THE UNIVERSITY OF MANITOBA

BUOYANCY RESPONSE TO WATER VELOCITY BY CREEK CHUB (*SEMOTILUS ATROMACULATUS*) AND ITS FUNCTION

BY

GORDON BEREZAY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

OCTOBER, 1977

"BUOYANCY RESPONSE TO WATER VELOCITY BY CREEK CHUB (SEMOTILUS ATROMACULATUS) AND ITS FUNCTION"

by

GORDON BEREZAY

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

MASTER OF SCIENCE

ABSTRACT

Effect of size, acclimation temperature and water velocity on reduction of buoyancy is described. Creek chub reduced buoyancy when increased water velocity was encountered. Fish were near neutral buoyancy (0.956-0.997ml/g) in still water but in current small fish (<73mm) were more buoyant (0.668ml/g) than larger fish (0.540ml/g). Extent of buoyancy adjustment was dependent on an interaction of acclimation temperature and water velocity. At water velocities of 15 and 25cm/s fish acclimated to 6 and 12°C were more buoyant than those at 20 and 27°C. At 35cm/s differences were reduced and at 45cm/s differences were reversed. A reduction in buoyancy required 3-6h and rate of decrease (0.035-0.068ml/g/h) was independent of acclimation temperature and water velocity.

When initially exposed to current fish lowered the anterior portion of their body and assumed an angle of attack of 9-12° from horizontal. A horizontal (<1°) angle of attack was gradually assumed over the period of time that buoyancy was adjusted. Fish acclimated to moderate current for 12 or 24h tired less readily in strong current (80% took 43 and 44 min respectively) than those not acclimated or acclimated for 6h (80% took 33 and 25 min respectively). Fish induced to swim in still water reduced buoyancy to a similar extent as those in current. It is concluded that midwater fish such as the creek chub reduce buoyancy as water velocity increases to compensate for the lift created due to deflection of water over the dorsal surface of the body.

ACKNOWLEDGEMENTS

This research was supported by grants from the Northern Studies Committee of the University of Manitoba and the National Research Council of Canada. I am indebted to Dr. J.H.Gee for his suggestions and guidance throughout the study and during preparation of the manuscript.

Thanks are also expressed to Dr. E.Scherer and Mr. S.Nowak of the Freshwater Institute of Winnipeg, for equipment, expertise and guidance during the optomotor response experiment. I express appreciation for the technical aid during the study from Mr. M.Collins, Mr. E.Gyselman and Mr. R.Tallman.

TABLE OF CONTENTS

INTRODUCTION

When stream fish encounter an increased water velocity they reduce their swimbladder volume and thus buoyancy. When in still water the response is reversed and a near neutral buoyancy is attained (Gee 1977). Changes in swimbladder volume of between 30% and 60% are common. Most North American species display this response (Gee *et al*. 1974) and it is common amongst Central American stream fish (Gee and Gee 1976). Rates of adjustment vary between species with 6h to 4 days required to reduce buoyancy from a neutral level to a minimum level and 2-8 days required to inflate the swimbladder (Gee 1968, 1970, Machniak and Gee 1975, Gee 1977). Factors affecting the rate and extent of buoyancy adjustment remain largely unknown as do the precise reasons for the adjustment. The purpose of this study was to examine the effects of size of fish, water velocity and acclimation temperature on the rate and extent of buoyancy adjustment by creek chub, *Semotilus atromaculatus* (Mitchill) and to provide information on why this fish alters its buoyancy in response to changes in water velocity.

Water velocity is known to effect the extent of buoyancy adjustment as buoyancy was found to be inversely proportional to water velocity in juvenile Atlantic salmon (Saunders 1965, Neave *et al*. 1966, Pinder and Eales 1969), fathead minnow (Gee 1977) and juvenile rainbow trout (Gee unpublished data). The effect of water velocity on the rate of buoyancy change remains unknown.

Water temperature affects the extent of buoyancy adjustment with the greatest extent occurring at optimal temperatures in the fathead minnow (Gee 1977). At extreme high and low acclimation temperatures, the extent of buoyancy adjustment was reduced (Gee 1977). These results may be misleading as fish were tested at the maximum water velocity they could tolerate and this varied with acclimation temperature. Nothing is known of the interaction between water velocity and acclimation temperature in determining buoyancy response. Gee (1977) found in the fathead minnow, that rates of increase in buoyancy were temperature dependent but the rate of decrease was not, but here fish were examined at only two acclimation temperatures.

Alexander (1967) suggested that a reduced buoyancy in current increased the specific gravity of the fish resulting in greater frictional forces between the body of the fish and the substrate, affecting a substantial saving in energy. This may explain why demersal fish reduce buoyancy in current or have evolved a greatly reduced swimbladder volume, but it does not explain why fish swimming in midwater reduce buoyancy. Gee and Gee (1976) and Gee (1977) suggested that reduction of swimbladder volume may alter the hydrodynamics of the fish and result in greater swimming efficiency. Lift from the swimbladder (LB) of a neutrally buoyant fish, motionless in still water supports the weight of the fish (W) such that LB=W. When a fish holds position in current or swims steadily in still water, additional lift results from deflection of the water around the body of the fish (LS) and LS+LB>W. If uncorrected the fish would rise in the water column. The hypothesis tested here is that when fish hold a midwater position in current or swim steadily in still water they reduce the volume of the swimbladder to attain an equilibrium such that LS+LB=W. Thus energy would not be required to maintain vertical position in the water column.

This paper provides information on the effects of size of fish on extent of buoyancy adjustment, water velocity and acclimation temperature on extent of adjustment and rate of decrease in buoyancy. Tests of the above hypothesis were made by measuring the swimming angle of attack during buoyancy alteration, the time to fatigue when fish are exposed to current with different buoyancies, and the decrease in buoyancy when swimming in still water.

MATERIALS AND METHODS

Creek chub used in experiments were collected from Cypress Creek near Clearwater, Manitoba, between September 24 and November 7, 1975. They were held in the laboratory at 12±1°C under a photoperiod of ∠/D=12:12. In all experiments fish were tested in a photoperiod of ∠/D=14:10. Fish less than 75mm in length were fed Ewos Salmon Starter (size 3), while larger fish were fed Ewos Trout Starter (size 4P).

Fish were held in either still water or current for specific periods of time to attain maximum and minimum buoyancy respectively. Current was created in an aquarium (122x61x61cm) using the design of Gee and Bartnik (1969). No substrate was used in the swimming chamber and the bottom was opaque plexiglass. Water velocity (up to 85cm/s) was determined by averaging 5 measurements from different locations, taken 2.5cm from the bottom with an Ott current meter (Type C1). Still water conditions were created by holding fish in aquaria (122x61x61cm or 90x44x44cm) which contained a small gently-bubbling airstone.

To determine buoyancy fish were taken by net from either still water or current aquaria and anesthetized in a solution of MS-222 (tricane methanesulfonate). Fish were not observed to spit gas from their swimbladder during handling (Appendix 1). Buoyancy was expressed by dividing the swimbladder volume (±0.001ml) by the weight

(±0.001g) of the gas-free-fish in water (1.0ml/g=neutral buoyancy). Swimbladder volume was determined by taking the difference between the weight of the intact fish in water and the weight of the gas-free-fish in water. This difference in weight (g) equals swimbladder volume (ml), as at a given depth 1ml volume of air creates a buoyant lift of 1g.

EFFECT OF SIZE, ACCLIMATION TEMPERATURE AND WATER VELOCITY

To determine the effect of size of fish on buoyancy attained in still water and current (Experiment 1), three size groups (58-73, 74-150, 151-224mm, fork length) of fish were acclimated for two weeks at 20±1°C. Two batches of 10 fish were taken from each size group. One was held in still water for 72h, the other in current (35cm/s) for 24h before buoyancy was measured. Preliminary experiments showed that fish reached minimum buoyancy within 6h of exposure to current and maintained that level for 8 days (Appendix 2).

To study the effect of water velocity and acclimation temperature on extent of buoyancy adjustment (Experiment 2) four groups of fish were acclimated for at least 14 days at 6,12,20 and 27(±1)°C. At each temperature 5 batches of 7 fish were held at different water velocities (still water, 15,25,35 and 45cm/s) and buoyancy was measured after 24h. At 6° and 12°C, fish could not tolerate velocities in excess of 45cm/s.

To determine the effect of acclimation temperature on rate of decrease in buoyancy (Experiment 3), 4 groups of fish were acclimated for at least 2 weeks at 6,12,20 and 27 (±1)°C. For each group, batches of 7 fish were held in still water for at least 3 days and buoyancy measured after either 0,0.75,1.5,3,6,12 or 24h in current (45cm/s).

The effect of different water velocities on rate of buoyancy decrease (Experiment 4) was measured on fish at 20±1°C. Groups of fish were held in stillwater for 3 days and then exposed to water velocities of either 15,35, 45,60 or 75cm/s. Buoyancy was measured for batches of 7 fish after different periods of time in current (0,0.75, 1.5,3,6,12 or 24h).

WHY FISH REDUCE BUOYANCY WHILE SWIMMING

The swimming angle of attack was measured on creek chub following exposure to current (Experiment 5). An aspect meter mounted on a tripod (Fig.1) was used to measure the difference in angle between the horizontal axis of the fish and the axis of the water flow (Fig.2). Six fish were acclimated for 14 days at 12±1°C in still water. Swimming angle of attack was measured for fish exposed to current (45cm/s) after 0,0.25, 0.5, 0.75, 1,1.5,3 and 6h.

To determine if fish tired more quickly in current when not at the correct buoyancy (Experiment 6), a group of fish were acclimated at least 14 days at 12±1°C. Four batches of 10 fish were held in either still water for 72h or current (45cm/s) for either 6,12 or 24h prior to

Fig.1. Aspect meter for measuring swimming angle of attack. Viewing slit (A) on a moveable aspect pointer (B) is alligned with the horizontal axis of the fish and the angle of attack is read on scale (c).

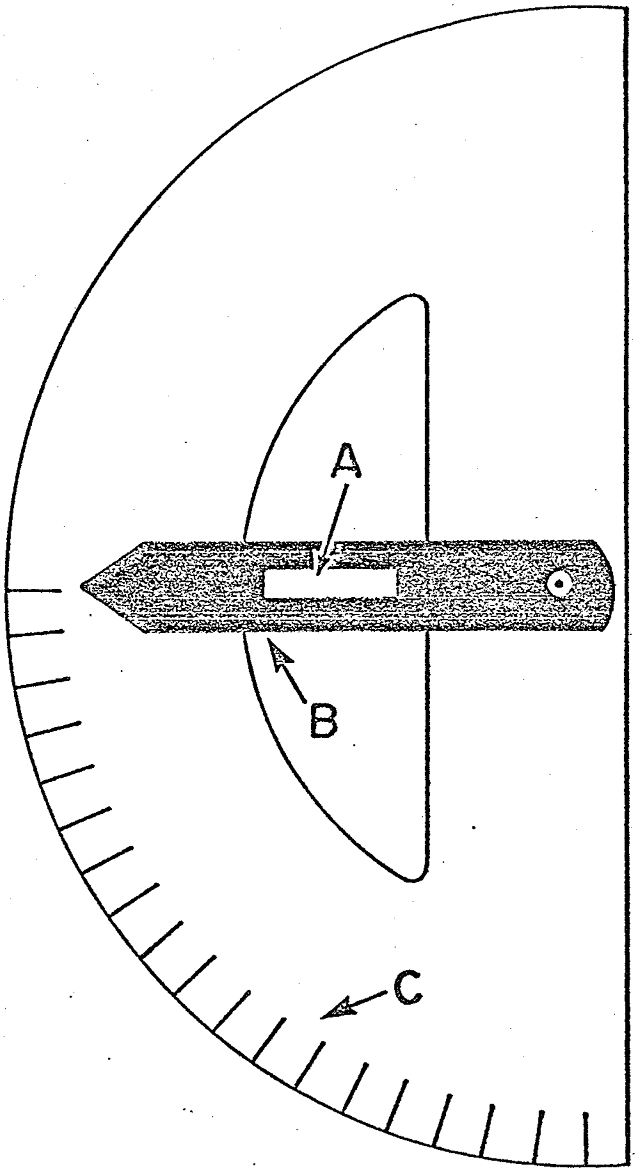
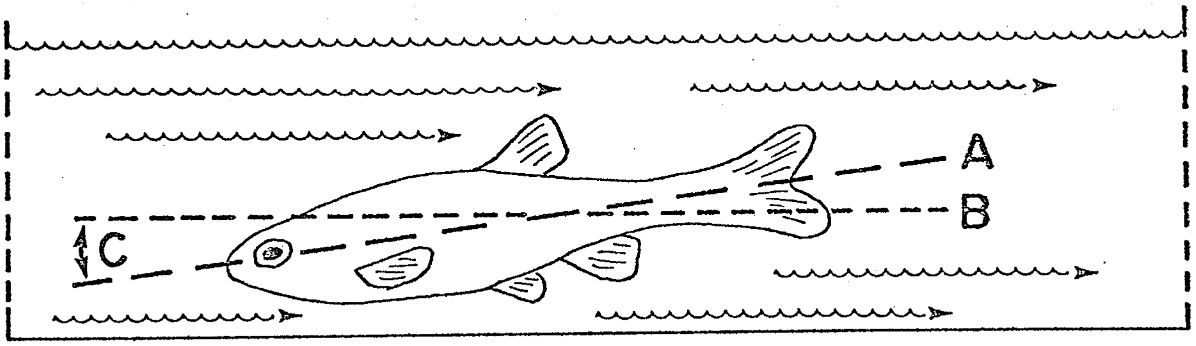

A
B
C

Fig.2. Relationship between horizontal axis of fish (A) and axis of current (B) resulting in angle of attack (C) of fish initially exposed to current.


A
B
C

exposure to a strong current (80cm/s). Time to fatigue was measured for each fish. It was expressed as the time exposed to strong current until the fish was unable to maintain position and rested against the outlet screen of the current chamber for 90s.

To determine the effect of swimming in still water on buoyancy (Experiment 7) a group of fish were acclimated at 20±1°C under a 12h photoperiod for 14 days. A batch of 7 fish were induced to swim at a mean speed of 15cm/s (range 12cm/s at inner rim and 18cm/s at outer rim) in an optomotor response system developed by Scherer and Nowak (in preparation) for 0.75h and their buoyancy determined. Still water buoyancy was determined for another batch of 7 fish.

RESULTS

EFFECT OF SIZE, ACCLIMATION TEMPERATURE AND WATER VELOCITY

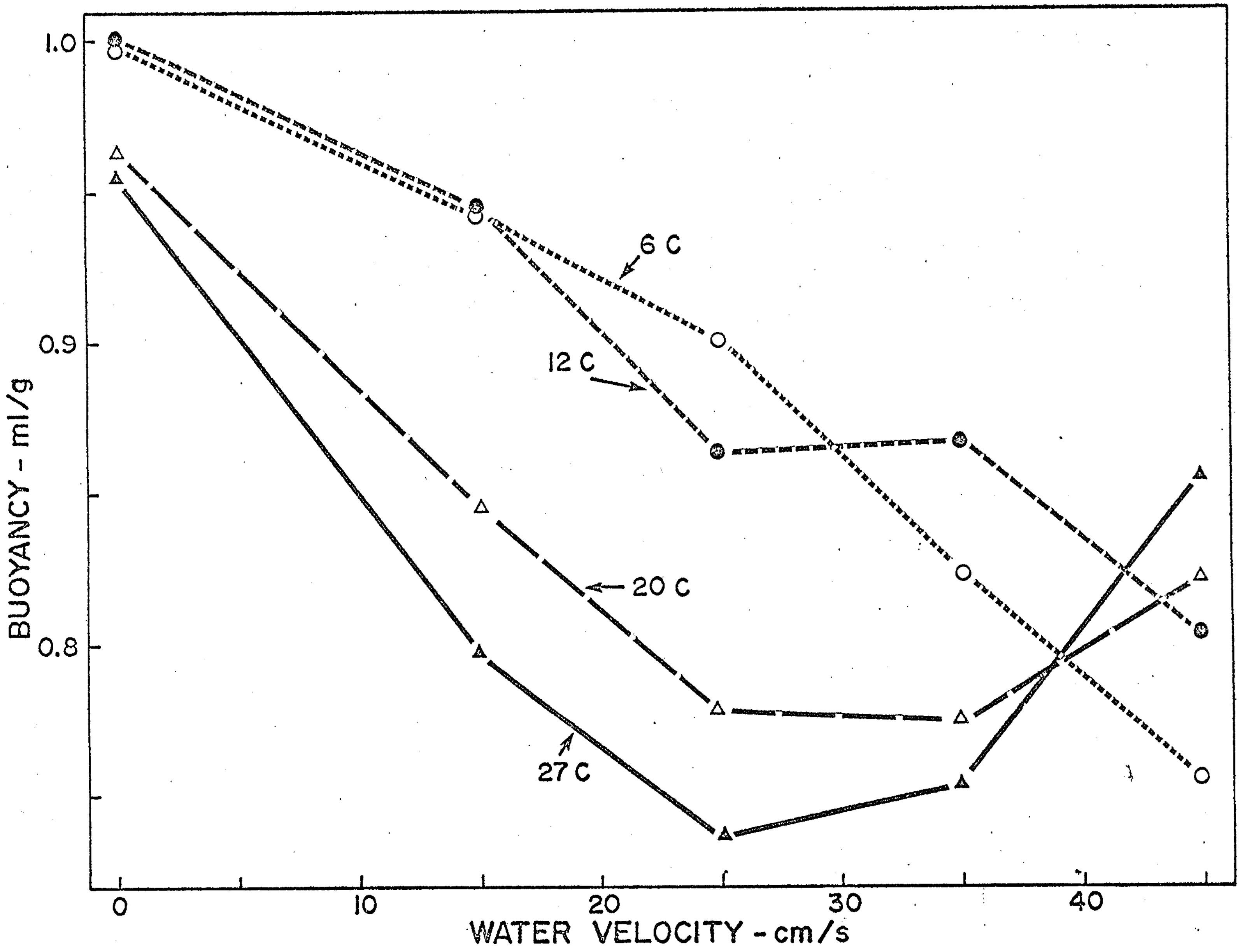
Experiment 1. Buoyancy did not vary significantly between size groups in still water and fish were at a near neutral buoyancy. In current, fish in the two larger size groups showed similar decreases in buoyancy, which were significantly ($p \leq 0.05$; analysis of variance; Appendix 3) greater than that of the smallest size group, shown as follows:

Fork length-mm		58-73	74-150	151-224
Mean buoyancy-ml/g(n=10):	Still	0.983	0.971	0.986
	Current	0.668	0.546	0.533

Experiment 2. The effects on buoyancy of water velocity, temperature and their interaction were significant ($p \leq 0.05$; two-way analysis of variance, Appendix 4). Buoyancy attained at 6 and 12°C diminished with increasing water velocity and the lowest buoyancy was achieved at the fastest water velocity (45cm/s; Fig.3). At 20 and 27°C the lowest buoyancy was attained at 25cm/s and at 20°C similar values were recorded at faster velocities, but at 27°C buoyancy increased significantly ($p \leq 0.05$; Student-Newman-Keuls test) from 25cm/s to 45cm/s (Fig.3).

Orthogonal comparisons showed that at each water velocity the mean buoyancy values were similar both at 6 and 12°C and at 20 and 27°C. In addition, when data in each of these pairs of temperatures were combined, differences between temperature groups were dependent on water velocity, shown as follows:

Fig.3. Effect of acclimation temperature (6,12,20 and 27^{o}C) and water velocity (0,15,25,35 and 45cm/s) on buoyancy response.


BUOYANCY - ml/g
1.0
0.9
0.8
WATER VELOCITY - cm/s
0
10
20
30
40
6 C
12 C
20 C
27 C

Water velocity-cm/s	0	15	25	35	45
Buoyancy-mean 6 and 12°C-ml/g	0.995	0.944	0.884	0.847	0.781
Buoyancy-mean 20 and 27°C-ml/g	0.960	0.822	0.760	0.076	0.840
Difference-ml/g	0.035	0.122	0.124	0.071	-0.059

<u>Experiment 3.</u> Fish reduced buoyancy at similar rates ($p \leq 0.05$; analysis of covariance; Appendix 5) when exposed to current at different acclimation temperatures, shown as follows:

Acclimation temperature	6	12	20	27
Slope of regression line	-0.00053	-0.00074	-0.00050	-0.00049

Differences between mean buoyancy values attained following 6h in current at 6 and 20°C were not significant but at 12 and 27°C fish increased buoyancy significantly ($p \geqq 0.05$; analysis of variance; Appendix 5) between 6 and 24h in current (Fig.4).

<u>Experiment 4.</u> When exposed to water velocity fish reduced buoyancy, reaching a minimum within 6h (Fig.5), independent of water velocity as slopes of regression lines calculated for each velocity were similar ($p \geq 0.05$; analysis of covariance, Appendix 6), shown as follows:

Water velocity-cm/s	15	35	45	60	75
Slope of regression line	-0.00030	-0.00048	-0.00048	-0.00050	-0.00051

Comparison of buoyancy values attained after 12h in water velocities of either 0,15,35,45,60 or 75cm/s indicate that velocity effects extent of buoyancy adjustment (one-way analysis of variance; $p \leq 0.05$; Appendix 6) and the still water buoyancy response is significantly different from all the others (Student-Newman-Kuels test; $p \leq 0.05$).

Fig.4. Effect of acclimation temperature on rate of decrease in buoyancy.

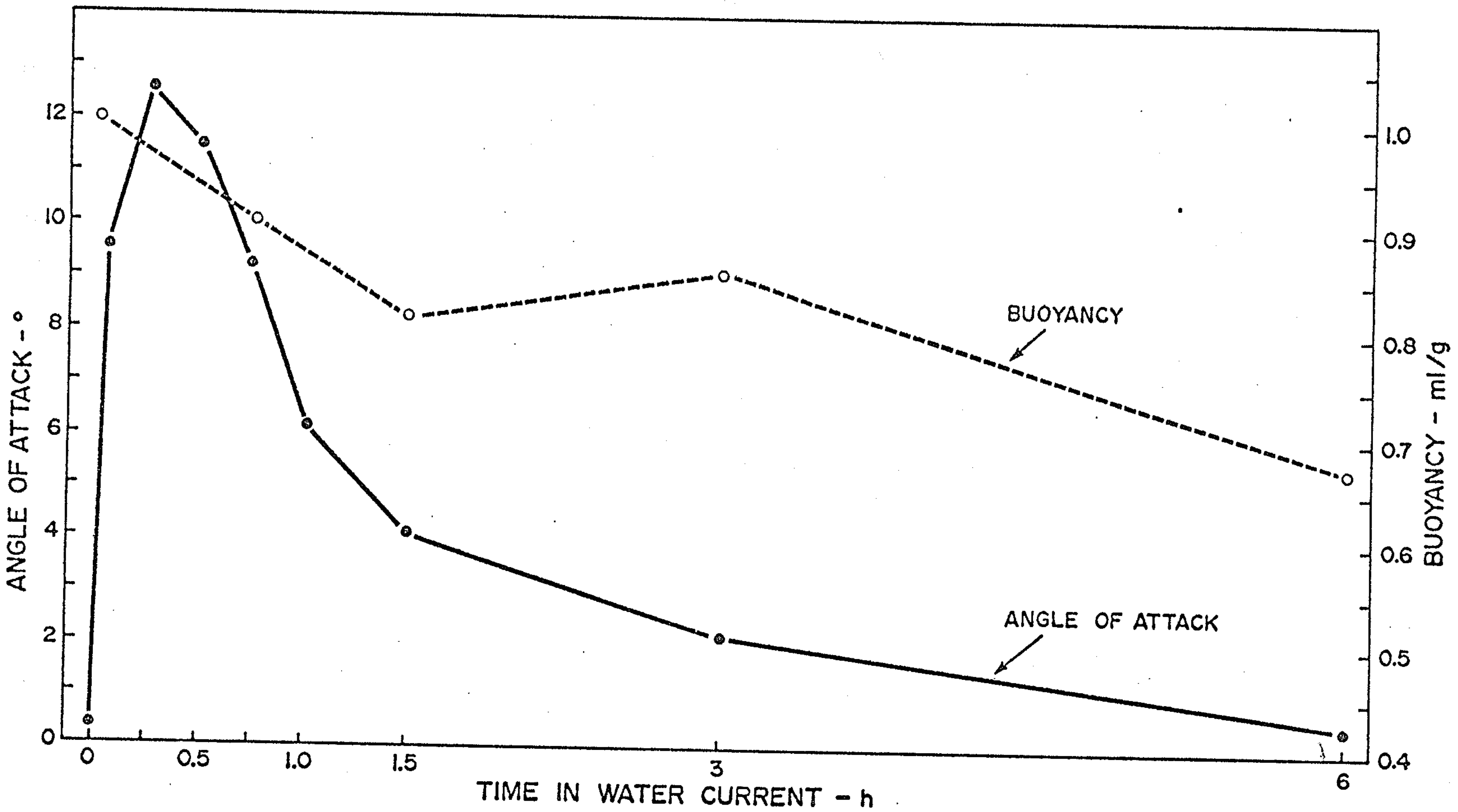
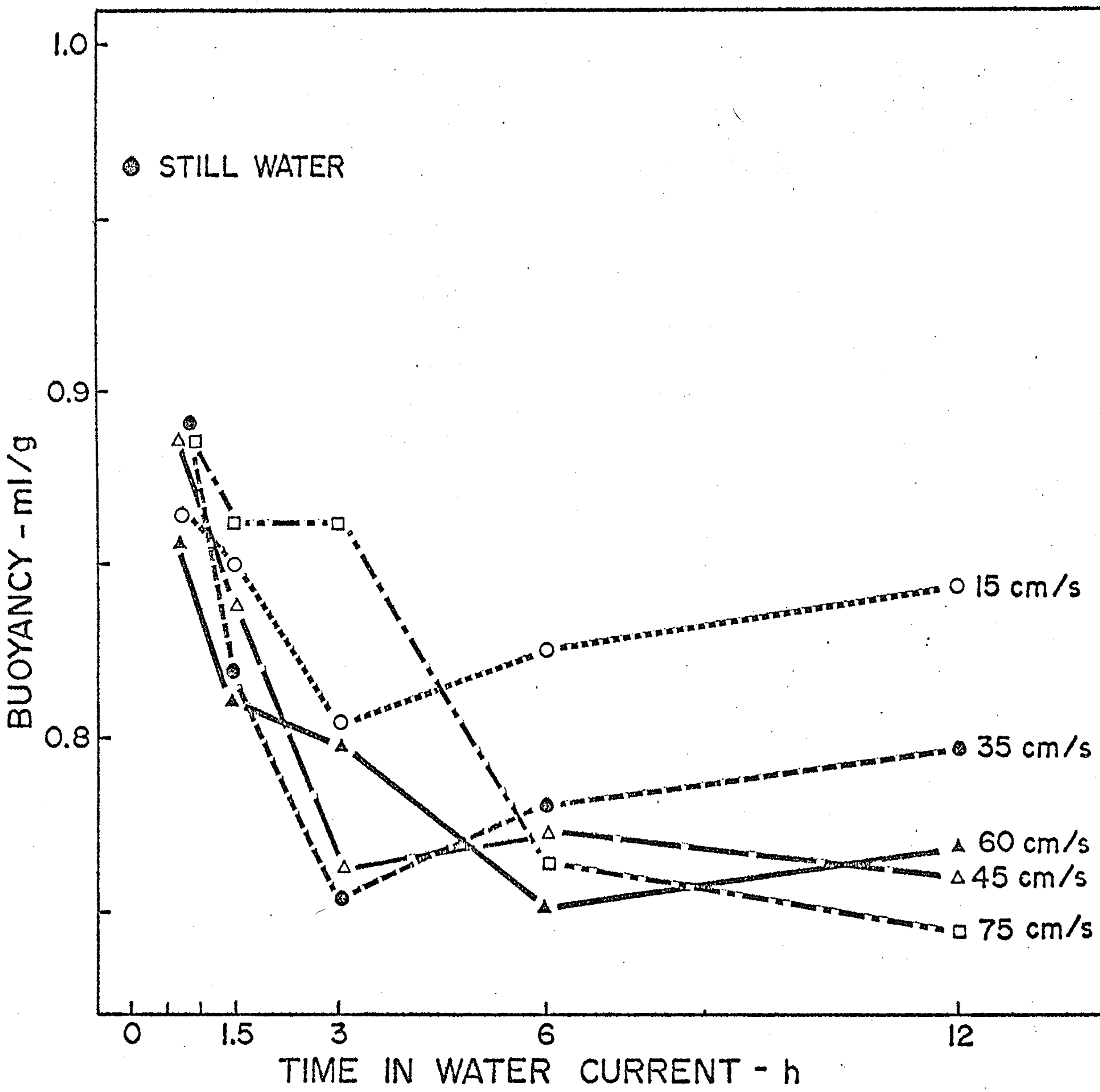

ANGLE OF ATTACK - °
0
2
4
6
8
10
12
BUOYANCY - ml/g
0.4
0.5
0.6
0.7
0.8
0.9
1.0
TIME IN WATER CURRENT - h
0
0.5
1.0
1.5
3
6
BUOYANCY
ANGLE OF ATTACK

Fig.5. Effect of water velocity on rate of decrease in buoyancy.


STILL WATER
15 cm/s
35 cm/s
60 cm/s
45 cm/s
75 cm/s
BUOYANCY - ml/g
1.0
0.9
0.8
0
1.5
3
6
12
TIME IN WATER CURRENT - h

WHY FISH REDUCE BUOYANCY

Experiment 5. When fish, acclimated to still water, initially encountered current (45cm/s) they showed an angle of attack in which the horizontal body axis was 9-12° from the horizontal axis of the water flow. This was reduced over time as buoyancy was reduced (Fig.6). After 6h in current, when buoyancy response was stabilized, the angle of attack was reduced to $\leq 1^{\circ}$ (horizontal axis of fish is almost parallel to current flow). These fish were visibly able to compensate more easily for turbulent fluctuations in the current and appeared to have decreased the frequency of their tail beats.

Experiment 6. Fish acclimated to moderate current (45cm/s) for 0 or 6h became fatigued more quickly in strong current (80cm/s) than those fish acclimated for 12 or 24h (Fig.7). In the former, 80% of the fish tired within 34 min exposure to strong current while the latter required 44 min before 80% of the fish were fatigued. Regression lines were calculated for the number of fish fatigued (up to 8) over time in the four periods of acclimation to current and were as follows:

0h; $Y= -1.60+0.042X$
6h; $Y=-19.66+0.065X$
12h; $Y= 2.86+0.028X$
24h; $Y= 1.16+0.027X$

Differences between slopes were significant (analysis of covariance; $p\leq 0.05$; Appendix 8).

Comparison between 0 and 6h lines and between 12 and 24h lines indicate that slopes of all lines are significantly different (analysis of covariance; $p\leq 0.05$; Appendix 8).

Fig.6. Relationship between angle of attack and buoyancy after exposure to current (45cm/s).

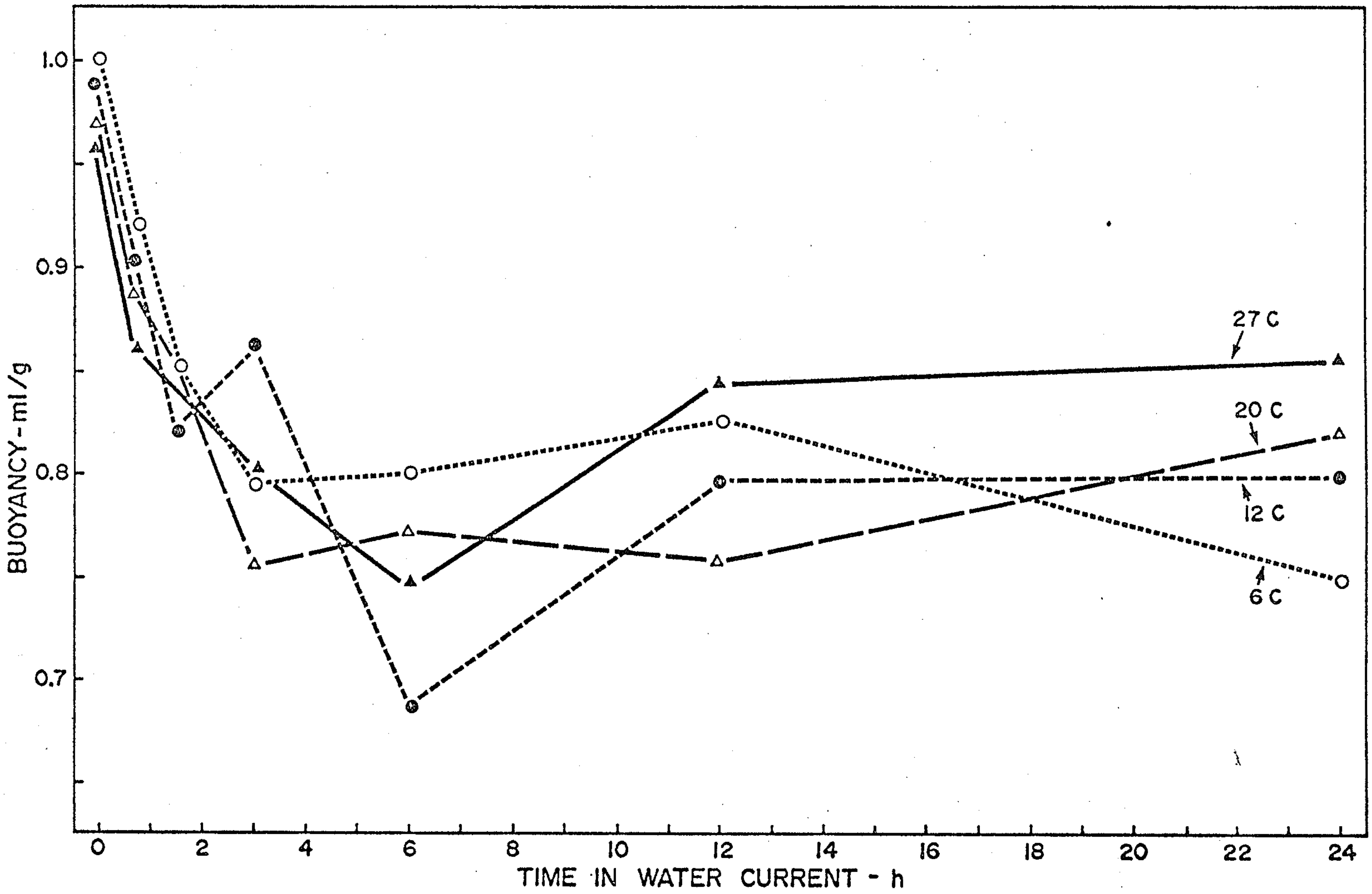
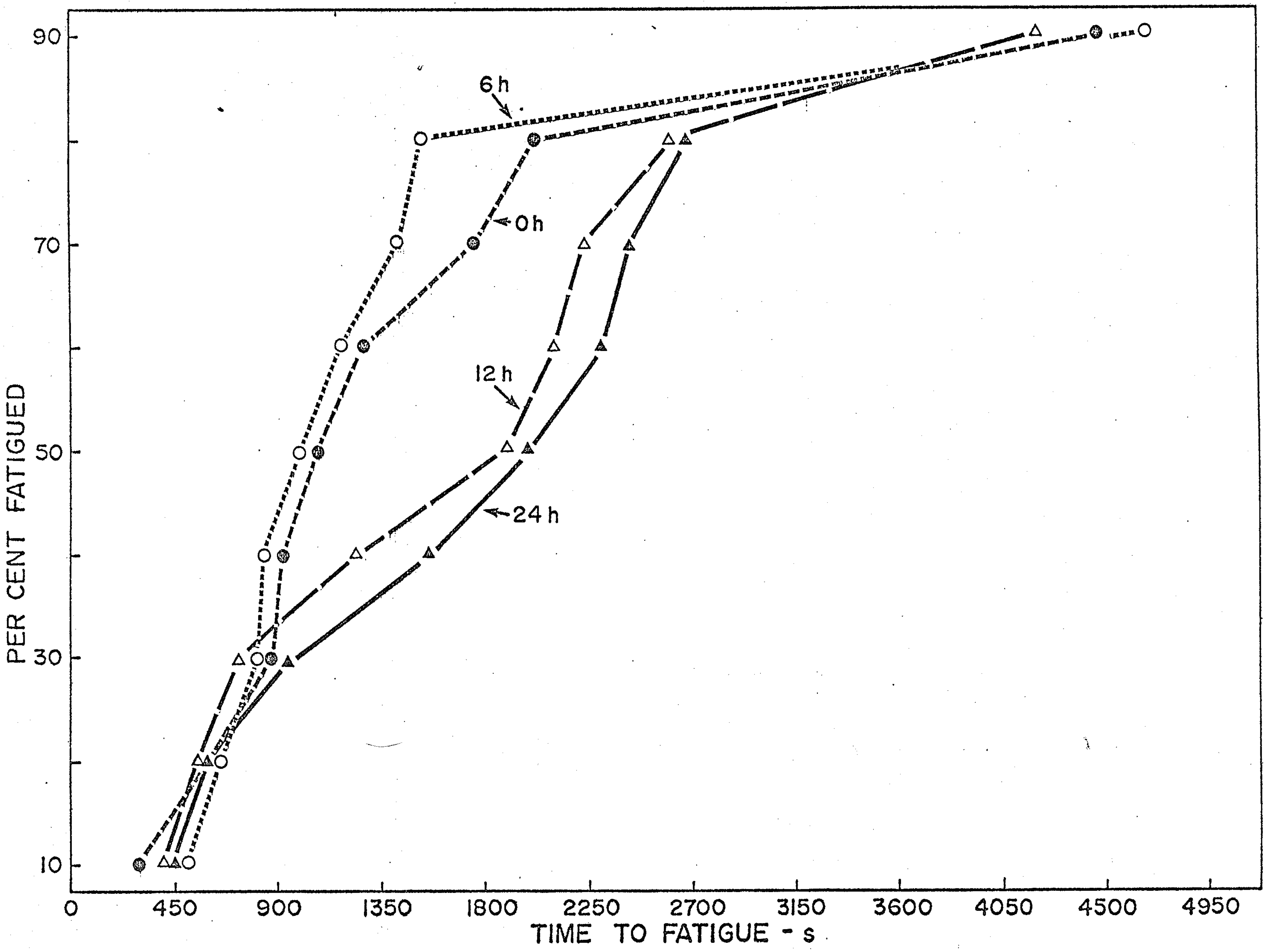

BUOYANCY - ml/g
1.0
0.9
0.8
0.7
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME IN WATER CURRENT - h
27 C
20 C
12 C
6 C

Fig.7. Effect of acclimation time in current on time to fatigue.


6 h
0 h
12 h
24 h
90
70
50
30
10
PER CENT FATIGUED
0
450
900
1350
1800
2250
2700
3150
3600
4050
4500
4950
TIME TO FATIGUE - s

Acclimation time in current (45cm/s) and thus buoyancy affect swimming efficiency or time to fatigue in strong current.

<u>Experiment 7.</u> When induced to swim at 15cm/s in still water fish reduced buoyancy significantly (one-way analysis of variance; $p \leq 0.05$). Extent of buoyancy adjustment in the optomotor tank and the current aquarium were similar with mean buoyancy values shown as follows:

Optomotor tank	-	resting	0.954
	-	induced to swim (45 min)	0.902
Current aquarium	-	still water	0.964
	-	current (45 min)	0.865

DISCUSSION

Creek chub attained a near neutral buoyancy in still water and reduced buoyancy when exposed to current. Extent of buoyancy adjustment in current was dependent upon size of fish, acclimation temperature and water velocity. The latter two factors interacted to produce a specific buoyancy. Rate of decrease of swimbladder volume was independent of acclimation temperature and water velocity. Fish acclimated to still water and then exposed to current had a nose-downward swimming angle of attack. The angle of attack returned to horizontal as buoyancy was decreased. Time to fatigue in strong current was dependent upon time acclimated to moderate water velocities.

EFFECT OF SIZE, ACCLIMATION TEMPERATURE AND WATER VELOCITY

The greatest reduction in buoyancy was made by the larger fish (>73cm)(Experiment 1). Such size dependent differences have been noted in several species (Gee 1968, 1972, 1977; Machniak and Gee 1975) and the range over which buoyancy is adjusted relates well to the water velocities encountered in the environment occupied by that particular species and size of fish. Smaller creek chub are most abundant in pools of streams and rivers where water velocity is least. All sizes would encounter increased rates of flow during spring runoff but the larger mature fish (>81cm) spawn over a two week period in May, at temperatures varying between

10-20^{o}C and in water velocities from 15-45cm/s (Moshenko and Gee 1973). Within these ranges of temperature and water velocity in the field, creek chub could adjust to the appropriate buoyancy level (Experiment 2) facilitating maintenance of territorial positions in current and thus enhancing success of spawning.

The rate of decrease in buoyancy was not affected by either acclimation temperature or water velocity (Experiment 4). The response was completed within 6h, a rate faster than that of Atlantic salmon parr and brook trout (36h; Saunders 1965), longnose dace (24-96h; Gee 1968), blacknose dace (132h; Gee 1970), fathead minnows (12-24h; Gee 1977) and tadpole madtom (96-192h; Machniak and Gee 1975). Gee (1977) also found that the rate of decrease in buoyancy in the fathead minnow was not affected by size, but the rate of decrease in the tadpole madtom was dependent on size (Mackhiak and Gee 1975). The mechanism of swimbladder reduction is not fully understood. Fish could either spit gas or it could be reabsorbed into the blood or both.

In Experiment 3 fish reduced buoyancy at all four acclimation temperatures in response to a water velocity of 45cm/s. The minimum buoyancy attained at 12 and 27^{o}C was not maintained after 6h and values reported at 12 and 24h were higher. The very low minimum buoyancy at 6h for 12^{o}C could have been caused by experimental error. There seems to be no physiological or hydrodynamical explanation for such a pattern in buoyancy reduction. At 27°C creek chub may have been stressed by high temperature resulting in an increase in buoyancy with time in current (discussed later).

The ability to reduce swimbladder volume at a rate independent of temperature and water velocity could be an advantage during times of increased flow, because a constant rate of decrease may allow a more sensitive regulation of swimbladder volume.

Extent of buoyancy reduction was influenced by the interaction of water velocity and acclimation temperature (Experiment 2). Gee (1977) found differences in minimum buoyancy at different acclimation temperatures in the fathead minnow. At 4.5 and 10°C buoyancy attained in current was significantly higher than at 20 and 25°C. Creek chub differ in their response in that minimum buoyancy achieved was independent of temperature. An acclimation temperature of 27°C appeared to influence the time over which minimum buoyancy could be maintained.

Fish acclimated at 27°C increased buoyancy significantly at higher water velocities (>25cm/s). In this treatment creek chub were near their upper incipient lethal temperature (30.3°C; Hart 1952) and at higher water velocities may have been metabolically stressed. Lactic acid would be produced (Black *ét al*. 1959) increasing gas input into the swimbladder and decreasing reabsorption due to the Bohr and Root effects (Steen 1963). Swimbladder volume would increase, resulting in loss of buoyancy control. The nonoptimal respiratory conditions which result would compound the stress on the metabolic processes of the fish.

Due to the unsteady swimming motion of fish (Lighthill 1970, 1971) there is an intrinsic drag force created at a horizontal angle of attack. As viscosity decreases at higher temperatures, frictional drag is decreased (Webb 1975) resulting in increased lift. Swimbladder volume could be reduced more at higher temperatures to compensate for the increased lift. This could explain the lower buoyancy values attained at higher temperatures at the same water velocity (Experiment 2) assuming the proposed hypothesis is correct.

WHY FISH REDUCE BUOYANCY

Various lines of argument support the hypothesis that lift due to swimbladder volume (LB) is reduced to compensate for increased lift from the body (LS) as an increased water velocity is encountered (LS+LB=W). First of all the extent of buoyancy reduction increases as water velocity increases in a number of species including juvenile Atlantic salmon (Neave et al. 1966), fathead minnows (Gee 1977) and rainbow trout (Gee unpublished data), as well as in creek chub. Lift would be increased at higher water velocities requiring greater reduction in swimbladder volume. Secondly, creek chub require 3-6h to reduce buoyancy to the minimum sustained buoyancy level (Experiments 3 and 4; Appendix 2). To compensate for the increased lift during the time buoyancy is adjusted, the swimming angle of attack is lowered as current is initially encountered and the body of the fish gradually approaches a horizontal position as buoyancy is adjusted (Experiment 5). This initial correction for lift is very inefficient as drag would be greatly increased, a factor that would favour a rapid reduction in buoyancy so that the fish can attain maximum benefit from its streamlined body shape.

Thirdly, fish at or near neutral buoyancy are less tolerant of fast water velocities than those which have a reduced buoyancy (Experiment 6). Fish which were not acclimated to current or were acclimated for only 6 hours, tired more quickly than those acclimated to current for either 12 or 24 hours. At 6 hours fish have adjusted buoyancy but still appear to be intolerant of current. This may be due to stress encountered while adjusting buoyancy resulting in lactic acid formation, which is removed very slowly from the body tissues (Black *et al*. 1959).

Fourthly, fish swimming in still water would generate similar lift forces as those holding position in current (Experiment 7). These fish reduced buoyancy suggesting that body shape does create lift.

Finally, indirect evidence supporting the hypothesis was observed in the northern pike (*Essox lucius*) which increased buoyancy when exposed to current (Gee *et al*. 1974).

The largest portion of the body is below midline, with a flat back, converse to that of the creek chub. Deflection of water around its body would create a downward force rather than an upward force requiring an increase in swimbladder volume to maintain the equilibrium between lift and weight (LS+LB=W).

Magnuson (1970) suggested that lift forces created by fins and swimbladder of *Euthynnus affinis*, swimming at a constant speed equals the weight of the fish in water. The swimbladder of continuously swimming Scombrids is generally lacking or too small to maintain neutral buoyancy (Magnuson 1970). Natural selection may have resulted in a reduced swimbladder volume conserving internal space in these fish. This suggests that buoyant lift required from the swimbladder of continuously swimming fishes may be reduced.

Water velocity and temperature interact to determine a specific buoyancy level although neither factor affects the rate of decrease in buoyancy as current is encountered. The hypothesis that creek chub reduced buoyancy in water velocity to overcome the lift produced by the hydrofoil shape of their body is substantiated.

LITERATURE CITED

Alexander, R.McN. 1967. Functional design in fishes. Hutchinson University Library, London. pp.1 to 160.

Black, E.C., W.G.Chi, F.D.Forbes and A.Hanslip. 1959. Changes in pH, carbonate and lactae of the blood of yearling trout, Salmo gairdneri, during and following severe muscular activity. J. Fish. Res. Board Can. 16:391-402.

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

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

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

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

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

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

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

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

Hart, J.S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. Biological Series No.60. University of Toronto Press. Toronto. pp. ii to 79.

Lighthill, M.J. 1970. Aquatic animal propulsion of high hydrodynamical efficiency. J. Fluid Mech. 44:265-301.

------.1971. Large amplitude elongate-body theory of fish locomotion. Proc. R. Soc. Lond. B. 179:125-138.

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

Magnuson, J.J. 1970. Hydrostatic equilibrium of Euthynnus affinis, a pelagic teleost without a gas bladder. Copeia 1970:56-85.

Moshenko, R.W. and J.H.Gee. 1973. Diet, time and place of spawning and environment occupied by creek chub (Semotilus atromaculatus) in the Mink River, Manitoba. J. Fish. Res. Board Can. 30:357-362.

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

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

Saunders, R.L. 1965. Adjustment of buoyancy in young Atlantic salmon and brook trout by changes in swim-bladder volume. J. Fish. Res. Board Can. 22:335-352.

Steen, J.B. 1963. The physiology of the swimbladder in the eel _Anquilla_ _vulgaris_ II. The reabsorption of gases. Acta Physiol. Scand. 68:138-149.

Verheijen, F.J. 1962. Gas spitting by alarmed fish disturbs their hydrostatic equilibrium. Science 137: 864-865.

Webb, P.W. 1975. Hydrodynamics and energetics of fish propulsion. Bull. Fish. Res. Board Can. 190:159 p.

Appendix 1. Effect of handling procedure on buoyancy adjustment of creek chub.

Verheijen (1962) reported gas-spitting in the physostomous creek chub when an alarm substance was released in the water. Due to handling procedures it was necessary to determine if handling influenced buoyancy.

A group of fish were acclimated at 20±1°C for 14 days. A batch of 8 fish were netted from the still water aquaria and their buoyancy measured. Another batch of 8 fish were netted from the holding tank and exposed to current (35cm/s) for 3 min before determing their buoyancy (Table 1A). Comparison of means (one-way analysis of variance; $p \geq 0.05$; Table 1A) indicated that transfer of fish from holding to current aquaria does not influence buoyancy significantly.

TABLE 1A. Buoyancy response of creek chub in still water and in current (35±2cm/s) for 3 min to determine if handling initiates gas-spitting.

	BUOYANCY (ml/g)	
	STILL WATER	CURRENT
	0.952	0.968
	0.948	0.981
	0.986	0.948
	0.946	0.993
	0.976	0.923
	0.984	0.972
	0.993	0.929
	0.982	0.918
MEAN	0.971	0.954
STANDARD DEVIATION	0.018	0.029

ANALYSIS OF VARIANCE TABLE

Source	df	ss	MS	F
Water velocity	1	0.0011	0.0011	1.94
Error	14	0.0082	0.0006	
Total	15	0.0093		

Appendix 2. Buoyancy response over an 8 day period, for fish acclimated at 6 and 20±1°C for 14 days and exposed to water velocities of 35cm/s and 45cm/s respectively.

TIME IN CURRENT-h	MEAN BUOYANCY-ml/g 6°C	20°C	95% Confidence Intervals 6°C	20°C
0.00	0.995	0.971	0.984-1.006	0.955-0.987
0.45	0.973	0.803	0.950-0.996	0.746-0.860
1.5	0.941	0.742	0.902-0.980	0.657-0.827
3	0.827	0.768	0.765-0.889	0.673-0.825
6	0.809	0.620	0.730-0.888	0.519-0.721
12	0.821	0.634	0.754-0.887	0.549-0.719
24	0.876	0.624	0.827-0.926	0.540-0.708
48	0.884	0.575	0.826-0.941	0.514-0.636
96	0.852	0.633	0.729-0.975	0.592-0.674
192	0.878	0.629	0.825-0.931	0.535-0.723

Fish reached minimum buoyancy within 6h and there was no significant change after that time. The swimbladder volume fluctuates randomly about the equilibrium buoyancy level, constantly absorbing and resorbing gas (Fänge 1966) and may be in a dynamic equilibrium with the ability to respond quickly to a fluctuation in water velocity.

Appendix 3. Effect of size on extent of buoyancy adjustment showing mean, standard deviation and 95% confidence intervals.

	STILL				CURRENT	
58-73	74-150	151-224		58-73	74-150	150-224
0.954	0.919	0.991		0.542	0.486	0.764
0.967	0.867	0.917		0.640	0.642	0.680
1.000	1.053	1.189		0.671	0.692	0.612
1.006	0.983	1.028		0.681	0.632	0.544
0.977	0.934	0.956		0.653	0.385	0.562
0.992	1.146	0.980		0.630	0.566	0.599
0.983	1.022	0.927		0.674	0.524	0.550
0.973	0.923	0.978		0.778	0.552	0.430
0.984	0.892	0.965		0.657	0.500	0.633
0.981	0.971	0.986	$\bar{x}$	0.668	0.546	0.533
0.015	0.084	0.079	S.D	0.065	0.092	0.198
0.003	0.064	0.057	95%C.1.	0.046	0.066	0.142

Appendix 3. continued.

ANALYSIS OF VARIANCE TABLE FOR STILL WATER

Source	df	SS	MS	F
Size group	2	0.011	0.001	0.126
Error	27	0.122	0.005	
Total	29	0.133		

ANALYSIS OF VARIANCE TABLE FOR FISH IN CURRENT

Source	df	SS	MS	F
Size group	2	0.079	0.040	5.49
Error	27	0.195	0.007	
Total	29	0.274		

Appendix 4. Effect of current velocity and acclimation temperature on buoyancy adjustment. Standard deviation and 95% confidence interval given for mean buoyance response after 24h exposure for each current-temperature combination.

Temperature	Water Velocity	Mean Buoyancy-ml/g	Standard Deviation	95% Confidence Interval for Mean
6°C	0	0.993	0.026	0.969-0.017
	15	0.943	0.048	0.899-0.987
	25	0.904	0.057	0.851-0.958
	35	0.824	0.101	0.731-0.917
	45	0.756	0.076	
12°C	0	0.997	0.007	0.991-1.003
	15	0.945	0.042	0.906-0.984
	25	0.863	0.059	0.808-0.918
	35	0.869	0.069	0.805-0.933
	15	0.805	0.121	0.693-0.917
20°C	0	0.964	0.027	0.939-0.989
	15	0.847	0.075	0.778-0.916
	25	0.780	0.035	0.748-0.812
	35	0.777	0.070	0.713-0.841
	45	0.822	0.090	
27°C	0	0.956	0.056	0.904-1.008
	15	0.797	0.073	0.729-0.865
	25	0.739	0.090	0.656-0.822
	35	0.775	0.077	0.704-0.846
	45	0.858	0.077	0.787-0.927

Appendix 4. continued.

2-WAY ANALYSIS OF VARIANCE TABLE

SOURCE	df	SS	MS	F
Current	4	5.019	1.255	204.295*
Temperature	3	0.962	0.321	39.168*
Interaction	12	1.221	0.102	49.685*
Error	120	3.443	0.025	
Total		10.645		

* Tested and found significantly different.

Appendix 5. Effect of acclimation temperature on rate of buoyancy adjustment. Mean, standard deviation and 95% confidence intervals, using groups of seven fish for each time-temperature combination at a current velocity of 45cm/s.

Hours In Current at °C	Mean Buoyancy -ml/g	Standard Deviation	95% Confidence Interval for Mean
6°C			
0.00	0.993	0.026	0.969-1.017
0.75	0.920	0.042	0.881-0.959
1.50	0.851	0.049	0.806-0.896
3.00	0.792	0.074	0.724-0.860
6.00	0.805	0.074	0.738-0.873
12.00	0.828	0.087	0.748-0.908
24.00	0.756	0.076	0.687-0.825
12°C			
0.00	0.997	0.007	0.991-1.003
0.75	0.906	0.069	0.842-0.970
1.50	0.820	0.090	0.737-0.903
3.00	0.862	0.061	0.806-0.918
6.00	0.687	0.091	0.603-0.771
12.00	0.793	0.102	0.699-0.887
24.00	0.805	0.121	0.693-0.917
20°C			
0.00	0.964	0.027	0.939-0.989
0.75	0.887	0.036	0.853-0.920
1.50	0.852	0.041	0.814-0.890
3.00	0.760	0.035	0.728-0.792
6.00	0.772	0.051	0.725-0.819
12.00	0.762	0.070	0.697-0.827
24.00	0.822	0.090	0.913-0.731
27°C			
0.00	0.956	0.056	0.904-1.008
0.75	0.862	0.038	0.827-0.897
1.50	0.842	0.043	0.802-0.882
3.00	0.805	0.045	0.0.763-0.847
6.00	0.748	0.079	0.675-0.821
12.00	0.842	0.105	0.745-0.939
24.00	0.858	0.077	0.787-0.927

Appendix 5. continued.

ANALYSIS OF COVARIANCE TABLE

		SS			ADJUSTED FOR COVARIANT		
Source	df	X^2	XY	Y^2	df	SS	MS
Temperature Groups	3	0.0	0.0	0.008			
Error	16	324,000.00	-182.970	0.136	15	0.033	0.002
Total	19	324,000.00	-182.970	0.144	18	0.041	
					3	0.008	0.003

$Ho_1 : \sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$

$\chi_3^2 = 0.559$ $\alpha = 0.05$

$Ho_2 : \beta_1 = \beta_2 = \beta_3 = \beta_4$

$F_{3,12} = 3.992$ $\alpha = 0.025$

$Ho_3 : \beta_0^1 = \beta_0^2 = \beta_0^3 = \beta_0^4$

$F_{3,1}{}^{5} = 1.116$ $\alpha = 0.05$

ANALYSIS OF VARIANCE TABLE-6°C DATA-45cm/s

Source	df	SS	MS	F
Between Time Groups	6	0.267	0.045	10.454
Error	42	0.168	0.004	
Total	48	0.435		

ANALYSIS OF VARIANCE TABLE-12°C DATA 45cm/s

Source	df	SS	MS	F
Between Time Groups	6	0.375	0.063	8.29
Error	42	0.336	0.008	
Total	48	0.711		

ANALYSIS OF VARIANCE TABLE-20°C DATA-45cm/s

Source	df	SS	MS	F
Between Time Groups	6	0.243	0.041	14.033
Error	42	0.126	0.003	
Total	48	0.369		

Appendix 5. continued.

ANALYSIS OF VARIANCE TABLE-27^{o}C DATA-45cm/s

Source	df	SS	MS	F
Between Time Groups	6	0.164	0.027	6.23
Error	42	0.168	0.004	
Total	48	0.332		

Appendix 6. Effect of water velocity on rate of buoyancy adjustment. Standard deviation and 95% confidence intervals for rate of change of mean buoyancy at 20°C at different current velocities.

Hours In Current At cm/s	Mean Buoyancy -ml/g	Standard Deviation	95% Confidence Interval for Mean
15cm/s			
0.00	0.964	0.027	0.939-0.989
0.75	0.865	0.052	0.817-0.913
1.50	0.839	0.054	0.789-0.889
3.00	0.802	0.084	0.772-0.832
6.00	0.824	0.028	0.798-0.850
12.00	0.843	0.045	0.801-0.885
24.00	0.847	0.075	0.778-0.916
35cm/s			
0.00	0.964	0.027	0.939-0.989
0.75	0.911	0.035	0.879-0.943
1.50	0.819	0.054	0.769-0.869
3.00	0.757	0.092	0.672-0.842
6.00	0.781	0.045	0.739-0.823
12.00	0.797	0.087	0.717-0.877
24.00	0.777	0.070	0.713-0.841
45cm/s			
0.00	0.964	0.027	0.939-0.989
0.75	0.887	0.036	0.853-0.920
1.50	0.852	0.041	0.814-0.890
3.00	0.760	0.035	0.728-0.792
6.00	0.772	0.051	0.725-0.819
12.00	0.762	0.070	0.697-0.827
24.00	0.822	0.090	0.731-0.913
60cm/s			
0.00	0.964	0.027	0.939-0.989
0.75	0.857	0.063	0.799-0.915
1.50	0.817	0.041	0.779-0.855
3.00	0.800	0.042	0.761-0.839
6.00	0.752	0.098	0.661-0.843
12.00	0.767	0.050	0.721-0.813
24.00	0.776	0.066	0.715-0.837
75cm/s			
0.00	0.964	0.027	0.939-0.989
0.75	0.901	0.057	0.848-0.954
1.50	0.863	0.069	0.799-0.927
3.00	0.864	0.056	0.812-0.916
6.00	0.765	0.097	0.675-0.855
12.00	0.744	0.106	0.646-0.842
24.00	0.748	0.042	0.709-0.787

Appendix 6. continued.

ANALYSIS OF COVARIANCE

		SS			ADJUSTED FOR COVARIATE		
Source	df	X^2	XY	Y^2	df	SS	MS
Current Groups	4	0.0	0.0	0.003			
Error	20	405,000.00	-183.780.	0.124	19	0.040	0.002
Total	24	405,000.00	-183.780	0.127	23	0.044	
					4	0.003	0.0008

$Ho_1: \sigma_1^2=\sigma_2^2=\sigma_3^2=\sigma_4^2$

$\chi_3^2=0.472$ $\alpha=0.05$

$Ho_2: \beta_1=\beta_2=\beta_3=\beta_4$

$F_{4,15}=2.588$ $\alpha=0.05$

$Ho_3: \beta_0^1=\beta_0^2=\beta_0^3=\beta_0^4$

$F_{4,19}=0.357$ $\alpha=0.05$

ANALYSIS OF VARIANCE TABLE-15cm/s-20°C

Source	df	SS	MS	F
Between Time Groups	6	0.114	0.019	6.061
Error	42	0.126	0.003	
Total	48	0.240		

ANALYSIS OF VARIANCE-35cm/s-20°C

Source	df	SS	MS	F
Between Time Groups	6	0.255	0.042	10.669
Error	42	0.167	0.004	
Total	48	0.422		

ANALYSIS OF VARIANCE-45cm/s-20°C

Source	df	SS	MS	F
Between Time Groups	6	0.243	0.041	14.033
Error	42	0.126	0.003	
Total	48	0.369		

Appendix 6. continued.

ANALYSIS OF VARIANCE TABLE-60cm/s-20°C

Source	df	SS	MS	F
Between Time Groups	6	0.225	0.038	10.604
Error	42	0.149	0.004	
Total	48	0.374		

ANALYSIS OF VARIANCE TABLE-75cm/s-20°C

Source	df	SS	MS	F
Between Time Groups	6	0.281	0.047	8.175
Error	42	0.241	0.006	
Total	48	0.522		

ANALYSIS OF VARIANCE TABLE COMPARING 12h BUOYANCY AT 0,15,35,45,60 and 75cm/s

Source	df	SS	MS	F
Current vel.	5	0.234	0.047	9.66
Error	36	0.175	0.005	
Total	41	0.409		

Appendix 7. Swimming angle of attach in response to current.

Standard deviation and 95% confidence interval of mean buoyancy response over time, at 12°C in current (45cm/s).

Hours In Current-h	Mean Buoyancy-ml/g	Standard Deviation	95% Confidence Interval for Mean
0.00	0.997	0.007	0.991-1.003
0.75	0.906	0.069	0.842-0.970
1.50	0.820	0.090	0.737-0.903
3.00	0.862	0.061	0.806-0.918
6.00	0.687	0.091	0.603-0.771

Standard deviation and 95% confidence interval of mean swimming angle over time, at 12°C in current (45cm/s)

Hours In Current-h	Mean Angle of Attack	Standard Deviation	95% Confidence Interval for Mean
0.00	0	-	-
0.03	8.14	4.60	3.13-12.97
0.25	12.50	1.38	11.05-13.45
0.50	11.50	1.38	9.95-13.05
0.75	9.17	2.32	6.74-11.60
1.00	6.17	1.72	4.36- 7.98
1.50	4.17	1.72	2.36- 5.98
3.00	1.86	1.21	0.68- 3.04
6.00	0.50	0.55	-0.07- 1.07

ANALYSIS OF VARIANCE

Source	df	SS	MS	F
Difference Between Time Measurements	8	1072.82	134.101	45.95
Error	45	131.355	2.919	
Total	53	1204.175		

Appendix 8. Effect of buoyancy response on time to fatigue.

Time (0h:00 min:00S) to fatigue at 12°C

	ACCLIMATION CURRENT CONDITION			
%Fatigued	0h-45cm/s	6h-45cm/s	12h-45cm/s	24h-45cm/s
10	5:02	8:28	7:03	6:33
20	9:53	10:04	9:42	9:46
30	14:20	13:12	12:08	15:31
40	15:10	14:21	20:45	26:14
50	18:16	17:03	31:52	34:47
60	21:24	20:13	35:33	38:41
70	29:12	23:59	36:41	40:26
80	33:22	25:34	43:47	44:54
90	1:14:20	1:17:26	1:06:41	1:30:00+
100	1:30:00	1:30:00	1:30:00	1:30:00+
Final Current Speed	80cm/s	80cm/s	80cm/s	81cm/s
Expected Buoyancy	0.997	0.687	0.793	0.805

ANALYSIS OF COVARIANCE FOR:0,6,12 and 24h

		SS			ADJUSTED FOR COVARIATE		
Source	df	X^2	XY	Y^2	df	SS	MS
Acclimation	3	2,172,040	0.0				
Error	28	13,801,900	451,650	16,800	27	2020.33	74.83
Total	31	15,973,940	451,650	16,800	30	4029.98	
				T ADJ	3	2009.65	669.89

$Ho_1: \sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$

$\chi_3^2 = 1.78$ $\alpha = 0.05$

$Ho_2: \beta_1 = \beta_2 = \beta_3 = \beta_4$

$F_{3,28} = 31.16$ $\alpha = 0.05$

$Ho_3: \beta_0^1 = \beta_0^2 = \beta_0^3 = \beta_0^4$

$F_{3,27} = 8.95$ $\alpha = 0.05$

ANALYSIS COVARIANCE FOR:0 and 6h

		SS			ADJUSTED FOR COVARIANT		
Source	df	X^2	XY	Y^2	df	SS	MS
Acclimation	1	42,539.10	0.0	0.0			
Error	14	3,245,280	159,675	8400	13	543.64	41.82
Total	15	3,287,820.1	159,675	8400	14	645.29	
				T ADJ	1	101.65	101.65

Ho$_1$: $\sigma_1^2=\sigma_2^2$

$\chi_1^2=1.16$ $\alpha=0.05$

Ho$_2$: $\beta_1=\beta_2$

$F_{1,14}=32.91$ $\alpha=0.05$

Ho$_3$: $\beta_0^1=\beta_0^2$

$F_{1,13}=2.43$ $\alpha=0.05$

ANALYSIS OF COVARIANCE FOR:12 and 24h

		SS			ADJUSTED FOR COVARIANT		
Source	df	X^2	XY	Y^2	df	SS	MS
Acclimation	1	84,245	0.0	0.0			
Error	14	10,556,600	291,975	8400	13	324.57	24.97
Total	15	10,040,845	291,975	8400	14	388.50	
				T ADJ	1	63.93	63.93

Ho$_1$: $\sigma_1^2=\sigma_2^2$

$\chi_1^2=0.000$ $\alpha=0.05$

Ho$_2$: $\beta_1=\beta_2$

$F_{1,14}=11.88$ $\alpha=0.05$

Ho$_3$: $\beta_0^1=\beta_0^2$

$F_{1,13}=2.56$ $\alpha=0.05$