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SWIMBLADDER FUNCTION AND BUOYANCY REGULATION IN THE KILLIFISH *FUNDULUS HETEROCLITUS*

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Summary

Killifish, *Fundulus heteroclitus*, subjected to artificial lift above their center of gravity (10 % of body weight) required a minimum of 7–8 days to resorb swimbladder gases completely. The swimbladders of some fish, however, did not fall below 50 % of normal volume. The rate of increase in swimbladder volume upon removal of lift varied little among individuals, with approximately 6 days required for complete refilling. Previous deflation of the swimbladder (by syringe) did not result in faster or more complete gas resorption when the fish were subjected to artificial lift. This suggests that the constraint to resorption observed in some fish is not mechanical, e.g. connective tissue, but may reflect individual variability in perception of the stimulus.

Swimbladder dry mass, which scaled as $(\text{body mass})^{0.79}$, was not affected by exposure to artificial lift. However, fish subjected to 7–11 days of artificial lift displayed slower rates of gas secretion upon removal of lift than control fish whose swimbladders had been evacuated by syringe. The initial rate was 65 % of that of control fish, with two additional days required to achieve normal buoyancy. Also, the rate of swimbladder gas resorption was 24 % faster the second time fish were exposed to artificial lift. These results demonstrate that the capacity for gas secretion and resorption can be altered by previous exposure to hydrostatic challenges.

Killifish buoyancy, expressed as swimbladder volume per weight of the gas-free fish in water, fell from 0.95 to 0.70 ml g^{-1} after 5 days of exposure to water current. Removal of the pectoral fins eliminated 70 % of this decrease, while removal of the pelvic fins had no effect. The rate of gas resorption by fish subjected to artificial lift was also not affected by removal of the pectoral fins. From these results it appears that the decrease in swimbladder volume in fish exposed to water currents is a consequence of lift forces produced by the pectoral fins, but that they are not required for regulation. Fish exposed to water currents or artificial lift swim with a head-down angle of attack. Theoretical estimates show that the vertical force component generated by this swimming behavior is of the appropriate magnitude to compensate for the additional lift.

Fish confined in transparent cages near the surface of the water were less

Key words: hydrostatic forces, buoyancy, swimbladder, *Fundulus heteroclitus*, weightlessness, fish density.

buoyant ($0.91\,ml\,g^{-1}$) than fish similarly maintained at the bottom of the tank ($0.98\,ml\,g^{-1}$). However, because this effect was small, 10% of swimbladder volume, visual perception of vertical position is apparently not the primary stimulus for volume regulation. Partial lift (2.65% of body weight) resulted in the resorption of twice as much swimbladder gas when attachment was anterior to the fish's center of gravity than when it was an equal distance posterior to the center of gravity. When equal amounts of partial lift and weight were added, lift anterior and weight posterior, no change in swimbladder volume occurred. With the position of these forces reversed, swimbladder volume increased by 31% to $1.27\,ml\,g^{-1}$. These results suggest that fish respond to pitching forces, i.e. longitudinal lift moments, as a stimulus for swimbladder gas secretion and resorption.

Introduction

Nearly all species of fish are heavier than water in the absence of swimbladder lift. Muscle density is close to $1.05\,g\,cm^{-3}$ (Alexander, 1959) whereas the density of the vertebral axial skeleton can range from 1.25 to $1.50\,g\,cm^{-3}$ (Webb, 1990). Fish, therefore, must generate a continuous upward force equal to approximately 6% of body weight if they are to remain in midwater. Hydrodynamic lift generated by swimming (either positive or negative) is an energetically costly solution, especially when swimming at a high angle of attack (Alexander, 1966). Lift forces can be produced by the flow of water over the paired fins (Magnuson, 1978), but since this also requires swimming it is only practical for active pelagic species. Using the pectoral fins to 'hover' appears to be advantageous only for fish of small body size (Alexander, 1990). For most fish species, neutral buoyancy can be achieved with the greatest economy by possessing a gas-filled swimbladder with a volume equal to 5.7–8.3% of body weight in fresh water or 3.1–5.7% in sea water (Alexander, 1966).

Adjustments in swimbladder volume occur in response to changes in water pressure (Tsvetkov, 1974), water density (i.e. salinity; Black, 1948) and water current (Gee, 1983). The influence of water current on buoyancy regulation is functionally different from that of either pressure or density. The effect of water current on swimbladder volume is believed to be the result of lift generated by the flow of water over the body and/or the fins (Stewart and Gee, 1981). It is not known precisely where on the body these forces are produced but, unlike pressure and density, they are likely to result in longitudinal instability during swimming (i.e. pitching).

The role of the swimbladder as a hydrostatic organ has been recognized since the early elegant work of Moreau (1874). In spite of much research on the biophysics of swimbladder function (Harden Jones and Marshall, 1953; Fänge, 1966; Steen, 1970; Blaxter and Tytler, 1978), little is known about what stimuli are involved in the regulation of overall buoyancy. Fish are able to adjust their swimbladder volume and buoyancy in response to pressure changes by means of proprioceptors in the swimbladder wall (Qutob, 1962). Mechanical sensors in the

swimbladder cannot be the only mechanism involved, however, since in most instances fish do not regulate to a constant swimbladder volume but rather to neutral buoyancy. The sizes of the secretory and resorbent areas are under the control of the autonomic nervous system and can be regulated by both adrenergic and non-adrenergic substances (Nilsson, 1971; Fänge *et al.* 1976; Ross, 1978; Lundin, 1991). Bianki (1964) reported that certain areas of the cerebellum are also required for normal swimbladder function, but again it is not clear what stimuli initiate an apparent series of neurosecretory events. Meesters and Nagel (1934) found that changes in swimbladder volume were accompanied by characteristic pectoral fin movements and concluded that gas secretion and resorption were coupled to the fin musculature. Other studies have confirmed the compensatory role of the pectoral fins (Harden Jones, 1952; McCutcheon, 1958) but suggest that they are not involved in regulation (Fänge, 1953).

It is also not known if the capacity of the swimbladder to secrete or resorb gases is affected by previous exposure to hydrostatic demands. This is an important issue for many migrating species, which experience vastly different hydrostatic conditions throughout their life-history. The production of swimbladder gas is largely the result of a localized acidosis in the gas gland produced by high glycolytic activity (D'aoust, 1970). The activities of enzymes, such as lactate dehydrogenase, in this pathway have been shown to be influenced by previous metabolic demand in other tissues (Sullivan and Somero, 1980, 1983), and it is possible that their activity can also be altered in the swimbladder by exposure to varied hydrostatic challenges.

In this study I examine swimbladder function and buoyancy regulation in a physoclistous fish by observing responses to artificial lift and weight. Specific objectives are (1) to describe the rate and extent of changes in swimbladder volume by fish with longitudinally stable artificial lift, (2) to determine the effects of previous hydrostatic demand on subsequent rates of swimbladder gas secretion and resorption, and (3) to investigate the stimuli used by fish to regulate buoyancy; in particular the role of vertical perception, the paired fins and longitudinal lift moments. I also examine the response of fish to water current to elucidate the role of the paired fins and unstabilizing longitudinal forces.

Materials and methods

Experimental fish

Killifish, *Fundulus heteroclitus* Linnaeus, were obtained from The Marine Biological Laboratory (Woods Hole, MA). They were gradually acclimated to fresh water at 17.5 °C in which they were maintained for at least 2 weeks prior to study. The fish were fed a combination of flaked commercial food and live worms (*Nadis*) during this period and during experiments lasting longer than 3 days.

Swimbladder volume

Swimbladder volume was estimated as follows. Fish were anesthetized in MS-

222 (ethyl *m*-aminobenzoate methanesulfanate; 300 mg l^{-1}) and weighed in water, making certain that no bubbles were trapped in the buccal cavity. An electronic balance and side-arm was used with the fish at a depth of approximately 5 cm. The weight of the fish in air was also determined at each sampling time. At the end of each of the experiments described below the fish were killed and dissected. The swimbladder was punctured with a scalpel, the gas-free weight of each fish in water was recorded, and its whole-body density was calculated. The difference between the weight (or lift) of the live fish in water at each sampling time and the weight of the gas-free fish in water equals the volume of the swimbladder (±0.001 ml). Gas-free weight in water at each sampling time was estimated for each fish using its weight in air at that time and its final density. To compare fish of different size, buoyancy was expressed as swimbladder volume divided by the weight of the gas-free fish in water (1.0 ml g^{-1}=neutral buoyancy).

General response to artificial lift

Experiment 1

Artificial lift, equal to 10 % of body weight in air, was attached above each fish's center of gravity. This is approximately 22 % greater than the lift produced by the swimbladder of a neutrally buoyant fish. The longitudinal center of gravity was estimated as the point along the fish from which it hung level when suspended in air (Fig. 1). Lift was provided by plastic 1.5 ml microcentrifuge tubes, adjusted to the desired lift force by changing the ratio of air to water inside them. Nylon thread (diameter=0.13 mm) was used to attach the tubes to the epaxial muscle of the fish, and a stainless-steel clip (66 mg in water) allowed the lift to be removed when the fish was weighed. Swimbladder volume was determined prior to adding lift and every 24 h thereafter for 7–11 days. The artificial lift was then removed and the increase in swimbladder volume monitored every 24 h until neutral buoyancy

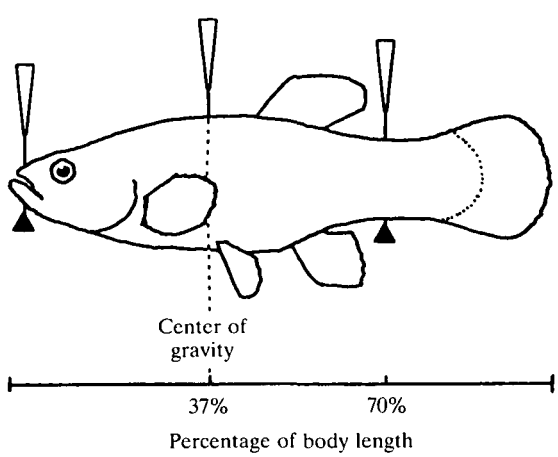

Fig. 1. Location of longitudinal center of gravity in the killifish *Fundulus heteroclitus* and attachment sites for artificial lift (dorsal positions) or weight (ventral positions).

was achieved. This and all following experiments were performed at 17.5 °C (range 17.0–18.0 °C).

Experiment 2

The response of the swimbladder to artificial lift was studied using a second method in which all of the gas in the swimbladder was removed prior to adding 10 % lift. Fish treated in this way were initially much closer to neutral buoyancy with lift attached than those in experiment 1. The fish were anesthetized and placed on a glass plate over highly focused light so that the swimbladder could be easily seen. A 1.0 ml syringe with a 25 gauge needle was then inserted through the muscle tissue to withdraw the gas. Changes in swimbladder volume were recorded every 24 h for 7–8 days following the addition of lift.

Experiment 3

The effect of previous gas removal and complete deflation of the swimbladder on the rate of gas resorption when the fish were exposed to artificial lift was also studied. The swimbladder lumen is attached to the visceral wall with connective tissue and, when gas is withdrawn by syringe, considerable negative pressure is required to collapse it. Because the swimbladders of some fish would not decrease to less than half of normal volume when exposed to 10 % lift, it was hypothesized that there might be mechanical limitations to deflation. To test this, swimbladder gas was completely evacuated from six fish, 8 days was allowed for the swimbladder to be refilled, and then 10 % lift was attached. The rates of swimbladder gas resorption from these treated fish were compared to that for control fish with 10 % lift sampled at the same time.

The effect of artificial lift on subsequent rates of swimbladder gas resorption and secretion

Experiment 4

A group of eight fish was exposed for 7–11 days to 10 % lift and then allowed approximately 1 week to completely refill their swimbladders. These same fish were then subjected to a second period of 10 % lift and their rate of gas resorption was compared to that during their first exposure.

Experiment 5

Eight fish previously exposed to 10 % lift had swimbladders which resorbed to less than 10 % of normal volume (three from experiment 1 and five from experiment 4). The gas secretion rates of these fish when lift was removed were compared to those of control fish to determine if 7–11 days without the demand for gas secretion resulted in atrophy of the gas-generating tissue. The controls were six fish whose swimbladders were evacuated by syringe and then monitored for rate of gas secretion.

Perception of vertical position

Experiment 6

Fish that are normally neutrally buoyant find themselves initially drawn to the surface of the water when lift is added or to the bottom of the tank when swimbladder gas is withdrawn. To examine the role of visual perception of vertical location on swimbladder gas secretion and resorption, two groups of five fish were confined in clear acrylic cages either to the surface of the water or to the bottom of the tank and monitored for changes in swimbladder volume. The cages, which had screened sides to allow for water circulation, were 23 cm × 30 cm and approximately 3 cm deep. These were placed in a larger tank (51 cm × 112 cm) filled to a depth of 37 cm and containing twelve free-swimming fish.

The effect of water current and the paired fins on swimbladder volume

Experiment 7

Fish were transferred from still water to flowing water and sampled every 24 h for changes in swimbladder volume. A current of 9.51 cm s^{-1} (approximately 1.07 body lengths s^{-1}) was generated in an oval tank by the force of the high-pressure intake water. The role of the paired fins in regulating buoyancy was of particular interest, so changes in swimbladder volume due to current were compared between control fish and those that had either their pectoral or their pelvic fins removed (under anesthetic). Five fish from each treatment group and five different control fish were used in each experiment.

To determine whether the pectoral fins are involved in buoyancy regulation in general, in addition to any role when the fish are swimming in a water current, the rate of swimbladder gas resorption of fish under 10 % artificial lift was also compared between control fish and those without pectoral fins.

Longitudinal lift moments

Experiment 8

The effect on swimbladder volume of lift forces acting along the length of the fish was first examined using artificial lift equal to 2.4 % of body weight in air (approximately 29 % of normal swimbladder volume). This lift was attached either above the upper jaw (3 % from the anterior end) or an equal distance posterior to the fish's center of gravity, at 70 % of the length of the fish (Fig. 1). The resorption of swimbladder gases was monitored every 24 h for these two groups of fish, which were experiencing the same overall hydrostatic forces but different pitching moments.

Experiment 9

The effect of longitudinal moments on swimbladder volume was investigated a second way. Equal amounts of lift and weight (2.4 % of fish weight in air) were

added to each fish such that the overall near-neutral buoyancy of the fish was unaffected. No change in swimbladder volume would therefore be expected. In one group of fish the lift was attached at the anterior position and the weight at the posterior position (Fig. 1); in a second group these positions were reversed. Coiled strips of galvanized steel wire were used as weights. Swimbladder volume was measured for fish from each group (N=6) after 24 h of exposure to opposite pitching moments.

Swimbladder dry mass

Swimbladder dry masses were obtained from control fish, from fish whose swimbladders had been evacuated by syringe, from fish exposed to 10 % lift and from those exposed to a current. The entire swimbladder, including the rete mirabili, was dried to constant mass, which was expressed as a percentage of wet body mass.

Analysis of data

All values are presented as means±S.E.M. Statistical comparisons were performed using the two-sample t-test unless otherwise stated.

Results

The mean length (L, cm) and mass (W, g) of experimental fish were 8.79±0.05 cm and 8.68±0.18 g, respectively. They were related by:

$$\ln W = -4.43 \pm 0.25 + 3.02 \pm 0.119(\ln L)\,. \quad (r^2=0.84;\ N=129)$$

The mean center of gravity for 22 killifish was 37.43±0.22 % of the distance from the anterior end of the fish. Mean whole-body density with swimbladder gas evacuated was 1.082±0.0004 g cm^{-3} (N=65). Buoyancy of control fish in still water was 0.964±0.002 ml g^{-1}. None of the control groups had buoyancies that differed significantly from one another (P>0.05).

General response to artificial lift

The fish did not appear to be stressed by the presence of the artificial lift and began feeding within several hours of the lift being attached. Feeding was initially most common at the surface of the water because of the fish's positive buoyancy. As their swimbladders resorbed gas, and they became more neutrally buoyant, their feeding also included foraging on the bottom of the tank.

Experiment 1

Swimbladder volume decreased in fish with 10 % artificial lift above the center of gravity; however, variability among individuals was large (Fig. 2A). Of nineteen fish monitored for both a decline in swimbladder volume and subsequent refilling, eight had swimbladders which resorbed to less than 10 % of normal

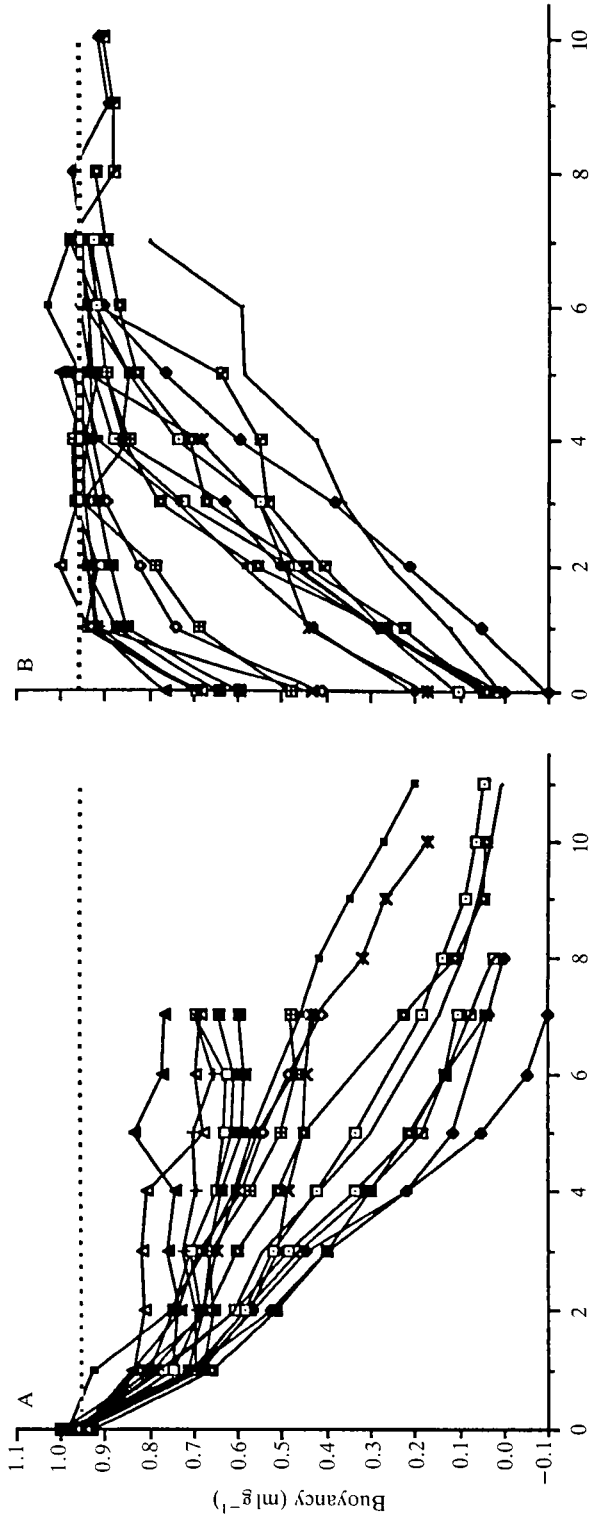

Fig. 2. (A) Rate of decrease in the swimbladder volume of killifish after the addition of artificial lift above the fish's center of gravity equivalent to 10 % of body weight (N=19). (B) Rate of increase in swimbladder volume for the same group of fish once the artificial lift has been removed. The dotted line shows the mean buoyancy of control fish.

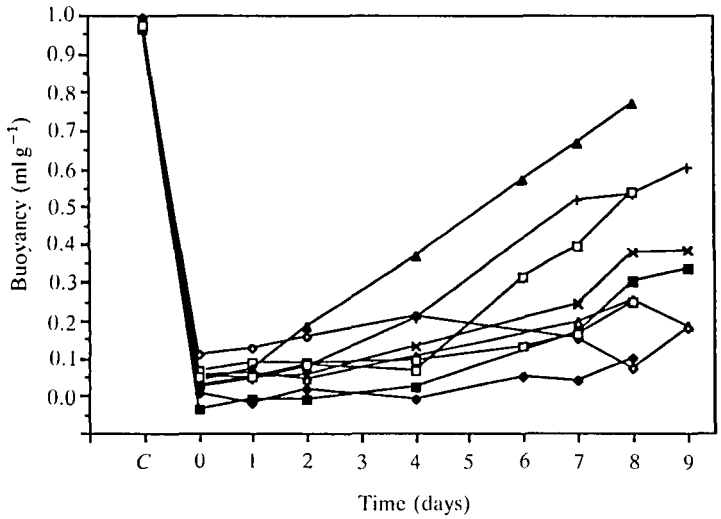

Fig. 3. The decrease, and subsequent increase, in buoyancy of killifish when swimbladder gas is evacuated by syringe. Artificial lift equivalent to 10% of body weight was attached above the fish's center of gravity at time zero. The symbol *C* represents control buoyancy before evacuation.

volume after 7–11 days. The swimbladder volumes of other individuals never decreased below 50% of normal volume. The rate of gas secretion upon removal of the lift was nearly the same for all initial swimbladder volumes. Therefore, the time required for complete refilling increased with the degree of resorption (Fig. 2B). When swimbladders volumes resorbed to less than 10%, approximately 6 days was required for complete refilling.

Experiment 2

The mean buoyancy of nine fish following evacuation of swimbladder gas was 0.039 ± 0.014 ml g^{-1}. At the same time as evacuation, 10% artificial lift was added to each fish above the center of gravity. No significant change in swimbladder volume occurred for 2 days, but by day 4 mean buoyancy was significantly greater than at time zero (0.133 ml g^{-1}; $P<0.05$) and it continued to increase thereafter (Fig. 3). The variability observed among fish in experiment 1 also occurred here; some fish maintained a nearly empty swimbladder while the swimbladders of others, even with 10% lift, increased to 75% of normal volume.

Experiment 3

The rate of gas resorption when exposed to 10% lift was not significantly different between control fish and those whose swimbladders had been previously deflated ($P=0.17$ for day 6). After 6 days the mean buoyancy of all fish decreased to 0.246 ± 0.048 ml g^{-1} (Fig. 4).

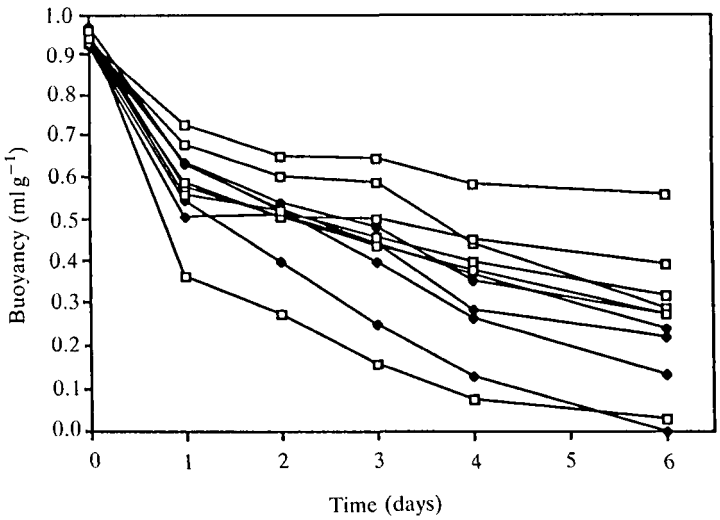

Fig. 4. The decrease in swimbladder volume of killifish exposed to 10% lift above the center of gravity; (◆) control fish; (□) fish whose swimbladders had been completely deflated by syringe and then allowed to refill.

The effect of artificial lift on subsequent rates of swimbladder gas resorption and secretion

Experiment 4

The rate of swimbladder gas resorption by fish previously exposed to 7–11 days of artificial lift was significantly faster the second time the fish were exposed to 10% artificial lift ($P<0.05$ for all sampling periods; paired t-test). The mean decrease in swimbladder volume after 3 days of lift was 24% greater during the second exposure (Fig. 5). The degree of resorption by individual fish was repeatable, i.e. fish that displayed slow rates of resorption during the initial exposure also resorbed most slowly the second time. Least-squares regressions of the first and second exposure were significant for each sampling period ($P<0.05$; $r^2=0.593$ at day 3).

Experiment 5

Eight fish exposed to artificial lift in experiments 1 and 4 had swimbladders which resorbed to less than 10% of normal volume. The mean buoyancy of these fish (0.020 ± 0.021 ml g^{-1}) was not significantly different from the buoyancy of six fish from which swimbladder gas was evacuated by syringe (-0.018 ± 0.019 ml g^{-1}). The rate of swimbladder filling by fish exposed to artificial lift, once the lift had been removed, was significantly slower than that for fish that had not been exposed to lift (Fig. 6; $P<0.05$ for the first 5 days). The initial rate for experimental fish was approximately 65% of that for control fish, and 2 additional days were required to achieve normal buoyancy.

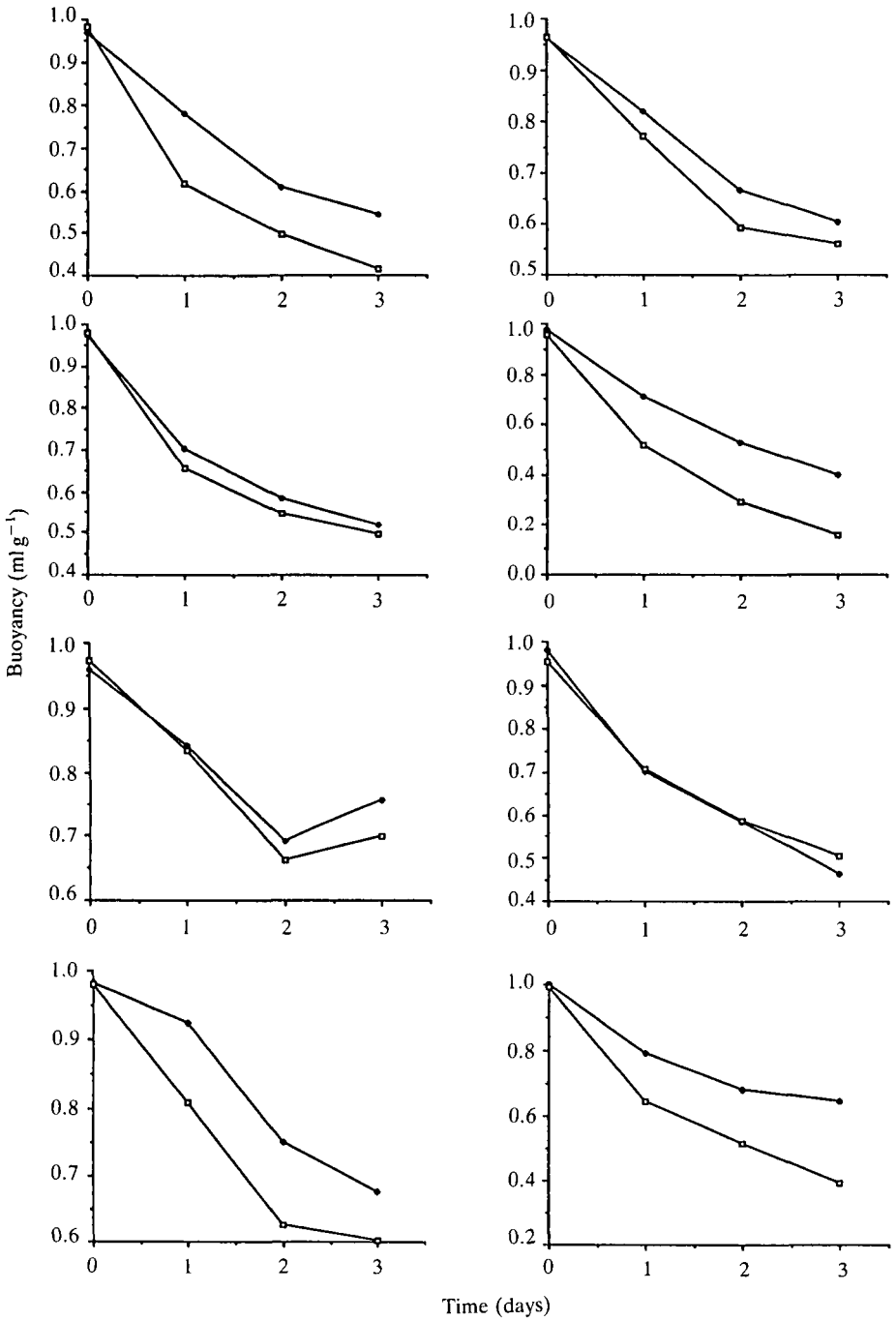

Fig. 5. The initial (◆) and secondary (□) rates of decline in swimbladder volume in eight killifish exposed to 10% artificial lift above their center of gravity.

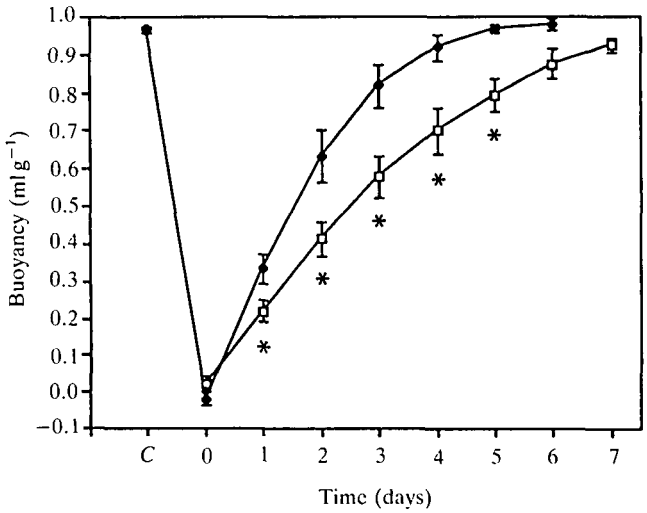

Fig. 6. The effect of exposure to 7–11 days of artificial lift (10%) on the rate of swimbladder filling by eight killifish once the lift has been removed (□). The control rate of filling (◆) is for six fish whose swimbladders were evacuated by syringe. Vertical bars indicate S.E.M. and asterisks indicate a significant difference between control and treatment groups ($P<0.05$). *C* represents control buoyancy.

Perception of vertical position

Experiment 6

The depth of the transparent cages was approximately twice the height of the fish, allowing them to swim normally. Those confined to the bottom made obvious attempts to swim up into the water column, while those at the surface made clear attempts to swim down. The mean buoyancy of fish confined to the surface decreased from 0.971 ± 0.008 to $0.910\pm0.008\,ml\,g^{-1}$ after 24 h (Fig. 7; $P<0.001$) and then remained at that value. Fish confined to the bottom showed a trend of increasing buoyancy, but the changes were not statistically significant after 3 days.

The effect of water current and the paired fins on swimbladder volume

Experiment 7

The mean buoyancy of control fish in current fell from 0.951 ± 0.014 to $0.793\pm0.018\,ml\,g^{-1}$ after 24 h and to $0.697\pm0.058\,ml\,g^{-1}$ after 5 days. The buoyancy of fish without pectoral fins was significantly higher than that of control fish at each sampling time (Fig. 8A; $P<0.01$). With pectoral fins removed, mean buoyancy fell only to $0.900\pm0.014\,ml\,g^{-1}$ after 24 h and remained unchanged thereafter. Of the decrease in buoyancy observed in control fish on day 5, approximately 70% had been eliminated by removing the pectoral fins. Removal of the pelvic fins had no effect on the decrease in swimbladder volume of killifish in current (Fig. 8B; $P>0.60$ for all sampling times).

Killifish experiencing 10% artificial lift without pectoral fins were still able to

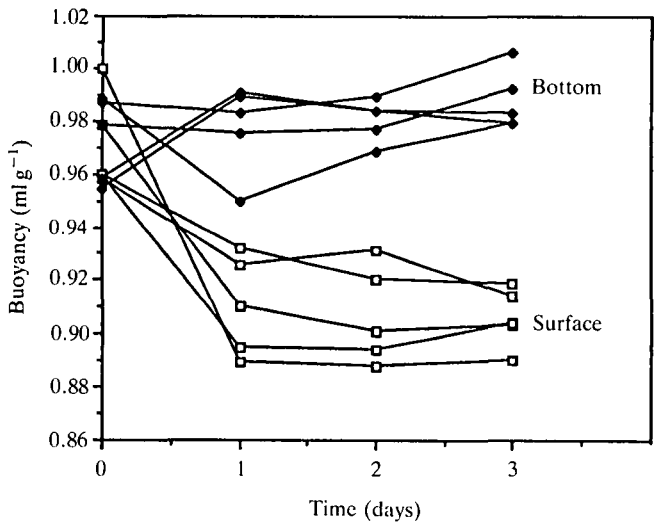

Fig. 7. The effect of perception of vertical location on the buoyancy of killifish. Fish were confined in transparent cages either on the bottom (◆) or at the surface (□) of a larger tank.

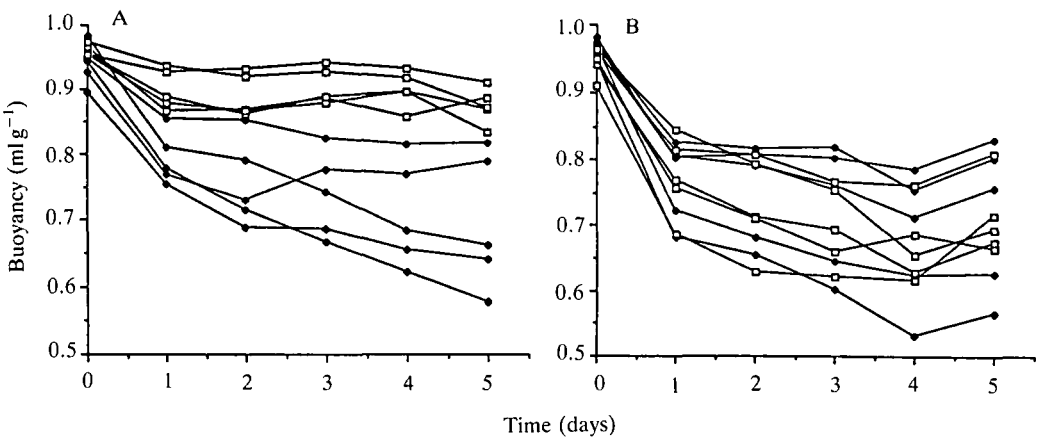

Fig. 8. The effect of exposure to water current on the swimbladder volume of control killifish (◆) and those from which either both pectoral fins (A; □) or both pelvic fins (B; □) had been removed.

orient themselves to swim in a head-down position and, therefore, to compensate for the additional lift. To do this, however, required exaggerated movements of the body musculature and fins other than the pectoral fins. The rate of swimbladder gas resorption was not significantly different between control fish and those without pectoral fins (Fig. 9; $P>0.25$ for all sampling times).

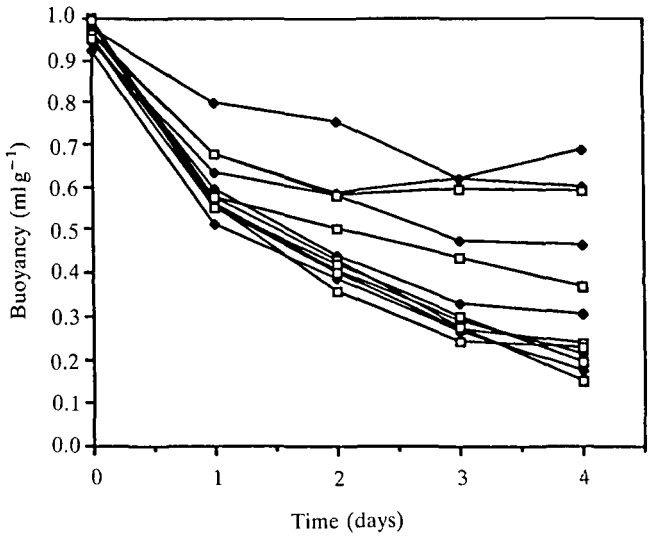

Fig. 9. The rate of change in swimbladder volume of control killifish (◆) and those with their pectoral fins removed (□) when both are exposed to 10% lift above their center of gravity.

Longitudinal lift moments

Experiment 8

Partial lift, 2.4% of body weight, resulted in a larger decrease in swimbladder volume when attached at the anterior position than when attached posteriorly (Fig. 10A; $P<0.01$ for all sampling times). The actual lift attached, accounting for fluctuations in lift and fish weight, was 2.65±0.08% of body weight. No change in buoyancy occurred from day 1 to day 4 in either group of fish ($P>0.05$). The mean values for buoyancy over this period were 0.733±0.013 and 0.847±0.010 ml g^{-1}, respectively, for fish with lift attached at the anterior and posterior positions.

Experiment 9

In this experiment equal amounts of lift and weight were attached to killifish so as to not alter the overall lift balance. They were positioned, however, to produce opposite pitching moments. Actual lift and weight were 2.38±0.050% and 2.41±0.027% of body weight, respectively. With lift attached at the anterior position and weight at the posterior position, no significant change in swimbladder volume occurred after 24 h ($P>0.10$). With the positions of these forces reversed, swimbladder volume increased by 31% to a mean buoyancy of 1.274±0.041 ml g^{-1} ($P<0.001$; Fig. 10B).

Swimbladder dry mass

The swimbladder lumen and rete mirabili together averaged 0.0348±0.0008% of killifish body mass (dry:wet). Exposure to 10% artificial lift had no effect on swimbladder weight, nor did any of the other treatments examined. Although the

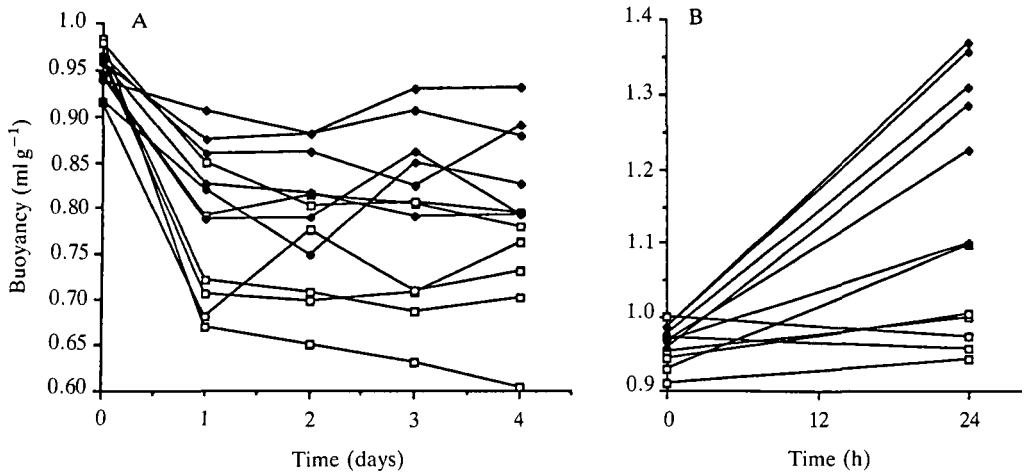

Fig. 10. The effect of unstable longitudinal forces on the swimbladder volume of killifish. (A) Partial lift, 2.65 % of body weight, was attached near the anterior of the fish (□) or an equal distance from, but posterior to, the center of gravity (◆). (B) Equal amounts of lift and weight added (2.4 % of body weight); lift anterior and weight posterior (□) or lift posterior and weight anterior (◆).

masses of fish ranged only from 5.7 to 13.9 g, significant negative allometry in swimbladder (SB, mg) dry mass was observed ($P<0.025$; H_o: slope=1):

$$\ln SB = -0.627 \pm 0.201 + 0.792 \pm 0.094(\ln W). \quad (r^2=0.64;\ N=43)$$

Discussion

General response to artificial lift

Fish which responded most rapidly to 10 % lift still required 7–8 days to resorb swimbladder gases completely. Variability in response among individuals was high, however, with some fish seemingly unable to decrease swimbladder volume below 60–70 % of normal volume. The particular responses observed among individuals were repeatable in subsequent exposures to artificial lift, and totally deflating the swimbladder by syringe prior to attaching lift did not result in more complete resorption of swimbladder gases (experiments 2 and 3). It appears that the constraint to resorption observed in some fish is not mechanical, e.g. restrictive connective tissue. The rate of filling of the swimbladder, when the artificial lift was removed or the swimbladder gas evacuated, displayed very little variability among the control and experimental fish. This suggests that physiological capacity for gas secretion and resorption when experiencing the same stimuli is not vastly different among individuals. The reason that some fish failed to resorb their swimbladder gas completely, therefore, may be due to individual differences in perception of the stimuli.

Actual rates of gas resorption and secretion can be calculated if the pressure of

swimbladder gas in these shallow-water fish is assumed to be 101 kPa (1 atm) (Gee *et al.* 1974). The maximal rate of gas resorption is estimated here from the decline in buoyancy of fish during the first 2 days of exposure to 10% artificial lift (Fig. 2A). The decrease in mean buoyancy over this 48-h period is from 0.969 to $0.652\,ml\,g^{-1}$. For a hypothetical 10-g fish this is equivalent to a decrease in swimbladder volume from 0.795 to 0.535 ml or a gas resorption rate of approximately $0.540\,ml\,kg^{-1}\,h^{-1}$.

The rate of change in buoyancy of fish following complete evacuation of swimbladder gas by syringe (Fig. 6) is used here to estimate the maximal rate of gas secretion. The increase in mean buoyancy over the first 2 days is from −0.018 to $0.634\,ml\,g^{-1}$. For a 10-g fish this is equivalent to an increase in swimbladder volume from 0.0 to 0.520 ml or a gas secretion rate of approximately $1.083\,ml\,kg^{-1}\,h^{-1}$. It should be noted when comparing these values with those of other species that the rate of gas secretion can be affected by body size. Although glycolytic capacity can increase with fish size in some tissues (Goolish 1989, 1991), it appears that the rate of gas secretion by the swimbladder decreases in larger fish (Gee, 1977; Harden Jones and Scholes, 1985).

Effect of prior exposure to lift or evacuation on the rate of swimbladder volume changes

The rates of swimbladder gas secretion and resorption were affected by previous exposure to artificial lift or swimbladder evacuation. Previous exposure to 10% lift resulted in a 24% increase in the rate of gas resorption during subsequent exposure. Conversely, exposure to 7–11 days of artificial lift resulted in a 35% decline in the rate of swimbladder filling once the lift was removed compared to the rate in control fish. Also, fish that had their swimbladders evacuated by syringe, and were therefore stimulated to secrete gas, showed a pattern of slower resorption when subjected to artificial lift (experiment 3; $P=0.06$ for days 4 and 6). These results indicate that the physiological processes involved in gas secretion and resorption can be altered to meet various hydrostatic challenges. This would, of course, be of advantage to fish that make large vertical migrations (Alexander, 1972; Harden Jones and Scholes, 1985), excursions into currents (Gee and Gee, 1976) or are exposed to varied salinity (Black, 1948). The ability to modify secretory capacity also cautions against extrapolating laboratory observations to natural populations (Kleckner, 1980), since acclimatization is likely to increase capacity and behavioral performance.

One of the motivations for the present study was to understand how the behavior of fish would be affected by the weightless conditions of space. There would be no need for swimbladder lift in the absence of gravitational forces and, therefore, atrophy of the gas-generating and volume-regulating tissues might be expected. If the artificial lift used here accurately simulates weightlessness, the results suggest that decreases in the capacity to generate swimbladder gases would occur in space and that behavior would be affected upon return to normal gravity. Juvenile killifish flown on the *Apollo–Soyuz* flight (Scheld *et al.* 1976) exhibited

swimming patterns, such as gravitating to the bottom of the aquaria, indicative of abnormal swimbladder function.

Buoyancy dynamics in water current

The swimbladder volume of most fish decreases when they are exposed to a current of water or are forced to swim in an optomotor tank (Berezay and Gee, 1978; this study). Under certain conditions the entire body of a fish may act as a lifting hydrofoil (MacKay, 1976), which led Stewart and Gee (1981) to argue that the decrease in swimbladder volume is a compensation for lift generated by the flow of water over the body. The results of the present study (experiment 7) suggest that most of the lift is not produced by the body but by the pectoral fins. Approximately 70 % of the decrease in swimbladder volume due to water current was eliminated when the pectoral fins were removed. Further indication that lift is produced by the pectoral fins, and not the body, is the head-down angle of swimming observed during the adjustment period (Berezay and Gee, 1978). This angle of attack would not generate a lift force but would produce, if anything, a downward force which would need to be compensated for with increased swimbladder volume. Furthermore, if lift produced by the flow of water over the body was the cause of decreased swimbladder volume, then increases in fish condition factor ($\text{weight} \times \text{length}^{-3}$) would tend to negate the effect of current. This is because the shape of the body would act increasingly like an inverted wing and thus generate a downward force. In fact, the opposite has been reported; individual fish with a higher condition factor show a significantly larger decrease in swimbladder volume when subjected to a current (Luoma and Gee, 1980).

The reason that fish in a current initially swim with a head-down orientation is probably not because of lift generation but to take advantage of the downward force component produced when swimming at an angle. In preliminary experiments, with current available and with artificial lift, *Fundulus heteroclitus* actively sought out the current and swam at a steep angle of attack (>20°) to keep from rising to the surface. This was in spite of the additional energetic costs of swimming. In the present study I also observed fish with artificial lift swimming in a head-down position in the absence of current; however, once their swimbladder gas had been resorbed, their swimming returned to normal. This suggests that the head-down swimming orientation is a general response to hydrostatic disequilibrium and that it is not to take advantage of hydrodynamic lift that may be produced by current.

The angle of attack assumed by fish subjected to current during the period when swimbladder volume is adjusted is reported to be 9–12° from the horizontal (Berezay and Gee, 1978). It can be shown that the vertical force component of total thrust produced by fish swimming at this angle is of the appropriate magnitude to compensate for observed changes in swimbladder lift. Theoretical estimates of thrust power produced by a 10-cm trout at maximum aerobic velocity are approximately 0.0058 W (Webb *et al.* 1984). At this velocity, $50\,\text{cm}\,\text{s}^{-1}$, total force production would be 0.012 N. The vertical component of this total for a fish

swimming at an angle of 12° would be sin12°×(0.012 N) or 0.0025 N. This is equivalent to a downward force of 0.255 g, or approximately 33 % of the swimbladder volume of a 10-cm fish. The magnitude of this force could be adjusted by altering either the angle of attack or the speed of swimming.

Compensation for artificial lift, or positive buoyancy in general, might also be achieved by active use of the pectoral fins. The maximum forces which can be generated this way, however, appear to be limited. Studies with perch, *Perca fluviatilis*, have shown that they are not able to use pectoral fin movements to maintain a vertical position following pressure reductions greater than 16 % (Harden Jones, 1952). This is equivalent to approximately 1.2 % of body weight. In the present study, therefore, even the partial lift used (2.4 % of body weight) would have been beyond what could be compensated for using pectoral fin activity. The results of experiment 7, showing that pectoral fin removal does not affect the rate of swimbladder gas resorption when fish are subjected to artifical lift, also suggest that fin activity plays a minor role in the overall lift balance. Changes in the angle of attack during swimming appeared to be more effective and more commonly used in the present study, and this method of compensation is probably also used for larger buoyancy perturbations in nature.

The stimulus for regulation of swimbladder volume

The response of killifish to artificial lift was a decrease in swimbladder volume, but it is still not clear what stimulus is involved in the regulatory mechanism. Experiment 6 demonstrates that visual perception of an individual's vertical position can influence swimbladder volume. However, because the magnitude of the effect was small (approximately 10 % of swimbladder volume), visual perception is not likely to be the primary stimulus for regulation. The pectoral fins are apparently not directly involved in regulation since removing them had no significant effect on the response of fish to artificial lift (experiment 7). Supporting this are studies of *Gadus callarias*, in which the pectoral fins were immobilized by sectioning the plexus brachialis (Fänge, 1953). Normal inflation of the swimbladder was observed following evacuation by syringe.

The results of experiments 8 and 9, in which lift was attached at various positions along the length of the body, provide some evidence of the stimulus used to adjust swimbladder volume. It is clear that in these experiments the fish are not regulating to an overall hydrostatic balance of zero, which would result in neutral buoyancy. When subjected to partial lift (2.65 % of body weight), twice as much swimbladder gas was resorbed when the lift force acted anterior, rather than posterior, to the fish's longitudinal center of gravity. This suggests that the fish are responding to rotational forces, i.e. pitching moments, as a stimulus for swimbladder gas secretion or resorption. Resistance to lifting the head appears to cause increased gas secretion, whereas conditions that require pulling the anterior of the fish downwards cause resorption. Under natural conditions this response would be adaptive and result in hydrostatic equilibrium. Consider, for example, a fish that has encountered decreased salinity (=density) and has become negatively

buoyant. By exerting the proper muscular control, the fish could raise its head, swim with a positive angle of attack and, thus, maintain its position in the water column. According to the mechanism above, this stimulus would result in gas secretion until the fish was at neutral buoyancy and could swim with normal longitudinal stability. Perceiving the resistance to swimming up or down would require vestibular function, and it has been reported that extirpation of the utriculus causes inappropriate deflation and over-inflation of the swimbladder (von Frisch, 1934).

It appears that under the experimental conditions of this study the fish were simply responding to rotational stimuli and that in some instances their responses were actually maladaptive. This can be seen most clearly in experiment 9, in which the fish were subjected to equal amounts of lift and weight. With the weight anterior to the center of gravity and the lift posterior, a condition requiring effort to lift the head, swimbladder volume increased by one-third. This response is in agreement with the mechanism above. However, these fish displayed extreme positive buoyancy and after 24 h were observed swimming head-down in a nearly vertical orientation to maintain their position. The thrust they were producing by swimming was, apparently, just equivalent to the buoyant force produced by their over-inflated swimbladders.

The response of fish to current is also consistent with rotational forces acting as a stimulus for gas secretion and resorption. The results of experiment 7 suggest that lift forces are produced by the pectoral fins of fish swimming in a current and that these forces, since they are anterior to the center of gravity, would generate a pitching moment. According to the mechanism proposed, the direction of this moment would result in gas resorption by the swimbladder, which is what is observed from fish exposed to current.

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