KARADENİZ TECHNICAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF FISHERIES TECHNOLOGY ENGINEERING

GASTRIC EVACUATION OF FORMULATED FEEDS IN BROOK TROUT

Ph.D. THESIS

Umar KHAN

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STATEMENT OF ETHICS

I declare that, this PhD thesis, I have submitted with the title “Gastric evacuation of formulated feeds in brook trout” has been completed under the guidance of my PhD supervisor Prof. Dr. Kadir SEYHAN. All the data used in this thesis were obtained experimental works done as parts of this thesis in our research labs. All referred information used in the thesis has been indicated in the text and cited in reference list. I have obeyed all research and ethical rules during my research, and I accept all responsibility if proven otherwise. 05/02/2020

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Gastric evacuation rate (GER) can be used to estimate stomach fullness in fish at any postprandial time, to predict return of appetite and optimize feeding regimes. The influences of meal size, fish size L (cm), temperature T (°C), dietary energy density E (kJ g^{-1}) and feed storage conditions (frozen, ambient conditions), on GER of brook trout fed a single meal of commercial pellets were studied. In addition, the effect of a second meal on GER was examined. Small (15 cm) and large (23 cm) fish were fed three meal sizes (close to satiation, 50% and 25%). GE was analyzed using a general power function, and data indicated that the square root function best described GE in brook trout independently of meal size. GER experiments performed at 12.5 °C, 13.2 °C, 15 °C, 16.8 °C, 18.6 °C and 20 °C revealed an exponential increased in GER from 12.5 °C to 18.6 °C and then a sharp decline at 20 °C. The effect of dietary energy density was determined using meals of 12.6 kJ g^{-1}, 17.7 kJ g^{-1}, and 22 kJ g^{-1}. GER increased with decreasing energy density of the meal. Neither feed storage conditions nor the arrival of a second meal influenced GER. The GER model for brook trout fed commercial pellets was $\frac{\mathrm{d}S_t}{\mathrm{d}t} = -0.000860L^{1.31}E^{-0.41}e^{0.08\,T}(1 - e^{1.82(T-20.6\,)})\sqrt{S_t}$ (g h^{-1}), where S_t is current stomach mass (g) and t is time (h). This GER model was used to calculate the amount of food that should be present in the stomach of a brook trout that had been fed on three consecutive days. The predicted values were similar to the observed (r^2 = 0.987). The GER model can be used to predict stomach content in fish that have been fed repeatedly and should have applications in the development of feeding routines for brook trout.

Key Words: Dietary Energy Density, Feeding Regime, Fish Size, General Power Function, Meal Size, Multiple Meals, Optimum Temperature

Doktora Tezi

ÖZET

FORMÜLE YEM İLE BESLENEN KAYNAK ALABALIĞINDA MİDE BOŞALTIMI

Umar KHAN

Karadeniz Teknik Üniversitesi
Fen Bilimleri Enstitüsü
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Danışman: Prof. Dr. Kadir SEYHAN
2020, 42 Sayfa, 12 Sayfa Ek

Balıklarda mide boşaltım oranı (GER) çalışmaları ile yetiştiriciliği yapılan balıkların yem alımının durduğu ve tekrar başladığı andaki mide doluluğu hesaplanarak optimum besleme sıklığı belirlenebilir. Bu çalışmada, ticari pelet yemlerle beslenen kaynak alabalıklarında; yem miktarı, balık boyu, sıcaklık, yemin enerji miktarı, yemin saklama koşulları ve ilave yemleme gibi faktörlerin GER üzerine etkisi belirlenmiştir. Bu amaçla; küçük (15 cm) ve büyük (23 cm) balıklar üç farklı öğün büyüklüğü ile beslenmiştir. Elde edilen verilerle mide boşaltımı basit üstel ilişki ile belirlenmiştir. Ayrıca besin büyüklüğünden bağımsız olarak mide boşaltımını en iyi tanımlayan modelin karekök model olduğu belirlenmiştir. Sıcaklığın etkisinin belirlenmesi amacıyla 12,5 °C, 13,2 °C, 15,0 °C, 16,8 °C, 18,6 °C ve 20,0 °C'de denemeler yapılmış; 12,5 °C-18,6 °C arasında GER'de eksponansiyel bir artış, 20 °C'de ise keskin bir düşüş gözlemnlenmiştir. Kaolin ile seyreltilerek 12,6 kJ g^{-1}, 17,7 kJ g^{-1} ve 22,0 kJ g^{-1} enerji miktarına sahip üç yem formüle edilmiş ve bunların GER üzerine olan etkileri belirlenmiştir. Yemdeki enerji miktarı azaldıkça mide boşaltımı artış göstermiştir. Bununla birlikte, GER üzerine depolama koşullarının ve ilave yemlemenin etkisinin olmadığı tespit edilmiştir. Ticari pelet yemle beslenen balıklarda GER modeli $\frac{\mathrm{d}S_t}{\mathrm{d}t} = -0.000860L^{1.31}E^{-0.41}e^{0.08\,T}(1 - e^{1.82(T-20.6\,)})\sqrt{S_t}$ (gr sa^{-1}) olarak belirlenmiştir. Modelin test edilmesi için üç gün boyunca düzenli olarak beslenen balıkların mide doluluk oranları model ile hesaplanmıştır. Hesaplanan değerlerin gerçek değerlere çok yakın olduğu tespit edilmiştir. Bu model yetiştiricilik faaliyetlerinde beslenme rutinlerinin geliştirilmesinde geniş bir kullanım alanı bulabilecektir.

Anahtar Kelimeler: Diyet Enerji Miktarı, Beslenme Rejimi, Balık Büyüklüğü, Genel Üstel Model, Öğün Büyüklüğü, Çoklu Öğün, Optimum Sıcaklık

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LIST OF ABBREVIATIONS

Symbol	Explanation	Unit
E	Dietary energy density	kJ g^{-1}
expno	Number of GE experiment	
L	Fish total length	cm
predlcm	Total length of brook trout	cm
predw	Weight of brook trout	g
stw	Mass of stomach contents recovered at time t	
sow	Meal size consumed by brook trout	
temp	Temperature	° C
T	Temperature	° C
S_o	Meal size	g
S_t	Stomach contents	g
t	Postprandial time	h
W	Fish weight	g
α	Shape exponent	
λ	Exponent of L	
γ	Exponent of W	
δ	Temperature exponent	° C^{-1}
μ	Coefficient of E	g kJ^{-1}
ρ	Rate parameter constant;	$g^{1-\alpha}$ h^{-1}
ρ_L	Rate parameter constant expanded by a function of L	$g^{1-\alpha}$ $cm^{-\lambda}$ h^{-1}
ρ_{LT}	Rate parameter constant expanded by a function of L and T	$g^{1-\alpha}$ $cm^{-\lambda}$ h^{-1}
ρ_{LE}	Rate parameter constant expanded by a function of L and E	$g^{1-\alpha}$ $cm^{-\lambda}$ h^{-1}
ρ_{LET}	Rate parameter constant expanded by a function of L, E and T	$g^{1-\alpha}$ $cm^{-\lambda}$ h^{-1}

1. GENERAL INTRODUCTION

Information about food intake of predators is needed for estimation of mortality rates of their prey species. Studies on gastric evacuation rate (GER) have been used to estimate rates of food intake in wild fish, which enable quantitative investigation of the trophic links between predators and their prey (Jobling, 1981; Seyhan and Grove, 1998; Andersen, 2001; Mychek-Londer and Bunnell, 2013). Studies on GER have also been used to develop optimal feeding regimes for farmed fish (Grove et al., 1978; Talbot, 1985; Booth et al., 2008), such as by examination of the progress of gastric emptying and the level of stomach fullness at return of appetite (Lee et al., 2000; Riche et al., 2004).

Providing feed to farmed fish at times that coincide with peak appetite may lead to improved growth and feed utilization, and reduce levels of feed waste (Bolliet et al., 2001; Dwyer et al., 2002; Booth et al., 2008). The effects of feeding regime on the production of farmed fish have been examined in many empirical studies (Andrews and Page, 1975; Hogendoorn, 1981; Wang et al., 1998; Xie et al., 2011). Several studies have revealed limits to fish growth with increasing feeding frequency (Jobling, 1983; Tsevis et al., 1992; Dwyer et al., 2002; Tian et al., 2015), and this may be an indication that there are limitations imposed by stomach capacity and fullness. If this is the case, the optimal level of stomach fullness at which fish should be fed could be estimated using GER models.

1.1. Effects of Potential Predictor Variables on GER

1.1.1. Meal Size

The impact of meal size on GER of fish is a much-disputed topic in the literature, and several authors reported different impacts of meal size on GER. Several studies have demonstrated a positive correlation between GER and meal size (Windell, 1967; Bagge, 1977; dos Santos and Jobling, 1995; Andersen, 1998). In contrast, no effect of meal size on GER was observed by Bromley (1988) for whiting (*Merlangius merlangus*) and cod (*Gadus morhua*). Ruggerone (1989) reported a negative correlation between GER and meal size when meal size was expressed relative to fish body size.

1.1.2. Fish Size

Compared to meal size, the effects of fish size on GER are less disputed, although the magnitude of the effect of fish body size on GER reported for different species have been found to vary considerably (Jobling, 1981; Bromley, 1994; Seyhan, 1994; Andersen, 1999). In contrast, other studies using the same meal size relative to fish body size, have not identified any effects of body size on GER, such as it was reported for brown trout (*Salmo trutta*) (Elliott, 1972).

1.1.3. Dietary Energy Contents

Several studies have reported an increase in GER when the energy content of the meal was reduced, and this is known as a compensatory response (Rozin and Mayer, 1961; Grove et al., 1978; Jobling, 1987). However, the magnitude of such changes in GER have been reported to be substantially higher by Andersen (2001, 2012) as compared to the results of Temming and Herrmann (2003).

1.1.4. Temperature

Temperature is known to be among the major variables affecting physiological process rates including GER in ectotherms. Generally, the relationship between temperature and GER has been described using an exponential. However, the GER of several fish species including brook trout (Sweka et al., 2004) and Atlantic cod (Tyler, 1970; Andersen, 2012) has been found to drop sharply between the optimum temperature and the upper thermal tolerance limit.

1.1.5. Prey Resistance to The Digestive Processes

The impact of prey resistance (e.g., robust exoskeletons, energy density of prey) are scarcely investigated in GER experiments. Generally, fish prey are considered homogenous with regard to resistance (Andersen and Beyer, 2005a,b).whereas prey such as crustaceans with robust exoskeletons are known to have two evacuation stages including an initial phase with slow decomposition of surface materials such as chitin, and a second phase known to have a significantly higher GER when more easily digestible materials are exposed (Berens and Murie, 2008; Couturier et al., 2013; Andersen et al., 2016).

1.1.6. Feeding Regime

According to a number of studies, the arrival of new food into the stomach of a fish that still contains a previous meal may accelerate the GER of the gastric residuum and slow down that of the new meal (Fletcher et al., 1984; Persson, 1984; dos Santos and Jobling, 1992). The inference that there is an acceleration of GER of the gastric residuum and a slowing down of GER of the second meal arises as a result of using of mass-dependent models of GER, rather than mechanistically driven, surface-dependent models, such as the one proposed by Andersen and Beyer (2005a,b).

1.2. Methodology and the Experimental Design

The GER experiments are mostly performed on individually held fish acclimated to laboratory conditions where the fish actively has started feeding in the testing environment. Several authors have also performed GER experiments on groups of fish such as sprat (*Sprattus sprattus*), Atlantic herring (*Clupea harengus*), and sardines (*Sardinops sagax*) since it was not possible to maintain such fish species individually (cited in Bernreuther et al., 2009). Furthermore, some GER studies on groups of fish have been performed to compare their results with those obtained from individually maintained fish (Bascinar et al., 2016; Bascinar et al., 2017).

The acclimated fish are then deprived food for a certain time (e.g., 72 h) prior to the GER experiment to let them empty their stomachs. They are then fed a known amount of food. Starvation of fish between one and six days did not affect GER in brown trout, whereas longer periods of starvation slowed down the GER (Elliott, 1972).

Several methods have been established to retrieve the stomach contents from experimental fish at different postprandial time to estimate their GER. The most frequently used approach is the so-called serial slaughter method, where the fish are killed using an overdose of anesthesia or by a blow to the head) and their stomachs dissected to recover the stomach contents (Bromley, 1994; Andersen, 2012). Also, stomach lavage on anaesthetized fish has been used to recover the stomach contents without killing the fish (Seyhan et al., 1998; Sweka et al., 2004). Another method for studying GER, and especially total gastric emptying time (GET) is using X-radiography technique whereby experimental fish are fed formulated diets or natural prey that were incorporated with radio-opaque marker such as barium sulphate ($BaSO_4$) powder or its suspension injected into prey body (Seyhan, 1994; Mazlum and Alabdullah, 2019). The movement of such feeds through the gut are then monitored by sequential X-ray photo of the gut at different postprandial times. However, the validity of radiographic methods depends on the markers passing through the gut of fish at a similar rate as the food, which is not always the case (Jørgensen and Jobling, 1988). Actually, GET rather than GER is monitored, and GER is inferred by the relationship between GET and meal size. Another issue with this method is the stress of fish that might slow down GER in the experimental fish (Talbot, 1985).

1.3. General Information About Brook Trout

Brook trout (*Salvelinus fontinalis*) is a commercially important fish species belonging to the family Salmonidae (Figure 1). As the genus name *Salvelinus* indicates, it is more closely related to chars such as dolly varden trout (*S. malma*) and arctic char (*S. alpinus*) than to species of the genus *Salmo*. Hence, it is also known as brook char (Ryther, 1997).

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Salmoniformes
Family:	Salmonidae
Genus:	Salvelinus
Species:	*S. fontinalis*

The species name *fontinalis* from the Latin means "living in springs" since brook trout prefers cold, well-oxygenated, clean water and is sensitive to high water temperature and acidity (Warren et al., 2017). Hence, the migration of brook trout within tributaries is strongly regulated by the ambient water temperature and they tend to migrate to the nearest cold-water refugia when the water temperature goes above 18° C (Petty et al., 2012). The upper lethal temperature limit reported for brook trout is 24° C to 25° C (Taniguchi et al., 1998; Wehrly et al., 2007).

Brook trout have been raised throughout Europe, Asia, Africa and South America by tribal, private, state and federal fish hatcheries for stocking purposes and fish markets (Fischer et al., 2009). This fish species was brought to Turkey from Europe in the 1990s and have been raised in rainbow trout (*Oncorhynchus mykiss*) farms (Başçinar et al., 2003; Innal and Erk'akan, 2006). The growth rate of brook trout is slower than rainbow trout, which negatively affects the viability of brook trout markets (Fischer et al., 2009). The commercial aquaculture market prefers fish with robust growth rate reaching market size in less time potentially increasing fish sale to the market (Fischer et al., 2009). The commercial viability of brook trout farming can be increased if the fish achieve a growth rate like rainbow trout acquiring a similar size as rainbow trout within the same time period (Fischer et al., 2009). This would eventually be advantageous for the fish-farmer and could expand the market share of brook trout. Consequently, further studies on the physiology and rearing techniques such as feeding regimes are needed to optimize the growth rate of brook trout.

1.4. Aims and Objectives

The major objectives of the work described in this thesis were to develop a GER model for brook trout subjected to experimental treatments involving differences in meal size, feeding regime, feed energy, fish size and water temperature. As such, the thesis incorporates studies that cover:

1. Modelling of the influence of meal size on the course of gastric evacuation with the aim of describing GER independently of meal size.
2. The influence of body size on GER
3. The influence of temperature on GER, with the aim of estimating the optimum temperature as well as the upper temperature limit for GER.
4. How GER is influenced by the energy density of the feed.
5. The effect of feed storage (frozen or at ambient temperature) on GER.
6. The GER of fish given single- and double-meals to examine the influence of the arrival of a second meal on GER of total stomach contents.

The results presented in the thesis should serve as a contribution to the development of improved feeding regimes for farmed brook trout. Additionally, the results may also provide a basis for the estimation of rates of food consumption of wild brook trout.

Figure 1. Brook trout (*Salvelinus fontinalis* Mitchill, 1814; Pisces, Salmonidae)

2. MATERIALS AND METHODS

2.1. Fish Acquisition

Farm raised brook trout of various body sizes were obtained from Prof. Dr. İbrahim OKUMUŞ Aquaculture Research & Production Unit at KTÜ Sürmene Faculty of Marine Sciences, Turkey. The fish were categorized in three groups according to their body size (small, medium and large). Each group was stocked in a separate holding tank (1200 l), and were kept under natural photoperiod. Fish had a continuous supply of fresh water and air bubbling to ensure the dissolved oxygen levels close to 100%. The fish in holding tanks were fed twice daily with commercial pellets (trout diets) acquired from Skretting Aquaculture (Table 1).

Table 1. Composition and energy density of the commercial pellets used for farmed brook trout (*Salvelinus fontinalis*). Diet-II was diluted by 20% and 40% kaolin for experiment 8 and 9, respectively

Feed batch	Used in experiments	Nutrients value (crude, %)				*Energy (kJ g^{-1})
		Protein	Fat	Fibre	Ash	
Diet-I	1– 6 + 10 – 18	44.0	21.0	3.9	9.0	22.10
Diet-II	7 – 9	37.0	23.8	2.5	6.3	22.32

*determined using bomb calorimetry

2.2. Acclimation Period to Experimental Conditions

Brook trout of required body size were transferred to the laboratory where each fish was held separately in individual tanks (~100 l) for the GER trials (Figure 2, Table 2). The fish were fed once a day for 10–15 days to allow acclimation to the experimental conditions. The experimental tanks were cleaned once daily by siphoning. After 10–15 days of acclimation, brook trout that feed regularly under the laboratory conditions were selected for the GER experiments.

Figure 2. Individual tanks used for stocking brook trout (*Salvelinus fontinalis*) for gastric evacuation experiments

2.3. Measuring of Satiation Meal Sizes and Time to Satiation

The acclimated brook trout were starved for 72 h to allow full evacuation of their stomachs. They were then fed individually from a pre-weighted initial meal sizes to apparent station when they stopped taking further food on their own. The uneaten pellets were retrieved by siphoning and were dried at 60 °C using Ecocell Drying Oven (Figure 3). Dry mass of uneaten pellets was subtracted from pre-weighted initial meal sizes to determine the satiation meal size for each fish. The time from the beginning of feeding to deliberate cessation of feeding was considered as satiation time (Brett, 1971).

Figure 3. Uneaten pellets collected from individual tanks were dried in an Ecocell Drying Oven

2.4. Gastric Evacuation Experiments

Brook trout, pre-acclimated to laboratory conditions, were fed a single meal of commercial pellets on empty stomachs (starved for at least 72 h) in all GE experiments excluding experiment 8. Brook trout in experiment 8 were fed with double meals wherein we provided the second meal 12 hours after the first meal. In majority of GE experiments, each brook trout quickly consumed all the offered feed, and uneaten pellets left after c. 30 minutes was recovered and subtracted from the initial meal size following the procedure explained in Khan et al. (2016) (Table 1).

Their stomach contents S_t (g) were then subsequently sampled by dissection of fish at predetermined postprandial times t until the first empty stomach observed (Figure 4). Prior to dissection of fish, the sampled fish were euthanized using benzocaine (150-350 mg/L), and the weight and total length (to the nearest cm) of each fish were measured. The retrieved S_t were dried at 60 °C using the same procedure mentioned-above.

Figure 4. Brook trout (*Salvelinus fontinalis*) were dissected to retrieve the contents of their stomachs

2.5. Effects of Meal and Fish Sizes

Experiments 1–3 on large fish and 4–6 on small fish were conducted to determine the patterns of GE in brook trout and to parameterize the effects of meal and body sizes on GER of brook trout. The fish were fed on meal sizes close to satiation meal in experiment 1 and 4, while 50% and 25% of this meal size were fed to brook trout in experiments 2, 5 and 3, 6, respectively (Table 2).

Table 2. Basic data (mean ± S.E.) on gastric evacuation experiments performed on brook trout (*Salvelinus fontinalis*) fed a single meal or two consecutive meals* of commercial pellets

		Brook trout		Pellet diet			Observation (n)	Predictor variable examined
Experiment number	**Temperature (°C)**	**Total Length (cm)**	**Body Mass (g)**	**Meal mass (g)**	**Kaolin (%)**	**Energy (kJ g^{-1})**		
Effects of body and meal sizes								
1	16.3 ± 1.7	23.9 ± 0.7	166.6 ± 14.6	3.79 ± 0.45			16	
2	17.1 ± 0.6	22.7 ± 0.8	145.4 ± 11.1	1.98 ± 0.05			15	
3	17.4 ± 1.1	23.0 ± 0.7	142.0 ± 9.8	0.79 ± 0.04			15	
4	16.2 ± 1.6	15.3 ± 0.6	39.8 ± 4.9	0.77 ± 0.06			13	
5	17.4 ± 1.1	15.0 ± 0.6	38.0 ± 5.3	0.40 ± 0.00			15	
6	17.1 ± 0.6	14.7 ± 0.9	31.9 ± 7.4	0.20 ± 0.00			16	
Effects of dietary energy								
7	12.0	30.24 ± 0.27	385.48 ± 17.00	4.02 ± 0.04		22.32	14	E, ρ_{LE}, ρ_{LTE}
8	12.0	30.42 ± 0.29	407.85 ± 8.11	4.05 ± 0.01	20	17.67	13	E, ρ_{LE}
9	12.0	28.78 ± 0.15	335.70 ± 8.66	4.04 ± 0.01	40	12.62	12	E, ρ_{LE}
Effects of temperature								
10	12.5	24.12 ± 0.17	161.91 ± 2.94	2.03 ± 0.01		22.10	17	T, ρ_{LTE}
11	13.2	23.80 ± 0.25	140.73 ± 4.67	2.03 ± 0.00		22.10	21	T, ρ_{LTE}
12	15.0	29.85 ± 0.18	367.04 ± 8.56	2.03 ± 0.00		22.10	17	T, ρ_{LTE}
13	16.8	25.91 ± 0.17	207.35 ± 4.55	2.03 ± 0.00		22.10	18	T, ρ_{LTE}
14	18.6	25.67 ± 0.23	211.25 ± 6.34	2.00 ± 0.00		22.10	17	T, ρ_{LTE}
15	20.0	28.67 ± 0.22	284.95 ± 8.20	2.02 ± 0.02		22.10	15	T, $\rho_{LTE,}$
Fed frozen pellets								
16	20	28.51 ± 0.22	287.21 ± 8.34	2.00 ± 0.03		22.10	16	$\rho_{LTE,}$
Single-meal experiment								
17	15.2	22.11 ± 0.20	115.09 ± 3.96	1.17 ± 0.02			15	ρ_L
Double-meal experiment*								
18	15.2	22.34 ± 0.34	118.25 ± 3.85	1.24 ± 0.06[a]			15	ρ_L
				1.20 ± 0.00[b]			15	ρ_L

[a]first meal fed at 0600 hours, [b]second meal at 1800 hours.

2.6. Effects of Dietary Energy

Kaolin as inert material was added to the previously crushed commercial pellets to obtain diets with different energy contents (Table 2). Three diets with different energy levels were prepared following the procedure explained in Khan and Seyhan (2019). These diets were used in experiments 7-9 to parameterize the effects of energy levels on the GER of brook trout.

2.7. Effects of Temperature and Feed Storage Conditions

Experiment 10-15 were performed at different water temperatures to parameterize the influence of temperature on GER of brook trout. The experiments were performed in ambient water temperature at different times of the year (Table 2). The effect of feed storage condition (stored either frozen or under ambient conditions) on GER of brook trout were examined in experiment 16 (Khan and Seyhan, 2019).

2.8. GER of Single- and Double- Meals

Brook trout in experiment 17 was fed a single meal while fed double meals in experiment 18 to compare the GER of single and double meals. Brook trout in experiment 18 was fed a second meal after 12 hours of the first meal (Khan and Seyhan, 2019).

2.9. Forecasting Capability of GER Model

The GER model summarized from the GE data of experiments 1–18 was used to estimate the stomach fullness of brook trout at time *t*. Brook trout of different body sizes were fed for three consecutive days, and their stomach contents were recovered on third day after two hours of the last meal (for details Khan and Seyhan, 2019).

2.10. Analysis of GE Data

The pattern of food evacuation from the stomach of brook trout independently of meal size was determined using the general power model:

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho S_t{}^{\alpha} \quad , \tag{1}$$

where ρ is the rate parameter constant ($g^{1-\alpha}\,h^{-1}$), α is the power parameter describing the course of food evacuation. The pattern of food evacuation is linear if $\alpha = 0$, a square root model if $\alpha = ½$, a so-called surface-area dependent model if $\alpha = ⅔$, and an exponential function if $\alpha = 1$.

The nonlinear regression and the iterative Marquardt method (NLIN procedure) of SAS, 9.04.01 was used to estimate the parameter of the general power model. Equation (1) was integrated over time t from the ingestion of the meal $(t = 0)$ to total evacuation of the meal:

$$S_t = S_0(1 - S_0{}^{(a-1)}\rho(1-a)t)^{1/(1-a)} + \varepsilon \quad , \tag{2}$$

where ε is an error term.

The rate parameter ρ was expanded to account for the effect of fish body size (i.e., total length) on the GER of brook trout. The relationship between rate parameter and fish size was described using a power function.

$$\rho = \rho_{LT} L^{\lambda} \; , \tag{3}$$

Experiments 1–6 were performed to describe the course of GE in brook trout together with the effects of meal and fish sizes. However, these experiments were completed at an unintended temperature range of 15.1 °C to 18.2 °C. Therefore, the relationship between GE and temperature at this range was described by an exponential function (Bromley, 1994, Seyhan, 1994) as:

$$\rho = \rho_{LT} L^{\lambda} \mathrm{e}^{\delta T} \ , \tag{4}$$

where λ and δ are parameters to be estimated.

The estimated value of δ from experiments 1–6 was close to ½ suggesting the square root model to adequately describe the GE of brook trout independently of meal size:

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho\sqrt{S_t} \tag{5}$$

The rate parameter constant ρ likewise to equation (3) and (4) was expanded to account to the effects of fish size and temperature:

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LT} L^{1.31} \sqrt{S_t} \tag{6a}$$

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LT} L^{1.31} \mathrm{e}^{\delta T} \sqrt{S_t} \tag{6b}$$

Equation (6) was integrated over time t from ingestion of meal ($t = 0$) to total emptying of the meal:

$$0 \leq t \leq 2\sqrt{S_0}\,(\rho_{LT} L^{1.31} \mathrm{e}^{\delta T})^{-1} \tag{7}$$

The data analyses for experiments 1–6 proved that square root model best described the course of GE in brook trout independently of the size of the meal.

For the effects of energy density, the rate parameter ρ_L was expanded to account to the effects of dietary energy density E (kJ g^{-1}) and temperature T (° C)

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LE} L^{1.31} E^{-\mu} \sqrt{S_t} \tag{8}$$

This equation was applied to GE data from experiment 7–9 to quantify the effects of E on GER by estimation of the exponent μ.

The rate parameter ρ_{LE} was further expanded by adding a simple exponential function to account for the effect of T within the lower temperature range 12.5–18.6° C (experiments 10–14):

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LET} L^{1.31} E^{-\mu} \mathrm{e}^{\delta T} \sqrt{S_t} \tag{9}$$

The relationship between temperature and GER throughout the experimental temperature range 12.5–20.0° C (experiments 10–14) was then described by a temperature optimum model following the method proposed by Andersen (2012):

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LET} L^{1.31} E^{-\mu} e^{\delta_1 T} (1 - e^{\delta_2 (T - T_u)}) \sqrt{S_t} \tag{10}$$

where δ_1 and δ_2 are temperature coefficients, and T_u is the upper temperature limit where GE is believed to zero. The optimum temperature T_{opt} was then calculated by:

$$T_{\mathrm{opt}} = T_{\mathrm{u}} - \delta_2^{-1} \ln\left[(\delta_1 + \delta_2)\delta_1^{-1}\right] \tag{11}$$

The parameter values of equations 5-10 were estimated by nonlinear regression and the iterative Marquardt method (NLIN procedure, SAS, 9.04.01). These equations were integrated over time t from ingestion of the meal $(t = 0)$ to total emptying of the meal:

$$\sqrt{S_t} = \sqrt{S_0} - \frac{1}{2}\rho t; \quad 0 \le t \le 2\sqrt{S_0}\,\rho^{-1}, \tag{12}$$

with the rate parameter ρ expanded according to equations 5–10.

The rate parameter ρ_L for experiment 17 and 18 was determined using equation (6b). For experiment 18, t in equation (1) was replaced by $(t - \tau)$ to provide the displacement in time between ingestion of the first and second meal where $\tau = 12$ h. Furthermore, the remains of the first meal at time τ (immediately after ingestion of the second meal) was estimated for the individual fish using the rate parameter value obtained from the single-meal experiment.

The one-way ANOVA was used to check for statistical differences among rate parameter values obtained from experiment 1–6, and 7–8. Furthermore, the *t*-test was used to test statistical differences between the rate parameter values obtained from experiments 15 and 16. The same procedure was applied to compare the GER of single-and double-meals experiments (experiment 17 and 18) to examine the influence of the arrival of a second meal on GE.

3. RESULTS

3.1. Satiation Meal Size

The mean satiation meal size estimated for large and small fish were 2% and 1.8% of their body weight. The preferred method of feeding for brook trout was to feed off the bottom, but it can also feed actively in the water column and from the surface (Table 3).

Table 3. Values (mean ± S.D.) of the major variables from experiments on brook trout (*Salvelinus fontinalis*) fed satiation meals of commercial pellets at 15,1 °C

Length (cm)	Body mass (g)	Satiation meal (g)	Satiation time (min)	Obs. (*n*)
23.7 ± 0.6	162.1 ± 13.4	3.46 ± 1.05	11.9 ± 2.5	20
15.4 ± 0.6	39.4 ± 5.5	0.71 ± 0.20	6.1 ± 2.1	20

3.2. GE Patterns

The use of general power model to combine GE data obtained with experiments 1-6 estimated 0.52 of the power parameter α suggested that the square root model best describe the GE in brook trout (Table 4). The estimated value of λ and δ were then fixed to estimate the α from experiment 1-3 and 4-6. Their estimated values of α were also closed to ½ that evinced the consistency of the results.

3.3. Effects of Temperature, Meal and Body Sizes

Using the square root function, the values of length exponent λ and temperature coefficient δ were estimated from combined data (experiment 1-6). Fixing λ and δ at the estimated values obtained from combine GE data, the rate parameter ρ_{LT} were determined for experiments 1-6, 1-3, 4-6 and for each individual experiment (Table 5). Their estimated values of ρ_{LT} ranged from 4.42 to 4.80 and did not differ significantly (ANOVA; $F_{5,\,84}$ = 0.485; P > 0.05). Thus, the course of GE in brook trout fed with commercial pellets can be adequately described by the square root model independently of meal size. The GE curves in Figure 5 and 6 were determined using the square root model $\sqrt{S_t} = \sqrt{S_0} - \frac{1}{2}\rho t$.

Table 4. Parameter estimates ± 95% CI using the general power function $\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LT}L^{\lambda}e^{\delta T}S_t^{\alpha}$ to combined data on gastric evacuation of meals of commercial pellets fed to brook trout (*Salvelinus fontinalis*)

Experiment number	α	δ	λ	ρ_{LT} ($\times 10^{-4}$)	Adj. r^2	Obs.
1-6 combined (all fish)	0.52 ± 0.08	0.029 ± 0.032	1.23 ± 0.44	8.45 ± 11.95	0.977	90
1-3 combined (large fish)	0.52 ± 0.10	0.029 (fixed)	1.23 (fixed)	8.56 ± 0.61	0.969	46
4-6 combined (small fish)	0.59 ± 0.13	0.029 (fixed)	1.23 (fixed)	9.48 ± 1.39	0.927	44

*L, total fish length (cm); T, temperature (°C); S_t , stomach content mass (g) at postprandial time t (h); α, δ, λ and ρ_{LT} , estimated model parameters

Table 5. Parameter estimates ± 95% CI using the square root function $\frac{dS_t}{\mathrm{d}t} = -\rho_{LT}L^{\lambda}e^{\delta T}\sqrt{S_t}$ to combined data on gastric evacuation of meals of commercial pellets fed to brook trout (*Salvelinus fontinalis*)

Experiment number	δ	Λ	ρ_{LT} ($\times 10^{-4}$)	Adjusted r^2	Obs.
1-6 combined (all fish)	0.052 ± 0.025	1.31 ± 0.17	4.61 ± 0.32	0.972	90
1-6 combined (all fish)	0.052 (fixed)	1.31 (fixed)	4.64 ± 0.13	0.972	90
1-3 combined (large fish)	0.052 (fixed)	1.31 (fixed)	4.64 ± 0.18	0.962	46
4-6 combined (small fish)	0.052 (fixed)	1.31 (fixed)	4.62 ± 0.23	0.919	44
1	0.052 (fixed)	1.31 (fixed)	4.70 ± 0.22	0.972	16
2	0.052 (fixed)	1.31 (fixed)	4.51 ± 0.46	0.854	15
3	0.052 (fixed)	1.31 (fixed)	4.65 ± 0.44	0.808	15
4	0.052 (fixed)	1.31 (fixed)	4.80 ± 0.34	0.918	13
5	0.052 (fixed)	1.31 (fixed)	4.52 ± 0.37	0.858	15
6	0.052 (fixed)	1.31 (fixed)	4.42 ± 0.61	0.551	16

*L, total fish length (cm); T, temperature (°C); S_t , stomach content mass (g) at postprandial time t (h); α, δ, λ and ρ_{LT} , estimated model parameters

Since the effect of temperature on GER of brook trout were estimated from a narrow range of temperature (15.1 to 18.2° C), therefore, the temperature dependency of GER was again parameterized with data from GE experiment numbers 10-15.

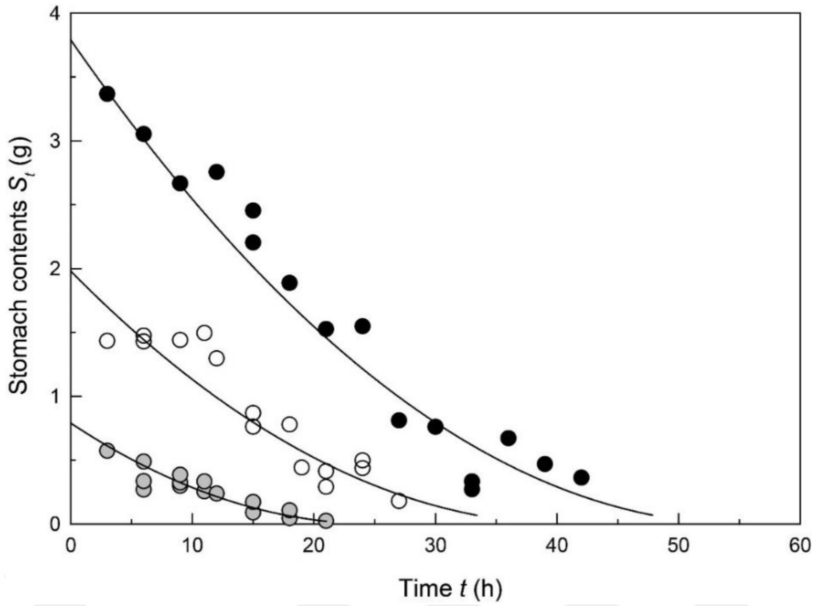

Figure 5. Gastric evacuation data and estimated curves using the square root function for large brook trout (*Salvelinus fontinalis*) fed meals of 4.0 g (●), 2.0 g (○), and 0.8 g (●)

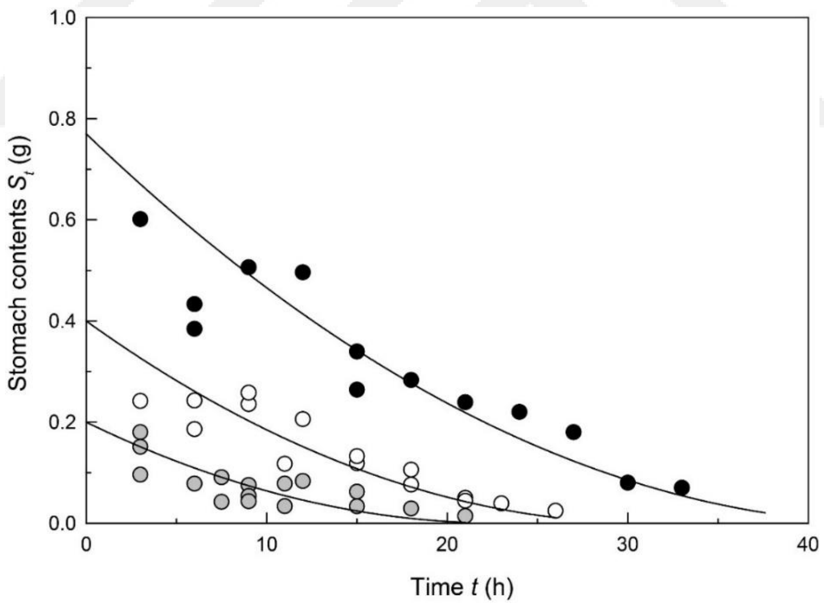

Figure 6. Gastric evacuation data and estimated curves using the square root function for small brook trout (*Salvelinus fontinalis*) fed meals of 0.8 g (●), 0.4 g (○), and 0.2 g (●)

3.4. Effects of Dietary Energy

The GER of brook trout has increased as the dietary energy density (kcal/g) of commercial pellets reduced by the addition of kaolin (Figure 7). The estimated value of the energy exponent μ obtained from the combined data of experiments 7 – 9 was 0.41 ± 0.16 (± 95% CI) (Table 6).

Table 6. Estimates ± 95% CI of the energy density exponent μ and the rate parameter ρ_{LE} using the square root function $\frac{dS_t}{dt} = -\rho_{LE} L^{1.31} E^{-\mu} \sqrt{S_t}$ to data on gastric evacuation of brook trout (*Salvelinus fontinalis*)

Experiment number	ρ_{LE} ($\times 10^{-3}$)	μ	Adjusted r^2	Observation (n)
7–9	2.97 ± 1.39	0.41 ± 0.16	0.990	39
7–9	2.94 ± 0.11	0.41 (fixed)	0.990	39
7	2.90 ± 0.22	0.41 (fixed)	0.986	14
8	2.98 ± 0.18	0.41 (fixed)	0.987	13
9	2.92 ± 0.21	0.41 (fixed)	0.989	12

The rate parameters ρ_{LE} estimated for individual experiment by fixing μ at 0.41 in equation (2) were not statistically different (one-way ANOVA; $F_{4, 116}$= 0.003; P > 0.05). Consequently, using by $\rho_L = \rho_{LE} E^{-0.41}$ a curve line was provided to combined data that depicted a close relationship (r^2 = 0.997) between the values ρ_{LE} estimated for individual experiment and dietary energy content. The GE curves to the individual experiments 7 – 9 were then provided by $S_t = \sqrt{S_0} - \frac{1}{2}\, 0.00297 L^{1.31} E^{-0.41} t$ Figure 8.

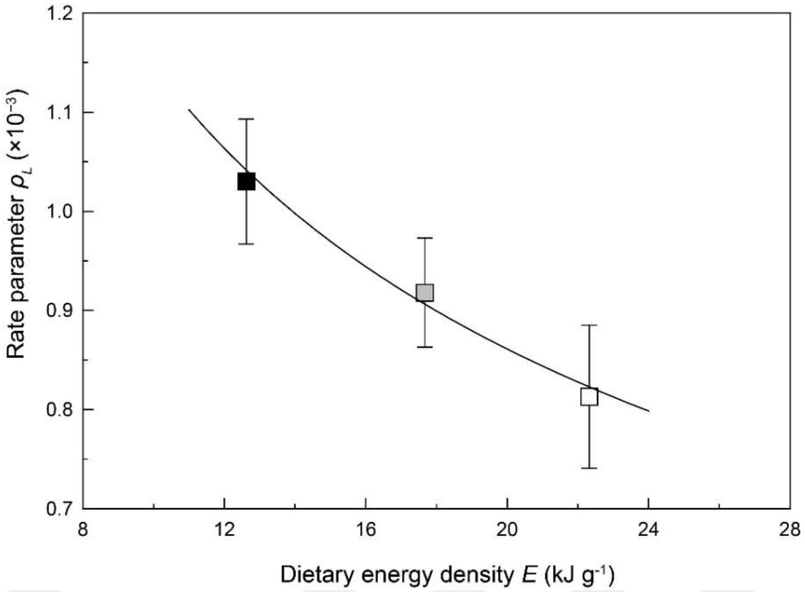

Figure 7. The influence of dietary energy density E on gastric evacuation rate as represented by the rate parameter ρ_L of brook trout (*Salvelinus fontinalis*). The three values of ρ_L (estimates ± 95% C.I.) are obtained from fish fed a formulated feed diluted with 0% (□), 20% (■) or 40% (■) kaolin. The gastric evacuation curve is achieved using the relationship $\rho_L = \rho_{LE} E^{-0.41}$ to all data

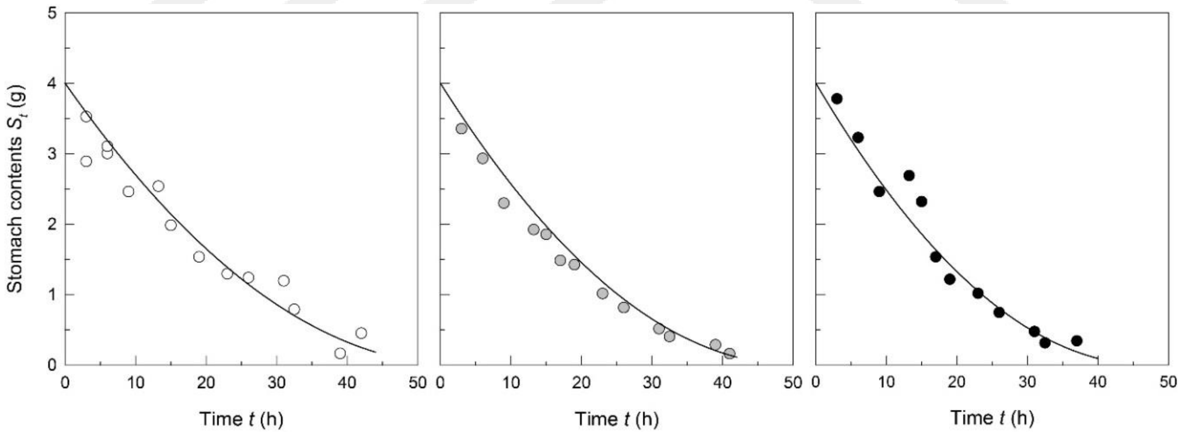

Figure 8. Gastric evacuation in brook trout (*Salvelinus fontinalis*) fed formulated feeds diluted with 0% (○), 20% (●) or 40% (●) kaolin. The gastric evacuation curves are obtained by use of the square root function $S_t = \sqrt{S_0} - \frac{1}{2}\, 0.00297 L^{1.31} E^{-0.41} t$ to evacuation data. S_0 (g) is the meal size, L (cm) total fish length, and E (kJ g^{-1}) energy density of the meal

3.5. Effects of Temperature and Feed Storage Conditions

The value of energy exponent μ was fixed at 0.41 in equations (9) and (10) before the impact of temperature on GER of brook trout was determined. The GER of brook trout increased as the temperature is raised from a minimum at 12.5° C to a maximum at 18.6° C followed by a sharp declined at 20.0° C (Table 7). Hence, the relationship between temperature and GER of brook trout within the lower range 12.5° C – 18.6° C (experiment 10–14) was described by the exponential model [equation (9)], which estimated 0.063 ± 0.013 for δ. This estimated value of δ corresponds to a Q_{10} value of 1.9. Using the parameterized exponential model $\rho_{LE} = \rho_{LET}\mathrm{e}^{0.063T}$ a curve line was provided to describe the relationship between GER of brook trout and temperature within the lower temperature range (r^2 = 0.999, Figure 9). In order to determine the relationship between GER of brook trout and temperature both at lower and higher temperature ranges, the temperature optimum model was applied which estimated a similar value of 0.063 for δ_1 without retrieving any value for δ_2. The value of δ_1 was then fixed at 0.08 in accordance to Andersen (2012) prior to running the temperature optimum model to estimate the values of δ_2 and T_u (Table 7).

The parameterized temperature optimum model $\rho_{LE} = \rho_{LET}e^{0.08T}\left(1 - e^{1.82(T-20.6)}\right)$ was used to provide a curve line to the GE data of brook trout obtained at lower and higher temperature ranges (Figure 10). This parameterized model provided a good fit to GE data (r^2 = 0.975). Furthermore, this parameterized model showed that the GER of brook trout increased exponentially as the temperature is raised to an optimum value at 18.9° C, and then began to decrease rapidly at higher temperatures, reaching zero near to 21° C (Figure 9).

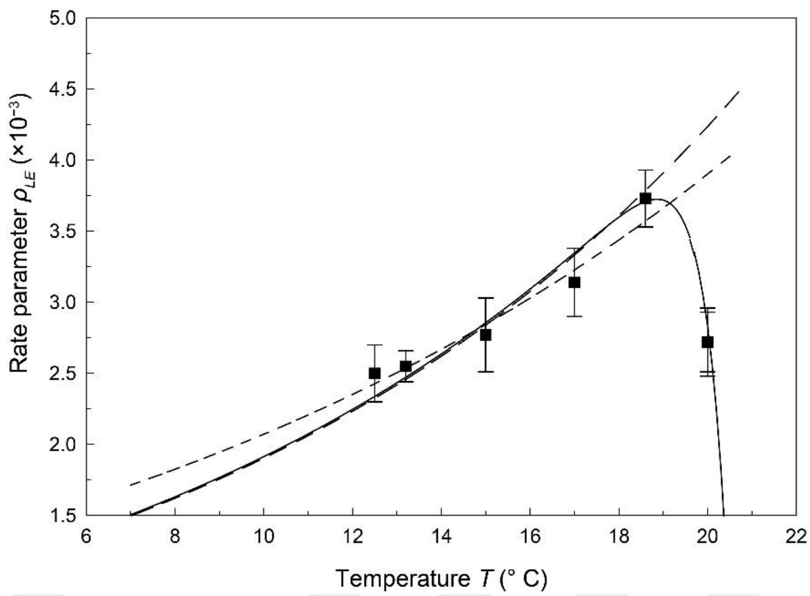

Figure 9. The relationship between temperature T and gastric evacuation rate as represented by the rate parameter ρ_{LE} (estimates ± 95% CI) of brook trout (*Salvelinus fontinalis*) fed a formulated feed. The exponential curves are acquired using the relationships $\rho_{LE} = \rho_{LET}\mathrm{e}^{0.063T}$(-----) and $\rho_{LE} = \rho_{LET}\mathrm{e}^{0.08T}$ (– – –) to all data except those obtained from the highest temperature, and the optimum by use of $\rho_{LE} = \rho_{LET}e^{0.08T}\left(1 - e^{1.82(T-20.6)}\right)$ (——) to all data

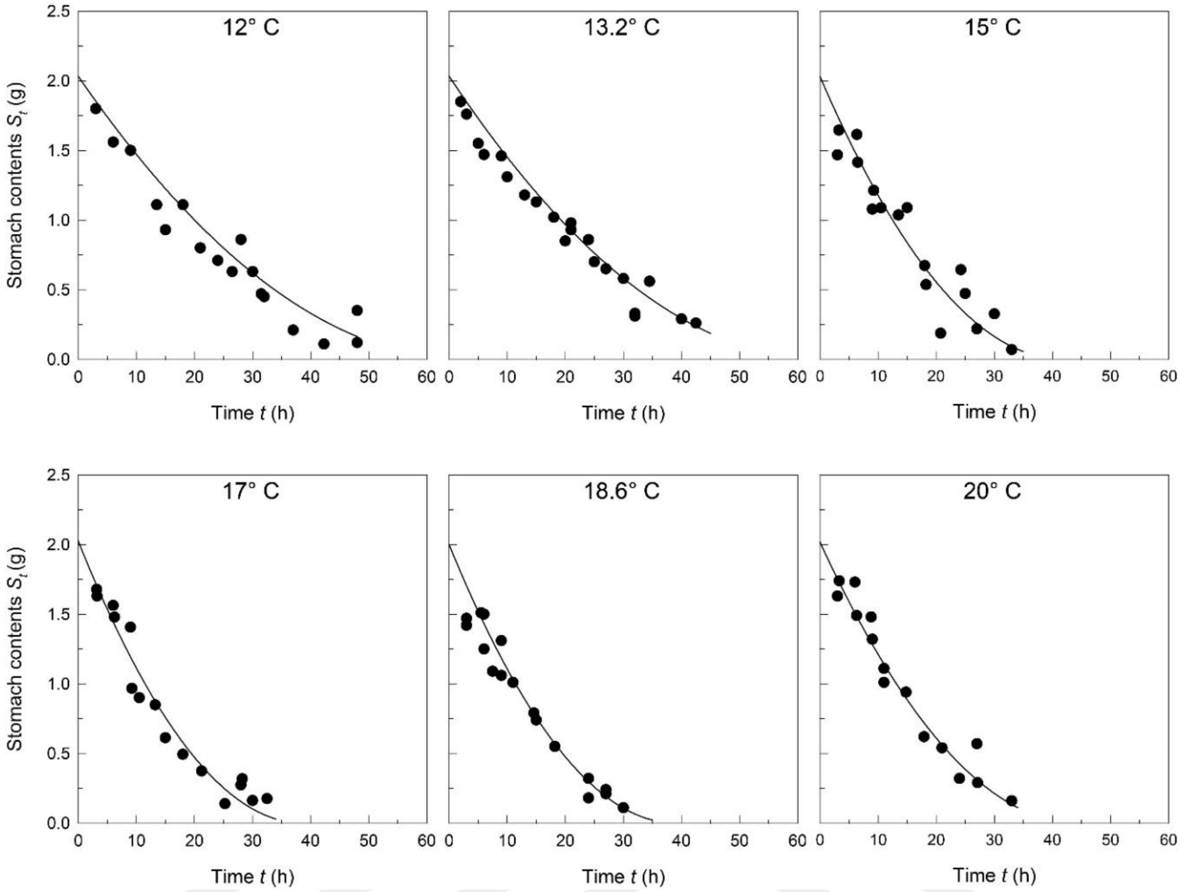

Figure 10. Gastric evacuation in brook trout (*Salvelinus fontinalis*) fed formulated feed at different temperatures. The curves are acquired using the relationship $S_t = \sqrt{S_0} - \frac{1}{2}0.000860L^{1.31}E^{-0.41}e^{0.08\,T}(1 - e^{1.82(T-20.6\,)})t$, where S_0 (g) is the meal size, L (cm) total fish length, E (kJ g^{-1}) feed energy density, and T (°C) temperature

Table 7. Parameter values (estimates ± 95% CI) obtained by use of equations (a), (b) and (c) to gastric evacuation data on brook trout (*Salvelinus fontinalis*) fed commercial pellets at different temperatures.

Experiment number	ρ_{LE} ($\times 10^{-3}$)	ρ_{LET} ($\times 10^{-3}$)	δ	δ_1	δ_2	T_u	Adjusted r^2	Obs. (n)
Equation (a)								
10	2.50 ± 0.20						0.950	17
11	2.55 ± 0.11						0.986	21
12	2.77 ± 0.26						0.943	17
13	3.14 ± 0.24						0.966	16
14	3.73 ± 0.20						0.981	17
15	2.72 ± 0.24						0.970	15
16	2.72 ± 0.21						0.973	16
Equation (b)								
10–15		1.10 ± 0.22	0.063 ± 0.013				0.970	88
10–15		0.85 ± 0.03	0.08 (fixed)				0.968	88
Equation (c)								
10–15		0.86 ± 0.03		0.08 (fixed)	1.82 ± 1.69	20.6 ± 0.69	0.970	103
10–15+16		0.86 ± 0.03		0.08 (fixed)	1.82 ± 1.69	20.6 ± 0.69	0.972	119

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LE} L^{1.31} E^{-0.41} \sqrt{S_t} \quad \text{(a)}$$

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LTE} L^{1.31} e^{\delta T} E^{-0.41} \sqrt{S_t} \quad \text{(b)}$$

$$\frac{\mathrm{d}S_t}{\mathrm{d}t} = -\rho_{LTE} L^{1.31} e^{\delta_1 T} (1 - e^{\delta_2 (T - T_u)}) E^{-0.41} \sqrt{S_t} \quad \text{(c)}$$

L (cm), total fish length; T (°C), temperature; S_t (g), stomach content mass at postprandial time t (h); δ, δ_1, δ_2, ρ_{LE} and ρ_{LTE}, estimated parameters.

The estimated rate parameter ρ_{LE} of brook trout fed on frozen commercial pellets (experiment 16) was found to be significantly similar to the rate parameter of experiment 15 (t-test, P > 0.05; Table 7). This did not result in any influence of feed storage conditions on the GER in brook trout.

3.6. GER of Single- and Double-Meals

There was also no significant difference between the rate parameters ρ_L estimated for experiment 17 (fed single meal) and experiment 18 (fed double meal) (t-test, P > 0.05). The total stomach content made up of two meals was therefore evacuated at the same GER as if it had come from a single meal (Table 8).

Table 8. Estimates ± 95% CI of the rate parameter ρ_L using the square root function $\frac{dS_t}{dt} = -\rho_L L^{1.31}\sqrt{S_t}$ to data from gastric evacuation experiments on a single meal or double meal.

Experiment number	ρ_L ($\times 10^{-3}$)	Adj. r^2	Observation (n)
11 Single meal	1.28 ± 0.11	0.827	17
12 Double meals	1.28 ± 0.15	0.807	18

L, total fish length (cm); S_t, stomach content mass (g) of either single meal or double meal (composed of the remains of two meals) at postprandial time t (h).

On the basis of the above results, the GER model for brook trout can be summarized by:

$$\frac{dS_t}{dt} = -0.000860L^{1.31}E^{-0.41}e^{0.08\,T}(1 - e^{1.82(T-20.6)})\sqrt{S_t} \tag{13}$$

3.7. Forecasting Capability of GER Model

The summarized GE model of brook trout (equation 13) was used to predict brook trout's stomach fullness that had been fed for three consecutive days. The values of stomach fullness predicted by GE model of brook trout were closely in line with the observed values (Figure 11).

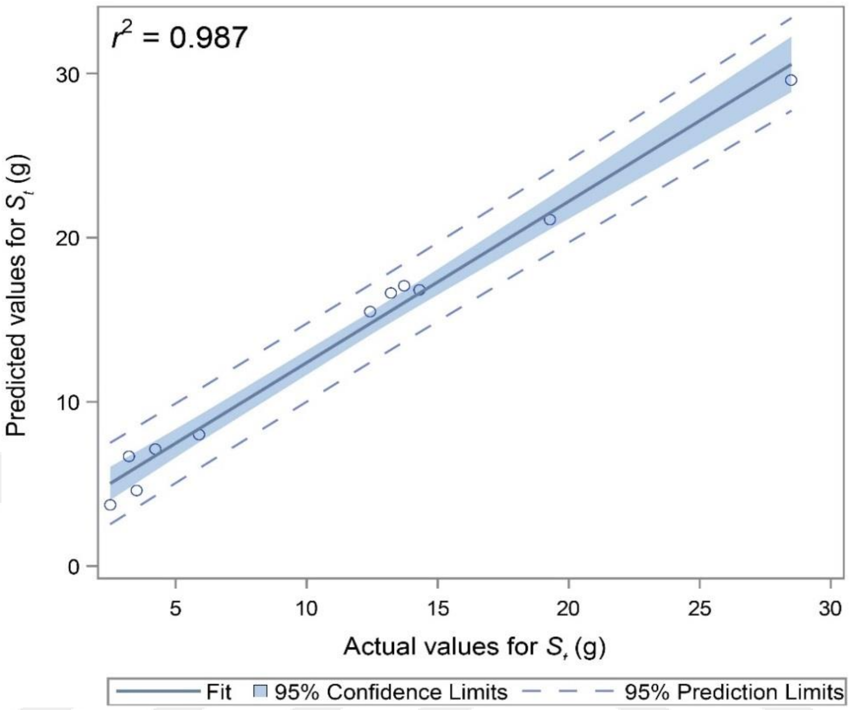

Figure 11. The stomach content mass S_t of formulated feed in brook trout (*Salvelinus fontinalis*) as predicted by the square root function $S_t = \sqrt{S_0} - \frac{1}{2}0.000860L^{1.31}E^{-0.41}e^{0.08\,T}(1 - e^{1.82(T-20.6\,)})t$ and plotted against actual values. The fitted curve of the relationship is $y = 0.982x + 2.592$ (r^2 = 0.987). S_0 (g) is meal size, L (cm) total fish length, E (kJ g^{-1}) feed energy density, and T (°C) temperature.

3.8. GER of Re-pelleted Feeds and Normal Pellets

The rate parameter of brook trout fed crushed re-pelleted feeds (experiment 7) was 0.00115 (± 0.00009) which was significantly different from the rate parameter of brook trout (0.00095 ± 0.00008) fed on normal pellets (*t*-test, $P < 0.05$). Though both types of pellets possessed the same moisture contents which did not differ significantly (*t*-test, $P > 0.05$).

4. DISCUSSION

4.1. GE Pattern

The use of general power model to combine GE data from experiments 1-6 evinced that the square root model adequately described the course of GE in brook trout. Similar to the present study, turbot (*Scophthalmus maximus*) were fed meals of different sizes composed of formulated diets and X-radiography were used to determine GE time (Grove et al., 1985). The power value estimated from the GE data of turbot was 0.55 suggesting the square root model to adequately describe the GE of turbot. Furthermore, several studies have proven the capability of the square root model to adequately describe the GE of different fish species such as pikeperch *Stizostedion lucioperca,* coho salmon *Oncorhynchus kisutch* and different gadoids which were fed a suite of natural prey species (cited in Khan et al., 2016). The capability of this model to describe the GE in fish has been supported by a mechanistical cylinder model based upon simple surface consideration of prey that evacuates isometrically by continuous successive peeling off (Andersen and Beyer, 2005b, 2005a). The cylinder model considers the prey as homogeneous in terms of resistance to the digestive process and biochemical composition throughout the body and formulated feeds are even more homogenous compared to natural prey. The cylinder model assumes that the meal is as a cylinder in the fish stomach where the radius of the cylinder gets reduced by successive peeling off without affecting the end of the cylinder (Andersen and Beyer, 2005a, b).

4.2. Effects of Body Size

The length exponent estimated for brook trout in this study was 1.31 which is consistent with earlier studies that ranged 1.3-1.4 estimated from different fish species such as haddock (*Melanogrammus aeglefinus*), Atlantic cod, whiting and saithe (*Pollachius virens*) (cited in Khan et al., 2016). Though several studies have also reported lower values of λ for Atlantic cod and pikeperch (Khan et al., 2016). The possible reason behind the lower estimation of fish size impact on GER of Atlantic cod and for pikeperch might be the fact that the larger fish were fed with larger size prey fish that generally had higher energy density compared to smaller prey (Pedersen and Hislop, 2001). Whereas, the smaller fish in these studies were fed with prey of smaller size. Hence, several studies including results from this thesis showed that GER gets depressed when the energy density of meal increased (Jobling, 1987). Along with length exponent, body mass exponent was also estimated for a possible comparison with values on body mass exponent from literature. The estimated value of mass exponent for brook trout was 0.40 ± 0.05 (95% C.I.) that is also in line with the findings of aforementioned authors. However, several other studies have obtained a lower estimate of mass exponent for turbot, Atlantic cod and pikeperch (*Stizostedion lucioperca*) (Khan et al., 2016).

4.3. Effect of Energy Density

The GER of brook trout were increased by decreasing the dietary energy density of commercial pellets through the dilution of kaolin. The GER was increased by 12.92% and 26.69% with 20% and 40% kaolin dilution respectively. Such an increase in GER is demonstrated as a compensatory response for decreases in the energy content of diet (cited in Khan and Seyhan, 2019) and this process is known to drive by feedback loops from the upper intestine (Jobling, 1987). The energy exponent μ estimated from experiment 7-9 is 0.41 which is in line with the finding of Temming and Herrmann (2003) reporting a value of $\mu = 0.45$ for cod. However these values of energy exponent μ are substantially lower than (Andersen, 2001; Andersen, 2012) who reported a value of 0.85 for whiting, Atlantic cod and saithe fed different fish prey and crustacean species. The lower estimation for μ from experiment 7-9 than (Andersen, 2001; Andersen, 2012) findings might be the types of food since commercial pellets disintegrate more rapidly in the fish stomach than natural prey which might cause fish to partly lose control on the process of evacuation and the intestine of fish becomes overloaded (cited in Khan and Seyhan, 2019).

4.4. Effects of Temperature

The application of temperature optimum model to describe the dependency of temperature on GER proved that the GER of brook trout increased with a raise in temperature up to around 18.9° C, and above this point the GER of brook trout tends to sharply decline until it stops near to 21° C. The optimum temperature limits estimated for growth rate is 16° C and 20.2° C for metabolic rate of brook trout and above these temperature limit the growth and metabolic rates of brook trout begin to sharply decline (cited in Khan and Seyhan, 2019). The lethal temperature range reported for brook trout is 24° C to 25° C (Taniguchi et al., 1998; Wehrly et al., 2007). The migration of wild brook trout within tributaries is also strongly regulated by the temperature of ambient water and the fish tend to migrate to the nearest cold-water refugia as the water temperature went beyond 18° C (Petty et al., 2012). Similar the findings of this study, an optimum temperature levels for the GER of Atlantic cod, Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*) have been reported (cited in Khan and Seyhan, 2019). Temming (1995) developed the first optimum temperature model for GER of fish to determine the temperature dependency of GER at low and high temperature. His model was latter modified by Andersen (2012) to fix a limitation (see Khan and Seyhan, 2019). The optimum temperature as well as upper temperature ranges estimated by Temming (1995)'s and Andersen (2012)'s models are identical.

Compared with the results ranging from 0.078 to 0.083 (Q_{10} = 2.18 – 2.29) that have been obtained by several other studies, the estimated temperature coefficient δ of 0.063 and corresponding to Q_{10} = 1.9 was in the low end. However, some other studies also reported lower values of δ from 0.032 (Q_{10} = 1.38) to 0.068 (Q_{10} = 2.0) (see Khan and Seyhan, 2019). The low value of δ in the present study might be due to missing GE data at lower temperatures since Seyhan (1994) obtained a significantly higher value of δ (Q_{10} increased from 0.14 to 1.97) when including data at further low temperatures. Thus, following (Andersen, 2012) the value of δ_1 was fixed at 0.08, which is in accordance with the version of temperature optimum function that best describes the effect of temperature on GER at low as well as high temperatures. Bernreuther et al. (2009) also used δ_1 fixed at 0.077 prior to application of the temperature optimum function on GER data of *S. sprattus* at temperatures between 7.5 °C and 21.5 °C.

4.5. Effects of Feed Storage Conditions

If not properly stored, formulated fish feeds rapidly deteriorate and develop molds (Robb et al., 2013). It is recommended to store them under cool, dry and well-ventilated conditions, and to avoid direct sunlight (Cruz, 1996; Craig et al., 2017). The possible effects of freezing the feed on GER was examined for brook trout fed frozen (–15.0 °C) and non-frozen commercial pellets. Meals of both treatments were evacuated at similar rates. No effect of storage of the formulated feed on the digestive rate could therefore be demonstrated.

Also, Andersen (2012) found that freshly killed and previously frozen (–20 °C) sandeel (*Ammodytes tobianus*) fed to Atlantic cod were evacuated at a similar rates. Further, feed storage conditions did not significantly influence food intake and growth performance in rainbow trout (*Oncorhynchus mykiss*) fed for 42 days with frozen feed (stored at 10° C, -1.1° C and -15° C) or non-frozen (stored at room temperature, 20.8° C) (Khan et al., 2018).

4.6. Effects of Second Meal on GER

The total stomach content composed of the two meals was evacuated by brook trout at a rate similar to that of a single meal, which is consistent with previously reported studies on other predator species (Elliott, 1972; El-Shamy, 1976; Temming, 2002). Therefore, GER of the total stomach content of brook trout fed pelleted feeds seems to be appropriately described independently of meal size and feeding regime by the parameterized square root function (Equation 13). Authors generally claim that a second meal in the stomach tends to speed up the evacuation of first meal and delay that of the second meal (see Khan and Seyhan, 2019). This apparent acceleration of GER of first meal and deceleration of the second one seems to be an artefact resulting from the use of mass-dependent rather than surface-dependent models (see Khan et al., 2016; Khan and Seyhan, 2019).

5. CONCLUSION

An expanded fully-parameterized GE model (equation 13), with a basis in the simple square root function, can be used to estimate the stomach fullness at return of appetite in brook trout subjected to a range of environmental and feeding conditions setting (feeding regime, feed characteristics, fish size and temperature). The model may have application in the development of feeding regimes for farmed brook trout, thereby reducing feed waste and the risk of pollution to recipient waters of farm effluent.

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7. APPENDIX

7.1. General Power Model

SAS program codes that were used to estimate the parameters of equation-4 (General power model):

```
FILENAME CSV "/folders/myfolders/PhD_GE all experiments.csv"
TERMSTR=CRLF;

PROC IMPORT DATAFILE=CSV
            OUT=Brooktrout
            DBMS=CSV
            REPLACE;
RUN;

title 'Gastric evacuation in brook trout. +AC model';
data exp;
set Brooktrout;
if expno=1 or expno=2 or expno=3 or expno=4 or expno=5 or expno=6;
run;

proc nlin data=exp method=marquardt;
parms A=0.08 B=0.5 C=1.5 RAC=0.0005;
delta=0.00000001;

R=RAC*EXP(A*temp)*predlcm**C;

s=sow**(1-B)-R*(1-B)*time;
if s>0 then stx=s**(1/(1-B));
else stx=0;
 model stw=stx;
```

```
output out=expp p=pstw r=stw_residual;
run;
proc summary data=expp;
    var stw_residual stw;
    output out=stats css(stw)=sstot uss(stw_residual)=ssres N=N;
run;
data expp;
    set stats;
    rsquared=1-(ssres/sstot);
    adjrsquared = 1-(1-rsquared)*(N-1) / (N- 4  -1);
run;
proc print data=expp;
run;
```

Here “a” stands for δ (temperature), “b” for α (shape exponent) and “c” for λ (exponent of fish length), and RAC for rate parameter constant ρ_{LT}.

7.2. Square Root Model

SAS program codes that were used to estimate the parameters of equation 10:

```
FILENAME CSV "/folders/myfolders/PhD_GE all experiments.csv"
TERMSTR=CRLF;

PROC IMPORT DATAFILE=CSV
            OUT=Brooktrout
            DBMS=CSV
            REPLACE;
RUN;

title 'Gastric evacuation in brook trout. Temperature effect+E';
data exp;
set Brooktrout;
```

```
if expno=10 or expno=11 or expno=12 or expno=13 or expno=14 or expno=15;
run;

proc nlin data=exp method=marquardt;

parms RLTE=0.0022 A=0.08 B=0.46 C=1.31 E=0.41 TU=20;

R=RLTE*predlcm**C*EXP(A*temp)*(1-EXP(B*(temp-TU)))*enerd**-E;

model sqrtstw=sqrtsow-0.5*R*time;

output out=expp p=psqrtstw r=stw_residual;
run;

proc summary data=expp;
    var stw_residual stw;
    output out=stats css(stw)=sstot uss(stw_residual)=ssres N=N;
run;
data expp;
    set stats;
    rsquared=1-(ssres/sstot);
    adjrsquared = 1-(1-rsquared)*(N-1) / (N- 3  -1);
run;
proc print data=expp;
run;
```

Here "a" stands for δ_1 (1st temperature coefficient), "b" for δ_2 (2nd temperature coefficient), "c" for λ (exponent of fish length), E for μ (coefficient of energy density), T_U upper temperature limit, and RLTE for rate parameter constant ρ_{LET}.

7.3. Raw Data Obtained from the Gastric Evacuation Experiments Listed in Table 2.

7.3.1. Experiment 1-6 for Parameterizing the Effects of Body and Meal Sizes on GE of Brook Trout

expno	temp	predlcm	predw	sow	stw	time
1	15.1	24.3	174.1	4	3.366	3
1	15.1	24.7	182.5	4	3.052	6
1	15.1	23.4	153	4	2.666	9
1	15.1	23.7	159.8	4	2.755	12
1	15.1	24.5	178.2	4	2.203	15
1	17.5	23.2	172.8	4	2.453	15
1	15.1	23.9	164	4	1.886	18
1	15.1	24.4	174.8	3.98	1.525	21
1	15.1	23.3	151.2	3.88	1.547	24
1	15.1	23.2	149.8	3.39	0.81	27
1	15.1	23.2	149.3	3.59	0.76	30
1	15.1	23.1	147.4	2.3	0.27	33
1	17.5	24.7	177.4	4	0.332	33
1	15.1	23.5	155	3.48	0.67	36
1	15.1	25.1	192.7	4	0.468	39
1	15.1	24.7	183.6	4	0.362	42
2	16.7	21.4	137.2	2	1.433	3
2	16.7	22.5	143.1	2	1.474	6
2	17.5	23.5	142.2	1.937	1.429	6
2	16.7	22.3	139.2	2	1.44	9
2	17.5	22.5	138.5	2	1.495	11
2	16.7	22.2	149	2	1.295	12
2	16.7	22.4	148.1	2	0.868	15
2	17.5	22	127.1	1.8	0.761	15
2	16.7	23	147	2	0.778	18
2	17.5	23	156	2	0.44	19
2	16.7	22.5	139.9	2	0.412	21

2	17.5	22.2	130.5	2	0.29	21
2	16.7	24	156.4	2	0.436	24
2	17.5	23.5	162.4	2	0.496	24
2	16.7	24.1	165.4	2	0.179	27
3	16.7	23.7	158.8	0.8	0.572	3
3	18.2	22	134.6	0.8	0.268	6
3	18.2	22.6	148.8	0.8	0.335	6
3	16.7	23	153.4	0.8	0.487	6
3	18.2	23	136.2	0.623	0.298	9
3	18.2	23.2	143.1	0.8	0.323	9
3	16.7	22.4	139.6	0.8	0.385	9
3	18.2	23.3	144	0.8	0.254	11
3	18.2	24.8	148.8	0.8	0.332	11
3	16.7	23.3	136.6	0.8	0.237	12
3	18.2	22.9	137.5	0.8	0.089	15
3	16.7	22.1	126.1	0.8	0.171	15
3	18.2	23	127.4	0.8	0.044	18
3	16.7	23.8	156.4	0.8	0.106	18
3	16.7	22.6	139.3	0.8	0.024	21
4	15.1	15.6	41.9	0.8	0.601	3
4	15.1	14.6	33.8	0.67	0.433	6
4	17.4	14.5	32.9	0.593	0.384	6
4	15.1	15.4	40	0.8	0.506	9
4	15.1	14.9	36.2	0.8	0.496	12
4	15.1	16.2	47	0.8	0.264	15
4	17.4	15.7	41.8	0.8	0.339	15
4	15.1	14.8	35	0.8	0.283	18
4	15.1	16.4	48.8	0.8	0.239	21
4	15.1	15.3	39.1	0.8	0.22	24
4	15.1	15.9	43.9	0.8	0.18	27
4	15.1	15.5	40.5	0.73	0.08	30
4	15.1	15	37	0.8	0.07	33
5	16.7	14.5	35.4	0.4	0.242	3
5	18.2	16.1	43.2	0.4	0.186	6
5	16.7	15.2	39.9	0.4	0.242	6

5	18.2	15.8	47.8	0.4	0.235	9
5	16.7	14.5	34.8	0.4	0.258	9
5	18.2	15.9	39.5	0.4	0.117	11
5	16.7	15.5	47.3	0.4	0.206	12
5	18.2	14.9	33.3	0.4	0.119	15
5	16.7	13.9	30.8	0.4	0.133	15
5	18.2	14.8	33.7	0.4	0.106	18
5	16.7	14.4	32.2	0.4	0.076	18
5	18.2	15	38.2	0.4	0.05	21
5	16.7	15.5	42.6	0.4	0.044	21
5	16.7	15	37.1	0.4	0.039	23
5	16.7	14.6	35.1	0.4	0.024	26
6	16.7	13.9	26.9	0.2	0.18	3
6	16.7	13.9	26.9	0.2	0.151	3
6	17.5	15.1	36.5	0.2	0.096	3
6	16.7	13.7	28.4	0.2	0.078	6
6	17.5	16.4	40.4	0.2	0.091	7.5
6	17.5	15.2	41.4	0.2	0.042	7.5
6	16.7	15.1	32	0.2	0.075	9
6	17.5	16.5	46.9	0.2	0.054	9
6	17.5	15.1	32.4	0.2	0.043	9
6	17.5	14.2	31	0.2	0.034	11
6	17.5	15.3	36.2	0.2	0.078	11
6	16.7	13.4	16	0.2	0.083	12
6	16.7	14.5	28.4	0.2	0.062	15
6	17.5	14.9	34.1	0.2	0.033	15
6	16.7	14.2	28.3	0.2	0.029	18
6	16.7	13.8	24.4	0.2	0.014	21

7.3.2. Experiment 7-9 for Parameterizing the Effects of Dietary Energy on Gastric Evacuation Rates of Brook Trout.

Kaolin %	expno	temp	predlcm	predw	sow	stw	time
0	7	12	32.4	511	4.047	3.524	3
0	7	12	29.7	343.5	3.534	2.889	3
0	7	12	31.1	414.2	4.034	3.001	6
0	7	12	28.5	278.8	4.042	3.103	6
0	7	12	29.8	431.6	4.073	2.459	9
0	7	12	30.4	419.8	4.024	2.536	13.25
0	7	12	29.9	336.4	4.088	1.981	15
0	7	12	29.3	334	4.063	1.531	19
0	7	12	29.6	386	4.005	1.291	23
0	7	12	30.6	402.6	4.087	1.237	26
0	7	12	29.6	341.2	4.071	1.192	31
0	7	12	30	329.6	4.081	0.786	32.5
0	7	12	31.3	481	4.08	0.161	39
0	7	12	31.1	387	4.017	0.449	42
20	8	12	29.5	412.2	4.053	3.351	3
20	8	12	30	400.3	4.029	2.93	6
20	8	12	29.7	400.298	4.075	2.296	9
20	8	12	29.6	392.29	4.057	1.92	13.25
20	8	12	28.2	388.225	4.018	1.851	15
20	8	12	31	399.67	4.095	1.481	17
20	8	12	30.5	410.55	4.025	1.421	19
20	8	12	30.5	396.86	4.07	1.012	23
20	8	12	31	483	4.022	0.812	26
20	8	12	31.8	445.75	4.08	0.512	31
20	8	12	31.3	420.9	4.016	0.401	32.5
20	8	12	32.1	372.01	4.044	0.282	39
20	8	12	30.3	380.01	4.02	0.157	41
40	9	12	28.3	312.8	4.043	3.777	3
40	9	12	29.1	321.1	4.071	3.227	6
40	9	12	29.1	332.6	4.039	2.46	9
40	9	12	28.6	388.85	4.007	2.687	13.25

40	9	12	29.2	354.407	4.016	2.318	15
40	9	12	29	288.25	4.046	1.531	17
40	9	12	28.5	319.975	4.018	1.213	19
40	9	12	29.6	381.011	4.049	1.013	23
40	9	12	28	325.146	4.071	0.742	26
40	9	12	27.9	306.902	4.02	0.471	31
40	9	12	29.2	357.35	4.064	0.31	32.5
40	9	12	28.8	340.026	4.056	0.339	37

7.3.3. Experiment 10-15 for Parameterizing the Effects of Temperature on GER of Brook Trout.

expno	temp	predlcm	predw	sow	stw	time
10	12.5	24.7	168.95	2.05	1.8	3
10	12.5	24.8	164.48	1.97	1.56	6
10	12.5	23.9	159.34	1.95	1.5	9
10	12.5	24.8	180.24	2.07	1.11	13.5
10	12.5	25	167.63	2.05	0.93	15
10	12.5	22.6	134.74	2.04	1.11	18
10	12.5	23.9	161.27	2.03	0.8	21
10	12.5	23.6	151.14	2.04	0.71	24
10	12.5	25	176.5	2.06	0.63	26.5
10	12.5	23	147.13	2.06	0.86	28
10	12.5	24.5	168.96	2.04	0.63	30
10	12.5	24.7	165.84	2.07	0.47	31.5
10	12.5	23.4	155.86	2.03	0.45	32
10	12.5	23.8	162.63	2.03	0.21	37
10	12.5	23.9	144.01	2.05	0.11	42.3
10	12.5	24.4	173.73	1.99	0.35	48
10	12.5	24.1	170.1	2.01	0.12	48
11	13	24.6	169.38	2.04	1.85	2
11	13	25.4	176.57	2.02	1.76	3
11	13	24.2	148.06	2.03	1.55	5
11	13.4	22	109.8	2.03	1.47	6

11	13.4	22.5	111.62	2.03	1.46	9
11	13.4	22.8	123.96	2.07	1.31	10
11	13.4	25.1	169.24	2.03	1.18	13
11	13.4	23.9	136.87	2.02	1.13	15
11	13.4	21.7	109.38	2.02	1.02	18
11	13	23.6	135.9	2.04	0.85	20
11	13.4	23	126.78	2.03	0.98	21
11	13.4	23.6	131.33	2.05	0.93	21
11	13.4	23.7	137.35	2.04	0.86	24
11	13.4	25.7	162.68	2.03	0.7	25
11	13	23.5	135.72	2.05	0.65	27
11	13.4	23.2	125.4	2.02	0.58	30
11	13.4	24.2	139.5	2.02	0.31	32
11	13.4	26	177.92	2.03	0.33	32
11	13	23	127.63	2	0.56	34.5
11	13	24.5	162.9	2.04	0.29	40
11	13	23.5	137.34	2.04	0.26	42.5
12	15	31	387.7	2.035	1.468	3
12	15	29.9	334.6	2.017	1.645	3.25
12	15	30.5	379.8	2.045	1.614	6.33
12	15	30.6	439.7	2.011	1.414	6.5
12	15	29.2	370.4	2.035	1.078	9
12	15	30	388.7	2.015	1.212	9.25
12	15	28	326.8	2.029	1.088	10.5
12	15	29.5	356.7	2.027	1.036	13.5
12	15	29.2	287.7	2.026	1.089	15
12	15	30	353.5	2.044	0.673	18
12	15	29.5	355.3	2.037	0.536	18.25
12	15	30.5	384.8	2.027	0.187	20.75
12	15	30.2	367.8	2.041	0.472	25
12	15	30.2	415.31	2.031	0.643	24.25
12	15	30.2	347.6	2.032	0.218	27
12	15	29	348.16	2.025	0.326	30
12	15	29.9	395.03	2.025	0.07	33
13	17	28	330.348	2.018	1.678	3.17

13	17	27.9	278.286	2.008	1.63	3.25
13	17	28.1	356.43	2.039	1.562	6
13	17	28.5	353.72	2.037	1.479	6.25
13	17	27.5	283.6	2.02	1.406	9
13	17	28	296.55	2.032	0.967	9.25
13	17	29.5	334.8	2.048	0.9	10.5
13	17	27.9	265.09	2.013	0.849	13.25
13	17	28.1	309.09	2.013	0.612	15
13	17	28.3	313.65	2.01	0.493	18
13	17	26.6	248.89	2.032	0.373	21.25
13	17	28.2	374.5	2.01	0.139	25.25
13	17	28.5	292.1	2.021	0.273	28
13	17	27.5	255.6	2.036	0.318	28.25
13	17	28.5	324.14	2.042	0.161	30
13	17	28.5	285.11	2.024	0.175	32.5
14	18.6	25.5	194.8	2	1.42	3
14	18.6	25	176.3	2	1.47	3
14	18.6	25.6	228.4	2	1.51	5.5
14	18.6	27.3	249.8	2	1.25	6
14	18.6	25.1	164.1	2	1.5	6
14	18.6	25.2	217.1	2	1.09	7.5
14	18.6	24.4	202	2	1.06	9
14	18.6	25.4	206	2	1.31	9
14	18.6	28.3	243.6	2	1.01	11
14	18.6	26.2	241.9	2	0.79	14.6
14	18.6	25.7	212.8	2	0.74	15
14	18.6	25.2	233.2	2	0.55	18.2
14	18.6	25	177.9	2	0.32	24
14	18.6	26	212.8	2	0.18	24
14	18.6	25.9	217	2	0.24	27
14	18.6	24.6	178.3	2	0.21	27
14	18.6	26	235.3	2	0.11	30
15	20	28	282.59	2.03	1.63	3
15	20	29	300.38	2.03	1.74	3.3
15	20	28.5	279.6	2.04	1.73	6

15	20	30.2	317.5	2.04	1.49	6.3
15	20	28.5	302.25	2.03	1.32	9
15	20	28.5	269.08	2.03	1.48	8.8
15	20	28.2	247.37	1.76	1.11	11
15	20	27.5	268.57	2.02	1.01	11
15	20	28.4	276.64	2.05	0.94	14.8
15	20	28.1	262.1	2.04	0.62	17.9
15	20	30	310.35	2.04	0.54	21
15	20	28.6	219.48	2.04	0.32	24
15	20	30	354.58	2.05	0.57	27
15	20	27.5	285.03	2.02	0.29	27.1
15	20	29	298.76	2.02	0.16	33

7.3.4. Experiment 16 for Parameterizing the Effects of Feed Storage Condition on GER of Brook Trout.

expno	temp	predlcm	predw	sow	stw	time
16	20	29.3	282.3	2.023	1.77	3.00
16	20	28.1	262.6	2.02	1.84	3.25
16	20	28.7	278.95	2.036	1.62	6.00
16	20	27.2	228.23	2.038	1.79	6.25
16	20	30.6	380.15	2.009	1.28	11.00
16	20	28.8	311.44	2.047	0.96	13.08
16	20	27.8	240.1	2.044	0.81	17.42
16	20	29	290.44	2.034	1.00	18.00
16	20	27.7	288.99	2.043	0.68	21.00
16	20	28.1	288.35	1.616	0.23	24.00
16	20	29.6	303.32	2.026	0.60	24.00
16	20	29	300.01	2.021	0.20	27.00
16	20	27.5	276.2	2.007	0.34	28.08
16	20	28.6	280.88	2.017	0.36	29.50
16	20	28.2	306.07	2.039	0.22	33.00
16	20	28	277.3	2.03	0.05	33.17

7.3.5. Experiment 17-18 for Comparing the GER of Single and Double Meals Fed to Brook Trout.

expno	temp	predlcm	predw	sow	stw	time
17	15.2	21.8	112.31	1.2	0.8035	3
17	15.2	23	135.18	1.211	0.7231	6
17	15.2	22.5	115.03	1.166	0.6799	9
17	15.2	21	97.88	1.203	0.4866	12
17	15.2	21.5	104.68	1.203	0.3154	12.5
17	15.2	22.9	143.21	1.201	0.2354	15
17	15.2	21.5	107.34	1.189	0.1289	18
17	15.2	21.8	121.17	0.972	0.0366	18.5
17	15.2	20.5	85.15	1.161	0.1391	20
17	15.2	22	107.6	1.198	0.0869	20
17	15.2	23.1	123.06	1.195	0.0927	21
17	15.2		130.7	0.997	0.0061	21
17	15.2	22.8	119.26	1.195	0.1612	23
17	15.2	23	123.95	1.205	0.1605	23
17	15.2	22	99.83	1.207	0.0277	25
18	15.2	21.6	115.6	2.421	1.3415	15
18	15.2	24.2	149.5	2.411	0.7473	18
18	15.2	23.1	130.3	2.407	0.6791	21
18	15.2	23.5	118.3	2.182	0.2143	24
18	15.2	19	91.94	2.416	0.7242	24
18	15.2	21.5	108.78	2.407	0.4905	27
18	15.2	21.5	136.5	2.396	0.2054	30.5
18	15.2	21.6	108.15	2.404	0.3827	30.5
18	15.2	22.8	117.06	2.397	0.3993	33
18	15.2	23.5	127.8	2.404	0.1452	33
18	15.2	22.3	120.1	2.408	0.1361	34
18	15.2	22	110.8	2.407	0.1488	34
18	15.2	23.5	126.5	3.185	0.3809	34
18	15.2	94.97	21.5	2.399	0.3516	35
18	15.2	23.5	117.52	2.403	0.186063	35

CURRICULUM VITAE

Umar KHAN graduated from the Government Higher Secondary School No.1 Peshawar Cantt., Pakistan in 2005. He received his B.Sc. in Biological Sciences and M.Sc. in Zoology (Specialization: Fisheries) from the University of Peshawar, Pakistan in 2009 and 2011, respectively. Until 2013, he worked as a senior biology teacher in a higher school where he taught GCSE O & A level. In 2013, he received a fully funded Ph.D. Scholarship from Türkiye Bursları (government-funded, competitive scholarship program), awarded to outstanding students to pursue a degree full-time at Turkish universities. His research interests include aquaculture and quantification of trophic links between predators and prey. He is fluent in five languages: English, Turkish, Pashto, Persian, and Urdu/Hindi. His publications which are SCI indexed include:

From current PhD thesis:

1. **Khan, U.,** Seyhan, K. (2019). Gastric evacuation rates in farmed brook trout subjected to a range of feeding conditions fed commercial pellets. Aquaculture, 513, 734390. http://bit.ly/2QL22pD
2. **Khan, U.,** Seyhan, K., Başçinar, N. and Başçinar, N. S. (2016). Satiation meal and the effects of meal and body sizes on gastric evacuation rate in brook trout *Salvelinus fontinalis* fed commercial pellets. *Journal of Fish Biology*, 89, pp.1227–1238. http://bit.ly/2CTtPwP

Some other publications:

3. Seyhan, K., Başçinar, N. S., Başçinar, N. and **Khan, U.** (2020). Gastric Evacuation in Brook Trout (*Salvelinus fontinalis*) Fry: Effect of Body Size. *Pakistan Journal of Zoology*. http://bit.ly/34xfUr6

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