The Contribution of the Swimbladder to Buoyancy in the Adult Zebrafish (*Danio rerio*): A Morphometric Analysis

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ABSTRACT Many teleost fishes use a swimbladder, a gas-filled organ in the coelomic cavity, to reduce body density toward neutral buoyancy, thus minimizing the locomotory cost of maintaining a constant depth in the water column. However, for most swimbladder-bearing teleosts, the contribution of this organ to the attainment of neutral buoyancy has not been quantified. Here, we examined the quantitative contribution of the swimbladder to buoyancy and three-dimensional stability in a small cyprinid, the zebrafish (*Danio rerio*). In aquaria during daylight hours, adult animals were observed at mean depths from 10.1 ± 6.0 to 14.2 ± 5.6 cm below the surface. Fish mass and whole-body volume were linearly correlated (r^2 = 0.96) over a wide range of body size (0.16–0.73 g); mean whole-body density was 1.01 ± 0.09 g cm^{-3}. Stereological estimations of swimbladder volume from linear dimensions of lateral X-ray images and direct measurements of gas volumes recovered by puncture from the same swimbladders showed that results from these two methods were highly correlated (r^2 = 0.85). The geometric regularity of the swimbladder thus permitted its volume to be accurately estimated from a single lateral image. Mean body density in the absence of the swimbladder was 1.05 ± 0.04 g cm^{-3}. The swimbladder occupied 5.1 ± 1.4% of total body volume, thus reducing whole-body density significantly. The location of the centers of mass and buoyancy along rostro-caudal and dorso-ventral axes overlapped near the ductus communicans, a constriction between the anterior and posterior swimbladder chambers. Our work demonstrates that the swimbladder of the adult zebrafish contributes significantly to buoyancy and attitude stability. Furthermore, we describe and verify a stereological method for estimating swimbladder volume that will aid future studies of the functions of this organ. J. Morphol. 000:000–000, 2008.

KEY WORDS: teleost; cyprinid; gas bladder; stereology; anatomy; morphology

A significant portion of the body mass of teleost fishes is composed of tissues such as bone and muscle that are denser than water (Alexander, 1993). Consequently, compensatory mechanisms are required to reduce the overall density of the body, in order to decrease the energetic cost of swimming to maintain vertical position in the water column. Pelagic teleosts have evolved several strategies to overcome the inherent negative buoyancy related to the density of body tissues. These include the synthesis of large amounts of lipid, the development of "watery" muscles, the reduction of bone mass, and the presence of an internal gas-filled chamber, the swimbladder (Alexander, 1972, 1989; Lefrancois et al., 2001). Of all the mechanisms used by fish to reduce total body density, the swimbladder has been proposed to be the most energy efficient (Alexander, 1993). The volume of low-density gas in the swimbladder offsets the higher density of body tissues so that fish possessing swimbladders are very close to neutral buoyancy at a specific depth in the water column. However, the quantitative contribution of the swimbladder to the attainment of neutral buoyancy has been established for only a few species of teleosts, including some cyprinids (Alexander, 1959) and the toadfish (Fine et al., 1995). Here we investigated the contribution of the swimbladder to buoyancy in a small fresh-water cyprinid, the zebrafish (*Danio rerio*).

The zebrafish is a model species used extensively for investigations of developmental, genetic, and molecular questions in vertebrate biology (Grunwald and Eisen, 2002) and has recently gained popularity in studies of integrative organ function and behavioral neurobiology (Briggs, 2002; reviewed by Miklósi and Andrew, 2006). Furthermore, the zebrafish has been the subject of recent studies aimed at understanding the control of buoyancy. We have described the morphology of the swimbladder together with its innervation, musculature, and vasculature both in adults (Finney et al., 2006) and during development (Robertson et al., 2007). The swimbladder is double-chambered

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in zebrafish; the anterior chamber is believed to be specialized for audition, having a connection to the inner ear via the Weberian ossicles (Bang et al., 2002), whereas the posterior chamber is believed to be hydrostatic in function. Anterior and posterior chambers are joined by the ductus communicans, a narrow passage isolating the contents of the two chambers. The zebrafish is physostomous: a pneumatic duct connecting the posterior chamber to the esophagus remains patent in the adult, allowing swimbladder volume to be altered by passing gas through this duct.

Field observations indicate that zebrafish inhabit small, shallow streams and feed primarily upon terrestrial insects from the surface of slowly running waterways (McClure et al., 2006). Observations of groups of adult zebrafish freely swimming in our laboratory aquaria suggest that these animals range from the middle to upper half of the available tank depth, and do not show marked tendencies to sink or rise when they briefly stop swimming. Given the presumptive hydrostatic function of the swimbladder, it would be expected that the volume of this organ would be regulated to maintain neutral buoyancy over the preferred depth range of the zebrafish, as suggested for other teleosts (Fange, 1983). Furthermore, it would be predicted that the swimbladder is positioned within the coelomic cavity to help the fish maintain a horizontal attitude, so that locomotory energy is not expended unnecessarily in counteracting tendencies to pitch or roll. In addition, the pressure inside the swimbladder must also be maintained above ambient for this organ to fulfill its acoustic and hydrostatic roles. Thus it has been hypothesized that the pressure in the swimbladder is regulated at a fixed value above ambient water pressure at the preferred depth (Alexander, 1959).

Our preliminary observations of zebrafish behavior support these general suppositions about the hydrostatic role of the swimbladder, but the specific contribution of this organ to buoyancy has not been measured in this species. From an ecological standpoint, the ability to maintain neutral buoyancy in the water column is critical and facilitates such daily activities as feeding, reproduction, and predator avoidance (Gee, 1983). To determine the potential hydrostatic contribution of the swimbladder to these behaviors, a detailed description of swimbladder morphology as well as measurements of whole animal density and swimbladder gas volume are required. Here, we present a noninvasive method of estimating swimbladder volume from a single lateral view using morphometric measurements and stereology, as well as techniques for directly measuring gas volume and whole-animal density. This study is the first morphometric analysis of the contribution of the swimbladder to whole-body density and thus to buoyancy in the adult zebrafish.

Our results showed that the presence of a swimbladder permitted zebrafish to attain nearly neutral buoyancy over their preferred range of depth in an aquarium. We have also shown that the position of the swimbladder in the body was close to optimal for horizontal stability, thus reducing the locomotory energy required to counteract changes in pitch and roll. By direct measurement, we found that the internal pressure of the zebrafish swimbladder, while above ambient, was considerably less than that reported in most other cyprinids.

MATERIALS AND METHODS

Animals

A total of 120 adult zebrafish *D. rerio* (Hamilton-Buchanan) of both sexes were used in this study. Fish were purchased from a local pet store (Aqua Creations Tropical Fish, Halifax, Canada) and kept in aerated, dechlorinated tap water in 75 l aquaria maintained at 28–30°C on a 14:10 h light:dark cycle for at least 3 days before experiments were done. Procedures for fish care and usage followed the *Guide to the Care and Use of Laboratory Animals* as established by the Canadian Council for Animal Care. Institutional approval was obtained from the University Committee on Laboratory Animals at Dalhousie University. Fish were fed Nutrafin staple fish food (Rolf C. Hagen, Montreal, Canada) two to three times daily. Behavioral observations were performed in aquaria maintained under the same conditions as the holding tanks. Experiments on isolated swimbladders were performed at ambient laboratory temperature (21–22°C). Local barometric pressure was recorded before each experiment during the first part of this study, but variations in ambient pressure (range 99.2–103.2 kPa) were too small to significantly affect swimbladder volume, so this factor was disregarded in later experiments.

Mean Observed Depth of Fish in Aquarium

To quantify the mean observed depth of adult zebrafish during daylight hours (09:00–19:00), 10 groups of five adult fish were monitored in a 75 l observation tank with a maximum depth of 36 cm. Animals of both sexes, ranging in total length from 20 to 40 mm, were randomly chosen from the holding aquaria, transferred to the observation tank, and allowed 12 h to acclimate. Fish depth was recorded with a monochrome CCD video camera (Honeywell Model HCM574E, Syosset, NY), aimed into the tank from one side, and connected to a computerized recording system (Astra 8 Video Surveillance System, Pace Setter Technologies, Dartmouth, NS, Canada). Observations were made for 1-h periods every second hour during the recording schedule. The tank was divided into 12 horizontal bins, each representing 3 cm of depth. Fish position within these bins was noted in single frames selected at 4-min intervals throughout each hour of recording. The presence of fish within a particular bin was noted by recording the middle depth of that bin (i.e., fish within the bin extending 0–3 cm in depth were recorded at 1.5-cm depth). For each hour of all trials, the overall mean depth of all fish in the group was calculated. Given the relatively small depth interval included in each of the bins and considering that fish were often oriented at an angle spanning the entire depth of a bin during sampling, we considered these data to be continuous over the whole depth of the tank for the purpose of estimating mean observed depth. A one-way ANOVA was performed to test for differences in mean observed depth among the different hours of recording.

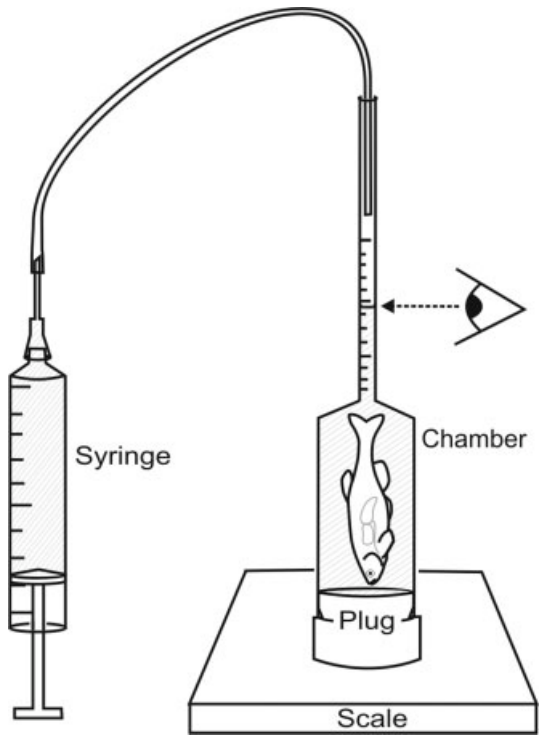

Fig. 1. Schematic diagram of apparatus for measuring zebrafish whole-body volume. See text for details.

Fish Mass, Length, Volume, and Density

Measurements were made on 48 fish of both sexes, ranging in total body length from 20 to 40 mm. The sample included some gravid females. Animals were anaesthetized with 0.02% MS222 (Ethyl 3-aminobenzoate methanesulfonate salt; Sigma Chemical Co., Mississauga, ON, Canada) and whole-body mass was determined on an electronic scale (resolution ± 10 mg) after blotting the fish with gauze to remove excess water. Fish length was measured from the protruding tip of the lower mandible to the end of the tail fin. To measure whole-body volume, a chamber was constructed by mounting a graduated 2 ml-pipette on the needle end of a 10-ml plastic syringe; the opening in the plunger end of the syringe body was then closed with a removable plug (Fig. 1). The empty chamber assembly was placed vertically on the scale pan and tared. A length of polyethylene tubing (PE 50, Clay Adams, NY) was then inserted into the open end of the pipette and positioned so that the tip of the tubing entered the chamber body. The chamber was then filled with water from a syringe attached to the tubing until the meniscus reached the 1-ml graduation on the pipette, and the tubing was withdrawn from the pipette. The mass of this volume of water was recorded. The chamber was then emptied, air-dried, and the fish was placed inside before replacing the plug and taring the assembly again. The chamber was then refilled with the same mass of water used to establish the initial volume. The difference between initial and final volumes as indicated on the pipette scale was taken as the volume of the fish. Whole-body density was then calculated as the dividend of mass and volume.

Swimbladder Volume

Swimbladder volume was either measured directly or estimated stereologically. For a subset of animals, both techniques were employed on the same swimbladder.

Measurement of swimbladder volume. Swimbladders were dissected intact from the coelomic cavity of anesthetized fish, ensuring that no gas escaped. These organs were then immersed in normal zebrafish Ringer's solution (Westerfield, 1995) in a Petri dish under a gas-collecting funnel (Fig. 2). The funnel was cut from the shoulder region of a glass Pasteur pipette and was connected to a 10-ml syringe by a 20-cm length of PE 90 polyethylene tubing. The segment of tubing closest to the collecting funnel was aligned with the edge of a ruled scale oriented vertically. The tubing and collecting funnel were filled with Ringer's solution and the open end of the funnel was submerged in the dish. Volume calibrations were performed before each swimbladder measurement by injecting a standard 50-µl bubble of air into the funnel from a 100-µl Hamilton syringe. This bubble was then drawn into the tube and its length measured. To measure swimbladder volume, both chambers of the swimbladder were punctured and the total volume of gas captured by the funnel was then drawn into the tube. Gas bubble length was measured to calculate the volume of the swimbladder.

Estimation of swimbladder volume. Volume estimations were made from photographs of the lateral aspect of swimbladders dissected from anesthetized animals. Each image was partitioned into regions representing the five volumes shown in Figure 3A; the linear dimensions used in the geometrical analysis below are illustrated in Figure 3B. All geometrical formulas follow the usage of Beyer (1985).

Anterior chamber volume was approximated by that of a rotary prolate ellipsoid:

$$\text{Volume } V_1 = 4/3\pi ab^2 \quad (1)$$

where $a = 0.5$ major axis and $b = 0.5$ minor axis.

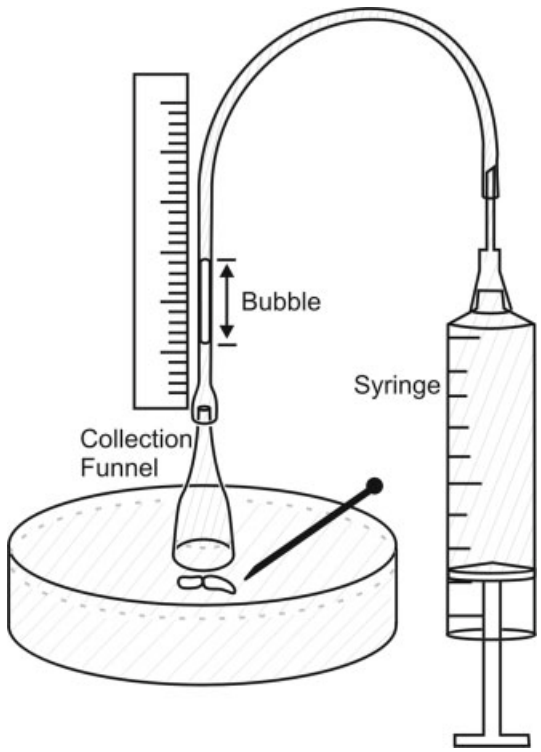

Fig. 2. Schematic diagram of apparatus for measuring volume of gas in swimbladder. Swimbladder was ruptured under collecting funnel and released gas was drawn into tubing by syringe. Volume was proportional to gas bubble length.

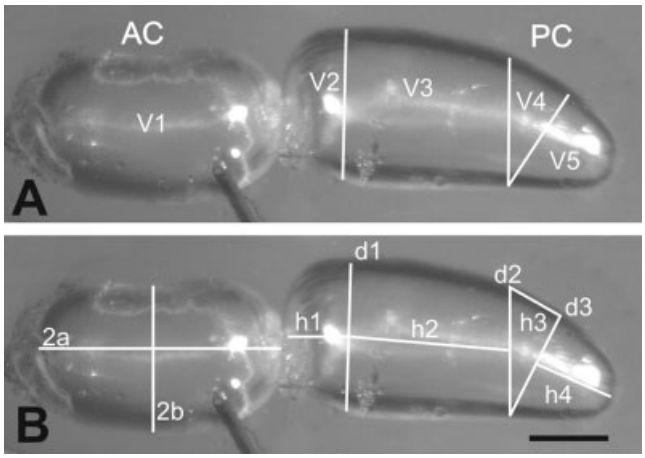

Fig. 3. Photographs of lateral view of swimbladder (anterior to left, dorsal toward top) illustrating geometric components used in estimating volume. (**A**) Anterior chamber (AC) volume approximated by rotary prolate ellipsoid (volume V_1). Posterior chamber (PC) volume approximated by sum of volumes $V_{2–5}$. (**B**) Linear dimensions used in estimating volumes V_1–V_5 (see text for explanation). Scale bar represents 1 mm.

Posterior chamber volume was approximated by the sum of the volumes of four regular geometric shapes.

Volume V_2 was a portion of a sphere:

$$\text{Volume } V_2 = 1/6\pi h_1(3r_1^2 + h_1^2) \quad (2)$$

where $r_1 = 0.5\ d_1$, measured at the widest point of the rostral portion of posterior chamber.

Volume V_3 was a cone frustum:

$$\text{Volume } V_3 = 1/3\pi h_2(r_1^2 + r_2^2 + r_1 r_2) \quad (3)$$

where $r_1 = 0.5\ d_1$, $r_2 = 0.5\ d_2$, d_2 taken as $0.9\ d_1$.

Volume V_4 was a half-cylinder:

$$\text{Volume } V_4 = 1/2\pi r_2^2 h_3 \quad (4)$$

Volume V_5 was a cone:

$$\text{Volume } V_5 = 1/3\pi r_3^2 h_4 \quad (5)$$

where d_3 was taken as the base of the cone; $r_3 = 0.5\ d_3$.

After swimbladder volume had been obtained, the contribution of the swimbladder to buoyancy was determined for individual animals by calculating fish density using the total volume of the fish with the swimbladder included (D_1, below) and comparing this value with that of the same fish after swimbladder volume had been subtracted from whole-body volume (D_2).

$$D_1 = m/v \quad (6)$$

$$D_2 = m/(v - v_{sb}) \quad (7)$$

where m is the whole-body mass, v is the whole-body volume, v_{sb} is the swimbladder volume.

Swimbladder Internal Pressure

Given that the swimbladder is a relatively compliant organ, if its internal pressure were substantially greater than the pressure within the coelomic cavity, as has been shown for a number of cyprinids ["excess" pressure, Alexander (1959)], the volume of the zebrafish swimbladder might have increased when the coelomic cavity was opened in this study. This would have introduced a systematic error into our measurements and estimations of swimbladder volume. To determine the magnitude of this potential error, we used X-ray images of whole fish to visualize the swimbladder *in situ* to test whether its volume increased when the coelomic cavity was opened (Chang and Magnuson, 1968; Bang et al., 2002). X-rays were taken of the lateral aspect of the bodies of 10 anesthetized, intact fish in shallow Ringer's-filled dishes. The coelom was then opened, X-ray exposure was repeated with the fish in the same position, and the estimated volumes were compared using the stereological method described earlier. X-ray images were made on a Belray Dental X-ray machine (Model 096; Takara Belmont Co., Mississauga, ON, Canada; 70 kVp, 10 mA at 0.1 s exposure). The swimbladder from each of these fish was then removed, photographed, and its volume was again estimated stereologically. The volumes of eight of these swimbladders were then measured directly by capturing their gas content.

Internal pressures of both the anterior and posterior chambers of the swimbladder in a separate group of nine fish were measured directly with a pressure transducer (Statham Model p23Dc, Hato Rey, Puerto Rico). Swimbladders were exposed by opening the coelomic cavity and gas in the two chambers was isolated by ligating the ductus communicans. A fluid-filled 30-gauge needle, connected to the pressure transducer via a length of PE 10 polyethylene tubing, was inserted through the wall of each chamber and internal pressure was read from the screen of an oscilloscope. We found that rapid penetration of the wall with the needle was essential to ensure that the wall did not tear. Transducer calibration was performed with a 10-cm water column.

Estimating Relative Positions of Centers of Mass and Buoyancy

A variation of the technique of Bone (1973) was used to determine the position of the center of mass along the rostro-caudal axis. A shallow dish was fitted with a pin anchored to the dish bottom and projecting upward at 90° to the plane of the bottom. An anesthetized fish was laid into the dish on its side with its ventral aspect touching the pin. Sufficient water was added to cover the fish and the dish was tilted gently to one side to balance the fish ventrally on the shaft of the pin. The position of the fish on the pin was adjusted along the rostro-caudal axis until the body was evenly balanced. The point at which the ventral surface of the fish touched the pin was then marked as the surface representation of the rostro-caudal center of mass. The distance from the tip of the lower jaw to the estimated center of mass was measured and the fish was then transected in the transverse plane at this point. The two resulting parts were then weighed to confirm the anterior–posterior distribution of mass around this point.

To localize the center of mass relative to the center of buoyancy in the dorso-ventral axis, each anesthetized fish was placed in a beaker of water to observe whether it came to rest with the dorsal, ventral, or lateral aspect uppermost.

Statistical Analyses

Values are expressed as means ± 1 standard deviation. The level of significance for all comparisons of means was set at $P \leq 0.05$. Pairs of means were compared using a t-test; multiple-means comparisons were performed using one-way ANOVA. Statistical calculations were done using Minitab 14 (Minitab, PA) or SPSS 14.0 (SPSS, Chicago, IL).

RESULTS

Mean Observed Depth of Fish in Aquarium

During the day, fish were located most frequently in the upper middle region of the 36-cm deep observation tank, although they occasionally

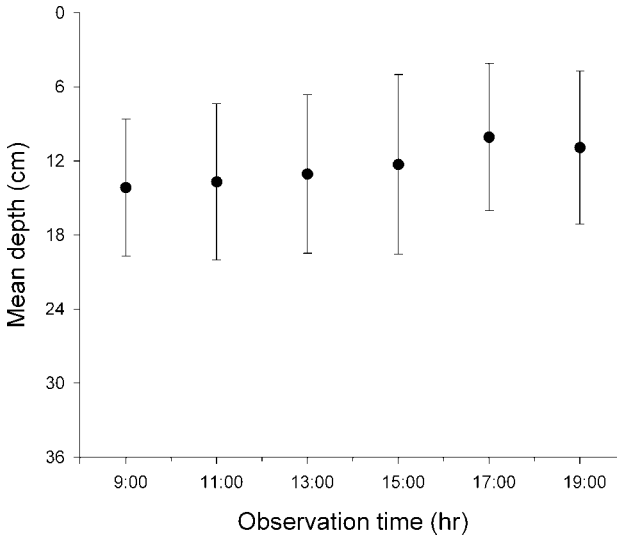

Fig. 4. Mean observed fish depth from surface in a 75-l tank (maximum depth 36 cm) for 1 h samples taken every second hour throughout the daylight period. There was no significant difference in mean observed depth over the sampling period.

swam to the surface or bottom. The mean depth of fish ranged between 10.1 ± 6.0 and 14.2 ± 5.6 cm from the surface (Fig. 4) with no significant differences in the mean observed depth at different times during the day (one-way ANOVA). The overall mean depth over the entire daily observation period was 12.4 ± 6.3 cm.

Mass, Density, and Volume

Body mass and volume were linearly correlated (r^2 = 0.96, n = 48) over a wide range of body size (0.16–0.73 g, Fig. 5). Mean whole-body density was 1.01 ± 0.09 g cm^{-3}, indicating that fish were nearly neutrally buoyant. This group included both males and females; 13 of the females were gravid (represented by dashed line in Fig. 5). Mean density of the gravid females was not significantly different (t-test) from that of the other fish. Mean density of body tissues in the absence of the swimbladder was 1.05 ± 0.04 g cm^{-3} for both gravid and nongravid fish, a value significantly greater (paired t-test) than mean whole-body density of intact fish. The gas volume in the swimbladder therefore made a significant contribution to zebrafish buoyancy, bringing body density to within 1% of that of the surrounding water.

For the same swimbladders, volumes estimated from linear dimensions of photographs of lateral views of both chambers and measured directly by gas recovery were highly correlated (Fig. 6: r^2 = 0.85, slope 0.91). This finding indicated that estimations of swimbladder volumes obtained stereologically were very close to the line of unity slope (Fig. 6, dashed line).

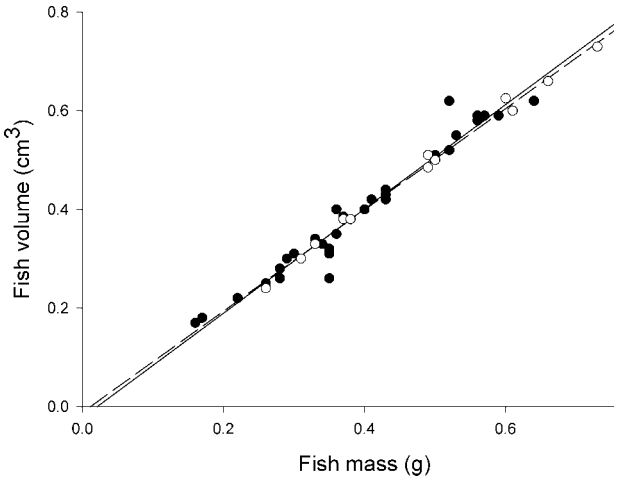

Fig. 5. Plots of co-relationship between whole-body mass and volume for male and nongravid female fish (closed circles, solid line) and for gravid females (open circles, dashed line). Both co-relationships were linear and there was no significant difference between these lines; r^2 = 0.96 for pooled data.

On the basis of such estimations, we calculated that the swimbladder occupied 5.1 ± 1.4% of whole-body volume (n = 27). However, this calculation may have been confounded by the possibility that the internal swimbladder pressure was sufficient to have caused this organ to expand when the coelomic cavity was opened. Alexander (1959) found that swimbladders of many cyprinid species contain gas at pressures estimated to be from 20 to more than 80 mmHg in excess of ambient pressure. We tested this possibility in the zebrafish using two methods.

In a group of 10 zebrafish, swimbladder volumes were estimated stereologically from X-ray images

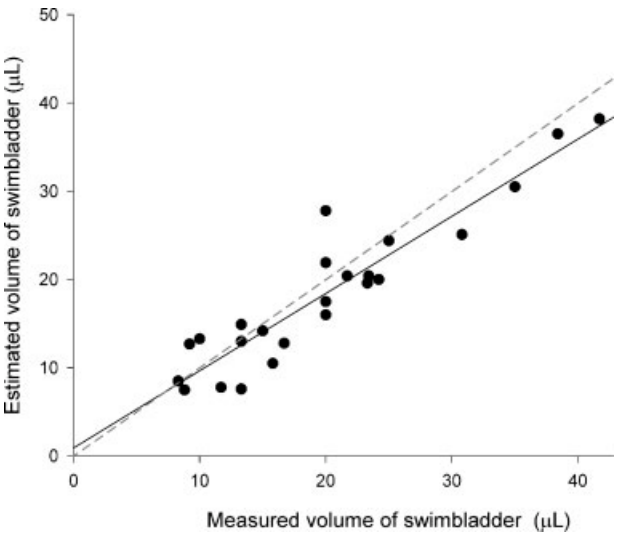

Fig. 6. Plot of co-relationship between measured and estimated swimbladder volumes; this relationship was linear (r^2 = 0.85, solid line) with a slope of 0.91. The dashed line has a slope of unity.

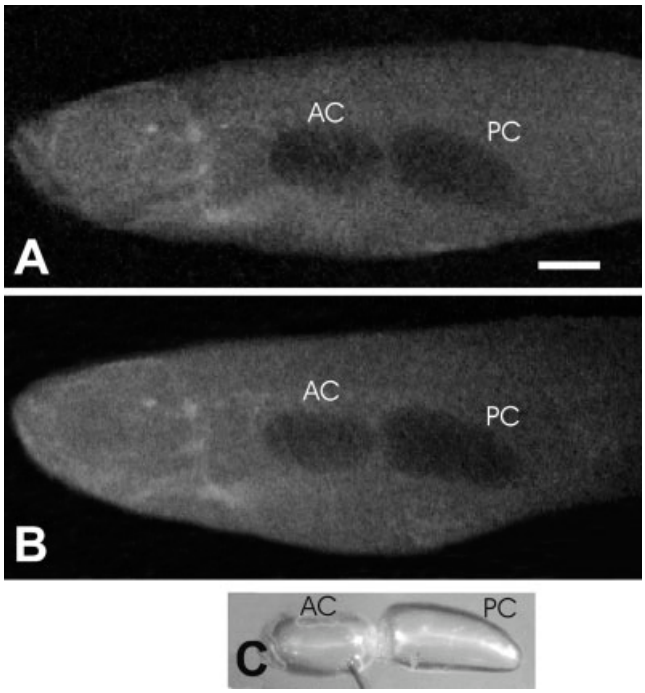

Fig. 7. Three lateral views of swimbladder from the same fish. (**A**) X-ray image of swimbladder in intact animal (AC, anterior chamber; PC, posterior chamber). (**B**) X-ray image after opening coelom. (**C**) Photograph of swimbladder after removal from the body. Scale bar in A represents 2 mm for all images.

of the lateral aspect of the swimbladder in intact, anesthetized fish (Fig. 7A) and again after opening the coelomic cavity of the same fish (Fig. 7B). Next, swimbladders were removed from these animals, photographed, and volumes were estimated stereologically from these images. Finally, gas volumes of eight of these swimbladders were measured directly. There were no significant differences (ANOVA) among estimated and measured volumes, indicating that the swimbladder did not expand significantly when the coelom was opened. This result suggested that internal swimbladder pressure was not greatly in excess of ambient pressure. Direct measurements of swimbladder pressure confirmed this: the mean pressure of the posterior chamber was 8 ± 1 mmHg (n = 9) and pressure in the anterior chamber was 7 ± 1 mmHg (n = 7) above ambient pressure. Thus, pressures in both chambers of the zebrafish swimbladder were relatively low and would not have likely introduced significant errors into our volume estimations.

Swimbladder Position and Relation to Center of Mass

The zebrafish swimbladder was consistently located in the same relative position in the body irrespective of body size. The mean distance from the protruding tip of the lower jaw to the rostral tip of the anterior chamber of the swimbladder was 22 ± 1% of the total body length (n = 11, r^2 = 0.94). The mean distance from the tip of the lower jaw to the ductus communicans was (33 ± 2)% of total body length (n = 11, r^2 = 0.94). Total swimbladder length from the rostral tip of the anterior chamber to the caudal end of the posterior chamber was 25 ± 1% of total body length (n = 10, r^2 = 0.93, range 27–39 mm).

The mean rostro-caudal center of mass was located at a distance of 33 ± 2% (n = 10, r^2 = 0.96) of total body length caudal to the tip of the lower jaw. Transections through the bodies of eight of these fish at the estimated center of balance were found to run directly through the ductus communicans; in two fish the plane of transection was through the anterior chamber within 1 mm of the ductus communicans. The relative masses of the anterior and posterior portions of all fish that were transected at the balance point matched within 2%, thus verifying that estimations of the center of mass derived from balancing the body on a pivot provided an accurate index of rostro-caudal mass distribution. These results demonstrate that the rostro-caudal center of mass was located one-third of the distance from the tip of the lower jaw to the tail and very close to the ductus communicans.

To evaluate the dorso-ventral center of mass, we observed the attitude of 12 anesthetized fish placed in a beaker of water. Ten fish sank onto their sides, one sank belly up, and one floated belly up. Each of the 10 fish that sank laterally was also observed to sink onto the opposite side at least once in succeeding trials, thus suggesting that the center of mass was at, or near, the center of buoyancy in this plane. In this context, measurements from our X-ray images showed that the ductus communicans was positioned approximately halfway between the dorsal and ventral surfaces of the fish (47 ± 5% of the distance from the dorsal to ventral surface, n = 10). Data from the experiments on the locations of the center of mass in the rostro-caudal and dorso-ventral axes are summar-

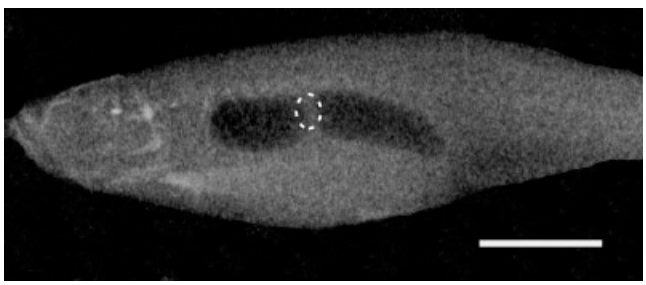

Fig. 8. Region of overlap (dashed oval) of centers of mass and buoyancy in the rostro-caudal and dorso-ventral axes, superimposed on lateral X-ray image of whole zebrafish. Scale bar represents 5 mm.

ized schematically on a lateral X-ray image of a zebrafish in Figure 8 to show that in both of these axes the center of mass was likely to be located in a small ellipse in the region of the ductus communicans.

DISCUSSION

Zebrafish maintained a mean depth of ~12 cm with occasional short forays to deeper or shallower regions. Most fish did not rise or sink appreciably when they stopped swimming, suggesting they were close to neutral buoyancy. This observation is supported by our measurements for whole-body density of 1.01. Our results are consistent with reports that zebrafish live in shallow, slowly-moving streams, where they feed primarily upon terrestrial insects that fall on the water surface. Occasionally fish swim deeper to ingest free-swimming aquatic invertebrates (McClure et al., 2006). It thus appears that zebrafish density may be optimized to maintain a depth just below the surface.

A predicted swimbladder volume of 4.8% of total body volume would be required to reduce zebrafish density to 1.00 g cm^{-3} using Alexander's (1966) mathematical model. The actual swimbladder volume for zebrafish in our study was 5.1% of body volume, a value very close to the predicted value and within the range of proportional swimbladder volumes reported for other cyprinids [5.0%–10.7%, Alexander (1959); 5.0%–7.1%, Overfield and Kylstra (1971)].

The position of the swimbladder within the body of a fish is critical to attitude stability. If the center of buoyancy were located rostral or caudal to the center of mass, the head of a hovering fish at neutral buoyancy would pitch upward or downward, respectively. Similarly, if the center of buoyancy were located ventral to the center of mass, the fish would tend to roll to one side. Counteracting either of these tendencies would require energy-expensive fin movements (reviewed by Alexander, 1966). Zebrafish swim almost continuously and do not tend to hover; yet even when they are occasionally nearly motionless these animals neither appear to have difficulty maintaining a horizontal attitude nor do they exhibit a tendency to roll. Our analysis of the location of the swimbladder within the body showed that the centers of mass and buoyancy were nearly coincidental in a circumscribed region of the body encompassing the ductus communicans. Furthermore, anesthetized zebrafish tended to sink slowly to the bottom, coming to lie most frequently on their sides. It therefore appears that the center of buoyancy in this species may be closer to the center of mass than it is in other teleosts, which usually come to rest ventral surface upward when anesthetized or dead (Alexander, 1966). Regardless of such a difference between species, there is evidence to suggest that in some teleosts the location of the center of buoyancy may be actively regulated in relation to the location of the center of mass. For instance, Goolish (1992), using externally applied weights and buoys to artificially change the center of buoyancy relative to the center of mass in *Fundulus* noted that fish that were artificially maintained at a head-downward pitch secreted gas to increase swimbladder volume, whereas fish pitched head-upward decreased swimbladder volume.

Finally, our study addressed whether the zebrafish swimbladder contained gas at a pressure in the range of values reported in other teleost swimbladders. In many cyprinids, the internal swimbladder pressure is at least 20–30 mmHg greater than ambient pressure at the depth to which fish are acclimated [so-called "excess" pressure, Alexander (1959)]. Such a high pressure in the zebrafish swimbladder might have caused an increase in the volume of the swimbladder when the coelomic cavity was opened, thus potentially confounding estimations and measurements of volume. Our estimates of swimbladder volumes from X-ray images were not, however, significantly different in fish with closed and open coeloms. This finding suggested that swimbladder pressure in this species was relatively low, and we confirmed this with direct pressure measurements. Pressures in the anterior and posterior chambers of the zebrafish swimbladder (7–8 mmHg) were considerably lower than those reported for almost all other cyprinids tested (Alexander, 1959).

This difference between zebrafish and other cyprinids may be due to a difference in mechanisms for filling the swimbladder. Cyprinids, including the zebrafish, can fill the swimbladder by gulping air at the surface and passing small air bubbles along the pneumatic duct: these species may in fact employ this mechanism exclusively when near the surface. However, most cyprinids also possess a gas gland for filling the swimbladder with gas from the bloodstream, presumably allowing these fish to generate internal swimbladder pressures 3- to 5-fold greater than those we observed in the zebrafish, albeit at considerable metabolic cost. As shown by McClure et al. (2006) and confirmed in this study, zebrafish appear to prefer relatively shallow depths, an optimal situation for filling the swimbladder with surface air via the pneumatic duct. Since this species does not possess a gas gland (Finney et al., 2006), it might thus be expected that maximum internal swimbladder pressure would be limited to the range we observed.

We found that gravid and nongravid fish had identical densities after subtraction of the swimbladder. Furthermore, we showed that the co-relationship between fish volume and density was the

same in both of these groups. Therefore, zebrafish eggs must have had densities close to 1.05 g cm^{-3} despite containing a considerable amount of lipid. Moreover, although we did not determine the specific gravity of egg masses, we observed that both immature egg masses and eggs that were laid by sexually mature zebrafish sank rapidly, confirming that egg density was greater than 1.00. In this respect, then, zebrafish eggs are similar to those of the toadfish (Fine et al., 1995). Nonetheless, gonad development (Ona, 1990), the increased total mass of gravid fish and the pressure of eggs on the swimbladder in the coelom all likely affect buoyancy control and warrant further investigation in this context.

This study in the zebrafish establishes for the first time the specific contribution of the swimbladder to the overall buoyancy and attitude stability in a small cyprinid. Moreover, this work demonstrates a stereological method of estimating swimbladder volume from linear dimensions taken from a single lateral view of this organ. We showed that values obtained stereologically matched very closely those obtained by direct volume measurement for the same swimbladders. Our results thus indicate that the volume of the swimbladder can be established accurately and noninvasively in intact, opaque wild-type fish using X-ray images. This technique should also be applicable to photographic images of nonpigmented stains of zebrafish (Lister et al., 1999) in which the swimbladder can be observed directly through the transparent body wall or to those obtained by magnetic resonance microscopy, as proposed by Kabli et al. (2006) for noninvasive studies of internal zebrafish anatomy. Swimbladder stereology, as described here, will facilitate further studies on the effects of ambient pressure, whole body fat content, and gut distension (Ona, 1990) on buoyancy of intact fish. We have also shown that accurate estimations of the volume of isolated swimbladders can be made stereologically without rupturing or otherwise damaging the organ, thus opening the way to further experiments on whole swimbladders *in situ* and *in vitro* to study physiological and pharmacological mechanisms of autonomic control of effectors within the swimbladder system.

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