



Effects of meal and body sizes on gastric evacuation rate in brook trout *Salvelinus fontinalis* (Mitchill, 1814) fed commercial pellets in group feeding

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ABSTRACT

The effects of meal and body sizes on gastric evacuation (GE) of brook trout *Salvelinus fontinalis* (Mitchill, 1814) were determined following group feeding instead of feeding individually maintained fish. The GE experiments included small (ranging from 64.75 to 69.72 g) and large fish (ranging from 161.59 to 170.95 g). Fish in each size group was fed with three different meal sizes under similar conditions. The stomach contents were then recovered at predetermined postprandial times by serial slaughtering. The square root model adequately described the course of GE in *S. fontinalis* independent of meal size. The estimates of mass and length exponent obtained from *S. fontinalis* fed individually are in line with the present estimates. The result of this study would facilitate the planning and management of feeding regimes for *S. fontinalis* to minimise food waste and optimise growth.

Keywords: Fish size, Group feeding, Meal size, Salmonidae, Square root model

Introduction

Gastric evacuation (GE) experiments have been used to quantify the daily ration of fish fed either natural prey (Elliott and Persson, 1978; Bromley, 1994; Seyhan, 1994; Seyhan and Grove, 1998; Andersen, 2001) or formulated diet (Windell *et al.*, 1972; Talbot and Higgins, 1983; Riche *et al.*, 2004; Bascinar *et al.*, 2016; Khan *et al.*, 2016). GE experiments are often performed by maintaining individual fish which is considered to be a standard practice (Jobling, 1981; Persson, 1981; Bromley, 1987; Andersen, 1999; Temming and Herrmann, 2001); however, some of the fish species such as Atlantic herring *Clupea harengus*, Atlantic mackerel *Scomber scombrus*, Sprat *Sprattus sprattus* and sardines *Sardinops sagax* (Jenyns), cannot be maintained individually. Therefore, GE experiments in such species may only be performed using groups of fishes (Bernreuther *et al.*, 2009).

GE experiments in brook trout *Salvelinus fontinalis* (Mitchill, 1814) based on fish fed individually have been conducted to determine the effects of body and meal sizes on GE rate (Khan *et al.*, 2016). GE experiments in *S. fontinalis* fed in group might be interesting to compare the results with that of individually maintained experiments. In this study, small and large size groups of *S. fontinalis* were fed with three different ranges of meal sizes composed of commercial pellets similar to Khan

et al. (2016). The main objectives of this study were to determine the effects of meal and body sizes on GE rate of *S. fontinalis* under group feeding.

Materials and methods

Fish and experimental conditions

Small and large sized fish of *S. fontinalis* were procured from the Sürmene Faculty of Marine Sciences, Trabzon (Table 1). They were stocked in six separate holding tanks (three tanks for each size fish) which were facilitated by a recirculating water system where the oxygen saturation was ensured by means of continuous air bubbling. The fish were fed twice daily with high grade commercial pellets provided by Skretting Aquaculture (protein 44%; fat 21%; fibre 4%; ash 9%) for one month before conducting the experiments. The same batch of commercial pellets was used in all GE experiments.

GE experiments

All GE experiments were performed at the same time under similar environmental conditions. The experimental fish were deprived of food for 72 h to ensure complete emptying of the stomachs before providing the experimental diet. Groups of large and small size fish (exp. 1 and 4 in Table 1) were fed from a known amount of food to the apparent satiation. The uneaten pellets were

retrieved from tanks using a water pipe and the mass was determined after drying in Ecocell drying oven at 60°C until constant weight and the same were subtracted from the initial known amount of food. The other experimental groups (exp. 2, 3 of small fish; 5, 6 of large fish) were fed with two different minimum satiation sizes of meal (Table 1). All the experimental fish were handfed.

At the end of feeding session (after 15 min), four fish were carefully removed from each tank to estimate the current stomach mass S_t (g) for t_0 and afterwards the stomach contents of four fish (in most of the

experiments) (Fig. 1) were recovered at predetermined postprandial times by serial slaughtering until the first empty stomach was observed. The stomach contents (S_t) were dried to constant weight. The fish were killed by an overdose of benzocaine (150-200 mg l⁻¹).

GE data modelling

Linear, square root and exponential models were applied to the GE data to determine the best fit model for GE of *S. fontinalis* (He and Wurtsbaugh, 1993; Paakkonen and Marjomaki, 1997; Sweka *et al.*, 2004).

Table 1. Basic experimental data (mean±S.E.) from gastric evacuation experiments in brook trout *Salvelinus fontinalis* fed on commercial pellets

Exp. no.	Body mass (g)	Total length (cm)	Meal size (g)*	No. of observations (n)
1	64.75 ± 0.76	18.35 ± 0.09	1.36 ± 0.10	70
2	65.17 ± 1.39	18.47 ± 0.12	0.92 ± 0.09	49
3	69.72 ± 1.48	19.16 ± 0.14	0.56 ± 0.12	49
4	167.43 ± 1.49	24.35 ± 0.19	3.31 ± 0.07	65
5	170.95 ± 1.98	24.57 ± 0.11	2.23 ± 0.14	43
6	161.59 ± 3.57	24.38 ± 0.15	1.23 ± 0.11	44

*Stomach content recovered at time 0

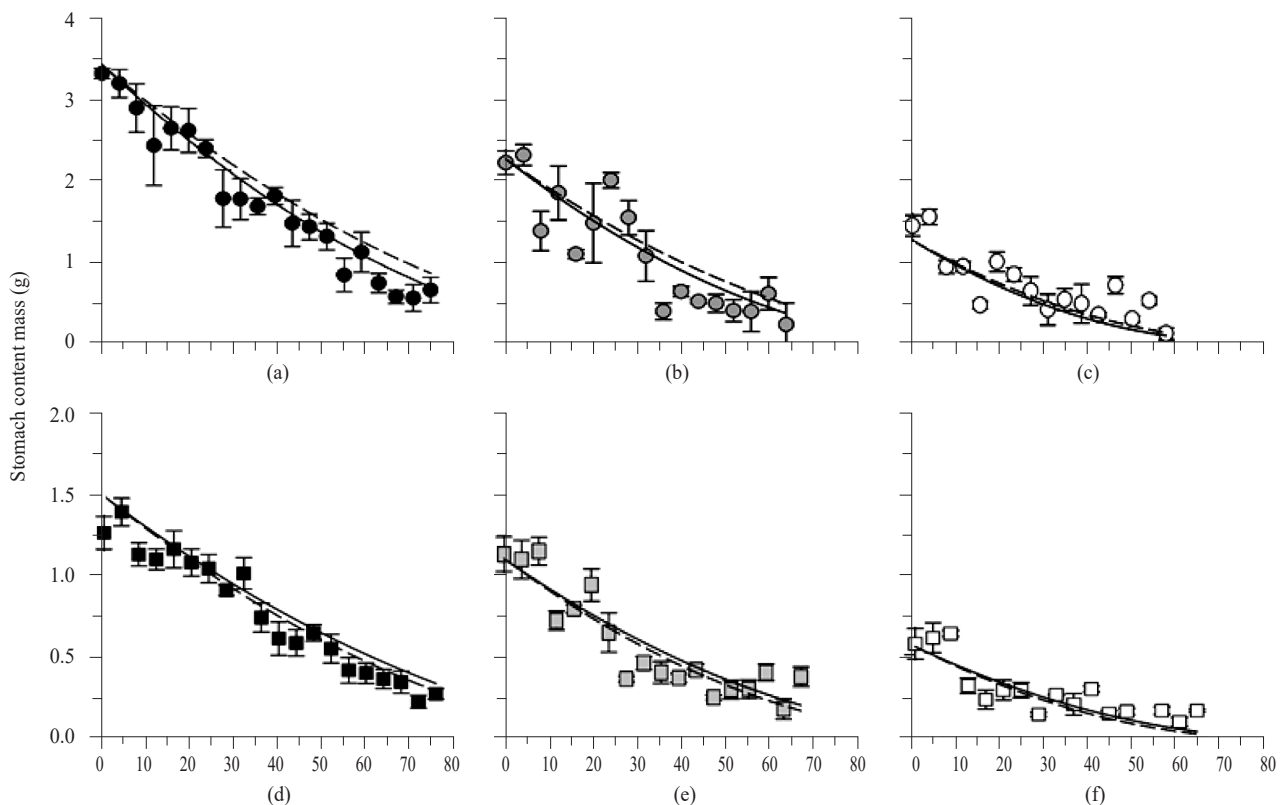


Fig. 1. Gastric evacuation of brook trout *Salvelinus fontinalis* fed three different meals of 3.31 g (●), 2.23 g (◐) and 1.23 g (○) to large size and 1.36 g (■), 0.92 g (◑) and 0.56 g (◒) to small size fish. The curves were provided with $\frac{dS_t}{dt} = -0.0016W^{0.553}\sqrt{S_t}$ (—) and $\frac{dS_t}{dt} = -0.0004L^{1.29}\sqrt{S_t}$ (---). Error bars indicate S.E.

$$\text{Linear model } \frac{dS_t}{dt} = -\rho t \text{ integrated: } S_t = a - \rho t \quad (1)$$

$$\text{Square root model } \frac{dS_t}{dt} = -\rho \sqrt{t} \text{ integrated: } \sqrt{S_t} = \sqrt{a} - 0.5 \rho t \quad (2)$$

$$\text{Exponential model } \frac{dS_t}{dt} = -\rho t \text{ integrated: } S_t = a e^{-\rho t} \Rightarrow \ln(S_t) = \ln(a) - \rho t \quad (3)$$

where S_t is the recovered stomach content mass (g), t is the time after ingestion of the meal (h), the parameter a is the intersection of the regression line with Y-axis and represent some sort of the mean ingested meal size S_0 and ρ is the rate parameter (g h^{-1}). This might be an optimal way of parameterising the evacuation rate when there is no information available about the ingested meal size of the individual fish.

Model selection

The best fit model was selected by comparing the values of the adjusted r^2 and the residual sum of squares (RSS) and the one consistently providing a higher value of adjusted r^2 and the lowest value of RSS was determined as a best fit model to the data.

Effects of body size

The square root model adequately described the GE of *S. fontinalis* independently of meal size since it consistently provided a lower value of RSS and higher value of adjusted r^2 . After determining the best fit model to the GE data, the effect of body size on GE rate was then analysed using simple power functions.

The rate parameter ρ in equation (2) was expanded to account for the effect of fish mass (g)

$$\sqrt{St} = \sqrt{a} - 0.5 \rho_W W^\beta t + \varepsilon \quad (4a)$$

where β is the mass exponent to be estimated.

For the purpose of comparison with literature values, the length exponent was also estimated as well in the present study:

$$\sqrt{S_t} = \sqrt{a} - 0.5 \rho_{TL} L^\lambda t + \varepsilon \quad (4b)$$

where λ is the length exponent (total length, cm) to be estimated. The parameter β and λ were estimated using equation (4a) and (4b) to all data combined. ε is an error term.

The parameters of equation (1), (2) and (3) were estimated using PROC GLM while the parameters of the equations (4a) and (4b) were estimated using NLIN procedure (SAS, 9.04).

Results

A total of six GE experiments were performed (exp. 1-3 for small fish; exp. 4-6 for large fish (Table 1) on *S. fontinalis* by feeding a range of different meal sizes. In these experiments a total of 320 *S. fontinalis* (168 small fish, 152 large fish) were used to recover stomach contents at predetermined postprandial times t after ingestion of the meal. Out of these 320 fish, none of the experimental fish was found with empty stomach during the recovery of stomach contents which strongly indicates that all experimental fish were facing very low stress under experimental conditions.

GE pattern and effect of meal size

The evacuation of meal from the stomach of *S. fontinalis* was adequately described by square root model independent of meal size (Table 2). The difference between the adequacy of square root and linear model to describe the course of evacuation in *S. fontinalis* was smaller than exponential model. The exponential model poorly described the GE of *S. fontinalis* in all experiments.

Effect of body size

The effect of body size was estimated using both fish mass as well fish length separately (Table 3). The estimated mass exponent β was 0.55, whereas the length exponent λ was 1.29. The effect of body size on GE rate of *S. fontinalis* could be summarised by:

$$\frac{dS_t}{dt} = -0.0016W^{0.553} \sqrt{S_t} \text{ (g h}^{-1}\text{)} \quad (5a)$$

$$\frac{dS_t}{dt} = -0.0004TL^{1.29} \sqrt{S_t} \text{ (g h}^{-1}\text{)} \quad (5b)$$

where S_t is current stomach mass (g), W is body weight (g), L total length (cm) and t is time (h).

Table 2. Estimates (\pm S.E.) of the intercept a and rate parameter ρ in the square root model: $\sqrt{S_t} = \sqrt{a} - 0.5\rho t$, linear model: $S_t = a - \rho t$ and exponential model: $\ln(S_t) = \ln(a) - \rho t$ from gastric evacuation data of brook trout *Salvelinus fontinalis* fed meals of commercial pellets

Exp. no.	Square root model				Linear model				Exponential model			
	a	$\rho (\times 10^{-2})$	RSS	Adj. r^2	a	$\rho (\times 10^{-2})$	RSS	Adj. r^2	a	$\rho (\times 10^{-2})$	RSS	Adj. r^2
1	1.22 \pm 0.02	-0.93 \pm 0.05	0.502	0.857	1.41 \pm 0.03	-1.61 \pm 0.07	1.286	0.875	0.50 \pm 0.06	-2.26 \pm 0.13	4.237	0.807
2	0.95 \pm 0.03	-0.87 \pm 0.08	0.574	0.724	0.91 \pm 0.05	-1.28 \pm 0.12	1.330	0.712	-0.08 \pm 0.09	-2.51 \pm 0.24	5.575	0.691
3	0.74 \pm 0.03	-0.71 \pm 0.08	0.517	0.639	0.56 \pm 0.03	-0.83 \pm 0.09	0.821	0.605	-0.60 \pm 0.09	-2.59 \pm 0.28	6.808	0.643
4	1.85 \pm 0.04	-1.52 \pm 0.08	1.530	0.838	3.22 \pm 0.08	-3.82 \pm 0.19	8.398	0.858	1.35 \pm 0.08	-2.58 \pm 0.18	7.396	0.758
5	1.51 \pm 0.06	-1.73 \pm 0.16	1.620	0.721	2.21 \pm 0.12	-3.47 \pm 0.31	6.892	0.742	0.90 \pm 0.13	-3.39 \pm 0.37	8.944	0.666
6	1.04 \pm 0.05	-1.06 \pm 0.15	1.319	0.551	1.08 \pm 0.08	-1.64 \pm 0.22	3.159	0.551	0.14 \pm 0.16	-3.15 \pm 0.49	15.337	0.482

Table 3. Summary statistics for parameter estimation of the expanded empirical model of gastric evacuation equations (3a) $\sqrt{S_t} = \sqrt{a} - 0.5\rho_w W^{\beta} t + \epsilon$ and equation (3b) $\sqrt{S_t} = \sqrt{a} - 0.5\rho_L L^{\lambda} t + \epsilon$ for combined data on gastric evacuation of meals of commercial pellets fed to brook trout *Salvelinus fontinalis*

Parameter	Estimate	95% CL		Adj. r^2	Obs. (n)
		Lower	Upper		
ρ_w	0.0016	0.0004	0.0019	0.722	321
B	0.553	0.3485	0.6337		321
ρ_{TL}	0.0004	0.0003	0.0009	0.301	321
λ	1.2901	0.9217	1.4340		321

Discussion

GE pattern in *S. fontinalis*

The square root model adequately described the course of evacuation in *S. fontinalis* which is in accordance with the previously published data (Bascinar *et al.*, 2016; Khan *et al.*, 2016). Moreover, the course of meal evacuation in *S. fontinalis* fry has also been found to be best described by square root model (Seyhan, K., unpublished data). Sweka *et al.* (2004) examined the effect of temperature on GE rate of *S. fontinalis* at five temperatures (*i.e.* 4.3, 9.1, 12.1, 15.6 and 17.0°C) and observed that square root model could adequately describe the GE of *S. fontinalis* at 12.1 and 17.0°C whereas at other temperatures (4.3, 9.1 and 15.6), linear model was found to best fit to the GE data. They fed the fish on a mixed diet of larval fly *Sarcophaga bullata* and larval beetle *Zophobus morio* with a standard meal size. The nature of diet has been found to affect the shape of evacuation (Bromley, 1994) and the deviation from the square root model in the study by Sweka *et al.* (2004) for individual meals may reflect prey heterogeneity (robust exoskeleton of the prey *i.e.*, insect). According to Khan *et al.* (2016) such deviation from the square root function could be removed using combined data on different meal sizes such as in Andersen (2001) where the square root model adequately describes the GE of shrimp (type of prey) independently of meal size; though for individual meals, the curve is more linear. The general applicability of the square root model properly describes the evacuation of different fish and crustacean prey from the stomachs of whiting *Merlangius merlangus*, saithe *Pollachius virens* and cod *Gadus morhua*, fed different meal sizes (Andersen, 2001). The square root model has been used to describe the pattern of evacuation in various other fish species such as pikeperch *Stizostedion lucioperca* (Koed, 2001) and coho salmon *Oncorhynchus kisutch* (Andersen and Beyer, 2007); different gadoids (Jones, 1974; Temming and Herrmann, 2003) and turbot *Scophthalmus maximus* (Grove *et al.*, 1985).

Effect of meal size

The estimated rate parameters obtained from different meal sizes for large and small size groups of *S. fontinalis* do not seem to differ greatly within exp. 1-3 and 4-6 which suggests that the square root model independently of meal size described the course of evacuation of *S. fontinalis* (Fig. 1). In contrast to individual fish experiment, group feeding data have some variation that could result in such minor variation in rate parameters (Table 2). Khan *et al.* (2016) tested the effect of meal sizes in small and large size *S. fontinalis* following individual fish experiments and reported square root to describe the course of GE independent of meal size.

Effect of body size

The estimated value of length and mass exponents were within the range of previously reported values [($\beta = 0.40 \pm 0.05$; $\lambda = 1.31 \pm 0.17$ ($\pm 95\%$ c.i.)] by Khan *et al.* (2016) for *S. fontinalis*. The estimated length exponent also lies within the range of 1.3-1.4 obtained for different gadoids including Atlantic cod (Jones, 1974; dos Santos and Jobling 1992; Andersen, 2001, 2012). Also, the mass exponent reported by these authors was in line with the present one on *S. fontinalis* just like the length exponent. However, some studies have reported a lower value of the length exponent for pike perch (Koed, 2001) and Atlantic cod (dos Santos and Jobling, 1995; Temming and Herrmann, 2003). The lower length exponent was due to the fact that the larger fish were fed with larger size prey containing higher energy (Pedersen and Hislop, 2001). Diet with higher energy has been found to depress the GE rate of fish (Jobling, 1987). Similar to length exponent, a lower value of mass exponent was also reported by Grove *et al.* (1985) for turbot fed pellet feed.

The present study should add a confidence to the belief that the results obtained from individually maintained fish could be applied to group study and *vice versa*. Using simple regression to converted data (for square root and exponential model) might give adequate results for the GE data where the initial meal ingested by each fish is unknown.

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