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TETRACYCLINE LABELLING OF OTOLITHS IN PLAICE

by

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Abstract

In this paper the results of a tagging experiment will be presented in order to examine the validity of the back calculation of previous lengths in the growth history of individual plaice.

To this end an experiment was conducted in the southern North Sea in July 1985, in which 1039 plaice between 15 - 25 cm were tagged with a Peterson tag and injected with oxytetracycline. The oxytetracycline gives a time-mark in the otoliths. From the diameter of the tetracycline ring the length at tagging could be back-calculated. 43 otoliths of plaice recaptured within 2 and 19 months after tagging were available.

The observed and the back-calculated growth showed a significant correlation. The average observed growth was 5.29 cm and compared to an average back-calculated growth of 5.90 cm. The back-calculated length thus were on average 0.61 cm lower than the observed length at tagging. This deviation between observed and back-calculated length is close to the shrinkage of dead plaice after capture (between 0.4 and 1.0 cm). The deviation between observed and back-calculated length at tagging was related to the absolute growth increment: highest deviations occured at low growth increments. The plaice that were recaptured in the month of release showed a negative growth of 1.7 cm for which no explaination could be given.

It was concluded that the tetracycline experiment showed that the back calculation technique could be applied in the study of individual growth in plaice, provided that the growth increment sufficiently exceeds the measurements error of fish-size and otolith measurements.

In addition the variability in growth between seasons and individual plaice was studied. Growth in length occured between April-May and October-November and showed a great variability between individual plaice.

1. Introduction

Back-calculation of the growth history of fish from annulus diameters can not only yield important information on the growth of fish, but also opens the possibility to study the allocation of energy over somatic growth and reproduction in individual fish (Rijnsdorp 1986). These applications of the back-calculation technique depend heavily on the accuracy of the back-calculations. Till so far no test of the accuracy of the back-calculation technique in analysing the growth of individual fish are available.

Labelling otoliths by chemical markers as tetracycline (Weber and Ridgway 1962, 1967; Jones and Bedford 1968) give us the possibility to study this accuracy directly. Tetracycline injected into the body cavity is assimilated and appears as a fluorescent band in calciferous material as otoliths and bones. In this paper the results of a tagging experiment are reported in which plaice were tagged with the normal Peterson tag and injected with oxytetracycline. A total of 43 otoliths taken from recaptures between 2 and 19 months after tagging were analysed.

In addition the tagging experiment will be analysed for the individual variation in growth in length and the seasonal pattern in growth.

2. Methods

2.1. Tagging.

Plaice with a length between 15 and 25 cm were tagged with a Peterson tag between 9 and 11 July 1985 at position 5240 N, 420 E (N=332) and position 5325 N, 515 E (N=707). In addition each plaice was injected with oxytetracycline in the body cavity at a dose of 50 mg per kg fishweight. Tetracycline was dissolved in Ringers solution in a concentration of 15 g/l. Length at tagging was measured to the mm below.

2.2 Recaptures.

The recaptures were collected in several fish auctions in the Netherlands and collected by the RIVO afterwards. Date and position of recapture was recorded. Length (mm below) and weight (nearest gram) were recorded at the RIVO and for part of the recaptures at the fish auction directly after landing. Because of the time interval between recapture date and landing, c.q. measuring date the length at recapture is underestimated (c.f. 2.5.).

2.3 Otolith preparation.

The otoliths were viewed with a Zeiss microscope with UV light (wavelength 420 nm) using filters (G365,FT395,LP420) Otoliths were first inspected for the presence of a tetracycline band.

A total of 218 recaptures was available. However in a great number of these the growth was negligable or the otoliths were broken. In 25% of the complete otoliths the tetracycline band was invisible or very faint. Finally 52 complete otoliths showed a clear fluorescent band of approximately 0.05 mm width. These otoliths were imbedded in resin, sectioned through the nucleus, photographed and finally the diameter of the tetracycline band and the total otolith were recorded. 43 were sectioned along the longitudinal axis. Of 9 others a cross section was made.

2.4. Back-calculation.

It was assumed that the growth of otolith-size and fish-size is strictly proportional. This was checked by studying the relation between otolith-size and fish-size in a sample of 118 otoliths from plaice sampled in the southern North Sea in 1985 and 1987. Only symmetrical otoliths were selected and the otolith-size was determined along the logitudinal axis. The results of the regression analysis are shown in Table 1 and in Fig.1. The functional regression line does not pass through the origin but gives an intercept with the Y-axis of 0.5 mm.

The length at tagging was back-calculated according to the following relation:

$$Lt = Lr \cdot (Otc - 0.50) / (Otot - 0.50)$$

where Lt = Length at tagging in mm,
Lr = Length at recapture in mm,
Otc = diameter of tetracycline ring in mm,
Otot = diameter of total otolith in mm.

In applying the correction factor of 0.50 mm in the back-calculation the assumption is made that each individual plaice of zero length has an otolithsize of 0.5 mm. This correction reduces the back-calculated length of recaptured fish of 30 cm by about 0.5 cm.

2.4 Change in length of plaice after catching

In order to estimate the change in length of plaice after catching, two groups of 25 plaice each were measured individually to the mm below directly after catching. Group A plaice were put in a plastic bag and deepfrozen at -20 degrees C for 3 weeks. After defrosting overnight length measurements were taken. Group B plaice were left on board for about 12 hours (T = +/- 14 oC) in an open fish basket before measuring the length. Finaly both groups were put at 4 degrees in an open fish basket and remeasured after 24 hours.

3. Results

3.1. Comparison of back-calculated and observed growth.

In Fig.2 the relation between the back-calculated and observed growth is shown. The regression coefficients of the functional regression (r=0.918, n=43) are: intercept u = 0.791 (95% c.l. 0.081 - 1.501) and slope v = 0.966 (95% c.l. 0.846 - 1.086). Because the slope does not differ significantly from 1.0, the functional regression line runs parallel to the line representing strict equality between back-calculated and observed growth. The adjusted means however are significantly different by 0.6 cm ($P<0.05$).

The frequency distribution of the deviations between the observed and the back-calculated growth also show that the back-calculated growth is on average slightly higher than the observed growth (Fig.3). In Fig.4. it is shown that these deviations especially occur in fish with little growth. The individual variations in the deviations are rather high but the mean deviation stabilize at a difference of less than -0.5 cm at overall growth increments of >= 4cm.

3.2. Change in fishlength after capture

In Table 2 the results of the change in length at different storing regimes are given. The mean length of 25 plaice deepfrozen for 3 weeks at -20 oC (group A) decreased with 2.4% from 23.73 cm to 23.15 cm. After prolonged storing for 24 hours in an open fish basket at 4 oC the average length decreased to 23.04 cm.

The mean length of Group B plaice stored for 12 hours in an open fish basket at +- 14 oC decreased by 1.2% from 23.13 cm to 22.86 cm. prolonged storing for 24 hours in an open fish basket at 4 oC reduced the mean length by 0.6% to 22.72 cm.

The overall decrease in mean length of group A and B plaice was 0.41 and 0.69 cm and 1.8% and 2.9% respectively.

3.3. Seasonal and individual variability in growth of tagged fish

In Fig.5. the observed growth of all 218 recaptures have been plotted against the number of months after tagging. The period over which growth in length occur reaches from April-May till October-November. Between November and April growth in length virtually stops. The mean growth by month is given in Table 3.

The plaice recaptured within the same month after tagging (= within three weeks) show a negative growth. Even of the plaice recaptured in August about 50% show a negative growth.

The great individual variation in growth is striking throughout the observation period. Twelve months after tagging the difference in growth ranges from a minimum of 2.5 cm to over 10 cm..

4. Discussion

The deviation of the back-calculated length and the length at tagging is influenced by:
1) measurement errors in length determinations.
2) shrinking of fish after recapture before length measurement.
3) variability in direct proportionality of otolith growth and growth of fish size.

ad 1. No estimate of the error in the length determinations is available. A systematic bias in length determination seems unlikely. This error term is assumed to be responsible for part of the variation in individual growth. Length determinations of live and moving fish will be less precise and even may have a systematic bias. This possibility should be checked in future.

ad 2. The order of magnitude of the shrinking of plaice after capture is about 0.5 cm or 2%. Johansen (cited in Hickling 1937) reported an average shrinkage in plaice between 0.5 and 1.0 depending on the time. Ouwehand (1973) reported an average shrinkage of 0.54 cm in soles.

Although in practice the treatment of recaptured plaice is variable before length measurements are taken: storing on deck in different seasons, storing on ice, deepfreezing, variable time-periods etc, the figures above are in close agreement and will therefore give an acceptable estimate of the average shrinkage of recaptured plaice between 0.5 and 1.0 cm.

The average difference between the back-calculated and observed length (0.6cm) are in close agreement with the shrinking of plaice after capture. The influence of shrinking on the acuracy of the back-calculated length will be smaller as growth is high. In a tagged fish with zero growth shrinking of 1 cm after capture will lead to a deviation of 1 cm between the back-calculated and observed length at tagging. In a fish that grew from 20 cm to 25 cm the shrinking of 1 cm will lead to a deviation of 0.8 cm (20-20/25*24). This explains a part of the higher deviations at low growth increments in Fig.4.

The observed negative growth of plaice recaptured within the month of release is much too large to explain by shrinking alone. If the observed negative growth of -1.7 cm (Table 3) is related to the bias in measuring the length of live plaice at tagging, this bias will be about 0.7 - 1.2 cm after correction for shrinking. Consequently the deviations of the back-calculated and observed

length at tagging will change by 0.7 - 1.2 cm.

ad 3. The back-calculations are based on a direct proportionality of otolith-size and fish-size as illustrated in Fig.1. Because the relation does not go through the origin we have assumed that for each individual a similar otolith-size at zero length applies (0.5 mm). Variation in this otolith size will influence the back-calculated length.

From the available information we can conclude that back-calculated length in plaice can be used to study the individual growth giving an unbiased estimate of the growth increment. However in the case of small growth we should be cautious because the measurement errors are likely to exceed the realised growth.

5. Literature

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Weber, D.D. and G.J. Ridgway, 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. The Progressive Fish Culturist 29(3):150-155.

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Table 1. Regression coefficients of the functional regression of otolith-size (Y) and length of plaice (X): $Y = u + v X$ with 95% confidence limits.

u	95% c.l.	v	95% c.l.	r	n
0.50	0.24-0.76	0.195	0.187-0.203	0.977	118

Table 2. The change in length of plaice in relation to the method of storing.

Group	A		B	
	mean	sdev	mean	sdev
Length after capture	23.73	1.95	23.13	1.24
Length after treatment 1	23.15	2.01	22.86	1.19
Length after treatment 2	23.04	1.97	22.72	1.26
Overall shrinkage	0.69		0.41	

Table 3. Growth increment by month after tagging plaice between 9 and 11 July 1985.

	Total			Male			Female		
1985	mean	sdev	n	mean	sdev	n	mean	sdev	n
July	-1.7	1.47	16	-1.3	0.95	8	-2.1	1.98	7
August	0.2	1.84	20	0.3	2.14	12	0.1	1.37	8
September	1.5	1.61	29	1.7	2.27	11	1.4	1.13	17
October	2.5	2.14	26	3.5	1.92	10	1.9	2.07	16
November	3.6	2.39	28	3.4	2.30	10	3.5	2.54	16
December	1.6	1.58	11	2.0	1.83	5	1.3	1.44	6
1986									
January	3.6	1.82	7	2.2		1	3.8	1.88	6
February	2.5	0.54	4	2.9	0.07	2	2.1	0.57	2
March	3.8	0.67	3				3.8	0.66	3
April	3.7	1.61	4	4.8		1	3.3	1.76	3
May	4.5	1.69	9	3.6	1.35	4	5.3	1.70	5
June	5.7	2.85	12	3.7	0.59	4	6.7	3.02	8
July	4.7	3.02	11	6.9	2.74	5	2.9	1.86	6
August	5.5	1.92	6	4.3	1.49	3	6.6	1.74	3
September	7.2	2.08	6	7.3	1.63	2	7.3	2.52	4
October	7.6	2.13	9	7.0	2.47	3	7.9	2.11	6
November	8.2	4.53	5	8.3	5.22	4	7.7		1
December	9.3	1.79	5	10.9		1	8.9	1.78	4
1987									
January	10.1	3.23	4	7.4	0.78	2	12.9	0.28	2
February	8.4	1.51	3	7.7	1.27	2	9.8		1

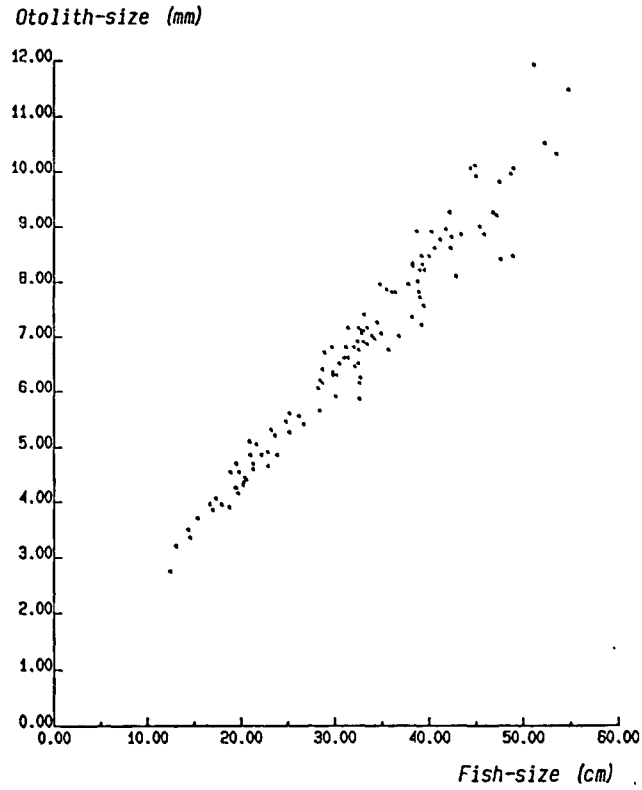


Figure 1. North Sea plaice
Relation between otolith-size (mm) and fish-size (cm)

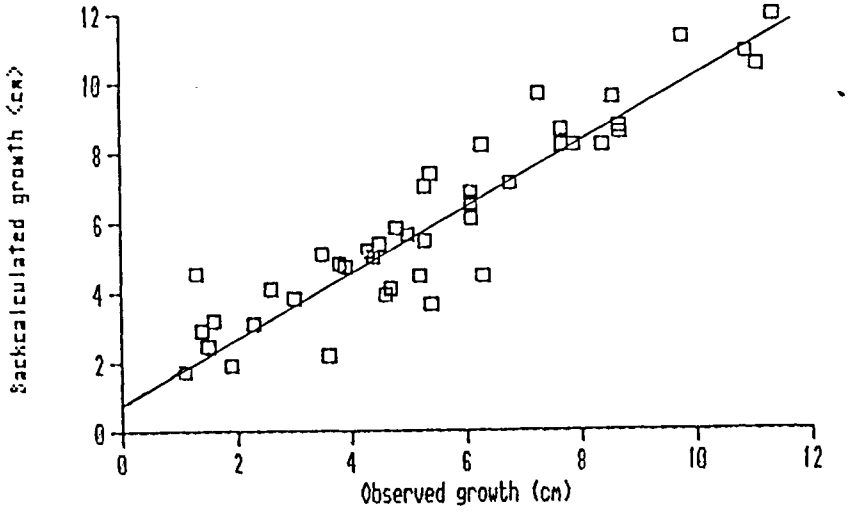


Figure 2. The relation between backcalculated and observed growth.

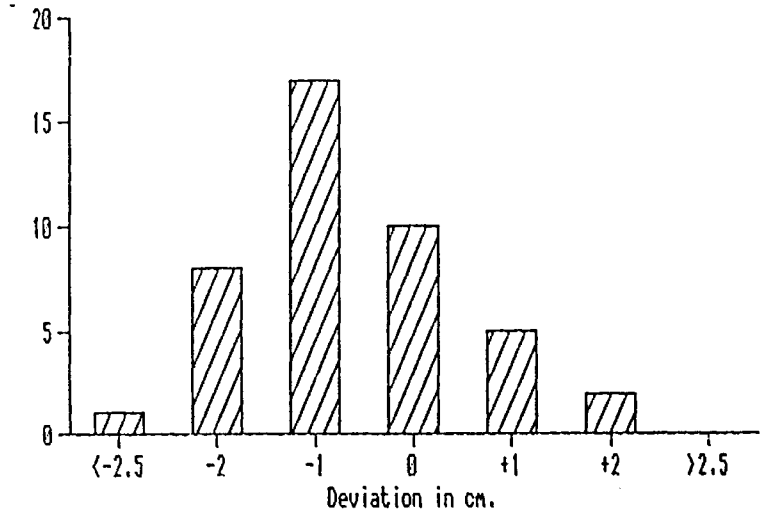


Figure 3. Frequency distribution of deviations between backcalculated and observed length.

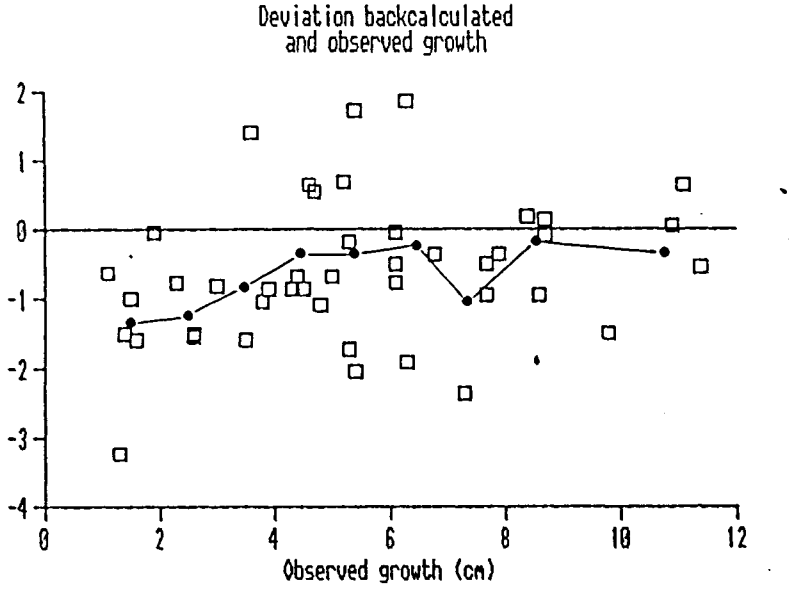
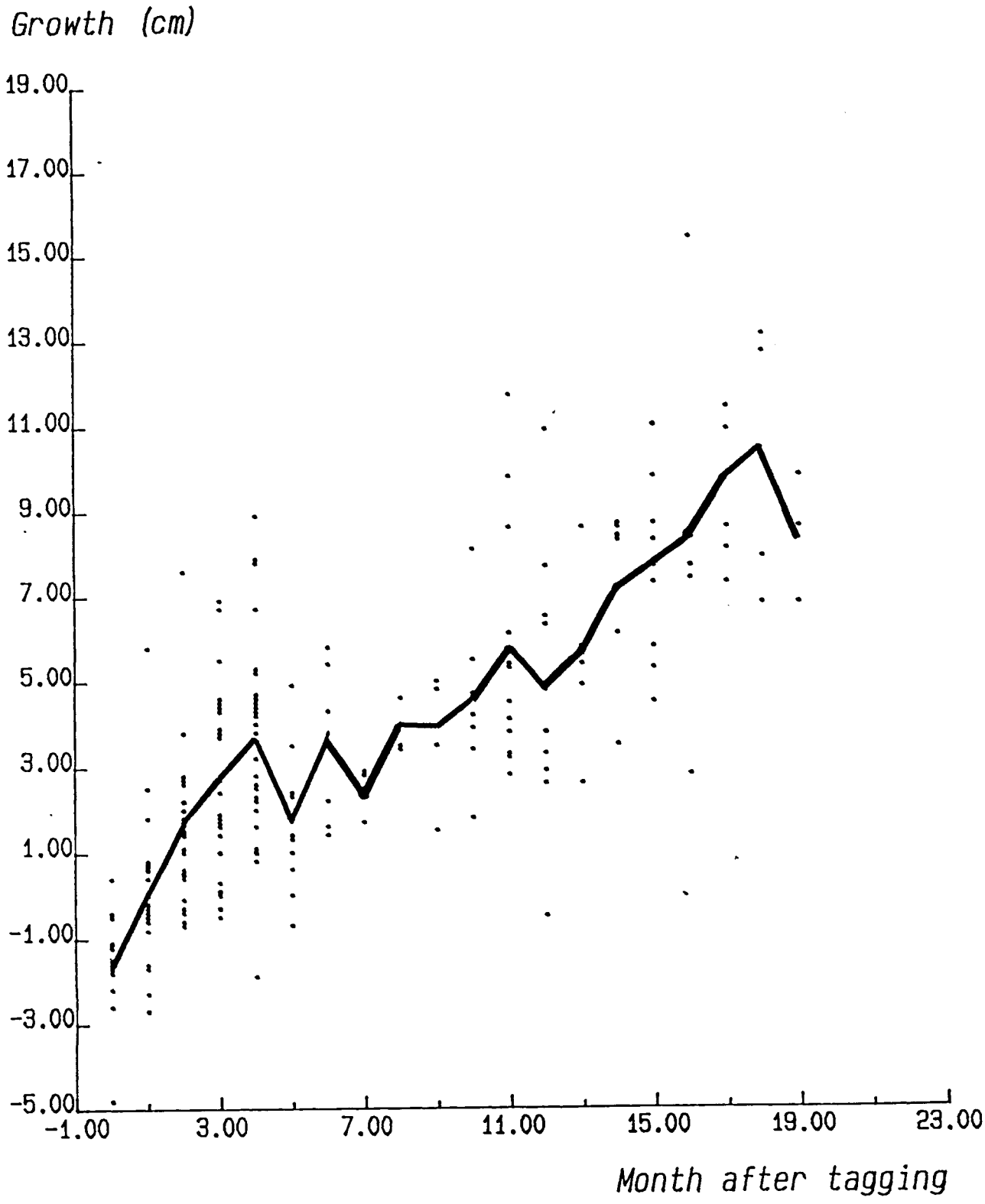


Figure 4. The relation between the deviation of the backcalculated and observed growth and the observed growth.



NORTH SEA PLAICE 1985, 1986

Observed growth increment after tetracycline tagging