
4 Oct	0.968 ± 0.011	0.013	0.595 ± 0.043	0.052
1 Nov	0.932 ± 0.038	0.045	0.587 ± 0.082	0.098
17 Jan 1977	0.944 ± 0.050	0.060	0.590 ± 0.100	0.120
21 Apr	0.990 ± 0.038	0.045	0.857 ± 0.063	0.075
30 May	0.833 ± 0.109	0.130	0.556 ± 0.129	0.154
13 Jul	0.985 ± 0.013	0.015	0.933 ± 0.066	0.079
8 Aug	1.006 ± 0.013	0.015	0.909 ± 0.125	0.149
15 Sep	0.990 ± 0.007	0.008	0.783 ± 0.109	0.130
17 Oct	0.974 ± 0.017	0.020	0.668 ± 0.088	0.106
10 Apr 1978	0.953 ± 0.030	0.035	0.653 ± 0.100	0.119
29 May	0.981 ± 0.040	0.048	0.576 ± 0.090	0.108

One-Way Analysis of Variance for Fish in Still Water

Source	df	SS	MS	F
Time	14	0.196	0.014	5.959*
Error	105	0.246	0.002	
Total	119	0.442		

* Tested and found significantly different ($p < 0.05$).

One-Way Analysis of Variance for Fish in Current

Source	df	SS	MS	F
Time	14	1.972	0.141	12.258*
Error	105	1.207	0.012	
Total	119	3.179		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test) in still water, however the analysis of variance is robust due to equal sample sizes (Glass et al. 1972).

Appendix 6.

Regression analyses for group B fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

Single Linear Regressions of Buoyancy in Still Water on Various Factors

Photoperiod

Source	df	SS	MS	F
Regression	1	0.0000016	0.0000016	0.0004
Residual	118	0.4418	0.0037	
Total	119	0.4418		

Coefficient of Condition

Source	df	SS	MS	F	r^2
Regression	1	0.0367	0.0367	10.698*	8.3%
Residual	118	0.4051	0.0034		
Total	119	0.4418			

* Tested and found significantly different ($p < 0.05$).
Regression line is $Y = 1.078 - 0.1099 X$.

Single Linear Regressions of Buoyancy in Current on Various Factors

Photoperiod

Source	df	SS	MS	F	r^2
Regression	1	0.3568	0.3568	14.922*	11.2%
Residual	118	2.8216	0.0239		
Total	119	3.1784			

Regression line is $Y = 0.382 + 0.0225 X$.

Coefficient of Condition

Source	df	SS	MS	F	r^2
Regression	1	0.2611	0.2611	10.559*	8.2%
Residual	118	2.9174	0.0247		
Total	119	3.1785			

* Tested and found significantly different ($p < 0.05$).
Regression line is $Y = 1.086 - 0.3619 X$.

Appendix 7.

Effect of constant environmental conditions (group C fish) on buoyancy over a 16 month period. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

Date	Still: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Still: SD	Current: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Current: SD
27 Jul 1976	0.982 ± 0.010	0.011	0.661 ± 0.091	0.109
21 Aug	0.980 ± 0.015	0.018	0.656 ± 0.093	0.112
24 Sep	0.994 ± 0.015	0.019	0.636 ± 0.065	0.078
15 Oct	0.982 ± 0.013	0.016	0.713 ± 0.090	0.107
12 Nov	0.935 ± 0.065	0.078	0.674 ± 0.121	0.145
13 Jan 1977	1.010 ± 0.010	0.012	0.709 ± 0.087	0.104
2 Feb	0.986 ± 0.020	0.024	0.647 ± 0.102	0.122
16 Mar	0.973 ± 0.015	0.017	0.750 ± 0.092	0.110
3 May	0.995 ± 0.020	0.024	0.754 ± 0.123	0.148
9 Jun	0.976 ± 0.023	0.028	0.642 ± 0.096	0.115
15 Jul	0.964 ± 0.032	0.038	0.713 ± 0.075	0.090
18 Aug	1.000 ± 0.008	0.010	0.674 ± 0.084	0.100
26 Sep[a]	0.992 ± 0.016	0.019	0.861 ± 0.062	0.074
27 Oct[a]	0.987 ± 0.010	0.011	0.951 ± 0.112	0.134
Overall mean (n = 12)	0.981		0.686	

a Not included in overall mean or in analysis of variance due to the effect of old age on buoyancy.

Two-Way Analysis of Variance

Source	df	SS	MS	F
Water velocity	1	4.198	4.198	610.932*
Time	11	0.093	0.009	1.235
Interaction	11	0.087	0.008	1.149
Error	168	1.155	0.007	
Total	191	5.533		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test), however the analysis of variance is robust due to equal sample sizes (Glass et al. 1972).

Appendix 8.

Effect of length of exposure to various spring photoperiods on buoyancy attained by small (3.3 - 4.5 cm, fork length) and large (5.5 - 6.5 cm, fork length) fish. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in current (35 $cm \cdot s^{-1}$) at $15^{o}C$. Deviations in sample size in parentheses.

	1 day exposure		7 day exposure	
Photo-period (h)	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
Small fish				
8.5	0.871 ± 0.111 (7)	0.120	0.835 ± 0.122 (5)	0.098
10.5	0.965 ± 0.070 (6)	0.067	0.869 ± 0.157 (7)	0.169
12.5	0.789 ± 0.169	0.203	0.732 ± 0.094	0.112
14.5	0.912 ± 0.056	0.067	0.939 ± 0.058	0.069
Large fish				
8.5	0.731 ± 0.067	0.080	0.707 ± 0.073	0.087
10.5	0.745 ± 0.094 (7)	0.102	0.699 ± 0.073 (7)	0.079
12.5	0.598 ± 0.073	0.087	0.714 ± 0.066	0.079
14.5	0.597 ± 0.055	0.066	0.606 ± 0.076	0.091

Two-Way Analysis of Variance for Small Fish

Source	df	SS	MS	F
Days	1	0.0205	0.0205	1.323
Photoperiod	3	0.2675	0.0892	5.755*
Interaction	3	0.0293	0.0098	0.632
Error	49	0.7574	0.0155	
Total	56	1.0748		

* Tested and found significantly different ($p < 0.05$).

Two-Way Analysis of Variance for Large Fish

Source	df	SS	MS	F
Days	1	0.0037	0.0037	0.521
Photoperiod	3	0.1553	0.0518	7.296*
Interaction	3	0.0600	0.0200	2.817[a]
Error	54	0.3830	0.0071	
Total	61	0.6020		

* Tested and found significantly different ($p < 0.05$).

[a] Tested and found only marginally different ($F_{3,54,0.05} = 2.79$, table value) and therefore failed to reject $Ho{:}\alpha\beta = 0$.

The effect of length of exposure to photoperiod on buoyancy was not significantly different ($p > 0.05$) in either small or large fish and therefore, data for 1 and 7 day exposures were grouped and re-analyzed.

Effect of various spring photoperiods on buoyancy attained by small and large fish. Mean buoyancy ($n = 16$) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in current ($35\ cm \cdot s^{-1}$) at 15^oC. Deviations in sample size in parentheses.

	Small fish		Large fish	
Photoperiod (h)	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
8.5	0.856 ± 0.069 (12)	0.108	0.719 ± 0.043	0.082
10.5	0.913 ± 0.082 (13)	0.137	0.722 ± 0.053 (14)	0.091
12.5	0.760 ± 0.086	0.161	0.656 ± 0.053	0.100
14.5	0.926 ± 0.036	0.067	0.601 ± 0.041	0.077

Two-Way Analysis of Variance for Grouped Data

Source	df	SS	MS	F
Size	1	1.0835	1.0835	97.613*
Photoperiod	3	0.1969	0.0656	5.910*
Interaction	3	0.2234	0.0745	6.712*
Error	111	1.2323	0.0111	
Total	118	2.7361		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test), however the analysis of variance is robust due to relatively equal sample sizes and a relatively small difference between largest (0.0259) and smallest (0.0045) variances (Glass et al. 1972).

Appendix 9.

Effect of long-term exposure to various photoperiods on buoyancy. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

Photo-period (h)	Still		Current	
	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
9.0	0.986 ± 0.016	0.019	0.707 ± 0.046	0.056
10.5	0.996 ± 0.018	0.021	0.741 ± 0.060	0.072
12.0	0.968 ± 0.015	0.018	0.596 ± 0.081	0.097
13.5	0.997 ± 0.014	0.016	0.667 ± 0.093	0.111
15.0	0.998 ± 0.007	0.008	0.727 ± 0.097	0.116
16.5	0.989 ± 0.021	0.025	0.746 ± 0.086	0.103

Two-Way Analysis of Variance

Source	df	SS	MS	F
Water velocity	1	2.046	2.046	437.196*
Photoperiod	5	0.088	0.018	3.775*
Interaction	5	0.049	0.010	2.085
Error	84	0.393	0.005	
Total	95	2.576		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test), however the analysis of variance is robust due to equal sample sizes (Glass et al. 1972).

Appendix 10.

Effects of decreasing and increasing length of photoperiod on buoyancy. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

	Still		Current	
Photoperiod (h)	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD	Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	SD
Decreasing				
16.5	1.010 ± 0.016	0.019	0.819 ± 0.144	0.172
15.5	1.002 ± 0.015	0.018	0.850 ± 0.066	0.079
14.5	1.017 ± 0.020	0.024	0.836 ± 0.067	0.080
13.5	0.969 ± 0.042	0.050	0.576 ± 0.052	0.063
12.5[b]	0.986 ± 0.026	0.031	0.785 ± 0.123	0.148
11.5	0.999 ± 0.021	0.025	0.902 ± 0.088	0.105
10.5	0.983 ± 0.025	0.030	0.905 ± 0.163	0.195
9.5	0.992 ± 0.004	0.005	0.608 ± 0.050	0.060
8.5	1.000 ± 0.005	0.006	0.808 ± 0.097	0.117
Increasing				
8.5	0.974 ± 0.034	0.041	0.675 ± 0.086	0.103
9.5	1.001 ± 0.010	0.012	0.849 ± 0.113	0.135
10.5	1.003 ± 0.015	0.018	0.748 ± 0.074	0.088
11.5	0.986 ± 0.019	0.022	0.752 ± 0.101	0.120
12.5[b]	0.996 ± 0.012	0.015	0.732 ± 0.113	0.136
13.5	1.004 ± 0.015	0.018	0.768 ± 0.114	0.137
14.5	0.995 ± 0.015	0.018	0.728 ± 0.103	0.124
15.5	0.992 ± 0.025	0.029	0.621 ± 0.114	0.136
16.5	1.008 ± 0.011	0.013	0.742 ± 0.146[a]	0.139

[a] Sample size was 6.

[b] Mean buoyancies of fish tested at a decreasing (in group I and II) or increasing (in group III and IV) photoperiod of 12.5 h were similar. Therefore, eight fish were randomly chosen from the two batches in order to join group I to II and III to IV in still water and in current.

Two-Way Analysis of Variance for Decreasing Photoperiod

Source	df	SS	MS	F
Water velocity	1	1.5531	1.5531	199.115*
Photoperiod	8	0.5107	0.0638	8.179*
Interaction	8	0.3946	0.0493	6.321*
Error	126	0.9874	0.0078	
Total	143	3.4458		

* Tested and found significantly different ($p < 0.05$).

Two-Way Analysis of Variance for Increasing Photoperiod

Source	df	SS	MS	F
Water velocity	1	2.4027	2.4027	300.338*
Photoperiod	8	0.1496	0.0187	2.338*
Interaction	8	0.1083	0.0135	1.688
Error	124	0.9914	0.0080	
Total	141	3.6521		

* Tested and found significantly different ($p < 0.05$).

Variances were unequal ($p < 0.05$; Bartlett's test), however the analysis of variance is robust due to equal sample sizes for decreasing photoperiods (Glass et al. 1972). For increasing photoperiod, sample sizes were equal ($n = 8$) except for one sample ($n = 6$).

Appendix 11.

Analysis of covariance on the effects of sexual development on buoyancy attained by female and male fish tested in still water or in current (40 $cm \cdot s^{-1}$) at 21°C.

Analysis of Covariance for Female and Male Fish in Still Water

Source	df	YY	XY	XX	b	Due regression		Deviations from regression	
						df	SS	df	SS
Residual 1 (♀)	27	0.0700	-6.3489	7216.5535	-0.00088	1	0.0056	26	0.0644
Residual 2 (♂)	37	0.0536	-7.5745	11703.4313	-0.00065	1	0.0049	36	0.0487
Sum	64	0.1236	-13.9234	18919.9848		2	0.0105	62	0.1131
						Adjusted for covariate			
						df	SS	MS	F
Sex	1	0.000024	0.1426	807.2103	0.00018				
Residual	64	0.1235	-13.9208	18920.1870	-0.00074	63	0.1133	0.0018	
Total	65	0.123524	-13.7782	19727.3973	-0.00070	64	0.1139		
					Treatment adjusted	1	0.00064	0.00064	0.358

1. Ho: $\beta_1 = \beta_2$
   $F_{1,62} = 0.133$ Equal slopes ($p > 0.05$).

2. Ho: $\alpha_1 = \alpha_2$
   $F_{1,63} = 0.358$ Equal treatments (sex) ($p > 0.05$).

3. Ho: $\beta_o^1 = \beta_o^2$
   $F_{1,63} = 5.695$ Regression lines are not horizontal ($p < 0.05$).

A single regression line for both female and male fish in still water is $Y = 0.979 - 0.0007 X$.

Analysis of Covariance for Female and Male Fish in Current

Source	df	YY	XY	XX	b	Due regression df	Due regression SS	Deviations from regression df	Deviations from regression SS
Residual 1 (♀)	32	1.4812	-90.6179	15737.9887	-0.0058	1	0.5218	31	0.9594
Residual 2 (♂)	39	1.0663	-48.1898	16809.8474	-0.0029	1	0.1381	38	0.9282
Sum	71	2.5475	-138.8077	32547.8361		2	0.6599	69	1.8876

Source	df	YY	XY	XX	b	Adjusted for covariance df	SS	MS	F
Sex	1	0.0028	0.8905	357.5760	0.0025				
Residual	71	2.5681	-138.0321	32547.8300	-0.0042	70	1.9827	0.0283	
Total	72	2.5709	-137.1416	32905.4060	-0.0042	71	1.9993		
Treatment adjusted						1	0.0166	0.0166	0.587

1. Ho: $\beta_1 = \beta_2$
   $F_{1,69} = 3.479$ Equal slopes ($p > 0.05$).

2. Ho: $\alpha_1 = \alpha_2$
   $F_{1,70} = 0.587$ Equal treatments (sex) ($p > 0.05$).

3. Ho: $\beta_o^1 = \beta_o^2$
   $F_{1,70} = 20.663$ Regression lines are not horizontal ($p < 0.05$).

   A single regression line for both female and male fish in current is $Y = 0.784 - 0.0042\ X$.

Variances were unequal for fish tested in current ($p < 0.05$; Bartlett's test), however the analysis of covariance is robust since the ratio of variances of the covariate (0.941) is close to 1 (Glass et al. 1972).

Calculation of sexual development

The gonad:body-weight ratio in male fish was smaller than in females. In order to have female and male fish over the same range of X - values for a covariance analysis, the gonad:body weight ratio was converted to sexual development.

$$\%\ \underset{\text{(females)}}{\text{sexual development}} = \frac{\text{gonad:body-weight ratio}}{0.208} \times 100$$ where 0.208 was the largest ratio of gonad:body-weight for females.

$$\%\ \underset{\text{(males)}}{\text{sexual development}} = \frac{\text{gonad:body-weight ratio}}{0.033} \times 100$$ where 0.033 was the largest ratio of gonad:body-weight for males.

Appendix 12.

Effect of starvation on buoyancy. Mean buoyancy (n = 8) with 95% confidence limits (CL) and standard deviation (SD) for fish tested in still water or in current (35 $cm \cdot s^{-1}$) at 15°C.

Days of starvation	Still: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Still: SD	Current: Mean buoyancy ($mL \cdot g^{-1}$) and 95% CL	Current: SD
0	0.978 ± 0.019	0.023	0.735 ± 0.090	0.108
4	0.986 ± 0.011	0.013	0.813 ± 0.058	0.070
8	0.986 ± 0.022	0.026	0.765 ± 0.062	0.074
12	0.998 ± 0.011	0.014	0.843 ± 0.096	0.115
16	1.000 ± 0.008	0.009	0.817 ± 0.069	0.082
20	0.944 ± 0.017[a]	0.020	0.862 ± 0.121	0.145
24	0.993 ± 0.008	0.010	0.733 ± 0.097	0.116
36	0.990 ± 0.009	0.011	0.836 ± 0.093	0.111
60	1.013 ± 0.005	0.006	0.906 ± 0.106	0.127

a Outlier not included in regression analysis due to possible effect of fluctuating water temperature.

Single Linear Regression with Replicated Y Values and Lack of Fit Analysis for Fish in Still Water

Source	df	SS	MS	F	r^2
Regression (Days)	1	0.0039	0.0039	15.246*	19.7%
Residual	62	0.0157	0.0003		
Total	63	0.0196			
Lack of fit	6	0.0024	0.00040	1.697	
Pure error	56	0.0133	0.00024		

* Tested and found significantly different ($p < 0.05$).
Regression line is $Y = 0.985 + 0.0004\ X$.

Single Linear Regression with Replicated Y Values and Lack of Fit Analysis for Fish in Current

Source	df	SS	MS	F	r^2
Regression (Days)	1	0.0926	0.0926	7.524*	9.7%
Residual	70	0.8615	0.0123		
Total	71	0.9541			
Lack of fit	7	0.1260	0.0180	1.541	
Pure error	63	0.7355	0.0117		

* Tested and found significantly different ($p < 0.05$).
Regression line is $Y = 0.771 + 0.0021 X$.

Mean coefficient of condition (K) ($n = 8$) with 95% confidence limits (CL) of fish starved over 60 days and tested in still water or in current.

Days of starvation	Mean K and 95% CL in still	Mean K and 95% CL in current
0	1.229 ± 0.096	1.153 ± 0.060
4	1.006 ± 0.075	0.994 ± 0.085
8	1.040 ± 0.117	0.957 ± 0.061
12	1.025 ± 0.063	1.017 ± 0.084
16	0.963 ± 0.119	0.971 ± 0.099
20	0.988 ± 0.053	0.925 ± 0.094
24	1.052 ± 0.076	1.045 ± 0.079
36	0.974 ± 0.097	0.932 ± 0.051
60	0.892 ± 0.159	0.863 ± 0.087

Appendix 13.

Regression analysis on the effect of coefficient of condition on buoyancy attained by starved fish tested in still water or in current (35 $cm \cdot s^{-1}$) at $15^{o}C$.

Single Linear Regression Analysis for Fish in Still Water

Source	df	SS	MS	F
Regression (K)	1	0.0005	0.0005	1.773
Residual	62	0.0190	0.0003	
Total	63	0.0195		

Non-significant regression line is Y = 1.013 - 0.0197 X.

Single Linear Regression Analysis for Fish in Current

Source	df	SS	MS	F	r^2
Regression (K)	1	0.1908	0.1908	17.498*	20.0%
Residual	70	0.7633	0.0109		
Total	71	0.9541			

* Tested and found significantly different ($p < 0.05$).
Regression line is Y = 1.240 - 0.4348 X.

Appendix 14.

Regression analysis on the effect of fat content on buoyancy attained by starved fish tested in still water or in current (35 $cm \cdot s^{-1}$) at $15^{o}C$.

Single Linear Regression Analysis for Fish in Still Water

Source	df	SS	MS	F
Regression (% fat)	1	0.00006	0.00006	0.203
Residual	62	0.0195	0.0003	
Total	63	0.01956		

Single Linear Regression Analysis for Fish in Current

Source	df	SS	MS	F
Regression (% fat)	1	0.0027	0.0027	0.201
Residual	70	0.9513	0.0136	
Total	71	0.9540		