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Anadromous and Catadromous
Fish Committee

AGE READING OF EELS USING TETRACYCLINE LABELLED OTOLITHS

by

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ABSTRACT

Approx. 3000 eels from 9 to 35 cm length were injected with tetracycline (75 mg /kg body weight) and released in a 17 ha pond. After one year, otoliths of recaptured eels (22) were cut and examined for the in vivo marker.

The paper discusses the possibility of a correct age reading, and consequences for the development of specialized assessment methods for the eel are discussed.

INTRODUCTION

The dutch eel fishery (exact catches unknown, assumed about 3000 ton/yr) is scattered over quite different waters: coastal areas, present and former estuarine waters, larger rivers and numerous lakes and polder waters. Because of this scattered nature of the fishery, a simple regulation of the fishery by the governement is rather difficult: directives should be adjusted to every local situation. For instance, the statutory minimum length and minimum mesh size is recently accompanied on the IJsselmeer (188000 ha) by a limit to the number of fyke nets per fishing permit. However, despite these governemental actions to optimize the fishery, catches show a rapid decline: up till 1970 recorded catches on the IJsselmeer were over 5 kg/ha/yr (often up to 10 kg), after that year recorded catches have reached the 5 kg only once (1976). Causes for this decline of the eel production are unknown. A shortage of glasseels during the last five years will accentuate this downward trend, but cannot be the cause: production went down in earlier years, and glasseels have been even less abundant before (Dekker, 1985) without an enduring drop in production. A shortage of food for the eel, possibly by means of interspecific competition with bream (see Lammens et al, 1985), is sometimes assumed, but not very likely given the abundance of the main food organisms and the slowly increasing growth rate of the bream in the IJsselmeer. Finaly, a straightforward overfishing seems very likely, but lack of data on fishing effort and exact catches makes an objective judgement completely impossible.

This uncertain basis severely hampers the development and enforcement of the undoubtly necessary management policy. More basic research for assessment methods of the eel fishery is therefore urgently needed.

Most of the more sophisticated assessment methods take their basis in the work of Beverton and Holt, 1957: the fate of an individual fish is partly described in deterministic terms (growth, recruitment...), partly in terms of chance (chance of being caught, chance of dieing, of getting stuck in a mesh; these chances, however, are not interpreted in a stochastic sense). These methods require a lot of detailed data, such as catch volume, total effort, catch composition, trends in recruitment and effort, etc.

On the other side, more generalizing models like that of Schaefer, 1954, just observe a direct relationship between a limited number of variables, e.g. total effort versus total catch volume. Although this kind of models miss the intrinsic accuracy of the analytical models, they do not require such an elaborate data set.

Therefore, the first step in selecting an appropriate assessment model for the eel fishery, is an evaluation of which data are available, and how reliable they are.

Productivity of fish stocks is basically the net outcome of two production processes: recruitment and growth in weight per individual. Since the reproduction of the eel is unknown, growth in numbers can not be purposely manipulated by management actions: monitoring the growth in numbers is the most we can do. This has been done in the Netherlands (Dekker,1985) and many other countries (Moriarty, 1985).

The second production process, growth per individual, is most often quantified by ageing individuals, and calcualting growth as the length (or weight) increment from the difference in population means for age a and age a+1, or alternatively from backcalculated lengths of individuals.

Age determination of eel has been intensively studied (see Deelder, 1984 for many references) , mostly ageing otoliths. However, it is generally accepted that the problem is not definitively solved: both the preparation method of the otolith and the way of counting rings are not uniformely conducted (Moriarty and Steinmetz, 1979, Boetius, 1985). Several attempts have been made to validate otolith reading techniques, either by marking individual eels (Tesch,1983, Moriarty,1983, Helfman et al., 1984) or by stocking virgin waters with glass eels (Dahl, 1967, Moriarty and Steinmetz, 1979). However, all of them evaluated the net reliability of their technique, without considering variations of preparation method or counting interpretation.

Therefore, the present study aimed to produce known otolith patterns, rather than eels of known age, by marking eels with tetracycline and to develop age reading methods which match the acquired data as best as can. Additionally, some data on growth can be collected directly.

MATERIALS AND METHODS

In October 1984, more than twothousand eels (Anguilla anguilla L.) were caught in the IJsselmeer, partly by fyke nets from a commercial fisherman, partly by electrified trawl (mesh 1 mm). These eels were transported to the laboratory, and held in aerated bassins for two weeks, to select the most viable eels. From 9 to 14 November, 2064 eels were injected intraperitoneally with tetracycline (oxytetracycline, purum, 4.5 mgr dissolved in 1 liter Ringers solution by long and intensive shaking) giving approximately 75 mg per kg body weight. Tetracycline deposits in newly formed bone tissue, and may be detected by its fluorescense for several years after marking (Weber and Ridgeway, 1962). The length distribution of the sample (measuring total length, rounding to centimeter below) is given in figure 1. Additionally, centimeter groups 15, 20, 25 and 30 were marked with a single spot of alcian blue, injected in the belly behind the anus, on 4 different spots characteristic for their length group. These marks enabled a direct observation of growth of the double marked eels.

The mortality of the bassin held eels went abruptly down after the tetracycline injections, nor did further transport cause significant deaths. Tetracycline is often used as an antibiotic drug, both applied to man and many animals.

This batch of marked eels was released on November, the 15th, in the "Kuinderkuilen", a 17 ha pond in a nature reserve in one of the polders in the IJsselmeer. The pond has a sandy bottom, about 1 to 3 meter deep, some vegetation on the bottom and a fringe of reed along the shore. Water is only supplied by rain; excess water flows out through a small channel, having a very dense reed vegetation throughout the channel. Fishing, and even walking along the shore is prohibited. It was assumed that the pond was almost eel free, but small samples caught during the summer of 1985 with a couple of small fyke nets contained a rather high number of bigger eels (over 50 cm) which undoubtly did not originate from the batch of marked eels. On a subsequent visit a worker of the governement of the polder told that undersized eels from cleaned ditches had been released in the Kuinderkuilen, up till 8 years before.

Additionally, on June the 6th 1985, approximately thousand glasseels were caught in front of the shiplocks in the dam between the Waddenzee and the IJsselmeer. These glasseels were gradually accustomed to fresh water. On June, 10th, tetracycline was added to the water, 0.05 gram/liter for 24 hours, and 0.1 gram/liter for another 24 hours. Again, losses declined after the addition of the tetracycline. The glasseels were released in the Kuinderkuilen on June, 13th.

On October, 15th, 1985, 433 eels were caught in the Kuinderkuilen, using an electric fishing unit. Their length frequency is given in figure 2. Lengths and weights were recorded and both otoliths (sagittae) collected. One of each pair of otoliths was embedded in polyester and sawn according to Deelder, 1976. These otolith slices were examined under a Zeiss fluorescens microscope with low magnification. Filters used were BP 400-440, FT 460 and LP 470.

Marked oitoliths were measured, using an ocular-micrometer with arbitrary, but constant scale.

RESULTS

Of the 433 eels recaptured, 6 eels had an alcian blue spot on the belly; 4 of these 6 eels showed a clear tetracycline mark in the otolith. Additionally, 15 eels without alcian blue spot showed a clear tetracycline ring. It should be noted, that two eels marked with alcian blue, were certainly marked with tetracycline, but did not show any fluorescent ring in their otolith. Therefore, some marked otoliths must have been missed.

Two of the otoliths showing tetracycline marks are reproduced in figure 3 and 4 under ordinary illumination, with arrows indicating the site of the tetracycline ring. These otoliths were selected to show a very distinct winterring and an undetectable winterring; the rest of the marked otoliths showed intermediate distinct winterrings.

Table I summarises all data collected from marked eels and otoliths.

Figure 5 shows the fish length/otolith radius relationship for the data of table I. The regression line ($O = (7.4 \pm 11.6) + (3.3 \pm 0.4) * L$, $R2 = 0.771$ with O = otolith radius and L = fish length) suggests a direct proportionality of fish length and otolith radius, i.e. no intercept. Release lengths have been backcalculated, based on this direct proportionality of lengths and otolith radii. It should be noted that these backcalculated lengths do not depend on the reading of winterrings, since the tetracycline ring was an exactly dated mark.

Given this set of known otoliths, it was intended to test several ageing methods. However, time did only allow a straightforward visual inspection of the slides. Other methods will be considered in due course.

To test the straightforward visual inspection, otolith slides were examined under ordinary filament illumination, to locate the last winterring. After the observer had decided (on subjective grounds), which ring it was, the illumination was changed to ultraviolet. Either the identification of the winterring was correct, or not, reducing the test to a simple binomial count. Two different observers did this test indepently, but both of them had been involved in selecting the tetracycline marked from the unmarked otoliths.

DISCUSSION

The aim of the present study is to select and confirm an age reading method for eel otoliths. To do so, marked otoliths were produced. However, there are two objections that can be put forward to criticize this method:

Firstly, during the application of the marker, the eels were confronted with a sudden and brute change in habitat and one or two marking agents. Inspection of table I shows that double marked eels (tetracycline and alcian blue) grew only 2.6 cm (based on 4 eels, growth from backcalculation), while the mean growth of the total sample was 3.3 cm/year. The tetracycline, being an antibiotic, may have suppressed any latent bacterial infection (thereby reducing the death rate during the experiment). Therefore, it is thought to have a minor influence on growth and winterring formation.

Secondly, the transplantation to a smaller water body may have induced an extra heavy ring in the otolith. If this is true, the marked winterring would have been more distinct than the winterring of an undisturbed eel. Therefore, the presented data should be regarded as giving the maximum reliability of the visual inspection method. Furthermore, only the last formed winterring was examined, again facilitating the correct detection. Knowing that only about half of the otoliths were read correctly, this means that straightforward visual inspection is a very disappointing method ! (see also Boetius, 1985).

To overcome this negative result, two ways are open: an other interpretation method, or an other preparation method. Deelder, 1975, proposed a scanning device, to facilitate age readings. However, he did not overcome the subjective element in the interpretation. Furthermore, his construction was incorrect in having a slit between ocular and sensor: the slit should restrict the area seen by the sensor, but in this position it only acts as a diaphragm.

Time did not allow tests of other methods, but at least two methods should be considered: scanning with subsequent automated image analysis, and burning of the otoliths (the second of every pair) following Moriarty, 1973.

If further validation studies do not confirm any age reading method, then there is no basis for sophisticated age centered assessment models.

In the mean time, the data presented here, can be analyzed with respect to growth. Penaz and Tesch, 1970, examined the possibility of backcalculation of lengths from winterrings. They concluded that lengths could be backcalculated reliably, but that one should allow for a disproportionate growth of the otolith (i.e. the intercept of the regression line). This contrast with the present findings. Although their work is based on much more data than the present work, it was prefered to use the intrinsic relationship of the present data, i.e. a direct proportionality.

Comparing the backcalculated lengths of eels marked with alcian blue, with their length deduced from the alcian blue spot markage (13.8 vs 15, 19.9 vs 20, 19.5 vs 20, 18.8 vs 20) shows that the backcalculation underestimates the release length. Using an intercept, following Penaz and Tesch, 1970, would have enlarged this discrepancy. However, the number of checked backcalculations is to few to reject the backcalculations at all.

The next step in the analysis of the growth data of table I is the well known Ford-Walford-plot of figure 6. This plot enables a quick analysis of the growth data, and can be used to fit different growth models. It should be noted that the initial lengths L1 in this graph are calculated from otolith radii and the final lengths L2, and therefore L1 and L2 are not independently estimated. Neglecting this dependency, and fitting regression lines to the data gives

L2 = 0.9859 + 1.098*L1 regressing L2 on L1, or
L2 = 0.3747 + 1.155*L1 regressing L1 on L2.

The observation that growth rate expressed as absolute length increments increases with length of the eel, is quite remarkable: as far as I know, no other commercially exploited fish shows this phenomenon. Presumably, the defering of the costs of spawning and changes in food composition (especially larger eels eating fish) may cause this aberrant growth pattern. Whatever the cause, it means that fitting any of the conventional growth models to this data is meaningless, and yields irrealistic parameters (Sparre, 1979, Moriarty, 1983). But, as a consequence, the interpretation of conventional growth parameters like L-infinity and k of the von Bertalanffy curve (Moriarty, 1983, Rossi and Colombo, 1976) may be quite misleading. Their role as summary statistics is based on the assumption that growth is ultimately limited by physiological factors, while in the case of the eel, the initial exponential growth phase may be broken off by sudden maturation and emigration. Therefore, growth comparisons should be restricted to absolute or relative length/weight increments.

Several authors have estimated growth of eels. Their work can be classified into three distinct groups: growth measured from tagged eels, growth from stocked eels in eel free waters and growth calculated from age readings (see table III). The literature data presented in table III should be used with care, since some length increments are given by the authors themselves, others were calculated from total length and age.

Although the range of estimated growths is quite large (1.7 to 25 cm/year), one can easily trace the causes for the outliers (Helfman et al, 1984: american eel; Dahl, 1967 and Deelder, 1981 and Boetius, 1985: cultured eels; Deelder, 1978: errors in method; Rossi and Colombo, 1976 mention the high temperature themselves; present work, from alcian blue marks: few eels, with wide confidence interval). Disregarding the obvious and explainable outliers narrows the range of estimates to 2 to 4.6 cm/year, which matches the present work very well.

Finally returning to the ultimate aim, assessment of eel fisheries, it should be noted that the present work has contributed no positive result to the problem of age readings of eels, but that it has confirmed the estimates of growth in temperate waters.

Although most assessment methods are based on age readings, one should realize that they do not critically depend on them: growth itself should be regarded as the key factor (e.g. Pope, 1985). Therefore, the first step in the development of assessment methods for the eel is more or less taken ! Whether the found differences in growth rate are determined accurate enough, and should be considered to be significant (their order of magnitude being equal to the interindividual differences found in the present study) solely depends on which fishery one studies, and which management actions should be adviced on. Noting the recent development of new methods (e.g. Shepherd and Nicholson, 1985) for the assessment of less-data-ed fisheries, the further development of eel assessments has a fair chance.

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Table I

Basic data of recaptured marked eels.

identification number	release length. cm	recapture length. cm	recapture weight. gr	tetracycline ring radius	otolith radius	backcalculated release length. cm
26	15	15.4	5	45	50	13.9
48	-	29.3	41	82	101	23.8
84	-	30.0	46	95	109	26.1
97	-	31.3	59	116	126	28.8
116	-	22.6	29	80	93	19.4
120	20	23.7	23	62	74	19.9
141	-	34.0	55	103	114	30.7
155	-	37.6	88	110	128	32.3
196	-	28.0	42	96	104	25.8
210	20	22.2	17	76	86	19.6
235	-	27.4	35	73	82	24.4
243	-	26.0	37	101	109	24.1
255	-	22.2	17	69	83	18.5
258	-	20.6	11	57	63	18.6
269	20	21.1	13	70	78	18.9
299	-	24.3	22	83	93	21.7
318	-	31.5	53	108	121	28.1
327	30	34.8	74	broken		
350	-	31.8	49	95	111	27.2
387	-	32.0	49	87	94	29.6
411	30	31.0	46	not visible		
433	-	32.3	55	84	106	25.6

Table II

Comparison of winterring readings of the marked otoliths, for different observers.
(+ = winterring correctly identified, - = winterring misidentified)

identification number	observer 1	observer 2
26	+	+
48	-	+
84	-	+
97	+	-
116	-	+
120	+	+
141	+	+
155	-	-
196	+	-
210	-	+
235	+	-
243	+	-
255	-	+
258	-	-
269	+	-
299	-	-
318	-	-
327		
350	+	-
387	-	-
411	-	-
433	-	-

Table III

Summary of literature data on growth of eel.
Note that for comparison growth had to be calculated from total length and age in many cases. Therefore, these data should not be viewed as a thorough review.

source	growth in cm/year
growth measured from tagged eels	
Penaz and Tesch, 1970	2-3
Tesch, 1977	2
Moriarty, 1983	2.5-4.6
Helfman et al., 1984, for the american eel	5.7
present study, from 6 eels marked with alcian blue	1.7±1.7
growth estimated from stocked eels in formerly eel free waters	
Dahl, 1967, in carp ponds	12.1
Moriarty and Steinmetz, 1979	2.5-3
Deelder, 1981, in eel farm ponds	males 12, females 17
Boetius, 1985, in eel farm bassins	15-25
growth estimated from age readings	
Ehrenbaum and Marukawa, 1914	3-4
Frost, 1945	4
Deelder and de Veen, 1958	4
Sinha and Jones, 1967	3.5-4
Deelder, 1978	15
Rossi and Colombo, 1979	6.6
Rasmussen and Therskildsen, 1979	4.5
Moriarty, 1983	2.2-3.2
Helfman et al, 1984, for american eel	4.4
present study, from backcalculations	3.3±1.3

Figure 1

Length distribution of sample of marked and released eels.

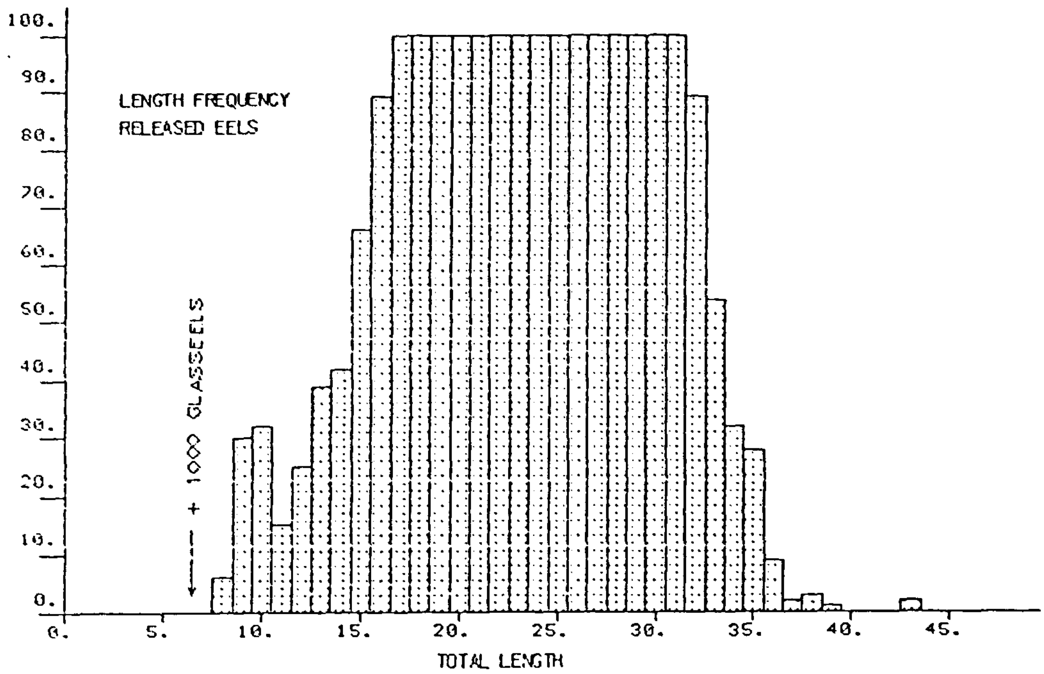

Figure 2

Length distribution of recaptured eels, marked (black) and not marked (grey).

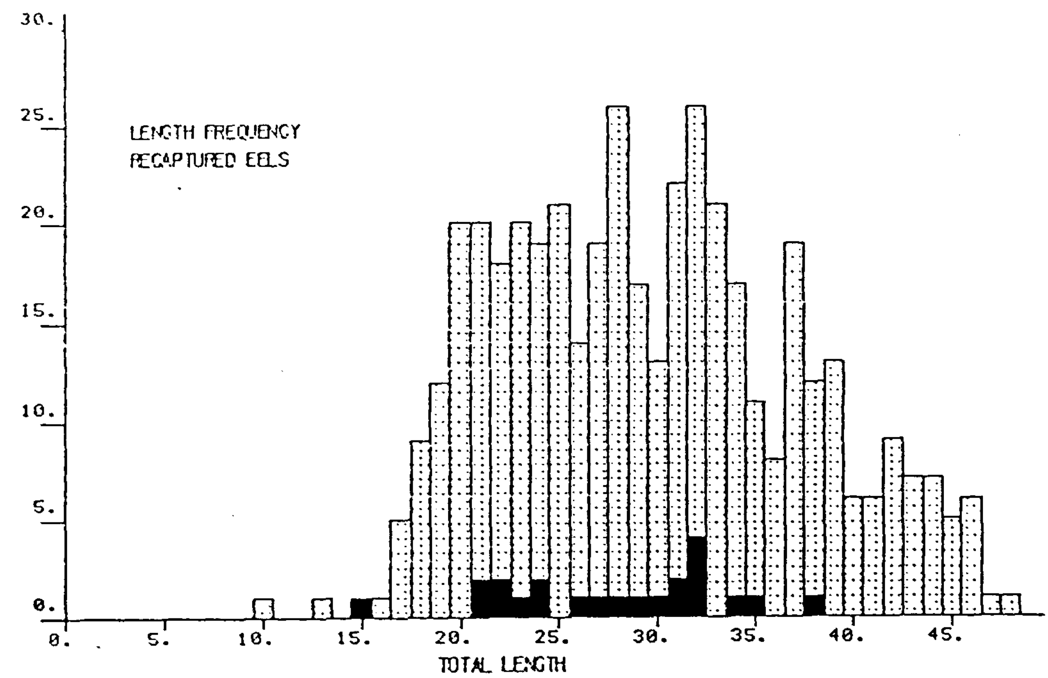

Figure 3

Photograph of otolith no 871 , with arrows indicating the ring marked with tetracyline

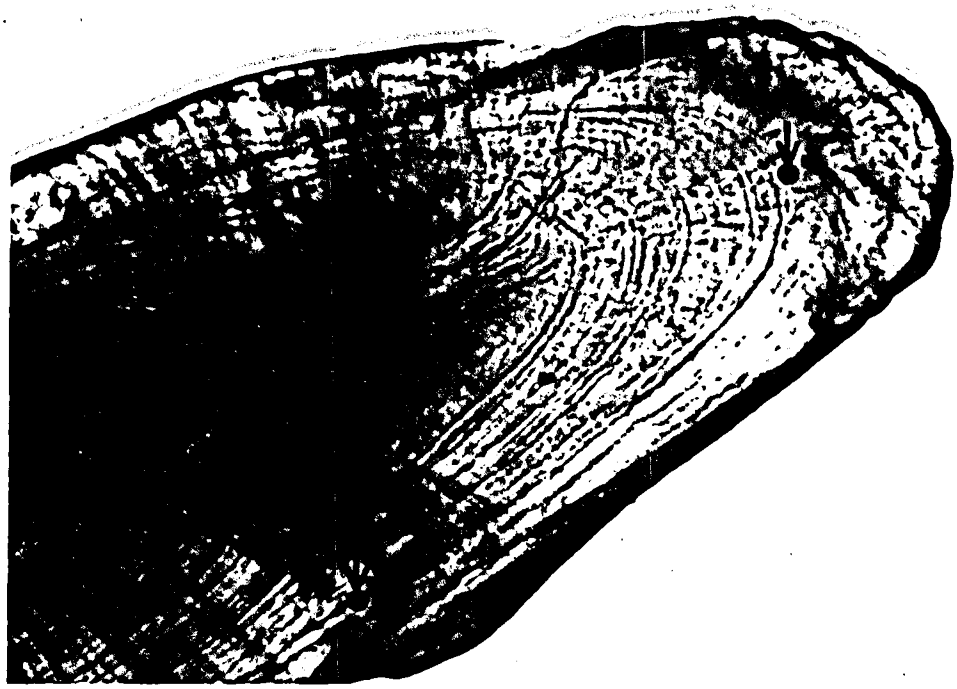

Figure 4.

Photograph of otolith no 318, with arrows indicating the ring marked with tetracyline

Figure 5

Plot of total eel length versus otolith radius.

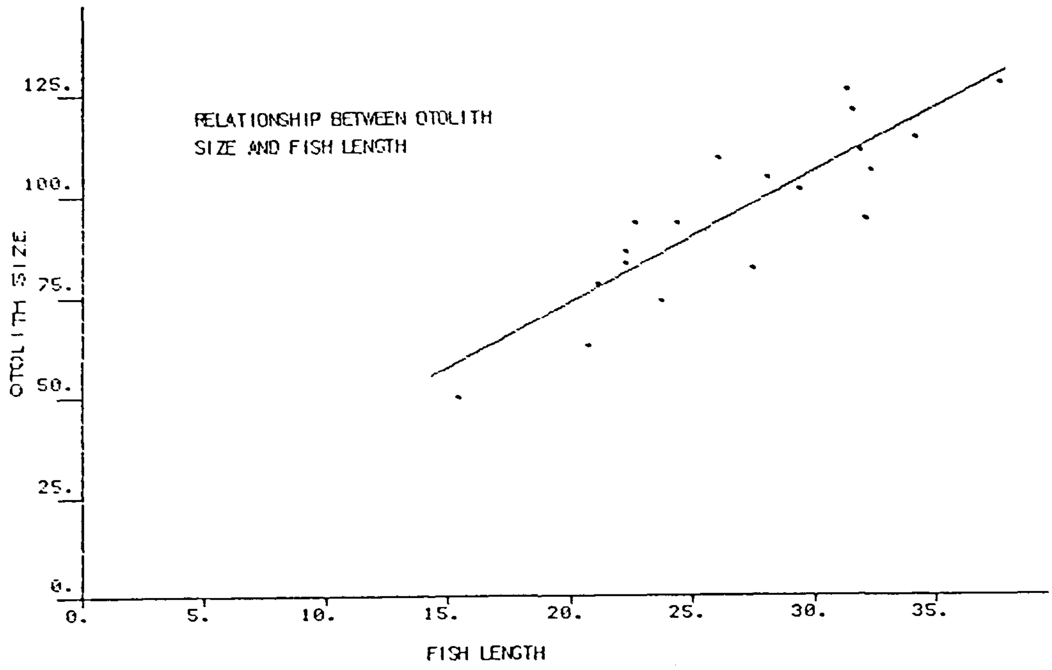

Figure 6

Ford Walford plot: length of recaptured eels versus backcalculated length at markage one year before.