



## Consumption of food and evacuation in dab (*Limanda limanda*) related to saturation and temperature. Preliminary results.

by

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### Abstract:

Dab (*limanda limanda*) acclimatized to 4 different levels of oxygen saturation have been fed to satiety in intervals of 24 hours with food of known weight. The food given was cooked *Mytilus edulis* and raw flesh of cod.

The levels of oxygen saturation were 20-40 %, 35-50 %, 65-85 % and 80-90 %, the temperatures were 11.5-12.0°C, 11.0-11.5°C, 10.0-10.5°C respectively and the salinity 30 ‰. The 3 lower levels of saturation were obtained by addition of nitrogen adjusted by a computer program connected to 3 WTW OXY 196 and 3 valves.

At the saturation level of 80-90 % the mean consumption per 100 g consumer/day or 0-4.5 % of the mean body weight/day. The amount of food eaten a given day seems to be related to the amount eaten the day before. At the lowest level of saturation (20-40 %) the consumption may stop for several days in between varying between 0 and 3.0 g/100 g consumer/day or 0.3.0 % mean body weight. The mean consumption per 100 g consumer/day at 80-98 % saturation in 94 consecutive days was 4.5 times higher than at a saturation of 20-40 %.

After acclimatization until normal feeding at two different temperatures 7.5-8.0°C and 10.5-10.6°C the dab have been starved for 24 hours and then offered pieces of cod flesh of known weight each with a numbered internal plastic tag. The food was offered to satiety. The oxygen saturation was 90-100 % and the salinity 33 ‰.

With intervals of 6 hours (6-30 hours) 3-9 fish were killed. The content of food in the stomach and the first part of the intestine and in the last part of the intestine have been removed and weighed separately, and the initial weight estimated from the numbered tags. The rates of evacuation the first 18 hours at the two different temperatures were at

the same level but faster at the highest (10.4-10.6°C) during the last periods. Assuming a linear evacuation the total emptying seems to last 49 hours and 30 hours at 7.5°C and 10.5°C respectively.

The results of the experiments have been compared with data on stomach content of dab in areas exposed to hypoxia, the southern Kattegat and Århus Bay.

## 1. Introduction:

In the transition area between the North Sea and the Baltic, the Kattegat and the Belt Sea, hypoxia regularly occurs in summer and autumn due to a distinct halocline between the brackish Baltic surface water and the saline North Sea water, along the bottom which do not allow oxygen from the atmosphere to supply the bottom water.

These oxygen conditions mainly influence bottom invertebrates and demersal fish like plaice and dab.

In dab a reduction in growth has been indicated in the southern Kattegat, Bagge and Nielsen, 1987. Bagge et al., 1993 and in Århus Bay, Bagge et al 1994.

It may be assumed that hypoxia reduces the appetite of the fish, which may be connected with a reduced evacuation of the stomach. Gwyther and Grove (1981) confirmed for dab that stomach fullness is a major factor controlling appetite in dab. That is shown earlier for other species by several authors, Grove et al 1978, Elliot and Persson, 1978 and Brett (1971). This report is very preliminary.

## 2. Material and Methods.

The experiments on gastric evacuation (A.) have been carried out in the laboratory of Danish Institute of Fishery and Marine Research in Charlottenlund and the experiments food optake related to oxygen saturation (B) has been carried out in the Marine Biological Laboratory (University of Copenhagen) in Helsingør.

### A. Gastric evacuation.

Dabs were trawled in 10 minutes hauls October 1994 and January 1991 in the northern Sound (Subdivision 23) and kept in three 700 l-tanks with recirculated aerated Sea water, salinity 33 o/oo. The temperature was in the first experiment 7.5°C and in the second 10.5°C. The illumination during day lasted 10 hours in both experiments. 30 individuals were put in each tank in the first experiment but after 1 month reduced by mortality to a total of 25 which were feeding regularly on raw cod meat. In the second experiment only 15 individuals were put in each tank which yielded 30 feeding individuals after 2 weeks.

In order to estimate the individual food intake the fish were offered lumps of cod meat of

known weight tagged with internal numbered yellow plastic tags 3 x 5 mm. These tags were easy to recognize in the stomachs and the intestines and not regurgitated as in cod, possibly because in dab the stomach is not that distinctly separated from the intestine.

Before the experiment on stomach evacuation at 10,5°C was started with tagged food the dab in the 3 tanks was fed with cod meat of known weight to estimate the mean food uptake per day relative to the mean body weight. The experiment was run at 10,5°C to make it comparable with the experiment (B) in Helsingør run at that temperature.

The following procedure was used to estimate the evacuation over time: the fish were starved 24 hours to secure empty stomachs and then fed until satiation with tagged food of known weight. After 6, 12, 18 and 24 hours respectively 3-9 dab (table 2) were captured, weighted and measured individually and then killed. The content of stomachs and guts were then weighted separately and the original intake of food estimated from the numbered tags.

### 3. Results.

#### 3.1 The daily consumption:

In the experiment run at 10,5°C. the dab have been offered food until it was sure that all of them had started feeding (about 4 weeks). The fish were then fed until satiation 6 days per week with food of known weight the last 14 days before the evacuation experiment started in order to estimate the daily consumption relative to body weight. The results are shown in table 1 as weight of food relative to body weight in percent.

It appears from the table that the food intake varies from 0,64 to 3,97 % of b.w. (body weight) and that a high food intake is followed by a day with a lower one. The values are means of 18,10 and 10 specimens in tank 1,2 and 3 respectively. These day to day fluctuations are also observed by Pandian (1970) and in the parallel experiments in Helsingør (B).

#### 3.2. The gastric emptying over time.

The gastric evacuation at 7.5°C and 10.5°C as percentage of the food intake after 6, 12, 18, 24 and 30 hours is shown in figure 1 and table 2 together with the means and the standard deviations of the weights of stomach content relative to body weight (b.w.) as percentage. The means and standard deviations of the body weights and the standard deviations are given as well. Fitting a straight line to the percentual evacuation of stomachs over time at the two temperatures it appears from figure 1 that at 7.5°C the emptying time is about 49 hours (48.9) and at 10.5 C. about 30 hours (30.4). Gwyther and Grove (1981) have given a none linear multiple regression which describes the relations between the gastric emptying time (GET hours), temperature, body weight and meal size relative to body weight:

$\ln(\text{GET}) = 1.46 + 0.68(\ln M) + 0.39(\ln W) - 0.035T$  where M is the relative meal size

$\ln(\text{GET}) = 1.46 + 0.68(\ln M) + 0.39(\ln W) - 0.035T$  where M is the relative meal size (M%b.w.), W the body weight (W grams) and T the temperature. Fitting the data in table 2 to that model with  $T=7.5^\circ\text{C}$ ,  $M=1.73$  and  $W=1.68.9$  (mean of the five mean body weights in table 2) and  $T=10.5^\circ\text{C}$ ,  $M=2.76$ ,  $W=207.5$  (mean of the four mean body weights in table 2) the GET of  $7.5^\circ\text{C}$  will be 35.5 hours and GET of  $10.5^\circ\text{C}$  47.6. The reason is that the impact of temperature in Gwyther and Grove's equation is negligible while the meal size relative to body weight is important.

The data from table 2 have been fitted to the none linear multiple regression model as used by Gwyther and Grove (1981), (REG in SAS) and a R selection used to control the relevance of all the variables:

Variables	R <sup>2</sup>
$\ln(M)$	0.2242
T	0.0773
$\ln(W)$	0.0110
$\ln(M)T$	0.2792
$\ln(M)\ln(W)$	0.2263
$\ln(W)T$	0.1099
$\ln(M)\ln(W)T$	0.2799

The regression is mainly influenced by  $\ln(M)$  and T. The weight of the fish is of negligible importance. Omission of  $\ln(W)$  yields the following expression:

$\ln(\text{GET}) = 3.65 + 0.41 \ln(M) - 0.087T$  ( $R^2 = 0.2792$ ,  $n = 50$ )  
 GET at  $7.5^\circ\text{C}$ . and  $10.5^\circ\text{C}$ . is then 25.1 hours and 23.5 hours respectively.

## B. Feeding and Oxygen Saturation.

### 4. Material and Methods.

The experiments at the Marine Biological Laboratory (University of Copenhagen) in Helsingør were started in the beginning of December 1994. The dab used in the experiments were obtained as described in (A.).

four tanks height 45 cm, width 49 cm and length 100 cm were used, covered on the bottom with 5 cm cleaned sand and on the surface with a plate of PVC foam. The volume of water in 3 of the tanks ( $A_1$ - $A_3$ ) was 100 l and in the fourth ( $A_4$ ) 170 l. Tanks  $A_2$  and  $A_3$  were connected so that the water was renewed from a recirculated system in  $A_1$  and returned to the system from  $A_3$  in order to create 3 different oxygen saturations. The water in  $A_0$  was renewed from the recirculated system and returned directly to the system. 4 dab (21-26 cm) (95-175 g) were put in each of the tanks  $A_1$ - $A_3$  and 5 in  $A_0$ . The salinity was 30 0/00 and the temperature in  $A_1$  was  $10.0$ - $11.0^\circ\text{C}$  increasing to  $11.0$ - $12.4^\circ\text{C}$  in  $A_3$ . In  $A_0$  the temperature was  $9.2$ - $9.8^\circ\text{C}$ .

A<sub>1</sub>-A<sub>3</sub> and the temperature and oxygen saturations were continuously down loaded in file format. 3 different oxygen saturations were obtained by covering the surface of the water in all tanks by plates of PVC foam and by admission of nitrogen to A<sub>2</sub> adjusted by a computer program to keep the saturation at a level of 42%. In that way a saturation of 35-50% was obtained in A<sub>2</sub> and a saturation of 20-35 % in A<sub>3</sub>.

The temperature and oxygen saturation was also registered manually every morning. In A<sub>0</sub> only manual registration has been done.

The fish were acclimatized in the period 1.12.94-26.01.95 to eat at saturations: 80-90% in A<sub>0</sub>, 20-35% in A<sub>3</sub>, 35-50% in A<sub>2</sub> and 65-85% in A<sub>1</sub>, being offered food of known weight (boiled *Mytilus edulis*) every morning at 8 o'clock GMT. The registration of the daily feeding started 27.01.95.

## 5. results.

### 5.1 Saturations and temperature.

The daily level of oxygen saturations in A<sub>0</sub> (the highest level) and A<sub>3</sub> (the lowest level) is shown in fig. 2 and the levels in between A<sub>1</sub> and A<sub>2</sub> in fig. 3. It appears that there are fluctuations but that the lowest level A<sub>3</sub> and the two highest levels A<sub>0</sub> and A<sub>1</sub> are well separated.

The continuous registration of saturation and temperature is not given but the mean saturations of the daily manual observations (94 obs. in the period 27.01-5.02.95 are given below:

	Mean saturation	Std.	Temp.
A <sub>0</sub>	85,78%	3.64	9.2-9.8
A <sub>1</sub>	72.69%	12.53	10.0-11.0
A <sub>2</sub>	41.48%	7.50	10.5-11.8
A <sub>3</sub>	29.80%	7.81	11.0-12.4

The standard deviation in A<sub>1</sub> is high but the levels of saturation in A<sub>2</sub> and A<sub>3</sub> differ significantly as well from A<sub>0</sub> and A<sub>1</sub>.

### 5.2 The daily consumption.

The daily consumption according to oxygen saturation is given as percent of body weight (b.w.). It was not possible to register the amount of food eaten individually, which means that a mean body weights per tank (A<sub>0</sub> -A<sub>3</sub>) have been used. The daily consumption is then: mean weight consumed per specimen /mean weight of consumers.

The mean weight consumed being the total amount consumed divided by the number of consumers in the tank irrespective whether all have eaten. During the experimental period growth of the dab was observed and therefore the mean weight of the consumers was changed 3 times during the period by mean of a length-weight key.

In fig. 4a is shown the daily consumption per 100 g consumer in relation to oxygen saturation 20-40% and 80-90% and in fig. 4b in relation to oxygen saturations 35-50% and 65-85%.

At the 20-40% saturation many days (45) without feeding are observed between days with food uptake, but also at 80-90% saturation some few none feeding days occur (4).

At 35-50% and 65-85% saturations the fluctuations are observed with a higher zero observations in the lower level of saturation (21) being compared to saturation 20-40% the number of zero observations is reduced.

The day to day range of feeding rate and the mean feeding rate varies according to the oxygen saturation as follows.

	Saturation %	Mean	Feeding rate % b.w.	Mean	St.d.	CV	zero obs.	N
A <sub>0</sub>	80-90	85.8	0-7.5	2.23	1.64	73.5%	4	94
A <sub>1</sub>	65-85	72.7	0-6.5	1.59	1.17	73.5%	5	94
A <sub>2</sub>	35-50	41.5	0-4.5	1.12	1.12	100.0%	21	94
A <sub>3</sub>	20-40	29.8	0-3.0	0.49	0.52	106.0%	44	94

It appears that the maximum feeding rate and the mean feeding rate is decreasing with decreasing oxygen saturation and that the c.v. is increasing together with the increasing number of zero observations. The mean feeding rate of 80-90% and 20-40% differ significantly.

The day to day fluctuations in feeding rates are as described in 3.2. Days with high feeding rates are followed by days with low ones (Pandian, 1981).

### 5.3 Daily oxygen saturation and consumption.

Even if it was aimed to keep the 4 levels of oxygen saturation constant quite large fluctuations occurred except in the level 80-90%. The daily consumption expressed as % of body weight is compared with daily observations of oxygen saturation and correlated.

The variations are shown on fig. 5 a (80-90%), fig. 5b (65-85%), fig. 5 c (35-50%) and fig. 5 d (20-40%), and the results of the correlations are given below:

	Saturation	%Mean	R	N	(N-2)	P
K <sub>0</sub>	80-90	85.8	0.0091	94	92	-
K <sup>1</sup>	65-85	72.7	0.302	94	92	0.01
K <sub>2</sub>	35-50	41.5	0.489	94	92	0.001
K <sub>3</sub>	20-40	29.8	0.429	94	92	0.001

The strongest correlations are found for K<sub>2</sub> and K<sub>3</sub> which may indicate that below a saturation of 40-50% the appetite starts to be affected significantly.

#### 5.4 The feeding rates in nature.

During the years 1987-91 5928 stomachs of dab have been sampled in the southern Kattegat on 4 stations, (Bagge et al. 1993) in March, May, September and November.

The mean stomach content as percent of body weight according to length in half cm for March, May and November including empty stomachs 1987-91 is shown in fig. 6.

To compare with the data from the experiments the feeding rates correspond to the lengths from 45-55 scm should be chosen (120-220 g. b.w.). It appears that the mean feeding rates in that interval are between 0.2 and 0.6% corresponding to a mean rate of 2.2 in the experiment at 80-90% saturation or 4-10 times less. It should be mentioned that the temperature in the experiment at 80-90% saturation was 9.2-10.0°C compared to the mean temperature of the observations at the bottom (30 m) on the four stations in the southern Kattegat in March and May 1987-91 which was 5.85°C. However, the corresponding mean temperature in November was 12.4°C, and the stomach content relative to body weight does not differ in that month. (Fig. 7).

## References.

- Bagge, O. and E. Nielsen, 1987.  
Growth and recruitment of plaice in the Kattegat. ICES C.M. 1987/6:7,  
Demersal Fish Committee.
- Bagge, et al., 1993.  
Bundfaunaens betydning for bundlevende fisk i det sydlige Kattegat. Havforskning  
fra Miljøstyrelsen Nr. 27, 1993.
- Bagge, O., E. Steffensen, E. Nielsen and C. Jensen, 1994.  
Growth and abundance of plaice in Århus Bay in relation to oxygen conditions  
1959-1993. ICES, C.M. 1994/J:16.
- Gwyther, D. and J. Grove, 1981.  
Gastric emptying in *Limanda limanda* (L.) and the return of appetite. J. Fish.  
Biol. (1981) 18. 245-259.
- Pandian, T.J., 1970.  
Intake and conversion of food in the fish *Limanda limanda* exposed to different  
temperatures. Marine Biology 5, 1-17 (1970).



### Stomach evacuation in dab.

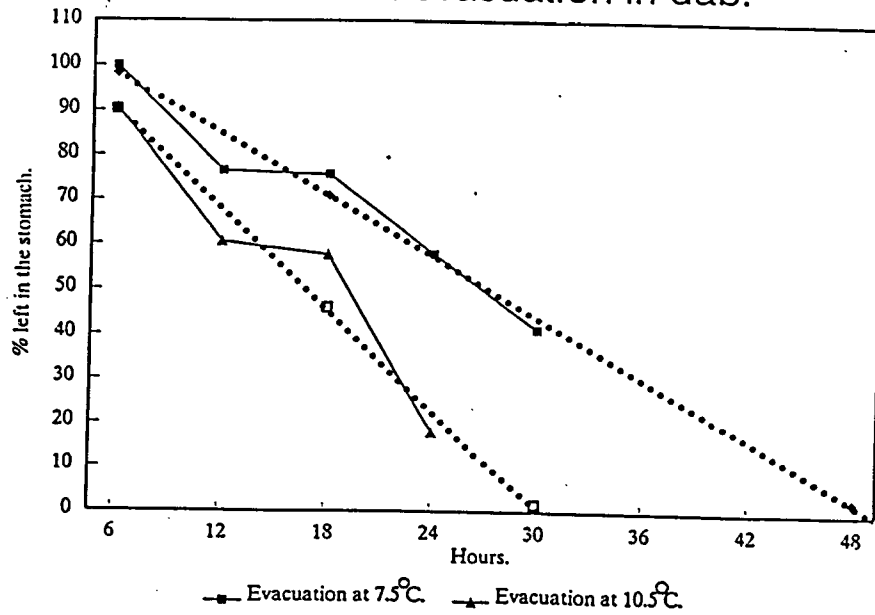
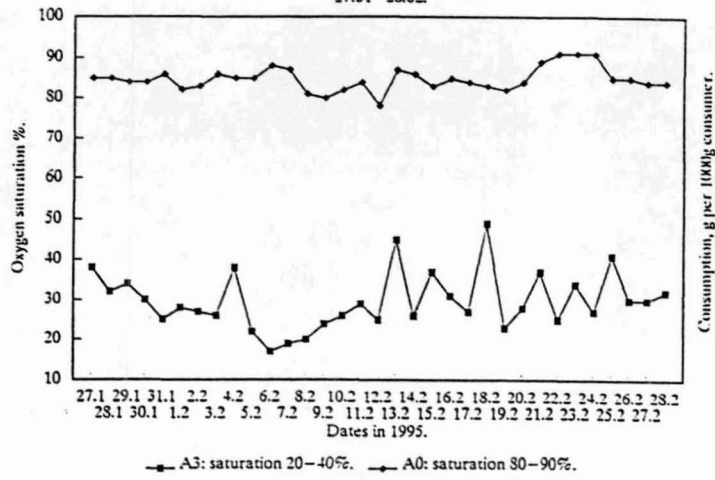


Figure 1. Evaluation of stomachs at 7.5°C and 10.5°C.

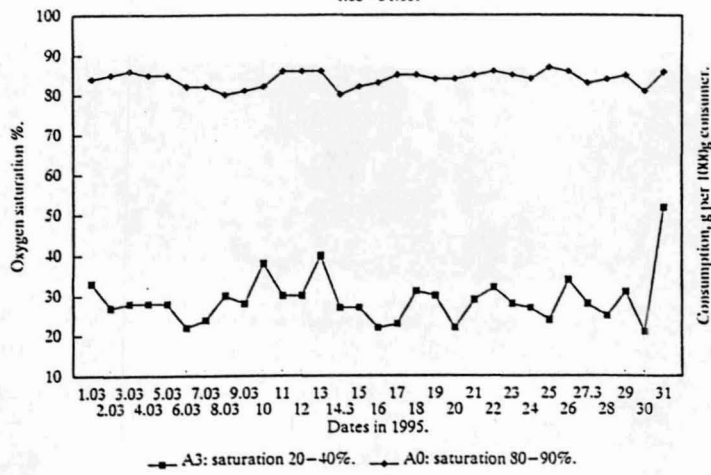
### Highest and lowest oxygen saturations.

27.01-28.02



### Highest and lowest oxygen saturations.

1.03-31.03



### Highest and lowest oxygen saturations.

1.04-2.05

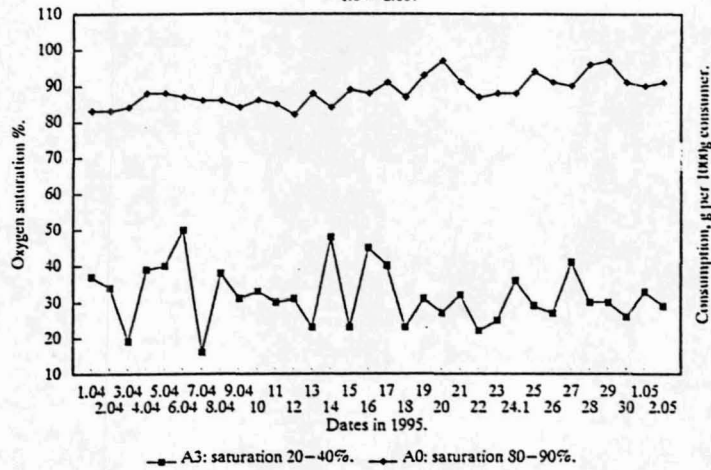
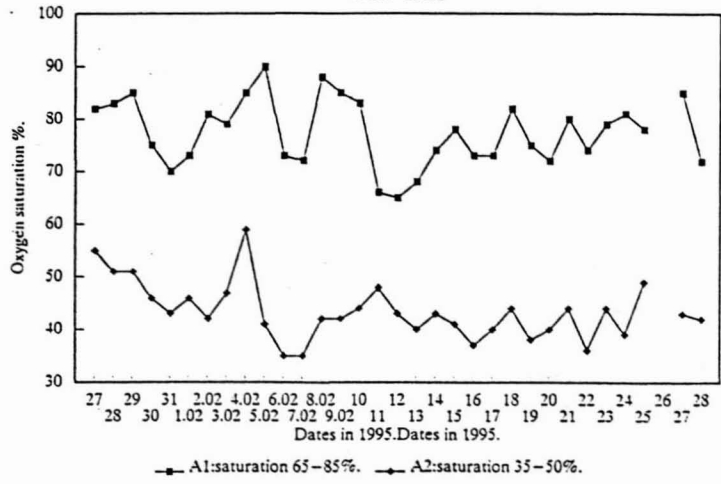
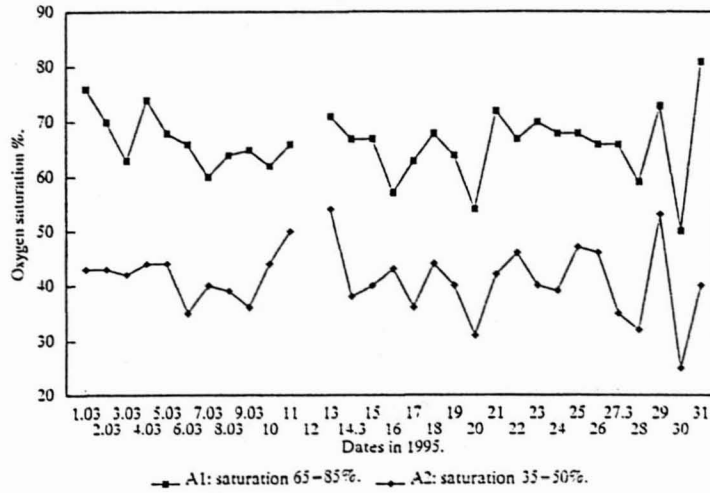


Fig. 2. Highest and lowest level of oxygen saturation.

Oxygen saturations between highest and lowest.  
27.01-28.02



Oxygen saturations between highest and lowest.  
1.03-31.3 1995.



Oxygensaturations between highest and lowest.  
1.04-2.05 1995.

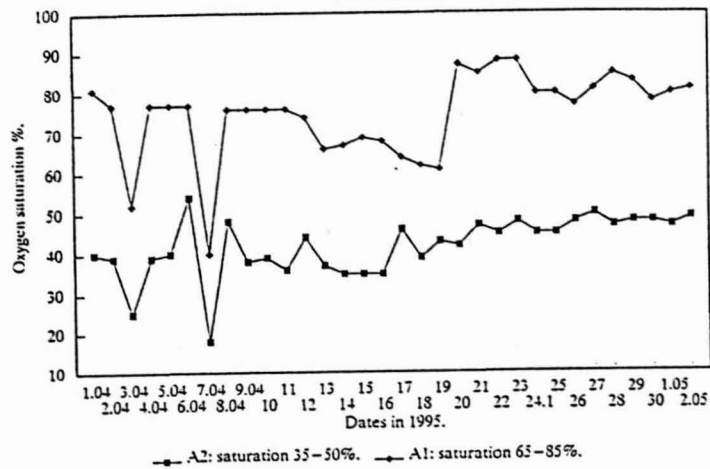
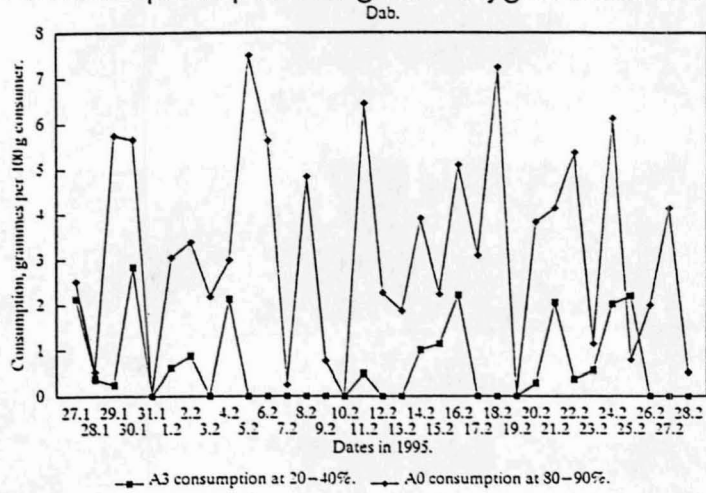
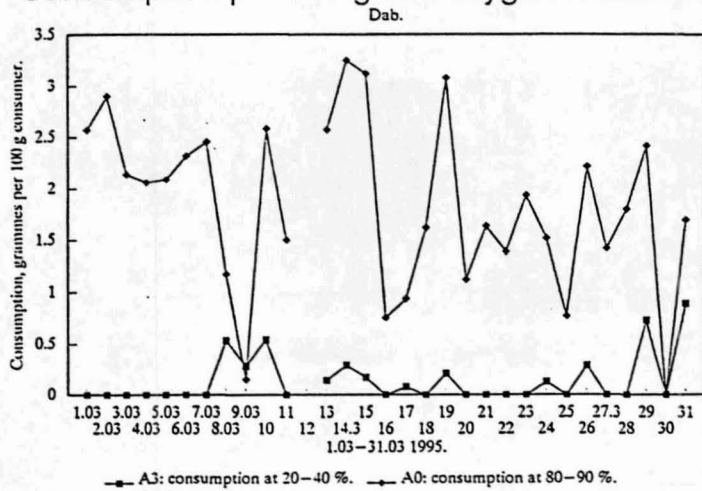


Fig. 3. Levels between highest and lowest oxygen saturation.

Consumption per 100 g and oxygen saturation.



Consumption per 100 g and oxygen saturation.



Consumption per 100 g and oxygen saturation.

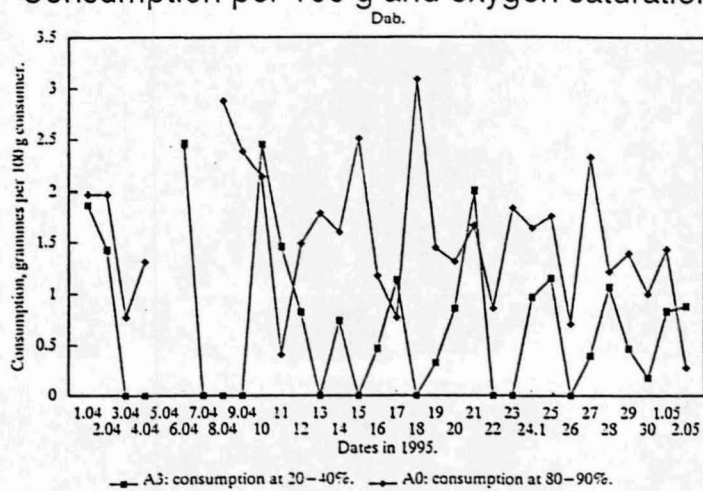
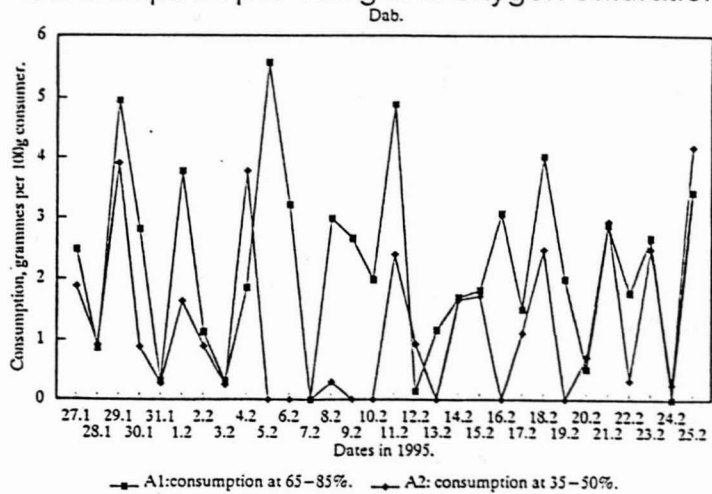
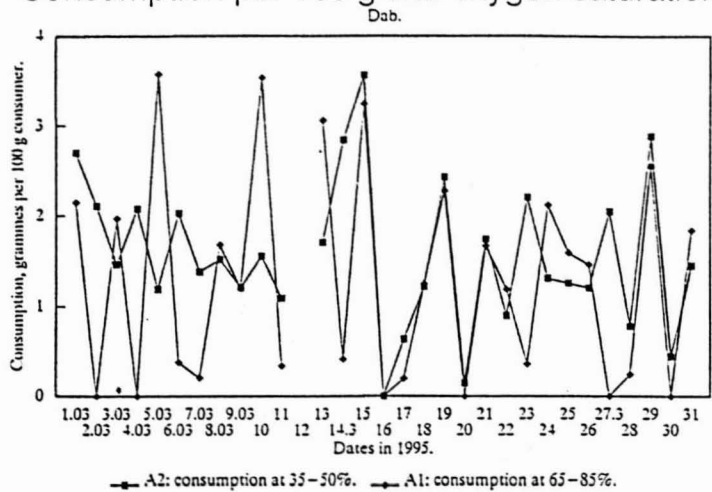


Fig. 4 a. Consumption relative to body weight at 80-90% and 20-40% oxygen saturation.

Consumption per 100 g and oxygen saturation.



Consumption per 100 g and oxygen saturation.



Consumption per 100 g and oxygen saturation.

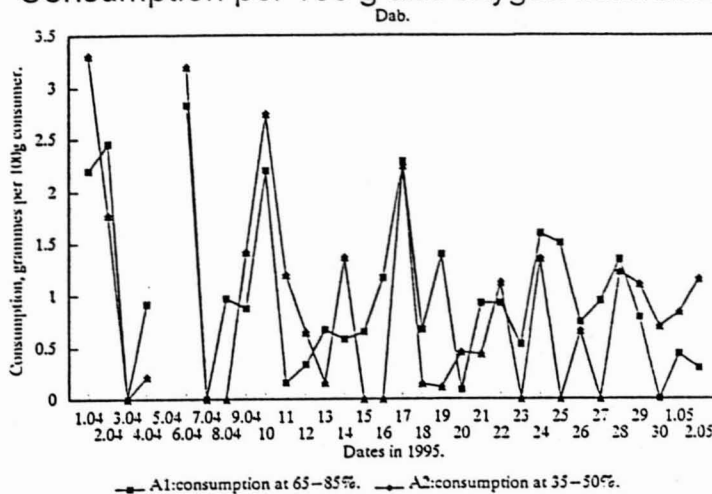
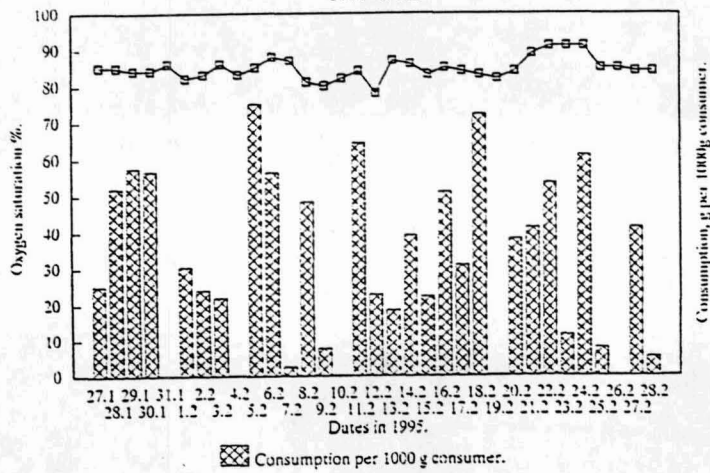


Fig. 4 b. Consumption relative to body weight at 35-50% and 65-85% saturation.

Fig 4b

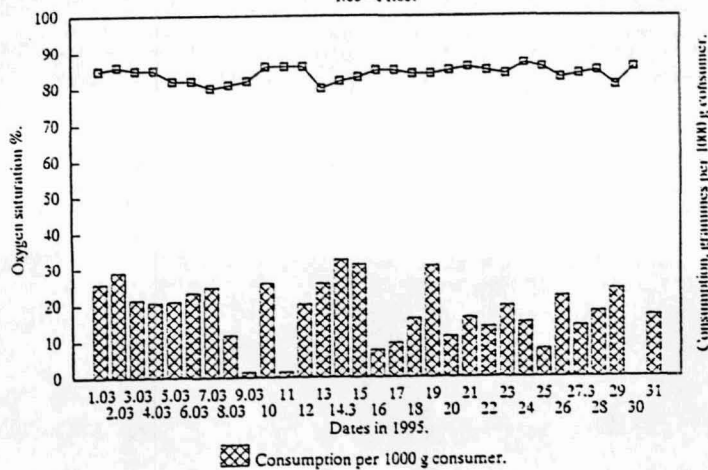
### Oxygen saturations and consumption.

27.01 - 28.02



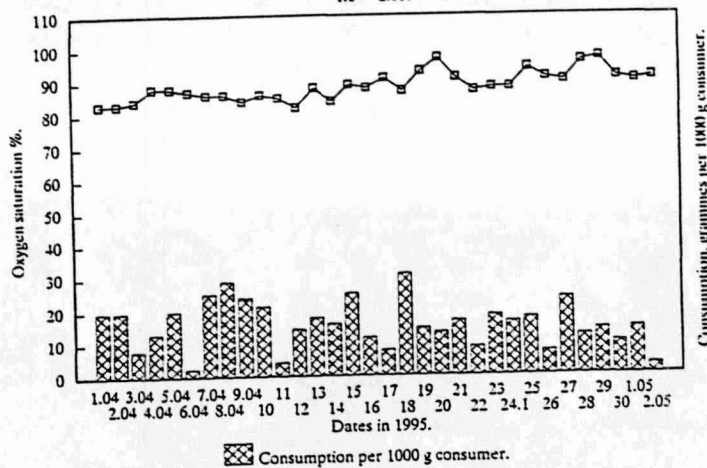
### Oxygen saturations and consumption.

1.03 - 31.03



### Oxygen saturations and consumption.

1.04 - 2.05



5 a. Daily consumption and daily oxygen saturation (80-90%).

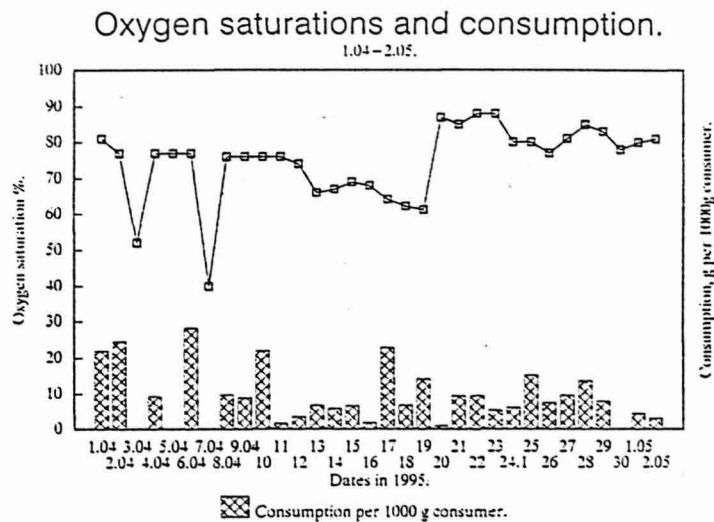
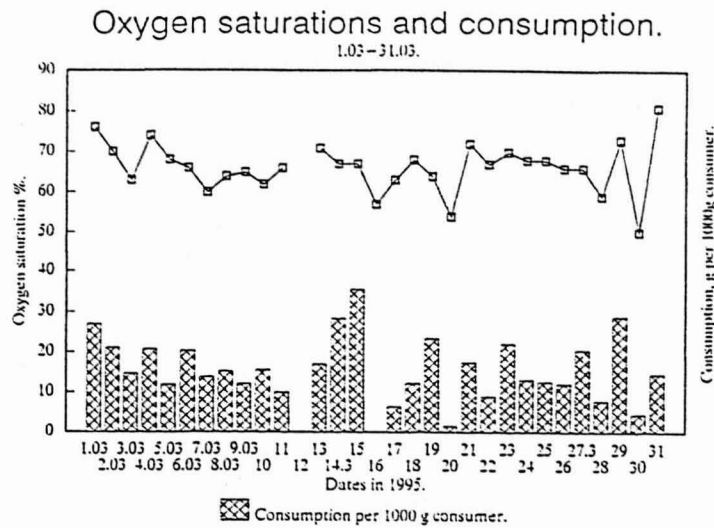
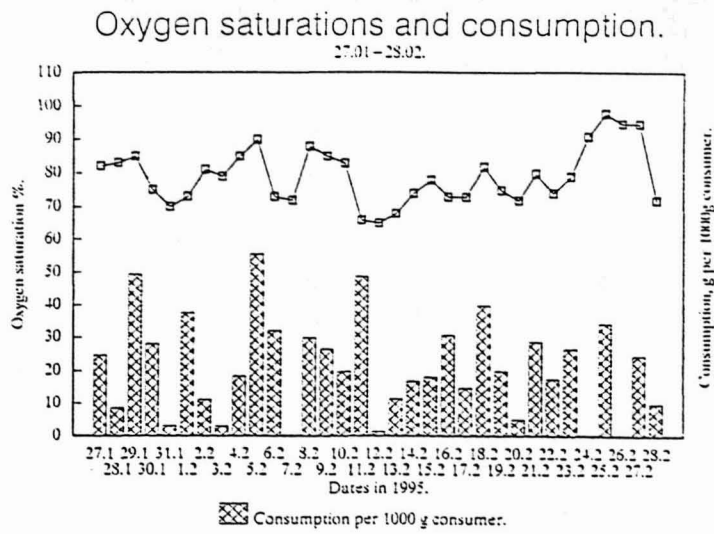
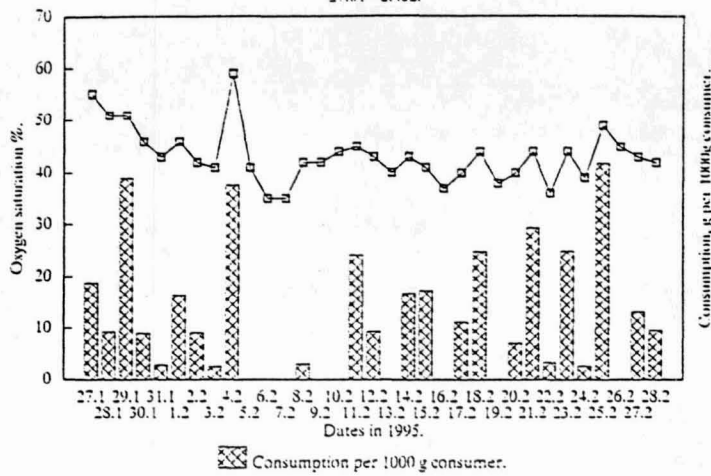


Fig. 5 b. Daily consumption and daily oxygen saturation (65-85%).



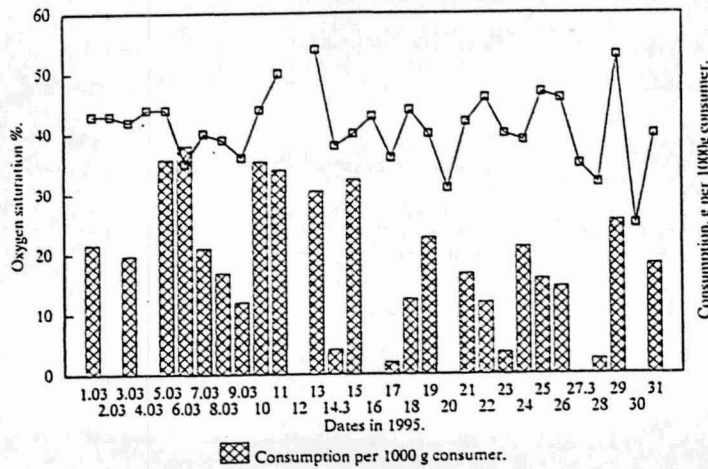
### Oxygen saturations and consumption.

27.01 - 28.02.



### Oxygen saturations and consumption.

1.03 - 31.03.



### Oxygen saturations and consumption.

1.04 - 2.05.

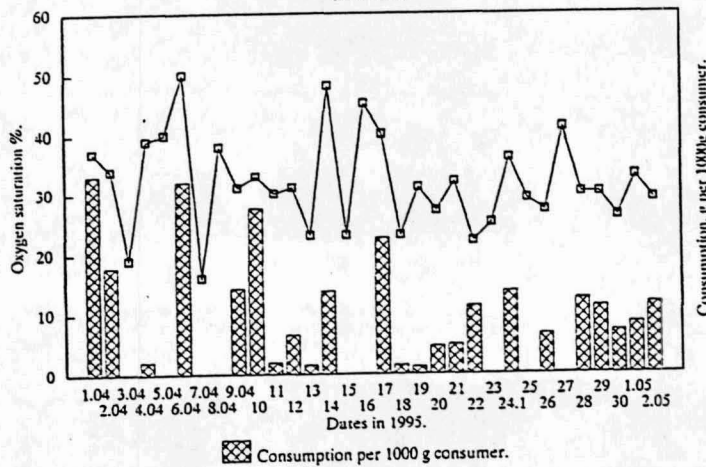
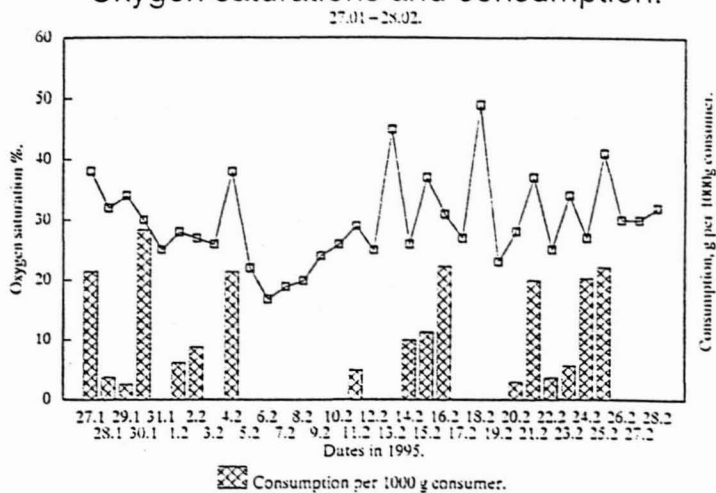


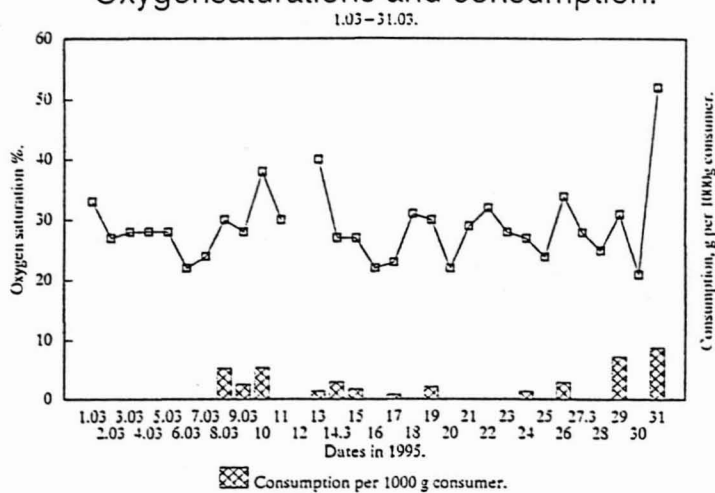
Fig. 5 c. Daily consumption and daily oxygen saturation (35-50%).



### Oxygen saturations and consumption.



### Oxygensaturations and consumption.



### Oxygensaturations and consumption.

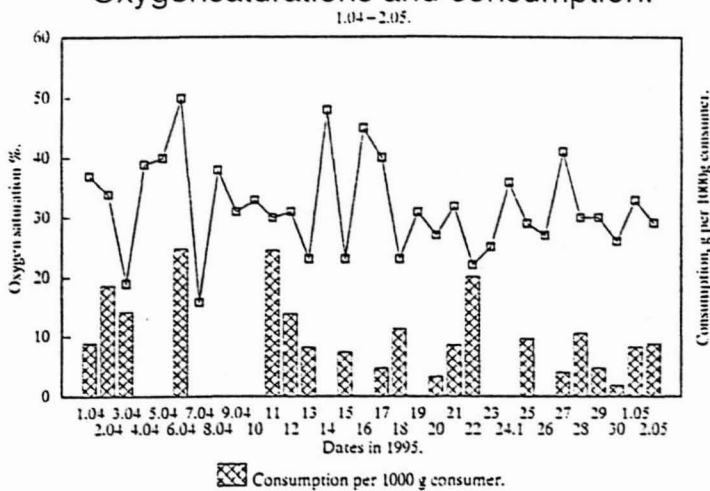


Fig. 5 d. Daily consumption and daily oxygen saturation (20-40%).

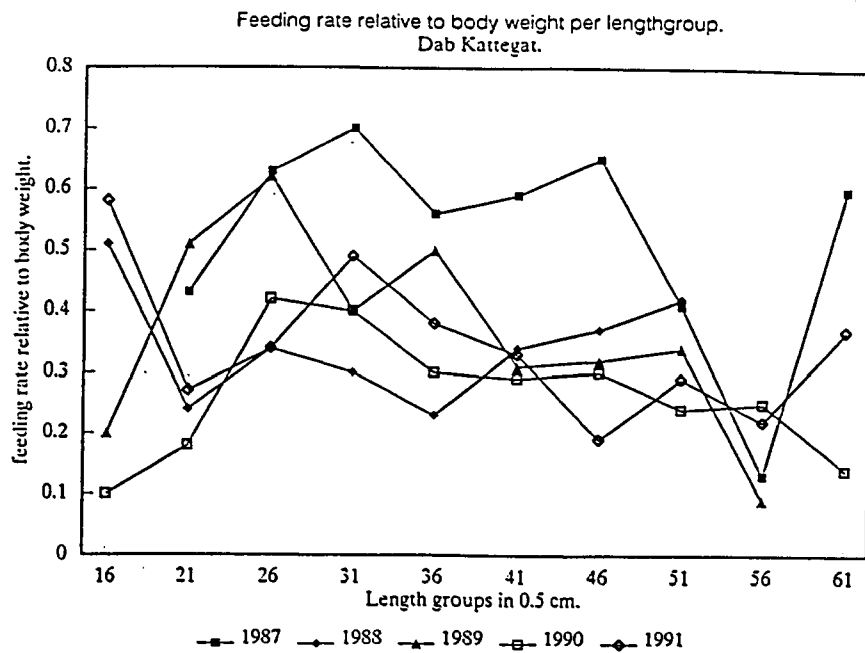


Fig. 6. Feeding rate relative to body weight per length group (%).

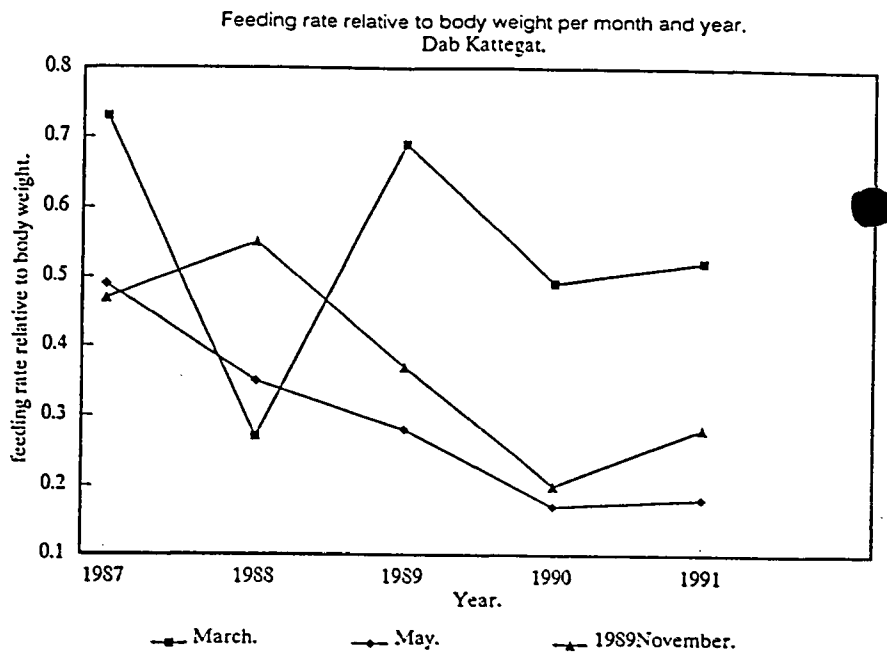


Fig. 7. Feeding rate relative to body weight (%) per month and year.

Day No.	Tank 1 (No. 18)	Tank 2 (No. 10)	Tank 3 (No. 10)
1	2.21	1.54	1.07
2	1.60	.77	1.15
3	Not fed	2.46	.64
4	3.97	2.24	1.35
5	1.75	Not fed	1.20
6	2.06	Not fed	Not fed
7	2.90	3.31	2.05
8	1.90	3.11	1.32
9	1.41	2.51	2.24
10	Not fed	2.03	2.12
11	2.80	2.55	.83
12	Not fed	Not fed	1.06
13	Not fed	2.16	Not fed
14	Not fed	1.76	2.86
15	Not fed	Not fed	Not fed
16	Not fed	2.67	2.61

Table 1. The daily feeding relative to body weight at 10.5°C (Charlottenlund).

7.5°C.

Hours	Number	Fish weight		Stomach content (% b.w.)		% left in stomach
		Mean	Std.	Mean	Std.	
6	3	159.0	9.8995	1.730	.8282	100.00
12	5	172.3	31.3564	1.6679	.6160	76.56
18	7	186.6	68.8458	1.1215	.7832	75.81
24	4	148.3	40.5134	2.6730	1.4459	57.83
30	6	178.3	52.8711	0.7512	.42246	41.18

10.5°C.

Hours	Number	Fish weight		Stomach content (% b.w.)		% left in stomach
		Mean	Std.	Mean	Std.	
6	9	191.2	41.7744	2.7265	0.7341	90.77
12	6	187.2	80.6874	1.732	1.0971	60.60
18	7	259.4	89.2185	1.812	1.6127	57.68
24	8	191.7	83.605	0.4167	0.3764	17.87

Table 2. Stomach content relative to body weight and time after feeding. Percentage of food left in the stomach.