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Food ingestion in juvenile cod estimated by inert lanthanide markers — effects of food particle size

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Development of formulated starter diets and improvement of diets for juvenile marine fish species are major challenges in aquaculture. The ingestion rate may be regarded as a parameter for evaluating whether a diet particle is available, recognized, of adequate size, palatable, and preferred by small fish. In this study, we evaluated the effect of food particle size (150−3425 μm) on ingestion rate in juvenile cod (36−826 mg wet weight). Lanthanide oxides were used as markers. Several mixtures of lanthanide marker-labelled diets were produced by combination of the mono-labelled size classes. Each combination was fed for one, four, or seven days to groups of juvenile cod in separate tanks. After termination by anaesthetic, the fish were collected, individually weighed, and frozen until analysis. The amount of lanthanides in the homogenized fish was measured by inductively coupled plasma-mass spectrometry (ICP-MS). There were no significant differences in ingestion of any of the markers when given in a mixture of mono-labelled particles. Thus, the markers Y_2O_3, La_2O_3, Nd_2O_3, Dy_2O_3, and Yb_2O_3 may be used as inert tools for evaluating diet ingestion. The total intake of dry diet was about 13 mg g^{-1} body weight. The particle size that caused the highest food intake was less than 1.2 mm in all experiments.

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Introduction

The optimal nutrient and physical properties of formulated start and weaning diets for marine fish larvae are not well established (NRC, 1993; Watanabe and Kiron, 1994). One of the first requirements in the chain of processes leading to growth is that the diet particle be available, recognized, of adequate size, palatable, preferred, and ingested by the fish. Several dietary characteristics, such as particle size, colour, taste, texture, initial water content, buoyancy, etc., may be of significance for the ingestion rate of marine fish larvae. Ingestion rates may be studied by visual observation of food intake, a time consuming and difficult method for larval and juvenile fish, or by analysis of gut content after a period of feeding. Markers like chromic oxide

(Austreng, 1978) and radioactive isotopes (Kolkovski et al., 1993) have been added to the diets to quantify the food content in the gut, often in connection with digestibility studies. An approach based on sets of several markers used simultaneously makes it possible to differentiate between ingestion of several diets offered together to fish in one tank, and thereby to establish selection criteria based on the diet characteristics. A prerequisite for doing these types of experiments is finding suitable non-toxic inert markers that can be incorporated in the diet without adding biasing factors, and which can be quantified by examination of the individual after ingestion. It is of particular importance that the markers do not influence the ingestion of the diet by acting as food repellents or attractors. Minimally, a set of markers used within one experiment should be equally attractive or repellent to the fish.

The 15 trivalent oxides of lanthanides and yttrium make it possible to carry out work with sets of several markers

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simultaneously within one experiment. Trivalent oxides of yttrium and rare earth metals have been evaluated as inert markers in apparent digestibility studies with salmonids (Austreng *et al.*, 2000). Such markers have been used to study the effects of several feeding stimulants on diet preference by gibel carp (*Carassius auratus gibelio*) (Xue and Cui, 2001), and used on juvenile cod (*Gadus morhua*) to study effects of dietary moisture content (Otterå *et al.*, 2003). The lanthanide oxides are finely powdered and water insoluble. It was concluded that La, Dy, and Yb may be safely used as nutritional markers (Luckey *et al.*, 1975), and lanthanide oxides have been used for nutrient studies in humans (Hutcheson *et al.*, 1979). However, inhalation of dust containing lanthanide during food production should be avoided (McDonald *et al.*, 1995; Hirano and Suzuki, 1996). The lanthanide oxides may be quantified in mixtures of individually labelled diets and in the stomach content of larvae and juveniles by inductively coupled plasma-mass spectroscopy (ICP-MS). In the present study, first, we aim to verify that lanthanide oxide markers are inert with respect to ingestion rate of juvenile cod, and second, to estimate food particle size preferences related to juvenile cod body size.

Material and methods

Two types of experiments were performed: a single experiment to test the marker inertness on cod juveniles, and then a series of six feeding experiments on juveniles of various sizes to test particle size preference. The basic content of the test diet (Table 1) is the same in all experiments. The basic test diet was divided into five batches, each batch labelled with a different lanthanide marker to get monolabelled pellets. Each pellet type was crushed and sieved into eight mono-labelled size classes. From this group of labelled size grades we produced seven multi-labelled diets (A–G) with five markers in each (Table 2).

Food production

As shown in Table 1, the test diet was formulated to be rich in protein, with herring (*Clupea harengus*) meal as the main component. Based on earlier experiments, the diet should be suitable for feeding juvenile cod (Lie *et al.*, 1989). The vitamins included were twice the level recommended by NRC (1993) in order to compensate for manufacturing losses and leakage. Squid mantle (*Gonatus fabricii*) and krill (*Euphasia superba*) were dehydrated by evacuation and ground together with the main mixture powder using a Retsch Ultra Centrifugal Mill, type ZM1. A base mixture of 20 kg was made and portioned into five batches of 2250 g. 4.5 g of one of the marker oxides was stirred vigorously into each batch followed by the addition of 320 g of water. Each batch of 2575 g moist mixture, with one marker included, was run through a 7-mm matrix on a pellet machine in the following marker sequence: La, Y, Yb, Dy, and Nd. The water content was measured to 19.4% before pelleting.

The markers Y_2O_3, La_2O_3, Nd_2O_3, Dy_2O_3, and Yb_2O_3 of 99.9% purity were purchased from Sigma Chemical Company. To reduce contamination of marker from the preceding batch, portions of 1.4 kg base mixture without marker were used to clean the pellet machine between each batch. This procedure resulted in approximately 2.2 kg of each batch of marked diet collected as pellets. The pellets were dried in air stream overnight to a dry weight of 95 g/100 g and crushed. A Fritsch Analysette rotatory shaker equipped with a staple of stainless steel sieves of decreasing apertures was used for grading the granulated mixture into eight particle classes, ranging from 0.0–0.3 to 3.35–3.5 mm. Then each particle size class was separately stored and analysed by ICP-MS to determine the marker element content.

The mono-labelled particle class 1.4–2.0 mm was used in a feeding experiment to verify that the markers' influence on the ingestion rate of a specific diet was not relative.

Table 1. Composition of feed mixture before addition of marker and pelleting.

Feed	Percentage of mixture			Percentage of dry weight			
	Wet weight	Ingredient dry weight	Dry weight	Lipid	Protein	Ash	Carbohydrate
Corn meal	11.0	90	11.0	0.2	0.9	0.1	10.0
Herring meal	75.3	93	77.9	7.7	61.3	8.6	
Gelatin	2.0	92	2.0		2.0		
Squid mantle	4.5	40	2.0	0.1	2.0		
Capelin oil	2.3	96	2.5	2.5			
Lecithin	1.0	96	1.1	1.1			
Krill paste	1.9	70	1.5	0.1	1.0	0.2	
Vitamins	0.9	97	1.0	0.3	0.4		0.3
Minerals	0.9	97	1.0			1.0	
Total	100		100	12.0	67.6	9.9	10.3

Table 2. Diet description and experimental conditions. A diet containing five markers was used in each experiment. In each of the experiments A−F, equal weights of five mono-labelled particle size classes, each with a different marker, were combined to a multi-labelled diet. In experiment G, mono-labelled particles of the same size were combined in form a multi-labelled diet. The mid-mesh opening (mm) is used as a nominal reference for the particle size. Experiment A is used as an example; three tanks of 180 l were stocked with 140 cod taken from a population with average body weight 36 mg sampled at day 0. The tanks were terminated on days 1, 4, and 7, respectively. At termination, 41 samples each with ten fish of similar size were sorted for ICP-MS analysis.

Name of diet and experiment				A	B	C	D	E	F	G
Size grades of feed particle class (mm)										
Mid	Upper	Lower	Span							
0.15	0.30	0.00	0.30	Dy						
0.50	0.70	0.30	0.40	Yb	Dy	La				
0.85	1.00	0.70	0.30	Nd	Nd	Yb	La	Dy		
1.20	1.40	1.00	0.40	Y	La	Nd	Yb	Y	Dy	
1.70	2.00	1.40	0.60	La	Y	Dy	Nd	Nd	Yb	La, Y, Dy, Nd, Yb
2.35	2.70	2.00	0.70		Yb	Y	Dy	La	Nd	
3.03	3.35	2.70	0.65				Y	Yb	La	
3.43	3.50	3.35	0.15						Y	
Total class span in experiment (mm)				2.00	2.40	2.40	2.65	2.65	2.50	0.60
Fish stocking density (individuals per tank)				140	150	100	95	32	28	29
Body weight of sample on day 0										
Mean (mg)				36	143	394	394	826	826	826
s.d.				6	25	89	89	161	161	161
Standard length at day 0										
Mean (mm)				17	24.8	35.4	44.1	44.1	44.1	
s.d.				1.1	1.6	2.9	2.9	2.6	2.6	2.6
n				22	30	40	40	40	40	40
Feeding and sampling during feeding experiment										
Daily supply of dry diet in tank (g)				10	25	25	33	33	29	29
Individuals in sample				10	10	5	5	2	2	2
Count of samples				41	45	59	43	46	41	41
s.d. as % of sample bw				16	12	11	11	8	8	7

Five fractions of this size class, with one marker in each fraction, were blended by equal weight to produce the test diet that was used for the assessment of possible marker taste preferences by the fish (Table 2, diet G).

To evaluate preferences for particle size, sets of five differently labelled size classes were blended by equal weight and offered to juvenile cod of various weight classes (Table 2, diets A−F).

Elemental analysis

For the analysis of ^{39}Y, ^{57}La, ^{60}Nd, ^{66}Dy, and ^{70}Yb concentrations in samples of the food and fish, the samples were digested by adding 2 ml concentrated nitric acid (HNO$_3$) and 0.5 ml hydrogen peroxide (H$_2$O$_2$). 1 g of diet was homogenized to measure marker concentration in the food samples, while two, ten-cod specimens were pooled in each analysis in order to have enough material for analysis of marker content in the fish. The digestions were run in tetrafluorine methoxil digestion vessels through a standardized temperature programme (S1, 250 W, 5.00 min; S2, 0 W, 1.00 min; S3, 250 W, 5.00 min; S4, 400 W, 5.00 min; and S5, 650 W, 5.00 min) in a microwave oven (Milestone MLS-1200, Italy) (Julshamn *et al.*, 1999). After digestion, the digests were diluted to 25 ml, and the elements were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin−Elmer SCIEX Elan 5000A). In ICP-MS, the ICP is established as an effective ion source for MS as detector for the lanthanide elements. The blank samples were prepared and run together with the sample solutions. The method of standard addition calibration was used for quantification of the element in question. The lanthanide content was calculated as milligramme elemental lanthanide per gramme total wet weight of fish in the vial.

Fish stocking, experimental conditions, and sampling

The juvenile cod used in the feeding experiments were collected from a population of pond-reared juveniles

([Blom *et al.*, 1991](#)). Newly hatched larvae had been released into the pond and preyed on naturally occurring zooplankton until they were collected for experiments. The fish were distributed randomly among black 180-l experimental tanks, and the feeding was started. The multi-labelled food was supplied with automatic disc feeders at 1-min intervals for 24 h d^{-1}. Feeding was in excess, approximately ten times the estimated requirement. Surplus food and dead fish sedimented in the conical bottom of the tanks were tapped out once a day. The water temperature during the experiments was approximately 10°C.

Three tanks were used for each feeding experiment, of which one tank was terminated at day 1, one at day 4, and the last at day 7 after start. One experiment (experiment G) was performed to verify that the marker was inert. Six experiments (A–F) were conducted to investigate the preference for diet particle size.

The fish in the tank were euthanized with an anaesthetic (metomediate) and gently tapped out through the outflow. The fish were rinsed in seawater, dried on a blotting paper, individually weighed (wet weight), put in scintillation vials, and frozen until analysis. Individuals of similar size (Table 2) were pooled in each vial. The experimental conditions, such as stocking of fish, labelling diets, feeding, and sampling, are specified in Table 2. Standard statistical tests ([Zar, 1999](#)) were used as specified in the appropriate figures and tables, specifically the Shapiro–Wilks W test of normality, two-way ANOVA, and chi-square for distribution fitting.

Results

Food production

The marker level (Table 3) and the corresponding level of contaminating markers (Table 4) in each mono-labelled food and size fraction were assayed. A contaminating marker is referred to as the remaining portion of the marker used to produce the preceding batch. The average contamination accompanying each main marker (Table 4) was used to calculate contamination correction factors (Table 5) to be applied to the ICP-MS measurements of lanthanide levels in the juveniles. An example of correction is that the level of observed Yb (mg kg^{-1}) in the fish from ingestion experiments should be reduced by 14.2% of the Dy value (mg kg^{-1}) (Table 5). Even though an identical amount of marker was used in each batch during diet production, the levels measured by ICP-MS after production were not the same. Most of the variation could be explained by marker (Table 3), while size fraction had less impact on the variability (Table 3). Only one ICP-MS analysis for each of the 40 feed batches was performed, as these analyses are very costly. Therefore, we chose to normalize the lanthanide level in each individual food particle batch for segregation (different lanthanide level for each size grade) and marker profile (different level of marker for each lanthanide used) in addition to corrections for contamination level when ingestion in cod was calculated. The normalizing procedure and resulting recalculated values are shown in Table 6.

In the case of feeding experiment G (Table 2), the size class 1.4–2.0 mm of mono-labelled particles was blended

Table 3. Apparent level of lanthanide main marker in 40 mono-labelled diets. The values are based upon a single ICP-MS analysis of each mono-labelled particle fraction. The marker ratio is the column mean as a proportion of the grand mean 1322.4 mg kg^{-1}, and the segregation ratio is the row mean as a proportion of this grand mean. The marker level refers to a dry diet with 5% water content.

Diet size range properties (mm)			Mono-labelled diet particle fraction with specific main marker							
			La	Y	Yb	Dy	Nd	Mean	s.d.	Segregation ratio
Mid	Lower	Upper	Single measurement: marker element level (mg kg^{-1})							
0.15	0.0	0.3	1 270	1 250	1 330	1 320	1 280	1 290	34	0.976
0.50	0.3	0.7	1 362	1 176	1 441	1 340	1 410	1 346	103	1.018
0.85	0.7	1.0	1 252	1 141	1 371	1 384	1 425	1 315	116	0.994
1.20	1.0	1.4	1 246	1 134	1 344	1 403	1 386	1 303	112	0.985
1.70	1.4	2.0	1 568	1 327	1 480	1 433	1 552	1 472	98	1.113
2.35	2.0	2.7	1 260	1 261	1 394	1 317	1 400	1 326	68	1.003
3.03	2.7	3.4	1 470	1 270	1 570	930	1 240	1 296	247	0.980
3.40	3.3	3.5	1 050	930	1 470	1 310	1 400	1 232	232	0.932
Mean			1 310	1 186	1 425	1 305	1 387	1 322.4	92	1.000
s.d.			158	124	81	158	95	69		
Main marker ratio			0.990	0.897	1.078	0.987	1.049	1.000		
Shapiro–Wilks W test (p)			0.59	0.23	0.68	0.002	0.36	0.69		

ANOVA: two way without return.

H$_{05}$: No segregation. $p = 0.156$.

H$_{qc}$: Equal lanthanide concentrations. Rejected. $p = 0.0047$.

Table 4. Level of the major contaminant (mg kg^{-1}) found in the single measurement of main marker (Table 3). Minor contaminants accompanying the main marker were below the threshold of detection.

	La*	Y	Yb	Dy	Nd
Diet size (mm)	None†	La	Y	Yb	Dy
0.15	0	56	43	130	20
0.50	0	11	1	120	0
0.85	0	63	38	164	22
1.20	0	46	26	174	18
1.70	0	47	15	107	0
2.35	0	10	8	214	9
3.03	0	8	4	390	20
3.43	0	88	4	180	10
Mean	0	42	17	185	12
s.d.	0	29	16	90	9

*The first row represents the main marker.
†The second row represents the major contaminants.

on an equal weight basis and fed to a group of juveniles with initial body weight of 826 mg.

The apparent food content associated with the cod juveniles was estimated from the ICP-MS analysis of marker oxides, corrected for cross-contamination, and normalized as described in the previous section. The corrected marker weight per cent distribution of each sample is shown in Figure 1. The hypothesis of marker inertness was not rejected (Table 7; chi-square = 0.6239, 4 d.f., p = 0.96 based on 41 samples). Therefore, we could use the investigated lanthanides and yttrium as inert markers, equally repellent or attractive if used together in the same experiment.

Table 5. Correction for contamination measured as a percentage of the main marker. The correction values are calculated from Table 4.

Main marker element	La	Y	Yb	Dy	Nd
Contaminating element	None	La	Y	Yb	Dy
% Weight of main marker	0	3.5	1.2	14.2	0.9
s.d.	0	2.4	1.1	7.1	0.6
n	8	8	8	8	8

Preferences for particle size (experiments A−F)

The fish in experiments A−F had the opportunity to ingest from a mixture of five particle size classes. The estimated amount of dry diet in the fish originating from each of the five particle size classes is reported in Figure 2. Ideally, the particles presented in each experiment should include the entire size range from which fish of a certain size are capable of ingesting. The experiments A, B, C, and E seem to fit this criterion. The smallest fish (experiment A; 33−41 mg) ingested particles from nominal size 0.15 to 1.7 mm, but most from size 0.15 mm. Fish weighing 146−178 mg (experiment B) had highest ingestion of particle sizes from 0.85 to 1.2 mm. In experiments C (fish; 400−506 mg) and E (fish; 848−1037 mg), the maximum (peak) ingestion came from particles near 1.2 mm. That was also the case for experiments D and F, where particle sizes from below 1.2 mm and up to 3.35 and 3.5 mm were offered to fish from 375 to 1180 mg. We conclude that small larvae ingested optimally small particles, and the particle size of "preference" increased up to about 1.2 mm at a body weight of approximately 600 mg. In none of the

Table 6. Normalized level of the lanthanide main marker in 40 mono-labelled diets. The normalization is derived from Table 3 by the formula: normalized lanthanide level = segregation ratio × element ratio × 1322.4.

| Main marker element	Mono-labelled diet particle with specific main marker							
	La	Y	Yb	Dy	Nd	Mean	s.d.	Segregation ratio
Nominal diet size (mm)	Normalized marker element level (mg kg^{-1})							
0.15	1 278	1 157	1 390	1 273	1 353	1 290	90	0.976
0.50	1 333	1 207	1 450	1 328	1 411	1 346	93	1.018
0.85	1 202	1 179	1 417	1 297	1 378	1 315	91	0.994
1.20	1 290	1 168	1 404	1 285	1 366	1 303	90	0.985
1.70	1 458	1 320	1 586	1 452	1 543	1 472	102	1.113
2.35	1 314	1 190	1 429	1 309	1 391	1 326	92	1.003
3.03	1 284	1 162	1 397	1 279	1 359	1 296	90	0.980
3.40	1 220	1 105	1 328	1 215	1 292	1 232	86	0.932
Mean	1 310	1 186	1 425	1 305	1 387	1 322.4	92	1.000
s.d.	68	62	74	68	72	69		
Main marker ratio	0.990	0.897	1.078	0.987	1.049	1.000		
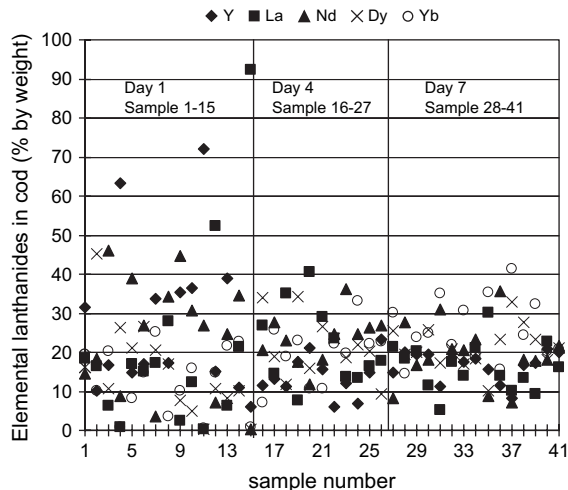
Figure 1. Percentage distribution (by weight) of five elemental lanthanides (Y, La, Nd, Dy, and Yb) in 41 samples of cod juveniles (two individuals per sample) fed using diet G. Values are corrected and normalized to the corresponding lanthanide levels in the diet.

experiments did we observe maximum (peak) ingestion from particles larger than 1.2 mm.

Small fish (below 500 mg bw) had not started eating on the first day (Figure 2, experiments A−D), but larger fish (800−1200 mg) had started. The ingested material associated with the fish from all experiments at days 4 and 7 was approximately the same (12.7 ± 2.6 and 13.4 ± 2.0 mg g^{-1} bw $\pm$ s.d., respectively) regardless of body weight, so it is not necessary to carry out feeding experiments of this type for more than four days.

Based on a dry weight composition of cod of 18% (Van Pelt *et al.*, 1997; Finn *et al.*, 2002), we can calculate the dry gut content as percentage of the larval dry weight as approximately $(12.7 + 13.4) \times 0.5 \times 100 \times 180^{-1} = 7.63\%$. Correspondingly, based on dissection and weighing, 6% gut content in cod larvae feeding on zooplankton has been reported (Gamble and Houde, 1984). The low feeding ratio for the smallest fish is further visualized when the entire data set from each day is plotted (Figure 3) with a linear trend forced through zero. The average magnitudes of ingestion calculated from the trend line at days 4 and 7 are 14.3 and 11.4 mg g^{-1} body weight, respectively ($r^2 = 0.78$ and 0.73).

Discussion

Even if no significant differences in marker concentrations among the particle size classes were observed, one should be cautious and aware of the possibility for marker segregation, which may arise as a result of incomplete blending or internal particle structures and breaking zones in the production of granulates from pellets. The use of normalization allows one to conduct a single measurement of the lanthanide concentration in each mono-labelled particle size fraction, which is time- and cost effective. With the exception of Yb as a contaminant of Dy, the contaminant correction was a minor adjustment. We recommend that cross-contamination during particle production be minimized or preferably avoided, which is feasible if thoroughly cleaned or separate equipment is used (Otterå *et al.*, 2003).
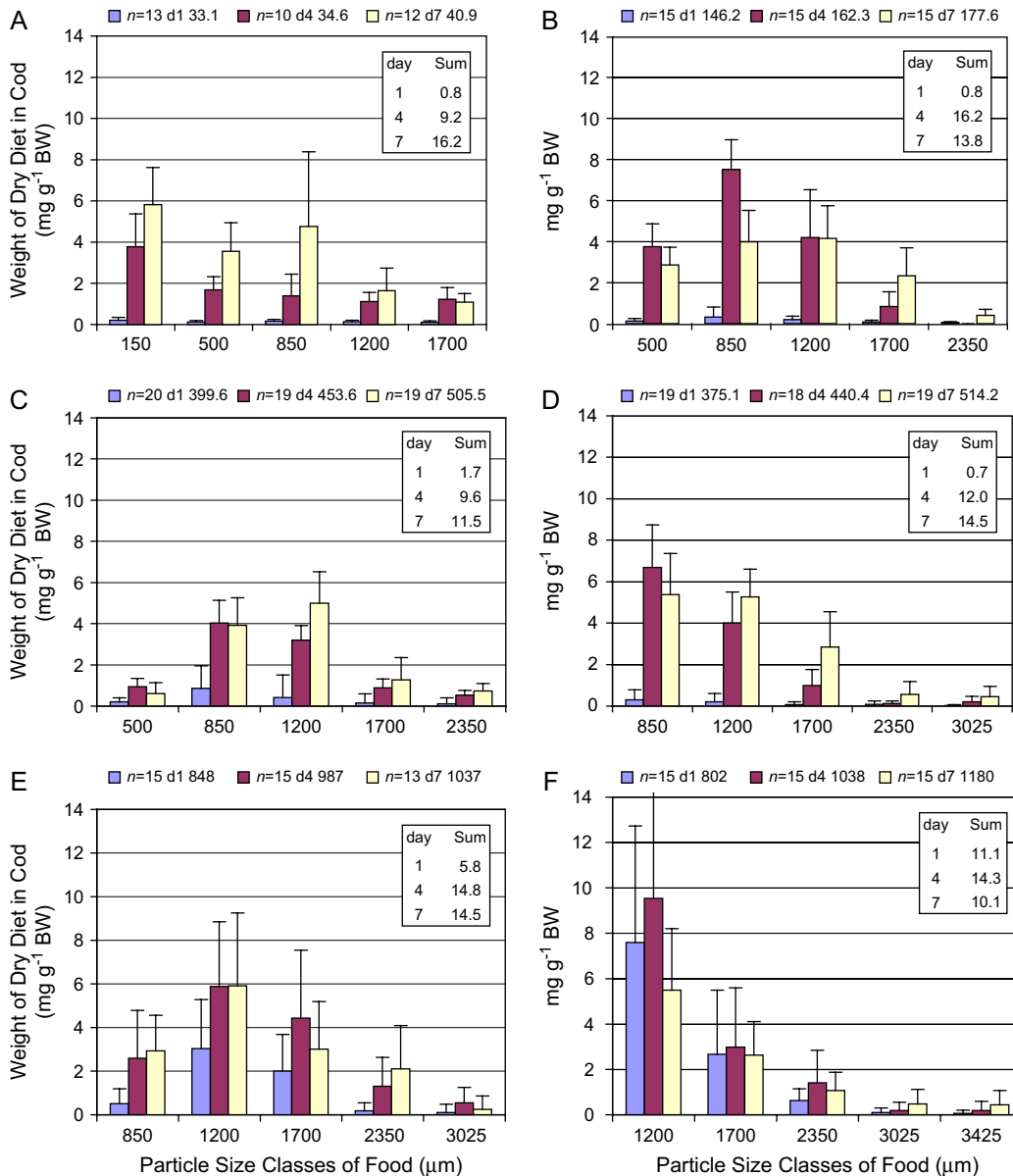
Table 7. Apparent ingested dry food originating from five simultaneously fed diets of nominal size 1.4 mm with one marker in each (experiment G). Hypothesis: Equal ingestion among markers.

Body weight (mg) in experiment G				
Day	1	4	7	All
Mean	850	977	1 215	1 010
s.d.	163	289	293	299
n	15	12	14	41

Ingested dry feed per body weight (mg g^{-1})								% Distribution				
Marker	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Day 1	Day 4	Day 7	All	Hypothesis
Y	2.96	2.4	3.01	1.8	2.36	1.1	2.78	1.9	27.6	16.0	18.8	20.2	20
La	1.96	1.8	4.34	2.6	2.07	1.3	2.71	2.2	18.3	23.1	16.4	19.7	20
Nd	2.65	2.0	3.75	1.7	2.49	1.4	2.93	1.8	24.7	20.0	19.8	21.3	20
Dy	1.68	1.8	4.18	2.6	2.65	1.2	2.76	2.2	15.7	22.3	21.1	20.0	20
Yb	1.48	1.3	3.48	1.7	3.00	1.3	2.59	1.7	13.8	18.6	23.9	18.8	20
Chi-square test on fit of % distribution with 4 d.f. (*p*)									0.13	0.78	0.81	0.96	
Ingested sum	10.7	2.6	18.7	4.4	12.6	2.8	13.8	1.5					

Figure 2. Dry diet (mg g^{-1}) in cod (mg wet weight) originating from each of five particle size classes (class midpoint μm). Mean and standard deviation shown for experiments A–F. n = number of samples; d1 = day 1; and average milligramme body weight sampled on that day. Inserted box: day and total sum of apparent ingested dry diet per wet body weight on that day.

We have assumed that hypothetical taste preferences are associated with the intended main marker in each diet particle, and that the effect of a minor contaminating marker on preference for ingestion is negligible. A rationale for this assumption might be that all the main marker sources may contribute to a low level lanthanide flavour in the tank, thus masking the presence of a minor contaminant in a certain particle. The variation in marker percentage distribution of experiment G seems to be largest in the tank terminated after one day as indicated by extreme outliers

above 40% or under 5% in Figure 1, and also if percentage standard deviations are calculated from Table 7. The reason may be that only a few particles are ingested the first day, causing statistical fluctuations. In retrospect, the particle range (1.4–2 mm) used in experiment G lies above the optimal particle size suited for ingestion (Figure 3, experiments E and F).

We did not find the maximum (peak) ingestion from particle classes of nominal size larger than 1.2 mm. The particles presented to the fish in experiments D and F were
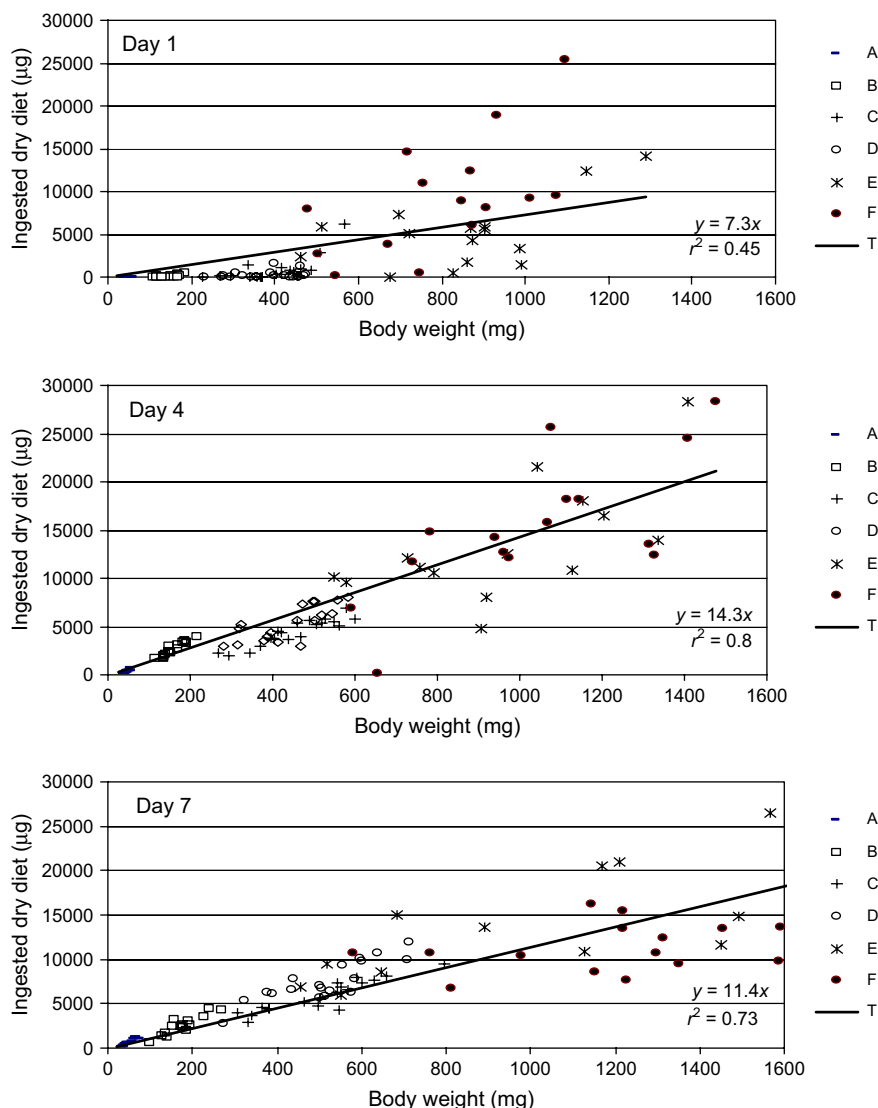
Figure 3. Scatterplot and linear relationship between apparent ingested dry diet (μg) and body weight of cod (mg) in experiments A−F on days 1, 4, and 7. Linear equations were forced through origin.

too large, and the ingestion maximum lies to the left in the histograms; 1.2 mm is less than 30% of the estimated mouth height of the fish (Otterå and Folkvord, 1993). However, from our experiments we cannot conclude that particles smaller than 1.2 mm were preferred. Larger particles may be less available owing to higher sinking rates than small particles. Although larger particles may be more easily seen and have more nutrition per particle, they are distributed in lesser concentration, and may be less available. More experiments with diets blended in unequal weight ratios should therefore be designed to evaluate preference, availability, and optimal ingestion of food.

It is likely that we overestimated the percentage gut content. The value 7.63% is based on the level of the marker in the diet at the time of ingestion, but the diet is digested, and

the marker is concentrated and finally evacuated after some time (Austreng *et al.*, 2000). Since the amounts of ingested materials associated with the fish at days 4 and 7 were about the same, it seems that a steady state equilibrium between ingestion and evacuation had been reached.

The large variation in the amount ingested among individual samples suggests a heterogeneous eating behaviour within each experiment. Such heterogeneity is of particular interest, since it may influence individual growth rates and lead to cannibalism. The heterogeneity is probably underestimated owing to the pooling of several individuals within a sample. An increase in the sensitivity of the detection method would significantly increase the usefulness of this method for larval and juvenile fish. This could be done by increasing the marker concentration and modifying the

dilution factor, or maybe by improving ICP-MS technology. The lanthanide marker methodology could then be used to generate ingestion data of single larva.

We conclude that yttrium and lanthanide labelling offers powerful tools for quantifying diet ingestion in fish and for comparing ingestion of differently labelled diets by the same group of fish. We also have used the technique to study effects of dietary water content (Otterå *et al.*, 2003) and future possibilities includes the study of the effects of food type, colour, taste, hardness, or other diet properties.

Acknowledgements

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