Gastrointestinal evacuation of particulate matter in polar cod *Boreogadus saida*

Bjørn-Steinar Sæther[1,*], Jørgen Schou Christiansen[2], Malcolm Jobling[1,2]

[1]Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø, Norway
[2]Norwegian Institute of Fisheries and Aquaculture, 9291 Tromsø, Norway

ABSTRACT: Gastrointestinal evacuation of inert particles was monitored in the polar cod *Boreogadus saida* (Lepechin), held at a temperature of 0.5°C. Fish (31.8 g ± 0.8 SD) were fed a moist diet, based on natural prey items, containing a particulate marker. Evacuation of marker from the gastrointestinal tract started 48 h after ingestion of a meal. The average time needed to evacuate 50% of the marker was estimated to be 132 h, whereas the total evacuation time was approximately 400 h. This may indicate that the polar cod does not feed on a daily basis at temperatures close to zero because of the prolonged time needed to evacuate meals. This work provides a methodological basis for studies of feeding and growth in polar cod using X-radiography.

KEY WORDS: Polar cod · *Boreogadus saida* · Food intake · Gastrointestinal evacuation · Radiography

INTRODUCTION

The polar cod *Boreogadus saida* (Lepechin) is an abundant cryopelagic species with a wide distribution in the Arctic ocean. It constitutes a key link between lower trophic levels and top predators such as other fish species, birds, and marine mammals (Hobson & Welch 1992). Knowledge of feeding and food evacuation rates in polar fish is scarce, and the central position of polar cod in the arctic food chain makes information about food turnover for this species of particular interest.

Evacuation rates of *Pagothenia borchgrevinki*, an Antarctic fish species with a similar ecological position to the polar cod, have been estimated using a sacrifice method (Montgomery et al. 1989), and this method has recently been used to study gastric evacuation rates in polar cod fed natural prey (Hop & Tonn 1998). The initial meal size is rarely known when this method is used; additionally, large sample sizes are needed to obtain reliable results and evacuation of meals by individual fish cannot be tracked. Further, the method is destructive, precluding repeated use of individuals.

We examined gastrointestinal evacuation rates of polar cod by feeding fish with food labelled with an inert particulate marker and following evacuation using X-radiography. The X-radiographic technique enables estimation of initial meal size, and permits repeated recording of the time course of evacuation at the individual level. Possible effects of handling and post-prandial feeding on evacuation were assessed.

MATERIAL AND METHODS

Individually tagged (FTF 69 fingerling tags, Floy®Tag & Manufacturing Inc. Seattle, Washington, USA), adult polar cod were randomly distributed among 3 tanks, each tank containing 15 fish (mean weight 31.8 g [0.8 SD, n = 45]) (Groups A to C). Fish were then left to settle without food for 2 d. The fish were held in full strength seawater at 0.5°C, under continuous light conditions. At the start of the experiment the fish were hand fed a moist food formulation (Christiansen & George 1995) containing inert X-ray dense marker (Ballotini lead glass beads; size 400 to 455 µm; Jencons Ltd., Leighton Buzzard). Ingestion and evacuation of the marker were monitored by X-radiography (Talbot & Higgins 1983, Jobling et al. 1995). One hour after

feeding, the fish were anaesthetised (benzocaine, 25 mg l^{-1} for 5 min) and radiographed (Siemens Nanodor 2, 12 mAs, 50 kV). The X-ray films (Agfa Structurix D7) were developed and the number of marker particles in the gastrointestinal tract (GI-tract) of individual fish was counted. Samples of food were also X-radiographed, and the number of ballotini regressed against weight of food by simple linear regression (Talbot & Higgins 1983, Wilkinson 1992):

$$F = 24 + 41b$$

where F is the weight of food in mg and b is the number of ballotini ($r^2 = 0.982$; $F_{1, 32} = 1761.1$; $p < 0.001$). The fish in Group A were radiographed sequentially at 0, 48, 120, 168, 264, 336, 408 and 504 h, and those in Groups B and C at 0, 168 and 408 h. Fish in Groups A and B were offered additional meals to satiation twice a week, whereas fish in Group C were deprived of food following the initial meal. The food provided in the later meals was identical to that given in the initial meal, except that it lacked particulate marker. Uneaten food was removed from the tank 15 min after feeding, enabling an estimate of group food intake to be made 'by difference'. Following the termination of the evacuation study, the fish were again fed a meal of marked feed and intake was estimated. When comparisons were made of amounts consumed in the initial and final meals estimated 'by difference' and from the X-ray technique, food intake recorded 'by difference' was higher (mean difference 1.65 g food fish^{-1} meal^{-1} [SD 0.27]). Pairwise correlation of food intake recorded by the 2 different methods were close to being significant (Pearson; $r = 0.784$; n = 6; p = 0.067). The discrepancy between food intake recorded by X-ray and 'by difference' may be due to the consistency of the formulated moist food; some food particles fragmented during ingestion, resulting in losses of food, thus leading to an overestimation of group food intake when using the 'by difference' method. Nevertheless, consumption estimated 'by difference' was considered a useful indicator of general trends in intake.

A Kolmogorov-Smirnov test, as modified by Lilliefors, was used to test the data for deviation from a normal distribution, and homogeneity of variance was assessed by visual inspection of data. Possible differences in fish body weights and food intake between groups were tested using an ANOVA (Wilkinson 1992). A Pearson correlation analysis was used to assess interaction between individual food intake and body weight (Wilkinson 1992). The evacuation of marker was described by iterative fitting of non-linear least square curve (Elashoff et al. 1982). For fish in Group A evacuation curves were plotted for individual fish, and the half time of evacuation of marker (T50%) was calculated for each individual. One fish was

excluded due to few observations (n = 2) before complete evacuation; the remaining 13 fish all had an coefficient of determination (r^2) of 0.936 or higher, and standard residuals homogeneously distributed around 0 through time. The relationship between T50% and initial food intake was examined using a linear regression analysis (Wilkinson 1992).

RESULTS

The distribution of fish body weights, but not evacuation of marker data, fulfilled the assumptions for parametric analyses (Kleinbaum et al. 1988); thus, results on gastrointestinal content of marker are presented as medians with 95% CI (Zar 1984). One fish in Group A died on Day 5 and data from this individual were excluded from analyses. There were no significant differences in initial body weights between the experimental groups ($F_{2,41} = 0.057$, p = 0.945). Initial food intake did not differ significantly between groups (Fig. 1; $F_{2,40} = 1.456$; p = 0.245; fish body weight as covariable [$F_{1,40} = 5.585$; p = 0.023]); median food intake (95% CI) Group A 0.8 (0.6 to 1.4), B 1.0 (0.7 to 1.7), C 1.3 (0.7 to 2.4) g food fish^{-1} meal^{-1}. There was a positive correlation between food intake and fish body weight in Group A (Pearson $r = 0.566$, n = 14, p = 0.035), but not in Group B ($r = 0.461$, n = 15, p = 0.084) or C ($r = 0.211$, n = 15, p = 0.451), although there was a tendency for the largest fish to consume most food.

Fish in Group A evacuated few marker particles from the GI-tract during the first 48 h, indicating a minimum passage time of marker through the GI-tract of approx-
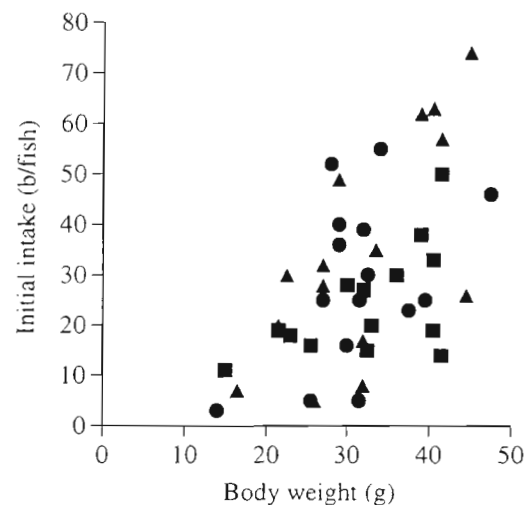

Fig. 1. Number of marker particles (b) consumed in the initial meal ingested by polar cod held at 0.5°C. Symbols: Group A (■), Group B (●) and Group C (▲). See text for details on group treatments
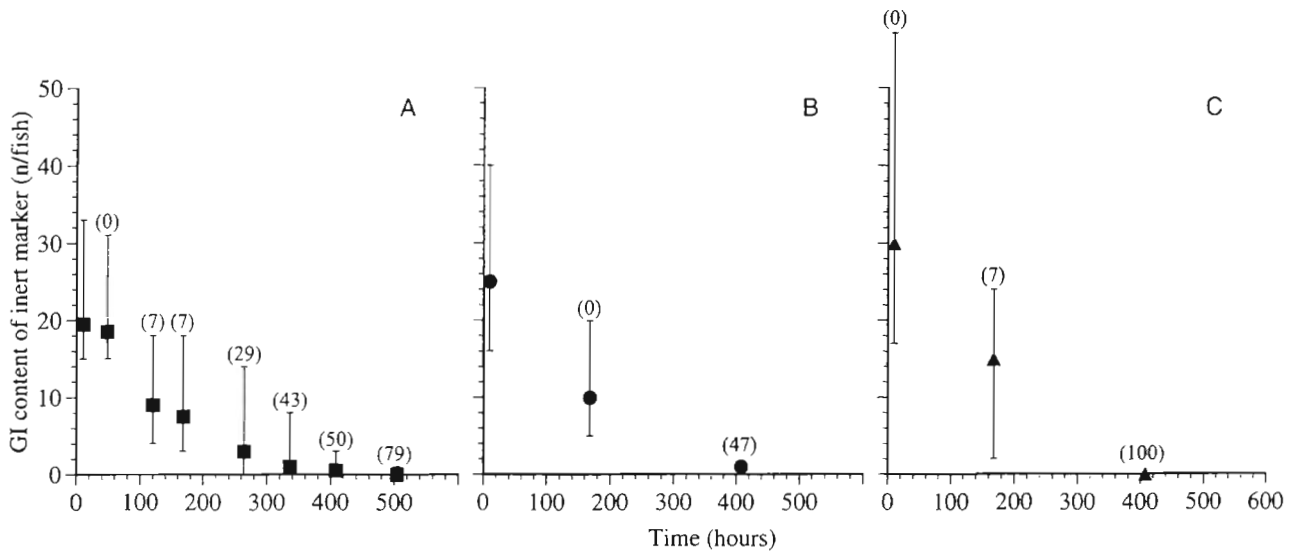
Fig. 2. Gastrointestinal (GI) evacuation of inert marker in polar cod kept at 0.5°C. Data are given as medians and variation is indicated by 95% confidence intervals. Numbers in parentheses denote the percent of fish devoid of marker. (A) Repeatedly fed, frequently handled fish, (B) repeatedly fed, less frequently handled fish, and (C) food-deprived fish handled as in B

imately 2 d (Fig. 2A). After this initial delay, evacuation of marker could be described by the least square exponential equation:

$$b = 27.89e^{(-0.009X)}$$

where b is the number of marker particles and X is time within the range 48 to 408 h after the initial meal ($F_{2,4} = 457.4$; $p < 0.01$). The fitted curve had a coefficient of determination (r^2) of 0.991, with residuals homogeneously distributed around 0. There were no differences among groups in numbers of marker remaining after 168 h, and fish in all groups had reduced the median marker numbers to 0 within 408 h (Fig. 2). However, only fish in Group C (deprived of food after the initial meal) were completely devoid of marker at this time (Fig. 2). When the amounts of food consumed by fish in Groups A and B after the initial meal were compared, the fish in Group B were found to have consumed 0.44 g fish^{-1} meal^{-1} more than those in Group A (95% CI 0.04 to 0.84 g fish^{-1} meal^{-1}). This indicates that the frequent handling to which fish in Group A were subjected resulted in a reduction in subsequent food intake.

The time for individual fish to evacuate 50% of the ingested marker (T50%) was estimated for Group A (Fig. 3); average T50% was calculated to be 132 h, but T50% was significantly correlated with numbers of ingested marker. The linear relationship (based on a least square stepwise backwards regression procedure; fish body weight and [weight × number of marker] excluded from the model at p = 0.15) was described by:

$$T50\% = 46.9 + 3.09b$$

where b is the initial number of marker ($r^2 = 0.587$; $F_{1,11} = 15.6$; $p < 0.01$; 1 outlier excluded). In addition, any confounding effect of fish body weight was considered and found to be negligible (Kleinbaum et al. 1988). The positive slope of the regression (3.09) demonstrates that evacuation time increases with increasing meal size, and the intercept (46.9 h) corresponds closely to the observed initial delay time for evacuation (48 h).
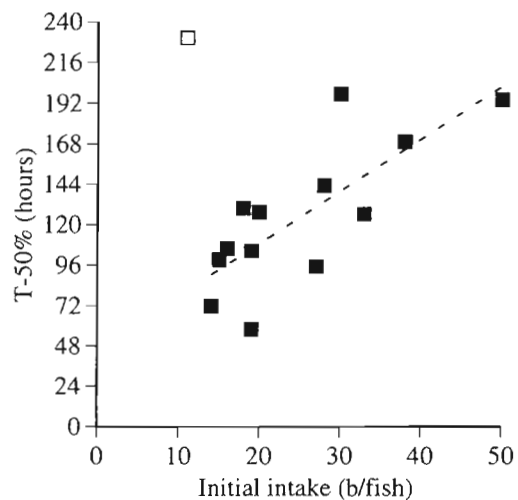

Fig. 3. Time required for polar cod to evacuate 50% of inert particles from the GI-tract. The open square indicates an outlier that is not included in the statistical analyses. The line was fitted using linear regression analysis

DISCUSSION

The passage of inert particles through the GI tract of polar cod occurs slowly at 0.5°C, the median evacuation time being approximately 400 h. This is similar to the findings of Hop & Tonn (1998), who reported that gastric evacuation required 12 to 13 d in polar cod held at a slightly lower temperature. Since return of appetite may be inversely correlated to gastric emptying (Grove et al. 1978), a long evacuation time may imply that food intake and growth in this species are limited by low GI evacuation rates when food is abundant (Hop et al. 1997). Such a long GI evacuation time implies that specific dynamic action (SDA) may elevate metabolism above the fasting rate for several days after ingestion of a meal (c.f. Saunders 1963). This should be taken into account when metabolic rate studies are performed on this species.

Evacuation of marker from the gut commenced after an initial delay of about 48 h (Fig. 2A), this delay representing the minimum time needed to transport the marker through the system. Following this initial delay, the evacuation appeared to be exponential, with a relatively rapid evacuation of marker during the first 4 to 5 d. The general pattern resembles that recorded for gastric evacuation in the rainbow trout *Oncorhynchus mykiss* (Grove et al. 1978) the Atlantic cod *Gadus morhua* (dos Santos & Jobling 1991a), and GI evacuation in the turbot *Psetta maxima* (Sæther & Jobling 1997). A similar pattern can also be implied from results of studies on polar cod fed natural prey (Hop & Tonn 1998). Polar cod in the present experiment required about 130 h to evacuate 50% of the inert matter from the gut following ingestion of a meal of 0.8 g food fish^{-1} meal^{-1} (Fig. 2A), but the time needed to empty half of the meal increased with increasing meal size (Fig. 3). Hop & Tonn (1998) reported a half time for gastric evacuation of approximately 150 h for polar cod (21.1 g body weight) held at −0.49°C; fish had consumed a single meal of natural prey (*Calanus* sp. and *Themisto* sp.) of approximately 2 g food fish^{-1} (9.25% of W). There is little information about GI evacuation of coldwater fish species, but long evacuation times seem typical, with several days being needed to empty the stomach after ingestion of a meal (*Pagothenia borchgrevinki* [Montgomery et al. 1989], *Theragra chalcogramma* [Smith et al. 1989], *G. morhua* [dos Santos & Jobling 1991a], *Lota lota* [Pääkkönen & Marjomäki 1997], *Boreogadus saida* [Hop & Tonn 1998]). Polar cod seem to have GI evacuation times that are longer than those reported for other coldwater species, although direct comparison is difficult because of differences in experimental protocols, food types and holding temperatures used in the various studies.

The time required to evacuate the gut increased with increasing meal size (Fig. 3), but the relationship between ingestion and T50% indicates that a doubling of food intake did not lead to a doubling of evacuation time. This is concordant with reports that GI evacuation is dependent on meal size, with higher evacuation rates as meal size increases (Jobling 1995, Pääkkönen & Marjomäki 1997, Sæther & Jobling 1997). Handling associated with repeated X-raying of fish resulted in a reduction in food intake, and this should be taken into account when consecutive food intake measurements are to be made. However, repeated handling was not found to significantly influence GI evacuation, as also reported in turbot *Psetta maxima* (Sæther & Jobling 1997). Repeated feeding did not seem to affect evacuation markedly, but as only the fish deprived of food after the initial meal were devoid of marker at the termination of the experiment, this might indicate that repeated feeding leads to some prolongation of the time needed to completely evacuate inert material from the GI-tract. dos Santos & Jobling (1991b) reported that when Atlantic cod *Gadus morhua* were fed at regular intervals there was a preferential retention of particulate marker in the stomach, and a similar response was described in turbot by Grove (1986). By contrast, Talbot et al. (1984) reported increased gastrointestinal evacuation of particulate marker in the salmon *Salmo salar* under conditions of repeated feeding.

The results of this study show that the X-ray method can be used to assess food intake in polar cod held at low temperatures (0.5°C), provided fish are X-rayed within 48 h after ingestion of a meal. Frequent handling of this species seems to reduce food intake. The discrepancy between food intake recorded 'by difference' and food intake measured by radiography implies that the former method is inadequate for accurate quantification of food intake, at least when using wet or moist formulated food. When using the X-ray method, a minimum of 400 h should be allowed to elapse between consecutive food intake measurements on polar cod held at low temperatures, because marker remaining from a previous meal would lead to overestimation of food intake. The results of the present study add to the evidence that X-radiography may be a valuable experimental tool to assess food intake-growth relationships in captive key species from natural populations, provided that some simple precautions are taken.

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