RESEARCH ARTICLE

A Noninvasive Method to Determine Fat Content in Small Fish Based on Swim Bladder Size Estimation

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ABSTRACT

The presence of fat stores in fish is widely used as a correlate of fish health and fitness. Techniques to measure fat content with some accuracy are available for medium-sized and large fish, but apart from morphometric indices, a noninvasive method to determine fat content in small fish has hitherto been lacking. In this study, we introduce a novel method to measure the fat content in live fish that can be applied also to small fish of less than 0.5 g of body mass. This approach relies on a precise measurement of the swim bladder volume, from which fat content can subsequently be deduced. As fat is positively buoyant, fish with larger fat stores require a smaller swim bladder to attain neutral buoyancy. To determine swim bladder volume, we developed a measuring device, which makes use of the differential compressibility of air and water. A fish is placed in a pressure-tight chamber to which a standardized amount of water is added. The resulting change in pressure Δp is inversely proportional to the volume of the swim bladder. Using juveniles and adults of *Simochromis pleurospilus* (Nelissen, '78; Pisces: *Tropheini*) a small cichlid fish, we show that Δp is tightly related to structural size, mass, and body condition. Most importantly, this approach allows to predict the visceral fat content of small fish more precisely than the six most commonly used morphometric body indices. *J. Exp. Zool. 313A, 2011.* © 2011 Wiley-Liss, Inc.

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The presence of fat stores in fish indicates the possession of surplus energy, as fat is the primary energy storage substrate in fish (Love, '70; Adams, '99; Tocher, 2003). Since fat content is a good index of future survival in some species (Simpkins et al., 2003) and a strong indicator of reproductive potential in some fish stocks (Marshall et al., '99), the amount of fat deposited by an individual has been used as a correlate of health or, more generally, of fitness (Adams, '99). Interests to quantify fat deposits of fish have thus emerged in different disciplines. A commercial fish breeder will use it as an indicator of health and animal welfare in his live stock (Novotny and Beeman, '90), ecologists may use it to judge the well being of wild populations

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(Liney et al., 2006; Stevenson and Woods, 2006) and in evolutionary studies the existence of energy reserves are an important determinant of optimal allocation decisions within trade-offs between growth, storage, and reproduction (Glazier, '99).

Many research fields have adopted small fish as model species, often requiring to obtain repeated measurements of the study animals. For example, marine biologists study dispersal of small reef fish (Mora et al., 2003), behavioral biologists investigate mate choice in sticklebacks (Bakker et al., '99; Milinski, 2003), evolutionary biologists study species radiation and adaptation in cichlids (Barlow, '98; Seehausen, 2006), guppies (Carvalho et al., '96) or sticklebacks (Schluter, '93), and toxicologists test the effects of chemical compounds on zebra fish (Bresch et al., '86). Existing techniques to measure the fat content in fish have a number of weaknesses, which render them unsuitable, to be used with live, small fish. Extracting fat from whole fish using solvents and a Soxhlet apparatus (Sawicka-Kapusta, '75) is the most accurate way of measuring fat content even in the smallest fish, but can only be performed in dead animals. However, it is inappropriate when working with small or endangered populations or when repeated measurements of the same individual are required. Condition indices are calculated from external measures of size and mass, and are potentially suitable for small fish (Bolger and Connolly, '89). They are easy to apply and therefore widely used, but often give only crude estimates of fat content (Hayes et al., 2001; De Robertis and Williams, 2008; Pardoe et al., 2008). Noninvasive techniques to measure fat content directly are based on electrical conductivity: bioelectrical impedance analysis (BIA) is a noninvasive method based on body conductivity; it determines the electrical impedance of an electric current through tissue (Ivorra et al., 2003). It is based on the fact that the electrical conductivity of animals is largely determined by the water content of the body, and that lean tissue consists of more water than fat tissue. During measurements, fish need to be anaesthetized and freed from external water. BIA has successfully been applied to freshwater fish of 12 g or more (Cox and Hartman, 2005). The "Total Body Electrical Conductivity" method (TOBEC) similarly uses body conductivity of animals placed in an electric field to deduce fat content (Angilletta, '99). And although Bai et al. ('94) successfully measured fish of 10–138 g, the usefulness of TOBEC for live animals is limited due to its limited repeatability (Robin et al., 2002). Although body conductivity methods are straightforward to use, they are often expensive, of limited applicability for field conditions, and they are too imprecise to be applied to very small fish (<10 g).

In this study, we introduce a novel, noninvasive method that allows measuring the total fat content in very small fish down to 1 g of body weight, if these fish possess a closed swim bladder (i.e. physoclists, which make up the majority of fish species). Our approach is based on the following considerations: (i) Bones and other fish tissues are negatively buoyant, whereas fat is positively buoyant, which means that fish with larger fat stores will require a smaller swim bladder to attain neutral buoyancy at a given depth. (ii) Gases are much more compressible than water. The method relies on a precise barometric measurement of the swim bladder volume from which we can deduce fat content. For that purpose, a fish is placed into a pressure-tight chamber to which a standardized amount of water is added. The increase in pressure is then inversely related to the volume of the swim bladder. This approach is easily applicable; it neither harms the fish nor requires anesthetising it, and it allows repeated measurements of fat content of the same individuals. Swim bladder volume can also be measured by computer tomography (Pees et al., 2010), but this method is costly and technically difficult to realize with the normal fish holding facilities. The method we present here can be easily used on each lab with some adjustments in such a way that it should be suitable for field studies and even for measurements under water. In this article, (i) we describe the underlying principles of this approach, (ii) we demonstrate the application of the method by comparing fat estimates of cichlids before and after a short period of enhanced feeding, and (iii) we show that swim bladder volume is an accurate predictor of visceral body fat mass.

MATERIAL AND METHODS

Measuring Chamber

We cut a cylindrical measuring chamber from a solid block of clear Perspex (Fig. 1). The chamber has an inner diameter of 73 mm, an outer diameter of 93 mm, a height of 78 mm, and a volume of 300 mL. The lid (1) closes the measuring chamber (2) with help of a 10-mm wide, 1-mm thick silicone gasket (3) to make it pressure-tight. A highly sensitive electronic manometer

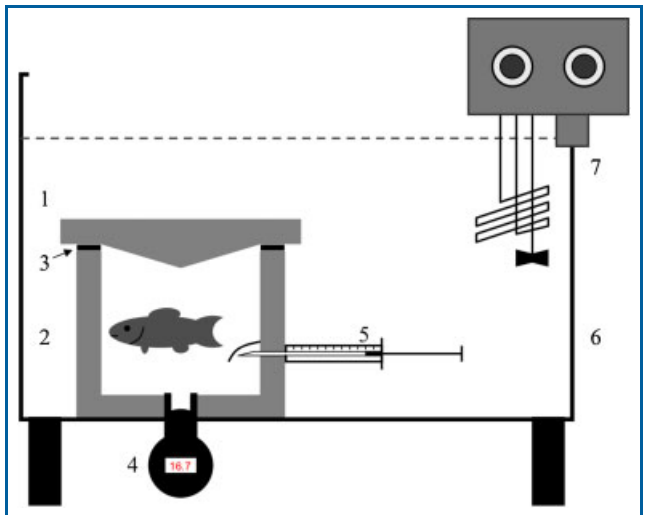

Figure 1. Schematic representation of the measuring apparatus consisting of a clear lid (1), measuring chamber (2), silicone gasket (3), electronic manometer (4), pressure-tight syringe (5), 60 l water tank (6), precision heater system (7).

(4) (Hirschmann PA430, −200.0 to+1,000.0 mbar; ±0.1 mbar) is mounted in a 15-mm hole in the bottom of the chamber. At the wall of the container, a 1-mm hole sealed with a 2-mm silicone gasket allows the insertion of a 50-µL pressure-tight syringe (5). During the measuring procedure, the measuring chamber is completely submerged in a 60-L-water tank (6), equipped with a precision heater system (7) (Lauda, Type T) to assure a constant temperature of 27°C (±0.1°C, Fig. 1). We kept the water level at 20 cm.

The cost of material for building the measuring chamber (including the electronic manometer, a pressure-tight syringe, a precision heater system, and a block of Perspex) totaled 1,500 USD, and the construction took about 25 working hours of a precision mechanic.

Association Between Air Volume and Pressure Change in the Measuring Chamber

To determine the relationship between pressure change Δp and swim bladder volume V_S, a series of gas measurements were carried out. First, we determined the pressure change in the chamber without any air present. Adding 50 µL of water to the completely filled measuring chamber increased the pressure by 488 mbar ± 0.5 SE. In a perfectly inflexible and completely water-filled measuring chamber, it would be impossible to insert further water, as water is not compressible. But since the lid is sealed with a compressible ring of silicone and the pressure sensor membrane bends inwards with increasing pressure, it is possible to insert a tiny volume of additional water into the chamber and increase the water pressure therein.

Then we measured Δp for different precise volumes of air injected in the chamber ranging from 50–1,300 µL. The air volumes were injected under an upside-down beaker placed in the chamber to prevent the air bubble from escaping until the chamber was closed. We repeated the measurement five times for each volume. As a next step we determined the functional relationship between air volume and pressure change Δp after adding 50 µL of water. Pressure change decreased with increasing air volumes (Fig. 2) following the power function

$$\Delta p(V) = a \times V^b + c. \quad (1)$$

The parameters $a = 204,\ 100$, $b = -1.205$, $c = -96.08$ fitted our data best (nonlinear least squares fitting, $R^2 = 0.999$). This function enables us to estimate the swim bladder volume of a small fish when knowing Δp. The function is very sensitive to variation of small swim bladder volumes, so that this method can obtain precise volumes also for very small fish. The parameters of Equation (1) have to be established first for each newly constructed measuring device before starting to measure any fish.

Measuring Protocol

To determine its relative swim bladder volume, a fish is placed in the measuring chamber. After visual inspection for possible air bubbles, we fixed the silicone gasket and pressure-proof lid to the chamber. Excess water leaves the chamber via the open 50-µL pressure-tight syringe (the plunger is not in the syringe). After 30 sec, the pressure in the chamber reaches ambient levels as visible on the manometer, the plunger is inserted into the barrel of the syringe, and 50 µL of ambient water is added to the chamber. We noted the resulting increase in pressure Δp and repeated this 10 times. When measuring smaller fish with higher pressure changes (probably due to inertia of the measuring chamber) the values of successive measurements increased over the first two to three measurements before being stable. Therefore, we used the mean of measurements 8–10 in all cases for further analysis. Afterwards we removed the lid, measured the fish's standard length (SL, i.e. tip of snout to end of caudal peduncle) with callipers to the nearest 0.1 mm, and determined the body mass (M) to the nearest 0.001 g. The measurement in the pressure chamber and taking morphometric measures outside of the tank takes approximately 180 and 20 sec, respectively. Given that all fish tissues apart from the swim bladder gas are as incompressible as water, they do not influence pressure change Δp, which is therefore determined by bladder volume V_S only.

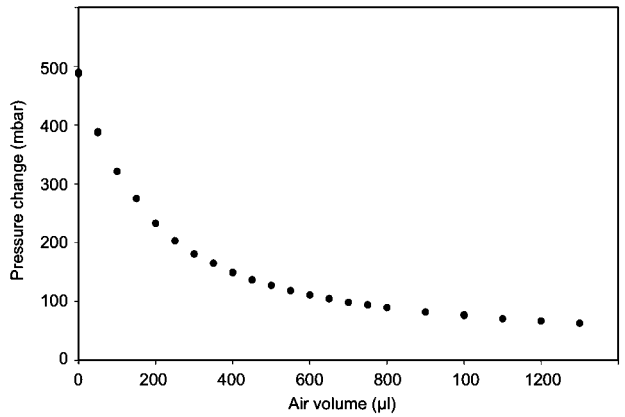

Figure 2. Relationship between pressure change and air volume in the measuring chamber. Pressure change for each volume was measured five times (data points of repeated measures are strongly overlapping).

Relating Δp to Body Fat

The measuring apparatus can determine pressure changes to the nearest 0.1 mbar. To determine the measurement error of the apparatus, we repeated all measurements of the 22 different air volumes five times (see Methods), and calculated the standard deviation of each set of repeats. The mean standard deviation of all measurement sets was 0.36 mbar ± 0.06 SE; therefore, all measurements in the range of ±0.72 mbar (2 SD) can be considered correct with a confidence of 95%. Translating our measuring accuracy into swim bladder volumes with help of

Equation (1), this translates to an error of 0.8 μL of swim bladder volume in a small fish (52 mm) and an error of 1.6 μL in a large (85 mm) fish, respectively, which indicates that our method is very powerful in detecting minute changes in swim bladder volume. As a fish actively controls buoyancy, the total density of the whole body of a fish should equal the density of water. The total density of a fish depends on the densities of its components, namely the swim bladder, the density of stored fat, and the density of all other tissues. Density is defined by weight per volume and the density of water is 1.0 kg dm^{-3}. For a fish in equilibrium (with zero buoyancy), the equation

$$\frac{V_A^* \rho_A V_M^* \rho_M + V_F^* \rho_F}{V_{total}} \quad (2)$$

must hold, where V_A is the volume of air (swim bladder), ρ_A the density of swim bladder gas (although swim bladder gas often differs in composition (Wittenberg, '58) gas composition in the swim bladder of shallow water fish mostly resembles atmospheric gas composition (Ostrander, 2000). Therefore here the density of air is used: 12,041.10^{-3} kg dm^{-3}), V_M is the volume of all other tissues (bones, muscles), ρ_M is the average density of remaining tissue, V_F is the volume of fat, ρ_F is the density of fat (0.91 kg/dm^3), and V_{total} is the volume of the whole fish. If we assume that the contribution of bone and muscle tissue to the total average density is constant, we can calculate how a change in fat volume corresponds to a change in swim bladder volume as

$$\Delta V_F = -\Delta V_A \frac{1-\rho_A}{1-\rho_F}. \quad (3)$$

For example, in a small fish (52.3 mm, 5.23 g) an increase in Δp of 2 mbar (swim bladder decreased by 3.2 μL (95% confidence interval: 2.4–4.0)) indicates a fat gain of 35.5 mg (26.6–44.4) or 0.68% (0.51–0.85) of body mass, if all other tissues apart from fat did not change.

We are aware that if the position of the swim bladder within the fish is fixed and positively buoyant (fat) and negatively buoyant (muscle and bones) tissues are not homogenously distributed along the body axis, the center of gravity may not be congruent with the center of buoyancy. This must then be counteracted by body or fin movements to ensure hydrostatic stability. However, as counteracting movements would expend energy, fish should be selected such that differently buoyant tissues are distributed homogenously along the body axis. It even seems as if fish can cope with a substantial variation of energy intake without changing their center of gravity (Brix et al., 2009). We therefore disregard potential effects of different centers of gravity or buoyancy in our analysis.

Validation Study

Relationship Between Δp and Body Size. We measured fat content in small fish using 72 individuals of the African mouthbrooding cichlid *Simochromis pleurospilus* (Nelissen, '78, Pisces: *Tropheini*), which is a suitable model species to investigate long-term effects of environmental variation on life history trade-offs (Taborsky, 2006a,b; Kotrschal and Taborsky, 2010a). It inhabits the rocky shores of Lake Tanganyika, where it feeds on epilithic turf algae. Males grow up to 11-cm SL and females reach sizes up to 10-cm SL (Taborsky, 2006a; Kotrschal and Taborsky, 2010b). Our study animals comprise 21 juveniles and 55 adults. The fish were housed individually at 27°C in 25-L tanks. Each tank was equipped with a layer of sand, a flower pot half for shelter, and an internal biological filter. Water depth inherently determines the swim bladder volume as fish adjust the volume to ambient pressure to be neutrally buoyant. Therefore, we kept the water level in holding tanks and measuring chamber at 20 cm throughout the experiment. Juvenile and adult fish were fed 6 days a week with standardized agarose cubes containing Tetramin flake food mixed with 5% *Spirulina* algae. The food amount in the cubes corresponded to 8% of mean body weight. To determine the relationship between body size and Δp in live fish all animals were measured once in the measuring chamber ($N = 72$).

Food Manipulation. To further evaluate the relationship between Δp and body condition, we carried out a feeding experiment. We used only the adult fish among our study animals to minimize potential growth effects ($N = 41$, see below). The first phase of the food experiment is aimed at reducing possibly existing fat stores of the adults to obtain a measurement of lean fish. Fish were mildly food restricted; they received a food amount corresponding to 4% of mean body weight (half a food cube) every other day during 8 days. Then the first Δp measurement was taken (AD LEAN). During the second phase of the experiment, we aimed to evoke fat storage in the same fish. During 25 days, they received a food amount corresponding to 12% of mean body weight (1.5 food cubes), and an additional 3% of pureed bovine heart. After 25 days, we re-measured the fish (AD FAT). We expected this manipulation to result in a marked increase in Δp, as the animals should compensate a higher fat storage with smaller swim bladders to retain neutral buoyancy.

Dissection. Additionally, we killed 12 adults that were of similar size and weight than the adults used in the food manipulation experiment (see Table 1) by an overdose of MS 222. These fish were not exposed to previous food manipulations, as we aimed to demonstrate the link between visceral body fat, Δp, and body size in a random, unmanipulated sample of adults. In these fish, we opened the body cavity and removed all visible visceral fat and weighed it to the nearest 0.01 mg with a high-precision electronic balance.

Morphometric Indices of Body Condition. From length and weight data, we calculated six other widely used indices of body condition (Peig and Green, 2010): (1) Fulton's index of body condition as $K = M/SL^3 \times 100\,g/mm^3$ (Bolger and Connolly, '89); (2) Quételet's index as $BMI = M/SL^2 * 1,000$; (3) Relative

Table 1. Body measurements of *Simochromis pleurospilus* juveniles, adults (AD) after food deprivation (lean) and after food supplementation (fat), and a separate group of adults that were dissected without previous food supplementation.

		SL (mm)			Mass (g)			Fulton's condition index K		
	N	Min.	Max	Mean ± SE	Min.	Max	Mean ± SE	Min.	Max	Mean ± SE
Juveniles	21	31.4	66.2	50.08 ± 2.14	0.84	8.18	3.78 ± 0.44	2.23	3.09	2.71 ± 0.048
AD LEAN	41	71.5	89.0	78.35 ± 0.69	9.97	20.22	13.35 ± 0.38	2.56	3.07	2.75 ± 0.019
AD FAT	41	71.0	91.5	78.60 ± 0.70	10.05	20.66	13.53 ± 0.39	2.59	3.01	2.76 ± 0.017
AD DISSECTED	12	73.5	87.6	80.17 ± 0.80	9.81	17.52	13.06 ± 0.52	2.27	2.93	2.52 ± 0.050

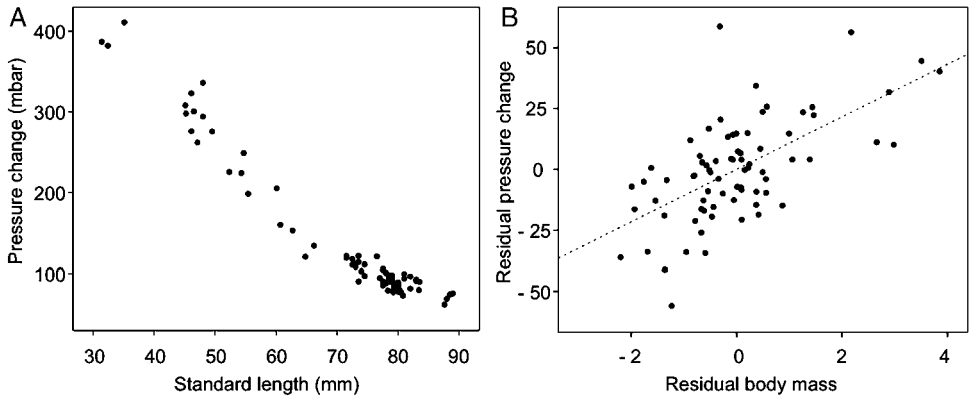

Figure 3. (A) Relationship between body size of juvenile and adult *Simochromis pleurospilus* and the resulting pressure increase after adding 50 µL of water to the measuring chamber. (B) Relationship between body mass and pressure change (residuals of quadratic regressions are shown after controlling body mass and pressure change for body size).

condition K_n (the observed individual mass divided by the predicted mass) $M^* = a*\mathrm{SL}^b$, where a and b are determined by ordinary least squares regression of M against SL; (4) Relative mass W_r, where a and b above are determined from a reference population; in our case from 550 wild *S. pleurospilus* caught for another study; (5) Residual index R_i, the residuals from a least squares regression of M against SL; (6) Scaled Mass index $\hat{M} = M[\mathrm{SL}_0/\mathrm{SL}]^{b_{SMA}}$, where SL_0 is the mean value of SL of the study population; the scaling exponent b_{SMA} can by calculated indirectly by dividing the slope from a regression by Pearson's correlation coefficient (Labarbera, '89; for details on the calculation of indices see Peig and Green (2009)).

Statistics

Curve fitting was carried out using MATLAB; all other analyses were carried out with SPSS 17.0 (SPSS Inc., Chicago, IL).

RESULTS

Association Between Pressure Difference Δ*p* and Body Size and Mass

The pressure change in our fish ranged from 69.1 to 401.0 mbar. According to Equation (1), this corresponds to swim bladder volumes V between 1,144 and 48 µL (see Appendix 1 supporting information for relationships between calculated swim bladder volumes and body size and mass). Swim bladder volumes were related to fish ranging from 31 to 89 mm SL (Table 1). The volumes increased with the SL of the fish, which is shown by the decrease in Δp with increasing body size (Fig. 3A; quadratic regression; juveniles, AD LEAN and AD DISSECTED: $N = 72$, $F = 888.4$, $R^2 = 0.97$, $P < 0.001$; to avoid pseudoreplication we chose not to include AD FAT fish in this analysis). Δp increased linearly with body mass after correcting both parameters for SL (Fig. 3B; juveniles, AD LEAN and AD DISSECTED; Pearson: $N = 72$, $r = 0.62$, $P < 0.001$).

Effect of Food Manipulations on Fulton's Body Condition and Δ*p*

During the period between the AD LEAN measurements and the AD FAT measurements, adults slightly increased in length (paired *t*-test: $N = 41$, $T = 1.87$, $P = 0.069$) and significantly gained weight (paired *t*-test: $N = 41$, $T = 3.73$, $P = 0.001$), but Fulton's body condition index did not change (paired *t*-test: $N = 41$, $T = 0.19$, $P = 0.86$).

AD LEAN fish yielded Δp values ranging from 69.1 to 121.7 mbar (97.8 ± 2.1 SE), which corresponds to swim bladder volumes of 1,144–531 µL (739 ± 22 SE). After 25 days of feeding

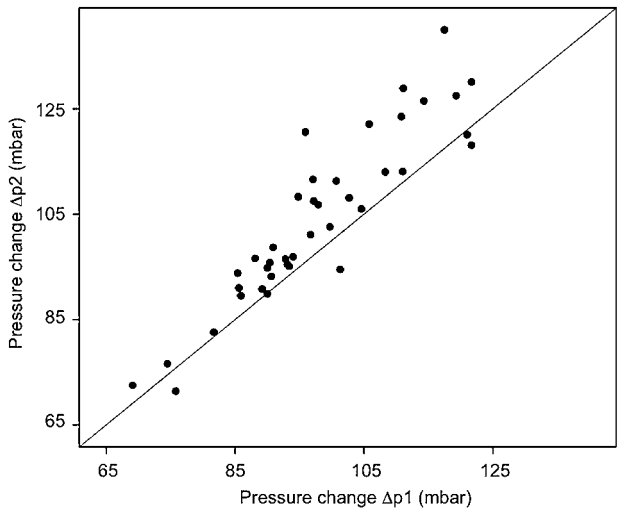

Figure 4. Relationship between pressure change before and after a period of enhanced feeding of adult *Simochromis pleurospilus*. The line indicates no change between measurements, dots above and below imply increased and decreased pressure change, respectively.

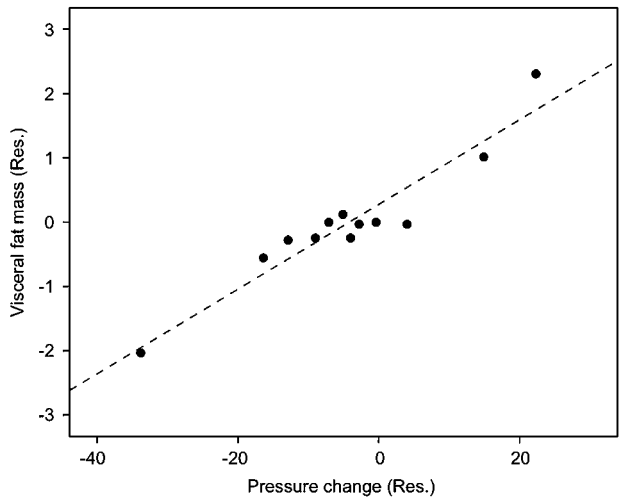

Figure 5. Relationship between pressure increase and visceral fat content (both controlled for body size) in *Simochromis pleurospilus*.

Table 2. Relationship between visceral fat mass and pressure change in *Simochromis pleurospilus*, compared to 6 traditional morphometric indices.

	R^2	F	P
Pressure change Δp	0.902	92.14	<0.001
Fulton's index K	0.491	9.68	0.011
Quételet's index	0.437	7.75	0.019
Residual index R_i	0.400	6.67	0.027
Relative mass W_r	0.381	6.16	0.032
Relative condition K_n	0.355	5.51	0.041
Scaled mass index $\hat{M}_i$	0.352	5.44	0.042

high energy food (AD FAT), Δp values had significantly increased (Fig. 4; paired t-test: $T = 5.87$, $P < 0.001$) now ranging from to 71.4 to 140.0 mbar (104.1 ± 2.6 SE), which corresponds to 1,096–433 µL (686 ± 24 SE) of swim bladder volumes. All but four individuals showed a marked increase in Δp, indicating a decrease in swim bladder volume over the course of the experiment (for body mass to weight relationships of experimental fish see Appendix 2 of supporting information).

In AD DISSECTED fish, the visceral fat mass (7.0–198.7 mg; see Appendix 3 of supporting information) significantly correlated with all morphometric body condition indices. The morphometric indices explained between 35 and 49% of variation (Table 2). In contrast, Δp corrected for SL explained 90% of the variation in visceral fat (Linear regression: $N = 12$, $F = 92.14$, $R^2 = 0.902$, $P < 0.001$; Fig. 5; Appendix 4 of supporting information).

DISCUSSION

In this study, we propose a new noninvasive method to estimate fat content in small fish, which relies on the measurement of swim bladder volume in neutrally buoyant fish. We measure swim bladder volume by obtaining the pressure change when adding small amounts of water to a pressure-tight chamber that holds the fish and is otherwise completely filled with water. The relationship between pressure increase after injecting a defined amount of water and gas volume in the measuring chamber can be accurately described (Equation (1)). When validating this method with the cichlid fish *S. pleurospilus*, we found pressure change Δp to be tightly related to body size and mass. Most importantly, pressure change predicted the visceral fat content of fish markedly better than six commonly used morphometric indices did.

Three lines of evidence indicate that Δp can be used to estimate fat content in live fish, namely (i) that Δp markedly increases with body mass, (ii) that a period of enhanced feeding resulted in a significant increase in Δp in most adults (indicating a decrease in swim bladder volume), and (iii) that Δp predicts well the mass of visceral fat, and it does so with a much higher accuracy than six commonly used morphometric condition indices.

Body Mass is Positively Associated With Pressure Change

It is save to assume that similar-sized conspecific animals have similar relative portions of skeletal and organ mass and more mass per length is therefore largely influenced by fat or muscle mass. As the density of fish muscle tissue tends to be greater than 1 (e.g. 1.06 g/dm^3 in cyprinids; Alexander, '59), an increase in muscle mass will lead to an increase in swim bladder volume.

In contrast, an increase in stored fat will produce uplift forcing the fish to reduce swim bladder volume in order to maintain equilibrium. The strong positive association between relative body mass and relative Δp (after accounting for structural size) indicates that relatively heavier fish have smaller swim bladders, which is most likely caused by a higher fat content of these fish.

Enhanced Feeding Increases Pressure Change

During 25 days of a high-caloric food treatment, most fish grew in length and gained weight. A fish growing in length should increase swim bladder volume proportionally but with a decelerating rate. This is reflected by a diminishing decrease in Δp with SL. Conversely, although fish grew slightly in length during 25 days of food treatment we found that Δp had *increased*, which can only be explained by a decrease in swim bladder volume due to increased fat storage.

The fact that Fulton's body condition index (which explained visceral fat best among the six morphometric indices) failed to show a difference between the measurements before and after 25 days suggests that, at least in *S. pleurospilus*, this index is of limited suitability to determine small changes in condition and, if at all, only gives a crude estimate of body fat (see also Davidson and Marshall, 2010). Estimation of swim bladder volume by measuring pressure change on the other hand did resolve the predicted difference between before and after a period of enhanced feeding. This indicates that our proposed method provides more accurate estimates of body condition.

Δp Predicts Visceral Fat Mass

Using Δp controlled for the fish's body size, it was possible to predict visceral fat mass with a much higher accuracy than by using morphometric condition indices.

When comparing two same-sized consepcifics, differences in fat content will only be reflected by differing swim bladder volumes, if the proportions of all other tissues are approximately constant. Our method will not detect swim bladder volume differences if, for example, a fat fish also has a much higher muscle mass compared with a lean fish. Our approach therefore may be of limited applicability when comparing individuals between different environments or across different life stages, as muscle mass may vary with the specific requirements of the respective environment and life stage. Furthermore, all gas contained within the tissues of a fish will influence the pressure change. Although minute quantities of digestion-related gas in the intestines cannot be ruled out, it is safe to assume that most gas in a healthy fish is stored in the swim bladder and the pressure change hence reflects swim bladder volume. If larger gas quantities were located in other tissues these would influence buoyancy and would be compensated by a reduction of the swim bladder volume.

In evolutionary, ecological and behavioral field studies researchers often would like to determine the body condition of live fish. So far, fish needed to be brought to the surface to apply any of the available fat-measurement methods or to obtain the body mass for morphometric indices. Physoclist fish inhabiting greater depths can often not be surfaced, however, without a difficult and time-consuming procedure of extremely slow ascend, which might potentially harm or distress the fish. Our method could even be used in the field to determine body condition *under* water: After fitting the measure chamber with a (commercially available) water proof manometer, researchers could determine body condition with higher accuracy and with considerable less stress inflicted on the animals due to greatly reduced handling time and immediate release at the place of capture. Our method should not distress the animals much more than catching and handling does, as handling time is short and the pressure applied on the fish resembles a vertical movement of 70–400 cm in the water column.

Given the strong positive relationship between body mass on Δp, the effect of enhanced feeding on Δp and its successful validation when comparing it with six condition indices, the pressure-based method can be considered as an improvement on existing condition indices working with morphometric data alone. To conclude, determining body fat noninvasively by measuring swim bladder volume is a suitable approach to measure body condition in small fish both in laboratory studies and, with additional adjustments, even under water.

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