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# A first test of a new modelling approach to estimate food consumption in particle-feeding fish

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## Summary

Models for estimating food consumption in fish by analysing changes in stomach fullness over time are invariably based on a stomach evacuation rate obtained when the fish is fasting, on the assumption that this rate also applies to when the fish is feeding. However, this often is not the case in fish that feed on small particles. A new modelling approach was therefore tested, which is based not only on stomach fullness but also on gut contents. To eliminate errors arising from assimilation in the gut, titanium(IV) oxide ($TiO_2$) was used as an indigestible marker. When applied to a dataset obtained from tilapia given several equal doses of pelleted feed over a 2.5-h period, the new approach gave a closer true consumption estimate than a conventional model. The evacuation rate proved to be a more sensitive parameter than the ingestion rate, but the former was no longer required by the new approach for estimating ingestion, thus liberating the food consumption estimate from any errors and dependencies inherent in the evacuation rate. The new approach assumes that the digesta of previous feedings can be distinguished from those of the feeding phase being analysed and therefore needs further refinement for those cases when this does not apply. Suggestions for such refinements are also given. This new approach is expected to be equally suitable for estimating consumption in stomachless fish.

## Introduction

In fish biology, much work has been done on food consumption estimation in the field, be it with the aid of bioenergetics models (Cui and Wootton, 1988; Arrhenius and Hansson, 1994; Owen et al., 1998) or by modelling the change in stomach contents over time (Thorpe, 1977; Lane et al., 1979; de Silva et al., 1996). The former requires a large database for implementation; the latter is based on only one previously determined parameter, namely, the stomach evacuation rate. In fish showing diel feeding periodicity, this parameter may even be obtained directly from field data pertaining to the non-feeding period, which is a standard feature of the MAXIMS model of Jarre et al. (1991), based on the model of Sainsbury (1986). Irrespective of whether the evacuation is studied independently or from the dataset used for calculating food consumption, the evacuation rate is almost always determined in a period where no feeding takes place but is used in conjunction with data from a feeding phase to calculate food consumption on the assumption that the evacuation rate is the same in both periods. However, Richter et al. (2002) demonstrated that this is not always the case, with the discrepancy probably particularly notable in fish that consume small particles. This is unfortunate, because many species in this category are phyto- or zooplanktivores, most notably milkfish *Chanos chanos* (Forsskål), silver carp, *Hypophthalmichthys molitrix* (Valenciennes), bighead carp, *Aristichthys nobilis* (Richardson), and the tilapias, all important to aquaculture worldwide.

Of the food consumption models developed to date, only Moriarty and Moriarty (1973) attempted a separate determination of the evacuation rate in feeding and non-feeding periods, so far applied only to tilapia. This assumed a constant ingestion rate and involved the analysis not only of the stomach but also of gut contents over time on the basis that the rate of evacuation from the former would be reflected by the rise in contents of the latter. The main disadvantage of this model, as well as subsequent applications (Harbott, 1975; Getachew, 1989), is that it failed to account for assimilation in the gut. It is therefore to be expected that the true level of food consumption was underestimated, possibly to a severe degree. This source of error could probably be eliminated by using an indigestible marker. A further possible source of error in the model is that it assumed stomach evacuation in tilapia to be linear, which contrasts with the findings of other authors (de Silva and Owojemi, 1983; Palomares and Pauly, 1996), who concluded that stomach evacuation in this species was probably exponential. Even the original data of Moriarty and Moriarty (1973) suggested that some form of curved evacuation function would probably have given a better fit.

The aim of the present work was therefore to restructure and mathematically redefine the Moriarty and Moriarty (1973) model to be based on exponential evacuation and to test this model concept with an appropriate dataset. Used in the present study was Nile tilapia, *Oreochromis niloticus* (L.), and the indigestible marker Titanium(IV) oxide ($TiO_2$), which had been tested for suitability in investigating digesta passage in this species (Richter et al., 2003a). For application in situations where natural food is consumed, an external marker (added by the experimenter) would of course not be applicable, but the multitude of internal markers (inherent in the diet) tested and verified to date (Tacon and Rodrigues, 1984; Leavitt, 1985; Galetto and Bellwood, 1994; Jones and de Silva, 1998; Goddard and McLean, 2001; Kavanagh et al., 2001; Sales and Britz, 2001) should make use of this model under field conditions possible. For the present purpose, an external marker that could be added in quantities controllable by the experimenter and easily determined with a great degree of accuracy was considered to be more suitable. In addition to the model with a constant ingestion rate, the mathematical basis of a model with ingestion inversely dependent on the stomach contents in conjunction with an exponential evacuation rate (Elliott and Persson, 1978; Sainsbury, 1986; Jarre et al., 1991) is also provided.

### Theoretical model basis

**Constant ingestion rate, linear evacuation.** The original Moriarty and Moriarty (1973) model was based on the increase in total intestinal tract content over the feeding period; the daily ration was taken to be the mass of the contents at the end of that period. Because the digesta reached the anus before this time, gut contents had to be extrapolated and stomach contents analysed separately to determine the precise time of the end of the feeding period, marked as the moment the stomach contents began to decline. The stomach and gut were assumed to be empty at the start of the feeding period. This was not the case for the gut, which on the sampling day still contained material from the previous feeding. It was possible to distinguish this from freshly ingested matter on the basis of colour, so that the old digesta could be eliminated from the analysis and the model made to work. Evacuation was assumed to be linear so that the mathematical basis of the model was as follows:

$$\text{when feeding:}\quad \mathrm{d}S/\mathrm{d}t = J_1 - E \rightarrow S = S_\mathrm{f} + (J_1 - E) \times (t - T_\mathrm{f}) \tag{1}$$

$$\mathrm{d}G/\mathrm{d}t = E \rightarrow G = G_\mathrm{f} + E \times (t - T_\mathrm{f}) \tag{2}$$

$$\text{when fasting}\quad \mathrm{d}S/\mathrm{d}t = -E \rightarrow S = S_\mathrm{n} - E \times (t - T_\mathrm{n}) \tag{3}$$

$$\mathrm{d}G/\mathrm{d}t = E \rightarrow G = G_\mathrm{n} + E \times (t - T_\mathrm{n}) \tag{4}$$

($S$ = stomach contents; $G$ = gut contents; $J_1$ = feeding rate; $E$ = stomach evacuation rate; $t$ = time; $S_\mathrm{f}$ and $G_\mathrm{f}$ = stomach and gut contents at start of feeding phase, respectively, both originally assumed to be zero; $S_\mathrm{n}$ and $G_\mathrm{n}$ = stomach and gut contents at start of non-feeding phase, respectively; $T_\mathrm{f}$ and $T_\mathrm{n}$ = time points when fish start and stop feeding.) The total intestinal tract contents during the feeding phase are then equal to the sum of stomach and gut contents:

$$(1) + (2) = S_\mathrm{f} + G_\mathrm{f} + J_1 \times (t - T_\mathrm{f}) \tag{5}$$

The theoretical stomach, gut and total intestinal tract trajectories are summarized in Fig. 1. Equation (4) is somewhat hypothetical, and will only apply if the digesta reach the anus after feeding has ceased.

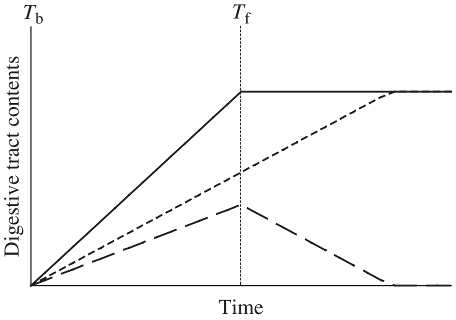

Fig. 1. Theoretical trajectories of stomach (— — —), gut (- - - - -) and total digestive tract (———) of original Moriarty and Moriarty (1973) model assuming linear stomach evacuation and constant ingestion rate. $T_\mathrm{b}$ and $T_\mathrm{f}$ = begin and end of feeding phase, respectively

**Constant ingestion rate, exponential evacuation.** The principal amendment in the model to be tested here concerns the form of the evacuation function. It is generally believed that stomach evacuation is not linear, but to some degree dependent on stomach fullness. Generally accepted is the exponential model ($B$ = 1 in the equation describing stomach evacuation: $\mathrm{d}S/\mathrm{d}t = -E \times S^B$). Richter et al. (2003a) presented evidence that the surface area model (B = $^2/_3$, Fänge and Grove, 1979) is more likely to apply in tilapia, at least when pelleted feed is given, but incorporation of this evacuation function into the model yields no arithmetical solution for the stomach contents, $S$ (Richter et al., 2003b). The following model is therefore based on exponential evacuation as a better approximation to the surface area function; mathematical basis of this model is as follows:

$$\text{when feeding:}\quad \mathrm{d}S/\mathrm{d}t = J_1 - E \times S \rightarrow S = S_\mathrm{f} \times \mathrm{e}^{-E\times(t-T_\mathrm{f})} + (J_1/E) \times (1 - \mathrm{e}^{-E\times(t-T_\mathrm{f})}) \tag{6}$$

$$\mathrm{d}G/\mathrm{d}t = E \times S = E \times (S_\mathrm{f} \times \mathrm{e}^{-E\times(t-T_\mathrm{f})} + (J_1/E) \times (1 - \mathrm{e}^{-E\times(t-T_\mathrm{f})})) \rightarrow G = G_\mathrm{f} + J_1 \times t + (J_1/E - S_\mathrm{f}) \times (\mathrm{e}^{-E\times(t-T_\mathrm{f})} - 1) \tag{7}$$

$$\text{when fasting}\quad \mathrm{d}S/\mathrm{d}t = -E \times S \rightarrow S = S_\mathrm{n} \times \mathrm{e}^{-E\times(t-T_\mathrm{n})} \tag{8}$$

$$\mathrm{d}G/\mathrm{d}t = E \times S = E \times S_\mathrm{n} \times \mathrm{e}^{-E\times(t-T_\mathrm{n})} \rightarrow G = G_\mathrm{n} + S_\mathrm{n} \times (1 - \mathrm{e}^{-E\times(t-T_\mathrm{n})}) \tag{9}$$

The rise in total intestinal tract contents during the feeding phase is given by the sum of stomach and gut contents:

$$(6) + (7) = S_\mathrm{f} + G_\mathrm{f} + J_1 \times (t - T_\mathrm{f}) \tag{10}$$

The theoretical stomach, gut and total intestinal tract trajectories are summarized in Fig. 2. Equation (9) is again expected not to apply, unless feeding ceases before digesta reach the anus.

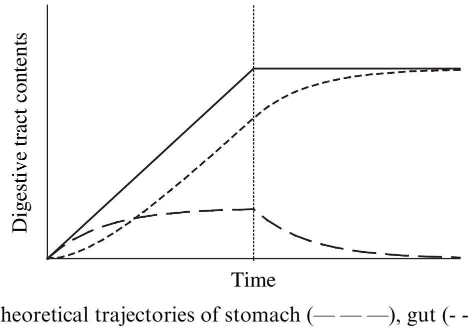

Fig. 2. Theoretical trajectories of stomach (— — —), gut (- - - - -) and total digestive tract (———) of new modelling approach assuming exponential stomach evacuation. $T_\mathrm{b}$ and $T_\mathrm{f}$ = begin and end of feeding phase, respectively

**Ingestion inversely dependent on stomach contents, exponential evacuation.** Several authors (Elliott and Persson, 1978; Sainsbury, 1986; Jarre et al., 1991) have presented a model in which the ingestion rate is not constant but declines as the stomach fills, reaching zero at a theoretical level of $S_{\max}$:

$$\text{when feeding:}\quad \mathrm{d}S/\mathrm{d}t = J_2 \times (S_{\max} - S) - E \times S \tag{11}$$

($J_2$ = instantaneous ingestion rate, *not* equivalent to $J_1$ in the constant ingestion rate model.) In practice, the overall level of ingestion ($J_2 \times [S_{\max} - S]$) never reaches zero during the feeding phase, but stabilizes at a level where it equals the overall level of evacuation ($E \times S$) so that the stomach fullness stabilizes at an asymptotic level of $S_\infty$ where:

$$S_{\max} = (J_2 + E) \times S_\infty / J_2 \tag{12}$$

Substituting Eqn (12) into Eqn (11) and integrating gives the following:

$$S = S_{\mathrm{f}} \times \mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})} + S_\infty \times (1 - \mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})}) \tag{13}$$

The rise in the level of gut fullness is then defined as follows:

$$\begin{aligned} \text{when feeding:}\quad \mathrm{d}G/\mathrm{d}t = E \times S = E \times [&S_{\mathrm{f}} \times \mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})} \\ &+ S_\infty \times (1 - \mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})})] \\ \Rightarrow G = G_{\mathrm{f}} + E \times S_\infty \times t &+ E \times (S_\infty - S_{\mathrm{f}}) \\ &\times (\mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})} - 1)/(J_2 + E) \end{aligned} \tag{14}$$

After feeding has ceased, the stomach and gut contents are described by the same equations as in the second model [Eqn (8) and Eqn (9) respectively]. The equation for the total intestinal tract contents (sum of stomach and gut content) during the feeding phase is somewhat more complex than in the other two models:

$$\begin{aligned} (12) + (14) = S_{\mathrm{f}} + G_{\mathrm{f}} + E \times S_\infty \times (t - T_{\mathrm{f}}) \\ + (S_\infty - S_{\mathrm{f}}) \times ([J_2 + E]/J_2) \times (1 - \mathrm{e}^{-(J_2+E)\times(t-T_{\mathrm{f}})}) \end{aligned} \tag{15}$$

The theoretical stomach, gut and total intestinal tract contents are summarized in Fig. 3.

## Materials and methods

In order to test the second model (constant ingestion, exponential evacuation), 42 tilapia (mean body mass: 357 ± 43 g SD) were placed into individual 40-L aquaria and allowed to acclimatize. The tanks were part of a circulation system which maintained dissolved oxygen in excess of 7.0 mg $\mathrm{L}^{-1}$ and water temperature at 27 ± 1°C. Fish were fed daily with pelleted feed and the experiment was delayed until all fish consumed the daily ration within 2 min of delivery. The eggs were removed from mouthbrooding females within 24 h of spawning in order to induce them to feed again as soon as possible. The day before the experiment, the fish were weighed individually (nearest 0.1 g) and not fed in order to adjust the experimental ration to a certain proportion of their body mass. Tilapia are robust fish and will usually take feed again within an hour of such treatment so that the experimental results were unlikely to have been influenced by stress.

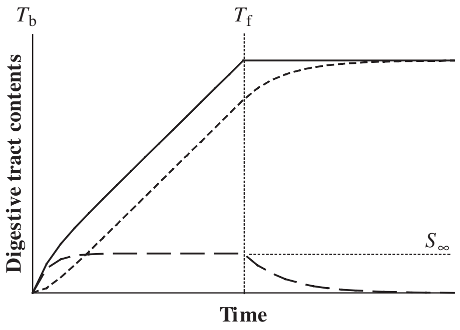

Fig. 3. Theoretical trajectories of stomach (— — —), gut (- - - - -) and total digestive tract (———) of new modelling approach assuming exponential stomach evacuation and an ingestion rate inversely dependent on level of stomach fullness. $T_{\mathrm{b}}$ and $T_{\mathrm{f}}$ = begin and end of feeding phase, respectively. $S_\infty$ = asymptotic stomach contents

On the day of the experiment, the fish were given five doses of marked pelleted feed (50% fishmeal, 41% wheat meal , 4% sunflower oil, 2% vitamin premix, 2% mineral premix, 1% $\mathrm{TiO_2}$). This multiple feeding approach was intended to simulate a continuous feeding period, which is normally observed in this species (Moriarty and Moriarty, 1973; Getachew, 1989; Richter et al., 1999). Each dose was calculated to constitute 0.1% of the fish body mass (0.1% BME, body mass equivalent) so that the total daily ration was 0.5% BME. Doses were given at half-hour intervals and three replicate fish were sampled for stomach and gut content analysis at intervals of 30 min to 1 h, with initial samplings timed to fall between feedings. The fish were immediately slaughtered and the innards (stomach, intestine, liver, visceral fat) dissected and preserved in 70% ethyl alcohol.

At a later date, the liver and visceral fat were separated from the intestinal tract. The digesta of each fish were then carefully flushed into two preweighed containers with distilled water, one each for stomach and gut contents. These were then dried by lyophilization and the dry weight of the contents determined as the difference between the full and empty container. The marker content of the digesta was then determined in the same manner as described in Richter et al. (2003a).

This involved the oxidation of organic material and dissolution of the marker in concentrated sulphuric acid at 400°C in a Kjeldahl digestion system, transfer to 25 ml graduated flasks and topping up with distilled water, the addition of 0.1 ml 35% hydrogen peroxide to a 1 ml aliquot of the test solution and the quantification of the yellow $\mathrm{TiO_2}$–$\mathrm{H_2O_2}$ complex by spectrophotometry at 405 nm. The standard curve was taken from Richter et al. (2003a):

$$\text{Marker } [\mu\text{g.ml}^{-1}] = 108.1 \times \mathrm{Abs}_{405} - 0.155$$

($\mathrm{Abs}_{405}$ = spectrophotometric absorbance at 405 nm).

Non-linear regressions were carried out using the NLIN (method = dud) routine of SAS® Vs.8.02 for Windows. All regressions were based on raw data rather than subsample averages. Marker data was multiplied by a factor of 100 to make the results comparable to those obtained from the dry weight data. The convergence criterion was the lowest sum of squared residuals.

## Results

Dry weights of the stomach contents are given in Fig. 4a, those of the stomach marker contents in Fig. 4b. The latter match the former fairly closely, but there is obviously more scatter in the marker data, particularly towards the end of the experiment. The data were analysed with Eqns (6) & (8), equivalent

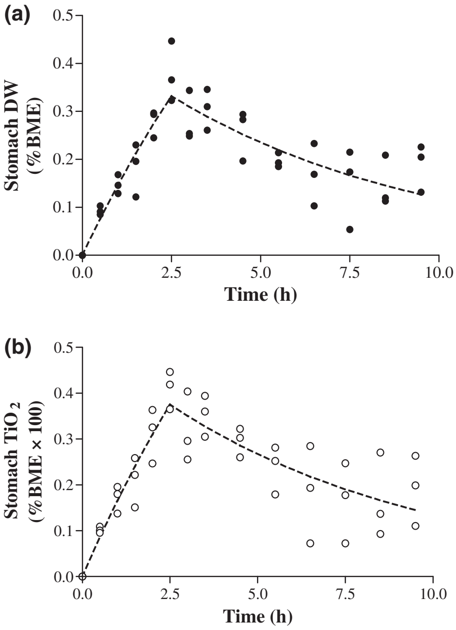

Fig. 4. Stomach content dry weights (a) and marker weights (b) of Nile tilapia (*Oreochromis niloticus*) given 0.5% BME ( = body mass equivalent) of marked pelleted feed in five equal doses. Dotted lines = predicted trajectories obtained by conventional MAXIMS Model. Marker inclusion level in feed: 1%; marker data multiplied by 100 to make results comparable to those from dry weights

Table 1
Parameter estimates and their 95% confidence limits for conventional MAXIMS model from stomach content dry weights (DW) and marker weights (TiO$_2$)

| | DW | TiO$_2$ |
|---|---|---|
| Ingestion rate, $J$ (% BME h$^{-1}$) | 0.157 (0.132–0.183) | 0.177 (0.157–0.197) |
| Evacuation rate, $E$ (h$^{-1}$) | 0.138 (0.096–0.179) | 0.136 (0.103–0.169) |
| End of feeding period, $T_f$ (h) | 2.50 (2.02–2.98) | 2.50 (1.99–3.01) |

to a conventional MAXIMS model (Jarre et al., 1991), with $T_f$ and $S_f$ fixed to zero. The resulting parameter estimates for $T_f$, $J_1$ and $E$ are given in Table 1. Trajectories of the best fits are included in Fig. 4a,b. Food consumption was known to be 0.5% BME spread over 2.5 h so that the expected value for $J_1$ was 0.2% BME h$^{-1}$. Clearly, the ingestion rate and consumption estimates obtained by the conventional modelling approach for both the dry weights and marker fell short of the true values, particularly when based on the former; in both cases the 95% confidence limits exclude the expected value, although for the marker data, the upper limit came very close.

The combined marker data for stomach and gut are shown in Fig. 5, together with the expected trajectory for the total intestinal tract. The marker recovery was obviously good, as demonstrated by the fact that the observed total marker content deviated only slightly from the expected level in the first 5 h of the trial. The weighted average recovery for the feeding period was 95.6%. A linear regression through the data for total marker content with the intercept fixed to the origin gave a slope (equivalent to the parameter $J_1$) of 0.190% BME h$^{-1}$ (±0.0026 SD). The lower and upper 95% confidence limits were 0.185 and 0.195% BME h$^{-1}$, respectively.

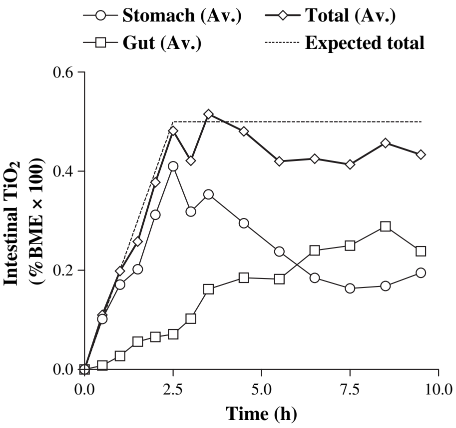

Fig. 5. Marker weights in stomach, gut and total digestive tract ( = sum of stomach and gut) in Nile tilapia (*Oreochromis niloticus*) given 0.5% BME ( = body mass equivalent) of marked pelleted feed in five equal doses. Data represent averages of three fish each; trajectory of expected total included for comparison. Marker inclusion level in feed: 1%; marker data multiplied by 100 to make results comparable to those from dry weights

Richter et al. (2002) presented evidence that when fish feed continuously or consume multiple meals, the evacuation rate $E$ is probably significantly higher in the feeding than in the non-feeding phase. For an estimate of the evacuation rate in the feeding phase, data for the stomach marker content was therefore remodelled with Eqns (6) & (8), with the parameters $J_1$ fixed to the known value of 0.2% BME h$^{-1}$ and $E$ permitted to differ between the feeding ($E = E_f$) and non-feeding ($E = E_n$) periods. The resulting estimates were 0.133 h$^{-1}$ for $E_n$, practically the same value as in the model with a uniform evacuation rate, and 0.252 h$^{-1}$ for $E_f$. This demonstrates not only that under the current experimental conditions, stomach evacuation when feeding was nearly twice as rapid as when fasting but also that an 11.5% increase in the feeding rate implies a much greater rise in the evacuation rate.

## Discussion

The marker recovery rates observed here for the feeding period may be regarded as high in view of the small absolute quantities of marker used. Both Kavanagh et al. (2001) and Titgemeyer et al. (2001) obtained lower values in pigs (92.3%) and cattle (92.8%), respectively, in which much larger marker quantities were used. In previous experiments under the same conditions, Richter et al. (2003a) determined recovery rates of 84.7–89.3% in Nile tilapia. The higher values observed here may be related to the method of analysing the gut marker content. Previous authors dried and ashed the gut, after which the ash was dissolved completely in acid by Kjeldahl digestion. The present method of dissecting out the contents and analysing subsamples, although more laborious, seems to give more accurate results.

Although the MAXIMS model and the new model concept both underestimated food consumption, the latter did so to a lesser degree. Indeed, if the difference in marker recovery rates from the expected value of 100% was due to systematic biological (e.g. assimilation in the digestive tract) rather than random analytical errors and the value of 95.6% recorded here was the obtainable maximum, this figure could be used to correct the ingestion rate, $J_1$, for the new model. This would then rise to 0.198% BME h$^{-1}$, equivalent to a consumption estimate of 0.49% BME. Assuming the error to be evenly distributed in stomach and gut, the appropriate figures for the MAXIMS model would also increase to 0.185% BME $h^{-1}$ and 0.46% BME. However, before such corrections are widely applied, more information on the true source of the error would be required; for the time being, the uncorrected ingestion and consumption values should be accepted.

The ingestion rate calculated with the aid of the new model concept was not only higher, but was also associated with a lower degree of variability. Paradoxically, this meant that the upper confidence limit of the lower absolute estimate of the MAXIMS model came closer to the expected value. Apart from the greater accuracy with which the ingestion rate was calculated, this new method of determining food consumption is wholly independent of the parameter $E$ when the ingestion rate is constant over the feeding period. This is demonstrated by the fact that, despite the complexity of the equations describing the stomach and gut contents [Eqns (6) & (7)], the rise in total digestive tract contents [Eqn (10)] is not based on this parameter. This means that all errors and uncertainty associated with the stomach evacuation rate, such as higher evacuation in the feeding compared to the non-feeding phase, have been eliminated from daily ration estimation. In view of the sensitivity and unreliability of parameter $E$ in fish feeding on small particles, this represents a distinct improvement on models used previously for estimating food consumption in such species.

In fish that show diel feeding periodicity, but in which the overall level of ingestion declines markedly over the feeding period, the evacuation rate cannot be eliminated from the determination of food consumption. Nevertheless, the longer the feeding period, the smaller the exponent in the equation describing total intestinal fullness [Eqn (15)] so that towards the end of the feeding period, this formula is mathematically reduced to a nearly straight line with a slope of $E \times S_\infty$. The latter parameter may be easily obtained from the stomach contents so that the evacuation rate can be determined from the total intestinal tract fullness, making the model workable although somewhat more difficult to put into practice than the constant ingestion model. It is probable that few fish species combine diel feeding periodicity with a declining level of ingestion. The only literature example known to the authors is the diamond turbot, *Hypsopsetta guttulata* Girard (Lane et al., 1979), a grazer feeding on particles larger than planktivores (Froese and Pauly, 2003) so that the gut reaches the asymptote ($S_\infty$) more quickly than in phytophagous fish such as the Nile tilapia.

The new approach performed well under the present set of experimental conditions but requires more rigorous testing and further refinement before field application. Thus, this approach is presented here as more of a model concept than a true, working model. The fact that $TiO_2$ can only be used in formulated feeds is unlikely to pose a problem, provided that a suitable alternative, inherent to the food consumed by the fish being investigated, can be found. A number of workers have investigated internal markers in natural foods (Tacon and Rodrigues, 1984; Leavitt, 1985; Galetto and Bellwood, 1994; Jones and de Silva, 1998; Goddard and McLean, 2001; Kavanagh et al., 2001; Sales and Britz, 2001), mainly analyses of crude fibre or various types of ash (standard, cell wall, salt-free or acid insoluble), but also of hydrolysis-resistant organic matter. The efficacy of these markers varied among studies, but low reproducibilities and large deviations from the results obtained by total collection methods were generally attributable to very low marker concentrations in the food or to soluble and digestible components in the marker. For example, Galetto and Bellwood (1994) investigated the marine green benthic alga *Enteromorpha flexuosa* when consumed by two species of damselfish and discovered that salt free ash was a better marker than standard ash, largely because the latter contained salts which could be assimilated by the fish. The model developed here is applicable mainly to planktivorous fish or grazers. Phytoplankton generally includes diatoms whose silicaceous shells contribute a large portion of acid insoluble ash towards overall dry matter. In benthic or planktonic green algae, the cellulose cell wall probably contributes enough indigestible fibre for this to be used as a marker. Where structural markers are absent, e.g. in blue-green algae, the other main food for tilapias, chemical substances could act as markers. Gudmundsson and Halldorsdotter (1995) studied *n*-alkanes as potential internal markers. It is known that the shorter chained forms (C-15, C-17 and C-19) are found in blue-green algae. Even more promising are the hopanoids, highly refractory cyclic carbon structures specific to the cyanobacteria (Kannenberg and Poralla, 1999; Summons et al., 1999). Although both *n*-alkanes and hopanoids are present at much lower concentrations than fibre or ash, the fact that they are determined by highly sensitive methods (gas chromatography or mass spectrometric gas chromatography) will probably make them viable markers.

The biggest potential source of error in the new approach is the fact that the gut is unlikely to be empty of the marker or other contents at the start of the feeding phase. Moriarty and Moriarty (1973) were able to eliminate this problem in the field by distinguishing between previously and newly ingested matter on the basis of colour, but this may not be possible in other fish or in tilapia feeding on other matter. It was hoped that the feeding scenario encountered by Moriarty and Moriarty (1973), in which the digesta reached the anus before feeding ceased, could be emulated, but this was not the case. One potential solution would be to analyse a certain fixed section of the hindgut separately, deeming the contents of any given subsample to be evacuated in the period before the next subsample is collected. More work would have to be done to determine whether this would provide an acceptable means of correction and how much of the posterior part of the gut would have to be analysed. It is possible that, since gut passage in fish is related to water temperature, the proportion of hindgut to be investigated separately would be related to this environmental factor.

Although further tests are necessary for confirmation, the new model also seems appropriate for estimating consumption in stomachless fish. The main problem with the analysis of stomachless fish is that there is no well-defined section of the anterior digestive tract, analogous to the stomach, in which no assimilation takes place. Some workers have therefore confined themselves to the anterior fifth of the intestine (de Silva et al., 1996) or the foregut (Grove and Crawford, 1980) in such species. However, it is unlikely that the intestine has the same capacity to expand as a stomach in order to store ingested matter, thus the precise form and rate of evacuation in the foregut of stomachless fish may be linked to other factors than in fish with a stomach. As the new model does not require an evacuation estimate when the ingestion rate is constant, it is likely to provide better estimates in fish where factors governing evacuation are unknown, making this model more widely applicable than previous models.

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## References

Arrhenius, F.; Hansson, S., 1994: *In situ* food consumption by young-of-the-year Baltic Sea herring *Clupea harengus*: a test of predictions from a bioenergetics model. Mar. Ecol. Prog. Ser. **110,** 145–149.

Cui, Y.; Wootton, R. J., 1988: Bioenergetics and growth of a cyprinid, *Phoxinus phoxinus* (L.): the effect of ration and temperature on growth rate and efficiency. J. Fish Biol. **33,** 763–773.

Elliott, J. M.; Persson, L., 1978: The estimation of daily rates of food consumption for fish. J. Anim. Ecol. **47,** 977–991.

Fänge, R.; Grove, D. J., 1979: Fish digestion. In: Fish physiology. Vol VIII. Bioenergetics and growth. W. S. Hoar, D. J. Randall and J. Brett (Eds). Academic Press, London, pp. 161–260.

Froese, R.; Pauly, D. (Eds), 2003: FishBase. World Wide Web Electronic Publication. Vers. 31 July 2003. Available at: http://www.fishbase.org (accessed on June 24, 2004).

Galetto, M. J.; Bellwood, D. R., 1994: Digestion of algae by *Stegastes nigricans* and *Amphiprion akindynos* (Pisces: Pomacentridae) with an evaluation of methods used in digestibility studies. J. Fish Biol. **44,** 415–428.

Getachew, T., 1989: Stomach pH, feeding rhythm and ingestion rate in *Oreochromis niloticus* L. (Pisces, Cichlidae) in Lake Awasa, Ethiopia. Hydrobiologia **174,** 43–48.

Goddard, J. S.; McLean, E., 2001: Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis niloticus*. Aquaculture **194,** 93–98.

Grove, D. J.; Crawford, C., 1980: Correlation between digestion rate and feeding frequency in the stomachless teleost, *Blennius pholis* L. J. Fish Biol. **16,** 235–247.

Gudmundsson, O.; Halldorsdotter, K., 1995: The use of *n*-alkanes as markers for determination of intake and digestibility of fish feed. J. Appl. Ichthyol. **11,** 354–358.

Harbott, B. J., 1975: Preliminary observations on the feeding of *Tilapia nilotica* L. in Lake Rudolf. Afr. J. Trop. Hydrobiol. Fish. **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 Mar. Sci. Symp. **193,** 99–108.

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). Aquac. Res. **29,** 487–493.

Kannenberg, E. L.; Poralla, K., 1999: Hopanoid biosynthesis and function in bacteria. Naturwissenschaften **86,** 168–176.

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. Anim. Feed Sci. Technol. **89,** 49–58.

Lane, E. D.; Kingsley, M. C. S.; Thornton, D. E., 1979: Daily feeding and food conversion efficiency of the diamond turbot: an analysis based on field data. Trans. Am. Fish. Soc. **108,** 530–535.

Leavitt, D. F., 1985: An evaluation of gravimetric and inert marker techniques to measure digestibility in the American lobster. Aquaculture **47,** 131–142.

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. J. Zool. (Lond.) **171,** 15–23.

Owen, S. F.; Houlihan, D. F.; Rennie, M. J.; van Weerd, J. H., 1998: Bioenergetics and nitrogen balance of the European eel (*Anguilla anguilla*) fed at high and low ration levels. Can. J. Fish. Aquat. Sci. **55,** 2365–2375.

Palomares, M. L.; Pauly, D., 1996: Models for estimating the food consumption of tilapias. In: The Third International Symposium on Tilapia in aquaculture. R. S. V. Pullin, J. Lazard and M. Legendre (Eds). ICLARM Conference Proceedings 41, ICLARM, Manila, pp. 211–222.

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. J. Appl. Ichthyol. **15,** 165–170.

Richter, H.; Lückstädt, C.; Schlechtriem, C.; Focken, U.; Becker, K., 2002: Evidence of violation of a basic assumption in models currently used for food consumption estimation in fish. Arch. Fish. Mar. Res. **49,** 171–188.

Richter, H., Lückstädt, C., Focken, U.; Becker, K., 2003a: Investigating the evacuation of pelleted feed and the suitability of Titanium(IV) oxide as a feed marker for gut kinetics in Nile tilapia. J. Fish Biol. **63,** 1080–1099.

Richter, H.; Lückstädt, C.; Focken, U.; Becker, K., 2003b: Some mathematical considerations in estimating daily ration in fish using food consumption models. Ecol. Modell. **171,** 381–393.

Sainsbury, K., 1986: Estimation of food consumption from field observations of fish feeding cycles. J. Fish Biol. **29,** 23–36.

Sales, J.; Britz, P. J., 2001: Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture **202,** 113–129.

de Silva, S. S.; Owojemi, A. A., 1983: Effect of dietary quality on the gastric evacuation and intestinal passage in *Sarotherodon mossambicus* (Peters) fry. J. Fish Biol. **23,** 347–355.

de Silva, S. S.; Amarasinghe, U. S.; Wijegoonawardena, N. D. N. S., 1996: Diel feeding patterns and daily ration of cyprinid species in the wild determined using an iterative method, MAXIMS. J. Fish Biol. **49,** 1153–1162.

Summons, R. E.; Jahnke, L. L.; Hope, J. M.; Logan, G. A., 1999: 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature **400,** 554–557.

Tacon, A. G. J.; Rodrigues, A. M. P., 1984: Comparison of chromic oxide, crude fibre, polyethylene and acid insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. Aquaculture **43,** 391–399.

Thorpe, J. E., 1977: Daily ration of adult perch, *Perca fluviatilis* L. during summer in Loch Leven, Scotland. J. Fish Biol. **11,** 55–68.

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. J. Anim. Sci. **79,** 1059–1063.

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