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Evacuation of pelleted feed and the suitability of titanium(IV) oxide as a feed marker for gut kinetics in Nile tilapia

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The present study assessed the suitability of titanium(IV) oxide, TiO_2, as a digesta passage marker in Nile tilapia *Oreochromis niloticus* and studied the shape of the evacuation curve in this species. In three separate trials, fish were given one dose of either 0·5, 0·25 or 0·1% of their body mass (% BME) of feed marked with 1% TiO_2 or 0·5% BME of the same feed without marker. The fish were serially slaughtered at intervals after feeding and the stomach contents analysed for dry mass and marker content. The data for individual trials were analysed with the linear, square root, surface area and exponential evacuation models and variable comparisons showed that, although the marker interfered slightly with the evacuation process, true meal size could be predicted more accurately from the marker data. The results of an analysis of the combined data sets suggested that stomach evacuation in this species is dependent more on food particle surface area (surface area model) than on stomach content mass (exponential model) as is generally assumed. On the basis of these results, it was concluded that TiO_2 at an inclusion level of 1% is an acceptable marker for quantifying evacuation with a view to predicting food consumption but should be used with caution in digestibility studies.

Key words: feed marker; mathematical modelling; Nile tilapia; stomach evacuation; titanium(IV) oxide.

INTRODUCTION

Fisheries biologists have in the past used a number of models to estimate food consumption in fishes (Bajkov, 1935; Eggers, 1977, 1979; Elliott & Persson, 1978; Olson & Mullen, 1986; Sainsbury, 1986). These work on similar assumptions, namely that changes in stomach fullness over time reflect the balance between ingestion and evacuation, that the evacuation rate is the same when the fishes are feeding or fasting and that different food items are evacuated independently of each other. Various forms of evacuation function have been proposed, most of which are based on the following generalized formulae:

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$$\frac{dS}{dt} = -ES^B \quad \text{for all } t \text{ before } S = 0 \tag{1}$$

$$\frac{dS}{dt} = 0 \quad \text{for all } t \text{ after } S = 0 \tag{2}$$

where S = stomach contents, t = time, E = instantaneous rate of evacuation and B = parameter quantifying the degree of dependency of evacuation on the level of stomach fullness

The most commonly applied form of the model assumes that $B = 1$ (simple exponential evacuation), in which case, S is mathematically always positive so that equation 2 never applies. Other versions have fixed B at values of zero (linear model, Olson & Mullen, 1986), 0·5 (square root model, Jobling & Davies, 1979; Jobling, 1981), 0·67 (surface area model, Fänge & Grove, 1979; Flowerdew & Grove, 1979; Salvanes *et al.*, 1995) or allowed B to vary as a variable (Jones, 1974; Temming & Andersen, 1994). There has been some debate as to which model is the most appropriate and it appears that none is universally applicable. Jobling (1987) presented evidence in favour of a theory that small particles of a low energy density, *e.g.* zooplankton, should be evacuated exponentially ($B = 1$) whereas large particles of high energy density, *e.g.* fish prey, should be evacuated linearly ($B = 0$) with intermediate forms possible ($0 < B < 1$).

Independent of which model is applicable to a certain combination of predator and prey type, Richter *et al.* (2002) demonstrated that when fishes show well defined feeding periodicity throughout the analytical period or consume multiple meals between which the stomach does not empty completely, the evacuation rate in the feeding period may be distinctly greater than that in the fasting period, leading to serious underestimations of food consumption. This is most likely to affect fishes consuming small particles such as zoo- or phytoplanktivores, which include some of the most important fish groups in international aquaculture, *e.g.* Nile tilapia *Oreochromis niloticus* (L.), milkfish *Chanos chanos* (Forsskål), silver carp *Hypophthalmichthys molitrix* (Valenciennes) and bighead carp *Aristichthys nobilis* (Richardson). It is unfortunate that in stomach content modelling, the evacuation rate is almost always determined in a period when the fish is fasting since it is not possible to assess the discrepancy by this approach. The only model attempting a separate determination of evacuation in feeding and fasting periods was that of Moriarty & Moriarty (1973) applied to Nile tilapia and *Enterochromis* (=*Haplochromis*) *nigripinnis* (Regan) which was based on the separate analysis of stomach and intestine, both empty at the start of the feeding period. Food consumption was assumed to be equivalent to the rise in total digestive tract content until defecation commenced, after which the gut contents were extrapolated until the end of the feeding phase. This was obtained by determining at what point in time the stomach contents started to decline. The model was also applied to Nile tilapia by Harbott (1975) and Getachew (1989).

The main source of error in the model of Moriarty & Moriarty (1973) is that it completely failed to account for assimilation in the gut. If this factor had been corrected for, the extrapolated level of gut fullness would have been rather higher towards the end of feeding, leading to higher ration estimates. The

model also assumed stomach evacuation in tilapia to be linear which is not supported by the findings of other workers on tilapia (Palomares & Pauly, 1996; Richter *et al*., 1999, 2002) as well as the general hypothesis of Jobling (1987) that small particles are evacuated exponentially. Nevertheless, the model is attractive to anyone estimating food consumption in fish species likely to have different evacuation rates in feeding and non-feeding periods and should not be discarded. It is probable that errors due to assimilation could be eliminated by using an indigestible marker. When assessing food consumption in wild fishes, a marker inherent to the food such as the various types of ash or fibre (Jones & de Silva, 1998) would have to be used. In testing the model under laboratory conditions, it would be easier to use pelleted feed, which would also allow the use of an external marker.

In studying fish nutrition, the most commonly applied external marker is chromium(III) oxide, Cr_2O_3, which is generally analysed spectrophotometrically after oxidation to Cr^{6+} (Marczenko, 1986). This, however, requires the use of powerful and potentially hazardous oxidizing agents such as perchloric acid, $HClO_4$, or ammonium persulphate ($[NH_4]_2S_2O_8$). Oxides of rare earth metals have also been used (Storebakken *et al*., 1999; Austreng *et al*., 2000) but these were included at low levels and analysed by atomic absorption spectrometry, a technique which may not be available to most fisheries scientists. Titanium(IV) oxide, TiO_2, determined photometrically, has been used as an alternative to Cr_2O_3 but so far only to assess digestibility, *e.g*. in Atlantic cod *Gadus morhua* L. (Lied *et al*., 1982) and rainbow trout *Oncorhynchus mykiss* (Walbaum) (Weatherup & McCracken, 1998).

The aim of the present study was to investigate TiO_2 as a potential marker for digesta kinetics and to analyse which form of evacuation most likely applies to Nile tilapia given pelleted feed. The criteria, which the marker would have to fulfil, and which were to be tested for were: (1) whether recovery was 100%, (2) whether the presence of a marker in feed influenced the evacuation of the other feed components and (3) whether the marker was evacuated at the same rate as the other feed components.

MATERIALS AND METHODS

FEED

Two types of pelleted feed based on the Hohenheim standard feed were used for the feeding experiments. The first included 1% TiO_2 as an indigestible marker (STD-M) whereas the other was made up without this substance (STD-U). Details of the other components and the proximate composition are given in Table I. The feed components were thoroughly mixed, the mixture made into 3 mm pellets after the addition of minimal amounts of water and the pellets freeze-dried. After drying by lyophilization, the feed was stored at −18° C until used.

EXPERIMENTAL PROCEDURE

Nile tilapia of *c*. 200–300 g body mass were kept individually in 45 l aquaria attached to a recirculating system. Four experimental runs were carried out and all the fish for one run were sampled on the same day. The fish were allowed to acclimatize for *c*. 2 weeks during which they were fed daily and monitored for their speed of food uptake. Each

TABLE I. Ingredients of feeds used in the feeding trials

	STD-M (%)	STD-U (%)
Fish meal (65% protein)	50	50
Wheat meal	41	42
Vitamin premix	2	2
Mineral premix	2	2
Sunflower oil	4	4
Titanium(IV) oxide	1	—

STD-M, 1% TiO_2 as an indigestible marker; STD-U, without the marker.

experiment was delayed until it was certain that all fish would consume all the food introduced to the aquarium without hesitation. In the case of mouthbrooding females, the eggs were flushed out of the oral cavity to induce the fish to resume feeding as soon as possible. The aquarium outflows were covered with a guard to make sure that no food would be swept out of the tank before being eaten.

Of the four trials, three were carried out with STD-M with ration sizes of 0·5, 0·25 and 0·1% body mass equivalent (% BME) and one with STD-U at 0·5% BME. The water temperature was kept constant at 27 range $\pm 1^\circ$ C throughout each trial as well as between experiments and the dissolved oxygen level was kept close to saturation by the recirculating system. The number of fish varied between trials; most were carried out with 24 fish but for the first trial (STD-U, 0·5% BME) 45 fish were available. Fish were sampled in groups of three replicate fish; on rare occasions, female fish had laid eggs the night before the experiment and had to be discarded from the analysis. Because of the necessity of the acclimatization procedure, it was not possible to replace these fish at short notice and only two replicate fish were sampled at the time for which the discarded fish had been intended. For each trial, the fish were starved for 36 h prior to the experiment to make sure that their stomachs were empty. During this fasting period, >12 h before the start of the experiment, they were also blotted dry and weighed to the nearest 0·1 g. This was done to be able to adjust the individual experimental ration to a certain percentage of their body mass in order to eliminate possible differences in stomach evacuation due to variations in body size. Since the range of body sizes was rather less than an order of magnitude, it would have been meaningless to include this variable in the evacuation model and adjusting the ration to the body size was considered the best way of eliminating this factor altogether. Nile tilapia are robust fish and will readily take food soon after return to the aquaria following such a weighing process (unpubl. obs.) so that it is highly unlikely that the results will have been influenced by stress.

On the day of the experiment, the aquaria were cleaned by siphoning off any visible faeces. About 15 min afterwards, the fish were simultaneously given their preweighed doses of feed (nearest 1·0 mg), following which they were killed at intervals of 0·5–2 h starting 15 min after feeding and the viscera removed and preserved in 90% ethanol. At a later date, the preserved stomachs were dissected from the viscera and the contents carefully flushed into preweighed containers with distilled water. These were then warmed overnight at 70° C to remove traces of alcohol, frozen and lyophilized and the mass of the stomach contents determined as the difference between the full and empty container.

TIO_2-ANALYSIS

Since no specific protocol for the analysis of TiO_2 in fish stomach contents appears to have been published, the following methodology was developed for quantifying this substance in freeze-dried gut contents derived from pelleted feed. In the case of the fish

given marked feed, two replicate subsamples of the dried stomach contents were weighed (nearest 0·1 mg), and each digested in 10 ml 96% sulphuric acid (H_2SO_4) in the presence of a Kjeltab catalyser tablet at 400° C in a Kjeldahl digestion unit for 3 h to oxidise organic matter and dissolve the marker. After cooling, the solution was transferred to a 25 ml flask and made up with distilled water. A 1 ml aliquot was transferred to a test tube, 0·1 ml hydrogen peroxide (H_2O_2) added, the solution mixed and allowed to stand for 1 h, after which the absorption of the yellow TiO_2-H_2O_2 complex was measured at 405 nm using a spectrophotometer. The marker quantity in the 1 ml aliquot was calculated from the absorption at 405 nm using the following equation calculated by linear regression from a standard solution: $Marker\ [\mu g\,ml^{-1}] = 108{\cdot}1\ (Abs_{405}) - 0{\cdot}155$. This quantity was then multiplied by 25 to obtain the total quantity of marker in the subsample after which the proportion of marker in the stomach contents was determined from the mass of the subsample. An average of the two subsamples was then calculated, allowing the determination of the mass of TiO_2 in the total stomach contents.

To test the marker recovery, it was essential to know how much TiO_2 had been evacuated into the gut. For this purpose, the whole intestine was dried at 105° C after the stomach, liver and most of the intestinal fat had been removed, ashed and the TiO_2 determined by digestion and spectrophotometry as for the stomach contents. This gave a value in total μg TiO_2 which was later recalculated as percentage BME; since the entire gut was used, no replication was possible here.

DATA ANALYSIS

The marker recovery data was analysed by linear regression to test for systematic loss of TiO_2 over time. The expected regression coefficient was zero and significant deviations from this value were tested for by means of a *t*-test (Sokal & Rohlf, 1995).

Equation 1 may be integrated to give:

$$\text{if } B \neq 1 : S = \left[S_0^{(1-B)} - (1-B)Et\right]^{[1-B]^{-1}} \quad \text{for all } t \text{ before } S = 0 \tag{3}$$

$$S = 0 \quad \text{for all } t \text{ before } S = 0 \tag{4}$$

$$\text{or if } B = 1 : \quad S = S_0 e^{-Et} \tag{5}$$

Andersen (1998) pointed out that, since the variability of stomach content data is greater at high initial meal sizes, the data should be divided by S_0 in order to improve homogeneity of variances to give:

$$SS_0^{-1} = \left[1 - (1-B)S_0^{(B-1)}Et\right]^{(1-B)^{-1}} \tag{6}$$

$$SS_0^{-1} = e^{-Et} \tag{7}$$

Despite the fact that meal size was known, however, it was decided to estimate this variable in some form in the present set of data in order to test for systematic deviations from the expected value. The variable S_0 was modified to S'_0M where M was the meal size so that S'_0 represented the predicted intercept on the *y*-axis as the proportion of the initial meal size, the expected value always being 1·0 regardless of meal size M. This gave the following general models:

$$SM^{-1} = S'_0\left[1 - (1-B)(S'_0M)^{(B-1)}Et\right]^{(1-B)} \tag{8}$$

$$SM^{-1} = S'_0e^{-Et} \tag{9}$$

Equations 8 and 9 were applied to the individual data sets for different meal sizes (both dry and marker masses), Equation 8 with B fixed to zero, 0·5 or 0·67. One important

aspect of the work was the comparison of the evacuation rates E for the same trial and evacuation model between dry mass and marker data. Since the E values obtained from Equation 8 are mass specific and the marker masses of the stomach contents were expected to be one hundred times lower than the dry masses, a comparison would not have been directly possible. To facilitate the comparison, the original S values for the marker masses were multiplied by 100 and the meal sizes M set to the same values as for the dry masses for the respective trials.

The combined data sets for different meal sizes were also used to investigate the shape variable B further. For this purpose, Equation 8 was applied to both the dry mass data (all four trials) as well as the marker data (three trials with STD-M) with B being allowed to vary (hereafter called the unexpanded model). A further analysis was done to analyse the effect of meal size on the evacuation rate E by splitting this into $E'M^D$ where D denoted the dependency of E on meal size, giving rise to the following (hereafter called the expanded model):

$$SM^{-1} = S_0' \left[1 - (1 - B) S_0'^{(B-1)} M^{(D+B-1)} E' t\right]^{(1-B)^{-1}} \tag{11}$$

All analyses were carried out using the NLIN procedure (method = dud) of SAS® (1989). The software determined the best fits on the basis of the lowest sum of squared residuals, which are included to facilitate a comparison between different model predictions related to the same data set. To test for differences between evacuation of marker and other stomach contents as well as the effect of marker inclusion on evacuation, the variable estimates were compared between data sets for marker and dry masses at the same meal size as well as for dry masses between marked and unmarked feed. The t-test for unequal sample sizes was used for these comparisons, applying the variable estimates and S.D. determined by multiplying the asymptotic S.E. given by the SAS® (1989) output by the square root of the number of data points. The significance of the deviation of the S_0 values from the expected value of 1·0 is reflected by whether this value falls in- or outside their 95% CL (equivalent to a t_s-test, Sokal & Rohlf, 1995).

RESULTS

The marker recovery differed between trials and sampling times (Table II). The low regression coefficients of marker recovery over time, none of which differed significantly from the expected value of zero (t-test, all $P > 0{\cdot}05$), rule out systematic errors such as a loss of marker due to assimilation. The average level of recovery was a little lower at 0·1% BME than at 0·25 or 0·5% BME. Although the marker had been included at a level of 1% in the feed, the concentration in the stomach contents was frequently found to be a little higher, *c.* 1·1%. The method of gut marker analysis did not permit an estimation of marker concentration, but any such figures would have been meaningless in themselves because of the confounding effects of the presence of gut contents from earlier unmarked feed on the one hand and assimilation of digested material on the other.

The stomach content dry masses recorded in the various trials are shown in Fig. 1. Despite the scatter around the means, it is possible in all cases to discern the general evacuation trajectory. One fish [at $t = 0{\cdot}25$ h given 0·25% BME, Fig. 1(b)] was found to have stomach contents greatly in excess of the amount of food provided and was discarded from the modelling analysis of the dry mass data. The model predictions are summarized in Table III; Fig. 1 also includes the trajectories of the best fit based on the model predictions. The predicted

TABLE II. Average marker percentage recovery values of Nile tilapia given different meal sizes of marked feed (marker inclusion level: 1%) and linear regression coefficients (±S.D.) of recoveries against time. All coefficients were not significantly different from zero (*t*-test, $P > 0{\cdot}05$)

	Recovery (%)		
Time after feeding (h)	0·5% BME	0·25% BME	0·1% BME
0·25	93·6	105·2	94·9
1·25	90·7	77·9	85·9
2·25	87·7	90·3	78·3
3·25	80·6	96·3	75·0
4·25	92·4	83·9	85·3
5·25	86·8	82·3	86·2
6·25	—	87·2	83·3
7·25	90·6	90·6	88·9
9·25	93·2	—	—
Overall average recovery	89·5	89·2	84·7
(±S.D.)	(±4·4)	(±8·6)	(±6·1)
Regression coefficient	0·18	−1·09	−0·25
(±S.D.)	(±0·58)	(±1·36)	(±1·02)

y-axis intercepts (S'_0 values) were consistently lower than the expected value of 1·0 and the deviation was statistically significant for all models at a ration level of 0·5% BME as well as in all cases when the linear model was applied. On the basis of the sum of squares residual (SSR) values, no model consistently gave the best fit. The exponential model gave the best fit at 0·1 and 0·5% BME when STD-M was used while the linear and square root models fitted best when STD-U was given at 0·5% BME and STD-M at 0·25% BME, respectively. There was an inverse relationship between evacuation rate and meal size, as shown by the fact that the values for E increased at lower meal sizes. A comparison between the dry matter evacuation rates for marked and unmarked feed showed that the E values were 10–20% higher for unmarked feed for all models except the linear model, the differences being significant in all cases except for the linear function (Table IV). The S'_0 values were higher when marked feed was used and this difference was significant for all models (Table IV).

The stomach marker masses are shown in Fig. 2, which also include the best fit trajectories of the various model predictions. In general, the data match that for stomach content dry masses closely, the exception being the aberrant fish in the data for 0·25% BME ration level, which had a stomach marker content closer to the expected value and was included in the analysis of the TiO_2 data. The results of the regression analyses are also given in Table III. The S'_0 estimates were clearly *c.* 5–10% higher than those obtained from the dry mass data, reflecting the fact that the marker concentration in the stomach content was 1·1% as opposed to an inclusion rate of 1% in the feed. These differences were all significant except at 0·1% BME for the exponential model (Table IV). The evacuation rates were also significantly higher when based on the marker

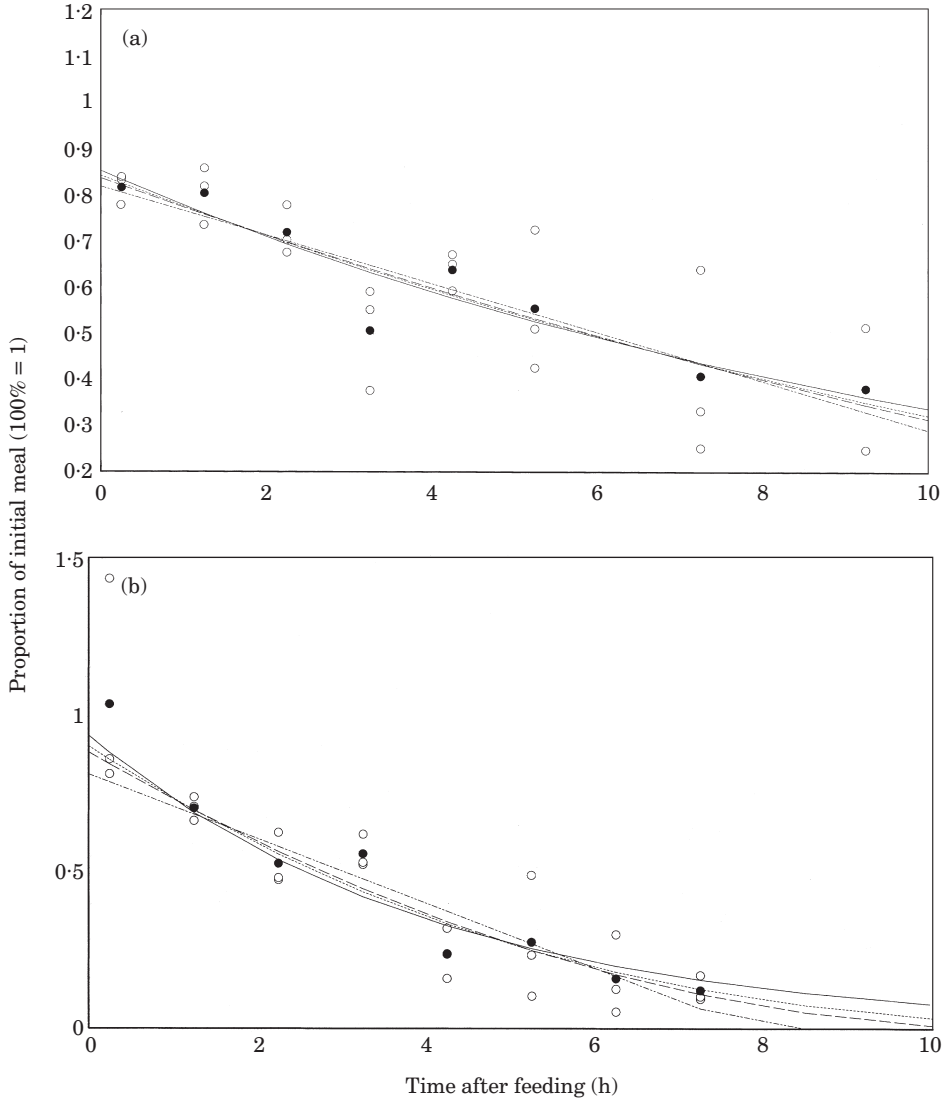

FIG. 1. Continued.

data except at 0·5% BME as well as for the exponential model at 0·25% BME (Table IV). The best fits were provided by the same models as for stomach content dry matter except at a meal size of 0·1% BME, when the surface area model gave a better fit than the exponential. The negative correlationship between meal size and evacuation rate was also found for the marker masses.

The results of the regression on combined data from the various feeding trials is summarized in Table V. The subsample averages and the trajectories determined from the model predictions are given in Fig. 3, which for the sake of clarity, excludes the data for individual fish. The shape variable B was clearly lower in the unexpanded model than in the expanded one, reflecting the

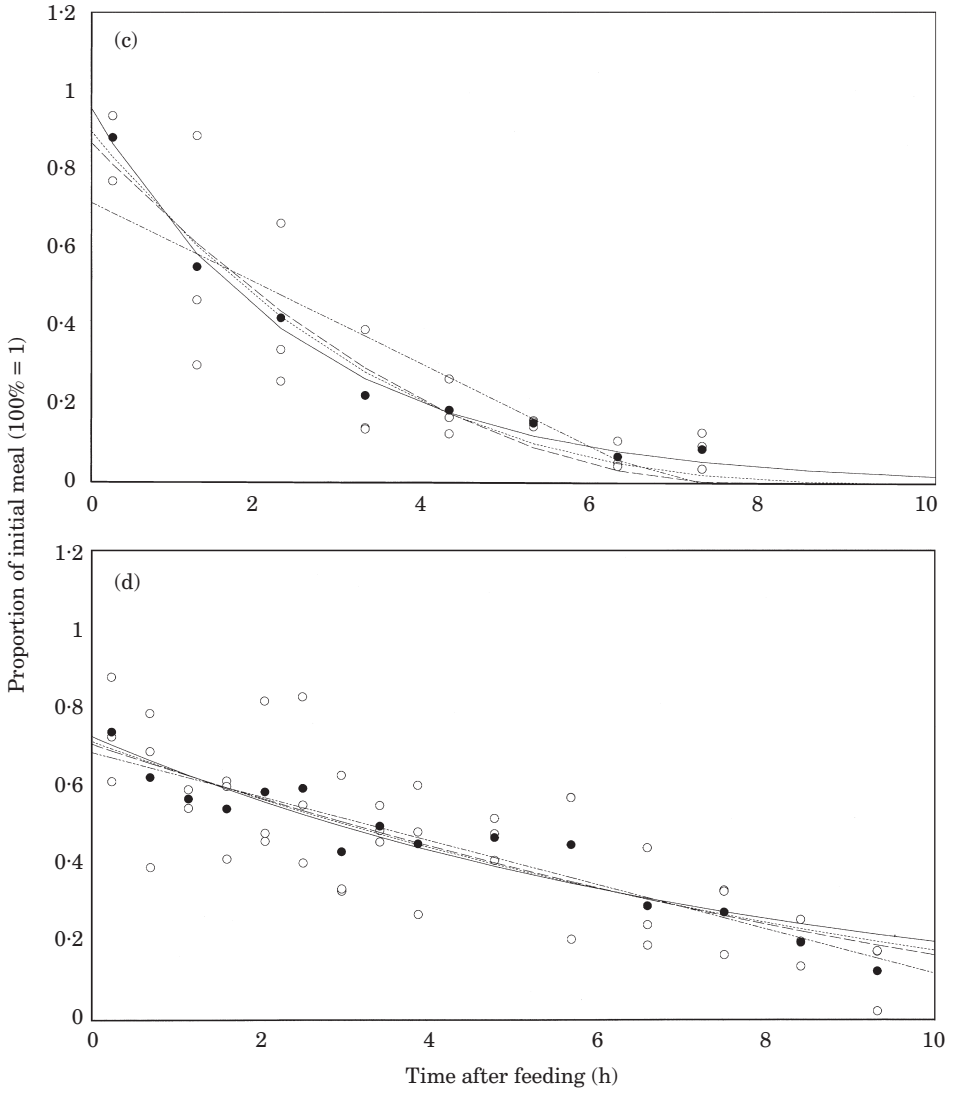

FIG. 1. Observed data (○), subsample averages (●) and trajectories of the best fits of the linear (— -- —), square root (— — —), surface area (------) and exponential (——) models for the stomach content dry masses of fish fed STD-M at (a) 0·5, (b) 0·25 and (c), 0·1% BME, or (d) STD-U at 0·5% BME. Single fish given 0·25% BME at $t = 0{\cdot}25$ h with abnormally high stomach contents were excluded from analysis (see text for details).

negative correlation of evacuation rate with meal size. The S'_0 estimate was again lower when calculated from stomach content dry matter than when calculated on the basis of the marker data. When the expanded model was applied, the shape variable obtained from both dry or marker mass data most closely approached the surface area model (expected $B = 0{\cdot}67$) although the 95% CL also included the expected values for the exponential and square root models.

TABLE III. Variable predictions, 95% CL (in parentheses) and goodness of fit (sum of squared residuals, SSR) of the analysis on individual data sets of stomach content dry masses (DM) or marker masses (TiO_2) for two feed types (STD-M, STD-U) at three different ration levels (0·5, 0·25 and 0·1% BME)

	STD-M						STD-U
	0.5% BME		0.25% BME		0.1% BME		0.5% BME
Model	DW ($n=23$)	TiO_2 ($n=23$)	DW ($n=23$)	TiO_2 ($n=24$)	DW ($n=24$)	TiO_2 ($n=24$)	DW ($n=44$)
Linear							
S_0	0·82 (0·73–0·91)	0·89 (0·80–0·99)	0·81 (0·72–0·91)	0·90 (0·78–1·02)	0·79 (0·58–0·85)	0·86 (0·58–0·90)	0·68 (0·61–0·75)
E	0·026 (0·017–0·036)	0·028 (0·018–0·039)	0·026 (0·020–0·031)	0·030 (0·023–0·036)	0·014 (0·010–0·019)	0·018 (0·011–0·025)	0·026 (0·019–0·032)
SSR	0·292	0·338	0·247	0·471	0·526	0·683	0·703
Square root							
S_0	0·84 (0·74–0·93)	0·91 (0·81–1·02)	0·88 (0·77–0·99)	0·98 (0·84–1·11)	0·87 (0·71–1·02)	0·92 (0·73–1·12)	0·70 (0·62–0·79)
E	0·050 (0·032–0·068)	0·052 (0·033–0·071)	0·084 (0·066–0·102)	0·093 (0·070–0·115)	0·076 (0·054–0·098)	0·089 (0·058–0·120)	0·056 (0·040–0·071)
SSR	0·286	0·323	0·224	0·428	0·430	0·634	0·720

Surface area							
S_0	0·84	0·92	0·90	1·00	0·90	0·95	0·71
	(0·74–0·94)	(0·82–1·03)	(0·78–1·02)	(0·85–1·14)	(0·74–1·05)	(0·74–1·15)	(0·63–0·80)
E	0·061	0·063	0·121	0·133	0·132	0·152	0·072
	(0·039–0·084)	(0·041–0·086)	(0·094–0·148)	(0·099–0·166)	(0·095–0·170)	(0·099–0·206)	(0·051–0·092)
SSR	0·284	0·320	0·227	0·428	0·402	0·629	0·727
Exponential							
S_0	0·85	0·94	0·93	1·04	0·96	1·00	0·72
	(0·75–0·96)	(0·82–1·05)	(0·80–1·07)	(0·87–1·20)	(0·78–1·13)	(0·77–1·24)	(0·63–0·82)
E	0·092	0·093	0·247	0·265	0·395	0·442	0·117
	(0·057–0·127)	(0·059–0·127)	(0·188–0·307)	(0·194–0·336)	(0·283–0·507)	(0·278–0·606)	(0·081–0·153)
SSR	0·283	0·314	0·243	0·440	0·371	0·645	0·742

TABLE IV. Level of significance of the differences in predicted *y*-axis intercept, S'_0 and evacuation rate, E, between dry mass (DM) and marker (Ti) data at the three different meal sizes as well as between marked (STD-M) and unmarked feed (STD-U) at 0·5% BME (*t*-test, d.f. $= n_1 + n_2 - 2$; NS, not significant; *, $0{\cdot}01 < P \leq 0{\cdot}05$; **, $0{\cdot}001 < P \leq 0{\cdot}01$; ***, $P < 0{\cdot}001$)

Model	0·5% BME (DM *v.* Ti)	0·25% BME (DM *v.* Ti)	0·1% BME (DM *v.* Ti)	0·5% BME (STD-M *v.* STD-U)
d.f.	44	45	46	65
Linear				
S'_0	***	***	**	***
E	N.S.	***	***	N.S.
Square Root				
S'_0	***	***	*	***
E	N.S.	**	**	**
Surface Area				
S'_0	***	***	*	***
E	N.S.	*	**	***
Exponential				
S'_0	***	***	N.S.	***
E	N.S.	N.S.	*	***

DISCUSSION

SUITABILITY OF MARKER

The high degree of scatter of the stomach content data around the means compared to trials in which fish are fed large particles (Andersen, 1998, 1999; Temming & Herrmann, 2001) can probably be attributed to the nature of the food. Soon after ingestion, pelleted feed is mixed with water or digestive juices so that it is quickly reduced to a semi-fluid paste with a dry matter content of only 20–25% (unpubl. data). If the stomach contractions of any fish sampled in a given sample are only slightly stronger than those in another replicate fish, it is likely that correspondingly more food will be evacuated, leading to more scatter in the data. It is possible that this problem could be reduced by analysing more fish per subsample. Nevertheless, despite the variability around the means, the S.E. to the variable estimates were low enough to highlight significant differences between data sets.

The use of a marker did not help to reduce this scatter which, if anything, increased when low quantities of TiO_2 were used. The single exception was the fish fed 0·25% BME, which was found to have stomach contents close to 150% of the meal size soon after feeding, probably due to the incidental ingestion of extra material such as faeces voided when ingesting the pellets or eggs remaining in the oral cavity after inefficient removal of ova from a mouthbrooding female. In such cases, as well as in fishes with substantial mucus secretion in the gut (*e.g.* common carp *Cyprinus carpio* L., unpubl. data), the use of a marker can be

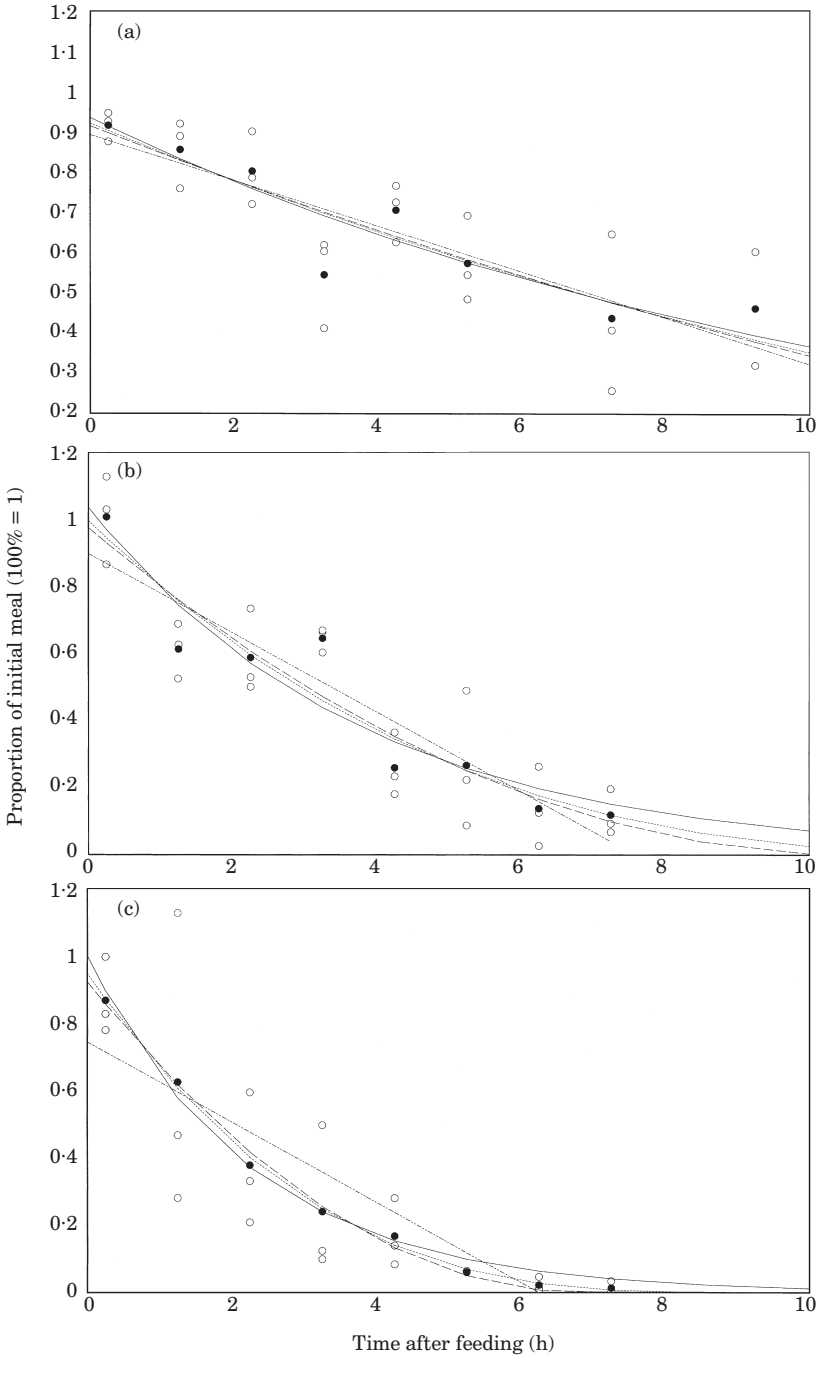

FIG. 2. Observed data (○), subsample averages (●) and trajectories of the best fits of the linear (— -- —), square root (— — —), surface area (------) and exponential (——) models for the stomach content marker masses of fish fed STD-M at (a) 0·5, (b) 0·25 or (c) 0·1% BME. Single fish given 0·25% BME at $t = 0{\cdot}25$ h with abnormally high stomach contents [*cf.* Fig. 1(b)] did not have abnormally high marker content and was included in the analysis (see text for details).

TABLE V. Variable predictions, 95% CL (in parentheses) and goodness of fit (sum of squared residuals, SSR) obtained by modelling the combined data of all relevant trials applied to stomach content dry masses, DM (all four trials) or marker masses, TiO_2 (three trials with STD-M) with or without meal size as a variable (expanded and unexpanded models respectively)

	Expanded		Unexpanded	
	DM	TiO_2	DM	TiO_2
S_0	0·81	0·97	0·79	0·93
	(0·74–0·88)	(0·87–1·07)	(0·74–0·85)	(0·85–1·01)
E	—	—	0·058	0·050
	—	—	(0·040–0·075)	(0·030–0·069)
E'	0·068	0·059	—	—
	(0·037–0·098)	(0·033–0·086)	—	—
B	0·70	0·73	0·44	0·28
	(0·13–1·27)	(0·16–1·31)	(0·32–0·56)	(0·13–0·43)
D	−0·30	−0·54	—	—
	(−0·96–0·36)	(−1·25–0·16)	—	—
SSR	1·999	1·489	2·016	1·535

helpful in distinguishing food from other material providing other prerequisites are met.

The marker recovery rates recorded here are of the same order as those found in some other, larger animals in which much greater absolute quantities were analysed. Short *et al.* (1996) recorded average values of 99·3% in chickens (0·5% TiO_2 in feed) but Titgemeyer *et al.* (2001) obtained a much lower average (92·8%) in cattle which was nearly identical to that observed by Kavanagh *et al.* (2001) in pigs (92·3%). Barton & Houston (1991) obtained such low values from combined faeces and regurgitated pellets in birds of prey (range: 44·0–81·8%) that they concluded that the marker was retained in the stomach, leading them to reject this substance as a marker in such animals. Weatherup & McCracken (1998) did not assess percentage recovery in rainbow trout but recorded higher Cr_2O_3 : TiO_2 ratios in faeces than in feed, indicative of some loss or incomplete assessment of TiO_2. Their inclusion level of Cr_2O_3, however, was higher (3%) than that of TiO_2 (1%) and the former was analysed by a more sensitive method (atomic absorption spectrometry) than the latter (spectrophotometry). The present results suggest that when low absolute quantities of TiO_2 are analysed, recovery is reduced, which could help to explain the findings of Weatherup & McCracken (1998). In the fish analysed here, the recovery was generally low in the middle of the trial when most of the marker was still in the stomach but some had already moved into the intestine. This suggests that detection of small quantities of TiO_2 in the stomach can be done with greater accuracy than that of similar quantities in the latter part of the intestinal tract. The gut analysis should probably also be carried out by dissection and subsampling, rather than by drying, ashing and analysis of the entire sample.

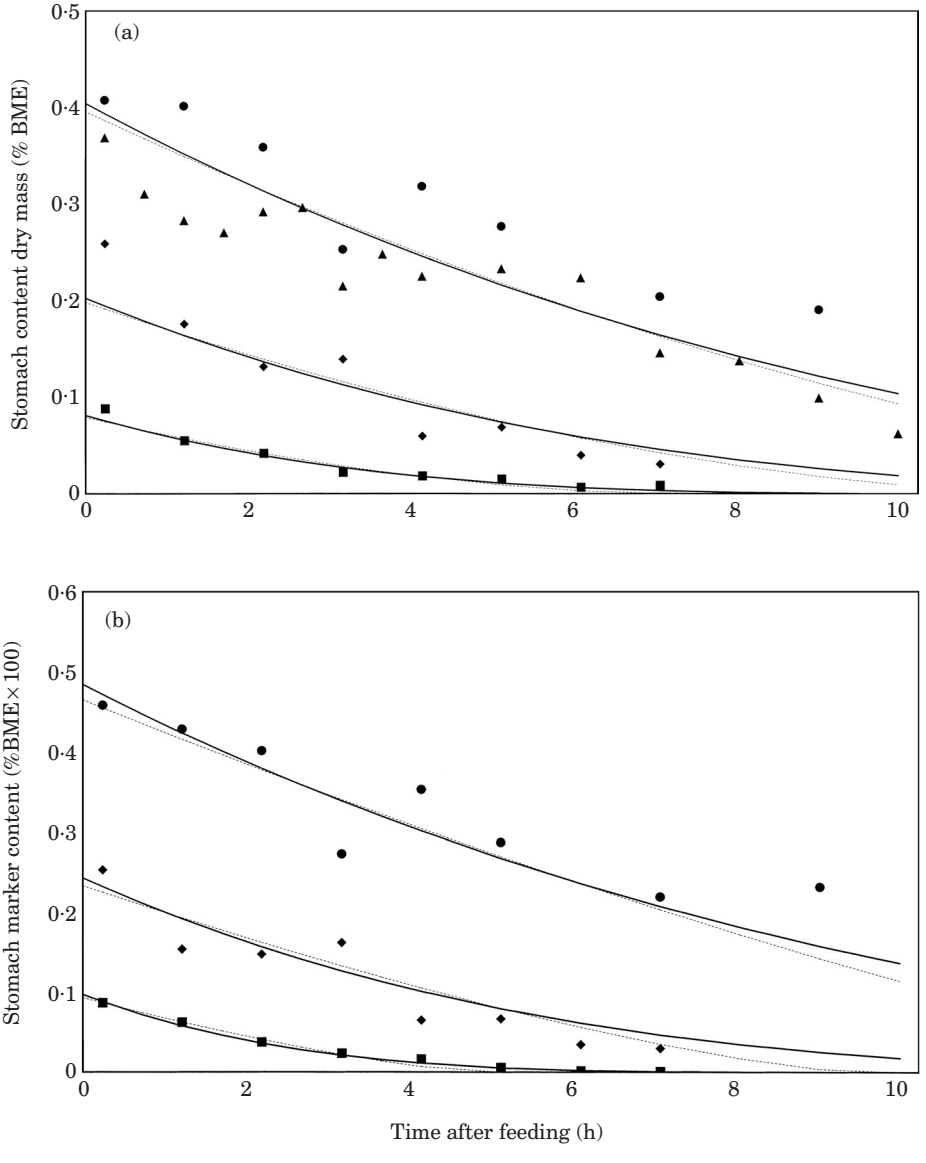

FIG. 3. Averages of observed data (●, 0·5% BME STD-M; ◆, 0·25% BME STD-M; ■, 0·1% BME STD-M; ▲, 0·5% BME STD-U) and trajectories of the predicted best fits of the expanded (——) and unexpanded (·····) models for stomach content (a) dry mass and (b) marker.

The increase in marker concentration in the stomach contents relative to that in the feed and the associated higher S_0 values obtained from the marker data compared to the dry masses poses some problems. It is possible that the preservation in alcohol may have removed lipids from the stomach contents before these were analysed. The crude lipid content of the feed was close to 10% so that the entire lipid fraction would have to have been removed for this to explain the increase in TiO_2 concentration. It is also known from studies on

other animals that the liquid fraction of the stomach contents, including the solubles and tiny, easily suspended particles (generally termed 'liquid phase') is evacuated at a faster rate than the large particles and indigestible matter ('solid phase') (Faichney, 1980, 1992; Caton *et al.*, 2000). In ruminants, this discrepancy is so great that different markers have to be used to label the two phases (Faichney, 1980, 1992). It is possible that the highly soluble components of the feed (*e.g.* some of the vitamins and mineral additives) were dissolved in the stomach soon after ingestion and were evacuated rapidly, in which case the E values measured here reflect only the evacuation of the less soluble matter. This would present a further difficulty in the general modelling of the evacuation of food consisting of small particles whose chemical constituents differ greatly in their digestibility. The possibility that some solubles leached from the pellets into the surrounding water cannot be ruled out but the fish were characteristically observed to take the feed well within a minute of it being given and all feed was consumed at the latest *c.* min after feeding. It should also be pointed out that as far as stomach content modelling is concerned, it is immaterial whether solubles are lost before ingestion or evacuated from the stomach soon after feeding.

A comparison of the evacuation rates for dry masses and TiO_2 determined for the individual data sets indicate that it is unlikely that the marker was retained in the stomach; if anything, the marker seemed to be consistently evacuated somewhat faster than the rest of the stomach contents. This discrepancy was most pronounced at lower meal sizes while at 0·5% BME, there was practically no difference in E values obtained from dry or marker masses. The higher evacuation rates for unmarked feed also suggest that the presence of the marker disturbs the digestive process to some extent so that evacuation is delayed. This effect would be expected to become more pronounced at higher marker inclusion levels, so that the marker recovery should probably not be raised by higher marker inclusion rates in the feed.

It is worth investigating the biological significance of the higher evacuation rates observed for either unmarked feed or for the marker when compared to the dry masses of marked feed. The fish were observed to take the feed readily so that unpalatability due to marker inclusion is unlikely to be a problem. The greatest difference in E values between respective dry mass and marker data was *c.* 15% (0·1% BME, surface area model) and that for dry masses between marked and unmarked feed *c.* 25% (exponential model). Richter *et al.* (2002) demonstrated that if the Bajkov model is used, a doubling of the evacuation rate would lead to a similar increase in the consumption estimate whereas in the case of the Elliott-Persson (Elliott & Persson, 1978) or MAXIMS (Sainsbury, 1986; Jarre *et al.*, 1991) models, such an increase in E would raise the consumption estimate by only *c.* 55% over a 5 h feeding period. The degree to which the differences in E will affect the consumption estimate therefore depends on the choice of model and in periodically feeding fishes, the Elliott-Persson and MAXIMS model are more likely to be chosen than the Bajkov model. More importantly, it should also be remembered that the accuracy of the daily ration estimate is linked to the ability of the model to predict the meal size at time $t = 0$ and this was found to be distinctly better when the marker data were analysed. In view of this, it would be expected that the evacuation rates based on dry

masses would underestimate food consumption. Moreover, the evaucation rate is merely a means to an end, that is to say an important variable in the ultimate goal of calculating food consumption, so its precise value is unimportant providing that E values determined from marked feed are not used on fishes given unmarked feed. The interfering effect which the marker seems to have on digestion is more likely to be of serious concern in studies in which digestibility is of importance. In view of these considerations, TiO_2 seems an acceptable marker for studying evacuation with a view to daily ration estimation but should be tested more thoroughly for use in digestibility studies.

FORM OF STOMACH EVACUATION FUNCTION

Although the respective fits to the data were nearly identical, the expanded model gave a rather different prediction for the variable B as well as for E than the unexpanded model, although the difference was less marked for E. This is understandable since the removal of the meal size dependency variable D from the equation mathematically forces alterations in B and E to compensate for the deficiency. Several workers have emphasized the need for practical evacuation models which may be applied to field data in order to estimate daily ration. Since the expanded model can only be used with prior knowledge of the variables that are being estimated, these scientists have concentrated their work on the unexpanded model (Temming & Andersen, 1994; Andersen, 1998; Temming & Herrmann, 2001), generally arriving at values of c. 0·5 for the shape variable B (Basimi & Grove, 1985; Grove *et al*., 1985). The results of the present analysis of the dry masses ($B = 0{\cdot}44$) is in line with the previous findings whereas the estimate obtained from the marker masses was lower ($B = 0{\cdot}28$). Nevertheless, by eliminating meal size and forcing other variables to mathematically explain the curvature of the evacuation function, a model with a sound physiological basis is not derived. The expanded model is better suited for this task and the B estimates obtained here and elsewhere (Flowerdew & Grove, 1979; Andersen, 1998) with this model suggest that evacuation is physiologically more surface related (expected B: 0·67).

In the present work, the CL of B calculated from both the expanded and unexpanded models ruled out only linear evacuation. The evacuation of small particles has frequently been thought to be physiologically exponential (Jobling, 1987; Temming & Herrmann, 2001) although the exponents obtained here satisfy a surface area model more closely. The physiology of evacuation, however, must be related to other, more complex factors. If the rate of digestion of a given food type at a given temperature by fish of similar mass of the same species is dependent only on the surface area of the food particles, a large number of food particles would require the same time span to be evacuated as a single particle of the same size. Since the pellets quickly crumbled in the stomach after ingestion, average food particle size in the present series of experiments may be assumed to be constant; nevertheless, it took longer to evacuate a large meal than a small one. The same reasoning may be used to question the exponential model: if, all other factors being equal, it always takes the same time to evacuate a given proportion (say 50%) of a certain type of food from the stomach, the instantaneous evacuation rates calculated with the

exponential model at different meal sizes should be equal, which was not the case. Some workers have concluded that the square root model generally gives the best fit (Jobling, 1981, 1986) and have attempted to provide a physiological basis for this model. Best fits given by the square root function, however, are often arrived at by eliminating meal size (Basimi & Grove, 1985; Grove *et al*., 1985; Andersen, 1998), making the physiological relevance dubious. Furthermore, the rationale of the square root model was tested by Persson (1986) and found to be questionable. Olson & Mullen (1986) put forward a model based on surface area proportional digestion in conjunction with a limitation in the availability of digestive juices during the earlier stages of the digestive process which resulted in a nearly linear evacuation function. This shows the negative effect of this limitation on the variable B and, if it was found to occur in practice, could explain better fits by models with variable values $<0{\cdot}67$.

While the variable estimates calculated with the unexpanded model could theoretically be used to calculate food consumption of Nile tilapia in the field, it is in practice probably easier to determine the evacuation rate directly from the field data in this species. Nile tilapia generally show clear feeding periodicity and this will become more pronounced in situations where pelleted feed is used since this is often given in distinct doses once or a few times over the daily cycle (unless demand feeders are used). This should result in long periods in which only evacuation takes place, allowing the relevant variables to be determined directly and eliminating the need for a temperature and fish size corrected evacuation rate. In view of this, the results of the unexpanded model are presented here only for the purpose of comparison with the expanded model and are not recommended for use in conjunction with field data to estimate daily rations.

In summary, TiO_2 appears to be an acceptable marker for investigating digesta passage in Nile tilapia with a view to estimating food consumption. Low absolute quanitites of marker can lead to poor recovery rates and higher inclusion rates should be investigated as a compensatory measure. Stomach evacuation in Nile tilapia is not linear but follows a curved pattern with the surface area model more likely than others to be appropriate. Before being used on this species, the model of Moriarty & Moriarty (1973) should therefore be amended to incorporate this feature.

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References

Andersen, N. G. (1998). The effect of meal size on gastric evacuation in whiting. *Journal of Fish Biology* **52,** 743–755.

Andersen, N. G. (1999). The effects of predator size, temperature and prey characteristics on gastric evacuation in whiting. *Journal of Fish Biology* **54,** 287–301 doi: 10.1006/jfbi.1998.0865.

Austreng, E., Storebakken, T., Thomassen, M. S., Refstie, S. & Thomassen, Y. (2000). Evaluation of selected trivalent metal oxides as inert markers used to estimate the apparent digestibility in salmonids. *Aquaculture* **188,** 65–78.

Bajkov, A. D. (1935). How to estimate the daily food consumption of fish under natural conditions. *Transactions of the American Fisheries Society* **65,** 288–289.
Barton, N. W. H. & Houston, D. C. (1991). The use of titanium dioxide as an inert marker for digestion studies in raptors. *Comparative Biochemistry and Physiology A* **100,** 1025–1029.
Basimi, R. A. & Grove, D. J. (1985). Gastric emptying rate in *Pleuronectes platessa* L. *Journal of Fish Biology* **26,** 545–552.
Caton, J. M., Lawes, M. & Cunningham, C. (2000). Digestive strategy of the south-east African lesser bushbaby, *Galago moholi*. *Comparative Biochemistry and Physiology A* **127,** 39–48.
Eggers, D. M. (1977). Factors in interpreting data obtained by diel sampling of fish stomachs. *Journal of the Fisheries Research Board of Canada* **34,** 290–294.
Eggers, D. M. (1979). Comments on some recent methods for estimating food consumption by fish. *Journal of the Fisheries Research Board of Canada* **36,** 1018–1020.
Elliott, J. M. & Persson, L. (1978). The estimation of daily rates of food consumption for fish. *Journal of Animal Ecology* **47,** 977–991.
Fänge, R. & Grove, D. J. (1979). Fish Digestion. In *Fish Physiology* Vol. VIII (Hoar, W. S., Randall, D. J., & Brett, J., eds), pp. 161–260 London: Academic Press.
Faichney, G. J. (1980). Measurement in sheep of the quantity and composition of rumen digesta and the fractional outflow rates of digesta constituents. *Australian Journal of Agricultural Research* **31,** 1129–1137.
Faichney, G. J. (1992). Application of the double-marker method for measuring digesta kinetics to rumen sampling in sheep following a dose of the markers or the end of their continuous infusion. *Australian Journal of Agricultural Research* **43,** 277–284.
Flowerdew, M. W. & Grove, D. J. (1979). Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. *Journal of Fish Biology* **14,** 229–238.
Getachew, T. (1989). Stomach pH, feeding rhythm and ingestion rate in *Oreochromis niloticus* L. (Pisces, Cichlidae) in Lake Awasa, Ethiopia. *Hydrobiologia* **174,** 43–48.
Grove, D. J., Moctezuma, M. A., Flett, H. R. J., Foott, J. S., Watson, T. & Flowerdew, M. W. (1985). Gastric emptying and the return of appetite in juvenile turbot, *Scophthalmus maximus* L., fed on artificial diets. *Journal of Fish Biology* **26,** 339–354.
Harbott, B. J. (1975). Preliminary observations on the feeding of *Tilapia nilotica* L. in Lake Rudolf. *African Journal of Tropical Hydrobiology and Fisheries* **4,** 27–37.
Jarre, A., Palomares, M. L., Soriano, M. L., Sambilay, V. C. Jr & Pauly, D. (1991). Some new analytical and comparative methods for estimating the food consumption of fish. *ICES Marine Science Symposia* **193,** 99–108.
Jobling, M. (1981). Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. *Journal of Fish Biology* **19,** 245–257.
Jobling, M. (1986). Mythical models of gastric emptying and implications for food consumption studies. *Environmental Biology of Fishes* **16,** 35–50.
Jobling, M. (1987). Influence of food particle size and dietary energy content on patterns of gastric evacuation in fish: test of a physiological model of gastric emptying. *Journal of Fish Biology* **30,** 299–314.
Jobling, M. & Davies, P. S. (1979). Gastric evacuation in the plaice, *Pleuronectes platessa* L.: effects of temperature and meal size. *Journal of Fish Biology* **14,** 539–546.
Jones, R. (1974). The rate of elimination of food from the stomachs of haddock *Melanogrammus aeglefinus*, cod *Gadus morhua* and whiting *Merlangius merlangus*. *Journal du Conseil International pour l'Exploration de la Mer* **35,** 225–243.
Jones, P. L. & de Silva, S. S. (1998). Comparison of internal and external markers in digestibility studies involving the Australian freshwater crayfish, *Cherax destructor* Clark (Decapoda, Parastacidae). *Aquaculture Research* **29,** 487–493.
Kavanagh, S., Lynch, P. B., O'Mara, F. & Caffrey, P. J. (2001). A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. *Animal Feed Science and Technology* **89,** 49–58.

Lied, E., Julshamn, K. & Braekkan, O. R. (1982). Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators chromium oxide, titanium oxide. *Canadian Journal of Fisheries and Aquatic Sciences* **39,** 854–861.

Marczenko, Z. (1986). *Separation and Spectrophotometric Determination of Elements*. Chichester: Ellis Horwood Ltd.

Moriarty, C. M. & Moriarty, D. J. W. (1973). Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. *Journal of Zoology, London* **171,** 15–23.

Olson, R. J. & Mullen, A. J. (1986). Recent developments for making gastric evacuation and daily ration determinations. *Environmental Biology of Fishes* **16,** 183–191.

Palomares, M. L. & Pauly, D. (1996). Models for estimating the food consumption of tilapias. In *The Third International Symposium on Tilapia in Aquaculture* (Pullin, R. S. V., Lazard, J. & Legendre, M., eds). *ICLARM Conference Proceedings* **41,** 211–222.

Persson, L. (1986). Patterns of food evacuation in fishes: critical review. *Environmental Biology of Fishes* **16,** 51–58.

Richter, H., Focken, U., Becker, K., Santiago, C. B. & Afuang, W. B. (1999). Analysing the diel feeding patterns and daily ration of Nile tilapia, *Oreochromis niloticus* (L.) in Laguna de Bay, Philippines. *Journal of Applied Ichthyology* **15,** 165–170.

Richter, H., Lückstädt, C., Focken, U. & Becker, K. (2002). Evidence of violation of a basic assumption in models currently used for food consumption estimation in fish. *Archive of Fishery and Marine Research* **49,** 171–188.

Sainsbury, K. (1986). Estimation of food consumption from field observations of fish feeding cycles. *Journal of Fish Biology* **29,** 23–36.

Salvanes, A. G. V., Aksnes, D. L. & Giske, J. (1995). A surface-dependent gastric evacuation model for fish. *Journal of Fish Biology* **47,** 679–695.

SAS (1989). SAS/Stat® *User's Guide*, *Version* 6, Vol. 2**.** Cary, NC: SAS Institute Inc.

Short, F. J., Gorton, P., Wiseman, J. & Boorman, K. N. (1996). Determination of titanium dioxide as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology* **59,** 215–221.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry*, 3rd edn. New York: W.H. Freeman & Co.

Storebakken, T., Kvien, I. S., Shearer, K. D., Grisdale-Helland, B. & Helland, S. J. (1999). Estimation of gastrointestinal evacuation rate in Atlantic salmon (*Salmo salar*) using inert markers and collection of faeces by sieving: evacuation of diets with fish meal, soybean meal or bacterial meal. *Aquaculture* **172,** 291–299.

Temming, A. & Andersen, N. G. (1994). Modelling gastric evacuation without meal size as a variable. A model applicable for the estimation of daily ration of cod (*Gadus morhua* L.) in the field. *ICES Journal of Marine Science* **51,** 429–438.

Temming, A. & Herrmann, J.-P. (2001). Gastric evacuation in the horse mackerel. I. The effects of meal size, temperature and predator weight. *Journal of Fish Biology* **58,** 1230–1245 doi: 10.1006/jfbi.2000.1531.

Titgemeyer, E. C., Armendariz, C. K., Bindel, D. J., Greenwood, R. H. & Löest, C. A. (2001). Evaluation of titanium dioxide as a digestibility marker for cattle. *Journal of Animal Science* **79,** 1059–1063.

Weatherup, R. N. & McCracken, K. J. (1998). Comparison of estimates of digestibility of two diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), using two markers and two methods of faeces collection. *Aquaculture Research* **29,** 527–533.

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