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

I am very grateful to my supervisor, Dr. J.H. Gee for his support, guidance and patience throughout this study. I express appreciation to Heather Smart and Bruce Stewart and all of the others who assisted me with the field work and preparation of this manuscript. I thank Brian Knudsen and Dr. A.N. Arnason for their advise on statistical problems. Also, I thank Drs. R.M. Evans, J.F. Klaverkamp and R.W. Newbury for their helpful criticism of this manuscript.

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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 (<u>Salmo salar</u>).

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 (<u>Pimephales promelas</u>) 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°C for 7 days prior to testing.	Held at a photoperiod of 12 h and 5°C. Fish were acclimated to 15°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 $cm \cdot s^{-1}$ for 24 h.	Tested at field photoperiod and 15°C. One batch in still water and one in a velocity of 35 ($\pm$ 2) $cm \cdot s^{-1}$ for 24 h.	Tested at a photoperiod of 12 h and 15°C. One batch in still water and one in a velocity of 35 ($\pm$ 2) $cm \cdot s^{-1}$ 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°C and therefore, fish collected from temperatures less than this were held and tested at 5°C. Fish were acclimated to desired temperatures by allowing at least 1 day for every 1.5°C change. Temperatures were controlled to within $\pm$ 0.5°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°C to slow down growth of the fish and then acclimated to 15°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 $5^{o}C$ 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 $15^{o}C$ 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 $15^{o}C$.

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.

Group	Photoperiod (h)		
	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·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

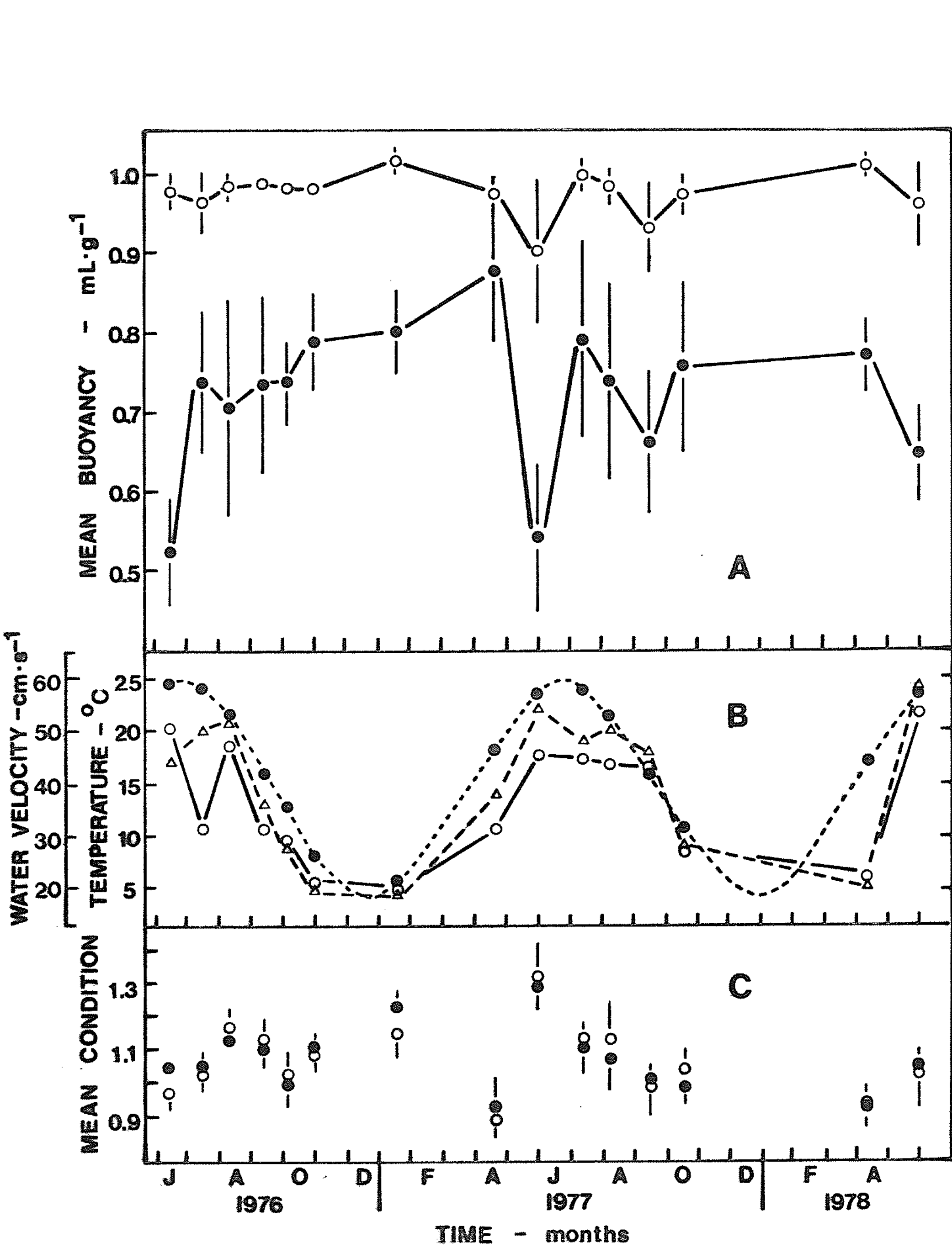
Seasonal factors

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

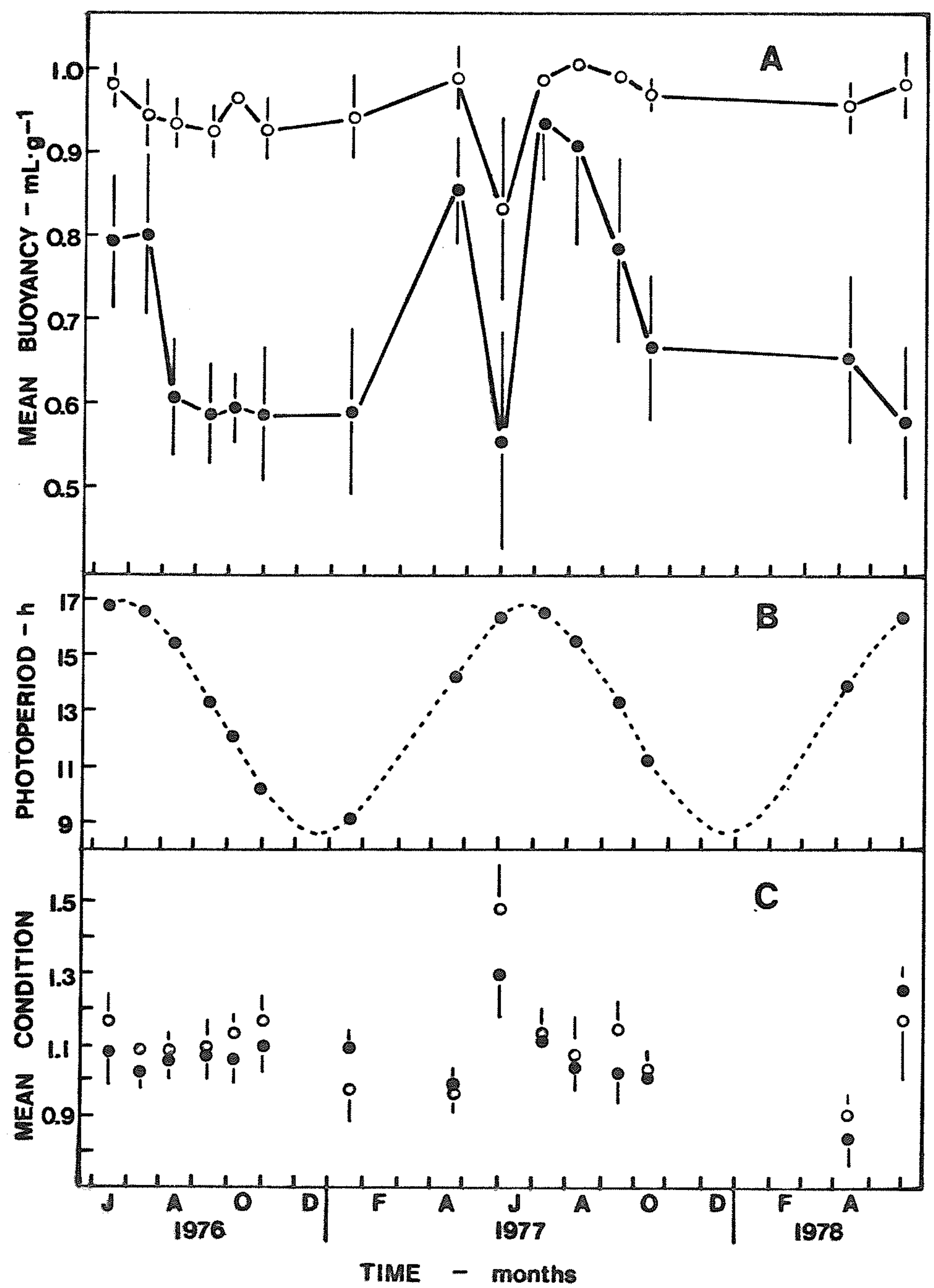
regression analyses; Appendix 4) in group A fish. In current, the regressions of buoyancy on water velocity, water temperature, photoperiod and coefficient of condition were also significant ($p < 0.05$, single linear regression analyses; Appendix 4). The slope of the regression line and the percentage 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.

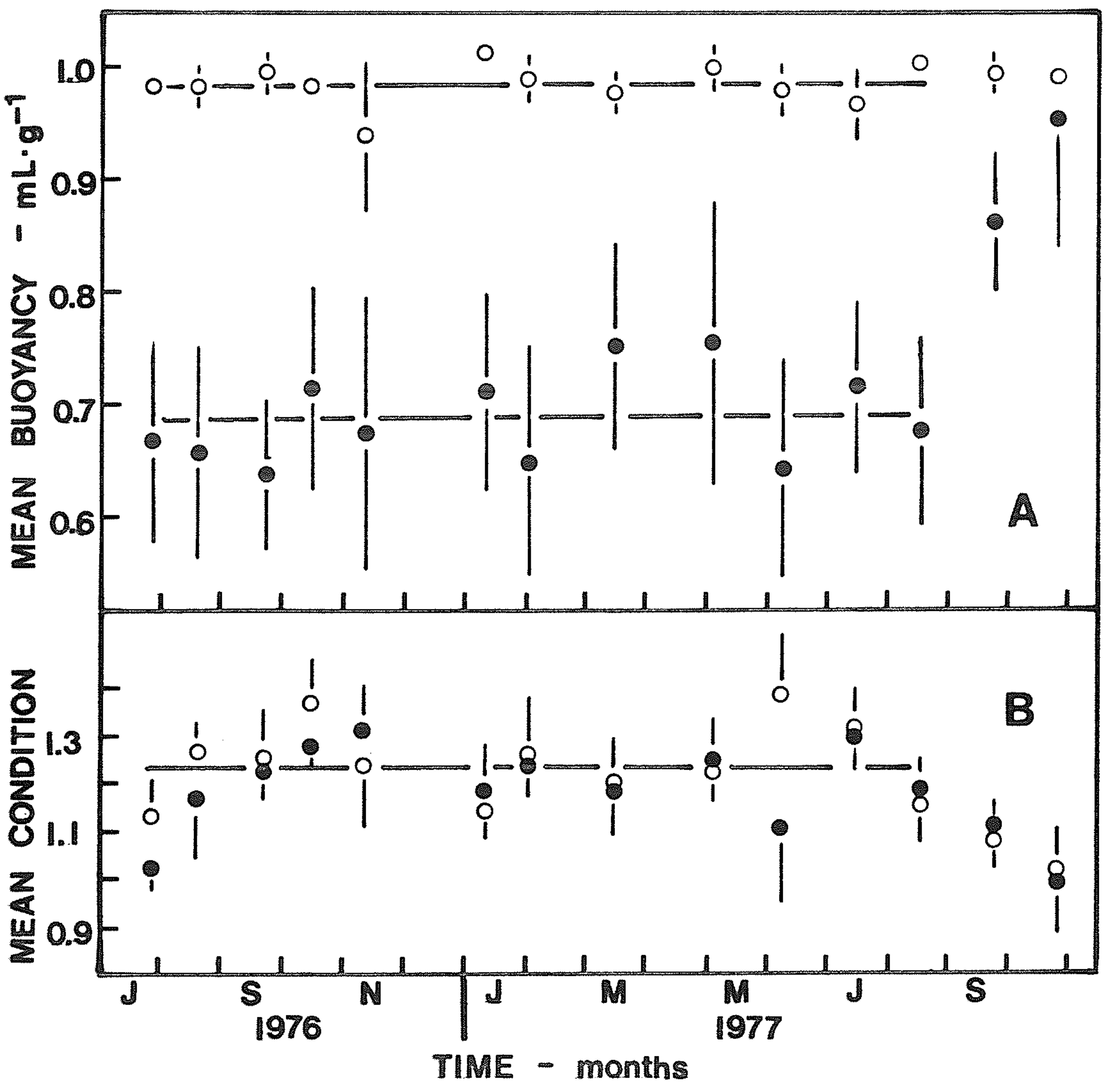

MEAN BUOYANCY - mL·g-1
1.0
0.9
0.8
0.7
0.6
0.5
A
PHOTOPERIOD - h
17
15
13
11
9
B
MEAN CONDITION
1.5
1.3
1.1
0.9
C
J A O D F A J A O D F A
1976
1977
1978
TIME - months

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

Group C fish. 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°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.


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