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Gastrointestinal evacuation of inert particles by turbot, *Psetta maxima*: evaluation of the X-radiographic method for use in feed intake studies

Bjørn-Steinar Sæther and Malcolm Jobling

NFH, University of Tromsø, N-9037 Tromsø, Norway.
E-mail: bjoerns@nfh.uit.no

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Abstract

Gastrointestinal evacuation of inert X-ray dense marker by turbot, *Psetta maxima*, was followed by use of radiography. Groups of individually tagged turbot aged 1 + years (136.5 g), were held at 16 °C and acclimatized to a feeding period of 4 hours per day. Fish were fed on pelleted feed in excess. The experimental design allowed a test of the effects of handling stress and post-prandial feeding on gastrointestinal evacuation. Repeated handling affected feed intake, but there were no significant treatment effects on gastrointestinal evacuation rate. Fish that ingested large meals displayed higher evacuation rates of markers than fish which ingested smaller meals, but total evacuation time was longer for the fish that consumed the largest meals. Marker particles started to be voided from the gastrointestinal tract approximately 10 hours following initiation of a meal, and evacuation of marker was completed within 52 hours under the holding conditions employed in the present study.

Keywords: Radiography, gastrointestinal evacuation, feed intake, turbot, *Psetta maxima*.

Evacuation gastrointestinale de particules inertes par le turbot, Psetta maxima *: évaluation de la radiographie pour les études d'absorption d'aliments.*

Résumé

L'évacuation gastrointestinale de marqueurs inertes, denses aux rayons X par le turbot, *Psetta maxima*, a été suivie par radiographie. Des groupes de turbots marqués individuellement et âgés d'un an (1 +; 136,5 g) ont été maintenus à 16 °C et acclimatés à une durée d'alimentation de 4 heures par jour. Les poissons ont été nourris de granulés distribués en excès. Le protocole expérimental permettait un test des effets du stress dû à la manipulation des poissons et à l'alimentation sur les déchets gastrointestinaux. Les manipulations répétées des poissons affectent l'absorption de nourriture mais il n'y a pas d'effets significatifs du traitement sur le taux d'évacuation gastrointestinal. Les poissons qui ingèrent de plus grandes quantités d'aliments présentent des taux plus élevés d'évacuation de marqueurs, mais le temps total d'évacuation est plus long pour les poissons qui consomment de plus grandes quantités. Les particules marquées commencent à quitter le tractus digestif environ 10 heures après le début de l'absorption d'un repas, et l'évacuation des marqueurs est achevée en 52 heures dans les conditions expérimentales de cette étude.

Mots-clés: Radiographie, évacuation gastrointestinale, alimentation, turbot, *Psetta maxima*.

INTRODUCTION

Although radiography is now widely used for estimating individual voluntary feed intake in large groups of fish (Jobling *et al.*, 1995), the method has several prerequisites (McCarthy *et al.*, 1993). One important prerequisite is that the particulate marker used for quantifying intake is retained in the gastrointestinal system until the fish are X-rayed. Any loss of marker will result in an underestimation of feed intake. The rate at which both food and marker are passed through, and evacuated from, the gastrointestinal tract is probably species specific (Fänge and Grove, 1979). Thus, information is required about gastrointestinal evacuation time before the X-radiographic method can be used for the estimation of feed intake by a given fish species under defined holding conditions.

Indigestible material may be retained in the stomach until the digestible part of the meal has been transferred to the intestine, after which the stomach is emptied of inert material (Jobling, 1995). Species specific differences in the evacuation of indigestible particles exist. Studies with cod, *Gadus morhua*, demonstrated preferential retention of particulate marker in the stomach when the fish were fed consecutive meals (dos Santos and Jobling, 1991), whereas in salmon, *Salmo salar*, there was an increase in gastrointestinal evacuation rate of marker under conditions of repeated feeding (Talbot *et al.*, 1984). Consequently, before using the X-radiographic method in feeding studies it is important that one is sure that the particulate marker is evacuated, rather than being retained for long periods of time under repeated feeding conditions.

Thus, an experiment was conducted to examine the gastrointestinal evacuation of inert particulate marker, and to test for possible effects of repeated handling and post-prandial feeding on evacuation and retention of the marker. The aim was to provide an evaluation of the radiographic method for routine use in feed intake studies with turbot.

MATERIAL AND METHODS

Four hundred individually tagged (FTF-69, Floy-Tag and Manufacturing, Seattle, WA) turbot aged 1 + year, with a median weight of 136.5 (131.0-140.5, 95 % c.l.) g, were used in this study. The fish were randomly distributed among 8 circular tanks (Ø = 80 cm, water height 26 cm), each tank containing 50 individuals (density 13.6 kg. m^{-2}). This gave 4 treatment groups with 2 tanks per treatment. The fish were held in sea water (33-35 salinity) at a constant temperature (mean 16.1 ± 0.6 °C) and photoperiod (16L:8D) for 4 weeks before the trial started. Oxygen concentrations were recorded weekly, and water flow was adjusted to maintain oxygen saturation at approximately 90 %. During acclimatization to test conditions food was offered in excess for 4 hours each day, during the light phase, using automatic feeders. The dry feed used was based on a commercial recipe (FK-Start, 2.5 mm, Felleskjøpet, Stavanger, Norway): protein 46 %, lipid 23 %, carbohydrates 13.7 %, ash 8.6 % and water 8.7 %.

In the last meal offered to the fish, immediately prior to the gastrointestinal evacuation study, the normal food was replaced by labelled food containing X-ray dense particulate marker. Inclusion of X-ray dense particulate marker (Ballotini glass spheres, Jencons Ltd., Leighton Buzzard, UK) in the feed, allows quantitative estimates of gastrointestinal content to be made (Talbot and Higgins, 1983; Jobling *et al.*, 1995). The labelled feed was formulated to be identical to the normal feed in every respect, with the exception of inclusion of ballotini (size no. 10, *i.e.* Ø = 230-320 µm) at low concentration (0.5% by weight). The relationship between the weight of the feed and number of ballotini is described by the following equation:

$$F = 8 \times b_{10} - 2 \quad (p < 0.001; r^2 = 0.996; n = 80)$$

where F is the weight of feed in mg and b_{10} is the number of ballotinis size 10. One hour after feeding had terminated, fish were anaesthetised (Benzocaine, 50 mg. l^{-1}, 2.5 minutes), X-rayed (Siemens Nanodor 2 X-ray machine, 4 s exposure time, 80 mAs, 50 kV, AGFA Structurix D7 film), and weighed to the nearest 0.5 gram. These procedures, from the time the fish were anaesthetised until they were returned to the holding tank, took 5-6 minutes. The exposed X-ray plates were developed and the numbers of ballotini present in the gastrointestinal tract of the fish counted. The weight of food consumed by individual fish was then calculated using the standard curve for the relationship between the weight of feed and number of ballotini.

Following measurement of initial ingestion, the 4 groups (2 tanks per treatment) were subjected to the following treatments: 1: The X-ray procedure was repeated every 6 hours and fish were fed every 24 hours (6F). 2: Fish were X-rayed after 24 hours and then every 18 hours, and fish were fed at 24 and 48 hours (24F). 3: Fish were X-rayed at intervals of 6 hours and deprived of food (6D). 4: Fish were X-rayed every 18 hours and deprived of food (18D). In order to distinguish between marker from the initial meal and to be able to assess the quantity of food consumed later, the food offered at 24 and 48 hours contained size 8.5 ballotini (Ø = 400-455 µm; 1% by weight);

$$F = 19 \times b_{8.5} \quad (p < 0.001; r^2 = 0.993; n = 82)$$

where F is the weight of feed in mg and $b_{8.5}$ is the number of ballotinis size 8.5.

Evacuation of the marker was determined by repeated counting of the numbers of X-ray dense bal-

lotini in the gastrointestinal tract of the individual fish, with numbers of marker particles being expressed relative to the weight of the fish (n. g^{-1}). The experiment was terminated when all fish had evacuated the size 10 ballotini marker completely. Different measurement frequencies were used to test for possible handling effects, whereas fed/deprived treatments were used to test for possible preferential retention of marker and effects of post-prandial feeding on evacuation rate.

Feed intake and weight results were compared between replicates and treatments by the Kruskal-Wallis test, using SYSTAT software (Wilkinson, 1992). Total emptying times were calculated using simple linear regression (SYSTAT), and slopes of regression lines were compared using the t-test as described by Zar (1984). Proportions of feeding fish were compared using chi-square analysis on frequency data (Zar, 1984).

RESULTS

There were no significant differences between replicates in feed intake amongst fish that fed ($p>0.05$; Table 1), and proportions of feeders were within the range 74-96 %. Non-feeding fish were excluded from the evacuation data, and results for replicates within treatments were pooled prior to further analyses.

Table 1. – Proportions of feeding fish (%) and median feed intake of feeding turbot subjected to different handling and feeding procedures. Feed intake is given in mg per gram of fish, with 95 % confidence limits in brackets. The data from the second meal summarises data collected when fish were fed after 24 hours. See text for description of treatments.

		Initial meal		Second meal	
Treatment	Replicate	% Feeders	Intake (mg. g^{-1})	% Feeders	Intake (mg. g^{-1})
6 F	1	92	3.97 (2.53 - 4.80)	12	0.17 (0.13 - 0.26)
	2	90	3.27 (2.05 - 4.60)	8	0.26 (0.18 - 0.47)
24 F	1	74	3.37 (2.22 - 4.21)	30	0.59 (0.22 - 1.64)
	2	96	3.83 (2.90 - 5.25)	16	0.16 (0.12 - 1.20)
6 D	1	84	4.78 (3.55 - 6.13)		
	2	94	2.43 (1.82 - 4.13)		
18 D	1	84	3.00 (1.85 - 4.21)		
	2	92	3.13 (2.06 - 4.85)		

There was a delay of approximately 10 hours from the beginning of feeding until the first marker particles were evacuated from the gut (Fig. 1*a, c*), this delay reflecting the time needed to transport the marker through the gastrointestinal tract. Following this initial delay, evacuation of marker appeared to be linear with time. Regression lines were fitted to the evacuation relationships for the groups monitored at 6 hour intervals (Fig. 1*a, c*):

$$6F: C = 0.62 - 0.02t \quad (p< 0.01; r^2 = 0.999; n = 4),$$
$$6D: C = 0.65 - 0.02t \quad (p< 0.01; r^2 = 0.999; n = 4),$$

where C is the gastrointestinal content of particulate marker in number per gram of fish, and t is the time elapsed in hours within the timespan 10-28 h. Linear regression lines were not fitted to the emptying patterns of fish measured less often, because of the low number of points. The fish monitored frequently had a total emptying time of 25-30 hours (Fig. 1*a, c*), and it is probable that evacuation of marker particles from the digestive tract of the other groups of fish occurred within a similar time interval.

Inspection of the X-ray plates revealed no signs of preferential retention of marker particles in the stomach, and a comparison of slopes of the regression lines for the groups differing in post-prandial feeding, (Fig. 1*a, c*), failed to detect any significant effect of consecutive meals ($p>0.05$). However, both feed intake in subsequent meals ($p<0.05$) and numbers of feeding fish were significantly reduced at 24 hours, as compared to the initial measurement (Table 1). In fish handled frequently the proportion of feeding fish was reduced from 91 % at initial feeding to 11 % at 24 h ($p<0.05$). In fish handled less frequently 85 % fed initially but only 23 % fed when food was offered 24 hours later ($p<0.05$). This suggests that the handling of the fish associated with taking the initial X-ray affected feeding on consecutive days. Further, the feeding of the fish 24 hours after the initial meal was influenced by handling frequency: groups of fish handled frequently had reduced numbers of feeding fish ($p<0.05$) but not lower feed intake ($p>0.05$) than those handled less frequently.

Fish comprising the group that was X-rayed at frequent intervals and offered additional meals (6F) were divided into three sub-groups according to initial feed intake: low (<1.5 mg. g^{-1} fish; n = 23), medium (4.0<5.5 mg. g^{-1}; n = 20) and high (7.0<10.0 mg. g^{-1}; n = 14). Emptying patterns differed among sub-groups (Fig. 2), the slopes of the regression lines being significantly different ($p<0.05$) between the high and medium feed intake sub-groups:

$$high: C = 1.29 - 0.04t \quad (p< 0.01; r^2 = 0.989; n = 5),$$
$$medium: C = 0.81 - 0.03t \quad (p< 0.05; r^2 = 0.978; n = 4),$$

where C is the gastrointestinal content of particulate marker in number per gram of fish, and t is the time elapsed in hours, within the timespan 10-32 h in the high, and 10-28 h in the medium feed intake subgroups, respectively.

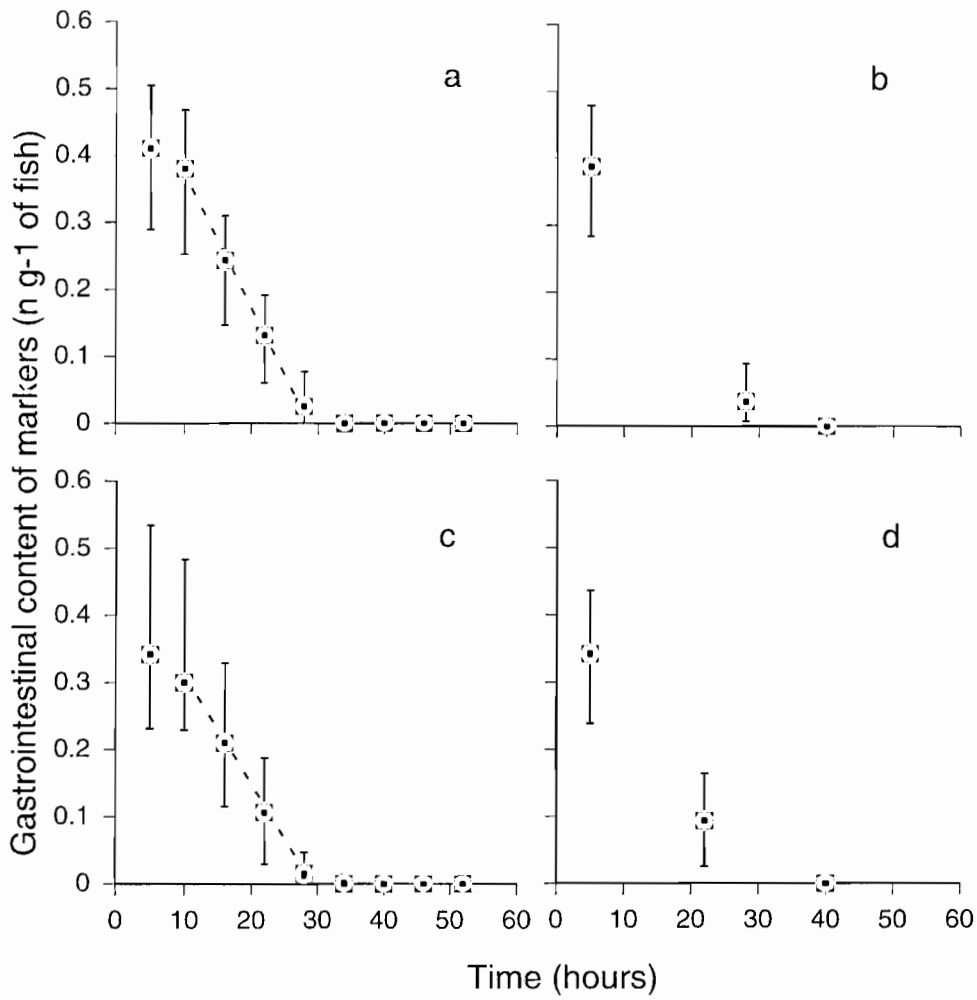

Figure 1. Gastrointestinal evacuation of inert particulate marker with time, when turbot were X-rayed every 6 h and fed every 24 h (a), X-rayed after 24 h then every 18 h and fed every 24 h (b), X-rayed every 6 h and not fed (c) and X-rayed every 18 h and not fed (d). Error bars show 95 % confidence limits.

This indicates that meal size affected emptying rate, the fish that fed the most having the highest emptying rate. This resulted in the time required to evacuate the markers from the gastrointestinal tract being only slightly longer than for fish which had consumed a medium-sized meal. The data were insufficient to perform any statistical analyses on fish that fed at a low level, but emptying rate seemed to decrease with decreasing meal size. Further, the time that elapsed until the evacuation of the first marker particles from the digestive tract tended to be reduced in the group that had ingested most food (Fig. 2).

DISCUSSION

The results show that the radiographic method can be applied in feed intake studies with turbot, provided that certain precautions are taken. There was an initial delay of about 10 hours from beginning of feeding until the median number of marker particles in gut of the fish started to decrease (Fig. 1). Both Grove *et al.* (1985) and Bromley (1987) report a retention of food in the stomach prior to passage into the anterior intestine. Thus, the observed delay in evacuation of marker in the present study may be due to a combination of retention in the stomach, and the time needed to transport the marker through the intestine. In fish that fed at the highest level this initial delay in particle evacuation was somewhat shorter than 10 hours (Fig. 2), so the time between the end of a feeding period until the fish are X-radiographed should not exceed 4-5 hours under the holding conditions of the present study. The median total evacuation time was close to 30 hours (Fig. 1), and all fish had completely emptied the marker from the gut after 52 hours. Thus, 52 hours is

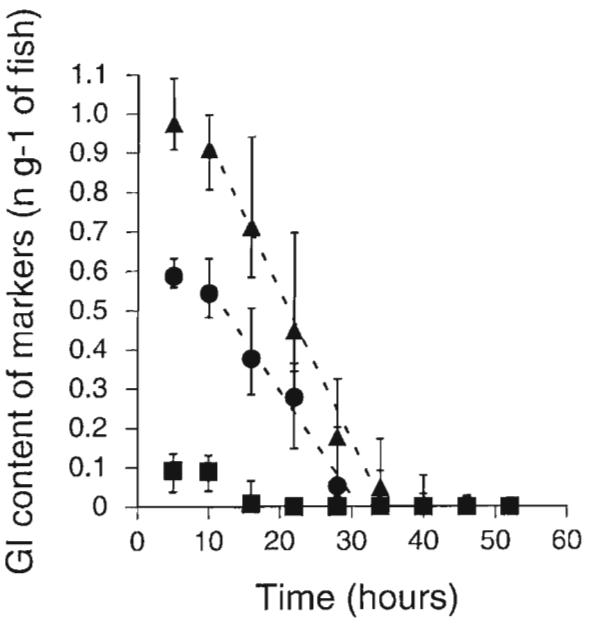

Figure 2. – Gastrointestinal evacuation of inert particulate marker by turbot with different initial feed intake (<1.5 mg g^{-1} of fish (■), 4.0<5.5 mg. g^{-1} (●) and 7.0<10.0 mg. g^{-1} (▲)). Error bars show 95 % confidence limits.

the minimum time that can be allowed to elapse between measurements of feed intake if only a single marker size is to be used. However, handling effects may have an influence on feed intake if measurements are made at so frequent intervals (McCarthy *et al.*, 1993; Jobling *et al.*, 1995). When evaluating the effects of handling on the feed intake in consecutive meals it must be taken into account that both 6 and 18 hours must be considered as extremely frequent handling. When X-radiography is used for the routine measurement of feed intake it is usual to allow 2-3 weeks to elapse between measurements, and such routine measurements are often combined with the monitoring of length and weight in a growth trial (Jobling *et al.*, 1995).

Several studies with fish have revealed that the evacuation of food may differ from that of inert markers, due to the preferential retention of particulate markers in the stomach (Grove, 1986; Jørgensen and Jobling, 1988; dos Santos and Jobling, 1991). In the present study the X-ray dense particulate marker appeared to pass relatively unhindered through the gastrointestinal tract, and post-prandial feeding did not seem to induce any prolongation of the time needed to evacuate the markers from the stomach and remainder of the gut. This contrasts with findings for the cod (dos Santos and Jobling, 1991), but in Atlantic salmon post-prandial feeding seems to initiate a rapid flow of marker particles along the gastrointestinal tract (Talbot *et al.*, 1984).

The results of the present study demonstrate an effect of meal size on evacuation rate and emptying time. Rates of gastric evacuation tend to increase as meal size increases, even though gastric evacuation time also increases (Jobling, 1995). This was probably also the case in the present study, because fish that fed most had significantly higher gastrointestinal evacuation rates than fish that fed less, and the times required for total evacuation of markers were similar amongst fish that ingested large and medium amounts of food. By contrast, Grove *et al.* (1985) reported that digestion rates of turbot tended to decrease as the meal size increased.

CONCLUSION

The X-ray method is suitable for feed intake studies in juvenile turbot, when certain conditions are met. A period of 8-10 h elapsed before particulate marker started to be evacuated from the gut. Evacuation of marker particles, and presumably also indigestible food remains, was completed within 52 h under the conditions of the present study, irrespective of feeding level. Fish that consumed most had the highest evacuation rates.

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