

The University of Manitoba

Buoyancy regulation by sticklebacks (Culaea inconstans
and Pungitius pungitius) in marsh and lake environments.

by

Barbara J. Beaver

A thesis

submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for
the degree of Master of Science

Department of Zoology

Winnipeg, Manitoba



June 1986

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ISBN 0-315-33953-5

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AND
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ABSTRACT

Buoyancy was examined in the field and laboratory in two species of sticklebacks; Culaea inconstans, the brook stickleback, and Pungitius pungitius, the ninespine stickleback. In the field, buoyancy was described for fishes captured from marsh and lake habitats, and the variability found was related to environmental factors and individual fish characteristics. Important environmental factors affecting buoyancy in both species were the presence of vegetation, water temperature, and current. In areas that were vegetated, buoyancy was higher. Buoyancy decreased with increasing water temperature and current, however, when increased wave action occurred, buoyancy increased. Buoyancy also decreased as sexual development increased in both species. Total length and density were also related to variability of buoyancy but their effects were dependent on species and sex. The consistently higher buoyancy values in Culaea, were attributable to its larger swimbladder volume and its lower density of tissues compared to Pungitius.

Comparable to field findings, laboratory experiments showed buoyancy to decrease in current and increase in still water in both species. However, when exposed to waves, both species reduced buoyancy. Water temperature affected buoyancy attained in current in Culaea only. At 6°, buoyancy attained in current was higher than at 10, 15, 20, and 25°C. The time required for buoyancy to decrease from maximum to minimum levels was 4 and 7 d for Pungitius

and Culaea, respectively. Rate of readjustment in still water required only 2 d for both species. Buoyancy in both species decreased as water velocity increased.

ACKNOWLEDGEMENTS

The advice and guidance given during the course of this study by my supervisor, Dr. J. H. Gee, is greatly appreciated. I would like to thank Ian Dixon and Katie Dyke for their assistance in the field. Ray Ratynski and Helen Cavadias were helpful in many aspects, from beginning to end of this endeavour. Special thanks also to Irene LaBrosse and Muriel Goodman (my mother) for typing this manuscript.

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada.

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INTRODUCTION

Water current is the dominant environmental variable in lotic communities (Gee 1986). To maintain local populations, fish must hold position and avoid downstream displacement to less favorable environments when velocities increase. Displacement can be avoided by temporarily seeking areas of reduced flow, swimming against the current, or reducing buoyancy (swimbladder lift), or all of these (Gee and Gee 1976).

Reducing swimbladder lift and replacing it with hydrodynamic lift when water velocity increases, and vice versa when still water is encountered, is a common response in fish (Gee et al. 1974; Gee and Gee 1976). This is because gas inclusions provide the greatest amount of lift with the least drag in still to slow moving water while hydrodynamic lift is most efficient in fast velocities or at high swimming speeds (Gee 1983).

Virtually all of the research on this response has taken place in the laboratory using physostomes. This study describes adjustment of buoyancy in two species of physoclists in the field and accounts for field variability by laboratory experiments.

The extent and rate of swimbladder volume adjustment are affected by individual characteristics (size, age, sexual development, condition) and environmental factors (photoperiod, hydrostatic pressure, water temperature, velocity, and acidity) (Saunders 1965; Neave et al. 1966; Pinder and Eales 1969; Gee 1968, 1972, 1977;

Machniak and Gee 1975; Berezay and Gee 1978; Luoma and Gee 1980; Stewart and Gee 1981; Jansen 1986). Many of these factors interact to influence buoyancy.

In this study I examined variation in buoyancy in two species of sticklebacks, Culaea inconstans (Kirtland), the brook stickleback, and Pungitius pungitius (Linnaeus), the ninespine stickleback. They commonly occur at Delta Marsh, Manitoba, during spring and early summer. Adults of both species migrate into the marsh in spring from adjoining Lake Manitoba (Schneider unpub. data). Although typical of still water, they encounter water currents at various times particularly during pre- and post spawning migrations.

Objectives of the study were, 1) to measure buoyancy in both species in the field at particular locations in the marsh and lake during spring and early summer, and relate buoyancy values to environmental variables and individual characteristics of the fishes, including their density, and 2) under laboratory conditions; (a) to describe effects of water temperature on buoyancy attained and on related variables in still water and current, (b) to determine the rate of adjustment in still water and current and (c) to describe the effect of waves and different water velocities on buoyancy, for both species.

MATERIALS AND METHODS

Field buoyancy measurements

The field study was conducted at Delta Marsh, located at the south end of Lake Manitoba. Sticklebacks were captured at 3 sites to describe variation in buoyancy and relate this to environmental factors. These sites differed from one another in some physical characteristics (Table 1).

Site 1 was sampled by seining. At each of the other sites, 10 to 15 poles were placed at and inside the edge of cattail stands. Two wire minnow traps were fastened to each pole, one near the surface and one near the bottom of the water column.

Site 1 was sampled at least twice weekly, usually in the afternoon. The minnow traps at the other sites were set for two 12 h periods during each week (0700-1900 and 1900-0700). At the time of sampling, water and air temperature, turbidity, wind speed and direction, depth of capture, and wave action (measured as the vertical distance between the crest and trough of a wave) and current (measured by timing a floating object over a known distance) were recorded.

Buoyancy was measured for each fish using the Cartesian diver principle. The fish was first anaesthetized in MS-222 (ethyl m-aminobenzoate methane sulfonate), placed in a plexiglass chamber (32 x 17 x 22 cm) filled with water to within 2 cm of the top,

Table 1. Environmental characteristics of each sampling site.

Environmental Characteristics				
Site	Depth ^a (m)	Secchi disc ^a transparency (m)	Wave action/ ^a Current	Vegetation Substrate
1. Mouth of Assiniboine Diversion (Lake Manitoba)	0.80	0.34	both wave action (0-70cm, crest to trough) and current flowing into the lake from the Diversion (0-17cm·s ⁻¹) were present	no submergent or emergent vegetation present hard sand/ gravel
2. Upstream end of Cram Creek (Delta Marsh)	0.25-0.50	to bottom	negligible wave action or current present at any time	both emergent (Typha spp.) submergent (Potamogeton spp.) vegetation present soft ooze
3. Man-made channel (connects a bay to an oxbow lake in the marsh)	1.50-2.00	0.25	no wave action present; current almost always present varying from (0-26cm·s ⁻¹) in two directions	only emergent (Typha spp.) vegetation present hard clay

^a Based on averages taken over the field season.

and made airtight. Pressure was reduced, using a handpump, until the fish rose off the bottom and remained suspended 5 cm below the water surface. This pressure of neutral buoyancy (PNB; Saunders 1965) was measured with a mercury manometer (± 1 mm Hg).

Once PNB was determined, buoyancy was calculated from Boyle's Law:

$$P_1 V_1 = P_2 V_2$$

where P_1 = PNB (mbars); V_1 = buoyancy of the fish at neutral buoyancy (1.0 mL.g^{-1}); P_2 = atmospheric pressure (mbars); V_2 = buoyancy of the fish at atmospheric pressure (mL.g^{-1}). The unknown, V_2 , could be solved from the above equation. Corrections to buoyancy were then made for depth of capture (Appendix 1).

After PNB determination, fish were placed in marked vials containing water soaked cotton and taken back to the laboratory. For each fish, total length (mm), weight of the intact fish in air (± 0.001 g), weight of the gas-free fish in water (± 0.001 g), and weight of the gonads in air (± 0.001 g) and water (± 0.001 g) were measured.

The weight of the gonads in air divided by the weight of the fish in air was used as an index of gonad development (after Luoma and Gee 1980). The largest observed gonad to fish weight ratio for each species and sex was assumed to be 100% sexual development. Sexual development was calculated as follows:

$$\% \text{ sexual development} = \frac{\text{gonad to fish weight ratio}}{\text{largest gonad to fish weight ratio}} \times 100$$

The weight density (g.mL^{-1}) of each fish was also determined. Weight density is defined as the weight per unit volume of a substance (Miller 1977). It was calculated by dividing the weight of the fish in air by fish volume. Fish volume was determined by taking the difference between weight of the fish in air and weight of the gas-free fish in water. This difference in weight (g) equals volume (mL), for 1 mL volume of gas supports a weight of 1 g of fish tissue in freshwater. A standard measurement of swimbladder volume to weight in air (mL.g^{-1}), was calculated in addition to density. Swimbladder volume was determined by multiplying buoyancy by weight of the fish in water.

Laboratory buoyancy measurements

Pungitius and Culaea were collected from Lake Manitoba and Delta Marsh in May and June 1984, and May 1985. Fish were held in the laboratory at 12 ± 1 °C and fed brine shrimp once a day, except 24 h prior to buoyancy measurements. Illumination was provided by 60 W light bulbs and maintained by time clocks on a 12L:12D photoperiod. Fish were acclimated to experimental temperatures at a rate of 1 °C.d⁻¹. Dechlorinated Winnipeg City water was continuously exchanged in all aquaria.

Laboratory testing conditions were designed to simulate two extreme conditions; still water and fast-flowing current, to determine their effects on buoyancy. Presumably buoyancy would be greatest in still water and least in current. Therefore not only

could the effect of variables such as temperature be determined in each situation but also their effect on the extent of adjustment (difference between maximum and minimum buoyancy) could be assessed.

Species were tested separately in either still water or current aquaria (90 x 45 x 45 cm). Still water conditions were created in an aquarium containing a gently-bubbling air stone. Sand and gravel covered the bottom of the aquarium and a few artificial strands of vegetation (green plastic) were randomly placed in the tank. Current was created in an aquarium using the design of Gee and Bartnik (1969). In current, fish were held in a chamber (60 x 40 x 20 cm) suspended in the aquarium constructed with stainless steel screens on the sides and ends and plexiglass on the bottom. Pieces of wood dowling (0.64 cm diameter, 5 cm long) were glued perpendicularly to the bottom of the chamber along the two sides running parallel to the water flow. This simulated emergent vegetation on the sides of a marsh channel. Each side encompassed an area of 60 x 15 cm, with approximately 35 dowling pieces per side. Water velocity was determined from the average of nine measurements taken at specific points free from dowling in the current chamber with an Ott current meter (type C1).

The effect of waves on buoyancy was also examined. Waves were created in a tank (188 x 31 x 47 cm) by an oscillating paddle using the design of Patee (in Stong 1968). The wave generating paddle was separated from the fish holding area (145 x 31 x 47 cm)

by a wire screen. In the absence of waves, water depth was 13 cm and when turbulence was generated, waves reached a height of 16 cm. Substrate in the holding area consisted of sand and gravel to simulate a beach. A gradient of the substrate began at approximately 50 cm from the screen and reached a height of 20 cm at the opposite end.

Effect of water temperature. The effect of water temperature on swimbladder variables in still water and current was described for each species. Five batches, each of Culaea and Pungitius (50 - 60 mm total length), were acclimated at 6, 10, 15, 20, and 25 ($\pm$) 1°C. At each temperature, 10 fish of each species were tested after 7 d in either current or still water. Current velocities at each temperature were the maximum tolerated (80% of the fish could hold position without resting against the outlet screen of the current chamber). These velocities (cm.s^{-1}) were as follows:

Temperature (°C)	6	10,15,20	25
<u>Culaea</u>	32	50	36
<u>Pungitius</u>	36	60	60

Rate of buoyancy adjustment. Rate of adjustment experiments were done at 10°C, in current velocities of 60 cm.s^{-1} for Pungitius (50 - 58 mm total length) and 50 cm.s^{-1} for Culaea (54 - 65 mm total length). Rate of decrease in buoyancy was measured by holding fishes in still water for 7 d to attain maximum buoyancy and then groups of 8 were examined after 0 (still water), 1, 3, 6, 12, 24, 48, 72, 96, and 120 h in current (both species)

and at 144 and 168 h (Culaea only). Other related swimbladder variables were also measured at each time (see below).

Rate of increase in buoyancy and changes in related variables were measured by holding fishes in current for 5 (Pungitius) and 7 (Culaea) d, to attain minimum buoyancy. Groups of 8 fish were examined after 0 (current), 1, 3, 6, 12, 24, 48, and 72 h in still water.

Effect of water velocity. The effect of different water velocities on swimbladder variables was tested on Pungitius (48 - 61 mm total length) and Culaea (49 - 50 mm total length) at 10°C. Groups of 8 fish of each species were acclimated to still water for 7 d, and then exposed for 7 d to velocities of either 0 (still water), 7, 16, 30, or 50 cm.s⁻¹.

Effect of waves. The effect of waves on swimbladder variables was tested on Pungitius (54 - 58 mm total length) and Culaea (54 - 66 mm total length) at 10°C. Two batches of each species were acclimated to still water. Groups of 8 fish of each species were examined after 7 d in either still water or waves.

Measurement of swimbladder variables. Swimbladder variables measured were buoyancy, standard volume, and internal pressure of the swimbladder gases. To measure these, fish were first anaesthetized in a solution of MS-222. Swimbladder volume (± 0.001 mL), weight of the gas-free fish in water (± 0.001 g), and the volume of gases released at atmospheric pressure (± 0.001 mL) were

measured following the procedure of Chiasson and Gee (1983)

Buoyancy was determined by dividing swimbladder volume by weight of the gas-free fish in water (1.0 mL.g^{-1} = neutral buoyancy). Internal pressure of gas in the swimbladder was measured by dividing volume of gas released at atmospheric pressure by swimbladder volume. Standard volume was determined by dividing volume of gas released, corrected to 101.3 kPa (standard pressure), by weight of the gas-free fish in water.

Statistical analysis

From all the variables measured in the field, a set was found that best described buoyancy in each species and sex. The criteria for choosing the best variables were the Cp statistic (Mallows') and R-squared value. The variables chosen maximized the R-squared value and minimized the Cp statistic. Field data were also analyzed using one-way analysis of variance (ANOVA), Student's t-test, and Tukey's multiple comparison test.

Laboratory experiments were analyzed using one and two-way ANOVA, analysis of covariance (ANCOVA), single linear regression analysis, and Tukey's multiple comparison test. In all statistical tests, differences were considered to be significant if the probability of error was less than 5% ($P < 0.05$). Significance in the appendices is indicated by an asterisk (*) and vertical lines from means on graphs represent the 95% confidence interval (CI).

RESULTS

Field buoyancy measurements

At all sites, Culaea was significantly more buoyant than Pungitius (t-test; Table 2: Appendix 2). Both species were significantly less buoyant at the Assiniboine Diversion than at all other sites (ANOVA; Tukey's test: Appendix 3). However, variability in buoyancy was high at all sites over the field season (Table 2). This variability can be partially explained by changes that occur in several variables, including both individual fish characteristics and environmental factors.

Variables affecting buoyancy in each species were similar, however, their order of importance, measured by the contribution to the R-squared value, differed slightly between species and sexes within species (Table 3). The R-squared value in multiple regression measures the proportion of the total variation that can be explained by the regression (Steel and Torrie 1980). In both species, water temperature and the presence or absence of vegetation contributed most to the R-squared value. Water current and related factors, wave action, wind speed and direction, appeared to be the next most important variables affecting buoyancy. Wave action was only an important factor in Culaea females. In both species, sexual development was more important in females and total length was more important in males. Fish density affected buoyancy more in Culaea than Pungitius.

The regression coefficient for each variable in the multiple

Table 2. Mean buoyancy ($\text{mL}\cdot\text{g}^{-1}$) pooled over May and June for each species at each site.

Species	Assiniboine Diversion			End of Cram Creek			Channel		
	N	$\bar{x}$	Range	N	$\bar{x}$	Range	N	$\bar{x}$	Range
<u>Culaea</u>	160	0.78	0.49-0.98	108	0.86	0.71-1.20	32	0.86	0.78-0.95
<u>Pungitius</u>	309	0.69	0.35-0.89	98	0.78	0.55-0.97	42	0.77	0.60-0.91

Table 3. Variables included in the multiple regression for predicting buoyancy in each species and sex. The overall R-squared value for the regression is listed, as well as regression coefficients and contributions to R-squared for each variable.

Species	Sex	Variable	Regression coefficient	Contribution to R-squared	R-squared
<u>Culaea</u>	female	water temperature	-0.0063	0.0838	0.38
		vegetation ^a	0.0710	0.0541	
		wave action	-0.0013	0.0366	
		sexual development	-0.0008	0.0239	
		wind direction ^a	-0.0289	0.0212	
		density	-1.1728	0.0111	
		water current	-0.0023	0.0061	
		wind speed	0.0002	0.0002	
		total length	0.0001	0.0004	
	male	vegetation ^a	0.1118	0.2372	0.60
		water temperature	-0.0076	0.1106	
		water current	-0.0029	0.0156	
		wind direction ^a	-0.0240	0.0086	
		total length	-0.0009	0.0019	
		density	0.3006	0.0004	
		wind speed	0.0003	0.0002	
		sexual development	-0.0002	0.0002	
		wave action	0.0001	0.0001	
<u>Pungitius</u>	female	water temperature	-0.0085	0.0809	0.37
		vegetation ^a	0.0637	0.0467	
		sexual development	-0.0007	0.0207	
		wind direction ^a	-0.0270	0.0084	
		water current	-0.0012	0.0031	
		wind speed	0.0006	0.0011	
		density	0.1408	0.0001	
		total length	0.0002	0.00005	
		wave action	0.0001	0.00003	
	male	vegetation ^a	0.0731	0.0822	0.41
		water temperature	-0.0086	0.0527	
		water current	-0.0021	0.0160	
		total length	-0.0031	0.0098	
		wind speed	0.0015	0.0060	
		wind direction ^a	0.0220	0.0053	
		sexual development	-0.0004	0.0041	
		wave action	0.0005	0.0032	
		density	0.5594	0.0019	

^a Codes vegetation: 1 = vegetation absent, 2 = vegetation present
wind direction: 1 = north, northeast, northwest.
 2 = south, southeast, southwest

regression, measures the changes in buoyancy attributable to changes in that specific variable, independent of the others. In both species, the regression coefficients showed buoyancy to 1) decrease with increasing water temperature, current, sexual development, total length (males only), and during south winds (except Pungitius males); 2) increase in the presence of increasing wind speed, density (except Culaea females), wave action (except Culaea females), and total length (females only).

To explain the consistently higher buoyancy of Culaea, the fish density variable was examined separately. When sites were combined over the field season, Pungitius was significantly more dense and its standard measurement of swimbladder volume to weight in air was also less (t-test; Table 4: Appendix 4). Culaea had a greater proportion of the body weight being supported by the swimbladder, and this combined with its low density produced a higher buoyancy.

Sex dependent differences in density and buoyancy occurred in Culaea but not in Pungitius. In Culaea, males were more dense and less buoyant than females (t-test; Table 4: Appendix 4). The standard measurement did not differ significantly between sexes in either species (t-test; Table 4: Appendix 4).

Laboratory buoyancy measurements

Effect of water temperature. Buoyancy and standard volume in Pungitius were significantly affected by water velocity (still vs. current) but the effect of temperature and its interaction with

Table 4. Mean buoyancy (mL.g⁻¹), density (g.ml⁻¹) and standard measurement (mL.g⁻¹), pooled from all sites over the field season for each species and sex. Asterisks represent significant differences at a level of $P < 0.05$.

Species	Sex	N	Buoyancy	Density	Standard
<u>Culaea</u>	female	137	* 0.83	* 1.056	* 0.044
	male	163	0.81	1.060	0.046
	both sexes	300	0.82	1.058	0.045
<u>Pungitius</u>	female	246	* 0.71	* 1.063	0.042
	male	203	0.72	1.064	0.043
	both sexes	449	0.72	1.063	0.043

Figure 1. Rate of change in mean buoyancy (mL.g^{-1}) (n=8) and mean internal pressure (atm) (n=7) on exposure to current (solid line and bars) and still water (brokenline and open bars) by Pungitius. Actual values are given on x axis but plotted on a logarithmic scale.

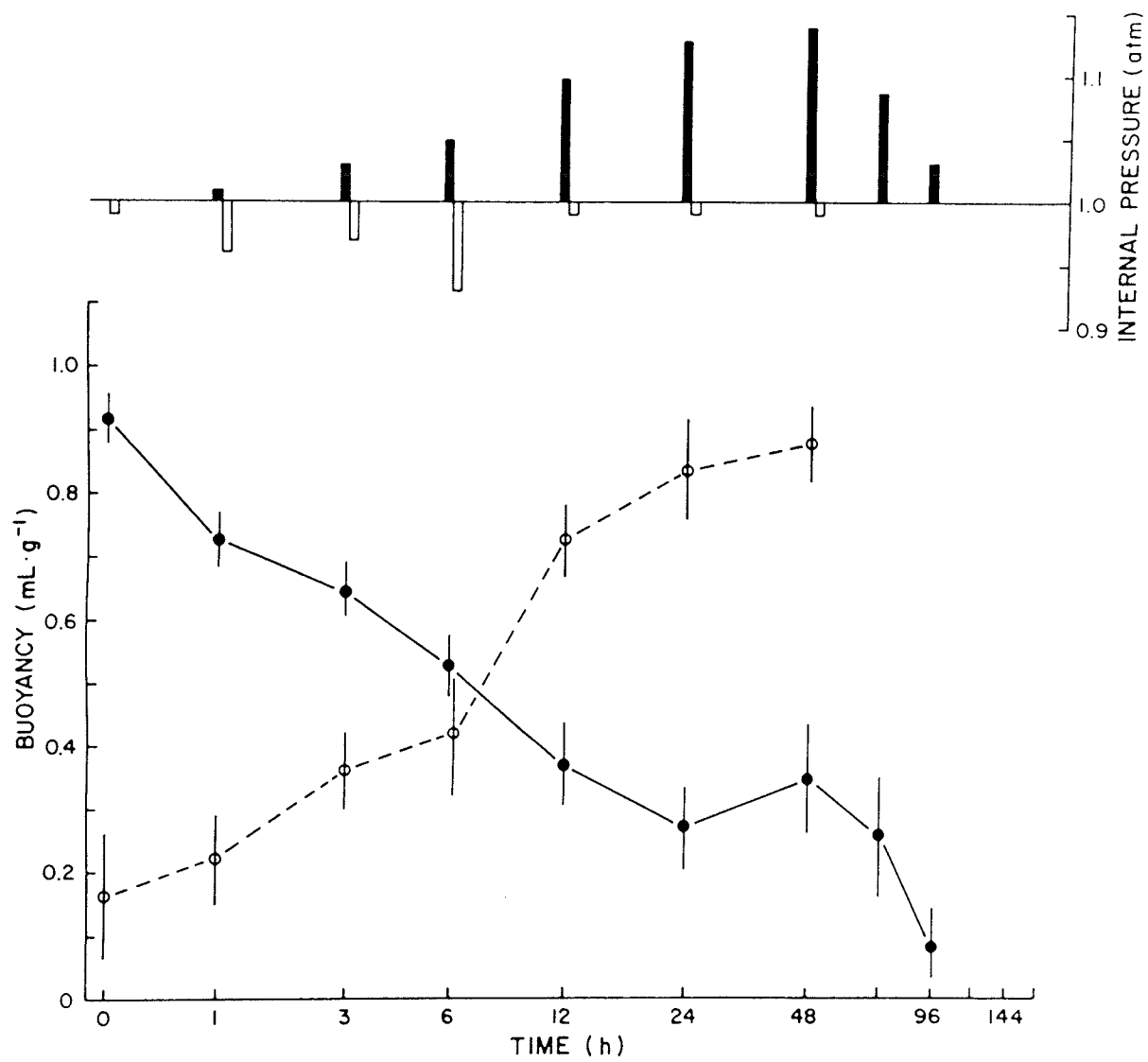
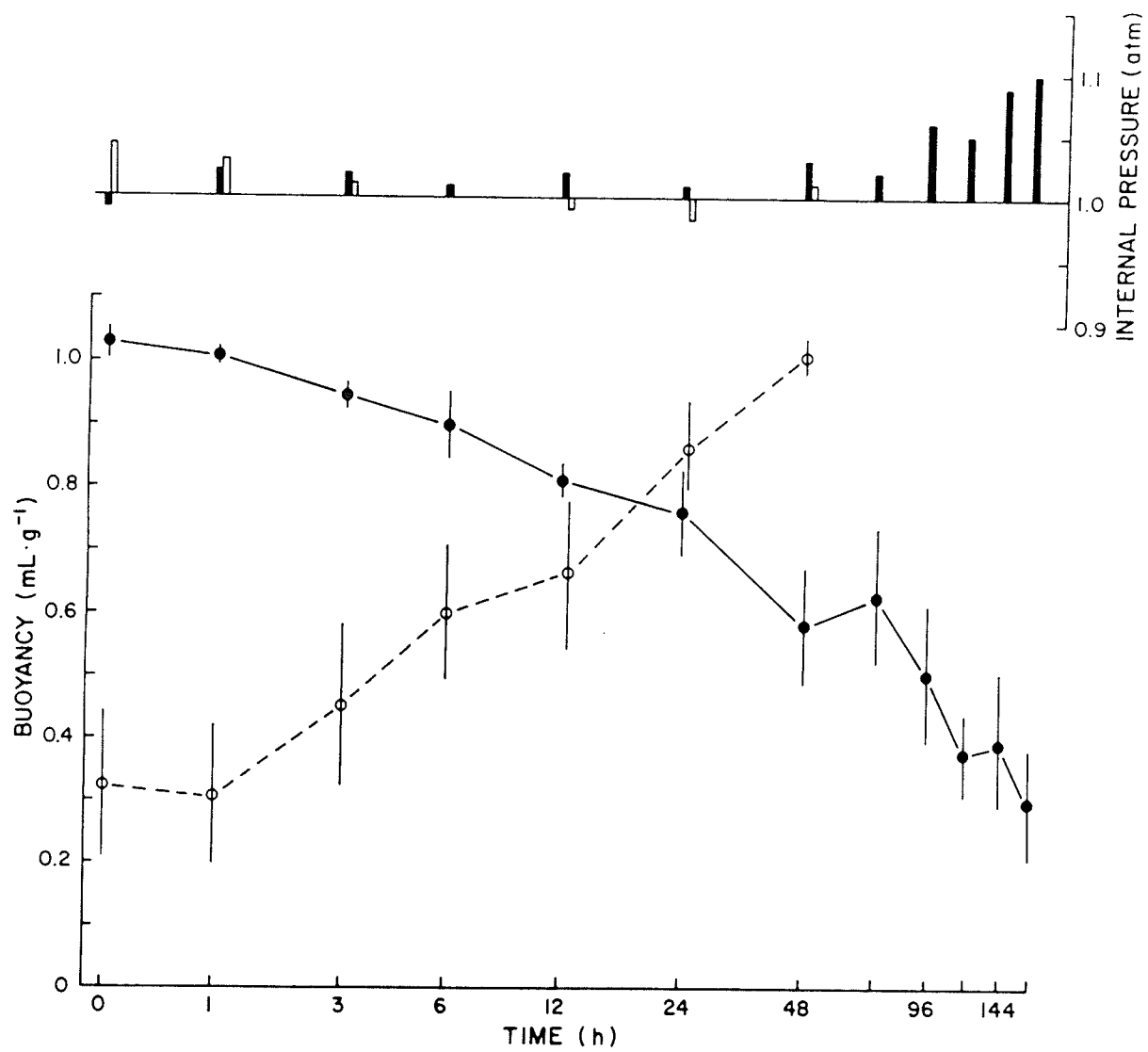


Figure 2. Rate of change in mean buoyancy (mL.g^{-1}) ($n=8$) and mean internal pressure (atm) ($n=8$) on exposure to current (solid line and bars) and still water (broken line and open bars) by Culaea. Actual values are given on x axis but plotted on a logarithmic scale.



water velocity were not significant (ANOVA; Appendix 5). Thus the extent of adjustment was not affected by temperature. Buoyancy in still water approached neutral at all temperatures (Table 5), while values in current were greatly reduced. Internal pressure was not affected by either temperature or water velocity (ANOVA: Appendix 5). At most temperatures internal pressure was slightly less than ambient (Table 6).

Water temperature, velocity (still vs. current), and their interaction significantly affected both buoyancy and standard volume in Culaea (ANOVA: Appendix 5). These values in current were less than those in still water but the extent of adjustment varied with temperature (Table 5). Both variables showed the least extent at 6°C and the greatest at 10°C. Values in still water did not differ significantly with temperature, but in current they were higher at 6°C than at all other temperatures (Tukey's test).

Water temperature significantly affected internal pressure in Culaea (Table 6), but water velocity and its interaction with temperature did not (ANOVA: Appendix 5). Internal pressure was significantly lower at 6° and 10° C and significantly higher at 20°C compared to all other temperatures (Tukey's test).

Rate of buoyancy adjustment. Pungitius and Culaea required 4 and 7 d, respectively, to reach minimum buoyancy on exposure to current (Figs. 1 and 2). For each species, regression lines were calculated with a logarithmic transformation of time for rate of

Table 5. Means ($n=10$) of buoyancy (Buoy) (mL.g^{-1}) and standard volume (Sv) (mL.g^{-1}) in both species held in still water and current for 7d. Extent of adjustment is the difference in means between still water and current.

Species	Water Temperature ($^{\circ}\text{C}$)									
	6		10		15		20		25	
	Buoy	Sv	Buoy	Sv	Buoy	Sv	Buoy	Sv	Buoy	Sv
<u>Culaea</u>										
still	1.00	1.03	0.89	0.97	0.99	1.04	0.93	0.96	0.91	0.92
current	0.64	0.65	0.34	0.34	0.47	0.47	0.37	0.39	0.40	0.42
difference	0.36	0.38	0.65	0.63	0.52	0.57	0.56	0.57	0.51	0.50
<u>Pungitius</u>										
still	0.98	0.98	0.86	0.86	0.81	0.79	0.88	0.89	0.86	0.89
current	0.20	0.20	0.05	0.05	0.16	0.17	0.14	0.13	0.19	0.18
difference	0.78	0.78	0.81	0.81	0.65	0.62	0.74	0.76	0.67	0.74

Table 6. Means (Culaea, n = 20; Pungitius, n = 17) and standard deviations (in parentheses) of internal pressure (atm) combined from still water and current at different water temperatures.

Species	Water Temperature (°C)				
	6	10	15	20	25
<u>Culaea</u>	1.00 (0.03)	0.99 (0.05)	1.02 (0.04)	1.05 (0.07)	1.03 (0.06)
<u>Pungitius</u>	0.99 (0.05)	1.02 (0.32)	0.98 (0.07)	0.93 (0.21)	0.97 (0.15)

decrease in buoyancy and standard volume (regression analyses; Table 7: Appendix 6). Slopes of the regression lines were not significantly different between species for rate of buoyancy decrease (ANCOVA: Appendix 6).

Internal pressure in the swimbladder of Pungitius did not change significantly over time when held in current but in Culaea it increased significantly (regression analyses; Figs. 1 and 2: Appendix 6).

Pungitius and Culaea attained maximum buoyancy in 2 d when water velocity changed from current to still (Figs. 1 and 2). Regression lines for rate of increase in buoyancy and standard volume were calculated with a logarithmic transformation of time for each species (regression analyses; Table 7: Appendix 7). There was no significant difference in the rate of buoyancy increase, expressed by the slopes of the regression lines, between species (ANCOVA: Appendix 7).

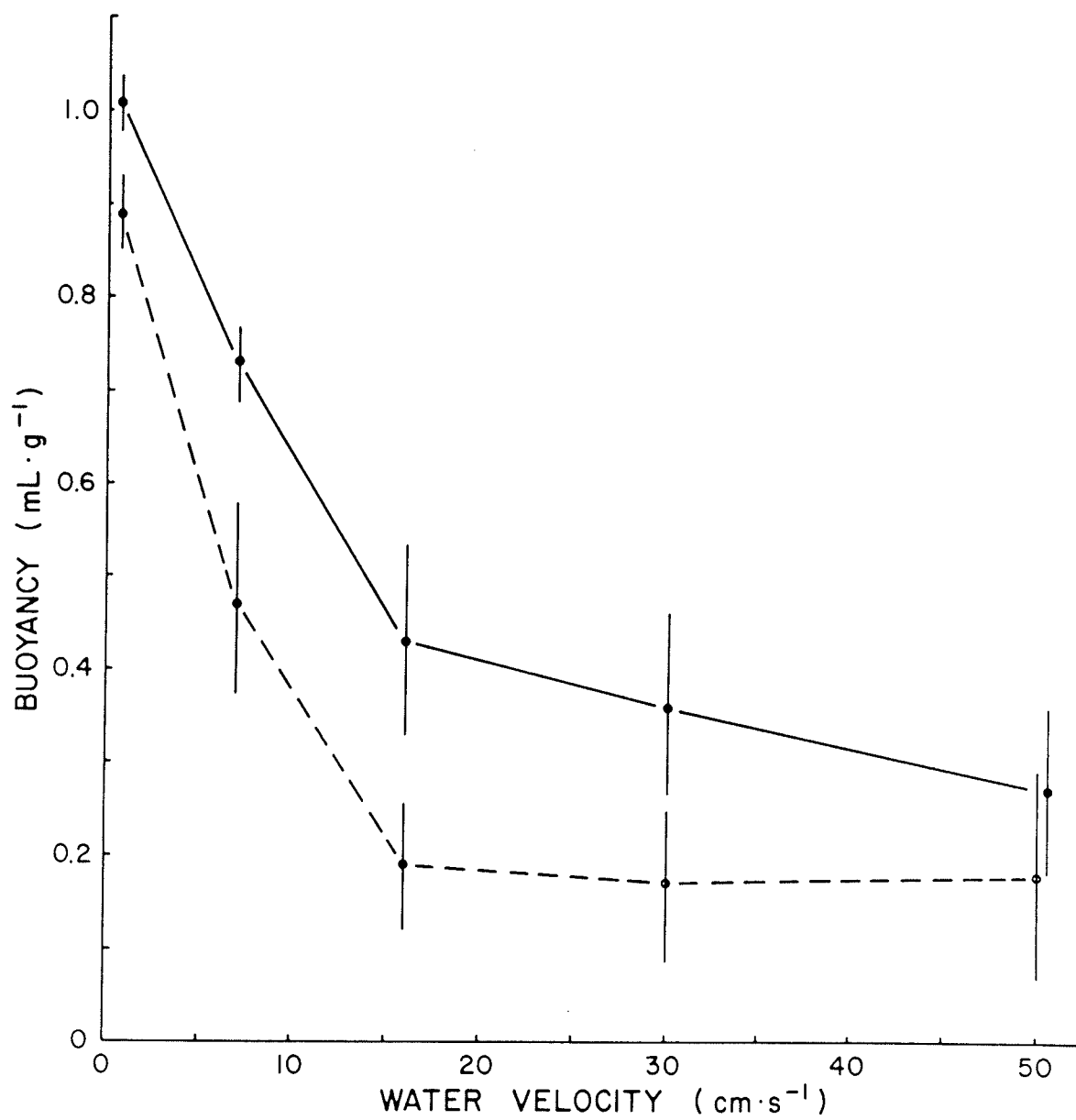
Internal pressure did not change significantly over time held in still water in either species (regression analyses: Appendix 7).

Effect of water velocity. Water velocity inversely affected buoyancy in both species (Fig. 3). It was significantly reduced between 0 and 7 cm.s⁻¹, 7 and 16 cm.s⁻¹, and 16 and 50 cm.s⁻¹ (Culaea only) (ANOVA; Tukey's test: Appendix 8). Internal pressure did not vary between treatments within species (ANOVA: Appendix 8)

Table 7. Regression lines describing rates of decrease (exposure to current) and increase (exposure to still water) of buoyancy (BUOY) ($\text{mL}\cdot\text{g}^{-1}$), standard volume (SV) ($\text{mL}\cdot\text{g}^{-1}$), and internal pressure (IP) (atm) in Culaea and Pungitius.

Species	Swimbladder variable	Experiment	
		Decrease	Increase
<u>Culaea</u>	BUOY	$y=1.130-0.328 \log x$ ($R^2=0.834$)	$y=0.230+0.433 \log x$ ($R^2=0.779$)
	SV	$y=1.129-0.318 \log x$ ($R^2=0.823$)	$y=0.232+0.430 \log x$ ($R^2=0.780$)
	IP	$y=1.005+0.0005 x$ ($R^2=0.439$)	no change
<u>Pungitius</u>	BUOY	$y=0.861-0.366 \log x$ ($R^2=0.866$)	$y=0.112+0.476 \log x$ ($R^2=0.873$)
	SV	$y=0.874-0.359 \log x$ ($R^2=0.853$)	$y=0.106+0.469 \log x$ ($R^2=0.846$)
	IP	no change	no change

Figure 3. Mean (n=8) buoyancy (mL.g^{-1}) attained by Culaea (solid line) and Pungitius (broken line) at different water velocities.



and was 0.99 atm for Pungitius and 1.01 for Culaea. As a result standard volume was similar to buoyancy.

Effect of waves. Buoyancy in both species significantly decreased when water conditions changed from still water to waves (ANOVA; Table 8: Appendix 9). Internal pressure did not differ between the two treatments in either species (ANOVA: Appendix 9) and was 1.01 atm for Pungitius and 1.00 atm for Culaea. Standard volume was similar to buoyancy.

Table 8. Means (n=8) and standard deviations (in parentheses) of buoyancy (mL.g⁻¹) attained after 5d in still water and waves.

Species	Still	Waves
<u>Culaea</u>	0.97 (0.03)	0.93 (0.02)
<u>Pungitius</u>	0.97 (0.03)	0.87 (0.05)

DISCUSSION

Buoyancy values of both species were extremely variable in the field, with Culaea consistently more buoyant than Pungitius. In both species water temperature and factors relating to water flow were most important, however sexual development, length, and density also influenced buoyancy. In the laboratory, both species attained near neutral buoyancy in still water and a greatly reduced buoyancy in current. In Culaea only, water temperature affected buoyancy attained in current. Time to decrease buoyancy from maximum to minimum levels was 4 and 7 d for Pungitius and Culaea respectively, while time to increase was only 2 d for both species. In both species water velocity and buoyancy were inversely related.

Field buoyancy measurements

In the field, many factors were found to affect buoyancy in both species. The two most important factors, indicated by their high contributions to the R-squared value, were water temperature and vegetation. The negative regression coefficient of water temperature, indicates that it and buoyancy are inversely related. Thus in early spring when water temperatures are cold in the lake and the marsh, buoyancy in both species is high. Water temperature is known to affect buoyancy in other species. In Atlantic salmon parr (Salmo salar) held in current in the field, buoyancy is significantly higher during winter months when water temperatures are lower (Neave et al. 1966; Pinder and Eales 1969).

Buoyancy in fathead minnows, Pimephales promelas, is significantly higher in both still water and current conditions at low water temperatures as opposed to high (Luoma and Gee 1980).

The regression coefficient for vegetation indicates that buoyancy increases in the presence of vegetation. In the field, vegetation was only present at the end of Cram Creek and the channel. Buoyancy did not significantly differ in either species between these two sites. It is postulated that the presence of vegetation is associated with still water. At the end of Cram Creek water current and wave action was not detectable, however in the channel, water currents occurred. Water flow within vegetation in the channel was likely minimal, where the sticklebacks were predominant. There would be no need for a low buoyancy under such conditions.

Water current and factors relating to it were also important in affecting buoyancy in both species. Water current was present in the channel and during south winds where the Assiniboine Diversion flows into the lake. Buoyancy in both species was inversely related to water current. Field studies on blackside darters, Percina maculata, also found buoyancy differences related to water current; buoyancy was less in fish captured from rapids than those from pools (Smart and Gee 1979; Cavadias unpub. data). Reducing buoyancy in water current and increasing it when still water is encountered, is a common adaptation in many north temperate fish species (Gee et al. 1974).

Wave action was present only at the Diversion. Buoyancy increased as wave action did, except in Culaea females. Wind speed and direction appeared to be related to wave action since increased wind speeds and northerly winds also produced increased buoyancy values in both species. In some other species, wave action produces an opposite effect on buoyancy. Gee and Machniak (1972) found that longnose dace, Rhinichthys cataractae, had much smaller swimbladder volumes on windy days than calm ones on beaches exposed to the wind. Buoyancy in river darters, Percina shumardi, and log perch, Percina caprodes, is also inversely affected by increasing wave action and wind speed (Cavadias unpub. data).

Water depth has an important effect on wave action. If depth is less than half the wavelength, then as it further decreases, a simultaneous reduction in wavelength and increase in wave height occurs until the wave crests and breaks (Wetzel 1975). The wave action extends to the bottom under these conditions creating horizontal movement (surge) of water. However if depth is greater than half the wavelength, there is no horizontal movement of water at the bottom.

The species mentioned above, Rhinichthys and Percina, are benthic shallow water fishes. They would almost always be exposed to surge conditions if waves were present. Therefore, buoyancy is reduced when wave action increases, as it is when current increases. In this study, wavelength was not measured in the field, thus it

was not known whether surge conditions were present. It can only be postulated that sticklebacks were in water deep enough that surge was not present for no reduction in buoyancy was observed in either species.

Sexual development and buoyancy were inversely related in both species. This factor was more important in females, probably owing to their more extensive development of gonad tissue. Similar results were observed in fathead minnows (Luoma and Gee 1980). Buoyancy in fatheads decreased as sexual development increased, with a greater decline occurring in current than in still water. The ability to reduce buoyancy on exposure to current during spring and summer freshets would clearly enhance the ability to hold position and ultimately contribute to reproductive success.

Body length was another factor affecting buoyancy in both species, however it was more important in males. In females, buoyancy increased with total length, but in the males the opposite occurred. Length and buoyancy are inversely related in several species (Gee 1983).

Density also affected buoyancy in both species but it was of low importance. The higher density of tissues in Pungitius helps to explain its consistently lower buoyancy values compared with Culaea. Lift can be generated by one or more of the following: 1) secreting gas into the swimbladder; 2) incorporating low density tissues

and fluids into the body; 3) swimming and obtaining hydrodynamic lift (Gee 1983). Therefore, Pungitius, which is lower in buoyancy than Culaea and more dense, should obtain more lift from hydrodynamic sources to maintain position at a given depth. The caudal keels on Pungitius may contribute to hydrodynamic lift.

Laboratory buoyancy measurements

Effect of water temperature. In still water both species were at or close to neutral buoyancy, while in current, buoyancy was greatly reduced. Water temperature affected buoyancy in Culaea only. At 6°C in current, buoyancy was higher than at the other temperatures, thus extent of adjustment was reduced at this temperature. In the field, temperature affected buoyancy in both species. Pungitius was not affected by temperature in the laboratory, suggesting that it has potential to attain low buoyancy in current at cold water temperatures in the field.

The maximum water velocity both species could tolerate in the laboratory at 6°C was less than that tolerated at higher temperatures. During spring runoff in north temperate areas, water velocities are generally at their greatest and waters are cold. Extent of buoyancy adjustment in Culaea was significantly reduced at 6°C. In the marsh, catches were very low for both species at water temperatures below 10°C. Possibly the inability to tolerate fast water velocities at those low temperatures in the channels connecting the marsh to the lake, restricted their movement into the marsh.

In a field study examining the movements of Culaea in the Roseau River, MacLean and Gee (1971) found water temperature and velocity to affect fish distribution within meltwater runoffs entering the river. In early spring, low water temperatures (3.3°C), combined with high water velocities (90 cm.s^{-1}) appeared to restrict movements into the runoff channels leading to spawning areas. Culaea cannot tolerate these velocities at low temperatures and thus they were most abundant downstream of these runoff channels under these conditions.

In studying the migration of several species of fishes into Delta Marsh, Schneider (unpub. data) reported major movements of both species of sticklebacks in early May (1983) when mean morning and evening water temperatures were 8° and 11°C , respectively. At these temperatures (10°C) in the laboratory, both species showed the greatest extent of buoyancy adjustment. Although water velocities are not known from this migration study, laboratory studies indicate that both species could tolerate velocities up to 50 cm.s^{-1} at 10°C . If velocities as strong as 50 cm.s^{-1} were encountered during migration, both species could adjust to a minimum buoyancy level, facilitating either movement upstream or maintenance of position in current.

MacLean and Gee (1971) noted upstream movements of prespawning Culaea occurring when water temperatures ranged between 15° and 21°C . However, Lamsa (1963) reported downstream movements of

Culaea in a small southern Ontario stream at 10°C. This suggests that water currents may provide a transport system for migrating sticklebacks into Delta Marsh. Fish could drift into the marsh with inward currents, holding position on outward flows in the spring. The opposite could take place in autumn when sticklebacks leave the marsh. In the plaice, Pleuronectes platessa, tidal currents provide transport to and from spawning grounds in a similar fashion, allowing energy savings of up to 40% (Arnold 1981).

Rate of buoyancy adjustment, Fish encountering variability in water velocity in time and space must regulate the amount and sources of lift to avoid displacement. Natural selection should favor the ability to alter swimbladder volume over an extensive range and accomplish the change as rapidly as possible (Gee 1986). In north temperate areas, most species can alter buoyancy rapidly and over extensive ranges (Gee and Gee 1976).

When exposed to water velocity in the laboratory, Pungitius and Culaea required 4 and 7 d, respectively, to make complete buoyancy adjustments. When still water was encountered, both species required only 2 d to adjust back to near neutral buoyancy. The difference between rates of deflation and inflation of the swimbladder in each species of sticklebacks, indicates that each species has a greater ability to secrete than resorb gas. However, since the rates were tested only at one temperature, 10°C, these results could reflect a temperature dependent response.

There is considerable variability in rates of buoyancy adjustment, within and between species. Rainbow trout, Salmo gairdneri, can complete an extensive adjustment in less than 1 h (Gee unpub. data), whereas the tadpole madtom, Noturus gyrinus, makes a moderate adjustment over a period of 8 d (Machniak and Gee 1975). Within a species, smaller size groups adjust faster than larger ones (Gee 1986). Water temperature is also an important factor affecting rates within species. For example, cold water temperatures slow down inflation rates in river darters (Balesic unpub. data) and fathead minnows (Gee 1977). In these same species, rates of deflation are unaffected by temperature, therefore they can respond with equal ability to increases in water velocity over a range of temperatures.

In physoclists, buoyancy alteration can be achieved by altering swimbladder gas volume and/or altering internal pressure of the swimbladder gas. Altering internal pressure is a rapid and precise mechanism, but only a limited change can be made (Gee 1983). Only Culaea used internal pressure adjustments as a mechanism for altering buoyancy. It was used as a means of reducing swimbladder volume near the final phases of buoyancy reduction in current. Pungitius increased internal pressure near the final phases of reduction, however no significant differences were found over time. Low volumes of swimbladder gas during these final phases of reduction in Pungitius increased error in internal pressure calculations and therefore may have masked its importance. In

the tadpole madtom, increases in internal pressure occurred only during the initial phases of buoyancy reduction (Machniak and Gee 1975). In blacknose dace, Rhinichthys atratulus, internal pressure increased during buoyancy reduction and decreased as buoyancy increased (Gee 1970). Internal pressure did not change in either species of sticklebacks during adjustment in still water.

Alteration of internal pressure may not only be involved in controlling the amount of lift in sticklebacks. It may also be used to adjust the shape of the swimbladder and control the center of buoyancy. This could affect body positioning and orientation used in feeding, reproductive, and escape behaviours. McCutcheon (1958) showed that in the pinfish, Lagodon rhomboides, alteration of internal pressure within certain areas of the swimbladder by muscular contraction, could produce changes in orientation.

The foraging behaviour in Culaea consists of a particular phase that appears to involve precise control of body positioning. Tompkins and Gee (1983) observed an 'aim' phase whereby the fish tilts head downwards from the horizontal position by about 45° and usually fixes on the prey on the substrate. It is possible that a combination of fin movements (hydrodynamic lift) and internal pressure alterations permit this orientation.

In each species of sticklebacks, the reproductive period includes establishment of territories, nest construction,

courtship dances, and parental care (Wootton 1976). Each phase involves finely controlled movements and postures that likely depend heavily on precise buoyancy control. Alteration of internal pressure may be particularly important in controlling postures in courtship dances and in the parental care phase where the male must fan the eggs held in the nest off the substrate.

Gee (1983) suggests that control and rapid alteration of buoyancy play an important role in escape once a predator has been detected. In the presence of a predator, Culaea and the three-spined stickleback, Gasterosteus aculeatus, stop normal activity and sink in the water column (Weselowski 1974; Wootton 1976). Sinking may be facilitated by fin movements combined with internal pressure alterations. The mudminnow, Umbra limi, reduces buoyancy by compressing swimbladder gases and drops to the substrate on detecting a predator (Gee 1981).

Effect of water velocity. In both species of sticklebacks, buoyancy was inversely related to water velocity. This relationship occurs in many pelagic species (Gee 1983). As water velocity increases, fish must either swim faster to maintain position or seek areas of reduced flow. Swimming faster results in increased drag. The amount of drag experienced depends on the combination of swimbladder and hydrodynamic lift used at particular velocities. Buoyant lift, obtained from gas inclusions into the swimbladder, produces the least amount of drag in still or slow moving water. In fast water velocities, hydrodynamic lift is most efficient (Gee 1983).

In the laboratory, buoyancy decreased when both species were exposed to waves. These observations contradict field observations of the relationship of buoyancy to wave action. The wave tank is designed to simulate shallow waves which travel in water that does not exceed in depth half the wavelength (Stong 1968). Under these conditions, horizontal movement of water (surge) occurs. Sticklebacks were likely responding to the surge by reducing buoyancy, as they do when water current is present. Therefore, the wave tank did not realistically simulate conditions in the field, where it was postulated that surge was not present.

The sticklebacks, Culaea and Pungitius, are species that typically inhabit vegetated areas but are not confined to them (Wootton 1976). This was evident in the field, for they were found to occupy both marsh and lake environments. Other studies have shown them to occupy pools and current areas of streams and rivers. The ability to successfully occupy each environment relates to their ability to alter swimbladder lift. Laboratory studies showed that both species could make extensive buoyancy adjustments over relatively short periods of time. Gee (1983) suggests that behavioural phenotypic plasticity of swimbladder volume is adaptive for it allows a fish to 1) occupy a variety of environments in space; and 2) adapt to changes in the dominant variable affecting lotic communities, water velocity and factors relating to it.

Culaea and Pungitius are both pelagic species. To maintain

position at depth within the water column, the forces of lift must equal weight. This can be achieved by a combination of 1) static lift, which is produced by incorporating lighter materials into the body such as gas inclusions in the swimbladder and/or incorporating low density tissues and fluids; and 2) dynamic (hydrodynamic) lift, which is produced by the movement of the body and fins through the water (Gee 1983). The amount of static and dynamic sources contributing to lift in each species differs slightly as evidenced by the consistently different buoyancy values between species under all conditions. Static lift in Culaea is greater than in Pungitius, owing to a larger swimbladder volume and lower density tissues. Therefore the contribution of hydrodynamic lift should be greater in Pungitius. Morphologically, Pungitius is equipped to use hydrodynamic lift by the presence of caudal keels, and in holding tanks, Pungitius appeared more active than Culaea.

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APPENDICES

APPENDIX 1

Buoyancy corrections for depth of capture: An example is used to show the steps involved for making a correction.

Example: If buoyancy of a fish equals 0.950 mL.g^{-1} and its depth of capture was 1.0 m, determination of buoyancy at depth is as follows:

Step 1 - if atmospheric pressure on the day of capture was 760 mm Hg, then:

$$\begin{aligned}\text{pressure at depth} &= \text{atmospheric pressure} + \text{water pressure at 1.0 m} \\ &= 10332.3 \text{ mm H}_2\text{O} + 1000.0 \text{ mm H}_2\text{O} \\ &= 11332.3 \text{ mm H}_2\text{O}\end{aligned}$$

(note: $760 \text{ mm Hg} = 10332.3 \text{ mm H}_2\text{O} = 1013.0 \text{ mbar}$)

Step 2 - buoyancy at depth of capture is then determined from Boyle's Law:

$$P_1 V_1 = P_2 V_2$$

where P_1 = atmospheric pressure (mm H₂O); V_1 = buoyancy of fish at atmospheric pressure (mL.g^{-1}); P_2 = pressure at depth (mm H₂O); V_2 = buoyancy of fish at depth of capture (mL.g^{-1})

$$\begin{aligned}V_2 &= \frac{(10332.3) (0.950)}{11332.3} \\ &= 0.866 \text{ mL.g}^{-1}\end{aligned}$$

APPENDIX 2

t-Test: Comparison of mean buoyancy ($\text{mL}\cdot\text{g}^{-1}$) attained by each species over the field season at each sampling site.

a) Assiniboine Diversion

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	0.78	160	0.007	equal	-10.33	467.0	0.0001
<u>Pungitius</u>	0.69	309	0.005				

b) End of Cram Creek

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	0.86	108	0.005	unequal	-10.22	173.2	0.0001
<u>Pungitius</u>	0.78	98	0.007				

c) Man-made Channel

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	0.86	32	0.008	equal	- 7.50	72.0	0.0001
<u>Pungitius</u>	0.77	42	0.009				

APPENDIX 3

One-Way ANOVA and Tukey's Test (means with the same letter are not significantly different): Buoyancy ($\text{mL}\cdot\text{g}^{-1}$) differences between sampling sites in each species.

a) Culaea

Source	df	ss	mss	F value	Prob>F
Site	2	0.5422	0.2711	50.21*	0.0001
Error	297	1.6036	0.0054		
Total	299	2.1458			
Tukey Grouping	Mean	n	Site		
A	0.86	108	End of Cram Creek		
A	0.86	32	Man-made Channel		
B	0.79	160	Assiniboine Diversion		

b) Pungitius

Source	df	ss	mss	F value	Prob>F
Site	2	0.6839	0.3419	51.43*	0.0001
Error	446	2.9656	0.0066		
Total	448	3.6495			
Tukey Grouping	Mean	n	Site		
A	0.78	98	End of Cram Creek		
A	0.77	42	Man-made Channel		
B	0.69	309	Assiniboine Diversion		

APPENDIX 4

t-Test: Comparison of mean buoyancy ($\text{mL}\cdot\text{g}^{-1}$), density ($\text{g}\cdot\text{mL}^{-1}$), and standard ($\text{mL}\cdot\text{g}^{-1}$) between species, with sites pooled over the field season.

a) Buoyancy

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	0.82	300	0.005	equal	-15.54	747.0	0.0001
<u>Pungitius</u>	0.72	449	0.004				

b) Density

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	1.058	300	0.0004	equal	8.83	747.0	0.0001
<u>Pungitius</u>	1.063	449	0.0004				

c) Standard

Species	Mean	N	Std Error	Variances	T	df	Prob>T
<u>Culaea</u>	0.045	300	0.0004	equal	-4.37	747.0	0.0001
<u>Pungitius</u>	0.043	449	0.0004				

APPENDIX 4 cont'd

t-Test: Comparison of mean buoyancy ($\text{mL}\cdot\text{g}^{-1}$), density ($\text{g}\cdot\text{mL}^{-1}$), and standard ($\text{mL}\cdot\text{g}^{-1}$) between sexes within species, with sites pooled over the field season.

a) Culaea, buoyancy

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	0.83	137	0.006	unequal	2.99	298.0	0.0030
male	0.81	163	0.007				

b) Culaea, density

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	1.056	137	0.0007	unequal	-4.33	266.4	0.0001
male	1.060	163	0.0005				

c) Culaea, standard

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	0.044	137	0.0006	equal	-1.49	298.0	0.1366
male	0.046	163	0.0006				

APPENDIX 4 cont'd

d) Pungitius, buoyancy

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	0.71	246	0.006	equal	-1.15	447.0	0.2520
male	0.72	203	0.006				

e) Pungitius, density

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	1.063	246	0.0005	equal	-0.78	447.0	0.4331
male	1.064	203					

f) Pungitius, standard

Sex	Mean	N	Std Error	Variances	T	df	Prob>T
female	0.042	246	0.0005	equal	-1.28	447.0	0.1991
male	0.043	203	0.0005				

APPENDIX 5

Two-Way ANOVA and Tukey's Test (means with the same letter are not significantly different): Effects on buoyancy ($\text{mL}\cdot\text{g}^{-1}$), standard volume ($\text{mL}\cdot\text{g}^{-1}$), and internal pressure (atm) in each species of exposure to still water and current at 6, 10, 15, 20, and 25°C.

a) Pungitius, buoyancy

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	13.3736	13.3736	650.81 *	0.0001
Temperature	4	0.1943	0.0486	2.36	0.0589
Interaction	4	0.0972	0.0243	1.18	0.3239
Error	90	1.8494	0.0205		
Total	99	15.5145			

b) Pungitius, standard volume

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	13.5214	13.5314	647.25 *	0.001
Temperature	4	0.2089	0.0522	2.50 *	0.0481
Interaction	4	0.1134	0.0284	1.36	0.2554
Error	90	1.8815	0.0209		
Total	99	15.7352			

	Tukey Grouping	Mean	n	Temperature (°C)
still water:	A	0.98	10	6
	A	0.89	10	25
	A	0.89	10	20
	A	0.86	10	10
	A	0.79	10	15

APPENDIX 5 - cont'd b) Pungitius, standard volume

	Tukey Grouping	Mean	n	Temperature (°C)
Current:	A	0.20	10	6
	A	0.18	10	25
	A	0.17	10	15
	A	0.13	10	20
	A	0.05	10	10

c) Pungitius, internal pressure

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	0.0297	0.0297	0.85	0.7052
Temperature	4	0.0761	0.0190	0.54	0.3602
Interaction	4	0.1838	0.0460	1.31	0.2740
Error	75	2.6301	0.0351		
Total	84	2.9197			

d) Culaea, buoyancy

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	6.8095	6.8095	613.74 *	0.0001
Temperature	4	0.3954	0.0989	8.91 *	0.0001
Interaction	4	0.2123	0.0531	4.78 *	0.0015
Error	90	0.9986	0.0111		
Total	99	8.4158			

APPENDIX 5 - cont'd

d) Culaea, buoyancy

	Tukey Grouping	Mean	n	Temperature (°C)
still water:	A	1.00	10	6
	A	0.99	10	10
	A	0.99	10	15
	A	0.93	10	20
	A	0.92	10	25
current:	A	0.64	10	6
	B	0.47	10	15
	B	0.40	10	25
	B	0.37	10	20
	B	0.34	10	10

e) Culaea, standard volume

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	7.0416	7.0416	610.06 *	0.0001
Temperature	4	0.4538	0.1135	9.83 *	0.0001
Interaction	4	0.1850	0.0463	4.01 *	0.0049
Error	90	1.0388	0.0115		
Total	99	8.7192			
	Tukey Grouping	Mean	n	Temperature (°C)	
still water:	A	1.04	10	15	
	A	1.03	10	6	
	A	0.97	10	10	
	A	0.96	10	20	
	A	0.92	10	25	

APPENDIX 5 - cont'd e) Culaea, standard volume

	Tukey Grouping	Mean	n	Temperature (°C)
current:	A	0.65	10	6
	B	0.47	10	15
	B	0.42	10	25
	B	0.39	10	20
	B	0.34	10	10

f) Culaea, internal pressure

Source	df	ss	mss	F value	Prob>F
Water Velocity	1	0.0029	0.0029	1.14	0.2885
Temperature	4	0.0427	0.0107	4.27 *	0.0033
Interaction	4	0.0222	0.0056	2.22	0.0733
Error	90	0.2251	0.0025		
Total	99	0.2929			

	Tukey Grouping	Mean	n	Temperature (°C)
	A	1.05	20	20
B	A	1.03	20	25
B	A	1.02	20	15
B		1.00	20	6
B		0.99	20	10

APPENDIX 6

Regression Analysis:a) Pungitius; buoyancy ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm of time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	4.2537	4.2537	453.17 *	0.0001
Error	70	0.6571	0.0094		
Total	71	4.9108			

Regression Equation: $y = 0.861 - 0.366 \log x$, R-squared = 0.866b) Pungitius; standard volume ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm of time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	4.0874	4.0874	406.79 *	0.0001
Error	70	0.7033	0.0100		
Total	71	4.7907			

Regression Equation: $y = 0.874 - 0.359 \log x$, R-squared = 0.853c) Pungitius; internal pressure (atm) vs. time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	0.0050	0.0050	0.311	0.5789
Error	61	0.9881	0.0162		
Total	62	0.9931			

Regression Equation: $y = 1.042 + 0.0003 x$, R-squared = 0.005

APPENDIX 6 - cont'd

d) Culaea; buoyancy ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm of time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	5.6360	5.6360	471.96 *	0.0001
Error	94	1.1225	0.0119		
Total	95	6.7585			

Regression Equation: $y = 1.130 - 0.328 \log x$, R-squared = 0.834e) Culaea; standard volume ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm of time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	5.2799	5.2799	436.29 *	0.0001
Error	94	1.1376	0.0121		
Total	95	6.4175			

Regression Equation: $y = 1.129 - 0.318 \log x$, R-squared = 0.823f) Culaea; internal pressure (atm) vs. time (h) in current

Source	df	ss	mss	F value	Prob>F
Model	1	0.0888	0.0888	73.68 *	0.0001
Error	94	0.1133	0.0012		
Total	95	0.2021			

Regression Equation: $y = 1.005 + 0.0005 x$, R-squared = 0.439

APPENDIX 6 - cont'd

Analysis of Covariance: Comparison of regression lines between species for rate of buoyancy decrease in current.

Source	df	yy	xx	b	Deviations from regression		
					df	ss	mss
<u>Pungitius</u>	71	20.2704	117.1071	-0.3661	70	0.6571	0.0094
<u>Culaea</u>	95	51.8192	228.8910	-0.3281	94	1.1225	0.0119
Pooled	166	72.0896	345.9981	-0.3424	164	1.7796	0.0213
					165	1.8082	0.0110
Difference between slopes					1	0.0286	0.0286
Between	1						
Total	167				166	5.7455	0.0346
Between adjusted means					1	3.9373	3.9373

1. Homogeneity of variances: $F = \frac{0.0119}{0.0094} = 1.2660$ (F0.05 94,70 1.35) N.SIG.

2. Comparison of slopes: $F = \frac{0.0286}{0.0213} = 1.3427$ (F0.05 1,164 3.84) N.SIG.

3. Comparison of intercepts: $F = \frac{3.9373}{0.0110} = 357.9364$ (F0.05 1,165 3.84) SIG

APPENDIX 7

Regression Analyses:

a) Pungitius; buoyancy ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	3.9126	3.9126	369.37 *	0.0001
Error	54	0.5720	0.0106		
Total	55	4.4846			

Regression Equation: $y = 0.112 + 0.476 \log x$, R-squared = 0.873

b) Pungitius; standard volume ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	3.8081	3.8081	295.94 *	0.0001
Error	54	0.6949	0.0129		
Total	55	4.5030			

Regression Equation: $y = 0.106 + 0.469 \log x$, R-squared = 0.846

c) Pungitius; internal pressure (atm) vs. time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	0.0046	0.0046	1.00	0.3211
Error	54	0.2440	0.0046		
Total	55	0.2486			

Regression Equation: $y = 0.965 + 0.0006 x$, R-squared = 0.019

APPENDIX 7 - cont'd

d) Culaea; buoyancy ($\text{mL}\cdot\text{g}^{-1}$) vs. logarithm of time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	3.2342	3.2342	190.03 *	0.0001
Error	54	0.9191	0.0170		
Total	55	4.1533			

Regression Equation: $y = 0.230 + 0.433 \log x$, R-squared = 0.779e) Culaea; standard volume ($\text{mL}\cdot\text{g}$) vs. logarithm of time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	3.1938	3.1938	191.10 *	0.0001
Error	54	0.9025	0.0167		
Total	55	4.0963			

Regression Equation: $y = 0.232 + 0.430 \log x$, R-squared = 0.780f) Culaea; internal pressure (atm) vs. time (h) in still water

Source	df	ss	mss	F value	Prob>F
Model	1	0.0049	0.0049	2.72	0.1046
Error	54	0.0966	0.0018		
Total	55	0.1014			

Regression Equation: $y = 1.017 - 0.0006 x$, R-squared = 0.048

APPENDIX 7 - cont'd

Analysis of Covariance: Comparison of regression lines between species for rate of buoyancy increase in still water.

Source	df	yy	xx	b	Deviations from regression		
					df	ss	mss
<u>Pungitius</u>	55	19.3919	57.7532	0.4757	54	0.5720	0.0106
<u>Culaea</u>	55	24.1325	57.7532	0.4325	54	0.9191	0.0160
Pooled	110			0.4541	108	1.4911	0.0266
					109	1.5072	0.0138
Differences between slopes					1	0.0161	0.0161
Between	1						
Total	111	115.5065	43.5244		110	1.6925	0.0154
Between adjusted means					1	0.1853	0.1853

1. Homogeneity of variances: $F = \frac{0.0160}{0.0106} = 1.5094$ (F0.05 54, 54 1.53) N.S.

2. Comparison of slopes: $F = \frac{0.0161}{0.0276} = 0.5833$ (F0.05 1, 108 3.92) N.S.

3. Comparison of intercepts: $F = \frac{0.1853}{0.0138} = 13.4275$ (F0.05 1, 109 3.92) SIG.

APPENDIX 8

One-Way ANOVA and Tukey's Test (means with the same letter are not significantly different): Effects on buoyancy ($\text{mL}\cdot\text{g}^{-1}$), standard volume ($\text{mL}\cdot\text{g}^{-1}$), and internal pressure (atm) in each species of exposure to increasing water velocity.

a) Pungitius, buoyancy

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	3.0583	0.7646	68.01 *	0.0001
Error	35	0.3935	0.0112		
Total	39	3.4518			

Tukey Grouping	Mean	n	Water Velocity ($\text{cm}\cdot\text{s}^{-1}$)
A	0.89	8	0
B	0.46	8	7
C	0.19	8	16
C	0.18	8	50
C	0.17	8	30

b) Pungitius, standard volume

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	2.9996	0.7499	57.63 *	0.0001
Error	35	0.4554	0.0130		
Total	39	3.4550			

APPENDIX 8 - cont'd b) Pungitius, standard volume

Tukey Grouping	Mean	n	Water Velocity (cm·s ⁻¹)
A	0.88	8	0
B	0.45	8	6
C	0.19	8	50
C	0.18	8	30
C	0.17	8	16

c) Pungitius, internal pressure

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	0.0475	0.0119	1.54	0.2154
Error	30	0.2309	0.0077		
Total	34	0.2784			

d) Culaea, buoyancy

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	2.9673	0.7418	80.70 *	0.0001
Error	35	0.3217	0.0092		
Total	39	3.2890			

APPENDIX 8 - cont'd d) Culaea, buoyancy

Tukey Grouping	Mean	n	Water Velocity (cm·s ⁻¹)
A	1.01	8	0
B	0.73	8	6
C	0.43	8	16
D C	0.36	8	30
D	0.27	8	50

e) Culaea, standard volume

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	2.8196	0.7049	79.75 *	0.0001
Error	35	0.3094	0.0088		
Total	39	3.1290			

Tukey Grouping	Mean	n	Water Velocity (cm·s ⁻¹)
A	0.99	8	0
B	0.74	8	6
C	0.43	8	16
D C	0.37	8	30
D	0.26	8	50

f) Culaea, internal pressure

Source	df	ss	mss	F value	Prob>F
Water Velocity	4	0.0070	0.0017	0.62	0.6540
Error	35	0.0991	0.0028		
Total	39	0.1061			

APPENDIX 9

One-Way ANOVA and Tukey's Test (means with the same letter are not significantly different): Effects on buoyancy ($\text{mL}\cdot\text{g}^{-1}$), standard volume ($\text{mL}\cdot\text{g}^{-1}$), and internal pressure (atm) in each species of exposure to still water and waves.

a) Culaea, buoyancy

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.0084	0.0084	13.21 *	0.0027
Error	14	0.0089	0.0006		
Total	15	0.0173			
Tukey Grouping	Mean	n		Treatment	
A	0.97	8		Still water	
B	0.93	8		Waves	

b) Culaea, standard volume

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.0166	0.0166	26.58 *	0.0001
Error	14	0.0088	0.0006		
Total	15	0.0254			
Tukey Grouping	Mean	n		Treatment	
A	0.98	8		Still water	
B	0.92	8		Waves	

APPENDIX 9 - cont'd

c) Culaea, internal pressure

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.00001	0.00001	0.02	0.88
Error	14	0.0053	0.0004		
Total	15	0.0053			

d) Pungitius, buoyancy

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.0430	0.0430	24.54 *	0.0002
Error	14	0.0245	0.0018		
Total	15	0.0675			
Tukey Grouping	Mean	n		Treatment	
A	0.97	8		Still water	
B	0.87	8		Waves	

e) Pungitius, standard volume

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.0511	0.0511	23.56 *	0.0003
Error	14	0.0304	0.0022		
Total	15	0.0815			

APPENDIX 9 - cont'd e) Pungitius, standard volume

Tukey Grouping	Mean	n	Treatment
A	0.98	8	Still water
B	0.87	8	Waves

f) Pungitius, internal pressure

Source	df	ss	mss	F value	Prob>F
Treatment	1	0.0003	0.0003	0.99	0.3362
Error	14	0.0043	0.0003		
Total	15	0.0046			
