Dana, vol. 4, pp. 19-39, 1985

Repeated induction of testicular maturation and spermiation, alternating with periods of feeding and growth in silver eels, *Anguilla anguilla* (L.)

Jens Dollerup & Chr. M. Graver
The Danish Institute for Fisheries and Marine Research,
Charlottenlund Castle, DK-2920 Charlottenlund, Denmark

Abstract

Sexual maturation was induced in a batch of 95 male silver eels (*Anguilla anguilla* (L.)) with human chorionic gonadotrophin (500 IU on days 0 and 7). After spermiation the eels were given food (from day 118) and started to eat and grow. Later two further sexual maturations were induced (injections on days 179 and 186 and on days 400 and 407). During the last part of the experiment (days 398 to 536) food was withheld.

The eels were kept in seawater, at 23.4 °C. They were marked individually (day 118), and body weight and total body length were measured at intervals. Groups of eels were sacrified at intervals for histological examination of the alimentary tract, weighing of organs and chemical determination of body composition.

During the first part of the period of feeding, food intake gradually increased, and the eels grew both in weight and length, their condition index increased, and their strongly atrophied alimentary tract regenerated. When the second maturation was induced, food intake decreased, growth stopped and the alimentary tract underwent atrophy. After the second period of maturation, food intake increased somewhat, and the alimentary tract again regenerated.

A marked increase in eye size was noted as a response upon both the first and the second maturation.

The sperm ducts showed a marked dilation at the first sexual maturation and continued to be dilated.

Protein and lipid (%) showed great individual variation, and so did liver weight, but there was a remarkable lack of correlation between these parametres and the rate of growth and the condition index. Furthermore, no correlation existed between the latter two parametres.

The male *Anguilla anguilla* is not destined to die after spawning and can be led through several successive phases of reproduction and atrophy of the alimentary tract, alternating with phases of feeding, regeneration of the alimentary tract and growth of the body.

Contents

continued next page

Introduction

The present study was carried out from April 1982 to October 1983 at the Danish Institute for Fisheries and Marine Research.

The background for our experiment was two papers: Fontaine *et al.*, 1982, which mentions a case of food intake by matured silver eels, *A. anguilla*, and Boëtius & Boëtius, 1982, in which re-maturation of sexually matured male silver eels *A. anguilla* is described. The experiment was planned in co-operation with Dr. Lis Olesen Larsen, Zoophysiological Laboratory A, University of Copenhagen, and Dr.s Inge & Jan Boëtius, the Danish Institute for Fisheries and Marine Research.

Experimental

The experimental batch consisted of 106 male silver eels, caught when leaving the Baltic for their seaward migration. The eels were brought to the laboratory shortly after capture. On their arrival on October 7, 1981, they were placed in a 1.5 m^3 tank. Temperature 14°C, salinity 28‰. The tank was connected to the seawater circuit (150 m^3) in Denmarks Aquarium.

At the start of the experiment, April 15, 1982, 95 eels were transferred to a 2 m^3 tank with circulating seawater. The temperature throughout the experiment was 23.4 ± 1.6°C and the salinity 28‰. 10 black PVC tubes (57×400 mm) were placed in the tank as hiding places for the eels.

As references, 9 male yellow eels were caught on September 7, 1982, in Roskilde Fjord and sacrificed soon after, as well as 10 newly caught male silver eels, which were sacrificed on October 27, 1982.

Eels are numbered in the following way: eels nos *1-9* are yellow, eels nos *10-19* are silver and nos 1-91 are the experimental eels. The number was assigned to the eel on the day it died.

Experimental days were numbered from day 0 (April 15, 1982) to day 536 (October 3, 1983).

The hormone used for sexual maturation was human chorionic gonadotrophin, HCG ('Physex', Leo). Two doses of 500 IU were given intramuscularly at one week's interval. Sexual maturation was induced on days 0 and 7, days 179 and 186 and days 400 and 407. Approximately three weeks after the first injection the eels were stripped three times at weekly intervals. When an eel failed to give off sperm it was excluded from the experiment as 'negative'. The 15 'negative' eels in the first maturation are excluded both from the experiment and from the primary table. After dissection, however, a few 'negative' eels proved to have matured and they were thus counted as mature in the statistics.

At the start of the feeding experiment (days 118 to 398), eels were individually marked by clippings in the pectoral and anal fins. The food used was krill and *Mytilus* at a ratio 1 : 1. Eels were fed every morning after removal of food left over from the day before.

Anaesthesia (immersion in 1.5 % solution of ethylurethane in seawater) was used for body weight and total body length determination on days 0, 118, 148, 179, 214, 365, 400 and 517, and for checking fin clippings. The reliability of the determinations of body weights and total lengths on anaesthetized eels was checked as follows: 10 control eels were anaesthetized and measured daily during five days. The relative error of the single determination was ±1.7 % for weight and ±0.5 % for length.

Eels for analysis were killed by a prolonged immersion in the ethylurethane solution, and body weights and total lengths were determined. The alimentary tract and liver were removed, cleaned for content and weighed. Tissue samples were taken from oesophagus, stomach and intestine and fixed in Susa-trichloroacetic acid (Heidenhain). The otoliths were then removed and the gutted eel plus the viscera were stored frozen at −18 °C.

On day 0 and day 118 eels were sampled for analysis by random picking. From day 118 and onward we selected eels in such a way that eels with both low, intermediate and high growth rates were represented, based on our knowledge of the eels' previous growth in the experiment. Eels which died or were killed because of disease or wounds (24 eels) were not analysed.

Dates of sampling and number of eels selected for histological and chemical analysis are given below.

			Day					
	yellow	silver	0	118	179	222	368	536
Number chosen for histological analysis	9	10	11	10	10	4	10	10
Number chosen for chemical analysis	6	6	7	6	6	4	6	0

Histological investigation of the alimentary tract were made in the following way: the tissue samples were embedded in paraffin, and 6 μm slices were cut and stained in hematoxylin (Ehrlich)-eosin. Measurement of diameter and width of layers were made under a Visopan microscope.

Chemical analysis was made on homogenized whole eels. Homogenates, about 2 grams each, were used for each determination of ash, dry matter, lipid, total nitrogen (N) and non-protein nitrogen (NPN). Samples for ash (6 per eel) were dried for 24 hrs at 105 °C and then heated to 600 °C for 4 hrs. Samples for dry matter (6) were placed for 24 hrs in an incubator at 40-50 °C and then for 24 hrs in vacuum desiccator. Lipid analysis (4) was made according to the chloroform/-methanol extraction method described by Bligh & Dyer 1959, followed by centrifuging at 3500 rpm for 30 min. N (3) was determined by Kjeldahls method using a Kjeltex System I equipment. NPN (3) was determined as follows: approximately 2 g homogenate in a volumetric flask was made up to 100 ml with 15 % trichloroacetic acid. After precipitation of protein, 40 ml filtrate was determined for N by Kjeldahl's method. Protein was calculated as (N − NPN) × 6.025, the conversion factor adopted from Love 1970 (footnote on p. 238).

Sexual maturation

Results

The maturation stages *1* to *7* (Boëtius & Boëtius, 1967) was used to describe testicular development in the male eels during artificial maturation.

We succeeded in inducing complete sexual cycles up to three times in individual male eels. The table below indicates the number of eels injected and the number of eels matured during the three experimental periods:

Days of injections	Number of eels injected	Number of eels matured	Per cent eels matured
0 and 7	95	86	91
179 and 186	46	38	83
400 and 407	10	7	70

During inspection of developmental stages of the testis, we observed that eels in *stage 7* showed a dilation of the *vas deferens* (sperm ducts), not present in immature eels. Due to this dilation the testicular lobes were dislocated from their original site (close to the body wall) to a more ventral position in the body cavity. This dislocation was most clearly recognized in the dorsolateral region of the air-bladder and was observed in all eels which had been through a complete maturation cycle. The difference in size in *stage 1* and *stage 7* is well demonstrated by injections of contrast fluid (e.g. methyl violet).

Discussion

It was expected that induction of a second maturation might necessitate a period of starvation before sensitivity to gonadotrophin would develop, because it is known that yellow eels do not respond to gonadotrophin (Boëtius & Boëtius, 1967). This was not necessary, and an inspection of the eels that matured in the second maturation, showed no relation between growth and the eels' ability to mature.

The tendency to a decline in sensitivity throughout the experiment may not be significant or may reflect some change related to the laboratory conditions or to ageing.

Feeding

Results

Fig. 1 shows the eels' daily consumption calculated as ingested food in grams per eel (IF).

From the start of feeding until day 179 the IF increased rapidly. The hormone injections on day 179 and day 186, seemed to suppress the eels' food intake. After stripping the eels for sperm the 'negative' eels were transferred to a separate tank and were fed separately. The 'negative' eels regain their appetite on approximately day 230, the matured eels, however, around day 280. Hereafter the matured eels show a steady increase in IF, but the increase is not as steep as was the case

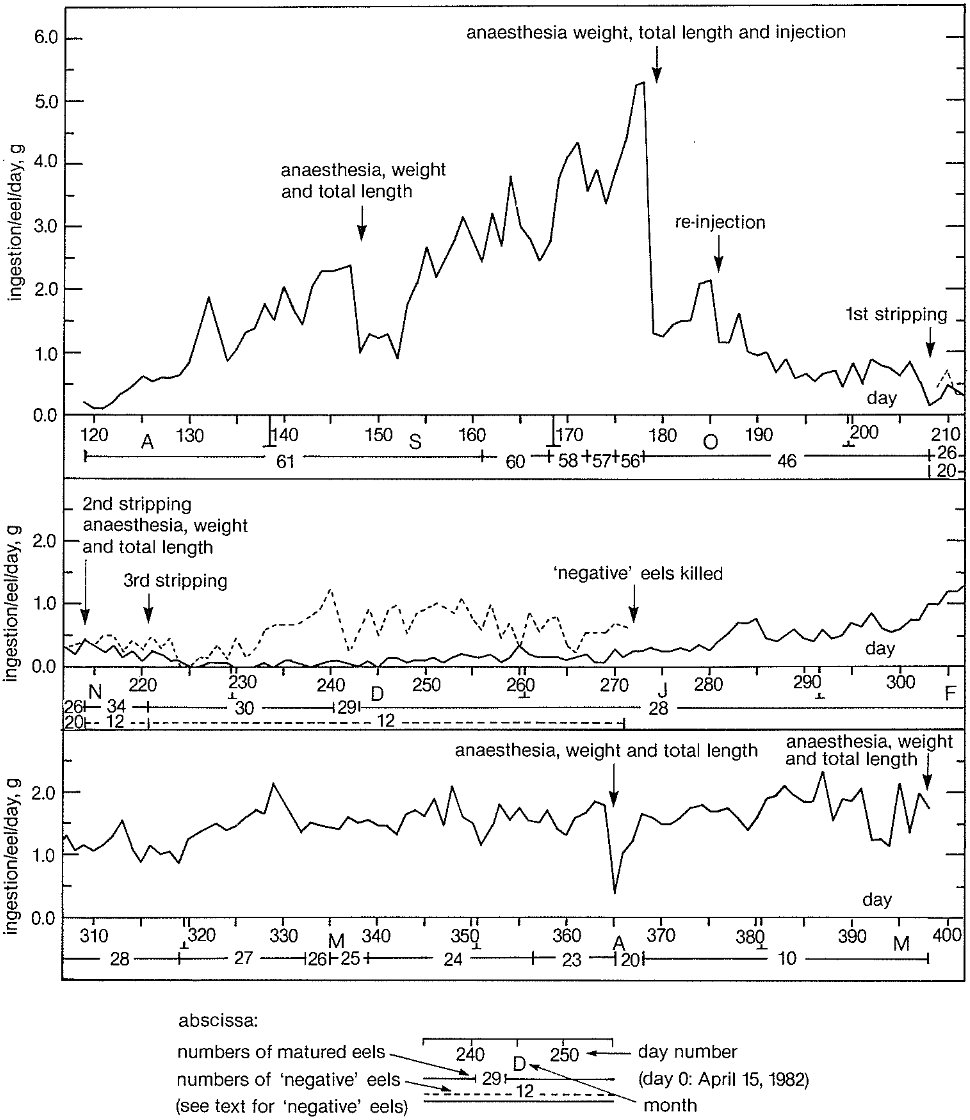

Fig. 1. Food consumption calculated as the difference between quantities of food offered and left over, plotted against time (from day 119 (August 12, 1982) to day 399 (May 19, 1983)). (Note that after the first stripping (day 207) the 46 eels matured in the first maturation are split into two batches fed separately: 26 which matured in the second maturation and 20 'negative'. After the second stripping 8 'negative' eels proved mature.)

between day 118 and day 179. The IF reaches a level between days 325 and 398 of 1.5-2.0 grams/eel.

Anaesthesia and/or injections strongly reduces IF for a few days.

Discussion

The gradual increase in food intake, from when feeding was started until the second gonadotrophin treatment was begun, is correlated with a regeneration of the alimentary tract. The abrupt decline after gonadotrophin injection is probably a result of unspecific reactions to anaesthesia and injections. The period of slowly decreasing food intake until day 220-230 probably reflects processes initiated by gonadotrophin, related to the induced spermatogenesis, since the biological half-life of the hormone is rather short (in toads 3-30 hours, Roos & Jørgensen 1974). Spermatogenesis was finished around day 222 (see primary table eel nos 46 to 49 all in maturation *stage 4*). The increasing food intake seen in 'negative' eels at that time may indicate that sexual maturation in those eels only leads to spermatogenesis and not to spermiation. In the matured eels the period of spermiation, which is finished around day 272 (see primary table eels nos 56, 57, 61 and 62 in maturation *stage* 6 or 7), is characterized by a continued low food intake, but then food intake increases again, although not to the level found before the second maturation.

Growth and condition index

Results

Body weights and total body lengths of the eels are shown in the primary table and in Fig. 2. In Table 1 the statistical parameters are given.

Table 1. Statistical parameters of experimental eels. G: growth rate, N.S.: not significant, r: correlation coefficient, SD: standard deviation, SE: standard error. Between day 0 and day 118 an ordinary t-test was used to compare mean values, from day 118 and onward a paired t-test was used.

day number	0	118	148	179	214	365	400	517	536
number of eels	95	61	61	56	46	23	10	10	10
mean body weight, $\bar{w}_b$, g	75.2	56.9	63.5	79.5	75.3	63.9	60.3	47.6	45.1
$2SE(w_b)$	2.65	2.50	3.30	4.93	4.49	7.01	7.44	5.72	6.25
level of significance, P (between successive days)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	<0.001	<0.025
mean total length, $\bar{l}_b$, cm	37.4	37.2	37.5	38.4	38.5	38.2	37.5	37.2	37.3
$2SE(l_b)$	0.43	0.48	0.49	0.51	0.62	0.82	1.19	1.15	1.18
level of significance, P (between successive days)		N.S.	<0.001	<0.001	<0.01	N.S.	N.S.	<0.001	<0.005
level of significance, P (day 118–179; 179–400; 400–536)			<0.001			N.S.		<0.005	
mean condition, $\bar{k}$	1.43	1.10	1.20	1.39	1.31	1.13	1.13	0.92	0.96
2SE(k)	0.03	0.03	0.04	0.06	0.05	0.08	0.08	0.10	0.10
$r(w_b, l_b)$	0.79	0.78	0.74	0.72	0.76	0.73	0.80	0.57	0.62
slope, b	4.83	4.11	4.94	6.90	5.54	6.25	4.98	2.85	3.28
SD(b)	0.39	0.42	0.59	0.91	0.71	1.26	1.34	1.65	1.48
$\bar{G}$, mg/g/day (day 0–118; 118–179; 179–400; 400–536)		−2.07	6.53			−0.86		−1.87	
SD(G)			4.42			0.51		0.47	

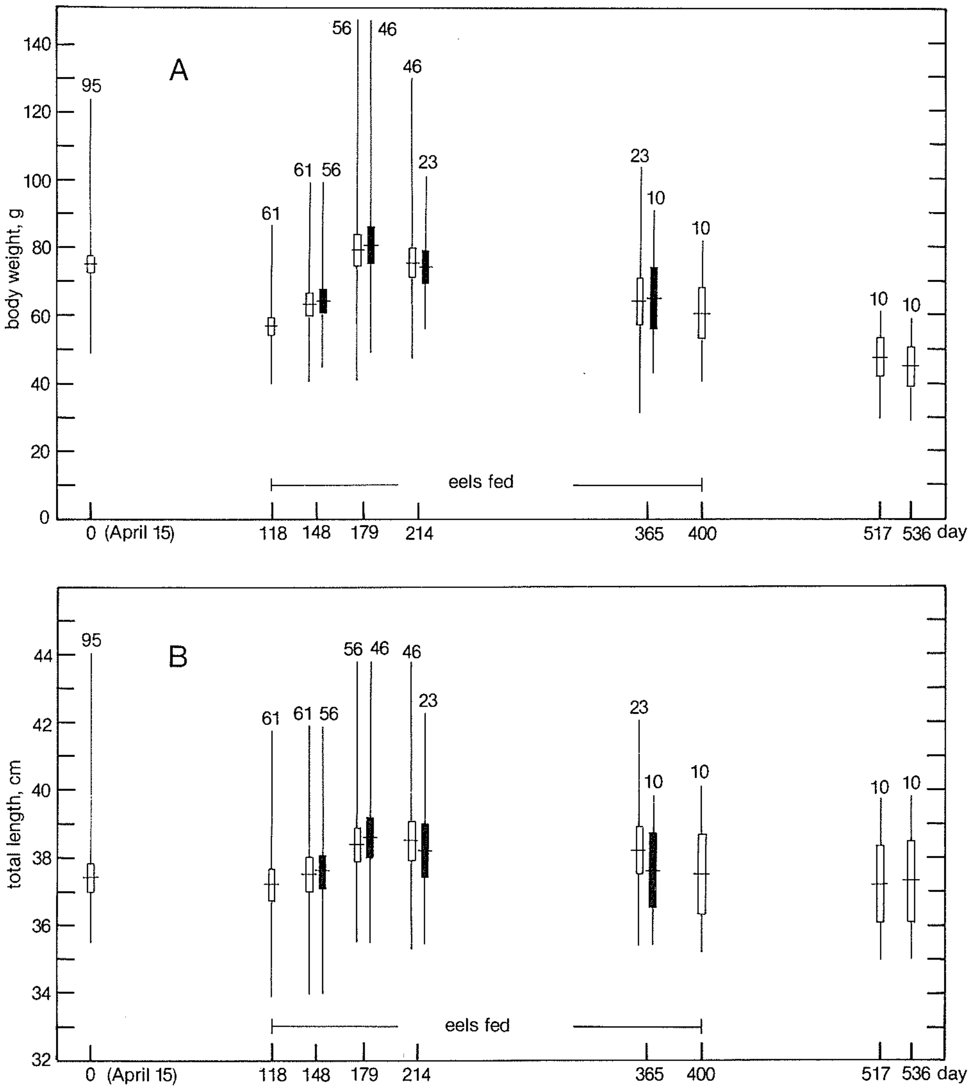

Fig. 2. A, body weights versus time. B, total lengths versus time. Vertical lines: range. Horizontal lines: mean. The figures indicate number of eels. White columns: 2SE values of all eels. Black columns: 2SE values of those eels that survived until next measurement of weights and total lengths.

Between day 0 and day 118 an ordinary t-test was used to compare mean values (we were not able to identify individual eels in this period), from day 118 and onward a paired t-test was used. Due to the narrow range in weights and lengths correlations were calculated from the raw data.

The growth rate of the individual eels (G) was defined as follows:

$$G = \frac{\text{change in body weight during the period}}{\text{initial body weight} \times \text{number of days in period}} \text{ mg/g/day}$$

As a standard expression of an eel's physical proportions we have chosen the condition index (k):

$$k = \frac{\text{body weight, g}}{(\text{total length, cm})^3} \times 10^3$$

Day 0 to day 118 (no feeding, 1st maturation). In this period body weights of the eels decreased significantly, whereas their total lengths only showed a slight reduction. The mean of G in this period was −2.07 mg/g/day.

Day 118 to day 179 (feeding). The eels in this period showed an increase in both body weights and total lengths. The mean of G is 6.53 mg/g/day. The maximum G (16.39 mg/g/day) is held by eel no. 77 with a gain in weight from 40 to 80 grams. Eel no. 52 has the minimum G (−3.01 mg/g/day) corresponding to a decrease from 60 to 49 grams. The maximum change in total length is 3.1 cm (an increase of 8.5 %) obtained by eel no. 72. None of the eels decrease in length in this period.

Day 179 to day 400 (feeding continued, 2nd maturation). There is a gradual decrease in body weights, whereas total lengths increase until day 214 and then slowly decrease.

Day 400 to day 536 (no feeding, 3rd maturation). Decrease in both body weights and total lengths is significant. Note that if the period is split into two parts, days 400-517 and days 517-536, a decrease in total length is present in the first period, but an increase in the second. The mean of G in this period was −1.87 mg/g/day.

Discussion

There was a significant growth in weight and length of the body of the fed eels, and the condition index increased in the beginning, but it never reached the initial level and declined in the later part of the feeding period. Condition index did not change in the same pattern as did food intake and the condition of the alimentary tract. Also lipid content and liver weight (see later) do not appear to be related to the nutritional condition.

A calculation of the correlation between the eels' condition index on day 118 and growth rate between day 118 and day 179 gave r = −0.15. However the feeding behaviour of the eels was as varied as their rate of growth. The eels left their tubes as soon as food was offered, and after 30 to 45 minutes they would all return to their tubes, even if left over food was present. Some eels fed continuously for up to 45 min. while others snapped food only a few times and still others were seen just to swim around outside the tubes without feeding. There was a great deal of fighting during feeding, but no relation between size and aggression was observed.

Note that the negative growth rate between days 0 and 118 equals the negative growth rate between days 400 and 536. In these two periods the eels underwent sexual maturation without feeding.

Alimentary tract and liver

Results

Fig. 3 shows the gross morphology of the alimentary tract and the sites at which tissue samples were taken. Fig. 4 demonstrates the histological changes. The well developed tract of the yellow eel is only somewhat reduced in the silver eels at the start of the experiment, after a period of starvation of half a year. During the next 118 days of sexual maturation there was a marked atrophy. On day 179 some eels which had grown very little, still had atrophied alimentary tracts, but those which had grown well had regenerated tracts. The histological changes are closely paralleled by changes in weight of the tract (Fig. 5A) (except yellow and silver eels), and growth and weight of the alimentary tract show a positive correlation (Fig. 5C).

The weight changes of the alimentary tract mainly reflect changes in epithelial height and in size of folds. The longitudinal muscle layer is almost unaffected. The circular muscle layer in the intestine is drastically reduced during the first period of starvation and this condition remains unaltered until the conclusion of the experiment.

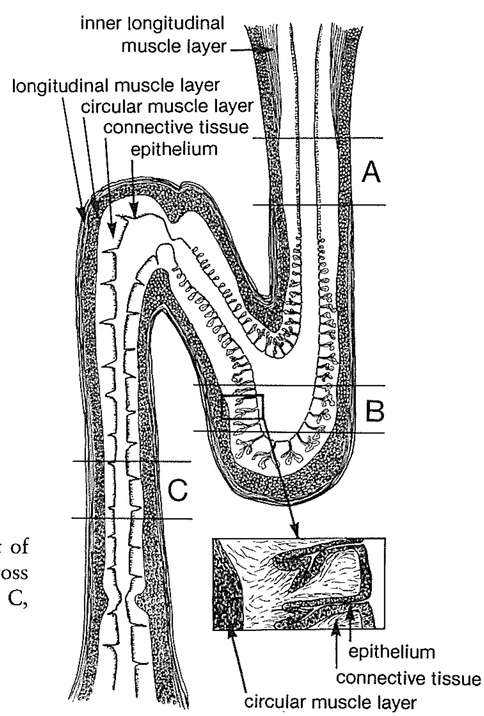

Fig. 3. Gross morphology of the alimentary tract of the yellow eel. A, B & C indicate regions where cross sections were made. A, oesophagus; B, stomach; C, intestine. Modified from Berndt 1938.

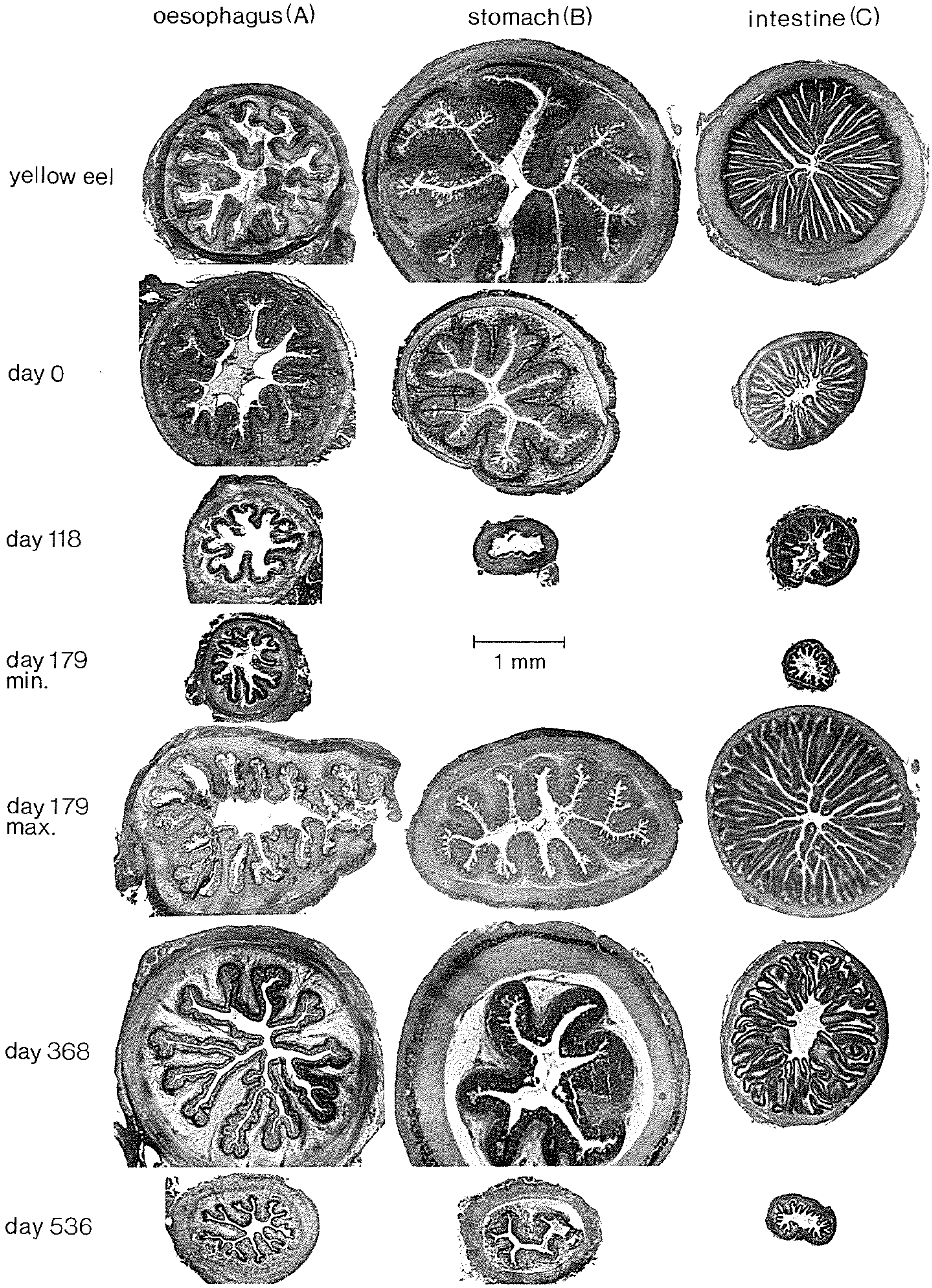

Fig. 4. Cross sections of alimentary tracts. Numbers indicate the day of sacrifice. The eels chosen had a gut weight close to the mean of the samples, except on day 179 where eels with minimum and maximum growth are shown. Individual numbers refer to the primary table as follow: Yellow eels: *3, 4, 8*. Day 0: 10, 9, 6. Day 118: 25, 21, 23. Day 179 min: 39, missing, 39. Day 179 max: 44, 44, 43. day 368: 76, 76, 76. Day 536: 91, 91, 91.

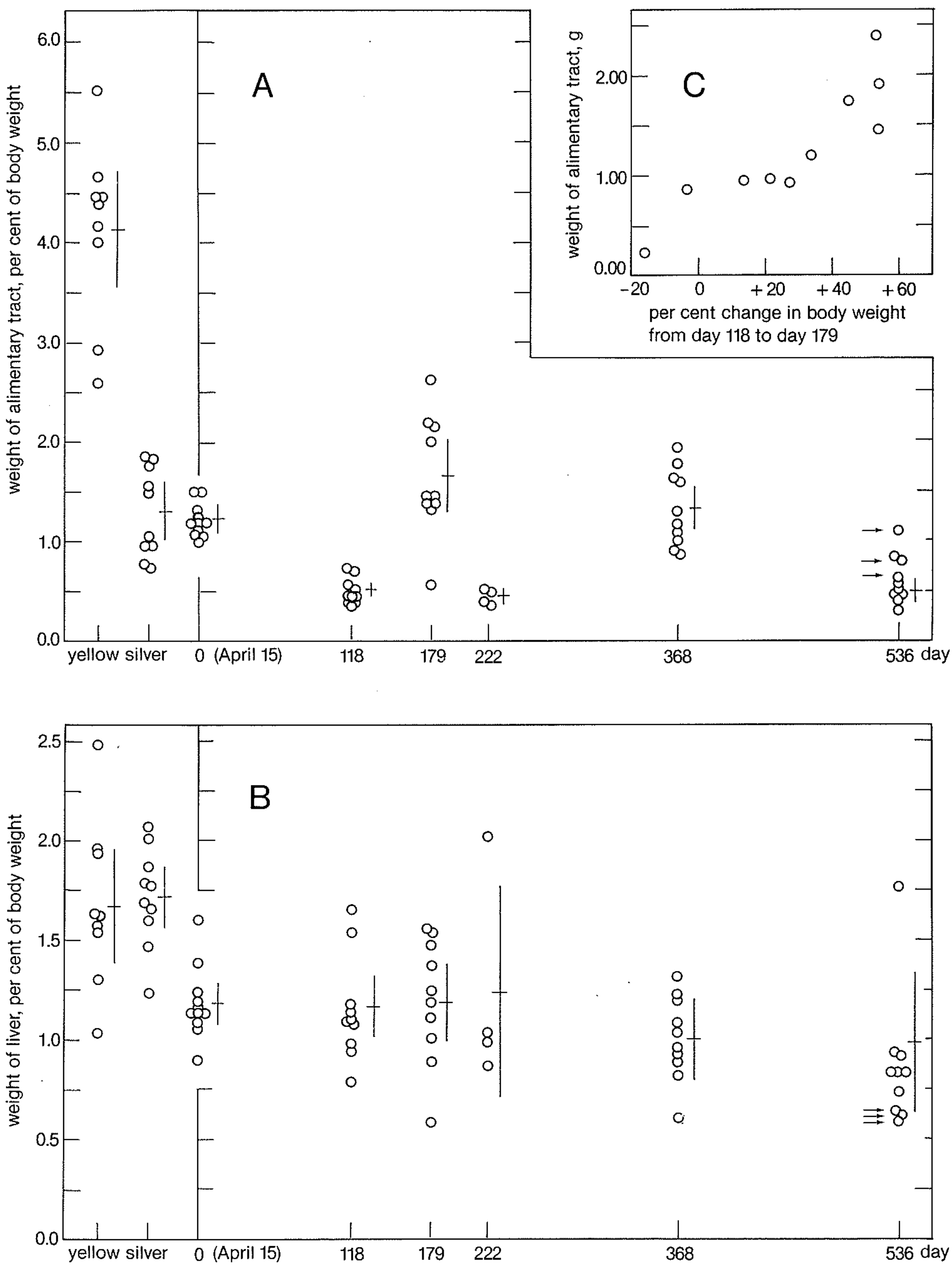

Fig. 5. A, weight of alimentary tract in per cent of body weight plotted against time. B, weight of liver in per cent of body weight plotted against time. Circles: individual eels. Horizontal lines: mean. Vertical lines: 2 SE. Arrows indicate eels not matured in the third maturation experiment. C, weight of alimentary tract on day 179 versus percentage change in body weight from day 118 to day 179.

During the periods of starvation and maturation certain characteristic changes in morphology and cell structure were noticed: 1. Heavy reduction in the number of folds in the stomach and in the intestine. 2. Boundaries between tissue layers disintegrate in several layers. 3. Cell to cell adhesion reduces.

The relative liver weight (Fig. 5B) is rather constant throughout the experiment, and only little lower than that of freshly caught yellow and silver eels.

Discussion

The eels were thus induced to show alternation between a phase dominated by reproduction and a phase dominated by food intake and growth, similar to the spontaneous 'Phasenwechsel' described for the Atlantic salmon by Mishlin 1941.

It may be of interest to note (Peters 1982) that 'stress' in form of 'unavoidable contact with a dominant eel' causes atrophy of the stomach, similar to the one described here during sexual maturation.

Protein, lipid and water

The data on protein, lipid, water, non-protein nitrogen and ash are given in the primary table. Fig. 6A-E shows the values plotted against time. Fig. 6F shows the calculated residuals ($R = 100 - (W + L + P)$). The results from freshly caught yellow and silver eels are given for comparison.

The relative amounts of protein and lipid was nearly the same in all examined groups. A strong negative correlation was found for lipid and water ($r = -0.97$). There was also a negative correlation between condition index and ash content ($r = -0.80$).

The extent of individual variation can be exemplified by the following figures:

Eel no.	Growth, days 118 to 179, mg/g/day	lipid, %	protein, %
39	−2.54	22.3	13.3
44	8.78	18.3	14.7
45	−0.53	10.3	16.6

The low degree of correlation between growth rate and lipid ($r = 0.27$) and between growth rate and protein ($r = 0.21$) of eels analysed from day 179 and onward, underline the large and chance individual variation.

Eye index and otoliths

Eye index

As an index of the area covered by the elliptical eye we have chosen the index (I):

$$I = \frac{\left(\frac{E_h \times E_v}{4}\right) \times \pi}{l_b} \times 100$$

where E_h and E_v are horizontal and vertical eye diameters (mm) and l_b is the total body length of the eel (mm).

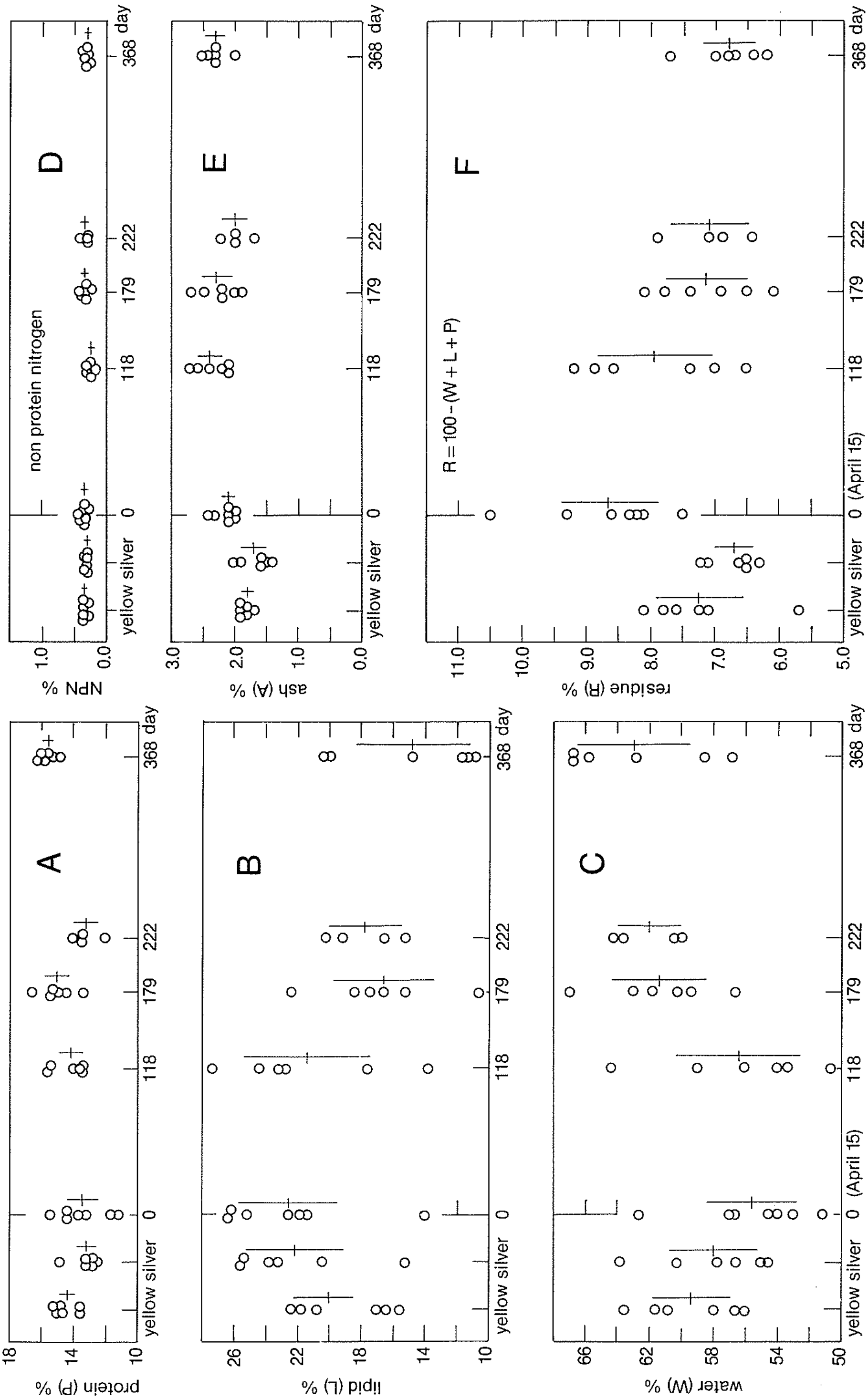

Fig. 6. Chemical composition of total eels expressed as per cent of body weight at the day of sacrifice plotted against time. A, protein; B, lipid; C, water; D, non-protein nitrogen; E, ash; F, residue. Circles: individual eels. Horizontal lines: mean. Vertical lines: 2SE.

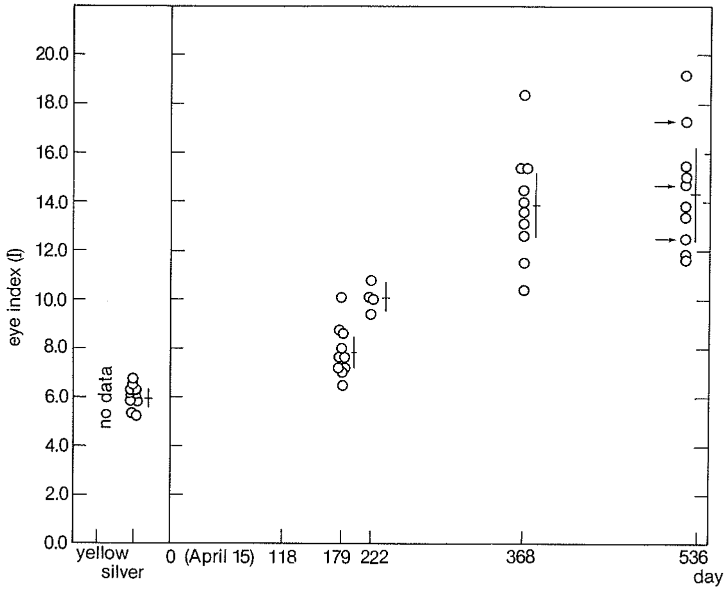

Fig. 7. Eye index (I) plotted against time. Circles: individual eels. Horizontal lines: mean. Vertical lines: 2SE. Arrows indicate eels not matured in the third maturation experiment.

The data are presented in the primary table and in Fig. 7 (only eels sacrified from day 179 and onward).

During the first period of sexual maturation the eye increases slightly (when the data from non-injected silver eels are used as a reference). Since data for day 118 are lacking we do not know whether the eye changed during the first feeding period between days 118 and 179.

However, during the second sexual maturation period (days 179 to 222: *stage 4*) a rapid increase in the eye area takes place. This increase continues to day 368.

During the third sexual maturation period (days 400 to 536) the eyes do not enlarge further, apparently limited by the dimensions of the cranium.

Otoliths

Otoliths were prepared and examined for possible structures reflecting periods of growth and sexual maturation. No such structures could be identified.

Concluding remarks

The results of experiments involving feeding and artificial sexual maturation of male silver eels are recapitulated in Fig. 8. Our experiments are given in the frame. The concept of 'Phasenwechsel' is demonstrated in the lower part of the frame.

The postmature feeding eels (day 179) have much in common with the yellow eel. Eels that feed intensively showed the colour of yellow eels and had well developed alimentary tracts. Moreover these eels were just as aggressive as feeding yellow eels. In two respects, however, our eels differ from yellow eels: their eyes are enlarged and they respond positively to HCG.

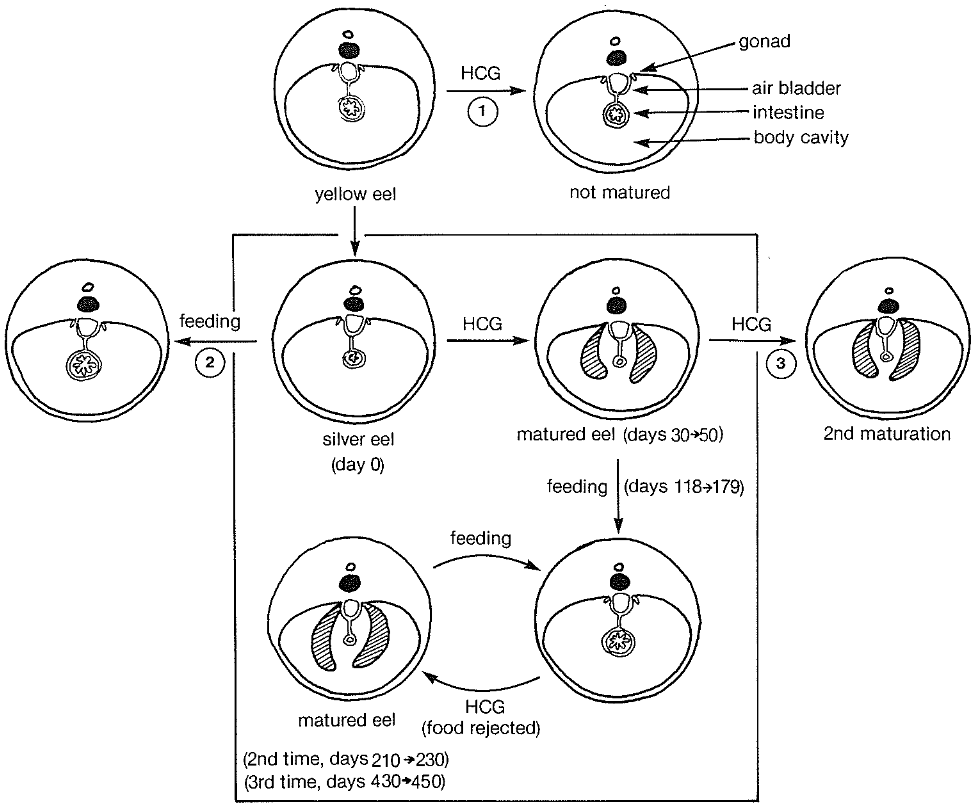

Fig. 8. Diagram demonstrating responses of male eels upon HCG and feeding. 1. Male yellow eels do not mature when treated with HCG (Boëtius & Boëtius 1967). 2. Male silver eels will feed after 6 to 7 months in captivity (Boëtius & Boëtius 1967). 3. Male silver eels can be matured twice with HCG, without intermediate feeding (Boëtius & Boëtius 1982). In frame: the present experiment.

A postmature feeding period of two months only resulted in a mean increase in body weight of 40%. A prolonged feeding period with no further induction of sexual maturation would probably have resulted in even larger eels.

The fact that male eels do not die after spermiation and are capable of taking up food and later on re-mature leads us to consider the eel as a potential multibreeder.

Acknowledgements

The authors wish to thank both the scientific and the technical staff at Zoophysiological Laboratory A, University of Copenhagen, for kind help and advice. Especially they wish to thank Dr. Lis Olesen Larsen for friendly and stimulating discussion and criticism, both during the experiment and in writing the manuscript. They also wish to express their gratitude to Dr. Povl E. Budtz and laboratory technician Susanne Binzer for their kind help in preparing the histological material.

References

Berndt, O., 1938: Morphologie und Histologie des Rumpfdarmes von *Anguilla fluviatilis* und die Veränderungen desselben im Individualzyklus. – Zool. Jb. (Anat.) *64*(4): 437-482.

Bligh, E.G. & W.J. Dyer, 1959: A rapid method of total lipid extraction and purification. – Can. J. Biochem. Physiol. *37*: 911-917.

Boëtius, I. & J. Boëtius, 1967: Studies in the European Eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to temperature and other factors. – Meddr Danm. Fiskeri- og Havunders. N.S. *4*: 339-405.

Boëtius, I. & J. Boëtius, 1982: Experimental maturation of European silver eels. – Proc. Int. Symp. Reproductive Physiology of Fish. Wageningen 1982: 174-176.

Fontaine, M., N. Delerue-Le Belle, F. Lallier & E. Lopez, 1982: Biologie Générale. – Toutes les Anguilles succombent elles après la reproduction et frayent elles nécessairement en mer? – C.R. Acad. Sc. Paris, t. 294, Série III: 809-811.

Love, R.M., 1970: The chemical biology of fishes. – Academic Press, London & New York. 547 pp.

Mislin, H., 1941: Der Phasenwechsel des Rheinlachses (*Salmo salar* L.) unter besonderer Berücksichtigung des Ernährungsapparates. – Rev. Suisse Zool. *48*, Suppl. 1: 1-181.

Peters, G., 1982: The effect of stress on the stomach of the European eel *Anguilla anguilla* (L.). – J. Fish. Biol. *21*: 497-512.

Roos, J. & C.B. Jørgensen, 1974: Rates of Disappearance from Blood and Biological Potencies of Mammalian Gonadotropins (HCG and Ovine LH) in the Toad *Bufo bufo bufo* (L.). – Gen. Comp. Endocrinol. *24*(4): 432-437.

Notes to primary table

Eels nos *1-9* are yellow and nos *10-19* are silver eels (controls).
Eels nos 1-91 are the experimental eels listed chronologically after time of death. Eels within frames are those chosen for analysis.
Eels outside the frames either died or were killed because of disease or wounds.
Blank means 'no data' and 0 (in column 32) means 'no longitudinal muscle layer present'.

1. no. Eel no.
2. w_{b118} Body weight at day 118, g.
3. l_{b118} Total length at day 118, cm.
4. w_{b148} Body weight at day 148, g.
5. l_{b148} Total length at day 148, cm.
6. w_{b179} Body weight at day 179, g.
7. l_{b179} Total length at day 179, cm.
8. w_{b214} Body weight at day 214, g.
9. l_{b214} Total length at day 214, cm.
10. w_{b365} Body weight at day 365, g.
11. l_{b365} Total length at day 365, cm.
12. w_{b400} Body weight at day 400, g.
13. l_{b400} Total length at day 400, cm.
14. w_{b517} Body weight at day 517, g.
15. l_{b517} Total length at day 517, cm.
16. date Date of death.
17. day Life-span in days after day 0 (April 15, 1982).
18. w_b Body weight at death, g.
19. l_b Total length at death, cm.
20. w_i Weight of alimentary tract, mg.
21. w_l Weight of liver, mg.
22. no. Eel no.
23. stage Stage of maturation. 2nd maturation indicated by one asterisk, 3rd maturation by two asterisks.
24. E_h Horizontal eye diameter, mm.
25. E_v Vertical eye diameter, mm.
26. I_dA Oesophagus diameter, μm.
27. I_dB Stomach diameter, μm.
28. I_dC Intestine diameter, μm.
29. M_cA Width of circular muscle layer in oesophagus, μm.
30. M_cB Width of circular muscle layer in stomach, μm.
31. M_cC Width of circular muscle layer in intestine, μm.
32. M_lA Width of longitudinal muscle layer in oesophagus, μm.
33. M_lB Width of longitudinal muscle layer in stomach, μm.
34. M_lC Width of longitudinal muscle layer in intestine, μm.
35. EA Width of epithelial layer in oesophagus, μm.
36. EB Width of epithelial layer in stomach, μm.
37. EC Width of epithelial layer in intestine, μm.
38. W Water in per cent of body weight, %.
39. L Lipid in per cent of body weight, %.
40. NPN Non-protein nitrogen in per cent of body weight, %.
41. P Protein in per cent of body weight, %.
42. A Ash in per cent of body weight, %.

Primary Table

1 no.	2 w_{b118}	3 l_{b118}	4 w_{b148}	5 l_{b148}	6 w_{b179}	7 l_{b179}	8 w_{b214}	9 l_{b214}	10 w_{b365}	11 l_{b365}	12 w_{b400}	13 l_{b400}	14 w_{b517}	15 l_{b517}	16 date	17 day	18 w_b	19 l_b	20 w_i	21 w_l
1															14-09-1982		58.9	35.2	2752	1469
2															14-09-1982		70.5	34.7	2829	1155
3															14-09-1982		76.6	36.1	1989	798
4															14-09-1982		67.5	35.1	3007	1310
5															14-09-1982		67.9	35.0	1995	1339
6															14-09-1982		71.3	34.8	2969	1116
7															14-09-1982		71.1	34.3	3135	1151
8															14-09-1982		71.5	34.8	3961	933
9															14-09-1982		66.1	34.6	2941	1011
10															27-10-1982		97.4	36.9	1816	1826
11															27-10-1982		121.2	39.6	2231	2160
12															27-10-1982		111.4	39.6	803	1634
13															27-10-1982		119.5	40.0	1266	1912
14															27-10-1982		91.4	39.3	882	1520
15															27-10-1982		87.0	38.0	652	1077
16															27-10-1982		103.9	36.3	1641	1843
17															27-10-1982		85.3	35.4	1498	1433
18															27-10-1982		99.4	37.4	948	2003
19															27-10-1982		80.1	34.1	1200	1653
1															15-04-1982	0	74.1	38.0	788	851
2															15-04-1982	0	100.8	40.0	1219	1393
3															15-04-1982	0	93.1	41.2	1033	1054
4															15-04-1982	0	75.9	32.3	1142	820
5															15-04-1982	0	87.7	38.9	1155	1087
6															15-04-1982	0	82.2	39.9	816	935
7															15-04-1982	0	95.3	40.4	1100	862
8															15-04-1982	0	75.1	35.8	944	887
9															15-04-1982	0	67.1	36.1	1010	765
10															15-04-1982	0	75.2	37.1	902	1202
11															15-04-1982	0	71.4	37.4	862	750
12															29-04-1982	14	77.6	36.1		
13															29-04-1982	14	66.8	37.6		
14															30-04-1982	15	68.8	35.2		
15															12-05-1982	27	62.9	37.7		
16															26-05-1982	41	40.3	35.2		
17															26-05-1982	41	78.8	39.0		
18															10-07-1982	86	58.7	35.5		
19															13-07-1982	89	51.2	34.5		
20															15-07-1982	91	69.4	40.4		
21															11-08-1982	118	64.9	39.0	231	743
22															11-08-1982	118	44.3	34.8	241	518
23															11-08-1982	118	52.9	37.5	251	817
24															11-08-1982	118	54.2	36.1	317	892
25															11-08-1982	118	44.7	38.6	309	416
26															11-08-1982	118	75.6	38.9	313	830
27															11-08-1982	118	64.6	41.0	254	704
28															11-08-1982	118	40.5	33.1	191	314
29															11-08-1982	118	49.3	35.8	366	471
30															11-08-1982	118	52.3	35.4	222	559
31	41	36.0	47	36.5											23-09-1982	161	44.2	36.7	1301	719
32	46	34.3	43	34.2											30-09-1982	168	40.6	34.2	244	630
33	42	34.6	41	34.5											30-09-1982	168	35.4	34.5	174	415
34	61	37.1	55	37.3											04-10-1982	172	53.1	37.4	317	698
35	87	40.2	82	40.7											07-10-1982	175	70.7	40.1	324	1789

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
no.	*stage*	E_h	E_v	I_dA	I_dB	I_dC	M_cA	M_cB	M_cC	M_lA	M_lB	M_lC	*EA*	*EB*	*EC*	*W*	*L*	*NPN*	*P*	*A*
1				1944	3056	2815	88	139	213	0	43	98	62	207	39					
2				1991	3148	3047	115	144	326	0	72	39	74	174	39	60.8	16.9	0.36	14.7	1.9
3				2269	3260	3010	86	170	246	51	70	70	69	262	59	56.6	21.8	0.33	14.5	1.8
4				2065	3500	2824	75	166	635	0	59	27	62	207	35	63.6	15.5	0.32	15.2	1.9
5				2121	2315	2111	96	90	262	20	47	52	57	180	31					
6				2250	3760	2611	161	141	215	23	68	62	68	213	35	56.0	22.3	0.32	13.6	1.8
7				1871	3010	3482	92	123	546	42	53	37	76	209	35	57.9	20.7	0.33	13.6	1.7
8				2250	3324	3871	115	125	500	54	49	105	61	238	65	61.5	16.4	0.34	14.9	1.9
9				2547	3482	2750	100	164	363	23	74	84	82	195	31					
10	1	5.6	5.2	2037	3244	2241	117	102	439	0	113	78	35	111						
11	1	5.5	5.5	1871	3185	3000	146	133	683	0	102	29	25	141						
12	1	5.6	5.2																	
13	1	6.1	5.6		1370	2009		30	410		57	35		57	23	55.0	25.3	0.29	13.1	2.0
14	1	5.6	5.5	2130	2685	1408	104	88	52	0	88	39	18	130	30	63.7	15.1	0.32	14.6	1.4
15	1	5.6	5.1	1889	1370		90	64		0	28		29	83		57.8	23.2	0.29	12.7	1.9
16	1	4.9	4.9	2778	3260	2334	113	137	495	0	115	39	39	166		56.6	23.8	0.29	13.1	1.5
17	1	5.0	4.8	2361	2972	2019	98	81	168	0	108	84	21	115		60.1	20.3	0.37	12.4	1.6
18	1	5.6	5.4	1806	1343	2389	86	52	471	0	70	49	10	55		54.6	25.6	0.35	12.7	1.6
19	1	5.2	4.8	2037	1389	2204	69	47	232	23	55	49	10	62	39					
1	1			2102	2204	1222	107	102	39	0	72	41	27	148	35					
2	1			2538		1426	103		36	65		39	150		29					
3	1			2778	1898	1713	88	55	47	0	50	37	59	70	36	51.2	26.2	0.35	14.3	2.0
4	1			1926	2185	1593	156	70	38	0	62	45	44	148	39	56.9	21.3	0.33	14.3	2.3
5	1			2593	1778	1482	237	86	53	0	64	55	74	76	29	53.9	26.3	0.32	11.2	2.0
6	1			2500	2408	1370	112	141	45	0	62	30	47	138	35	62.6	13.9	0.39	15.3	2.4
7	1			2778	2371	2408	156	117	168	0	47	31	78	168	44					
8	1			1991	2315	1389	151	133	53	0	78	21	39	127	31	54.5	22.6	0.35	13.6	2.1
9	1			2130	2472	1408	198	115	92	0	68	29	53	139	31	52.9	25.1	0.45	11.5	2.1
10	1			2408	1991	1519	103	96	88	0	66	29	98	107	35	56.6	21.9	0.35	13.4	2.1
11	1			2130	1932	1296	117	86	75	0	39	28	74	130	36					
12	1																			
13	1																			
14	2																			
15	2																			
16	1																			
17	4-5																			
18	5-6																			
19	6																			
20	7																			
21	6-7			1593	797		22	19		0	31		34	39		59.8	17.6	0.28	15.6	2.2
22	7				1445	820		78	39		43	27		27						
23	6-7				1296	1101		39	30		39	36		120	23	49.5	27.5	0.21	13.4	2.1
24	6					859			21			28			31					
25	7			1482		1296	62		23	60			31		23	55.9	23.1	0.23	13.6	2.7
26	7				1092	1014		39	16		59	27		78	37	54.0	22.8	0.27	14.0	2.1
27	6-7			1758		1480	59		43	59		63	93		47					
28	6			1665			124						94			64.3	13.8	0.30	15.4	2.6
29	7			2313			195			62			78			53.4	24.4	0.24	13.3	2.4
30	6					858			39			59			59					
31	7																			
32	7	6.2	5.6																	
33	7	6.6	5.6																	
34	7	7.2	6.3																	
35	7	6.5	6.3																	

continued next page

1 no.	2 w_{b118}	3 l_{b118}	4 w_{b148}	5 l_{b148}	6 w_{b179}	7 l_{b179}	8 w_{b214}	9 l_{b214}	10 w_{b365}	11 l_{b365}	12 w_{b400}	13 l_{b400}	14 w_{b517}	15 l_{b517}	16 date	17 day	18 w_b	19 l_b	20 w_i	21 w_l
36	55	37.3	66	37.8	80	38.1									11-10-1982	179	80.3	38.1	1733	1181
37	58	36.1	59	36.0	71	36.8									11-10-1982	179	71.3	36.8	954	841
38	50	36.3	57	36.9	64	37.4									11-10-1982	179	63.7	37.4	945	690
39	49	37.3	45	37.3	41	37.3									11-10-1982	179	41.4	37.3	234	636
40	59	36.8	65	37.1	90	38.6									11-10-1982	179	90.4	38.6	2389	521
41	59	38.1	61	38.2	67	38.4									11-10-1982	179	67.1	38.4	971	824
42	64	38.0	87	38.7	86	39.1									11-10-1982	179	85.6	39.1	1196	756
43	57	38.4	75	38.8	88	39.5									11-10-1982	179	87.5	39.5	1912	1186
44	48	34.1	53	34.4	74	36.0									11-10-1982	179	73.7	36.0	1459	1143
45	65	37.6	60	37.6	63	37.9									11-10-1982	179	62.9	37.9	873	631
46	54	36.2	50	36.1	58	36.3	59	36.2							23-11-1982	222	57.5	36.1	217	499
47	82	41.8	76	41.9	72	41.8	69	41.6							23-11-1982	222	67.1	41.4	329	1352
48	58	39.4	72	39.9	81	40.8	74	40.4							23-11-1982	222	71.2	40.2	385	696
49	55	37.2	70	37.4	90	38.5	78	38.1							23-11-1982	222	75.9	38.1	297	800
50	60	37.0	67	37.1	85	38.0	81	37.9							12-12-1982	241	66.3	37.3		
51	60	37.3	65	37.5	80	38.2	62	38.2							14-12-1982	243	55.7	37.1		
52	60	37.6	52	37.6	49	37.6	47	37.2							12-01-1983	272	37.8	37.5		
53	50	35.7	69	36.2	86	37.2	73	37.1							12-01-1983	272	64.0	37.0		
54	76	40.9	99	41.8	147	43.8	125	43.8							12-01-1983	272	144.0	44.8		
55	70	40.0	79	40.2	115	42.4	98	42.3							12-01-1983	272	107.4	42.9		
56	74	39.4	73	39.5	85	40.4	88	40.8							12-01-1983	272	75.8	40.4		
57	71	40.8	90	41.6	86	41.7	79	41.5							12-01-1983	272	67.9	41.3		
58	60	36.7	59	36.4	70	37.2	66	37.1							12-01-1983	272	55.9	36.8		
59	63	38.9	83	39.5	110	41.0	98	41.0							12-01-1983	272	97.2	41.2		
60	50	35.5	58	35.8	67	36.8	71	36.9							12-01-1983	272	83.0	38.9		
61	56	36.0	65	36.8	84	38.6	85	39.4							12-01-1983	272	72.3	39.0		
62	50	36.8	60	37.5	82	38.8	78	38.9							12-01-1983	272	65.0	38.7		
63	50	33.9	52	34.0	70	35.5	66	35.8							12-01-1983	272	53.8	35.7		
64	43	34.9	47	35.1	55	35.6	52	35.3							28-02-1983	319	29.9	35.3		
65	62	36.6	80	37.4	108	39.4	101	39.3							14-03-1983	333	79.8	38.9		
66	59	37.5	72	38.1	89	39.0	82	38.8							16-03-1983	335	41.4	38.0		
67	48	35.6	45	35.5	64	36.2	60	36.3							20-03-1983	339	37.2	35.1		
68	49	36.9	52	37.1	69	37.4	56	37.4							06-04-1983	356	34.1	37.8		
69	46	37.1	52	37.3	67	38.1	66	37.7	54	37.8					15-04-1983	365	54.4	37.8		
70	57	37.0	53	37.0	62	37.2	59	37.1	31	36.9					15-04-1983	365	30.8	36.9		
71	46	34.7	57	34.8	65	35.8	70	35.8	50	35.4					15-04-1983	365	49.5	35.4		
72	59	36.6	78	37.7	116	39.7	101	39.7	104	40.5					18-04-1983	368	102.2	40.9	1207	1063
73	58	35.7	69	36.4	87	37.6	77	37.3	67	37.5					18-04-1983	368	68.1	37.3	885	554
74	60	37.7	54	37.7	60	38.3	59	38.3	58	38.4					18-04-1983	368	57.4	38.4	930	695
75	45	37.3	56	37.8	71	38.8	69	38.4	53	38.3					18-04-1983	368	51.3	38.3	446	468
76	75	41.6	73	41.7	91	42.1	86	42.3	74	42.1					18-04-1983	368	73.0	42.2	1279	861
77	40	34.2	57	35.3	80	36.9	72	37.0	46	36.3					18-04-1983	368	46.2	36.2	419	409
78	57	37.8	86	39.1	111	40.6	96	41.0	92	41.4					18-04-1983	368	85.7	41.4	941	515
79	53	37.8	51	37.8	75	38.5	74	38.1	56	38.0					18-04-1983	368	53.8	37.9	862	589
80	64	40.2	75	40.4	101	41.4	96	41.4	67	40.8					18-04-1983	368	64.0	40.9	643	837
81	62	38.7	77	39.1	97	40.2	88	40.1	69	39.6					18-04-1983	368	66.2	39.4	1272	628
82	50	35.9	57	36.5	80	38.0	73	37.6	65	37.9	64	38.0	49	37.3	03-10-1983	536	47.1	37.3	211	437
83	46	34.9	59	35.8	70	36.3	64	36.1	50	36.2	52	36.0	45	35.7	03-10-1983	536	33.2	35.7	194	277
84	59	38.4	67	39.0	86	39.6	84	39.7	91	39.7	82	40.1	61	39.7	03-10-1983	536	59.3	39.8	651	375
85	47	38.4	48	38.3	67	39.4	70	39.7	66	39.8	56	39.7	41	39.4	03-10-1983	536	38.4	39.5	315	280
86	59	36.8	73	37.5	84	38.1	74	37.8	67	37.8	63	37.4	49	37.3	03-10-1983	536	47.5	37.4	231	398
87	60	38.2	68	38.3	84	39.4	78	39.2	80	39.8	72	39.6	54	39.4	03-10-1983	536	54.5	39.5	440	351
88	50	34.8	52	35.1	67	36.2	64	36.0	43	35.4	40	35.2	30	35.0	03-10-1983	536	28.9	35.0	138	511
89	60	37.0	60	37.0	71	37.7	69	37.5	70	38.1	66	38.2	57	37.6	03-10-1983	536	54.2	37.7	165	451
90	67	35.2	68	35.1	79	35.6	73	35.4	62	35.4	56	35.2	50	35.0	03-10-1983	536	48.5	35.0	192	445
91	50	35.5	51	35.6	56	36.1	56	35.7	55	36.1	52	36.0	40	35.8	03-10-1983	536	38.9	35.9	248	224

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
no.	stage	E_h	E_v	I_dA	I_dB	I_dC	M_cA	M_cB	M_cC	M_lA	M_lB	M_lC	EA	EB	EC	W	L	NPN	P	A
36	7	7.2	6.8	3917	3463	1945	204	148	39	39	111	33	115	209	52					
37	7	5.6	5.4	2296	1750	1695	122	52	25	39	35	17	62	138	23	59.4	17.4	0.30	15.1	2.2
38	7	6.1	5.9	2593	1926	2463	112	90	45	29	59	28	74	130	39	63.0	15.1	0.31	15.4	2.2
39	7	6.1	5.6	1172		562	38		16	29		21	26		19	56.6	22.3	0.26	13.3	2.7
40	7	6.0	5.8	2871	4074	2408	226	199	23	0	156	22	55	228	31	61.7	16.5	0.47	14.4	1.9
41	7	6.3	6.2	2334	2648	2454	117	156	56	29	88	27	62	156	39					
42	7	6.0	6.0	2963	2408	1390	180	143	45	43	122	28	55	127	27					
43	7	6.3	6.1	3148	3222	2334	229	187	39	0	127	30	96	185	39					
44	7	6.4	6.2	2685	2547	2111	117	125	32	19	86	28	70	141	47	60.1	18.3	0.34	14.7	2.0
45	7	6.5	6.4	1815	1741	1556	148	117	39	20	68	20	86	117	32	67.0	10.3	0.28	16.6	2.5
46	4*	7.2	6.9	1296	1211	1204	60	27	29	0	49	34	14	78	17	63.6	16.6	0.31	13.4	1.7
47	4*	7.4	7.2	1296		1045	39	19	33	14	37	33	20	12	16	60.0	20.2	0.39	11.9	2.2
48	4*	7.0	6.9	1019	619	1277	73	19	40	0	77	29	20	10	15	64.1	15.1	0.33	13.9	2.0
49	4*	7.2	6.7		871	1045		25	25		34	42		48	19	60.4	19.1	0.28	13.4	2.0
50	4-5*	8.3	7.8																	
51	4-5*	8.0	7.8																	
52	7																			
53	7																			
54	7																			
55	7																			
56	6*																			
57	6*																			
58	7																			
59	7																			
60	7																			
61	6*																			
62	7*																			
63	7																			
64	6*																			
65	7*	8.2	8.1																	
66	7*	9.0	8.6																	
67	7*	7.2	7.1																	
68	6*	8.2	7.5																	
69	6*	7.8	7.7																	
70	6*	8.8	8.4																	
71	7*	8.0	7.8																	
72	7*	8.2	8.0	2778	4130	1500	293	225	38	0	109	32	62	241	39	58.5	19.9	0.27	14.8	2.0
73	6*	8.8	8.3	2963	3648		161	269		0	105		68	185						
74	6*	8.4	7.9	2963	3334	1778	199	184	30	0	156	35	99	222	31	65.8	11.6	0.35	15.6	2.4
75	7*	7.7	7.3	2222	2037	1296	71	84	39	23	60	29	47	133	21					
76	6*	8.7	8.1	3056	3426	1945	203	273	41	39	121	25	74	72	23	66.7	11.2	0.30	15.7	2.3
77	7*	9.5	8.9	1852	2111	1389	141	75	27	0	70	39	39	113	21					
78	7*	7.6	7.2	2296	2741	1667	169	168	62	39	89	33	29	148	21	56.8	20.3	0.30	15.2	2.3
79	6*	8.4	8.3	2315	1889	2148	164	79	49	0	47	35	57	139	29	66.8	10.8	0.30	16.2	2.5
80	6*	8.7	8.4	2037		1172	122		23	39		32	35		32					
81	7*	8.9	8.7	3519	3797	2037	253	193	34	55	78	33	68	206	35	62.7	14.7	0.28	15.9	2.3
82	6**	8.5	8.4	1481	898	797	39	23	29	19	35	29	49	58	14					
83	6**	7.3	7.2	1204	742		29	31		29	39		27	25						
84	7*	9.7	9.0	2222	2870	1172	78	109	23	39	39	23	141	51	27					
85	5-6**	7.9	7.5	2037		703	74		23	0		18	25		18					
86	6**	8.2	7.8	1481	742	1074	51	12	10	0	23	23	55	53	25					
87	7*	8.8	8.4		1250	1204		19	18		49	31		78	19					
88	6**	8.3	7.4	1094		1016	27		16	0		16	53		18					
89	4**	9.8	9.4		492	586		21	10		29	18		14	21					
90	6**	8.4	8.2	1250		664	49		14	0		18	18		16					
91	7*	7.6	7.5	1481	586	703	78	66	18	0	49	18	31	63	18					