

Research Article

A direct and straightforward method for measurement real maximum fish stomach volume to improve aquaculture feeding research

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ABSTRACT. In fish feeding studies, stomach related parameters are mainly measured in order to have biometric relationships. To determine those parameters a direct and straightforward method for measuring real maximum stomach volume in fish based on Archimedes' principle was tested and compared against a traditional method. The new method is based on the measure of the real maximum stomach volume when fish are fed *ad libitum*. By contrast, the traditional method measures the forced expanded capacity of the stomach under an arbitrarily assigned hydraulic pressure head of a 50 cm column of water. The two methods were assayed in juvenile rainbow trout *Oncorhynchus mykiss*, and the results displayed significant different stomach volume and related indices. The best relationships for potential regression were obtained using the Archimedes' principle method, being between fish weight and stomach volume ($R^2 = 0.68$), and stomach content and stomach volume ($R^2 = 0.95$). These results indicate that the measurement of real maximum stomach volume in fish using the Archimedes' principle method could be a useful method of estimation of how much stomach can expand; this can be a suitable tool to improve fish feeding research by regulating the amount of feed that can be ingested by animals.

Keywords: Archimedes' principle; stomach volume, fish feeding, *Oncorhynchus mykiss*.

INTRODUCTION

A common method for investigating the diet of fish is the analysis of stomach measurement (Adeyemi & Akombo, 2012; Abdul *et al.*, 2016). Various methods have been used to measure the maximum stomach volume in fish (Kariya *et al.*, 1968; Kimball & Helm, 1971; Jobling *et al.*, 1977; Burley & Vigg, 1989), but the most commonly used method has been the measurement of volume under 50 cm pressure head, described by Jobling *et al.* (1977). The Jobling's method has been followed in research into compensatory growth in fish (Nikki *et al.*, 2004; Känkänen & Pirhonen, 2009; Mattila *et al.*, 2009) using radiography (Flowerdew & Grove, 1979), different diets (Ruohonen & Grove, 1996), studying indirect estimation of stomach volume (Pirhonen & Koskela, 2005) and determination of stomach fullness (Phelps *et al.*, 2007).

In different species, such brown trout *Salmo trutta* Linnaeus 1758, mountain whitefish *Proposium williamsoni* (Girard, 1856), white crappie *Pomoxis annularis* (Rafinesque, 1818), channel catfish *Ictalurus*

punctatus (Rafinesque, 1818), spotted bass *Micropterus punctulatus* (Rafinesque, 1819), bluegill *Lepomis macrochirus* (Rafinesque, 1819), black crappie *Pomoxis nigromaculatus* (Lesueur, 1829) and white bass *Morone chrysops* (Rafinesque, 1820), measurement of the stomach content has been achieved by gauging stomach volume (Kimball & Helm, 1971; Gosch *et al.*, 2009), which can also prove an effective strategy.

The main aim of the present study is to propose and validate a simple and direct measurement method based on Archimedes' principle, which provides real value for maximum stomach volume in fish. On the other hand, other methods are more complex to do, requiring more time and more factors have to be considered, which decrease the possibility to have a direct measurement in the determination of biometric parameters in fish feeding research. Likewise, Archimedes' principle is commonly used in fisheries and fishing biology since several years ago (Yañez-Arancibia, 1975). In this sense, the purpose of this study results is to apply this method to aquaculture fish feeding.

MATERIALS AND METHODS

Experimental conditions and stomach volume measurements

The experiment was carried out with thirty rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) juveniles. These fish were obtained from hatchery stock at the Catholic University of Temuco (Chile). During the acclimation period, the fish were kept in a 3,000 L fiberglass tank connected to a flow-through water system at 15°C and with natural photoperiod. Before sampling, the fish were starved for four days and then fed until satiation with commercial food, once per day (09.00 AM). Immediately after the fish stopped feeding, they were anesthetized with benzocaine (0.15 mL L⁻¹) and killed with a sharp blow on the head. Fish were weighted, and standard length was measured. Body cavity was opened, and the stomach was removed. Then, the stomach volume was measured using a method based on Archimedes' principle (the body immersed in a fluid displaces a liquid volume equal to the immersed body volume). In order to achieve this, the stomach was separated from the fish, starting in the esophagus and finishing in the pyloric sphincter (along with the food contained inside) and then it was introduced into a test tube (to 0.1 mL) filled with water. The stomach was subsequently emptied entirely and again introduced into a test tube filled with water. A subtraction (full - empty stomach) was performed in order to find the real capacity of the stomach, taking both volumes, according to Yañez-Arancibia (1975). We define in this paper, real maximum stomach capacity as the maximum volume of a fish to ingest food until it stops eating; in other words, what the fish wants or can ingest.

After those measurements, the volume of the same stomachs was measured according to Jobling *et al.* (1977). Shortly, taking the emptied stomachs, a string was tied around the pyloric sphincter, and the esophagus was fastened to a burette. Stomach volume (to the nearest 0.1 mL) was estimated as the volume of water required to expand the stomach with a pressure head of a 50 cm column of water. Once volume capacities had been measured, the stomach and stomach contents were weighed to determine ratio stomach volume/stomach weight.

Statistical analyses

Values were displayed as mean \pm SD. Statistical analyses were performed using Minitab version 17.0 for Windows, and a Student's *t*-test was used to test possible differences among parameters measured associated to assay method ($P < 0.05$). Regression analysis was used to test for possible relationships

between stomach volume and other measured variables of individual fish (fish weight, stomach and stomach contents weight). Therefore, a potential regression model was proposed according to higher R² indicative of the best curve adjustment.

RESULTS

Mean total weight and mean standard length of fish are displayed (Table 1). The average stomach volume using Jobling's method was significantly ($P < 0.001$) higher than values obtained by the Archimedes' principle method (Table 1). Consequently, the ratio stomach volume (mL)/stomach weight (g) was significantly ($P < 0.001$) higher when using Jobling's method than when measured using the Archimedes' principle method (Table 1).

Constants, potential models and determination coefficients according to relationships and methods are presented (Fig. 1). A good positive correlation between stomach volume and fish weight was observed in both the Archimedes' principle and Jobling methods, with the R² values of 0.68 and 0.56, respectively (Fig. 1a).

The same tendency was found in the correlation between stomach volume and stomach content, with the Archimedes' principle method being higher than the Jobling's method (R² values of 0.95 and 0.68, respectively; Fig. 1b). Likewise, a good correlation was obtained between stomach volume and stomach weight, where the R² values in the Jobling's method were slightly higher than those of the Archimedes' principle method (Fig. 1c).

The behaviour of the curves created by plotting the points (X, Y) of the various relationships is as follows: with the Jobling's method, the intersection of the curve with the Y-axis (0.0499, 4.3092 and 3.7867 in Figures 1a-1c, respectively) is higher in each case than with the Archimedes' principle method (0.0007, 1.4467 and 1.6138 in Figures 1a-1c, respectively). The slope values obtained with the Jobling's method (1.059, 0.6439 and 0.7575 in Figures 1a-1c, respectively) were lower than with the Archimedes' principle method (1.829, 1.827 and 1.1143 in Figures 1a-1c, respectively), in each case producing a lower curve position within the coordinate planes.

DISCUSSION

According to the present experiment, the Archimedes' principle method is more effective and provides greater data precision in the measurement of real maximum stomach volume in fish, based on higher R² (means best curve adjustment) compared to results obtained by

Table 1. Biometric and stomach parameters in juveniles of rainbow trout (*Oncorhynchus mykiss*) fed until satiation. Values are means ± SD, n = 12. Different superscript letters in the same raw indicate significant differences between assay methods ($P < 0.05$).

Measure methods	
Fish	
Total weight (g)	147.08 ± 17.73
Standard length (cm)	24.67 ± 1.03
Stomach	
Weight (g)	3.55 ± 0.63
Content (g)	3.66 ± 0.77
	Archimedes Jobling
Volume (mL)	6.74 ± 1.62 ^a 9.93 ± 1.70 ^b
Volume (mL)/weight (g)	1.9 ± 0.38 ^a 2.82 ± 0.36 ^b

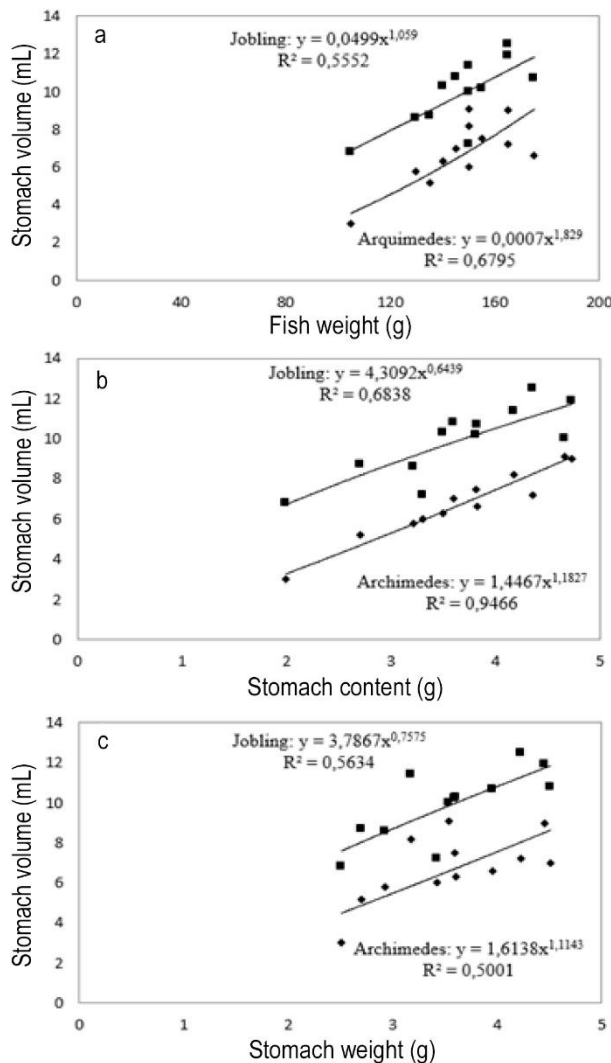


Figure 1. a) The relationship between stomach volume and fish weight, b) stomach content and c) stomach weight rainbow trout (*Oncorhynchus mykiss*) fed until satiation and following two assay methods. Each point represents an individual fish. Jobling method (■, n = 12), and Archimedes method (◆, n = 12).

Jobling method. The following aspects demonstrate the method's superiority: 1) it yields the maximum real value of stomach expansion in a situation where a fish wants to ingest as much as it can, 2) the measurement process is simple and quick, and 3) relationships with stomach volume observed when using the Archimedes' principle method present values that are optimally adjusted and have greater certainty than values obtained using the Jobling' method.

The higher stomach volume values obtained using the Jobling's method can be attributed to the forced expansion of the stomach, owing to the constant hydraulic pressure head of a 50 cm column of water applied by liquid entering the stomach and resulting in a higher curve position corresponding higher values of stomach volume (depending variable) for each independent variables (fish weight, stomach content, stomach weight) which is not the case with the Archimedes' principle method. Thus, the same explanation can be applied to the ratio of volume to weight: if the same weight of the stomach divides both measured volumes, the Jobling's method will yield a higher value.

According to the relationship between stomach volume and stomach content, the Archimedes' principle method presented a higher coefficient of determination than the Jobling's method; this could be because the first method considers the real maximum volume of the stomach, while the second considers the maximum forced expanded stomach volume. Also, Pirhonen & Koskela (2005), evaluating the same relationship in rainbow trout (using the Jobling's method) and experimenting with different feeding regimes, obtained an R² value of 0.75, like that registered in the present experiment. By another hand, Känkänen & Pirhonen (2009), evaluated different feeding regimes in whitefish *Coregonus lavaretus* (Linnaeus, 1758) displaying an R² value of 0.964, between stomach volume and stomach content with the Jobling's method higher than that

obtained in the present work. However, this difference is like due to the use of a different species as test subjects.

Furthermore, the relationship between stomach volume and fish weight was also stronger using the Archimedes' principle method. Several differing relationships have been reported between these variables, although differences could be the result of either fish physiology or method used (as the Jobling's method is not a direct measurement). Ruohonen & Grove (1996), testing different diets in *O. mykiss* and employing the Jobling's method, obtained an R^2 value of 0.49, this is a low value, and like results obtained by Pirhonen & Koskela (2005) with the same species and method. Burley & Vigg (1989), with coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) and northern squawfish *Ptychocheilus oregonensis* (Richardson, 1836), using their method based on a maximum expansion of the stomach which consisted of filling the stomach with air under pressure provided by a cylinder of gas. The stomach was inside of a chamber filled with water, where the displacement of the water was evaluated as it filled the stomach with air. These authors obtained an R^2 value of 0.70 in an exponential regression. In other fish such as dab *Limanda limanda* (Linnaeus, 1758), R^2 value was 0.974 (Jobling *et al.*, 1977). As we can observe there are also good positive values in studies using Jobling's method, but the main point that we try to suggest and evaluate is the simplicity of Archimedes' principle method to do this measurement, which makes it more suitable.

The reason the potential model was chosen for the present experiment was that it exhibits a better adaptation to plotted point values (X,Y), because fish weight, stomach weight, and stomach content cease to increase at a certain point in the fish life cycle, and this could result in an asymptotic stomach volume. The linear model would present a different situation: an infinite increase projection between the X and Y axes.

Because of this study, Archimedes' principle method is recommended to be applied in Aquaculture fish feeding research, due to its simplicity nature and accurately, which make more accessible the labor and time.

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