Indirect estimation of stomach volume of rainbow trout
*Oncorhynchus mykiss* (Walbaum)

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Abstract

The main hypothesis of this study was that if stomach volume is correlated with food intake it can be estimated without laborious and destructive direct measurement. Rainbow trout, *Oncorhynchus mykiss* (Walbaum), ca. 500–1300 g, were starved for 1, 4, 8 or 16 days at 15 °C after which they were fed in excess with dry pellets containing known amounts of X-ray-dense markers. Immediately after feeding the fish were killed, X rayed and weighed. Then the stomach was dissected, its contents removed and weighed, and stomach volume was measured. X-ray plates were developed and feed intake was estimated based on the amount of marker. All measured variables correlated positively with stomach volume. The best fit for linear regression models was obtained for fish starved for 4 days, where stomach content (dry mass) explained 94%, food intake (based on X-ray measurement) 77% and fish mass 62% of the variation in stomach volume. However, as stomach content measurement can be a lethal, or at least very stressful, event for the fish, the accuracy of food intake measurement (X-ray) could be increased using multiple regression. In multiple linear regressions, \( R^2 \)-values varied between 0.79 (16-day starvation) and 0.91 (1-day starvation) with food intake and fish mass as explanatory variables for stomach volume. These results indicate that the stomach volume in rainbow trout can be estimated satisfactorily using indirect methods, which are not detrimental to the fish, although feeding history may affect the accuracy of the estimates.

Keywords: X-ray, non-lethal, feed intake, salmonids, stomach capacity, stomach water content

Introduction

In many aquacultural experiments, especially related to feeding, estimates of stomach volume, preferably without laborious direct measurement would allow more meaningful conclusions. For example, some experimental data have suggested that increased meal size was accompanied by increase in stomach volume, but proof was lacking because stomach volume was not measured (Pirhonen & Forsman 1998; Ogata & Shearer 2000). Knowledge of stomach capacity could help to explain differences in food intake and food processing abilities between individuals. It would usually be desirable to measure stomach volume without killing the fish, firstly, for animal welfare reasons and secondly, because possible ontogenetic changes or changes because of feed manipulation could be measured repeatedly in the same individuals.

Stomach volume can be measured directly by dissecting the stomach and filling it with a known volume of air (Burley & Vigg 1989) or water (Jobling, Gwyther & Grove 1977). However, such methods are time consuming and large sample sizes cannot easily be handled. Jobling and colleagues (1977) and Jobling (1980) have shown with dab, *Limanda limanda* (L.), and plaice, *Pleuronectes platessa* L., respectively, that there is a strong linear relationship between fish mass and stomach volume, which would allow stomach volume to be obtained from an established equation simply by weighing the fish. However, with other species the relationship between fish mass and stomach volume does not appear to be linear and the correlation between the two variables is only modest or poor (Burley & Vigg 1989; Grove & Holmgren 1992; Ruohonen & Grove 1996). Koskela, Jobling and
Pirhonen (1997) estimated stomach volume indirectly by starving whitefish, Coregonus lavaretus (L.), for 4 days and then feeding them to satiation with X-ray-dense marker feed; feed intake by each individual was then estimated from X-ray plates. Feed intake after starvation was assumed to correlate with stomach volume, but no direct evidence of such a relationship was presented.

Because previous experiments indicate that fish mass is not a good indicator of stomach volume in rainbow trout, Oncorhynchus mykiss (Walbaum), the aim of the present experiment was to evaluate if stomach volume could be estimated indirectly in rainbow trout by correlating stomach volume with food intake or actual stomach content. Special emphasis was given to methods which could be used to estimate stomach volume without killing the fish. We also tested if starvation time before measurements would affect the accuracy of stomach volume estimates.

Materials and methods

The experiment was carried out at Laukaa Aquaculture Station of the Finnish Game and Fisheries Research Institute during October 2003. The fish (ca. 500–1300 g) were all female offspring from the rainbow trout of the national breeding programme. Prior to the start of the experiments the fish were held in cultivation tanks and fed with commercial dry feed. For the experiments, 10 fish were transferred to each of the 1.7 m², flow-through, circular, experimental tanks 2 weeks before the start of the first measurement. Water temperature was adjusted to 15 °C. The fish were fed by belt feeders throughout the day (2 h on) with extruded dry diet (protein 43.8%, fat 22.0%, energy 24.1 MJ kg⁻¹) in excess. For the feed intake measurement, feed was similar except that X-ray-dense lead glass beads (ballotinis, size 9) were mixed with the ingredients before compressing them into pellets in an extruder. Samples of marker food were X-rayed and a relationship between number of beads (Y) and amount of food (V) was established (Y = 0.1433 × V, P < 0.001, R² = 0.966). Photoperiod was 24 h:0 D and light was provided by fluorescent tubes.

There were three replicate tanks for each treatment (i.e. starvation period before measurements). For the measurements, the fish were first starved for 1, 4, 8 or 16 days and then fed the X-ray-dense diet for 45 min in excess with belt feeders. At the end of the 45 min feeding period, the fish were also offered food by hand to ensure that all individuals were satiated. Then five fish were netted from each tank killed by a sharp blow on the head. X-rayed (Kodak X-OMAT MA film, Bennett HFQ 3000P X-ray machine using 70 kV, 200 mA and 0.2 s for exposure) and weighed (to 1 g). The body cavity of each fish was cut open and the stomach and intestines were carefully removed. Stomach content was pressed out using fingers into a tared cup and weighed (to 0.01 g). A string was tied around the pyloric sphincter and the oesophagus was tied to a burette. The volume of the stomach was measured as the volume of water required to dilate (to 0.5 mL) the stomach with a pressure head of 50 cm water (Jobling et al. 1977). Dry and wet masses were calculated both from the stomach contents and from a sample of pellets (water content in feed 4.6%) after freeze drying them to constant mass, and dry masses were used in the calculations.

For the calculations of treatment effects, the data from the three replicate tanks were pooled because there were no significant differences between tank means as estimated with ANOVA, except for a slight difference (P = 0.047) in the amount of water in the stomachs of fish starved for 4 days. Linear regression analyses between two or three variables were performed using SPSS statistical software and P = 0.05 was taken as the level of significance. The results were calculated for the pooled data and also for each treatment separately. To evaluate the relationship between fish weight and stomach volume, the data were fitted to linear and various non-linear (logarithmic, quadratic, cubic, power and exponential) models. However, when the models were calculated for each treatment (n = 15) none of the non-linear models appeared to be superior to the linear model (as judged by the R²-value).

Results

If all the data were pooled, the best (in terms of R²-value) estimate of stomach volume with one explanatory factor was achieved by measuring stomach dry mass content (R² = 0.75, Fig. 1b) while the poorest estimate of stomach volume was obtained by measuring fish mass (R² = 0.28, Fig. 1c). Stomach volume could be estimated more accurately using a regression model with two explanatory factors. For example, when using both food intake (based on X-ray) and fish mass as variables, the R²-value for the pooled data was 0.83 or using stomach content and
fish mass as explanatory factors the $R^2$-value was 0.86 (Table 1). The explanatory variables used in the multiple regressions were not significantly correlated with each other ($P > 0.05$).

When the estimation of stomach volume was tested for each starvation period separately, wide variation in $R^2$-values between treatments was apparent when fish mass is used as an explanatory factor (from 0.09 to 0.62, Table 2) and for the fish starved for 1 or 2 days the relationship between fish mass and stomach volume was not significant. The variation in $R^2$-values between stomach volume and food intake (X-ray food), or between mass of stomach content between treatments was much smaller (0.67–0.77 and 0.70–0.94 respectively; Table 2). Irrespective of the explanatory factor for the stomach volume, the best fit for the regression model was always in the group starved for 4 days before measurements (Table 2). In that group stomach content dry mass explained 94% of the variation in stomach volume, and food intake (based on X-ray) explained 77% of that variation (Table 2). Somewhat higher $R^2$-values were achieved when two factors were used to explain variation in stomach volume in different treatments. When food intake and fish mass were used to explain stomach volume, $R^2$-values ranged between 0.79 (16 days) and 0.91 (1 day) in different treatments (Table 1). Slightly more accurate estimates were achieved (0.83–0.95) using stomach content and fish mass as explaining variables for stomach volume (Table 1).

Food intake based on X-ray measurement was found to correlate well with stomach content, irrespective of treatment (Fig. 2a). Also the amount of water in the stomach was highly correlated ($R^2 = 0.97$) with the dry mass of stomach contents, even if data from different treatments were pooled (Fig. 2b). The average ± SD water percentage in stomach contents was 42.2 ± 3.1%.

**Discussion**

Interestingly, very few data are available concerning the possible relationship between stomach volume

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**Table 1** Parameters to estimate stomach volume with food intake (g; X-ray method) and fish mass (g) or with stomach content (g; dry mass) and fish mass in ca. 500–1300 g rainbow trout *Oncorhynchus mykiss* after period of starvation

<table>
<thead>
<tr>
<th>Stomach volume (ml)</th>
<th>Food intake (x) and fish mass (z)</th>
<th>Stomach content (x) and fish mass (z)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intake (g); x-ray</strong></td>
<td>$R^2$</td>
<td>SEe</td>
</tr>
<tr>
<td>1</td>
<td>0.91</td>
<td>4.14</td>
</tr>
<tr>
<td>4</td>
<td>0.86</td>
<td>5.53</td>
</tr>
<tr>
<td>8</td>
<td>0.86</td>
<td>4.60</td>
</tr>
<tr>
<td>16</td>
<td>0.79</td>
<td>5.64</td>
</tr>
<tr>
<td>Pooled data</td>
<td>0.83</td>
<td>5.35</td>
</tr>
</tbody>
</table>

The model is volume = $a + bx + cz$, where $a$, $b$, and $c$ are constants, $x$ is food intake (g) or stomach content (g) and $z$ is fish mass (g). $P$ values are given for constants $b$ and $c$.

SEe: standard error of estimate; NS, not significant. $P > 0.05$; $n = 15$ in each fasting period, 60 for pooled data.
and voluntarily ingested ration size. Such a relationship has been suggested (Koskela et al. 1997; Pirhonen & Forsman 1998; Ogata & Shearer 2000) but it appears that only in one study has such a relationship been measured directly (Nikki, Pirhonen, Jobling & Karjalainen 2004). The results of the present study indicate that stomach volume and the amount of ingested food, as estimated using the X-ray method, have a significant positive relationship in rainbow trout irrespective of the length of the preceding starvation period. In other words, by measuring food intake by an individual fish it is also possible to get an estimate of the stomach volume in absolute terms. In accordance with this is the finding of Nikki and colleagues (2004) who reported a positive relationship between relative feed intake (g kg\(^{-1}\)) and relative stomach volume (mL kg\(^{-1}\)) in rainbow trout. However, in that experiment the relationship was not as good as in the present study, possibly because Nikki and colleagues (2004) reported feed intake over a period of 3 weeks, while in the present experiment food intake was measured only once. It appears that the length of the starvation period does not greatly influence the accuracy of the model (food intake explaining stomach volume), as shown by the \(R^2\)-values ranging from 0.67 to 0.77.

Stomach volume could be estimated with even higher accuracy in rainbow trout by measuring stomach content directly soon after the fish had been fed. Stomach content measurements in this study were made after killing the fish, by pressing the stomach content out with fingers. This method can be used for stomach volume measurements only on those occasions when the fish can be killed or when it is not possible to use an X-ray method. Stomach content could also be measured without killing the fish by flushing the stomach (Jobling, Covès, Damsgård, Kristiansen, Koskela, Petursdottir, Kadri & Gudmundsson 2001), but such a procedure is likely very stressful for the fish and is also quite laborious.

Table 2 Parameters to estimate stomach volume with food intake (based on X-ray method), stomach content (dry mass) or fish mass in ca. 500–1300 g rainbow trout *Oncorhynchus mykiss* after a period of starvation

<table>
<thead>
<tr>
<th>Starvation period (days)</th>
<th>Intake (g): X-ray</th>
<th>Stomach content (g)</th>
<th>Fish mass (g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(R^2)</td>
<td>SEe</td>
<td>a</td>
</tr>
<tr>
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<td>0.71</td>
<td>7.26</td>
<td>17.2</td>
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<tr>
<td>4</td>
<td>0.77</td>
<td>6.74</td>
<td>13.4</td>
</tr>
<tr>
<td>8</td>
<td>0.67</td>
<td>6.74</td>
<td>11.5</td>
</tr>
<tr>
<td>16</td>
<td>0.73</td>
<td>6.11</td>
<td>7.79</td>
</tr>
</tbody>
</table>

(a) stomach content = 1.16x - 0.91
\(R^2 = 0.88, P < 0.001\)
S.E. of estimate = 2.03

(b) stomach content = 1.30x - 0.56
\(R^2 = 0.97, P < 0.001\)
S.E. of estimate = 1.10

Figure 2 Relationship between stomach content dry mass and (a) food intake (based on X-ray method), (b) amount of water in stomach in rainbow trout *Oncorhynchus mykiss* (ca. 500–1300 g). Data points represent individuals starved for different periods before measurements: ◊, 1 day; △, 4 days; ○, 8 days and ×, 16 days of starvation.
indicates that, irrespective of the time elapsed (1–16 days) from the last meal, a rainbow trout is able to fill its stomach with new food. This suggestion is supported by the observations of Ruohonen, Grove and McIlroy (1997) that at least 98% of a satiation meal will leave the stomach within 24 h.

Even if fish mass alone was not a reliable parameter for estimating stomach volume, it did increase the accuracy of the stomach volume estimate when combined with food intake or stomach content measurements. Thus, using two explanatory factors it was possible to obtain a good estimate of the stomach volume by measurements which can be done without sacrificing the fish. The poorest fit (but still explaining ca. 80% of the variation) for the multiple regression equations of Table 1 was in both cases with the fish starved for 16 days. The lower R²-value in this group compared with other groups may indicate some changes in the digestive tract after relatively long starvation. Such changes can be expected because rainbow trout have been found not to maintain excess stomach capacity (Ruohonen & Grove 1996). However, possible changes in the stomach capacity in respect to starvation time were not evident when stomach volume was estimated from the simple regression models.

Results from this experiment indicate that ingested food absorbs water at the same rate in all treatment groups and that there is a linear relationship between water content of food in the stomach and dry mass of stomach contents. This finding indicates that rainbow trout can adjust their stomach water content very accurately, irrespective of the amount of food ingested. Rainbow trout have been reported to be able to moisturize ingested dry feed by stomach secretions, by swallowing water with the food and by drinking (Ruohonen et al., Kristiansen & Rankin 2001). The average stomach water content in this study was 42.2%, while 46.7% has been observed in 1.5 kg rainbow trout (Ruohonen et al., 1997) 3 h after feeding and 52% in 20 g rainbow trout (Kristiansen & Rankin 2001).

Acknowledgments

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References


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