

The University of Manitoba

Factors affecting swimbladder lift and swimbladder gas  
composition analysis in three species of darters:  
Percina shumardi, P. maculata, and P. caprodes.

by Eleni Cavadias

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

May, 1986



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FACTORS AFFECTING SWIMBLADDER LIFT AND SWIMBLADDER GAS  
COMPOSITION ANALYSIS IN THREE SPECIES OF DARTERS:

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## Abstract

Swimbladder lift (SL) was examined in the field and in the laboratory in three species of Percina (Percina shumardi, P. maculata, and P. caprodes) to determine its variation and to identify factors responsible. In the field measurements were made on fishes collected from lentic and lotic habitats and the results were related to environmental variables and fish characteristics. SL decreased with increased current or turbulence and wind action, while the remaining variability was partially explained by temperature, sex, body and gonad weight as well as length. Comparable results were obtained from laboratory experiments, namely SL decreased upon exposure to current or turbulence while it increased in still water. SL attained in either still water or current was dependent on temperature in all three species (positive relationship in still water and negative in current) and on fish size in the log perch, P. caprodes, (negative relationship in still water and positive in current). The rate at which SL adjustment took place in blackside darters, P. maculata, in either still water or current was not affected by fish size and was completed in 168 h with almost 80% of the change taking place within the first 48 h. The 3 main swimbladder gases (O<sub>2</sub>, N<sub>2</sub>, and CO<sub>2</sub>) were present in different proportions and behave differently during the inflatory and

deflatory reflexes. In log perch acclimated to still water, O<sub>2</sub> comprised 28% of total volume and was reduced to 8% after 48 h exposure to current. During the inflatory reflex O<sub>2</sub> increased to 69% by 24 h and then dropped to 28% by 168 h. CO<sub>2</sub> remained at less than 3% during the whole time.

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## INTRODUCTION

Fishes occurring in streams, rivers, and wave-washed beaches of large lakes are exposed to variation in turbulence from current and waves over time. Many species, both physostomes and physoclists, adjust the amount of lift (volume) from the swimbladder in response to this environmental change, reducing such lift in current or turbulence and increasing it when still or calm waters are encountered. Nearly all of the research on this response has been in laboratory experiments dealing primarily with pelagic species (Gee 1983) with little information available from the field. Observations by Smart and Gee (1979) in rivers and Gee and Machniak (1972) on wave-washed beaches suggest that fishes in the field respond in a manner predicted by laboratory observations.

The extent of adjustment of swimbladder lift (SL) is considerable. Fishes (physostomes and physoclists) acclimated to still water and exposed to current typically reduce gas volume by 40-60% and require 1-4 d to complete the adjustment. The reverse adjustment requires a similar time (Gee 1986). However studies on rate of adjustment are restricted to physostomes. Water temperature and velocity and photoperiod affect the rate and extent of this response which also varies with size of fish, condition, and sexual

development (Gee 1983). The purpose of this study is to measure SL on three species of benthic physoclistous darters (Percina) in the field from both lotic and lentic environments, to identify factors affecting buoyancy and to describe the rate of adjustment.

River darters, Percina shumardi (Girard), live in moderate to fast current in rivers with larger individuals inhabiting faster and deeper waters (Thomas 1970). In lakes they are most abundant in exposed beaches where more than 75% of the substrate is rubble (stones >64 mm in diameter). In mid summer they are also found in protected beaches with finer substrates. Some migrate into inlet streams in April and May where spawning occurs in shallow riffles (45 cm/sec) with fine pebble substrate. They move downstream after spawning, moving out of the rivers by September (Balesic 1971). Blackside darters, Percina maculata (Girard), inhabit pools and rapids of small to moderate sized streams and are uncommon in large rivers (Thomas 1970). Spawning occurs in April and May following upstream migration (Thomas 1970). They are most abundant in riffles (>25 cm/sec) but are also abundant in channels (5-25 cm/sec) and pools (<5 cm/sec) (Smart and Gee 1979). Log perch, Percina caprodes (Rafinesque), successfully inhabit a variety of environments ranging from streams and rivers of all sizes to mud, gravel or sandy bottom lakes (Thomas 1970) to depths of 39 m

(Trautman 1957). Spawning occurs from late March (Miller and Robinson 1973) to July (Winn 1958b) depending on location. All three species live on average 3 years and feed mainly on midges, mayflies, and small crustaceans (Thomas 1970). Some river darter populations feed heavily on snails (Thompson 1974) and log perch have been observed to overturn stones with their protruding snouts and feed on dislodged invertebrates (Keast and Webb 1966).

Specific objectives of this study were to measure:

1. in the field, SL of darters in a variety of habitats and to relate such measurements to environmental variables and individual characteristics of the fishes and
2. in the laboratory, (a) the effect of holding condition (still water, water current, waves), fish size and water temperature on SL, (b) the rate of change of SL in response to changes in water velocity and (c) the importance of gain and loss of specific gases during alteration of SL.

## MATERIALS AND METHODS

### Field buoyancy measurements

Darters for buoyancy measurements were captured in the field by seining. Seine hauls were made in environments where water velocity, depth and turbulence were relatively homogeneous. Fishes were examined from May to September in 1984 and 1985. Blackside darters were collected from the Brokenhead, Pembina and Cypress Rivers from depths between 15-80 cm, and velocities of 0-100 cm/sec. River darters were collected from the Red River (St. Norbert) and from Lake Winnipeg (Hecla Island; Gimli), from depths ranging from 0-100 cm, velocities of 0-100 cm/sec and along beaches ranging from calm to turbulent. Log perch were collected from Lake Winnipeg (as above) and Lake Manitoba at Delta Marsh. At the latter, depth varied from 70-90 cm and turbulence from wave action was always present. As water velocity and turbulence were considered to be factors potentially influencing buoyancy, darters were collected from a wide range of variation of these factors. Seining continued at each site until at least 10 fish of a particular species were collected from a given velocity or amount of wave action. After each sample was collected, water temperature, depth, windforce and current velocity were recorded and descriptions of substrate and vegetation

were made to determine if relationships existed between particular environmental factors and buoyancy.

Two procedures were used to measure SL in the field. The first was an application of the cartesian diver principle with the anaesthetized fish being the diver. The swimbladder volume and therefore the density of the fish can be changed by varying the pressure around it. Fish, within 20 min of capture were anaesthetized individually using MS-222 (ethyl m-aminobenzoate methane sulfonate) and placed in a rectangular plexiglass chamber (30x15x49 cm) open on the top, mounted on a wooden base. The chamber was filled with water from the sampling site to within 3 cm of the top, made airtight by sealing the top with a rubber stopper, and pressure inside was reduced by a handpump until the fish rose off the bottom and remained at a specific point in the water column about 5 cm from the surface, the pressure of neutral buoyancy. Using this value ( $\pm 1.00$  mm Hg) it is possible to calculate SL using the equation of Boyle's law:

$$P_1 \cdot V_1 = P_2 \cdot V_2$$

where  $P_1$  = pressure of neutral buoyancy (mm Hg)

$V_1$  = neutral buoyancy (1.00 ml/g)

$P_2$  = atmospheric pressure (mm Hg)

$V_2$  = SL of fish (ml/g) at atmospheric pressure.

No correction was made for depth of capture because it was not possible to determine at which depth in the water column fish were actually caught.

This procedure was only used for relatively buoyant fishes ( $> 0.250$  ml/g). If the handpump did not provide an adequate pressure reduction ( $< 190$  mm Hg) then a second method was utilised. A glass funnel was inverted over a mirror in a container filled with water. A graduated pipette ( $\pm 0.001$  ml) was attached to the funnel and a syringe was attached to the top of the pipette. The whole apparatus was filled with water. The body cavity of the fish was opened under water at the base of the funnel and swimbladder gas was released and collected in it and drawn into the pipette using the syringe. Swimbladder volume ( $\pm 0.001$  ml) at atmospheric pressure was measured. Fish then were placed in numbered vials partially filled with water and taken back to the laboratory where they were stored overnight in a refrigerator ( $3-5$  °C). The next day the following measurements were taken for each fish: total length, gonad weight, and fish weight in air as well as the weight of the gas free fish in water. This latter weight did not change significantly over 24 h (Appendix 1). SL was calculated using the following equation:

$$\frac{\text{Volume of gas released (ml)}}{\text{Weight of gas-free fish in water (g)}}$$

With the second method, if gas was held in the swimbladder under pressure, the volume released would not accurately reflect the swimbladder volume. Such an error however is slight compared to the actual differences between still water and current or turbulence. For individuals with small swimbladder volumes, like those used here, it would be <0.002 ml.

#### Statistical analysis

Of all the variables that were measured only a few were found to correlate with buoyancy. The criteria for choosing the best subsets were the Cp statistic (Mallow's) and the R<sup>2</sup> value. The subsets chosen maximized the R<sup>2</sup> value and minimized the Cp.

For log perch and river darters which were encountered in waves, a code indicating the presence or absence of turbulence was used. All independent variables except wind force and gonad weight were tested during laboratory experiments for significance. These two were not tested because of technical limitations. A discriminant function

analysis was performed for each species in an attempt to find the combination of variables that provides the best separation between groups on the basis of their origin (still or running-turbulent water) using only variables representing fish characteristics (total length, sex, buoyancy and body and gonad weight). Differences were considered significant at  $p < 0.05$ .

#### Laboratory buoyancy measurements.

Laboratory holding conditions were designed to simulate three habitats: still water pools, fast-flowing rapids, and wave-washed beaches, to determine the effect of presence or absence of current or wave turbulence on SL. SL attained in still water would be greatest while that attained in current or waves would be least. As a result not only could effects of size of fish and temperature be assessed in each holding condition but their effects on extent of adjustment (difference between maximum and minimum buoyancy) could also be determined. Blackside darters were examined in still water and current while the remaining species were examined under all three holding conditions.

Depending on their numbers each species was held separately in bare 80, 190, or 380 L tanks, equipped with air stones. A 12L:12D photoperiod was provided, using 40

watt lightbulbs, and temperature was kept at 13 °C ( $\pm 0.50$  °C). Fishes were fed frozen brine shrimp twice daily, and were treated occasionally with salt to prevent fungal infections. Some fishes were held for up to 1 year in the laboratory prior to testing but this had no apparent effect on SL (Appendix 2 ).

To measure SL in still water, fishes were held in aquaria (92x45x47 cm) with a capacity of 170 L. The bottom was covered with washed gravel and sand and aeration was provided through gently-bubbling air stones. Long strands of green plastic attached to rocks on the bottom simulated plant growth. Fish were held for 7 d in still water to attain maximum buoyancy. The number of fish in these tanks was limited to 50 as it was found that density affected SL (Appendix 3).

Current was created in a 92x45x47 cm aquarium using the design of Gee and Bartnik (1969). Fishes were contained in a rectangular basket (60x44x18 cm) in which water flowed in one end and out the other. Depth at the inlet screen was about 4 cm and 8 cm at the outlet screen. Coarse gravel was used for substrate. Water velocity was measured by averaging four current values each taken at six points in the stream tank, three on each side at about mid depth with an Ott current meter (type C1). Although current tanks were

arranged in such a way as to make the water velocity as uniform as possible, velocities were always higher in the front than in the back. The mean velocity was 1.6 m/sec and the standard deviation 0.2 m/sec. Fishes were introduced to current tanks and water velocity was increased gradually to avoid stressing them excessively. Increases in velocity were such that 75% of the fishes were able to hold position against the water flow. The maximum velocity was reached within 1 d. Fish were held in current for 7 d to reach minimum buoyancy.

Waves were generated in a 188x31x47 cm aquarium by an oscillating paddle using the design of Patee (in Stong 1968). The wave-generating paddle was separated from the fish holding area (145x31x47 cm) by a metal screen. Water depth was 13 cm when the paddle was idle. Wave height from trough to crest could be regulated from 0.50 to 3.0 cm. The bottom of the holding area was covered with gravel and sand to simulate a beach. The gradient started at about 50 cm from the screen and reached a height of 20 cm at the other end. Fishes were introduced into the tank at the lowest gear (minimum turbulence). Maximum turbulence was reached within 4 d.

To measure SL and related variables fish were removed from the experimental tanks, anaesthetized in MS222 and the

following measurements were taken using the procedure of Chiasson and Gee (1983): volume of the intact swimbladder, volume of gases in swimbladder at atmospheric and standard pressures, and weight of the gas-free fish in water. SL was expressed as the ratio of swimbladder volume ( $\pm 0.001$  ml) over the weight ( $\pm 0.1$  mg) of the gas free fish in water, where 1.0 ml/g represents neutral buoyancy. Internal pressure (atm) of swimbladder gas (IP) was calculated by dividing the volume of swimbladder gases at atmospheric pressure by the intact swimbladder volume. Finally the volume of swimbladder gas at standard pressure was divided by the weight of the gas-free fish in water to provide a measure of weight specific gas volume at standard pressure (SV).

Effects of holding condition, fish size and temperature.

Darters were divided into sizegroups as follows: 3 (31-40 mm), 4 (41-50 mm), 5 (51-60 mm), 6 (61-70 mm), 7 (71-80 mm) and 8 (>80 mm) to determine the effect of size on SL and IP attained in each holding condition and extent of adjustment of SL. Buoyancy was measured on river darters of sizegroups 4 and 5, held in still water, current, and waves. In blackside darters sizegroups 3 to 6 were tested in still water and current while log perch in sizegroups 5-7 were tested in still water, current and waves. These experiments were completed at 17 °C and analysed in a two way ANOVA with 8 replicates. Prior to testing in experimental conditions,

fish were acclimated to still water for at least 4 days.

The effect of temperature on SL attained in each holding condition and on extent of adjustment was determined at 7, 17 and 25 °C. River darters (sizegroup 4) and log perch (sizegroup 7) were tested in still water, current and waves at each temperature except at 7 °C fish could not tolerate waves so this treatment was omitted. Buoyancy of blackside darters (sizegroup 5) was measured in still water and current at each temperature. Results were analysed in a two-way ANOVA. Differences were considered significant if  $p < 0.05$ .

Rate of swimbladder adjustment. Blackside darters (sizegroups 4, 5, and 6) were held in still water tanks and after 7 d, 8 fish were removed and SL and IP measured (time 0 in current). The remaining fish were transferred to current tanks. Batches of 8 were removed at 6, 12, 24, 48 and 168 h and their buoyancy measured. A second group of fish was held in two current tanks for 7 d. A sample of 8 was removed (time 0 in still water) and the rest transferred to still water tanks. Batches of 8 fish were removed after 6, 12, 24, 48, and 168 h. This procedure was repeated for each sizegroup. Slopes of regression lines were compared using ANCOVA with sizegroup being the covariate. Differences were considered significant if  $p > 0.05$ .

Changes in gas composition during SL adjustment. Changes in gas composition were described in log perch during deflation (exposure to current) and inflation (exposure to still water) (sizegroup 8) at 17 °C. Log perch were acclimated to still water for 7 d and then exposed to current. A second batch was acclimated to current and then exposed to still water. SL and SV were measured in fish samples (n=16) that had been under each of these conditions for 0, 6, 24, 48, 96 and 168 h. Eight of the 16 fish were used for buoyancy measurements and the other 8 to describe the gas composition. To obtain swimbladder gases, fish were anaesthetized in MS222, the body cavity opened to expose the swimbladder, and a gas sample (70-90  $\mu$ l) was withdrawn using a 100  $\mu$ l gas tight Hamilton syringe (Hamilton Inc., Reno, Nevada). The needle was then sealed with a rubber cap to prevent leakage until the gas was analysed, a few seconds later. Before or after each set of measurements four calibration curves were made using a known mixture of 10% CO<sub>2</sub>, 43% O<sub>2</sub>, and 39.8% N<sub>2</sub>. For each gas, a mean value was calculated for the area/unit gas volume (mm/ $\mu$ l). This was used to compute the volume of each gas present in the swimbladder from the peak areas in the chart. The analysis was performed in a Carle basic gas chromatograph 8700 (Carle California), modified for respiratory gases (one poropack QST 50/80 mesh and one molecular sieve 5A 42/60 mesh).

Fifty ul of the gas were injected into the chromatograph and peaks for CO<sub>2</sub>, O<sub>2</sub> (plus Ar) and N<sub>2</sub> were resolved on a strip chart recorder as each fraction passed the thermal conductivity detector in the gas chromatograph. The area under the peaks was calculated using the following formula:  
Peak area=(max. peak height) \* (width of peak at 1/2 h).

The absolute amounts of the 3 gases in the SB, corrected for fish weight, were calculated using the standard volumes from the rate of adjustment experiments.

## RESULTS

### Field buoyancy measurements

The eight variables that best predicted buoyancy were identical for the 3 species (Table 1). Current velocity or turbulence and wind were the factors that contributed most to the  $R^2$  values for all three species, followed by temperature in blackside darters. Length was least important in river darters and blackside darters whereas in log perch the least important was gonad weight. The equation for log perch had the highest  $R^2$  value and that for river darters the lowest.

Buoyancy, weight, (blackside darters) and sex in the case of river darters or length in the log perch, were the variables that separated fishes from still water and current with the highest percentage of success (Table 2).

Differences in buoyancy between fishes coming from still water and current or waves were significant for all three species (Table 3) but no significant differences were found between current and waves with river darters. Furthermore, buoyancy differences within locations, between areas of slow and fast water flow were significant (Table 3; t-test Appendix 4).

Table 1. The best multiple regression equations for predicting buoyancy in blackside darters (BSD), river darters (RD) and log perch (LP). The three most important variables contributing to R<sup>2</sup> in each species are marked by asterisks.

Species	Variables	Range	Regression coefficient	Contribution to R <sup>2</sup>	R <sup>2</sup>
BSD	Length(mm)	22-81	0.001654	0.00026	.60
	Temperature(°C)	16-25	0.047605	0.07505**	
	Sex	0-2	-0.126060	0.06544*	
	Current(cm/sec)	0-100	-0.007222	0.28708***	
	Wind	0-2	0.054577	0.00921	
	Weight(g)	.2-5.2	-0.061542	0.00372	
	Gonad wgt(g)	0-0.4	0.943854	0.00870	
	Intercept		-0.171533		
RD	Length(mm)	26-29	-0.001230	0.00071	.250
	Temperature(°C)	10-26	-0.002848	0.00211	
	Sex	0-2	0.003152	0.00008	
	Turbulence	1-3	-0.195563	0.12351***	
	Wind	1-3	-0.048519	0.01013**	
	Weight(g)	.2-3.4	0.044545	0.00384	
	Gonad wgt(g)	0-.24	-0.507586	0.00895*	
	Intercept		0.694425		
LP	Length(mm)	31-119	-0.001282	0.00198*	.606
	Temperature(°C)	15-26	0.001988	0.00043	
	Sex	0-2	-0.004201	0.00018	
	Turbulence	1-3	-0.093348	0.06257**	
	Wind	1-3	-0.099938	0.07006***	
	Weight(g)	.3-15.9	-0.002457	0.00020	
	Gonad wgt(g)	0-1.22	0.016702	0.00020	
	Intercept		0.689486		

1.sex codes: 0=immature, 1=male, 2=female.

2.wind force: 0=calm, 1=light, 2=moderate, 3=strong.

3.turbulence: 1=still water, 2=current, 3=waves.

Table 2. Combinations of variables that best differentiate between fish from different environments (still water, current or waves) and the percentage of successful classifications for blackside darters (BSD) river darters (RD) and logperch (LP).

Species	Variables	% of successful classifications		
		still	current or waves	combined
BSD	Buoyancy, Weight	77.5	88.1	80.9
RD	Buoyancy, Weight, Sex	100	88.9	90.9
LP	Buoyancy, Weight, Length	94.4	88.9	92.5

Table 3. Sample size (n) mean buoyancy values (ml/g) ( $\bar{X}$ ) and standard deviations (s) for blackside darters (BSD), river darters (RD) and log perch (LP) from different environments.

Species	Environment											
	still			Current (cm/sec)						waves		
				< 30			> 30					
	n	$\bar{X}$	s	n	$\bar{X}$	s	n	$\bar{X}$	s	n	$\bar{X}$	s
BSD	40	.76	.16	25	.40	.11	22	.04	.07			
RD	10	.35	.05				26	.14	.17	19	.16	.14
LP	54	.40	.17							107	.12	.10

## Laboratory buoyancy measurements

Effects of holding condition, fish size and temperature. SL of river darters (sizegroup 4) was significantly affected by holding condition (still water vs current) and its interaction with temperature was also significant (ANOVA; Appendix 5). As a result the extent of adjustment was affected by temperature, the greatest adjustment occurring at 25 °C and the least at 7 °C (Table 4). Significant differences occurred only between 7 and 25 °C in either still water or current (Bonferroni's test). In still water, SL increased with temperature whereas in current it decreased. There was no significant difference between still and current at 7 °C. At 17 and 25 °C differences were significant. IP was not affected significantly by temperature in still water. In the comparisons involving all 3 holding conditions at 17 and 25 °C, holding condition significantly affected buoyancy, the differences being between still water and current or waves (Bonferroni), but the effect of temperature and its interaction with holding condition was not significant (ANOVA; Appendix 5).

The effects of sizegroup and its interaction with holding condition were not significant but holding condition (still water vs current vs waves) significantly affected

Table 4. Effects of holding condition and temperature on buoyancy (ml/g) and IP (atm; shown in parentheses) of sizegroup 4 river darters. Mean values are shown are shown (n=8).

Holding condition	Temperature, °C		
	7	17	25
Still	0.375(1.10)	0.442(1.06)	0.612(0.98)
Current	0.214	0.036	0.013
Waves	*	0.002	0.001

\* Fish did not survive this treatment.

SL (ANOVA;Appendix 5). Mean SL values of sizegroups 4 and 5 combined, (differences were not statistically significant) were 0.416 ml/g in still water 0.034 ml/g in current and 0.053 ml/g in waves. Differences in SL were significant only between still water and current or waves.

The effect of size on SL of blackside darters was not significant. The differences in SL between still water and current were significant but the interaction was not. In still water mean buoyancy of all sizegroups was 0.782 ml/g, IP was 0.986 atm. In current mean SL of all sizegroups was 0.052 ml/g. It tests performed at three temperatures (7, 17, 25 °C) the effect on SL of holding condition (still water vs current) was significant as was the interaction between this factor and water temperature (Table 5;ANOVA,Appendix 5). Thus the extent of adjustment of SL was affected by temperature with the greatest extent occurring at 25 °C. IP in still water was not significantly affected by temperature.

In log perch the effect of temperature and holding condition on SL were significant as was their interaction (ANOVA;Appendix 5). In still water SL increased with temperature whereas in current it decreased (Table 6). Thus the extent of SL adjustment was determined by temperature and was greatest at 25 °C and least at 7 °C. Comparisons of

Table 5. Effects of holding condition and temperature on buoyancy (ml/g) and IP (atm; shown in parentheses) of sizegroup 5 blackside darters. Mean values are shown (n=8).

Holding condition	Temperature, °C		
	7	17	25
Still	0.728(0.98)	0.761(0.99)	0.826(1.00)
Current	0.072	0.033	0.027

Table 6. Effects of holding condition and temperature on buoyancy (ml/g) and IP (atm; shown in parentheses) of sizegroup 7 log perch. Mean values are shown (n=8).

Holding condition	Temperature, °C		
	7	17	25
Still	0.264(1.09)	0.465(1.01)	0.683(1.00)
Current	0.043	0.039	0.016
Waves	*	0.081	0.038

\* Fish did not survive this treatment.

the means by Bonferroni tests showed significant differences between 7 and 25 °C. IP in still water did not vary significantly with temperature (Table 6). In comparisons involving all three holding conditions (still water, current, waves) at 17 and 25 °C holding condition significantly affected SL and its interaction with water temperature was also significant. According to Bonferroni's test the differences are restricted between still water and current or waves (Appendix 5). The greatest extent of adjustment occurred at 25 °C.

There was no significant effect of sizegroup on buoyancy but the differences between buoyancy attained at different holding conditions as well as the interaction were significant (Table 7; Appendix 5). The extent of buoyancy adjustment is, therefore, affected by sizegroup, smaller fish being capable of the greatest adjustment. Comparisons of the means within each treatment showed significant differences between sizegroups 5 and 7 in still water, and 5 and 6 in current (Bonferroni's test). IP in still water did not show any significant change during the experiment.

There were no significant differences between sexes in the extent of buoyancy adjustment in either still water or current in any of the three species examined (Table 8; t-test; Appendix 5).

Table 7. Effects of holding condition and size on buoyancy (ml/g) and IP (atm; shown in parentheses) of log perch tested at 17 °C. Mean values are shown (n=8).

Holding condition	Sizegroup		
	5	6	7
Still	0.686(1.05)	0.563(1.04)	0.465(1.01)
Current	0.012	0.051	0.039
Waves	0.020	0.037	0.081

Table 8. Sample size (n) standard error and mean buoyancy values for male and female blackside darters (BSD) and river darters (RD) in still water and current.

Species	Environment						
	sex	Still water			Current		
		mean	n	std.error	mean	n	std.error
BSD	males	0.785	19	0.0326	0.065	19	0.0134
	females	0.772	4	0.0706	0.003	11	0.0198
RD	males	0.478	14	0.0525	0.042	18	0.0192
	females	0.513	15	0.0553	0.068	5	0.0182

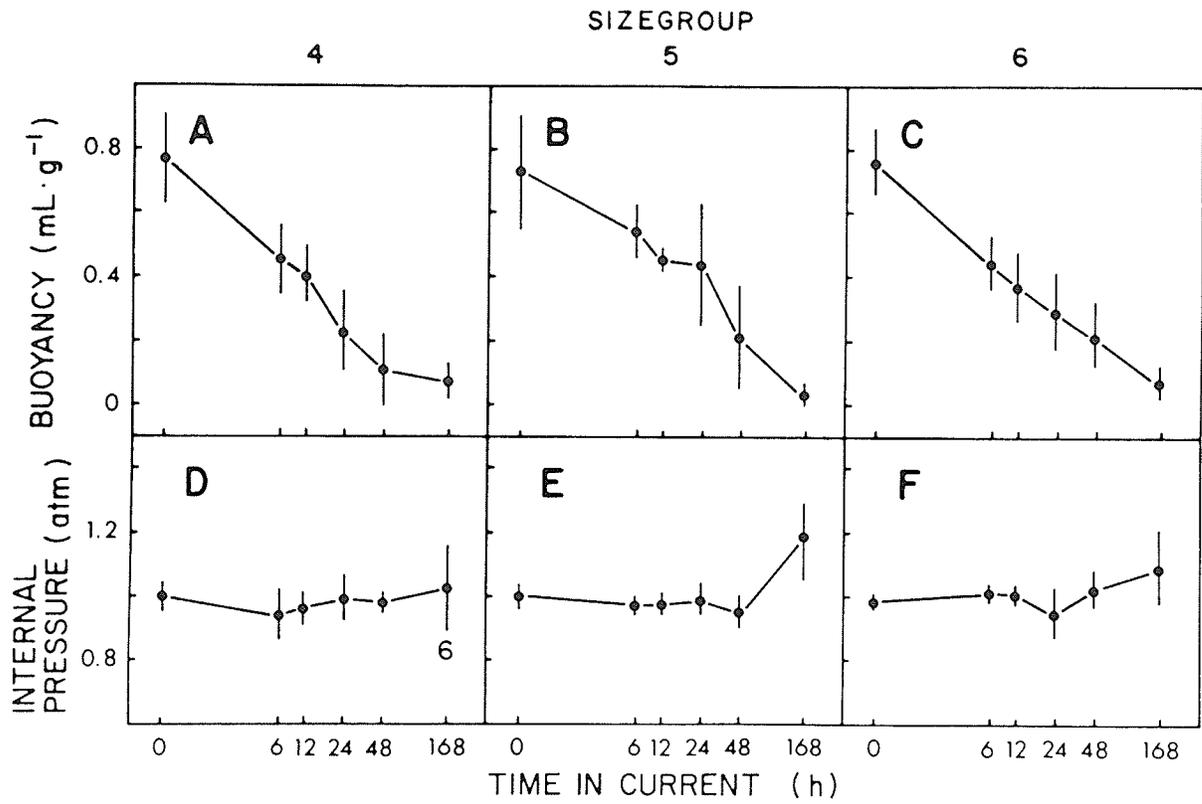
Rate of adjustment for blackside darters. The rate of change in SL and SV over time in response to either current (decrease in SL, SV) or still water (increase in SL, SV) was best described by a curvilinear regression model. However a logarithmic transformation of time gave almost as high  $R^2$  values therefore these equations were used to compare the rates (Appendix 6).

When exposed to current after acclimation to still water, blackside darters reduced their SL to a minimum over 7 d (Fig. 1 A to C). Because IP was close to 1.0 atm or varied from 1 atm at very low swimbladder volumes, SV was very similar to SL and these data are not shown. Comparisons, between sizegroups, of the rates of buoyancy decrease using the slopes of the regression lines showed no significant differences (Table 9; ANCOVA; Appendix 7). During the period of decrease, IP remained near 1 atm except in sizegroups 5 and 6 where it increased at 7 d in current (Fig. 1 D to F). A two-way ANOVA with IP as the dependent variable and time in current and sizegroup as the independent variables showed sizegroup to be insignificant but the effects of time as well as the interaction between time and sizegroup were significant (Appendix 8).

Table 9. Regression lines describing rates of decrease (exposure to current) and increase (exposure to still water) of SL and SV by three sizegroups of blackside darters. SL data are illustrated in Fig 1.

Experiment	Parameters	Sizegroup	Regression line	R <sup>2</sup>
Decrease	SL	4	$Y = .747 - .146 \log(\text{time})$	.760
		5	$Y = .783 - .136 \log(\text{time})$	.653
		6	$Y = .730 - .133 \log(\text{time})$	.780
	SV	4	$Y = .726 - .141 \log(\text{time})$	.750
		5	$Y = .780 - .134 \log(\text{time})$	.628
		6	$Y = .717 - .130 \log(\text{time})$	.764
Increase	SL	4	$Y = .002 + .165 \log(\text{time})$	.692
		5	$Y = .074 + .150 \log(\text{time})$	.661
		6	$Y = .069 + .140 \log(\text{time})$	.614
	SV	4	$Y = .005 + .161 \log(\text{time})$	.657
		5	$Y = .057 + .144 \log(\text{time})$	.651
		6	$Y = .045 + .134 \log(\text{time})$	.562

Figure 1. Rate of decrease in SL (A-C) and changes in IP (D-F) on exposure to current by blackside darters of sizegroups 4, 5, and 6. Mean values, n=8 unless otherwise indicated, and 95% confidence limits (vertical lines) are given. Time 0 h is in still water. Actual values are given on x axis but plotted on a log scale.



When exposed to still water after acclimation to current fish increased SL to a maximum over 7 d (Fig. 2 A to C) with a parallel increase in SV. For the first 6 h, sizegroups 5 and 6 showed very little change in SL (Fig. 2 B,C) and SV. There were no significant differences in the rate of buoyancy increase, expressed by the slopes of the regression lines, between the three sizegroups (Table 8; ANCOVA; Appendix 7). IP for these sizegroups remained above 1 atm during the first 12 to 24 h and then decreased close to atmospheric until the end of the experiment (Fig. 2 D to F). A two-way ANOVA with internal pressure as the dependent variable and time and sizegroup as the independent variables did not show any significance for the effect of sizegroup but both time and the interaction between time and sizegroup were significant (Appendix 8).

Swimbladder gas composition. When log perch were exposed to current after being acclimated to still water, SV declined from 0.791 to 0.077 ml/g in 96 h. This decline was best described as follows:  $SV^2 = 0.185 + 0.446(1/\text{time}) - 0.037(\log \text{time})$  ( $R^2 = .912$ ; Appendix 9). IP remained near 1 atm throughout the experiment. During that decrease,  $O_2$  and  $N_2$  declined at almost the same rate (Fig. 3). At 6 h the swimbladder was filled almost exclusively (91.84%) with  $N_2$ .

Figure 2. Rate of increase in SL (A-C) and changes in IP (D-F) on exposure to still water by blackside darters of sizegroups 4, 5, and 6. Mean values,  $n=8$  unless otherwise indicated, and 95% confidence limits (vertical lines) are given. Time 0 h is in current. Actual values are given on x axis but plotted on a log scale.

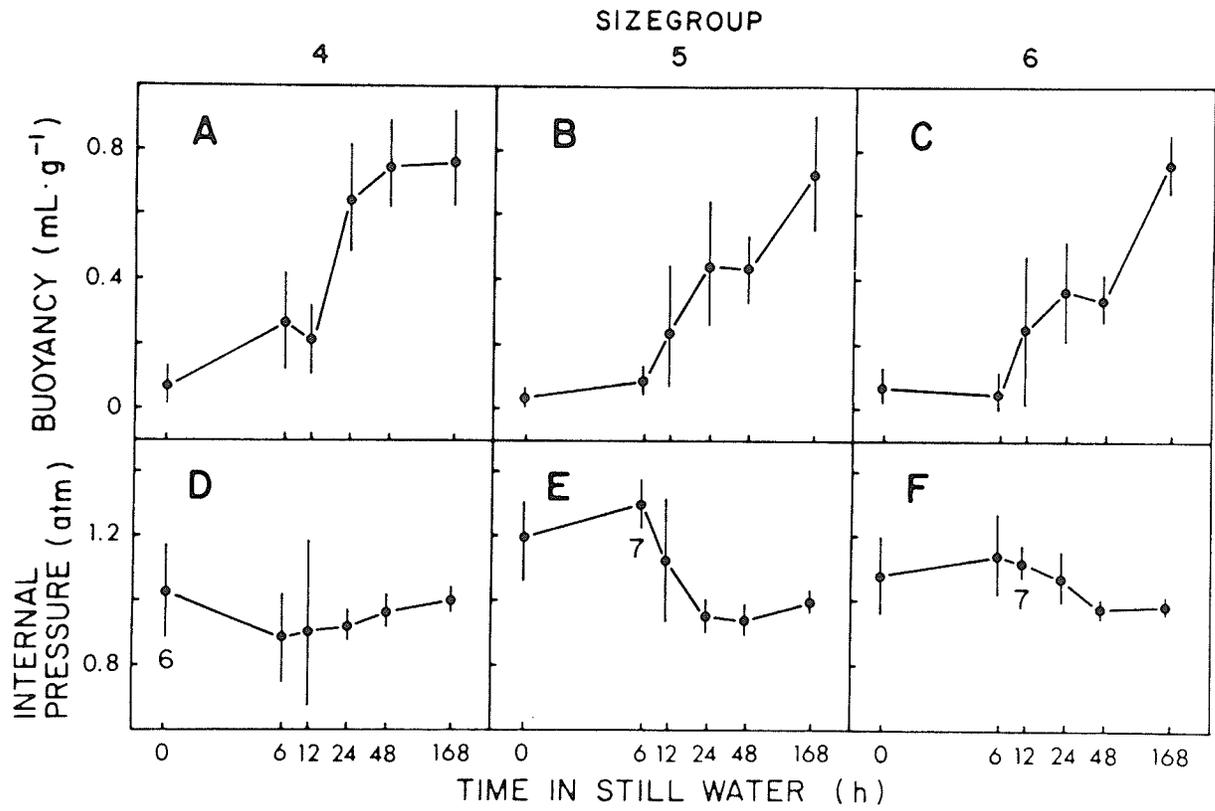
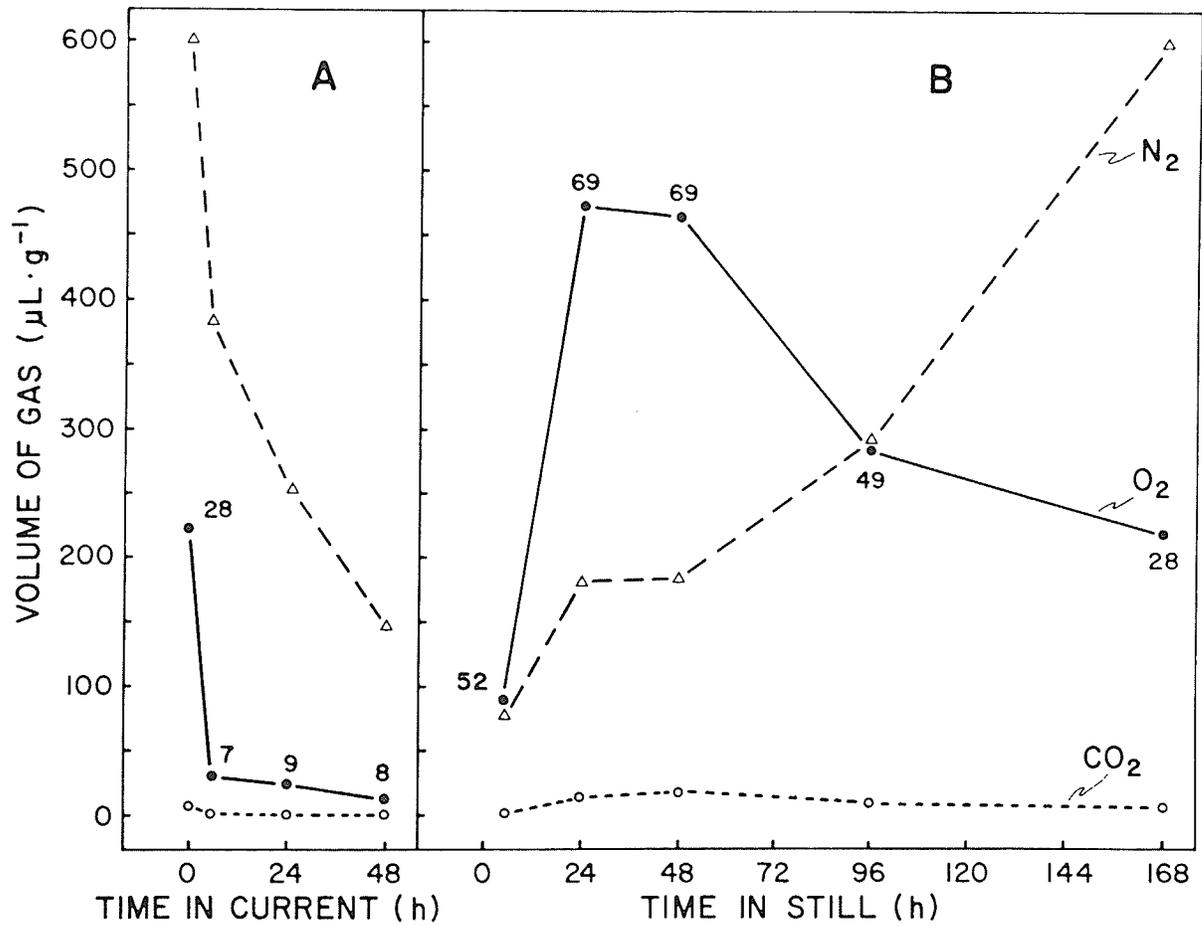


Figure 3. Changes in swimbladder gas composition of log perch during during decrease (A) and increase (B) of SL, with proportions of O<sub>2</sub> indicated. Gas volumes are given per unit weight of fish in water. Swimbladder gas volume after 48 h in current and prior to 6 h in still water were too small to determine gas composition.



No measurements were possible after 48 h in current because the amount of swimbladder gas was too low.

After being acclimated to current, fish exposed to still water increased SV in 24 h from 0.085 to 0.672 and between 96 and 168 h to 0.791 ml/g. Between 24 and 96 h there was little change. The equation of the line best describing the increase as follows:

$$1/SV=1.122+0.015(\text{time})-0.00004(\text{time}^2)-0.164(\sqrt{\text{time}})$$

( $R^2=.7473$ ; Appendix 9). IP remained near 1 atm at all times.

Between 6 and 24 h all three gases increased, O<sub>2</sub> at the highest rate and CO<sub>2</sub> at the lowest. Between 24 h and 48 h

O<sub>2</sub> remained at 69% of the volume and then decreased to 49% at 96 h. N<sub>2</sub> increased in proportion at a constant rate from 6 to 168 h when it comprised about 76% of the total volume. During both experiments CO<sub>2</sub> amounts never exceeded 2.8% of the total volume.

## DISCUSSION

Buoyancy values of the three species were highly variable in the field. Buoyancy (SL) was usually related to water velocity, turbulence and wind force, and varied with temperature, especially in blackside darters, and, in the case of the log perch, with size of fish. In the laboratory darters acclimated to still water responded to current or waves by reducing SL. After acclimation to current, they increased SL when still water was encountered. Buoyancy attained in either still, current or waves was dependent on water temperature and on size (log perch). Buoyancy adjustment in response to these variables required 7 d (blackside darters) and was accomplished primarily by gain/loss of O<sub>2</sub> and N<sub>2</sub> from the swimbladder (log perch).

Field buoyancy measurements.

Buoyancy of darters in the field was related to the same variables in each of the three species. The importance of these variables expressed as their relative contributions to R<sup>2</sup>, differed between species as did the total R<sup>2</sup> for the multiple regression equations. Water velocity (blackside darters) or turbulence and wind (river darters and log perch) were the most important variables in predicting buoyancy. Most of the variability was explained by these

factors while the rest could be partly explained by a combination of the remaining variables each making different contributions. For river darters and log perch, wind was an important factor since almost all of the fishes were caught in wave-washed beaches where turbulence and wind force are closely related. In blackside darters which were caught in rivers and streams, sites usually not directly exposed to wind action, this factor did not contribute much to  $R^2$ . Gee and Machniak (1972) found that longnose dace (Rhinichthys cataractae) captured on windy days along wave-washed beaches had much smaller swimbladders than those captured on calm days. Similarly blackside darters taken from rapids of rivers were less buoyant than those taken from pools (Smart and Gee 1979).

Length was an other important factor affecting buoyancy variability, contributing most to  $R^2$  in the log perch probably only because the wider range of lengths sampled made the trend more apparent. Furthermore there was a spatial separation, the larger individuals inhabiting more turbulent waters.

Sex was among the most important factors in blackside darters, females being less buoyant in current, but it was the least important in the other two species. There was no significant difference in buoyancy attained by the two sexes

in laboratory experiments. According to Thomas (1970) male and female blackside darters migrate separately upstream from pools to riffles to spawn, the females following the males, but there was no distinct separation of sexes between still and running water.

#### Laboratory buoyancy measurements

Effect of holding condition, fish size and temperature. All three darter species, acclimated to current, increased buoyancy over 7 d after exposure to still water. SL typically supported 25-80% of the body weight in still water depending on species, size and temperature. Darters are benthic species spending the majority of their time near or on the substrate, even in still water. Pelagic species described by Gee (1986) were typically more buoyant, with SL supporting 85-100% of the body weight in water.

Because these darters did not attain neutral buoyancy in still water they had to generate some hydrodynamic lift from swimming to move up off the substrate or along the bottom. However a somewhat negative buoyancy is compatible with the "sit and pounce" feeding behaviour common to darters. Percina feed by locating food items visually and when in range they dart towards them (Smart and Gee 1979). A negative buoyancy combined with periods of inactivity and

cryptic coloration make them inconspicuous to prey and also promotes avoidance of predators. In comparing the diet and feeding behaviour of johnny darters (Etheostoma nigrum; no swimbladder) and blackside darters, Smart and Gee (1979) found that the possession of a swimbladder permitted blackside darters to feed either just off the substrate or on the sides and tops of large stones protruding above the substrate. In still water in the laboratory darters remained motionless on the substrate and attacked food particles that sank towards the bottom, using short darts. However they often would swim up off the bottom some 30 cm, capturing food immediately after it was presented to them and then sinking passively towards the bottom.

After 7 d exposure to current, darters decreased their buoyancy becoming highly negative with the swimbladder supporting less than 10% of their body weight in water, and some individuals of all three species eliminated all detectable amounts of gas from the swimbladder. Pelagic species surveyed by Gee (1986) seldom reduced lift from the swimbladder below a level that would support 30% of their body weight in water.

Negatively buoyant fishes generate considerable frictional forces between the body and the substrate and this aids in holding position when exposed to currents

(Alexander 1966). The relationships between viscous and frictional drag were described by Arnold and Weihs (1978). Very low negative buoyancy is important for feeding in areas of current or turbulence where precise control of movements is vital. All three species observed while feeding in current behaved in a similar fashion. They kept position against the substrate and as drifting food was detected, they made a feeding dart and after capture, dropped to the substrate. Feeding darts were directed above or sideways. In waves, feeding darts were usually directed straight ahead and above. Because the wave motion was imparted to both the food and the fish it was harder to predict and anticipate the movement of food than it was in current. Occasionally fishes picked up items from the substrate in protected areas behind large rocks.

The ability to reach a low negative buoyancy is critical to maintaining position during spawning. Log perch and river darters either move into inlet streams and spawn in rapids/riffles just after spring runoff (Balesic 1971) or else they move onto exposed beaches where spawning also takes place (Winn 1958a,b; Trautman 1957). A negative buoyancy on wave-washed beaches is adaptive as indicated by observations in the wave tank. Here most of the time darters selected spaces between stones where they rested in contact with the substrate protected from turbulence. They

were not observed to orientate in any particular direction and their position appeared to be determined by that of the resting space.

The effect of size on buoyancy in still water and in current and on the extent of adjustment was variable among species. In these experiments fish size affected SL levels in both still water and current but the effect was more pronounced in still water where SL decreased with increasing size in all three species. The differences however were statistically significant only in the case of the log perch. Lack of difference could be related to the restricted size range sampled, at least in the case of the river darters. In other fishes there is considerable variation in the way size of fish influences buoyancy adjustment. Longnose dace (Rhinichthys cataractae) make extensive adjustments as juveniles SL ranging from negative to neutral values. Adults however make restricted adjustments over negative levels. Blacknose dace (R. atratulus) are just the opposite. The ability to make extensive buoyancy adjustments provides adaptation to a wide range of velocities and habitats. A narrower range of buoyancy adjustment indicates a restricted optimal habitat, either still to slow moving current or moderate to fast velocities. In log perch, juveniles may utilize a wider range of habitats since they are able to both increase their buoyancy

in still water and reduce it in current, while older fishes, being incapable of reaching high buoyancy values in still water, may be mostly restricted to current and turbulence.

Water temperature affected SL in each holding condition. Buoyancy increased with temperature in still and decreased in current and waves. As a result, the extent of adjustment was greatest at 25 °C. Gee (1977) found that the extent of adjustment by fathead minnows (Pimephales promelas) was greatest at an intermediate temperature 20 °C. Buoyancy of the stickleback Pungitius pungitius was not affected by temperature in either still or current while that of Culaea inconstans was, with the extent of adjustment being less at 6 °C than at 10-25 °C (Beaver unpub .data).

All three species potentially encounter water currents in spring during the spawning period when temperatures are 10-15 °C. It is to be expected then that the extent of adjustment would be greatest under such conditions. However the extent of adjustment was greatest at 25 °C. Possibly some other factors influence buoyancy at this time either with or in addition to temperature. Photoperiod is known to affect the extent of SL adjustment (Luoma and Gee 1980) in Pimephales promelas, with a 12L:12D (the same as used here) producing the greatest effect. However Luoma and Gee (1980) found that as gonad development increased in P. promelas so

did the extent of adjustment. Fish that were close to 100% development, just prior to spawning, could reduce buoyancy in current more than twice as much as those that were immature. Either photoperiod or development of the gonads could affect buoyancy in Percina to produce a more optimal SL during the colder temperatures of the spawning season. The ability to maintain a minimum buoyancy over time is an other factor that might be affected or interact with temperature. This ability was impaired under stress of high temperatures (Stewart and Gee 1981) or sublethal stress from toxicants (Jansen 1986). Maintenance of minimum buoyancy may be enhanced at cooler temperatures.

Neither log perch nor river darters could tolerate 7 °C in waves. They gradually lost their ability to hold position and were consequently dislodged by the waves and died in a few hours. This agrees with the observations in the Delta Marsh sampling site where fish were absent until temperatures were above 10°C at the shore. Apparently log perch populations spend the winter offshore in lakes and migrate onshore in the spring, some continuing their journey upstream (Winn 1958 a,b).

Measurements of SL in the field were not directly comparable to those in the laboratory. Fishes from either current or wave-washed beaches were not as negatively

buoyant as those in the laboratory. SL of fishes in the field is to a large extent a reflection of past environmental conditions because of the time required to make an adjustment. As a result, if environmental variables change frequently, as would wind direction and strength influencing wave action on exposed beaches, then the extremes of buoyancy would seldom be reached. Stream environments are different as changes in water volume flowing in a stream channel over time do not fluctuate as frequently. Here fish would have some time to acclimate to a particular velocity and the more extreme buoyancy values could be attained. These trends are observed when field and laboratory data are compared. Blackside darters from stream environments compared more closely in SL to those tested in the laboratory than did log perch or river darters coming from lakes.

Rate of buoyancy adjustment. Blackside darters reduced SL from near neutral in still water to highly negative over 7 d in current, with 80% of the change taking place during the first 48 h. The average rate of loss during that time was 12  $\mu\text{l/g/h}$ . This reduction was achieved primarily by gas resorption and to lesser extent by an increase in IP which occurred at lower swimbladder volumes. The rate of reduction is greater than that recorded for Percina shumardi (7.0  $\mu\text{l/g/h}$ ; Balesic unpublished data), and falls between the

two values available for P. caprodes (12.9  $\mu\text{l/g/h}$ ; Balesic unpublished data; 7.4  $\mu\text{l/g/h}$ -gas composition experiment) and is also higher than that of the physoclistous sticklebacks Culea inconstans (4.8  $\mu\text{l/g/h}$ ) and Pungitius pungitius (8.8  $\mu\text{l/g/h}$ ; Beaver unpub. data).

Blackside darters increased SL over 7 d to near neutral levels at an average rate of 9  $\mu\text{l/g/h}$  calculated over the first 48 h when 80% of the change took place. Initially this was accomplished by a slight decrease in IP to negative values (less than ambient) and by gas secretion. The rate of buoyancy increase is greater than that recorded for P. shumardi (8.1  $\mu\text{l/g/h}$ ; Balesic unpub. data), and falls between the two values available for P. caprodes (13.5  $\mu\text{l/g/h}$ ; Balesic unpub. data and 6.1  $\mu\text{l/g/h}$  gas composition experiment) and is also lower than the rate recorded for C. inconstans (14.2  $\mu\text{l/g/h}$ ) and P. pungitius (15.0  $\mu\text{l/g/h}$ ) (Beaver unpub. data).

These rates of buoyancy change are generally similar to physostomes although there is considerable variation between such species (Gee 1986). The extent to which physostomes gulp/spit relative to secretion/resorption determines the rate of change, the former being more important than the latter.

IP varied from positive to negative values. Positive values are common as most fishes hold the gases in the swimbladder under pressures just above ambient (Gee et al. 1974). McCutcheon (1966) suggested that such pressures were maintained by contraction of muscles in the swimbladder wall and that by contracting/relaxing these muscles fishes could control SL precisely but over a very narrow range. The occurrence of negative IP is rare but values as low as 0.93 atm were recorded by Sundnes and Gytre (1972). The causal mechanism however is not understood. According to Alexander (1959) excess IP combined with a swimbladder wall of limited extensibility reduces the rate at which SL changes with depth.

The decrease in buoyancy started immediately, a significant part taking place during the first 6 h, whereas the increase of buoyancy started after 6 h and even 12 h in the case of sizegroup 4. This either reflects physiological differences between the processes of gas secretion and resorption or it is related to behaviour and reflects the urgency to decrease buoyancy in order to be able to hold position against the current. An increase in buoyancy however would not be that urgent in the laboratory holding conditions since fish were amply fed twice daily and did not need to swim continuously to locate food.

Gas composition. Gas resorption in log perch began immediately after fish were exposed to current. Both O<sub>2</sub> and N<sub>2</sub> declined sharply, and by 6 h O<sub>2</sub> was reduced by 86%, N<sub>2</sub> by 31% and CO<sub>2</sub> by 78%.

When a physoclistous fish is in hydrostatic equilibrium the swimbladder is in a steady state with gases deposited and resorbed at the same rate. The constancy of the volume is the result of a continuous flux of gases through the membranes of the swimbladder wall that is finely adjusted by autonomic reflexes. During resorption the oval, which separates the swimbladder into secretory and resorbent areas, opens exposing the gas permeable resorbing part (Fange 1983).

Gases diffuse according to their partial pressure and the amount of resorption is equal to the arteriovenous difference in gas content times the volume of blood flow. Resorption is regulated partly by varying the proportion of the area exposed to the gas and partly by varying the blood flow through the resorbent area. The structures of importance for gas resorption, the resorbent capillary plexus and the muscularis mucosae, are controlled by autonomic nerve reflexes. During the deflatory reflex (increased resorption) the inner gas impermeable membrane (secretory mucosa-submucosa) contracts towards the gas gland

leaving the resorbent mucosa relaxed and thin. Simultaneously the resorbent capillaries dilate. The  $O_2$  resorbed for hydrostatic purposes is most likely utilized metabolically but some of it is inevitably lost to the ambient water (Steen 1970).

According to Krogh's measurements (1919)  $CO_2$  has the highest diffusion rate through biological membranes and  $N_2$  the lowest.  $O_2$  is the main gas that is exchanged during volume control of the swimbladder while  $N_2$  is least movable and  $CO_2$  diffuses so easily that the concentration within the swimbladder is usually close to zero (Fange 1983). In the results presented here,  $O_2$  diffuses more quickly than  $CO_2$  probably because of higher partial pressure. Nitrogen was the least movable gas, diffusing slowly despite its extremely high  $P(N_2)$  values. It does however diffuse out eventually in order to achieve the very low SV values observed after exposure to current.  $N_2$  was very active in the regulation of the swimbladder volume and its  $P(N_2)$  which was kept high during the whole of the experiment because of the sharper decreases in the other 2 gases, should be one of the reasons for that.

After exposure to still water following acclimation to current only very slight changes were observed in SV and presumably gas composition in the initial 6 h.  $O_2$  was

secreted mainly between 6 and 24 h while  $N_2$  was secreted at a steady rate from 6 to 168 h. During the inflatory reflex (increased gas secretion) the secretory mucosa relaxes and expands over the inside of the swimbladder covering the resorbent area to a varying degree, restricting gas exchange. At the same time the gas gland arterioles dilate, the gas gland cells increase their metabolism, and the resorbent capillaries contract. The decrease in pH caused by lactic acid produced in the gas gland causes the release of  $O_2$  from hemoglobin and converts carbonate and bicarbonate ions to dissolved  $CO_2$ . The combined effect of high concentration of lactate anions together with high  $CO_2$  values serve to salt out inert gases from their solution in the blood plasma. The concentration of the 3 main gases plus other inert gases is increased by a counter-current multiplication in the rete mirabile. Of the inert gases it is the more diffusible ones that are favored since they pass more rapidly from the outflowing to the inflowing capillaries of the rete (Kuhn et al. 1963; Wittenberg et al. 1964, 1981a,b; Wittenberg et al. 1981b).

There is some maximum  $P(O_2)$  value in the swimbladder at which fish must secrete  $O_2$  as fast as possible simply to keep up with the rate of loss by diffusion. This pressure (max. sustainable  $P(O_2)$  in the swimbladder) depends on the  $O_2$  conductance of the swimbladder wall and the maximum possible

rate of secretion (Lappenas and Schmidt-Nielson 1977). According to the results here, this point is reached at 24 h. The elevated rate of O<sub>2</sub> secretion continues up to 48 h, when O<sub>2</sub> starts to decrease. N<sub>2</sub> however continues to increase until 168 h. The processes of O<sub>2</sub> and N<sub>2</sub> secretion are closely linked. Protons arising from disassociation of lactic acid drive O<sub>2</sub> from its combination with blood hemoglobin and thus provide the link between O<sub>2</sub> secretion and N<sub>2</sub> secretion (Wittenberg et al. 1981b). Since the swimbladder is at a steady state with gases resorbed and secreted at the same rates, gas composition varies at different stages during secretion. The gases with the highest diffusion rates (O<sub>2</sub> and CO<sub>2</sub>) diffuse out faster than they are produced resulting in a net loss. N<sub>2</sub> having a very low diffusion rate and a low P(N<sub>2</sub>) at the first hours of secretion continues to increase since the rate of secretion is higher than the rate of resorption. This sequence of events results in differences in composition between the newly secreted gas and that present during the steady state. For example Hufner (1892; in Fänge 1983) reported that the swimbladder gases of the whitefish (Coregonus acronius) captured from depths of 60-80m contained 99% N<sub>2</sub>. According to Wittenberg (1958) if the SB of fish such as the freshwater cyprinids (which are normally filled with N<sub>2</sub>) are emptied experimentally, the swimbladder is refilled with a gas mixture containing up to 60% O<sub>2</sub> and 2-10% CO<sub>2</sub>. Oxygen

is removed from the swimbladder rapidly (Wittenberg 1958) N<sub>2</sub> slowly, thus N<sub>2</sub> is accumulated in th SB of those fishes that secrete gas slowly (Wittenberg et al. 1981b).

O<sub>2</sub> is the main gas deposited during the initial stages of secretion but CO<sub>2</sub> values of up to 85% have been calculated for the perch (Perca flavescens) (Jacobs 1932; Meesters and Nagel 1934; both in Fange 1983). Even though this figure is possibly somewhat exaggerated due to uncertainty in the calculations, values of CO<sub>2</sub> of up to 40% have been found in Pomatomus (Wittenberg et al. 1964). The highest values of CO<sub>2</sub> occur at the peak of gas secretion when resorption of gases is minimized due to the closure of the oval (Fange 1983). During that stage the cod (Gadus morhua) shows values of 12-13% CO<sub>2</sub> and up to 80% O<sub>2</sub>. In the barracuda gas bubbles collected under a plastic film on the surface of the gas gland contain 3.6-5.3% CO<sub>2</sub>. In the wrasse (Ctenolabrus) however CO<sub>2</sub> never exceeded about 3% during any phase of gas secretion (Fange 1953). The steady state composition is variable depending on species and depth, O<sub>2</sub> increasing with depth (Wittenberg 1961) while N<sub>2</sub> decreases (Fange 1983).

Log perch followed the general trend of high O<sub>2</sub> and low N<sub>2</sub> in the newly secreted gas but CO<sub>2</sub> remained low throughout the experiment. The high O<sub>2</sub> and low N<sub>2</sub> percentages were

reversed by the end of the experiment approaching the proportions of the gases present in water equilibrated with atmospheric air (35 O<sub>2</sub>:65 N<sub>2</sub>) and therefore the proportions present in the blood plasma which could help minimize losses through diffusion.

The Percina spp. studied here occupy a broad range of environments. Even though blackside darters are restricted to streams and rivers, they inhabit both pools and running waters. River darters and log perch select an even broader range, successfully occupying lotic and lentic waters, and within each occurring in a variety of environments. I conclude that much of the ability to do so relates to their ability to vary swimbladder lift. Such behavioural phenotypic plasticity of swimbladder volume (Gee 1974) is highly adaptive as it permits a fish to (1) occupy a variety of environments in space and (2) adapt to changes in the dominant variable within each environment, current or turbulence, over time.

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## APPENDICES

APPENDIX 1

t-test: Comparison of mean body weight at time of dissection (0) and after 24 h in a refrigerator.

Time	mean	n	std.error	Variances	T	df	prob>T
0	0.1372	32	0.008	unequal	0.27	62	0.7901
24	0.1342	32	0.008				

APPENDIX 2

t-test: Comparison of SL (ml/g) attained by blackside darters held in the laboratory for less than one month and those held for about one year. Time is in months.

a)current

Time	mean	n	std.error	Variances	T	df	prob>T
0	0.021	24	0.0085	unequal	0.87	42	0.378
12	0.033	24	0.0115				

b)still water

Time	mean	n	std.error	Variances	T	df	prob>T
0	0.721	24	0.0275	unequal	1.67	43	0.103
12	0.662	24	0.0215				

### APPENDIX 3

ANOVA used to compare SL (ml/g) attained by blackside darters in three levels of crowding in still water (10, 50, and 150 fishes) and two levels of crowding (10 and 20) in current.

#### a) Effects of crowding in still water.

Source	df	F	prob>F	variances
Model	2	16.90	0.0001	equal
Error	21			
Total	23			

#### b) effects of crowding in current

Source	df	F	prob>F	variances
Model	1	0.96	0.3442	equal
Error	14			
Total	15			

#### APPENDIX 4

t-tests between buoyancy values (ml/g) attained in different water conditions.

##### A. Blackside darters

###### a) 1/SL in current (31 vs 99 cm/sec)

Treatment	mean	n	std.error	variances	T	df	prob>T
current=31	0.735	25	0.0219	equal	8.42	45	0.0001
current=99	0.966	22	0.0151				

###### b) SL in still water vs current

Treatment	mean	n	std.error	variances	T	df	prob>T
still	0.585	42	0.0359	unequal	9.84	129	0.0001
current	0.169	89	0.0222				

##### B. River darters

###### c) SL in still water vs current and waves

Treatment	mean	n	std.error	variances	T	df	prob>T
still	0.352	10	0.0169	equal	3.89	53.0	0.0003
current							
or	0.151	45	0.0239				
waves							

###### d) SL in current vs waves

Treatment	mean	n	std.error	variances	T	df	prob>T
current	0.143	26	0.0335	unequal	0.39	43	0.694
waves	0.162	19	0.0340				

Appendix 4 continued

C. Log perch

e) 1/SL in still vs waves

Treatment	mean	n	std.error	variances	T	df	prob>T
still	0.722	54	0.0124	unequal	12.1	88.	0.0001
waves	0.895	107	0.007				

APPENDIX 5

ANOVA and means comparisons for SL values attained by river darters:

a) effects of temperature on SL in still water and current.

Source	df	F	prob>F	variances
Holding condition	1	87.19	0.0001	unequal
Temperature	2	1.16	0.3220	
Interaction	2	8.37	0.0007	
Error	41			
Total	46			

b) effects of temperature on SL in current and waves.

Source	df	F	prob>F	variances
Holding condition	1	14.06	0.0008	unequal
Temperature	1	3.91	0.0580	
Interaction	1	3.14	0.0871	
Error	28			
Total	31			

c) effects of sizegroup on SL in still water, current and waves

Source	df	F	prob>F	variances
Holding condition	2	37.76	0.0001	unequal
Sizegroup	1	0.13	0.7222	
Interaction	2	1.24	0.3006	
Error	42			
Total	47			

Appendix 5 continued

ANOVA and means comparisons for SL values attained by  
blackside darters.

a) effects of temperature on SL in still water and current.

Source	df	F	prob>F	variances
Holding condition	1	1180.35	0.0001	unequal
Temperature	2	0.80	0.4566	
Interaction	2	3.81	0.0301	
Error	42			
Total	47			

b) effects of sizegroup on SL in still water and current

Source	df	F	prob>F	variances
Holding condition	1	966.49	0.0001	unequal
Sizegroup	3	0.58	0.6380	
Interaction	3	1.22	0.312	
Error	56			
Total	63			

Appendix 5 continued

ANOVA and means comparisons for SL values attained by log perch.

a) effects of temperature on SL in still water and current.

Source	df	F	prob>F	variances
Holding condition	1	89.35	0.0001	unequal
Temperature	2	5.95	0.0053	
Interaction	2	7.75	0.0014	
Error	42			
Total	47			

b) effects of temperature on SL in still water current and waves.

Source	df	F	prob>F	variances
Holding condition	2	149.38	0.0001	unequal
Temperature	1	3.07	0.0870	
Interaction	2	8.36	0.0009	
Error	42			
Total	47			

c) effects of sizegroup on SL in still water current and waves

Source	df	F	prob>F	variances
Holding condition	2	253.62	0.0001	unequal
Sizegroup	2	1.34	0.2700	
Interaction	4	5.44	0.0008	
Error	63			
Total	71			

Appendix 5 continued

t-tests between buoyancy values (ml/g) attained by males and females in still water and current.

1. Blackside darters

a) still water

Sex	mean	n	std.error	variances	T	df	prob>T
males	0.785	19	0.0326	unequal	0.09	4	0.931
females	0.772	4	0.0706				

b) current

Sex	mean	n	std.error	variances	T	df	prob>T
males	0.065	19	0.0134	unequal	0.70	19	0.491
females	0.080	11	0.0198				

2. River darters

a) still water

Sex	mean	n	std.error	variances	T	df	prob>T
males	0.478	14	0.0525	unequal	0.46	27	0.651
females	0.513	15	0.0553				

b) current

Sex	mean	n	std.error	variances	T	df	prob>T
males	0.042	18	0.0192	unequal	1.01	14	0.330
females	0.068	5	0.0182				

APPENDIX 6

Regression equations

Blackside darters

Time (0-168 h) in still water vs SL (ml/g); Logarithmic transformation of time.

a) sizegroup 4

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	121.19	0.0001	0.692
Error	54			
Total	55			

Regression equation:  $Y = .002 + .165X$

b) sizegroup 5

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	105.51	0.0001	0.661
Error	54			
Total	55			

Regression equation:  $Y = -.074 + .150X$

c) sizegroup 6

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	86.02	0.0001	0.614
Error	54			
Total	55			

Regression equation:  $Y = -.069 + .140X$

Appendix 6 continued

Time (0-168 h) in current vs SL (ml/g); Logarithmic transformation of time.

a) sizegroup 4

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	145.39	0.0001	0.760
Error	46			
Total	47			

Regression equation:  $Y = .747 - .146X$

b) sizegroup 5

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	86.57	0.0001	0.653
Error	46			
Total	47			

Regression equation:  $Y = .783 - .136X$

c) sizegroup 6

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	163.34	0.0001	0.780
Error	46			
Total	47			

Regression equation:  $Y = .730 - .133X$

Appendix 6 continued

Time (0-168 h) in still water vs SV (ml/g); Logarithmic transformation of time.

a) sizegroup 4

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	103.63	0.0001	0.657
Error	54			
Total	55			

Regression equation:  $Y = -.005 + .161X$

b) sizegroup 5

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	101.93	0.0001	0.649
Error	54			
Total	55			

Regression equation:  $Y = -.055 + .144X$

c) sizegroup 6

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	70.19	0.0001	0.563
Error	54			
Total	55			

Regression equation:  $Y = -.043 + .133X$

Appendix 6 continued

Time (0-168 h) in current vs SV (ml/g); Logarithmic transformation of time.

a) sizegroup 4

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	138.06	0.0001	0.750
Error	46			
Total	47			

Regression equation:  $Y = .726 - .141X$

b) sizegroup 5

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	77.81	0.0001	0.627
Error	46			
Total	47			

Regression equation:  $Y = .779 - .134X$

c) sizegroup 6

Source	df	F	prob>F	R <sup>2</sup>
Log time;X	1	148.58	0.0001	0.763
Error	46			
Total	47			

Regression equation:  $Y = .716 - .129X$

APPENDIX 7

Analysis of covariance: standard volume vs time using sizegroup as the covariate; rate of decrease.

size	df	$\Sigma X^2$	$\Sigma Y^2$	Regression coefficients	Deviation from Regression df	SS	MS
4	47	250272	8.566	-0.0026835	46	2.11501	0.04598
5	47	250272	11.416	-0.0033350	46	1.73343	0.03768
6	47	250272	8.769	-0.0026652	46	1.55479	0.03380
					138	5.40323	0.03915
pooled (within)					140	5.45026	0.03893
differences between slopes					2	0.04703	0.00022
within and between					142	5.58518	0.03933
difference between adjusted means					2	0.13492	0.00040

Appendix 7 continued

Analysis of covariance:buoyancy vs time using sizegroup as the covariate;rate of decrease.

size	df	$\Sigma X^2$	$\Sigma Y^2$	Regression coefficients	Deviation from Regression	df	SS	MS
4	47	250272	8.998	-0.0028825		46	2.10966	0.04586
5	47	250272	11.269	-0.0034146		46	1.64025	0.03566
6	47	250272	8.999	-0.0027744		46	1.55234	0.03374
						138	5.30225	0.03842
pooled (within)						140	5.34007	0.03814
differences between slopes						2	0.03782	0.01891
within and between						142	5.43597	0.03828
difference between adjusted means						2	0.09589	0.04795

Appendix 7 continued

Analysis of covariance: standard volume vs time using sizegroup as the covariate; rate of increase.

size	df	$\sum X^2$	$\sum Y^2$	Regression coefficients	Deviation from Regression df	SS	MS
4	55	324000	18.645	0.00384365	54	3.04240	0.05634
5	55	324000	12.836	0.00383837	54	1.96182	0.03633
6	55	324000	12.025	0.00385691	54	1.90863	0.03534
					162	6.91286	0.04267
pooled (within)					164	6.91289	0.04215
differences between slopes					2	0.00003	0.00052
within and between					166	7.38737	0.04450
difference between adjusted means					2	0.47448	0.00235

Appendix 7 continued

Analysis of covariance:buoyancy vs log(time) using sizegroup as the covariate;rate of increase.

size	df	$\Sigma X^2$	$\Sigma Y^2$	Regression coefficients	Deviation from Regression	df	SS	MS
4	55	664.93	19.967	0.164993		54	1.76071	0.03261
5	55	664.93	13.260	0.150524		54	1.68324	0.03117
6	55	664.93	11.778	0.139884		54	1.78298	0.03302
						162	5.22694	0.03215
pooled (within)						164	5.27305	0.03215
differences between slopes						2	0.04611	0.02306
within and between						166	5.95879	0.03590
difference between adjusted means						2	0.68574	0.34287

APPENDIX 8

Effect of size (sizegroups 4, 5, 6) and time (0-168 h) in still water or current on internal pressure for blackside darters.

a)still water

Source	df	F	prob>F	variances
model	20	4.29	0.0001	unequal
sizegroup	2	5.86	0.0036	
time	7	7.06	0.0001	
interaction	11	2.23	0.0156	
Error	143			
Total	163			

b)current

Source	df	F	prob>F	variances
model	17	3.83	0.0001	unequal
sizegroup	2	1.83	0.1654	
time	5	9.37	0.0001	
interaction	10	1.45	0.1652	
Error	123			
Total	140			

APPENDIX 9

Regression equations

Log perch

Time (0-168 h) in current vs SL (ml/g); best equation  
( $Y=SL^2$ ).

Source	df	F	T	prob>F	prob>T	R <sup>2</sup>
model	2	243.83		0.0001		0.915
(1/time)X	1		7.57		0.0001	
(log time)X <sup>2</sup>	1		3.50		0.0011	
Error	45					
Total	47					

Regression equation:  $Y=.213+438X-.042X^2$

Time (0-168 h) in still water vs SL (ml/g); best equation  
( $Y=1/SL$ ).

Source	df	F	T	prob>F	prob>T	R <sup>2</sup>
model	3	38.36		0.0001		0.652
time;X	1		3.44		0.0014	
( $\sqrt{\text{time}}$ )X <sup>2</sup>	1		5.37		0.0001	
Error	41					
Total	43					

Regression equation:  $Y=1.007+.0034X-.076X^2$

Appendix 9 continued

Time (0-168 h) in current vs SV (ml/g);best equation  
( $Y=SV^2$ ).

Source	df	F	T	prob>F	prob>T	R <sup>2</sup>
model	2	234.32		0.0004		0.912
(1/time)X	1		7.80		0.0001	
(log time)X <sup>2</sup>	1		3.02		0.0041	
Error	45					
Total	47					

Regression equation:  $Y=.185+446X-.037X^2$

Time (0-168 h) in still water vs SV (ml/g);best equation  
( $Y=1/SV$ ).

Source	df	F	T	prob>F	prob>T	R <sup>2</sup>
model	3	36.33		0.0001		0.747
time;X	1		4.23		0.0001	
(time )X <sup>2</sup>	1		3.14		0.0032	
( $\sqrt{\text{time}}$ )X <sup>3</sup>	1		5.93		0.0001	
Error	40					
Total	43					

Regression equation:  $Y=1.122+.015X-.00004X^2-.164X^3$