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Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989

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ABSTRACT: Somatic growth, otolith growth and RNA/DNA ratios of larval plaice were studied in the southern North Sea during the years 1987 to 1989. Somatic growth rates were strongly correlated with seawater temperature. Both otolith growth and RNA/DNA ratios were correlated with somatic growth. Age at metamorphosis declined with increasing temperature, but there was no relationship between size at metamorphosis and temperature. Development seemed to be more related to temperature than to size. Large between-year differences were observed in the relationship between body size and otolith diameter. Otolith growth indices were used to compare back-calculated otolith growth between years or periods. In general growth was positively related to temperature, but early otolith growth was fastest at intermediate temperatures. It is proposed that the high temperature in 1989 had a negative effect on growth during the early larval stages, but a positive effect on the later stages.

INTRODUCTION

Growth and mortality are important processes during the development of fish larvae. Many marine fishes have an extremely high fecundity and a relatively long lifespan; consequently, mortality during the early stages must be very high (Rothschild 1986). This mortality takes place mainly during the egg and larval stages, and is sometimes up to 99.99 %. It has long been recognized that year-class strength, usually determined at the age of reaching maturity, is to a large extent determined during the early life stages (Hjort 1914). Mortality during these stages is thought to be mainly governed by 3 processes: (1) predation, (2) starvation, (3) hydrographic displacement, although only in few cases has one been able to assess the relative importance of these factors (Hewitt et al. 1985, Leak & Houde 1987). Accurate assessment is difficult however because all the underlying processes occur simultaneously and may interact with each other.

Plaice *Pleuronectes platessa* L. is a marine flatfish in which all these factors are likely to be important. It spawns in the open sea, and the eggs and larvae drift and migrate towards their nursery areas in the coastal zones of England, the Netherlands, Germany and Denmark (Simpson 1959, Zijlstra 1972, Talbot 1977, Rauck & Zijlstra 1978, Cushing 1990). Recruitment of plaice is remarkably constant from year to year (Bannister 1978, Rauck & Zijlstra 1978), and density-dependent regulation mechanisms have been suggested. Mortality and drift of the eggs and larvae were studied extensively for 11 yr between 1947 to 1971 (Harding et al. 1978). A significant correlation was found between the number of eggs in the last developmental stages and recruitment at 2 yr of age (Brander & Houghton 1982), but this correlation has not yet been confirmed by independent data. Larval mortalities were investigated for 4 yr (Harding et al. 1978) but at that time information on growth in the field and accurate aging techniques were lacking, so these mortality rates are not reliable. However, larval density is low, and intraspecific density-dependent mechanisms are hard to imagine.

The ecology of juvenile plaice in the nursery areas has been a subject of study in England, Germany and the Netherlands (see Bergman et al. 1988). Early juvenile mortality was shown to be density-dependent, but could only explain a part of the reduction in the between-year variation in year-class strength. It was concluded that year-class strength was mainly determined during the pelagic phase (Veer 1986). Recently it has been hypothesized that relatively small, or episodic, changes in daily growth and mortality rates may have a large influence on ultimate survival (Houde

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1986, 1989). At a constant rate of mortality a small change in growth rate may lead to large differences in recruitment, by changing the time needed to reach a certain stage. Detailed knowledge about growth is therefore essential for a better understanding of the generation of year-class strength.

In recent decades a number of methods have been developed to study larval growth in the field. The most important, and the easiest to apply, is the otolith increment technique (see reviews by Campana & Neilson 1985 and Jones 1986). Another method, which was recently improved and is now applicable to individual fish larvae, is the analysis of RNA/DNA ratios (Buckley 1984, Clemmesen 1988).

The present study was undertaken to gain more insight into the effect of temperature on the growth and development of plaice larvae in the open sea, and therefore into processes which may be important for the generation of year-class strength. Attention is focused on the variation in growth rate within years and between years from 1987 to 1989. Otolith based methods as well as analysis of RNA/DNA ratios were employed, and were compared with each other.

MATERIAL AND METHODS

Sampling. Samples were collected in 1987, 1988 and 1989 from the RV 'Aurelia' (For more details see Fig. 1 and Table 1). Double oblique tows were made with an Isaacs-Kidd mid water trawl (length 17 m, mouth opening 7.3 m^2, mesh size 1.4 mm, towing speed ca 2.5 knots). The Isaacs-Kidd trawl is highly selective for larger larvae, and no yolk-sac larvae were caught. In 1988 and 1989 additional hauls were made on locations where eggs and larvae were abundant. A bongo-type net and a plankton torpedo were also employed, but even then hardly any yolk-sac larvae were caught, so the youngest larval stages were not represented in this study.

In 1987 the first samples were stored in a buffered formaldehyde solution (4 %) but this caused partial dissolution of most of the otoliths within 24 h. Thereafter all samples were stored in 96 % ethanol. If possible samples were sorted on board, otherwise they were stored in 96 % ethanol. The ethanol was replaced within 24 h, and the samples were sorted in the laboratory within 1 wk. In 1988 several samples were stored in a 2 % buffered formaldehyde solution to determine a size-weight relationship (Hovenkamp 1990). About 50 % of the otoliths 'survived' this treatment and were included in the analysis. At several stations in 1988 and 1989 (Table 1), a subsample of ca 16 living larvae was taken and immediately stored in liquid nitrogen (−196 °C) for RNA/DNA analysis. In winter the water column of the southern North Sea is well mixed, so water temperatures from surface bucket readings were used.

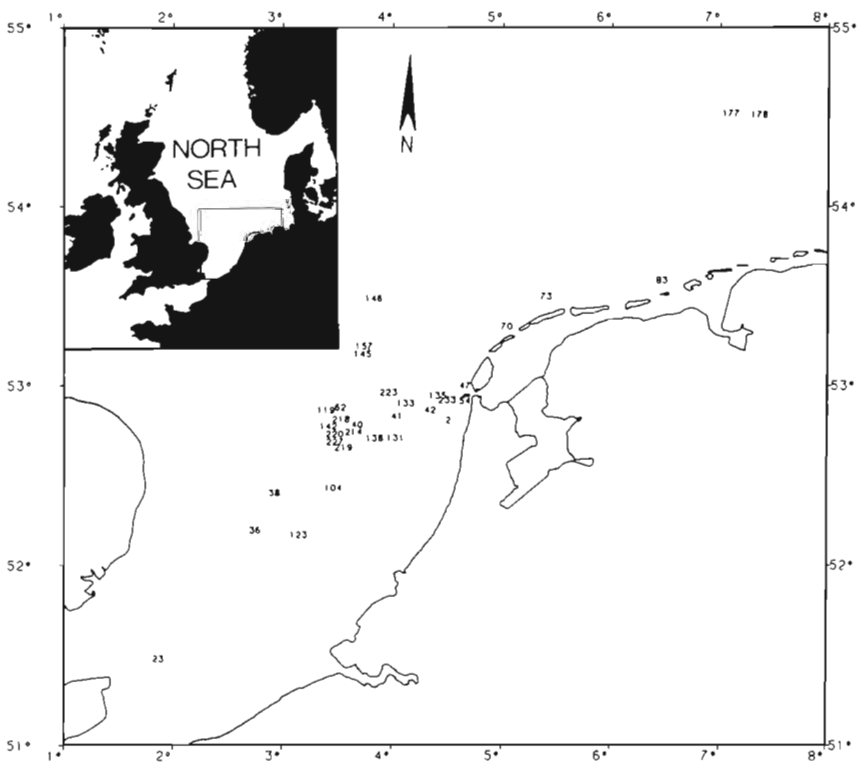

Fig. 1. The North Sea, showing location of sampling stations. Stations between 1–100 were sampled in 1987, 101–200 in 1988, 201–300 in 1989

Table 1. Larval plaice collection 1987 to 1989. Station, date and cruise, location and temperature. Hauls taken at approximately the same time and location are taken together as one station. At several stations, subsamples were taken for RNA/DNA analysis

Station	Date	Cruise	Latitude	Longitude	Temp. (°C)	
2	23 Feb	1987-1	52°47'34	4°29'33	4.8	
23	25 Feb	1987-1	51°27'84	1°52'48	5.1	
36	26 Feb	1987-1	52°10'49	2°45'46	5.0	
38	26 Feb	1987-1	52°22'78	2°56'35	4.9	
40	26 Feb	1987-1	52°45'72	3°40'31	5.1	
41	26 Feb	1987-1	52°48'54	4°02'99	4.8	
42	26 Feb	1987-1	52°50'63	4°21'11	4.9	
45–48	16 Mar	1987-2	52°57'75	4°40'16	4.7	
54	19 Mar	1987-2	52°56'61	4°41'00	4.6	
60–63	23 Mar	1987-3	52°51'56	3°32'19	4.5	
70–71	24 Mar	1987-3	53°18'68	5°02'84	4.5	
73–74	24 Mar	1987-3	53°28'73	5°24'55	4.4	
83	25 Apr	1987-3	53°34'27	6°28'38	4.3	
103–105	22 Feb	1988-1	52°24'71	3°26'68	7.0	RNA/DNA
119–120	8 Mar	1988-2	52°50'57	3°22'54	6.1	RNA/DNA
123	9 Mar	1988-2	52°03'08	3°07'69	6.4	RNA/DNA
131	10 Mar	1988-2	52°41'26	4°00'53	6.3	
132–134	10 Mar	1988-2	52°52'85	4°06'06	5.9	
135	10 Mar	1988-2	52°53'41	4°23'46	6.0	
138	22 Mar	1988-3	52°41'62	3°48'26	6.7	RNA/DNA
142	22 Mar	1988-3	52°45'25	3°23'92	7.0	
145	22 Mar	1988-3	53°10'36	3°42'49	6.8	
146	22 Mar	1988-3	53°08'19	3°49'12	6.4	
157	28 Mar	1988-4	53°11'09	3°43'28	7.4	
177	12 Apr	1988-5	54°30'14	7°05'04	6.4	
178	12 Apr	1988-5	54°29'84	7°21'00	5.8	RNA/DNA
214	24 Jan	1989-1	52°43'63	3°29'37	8.2	RNA/DNA
218	25 Jan	1989-1	52°45'61	3°30'77	7.8	
219	21 Feb	1989-2	52°38'07	3°32'00	7.7	RNA/DNA
220	21 Feb	1989-2	52°42'66	3°27'41	7.8	
223	7 Mar	1989-3	52°07'56	3°56'66	7.6	
225–227	7 Mar	1989-3	52°39'90	3°27'42	7.4	RNA/DNA
233–235	6 Apr	1989-4	52°52'97	4°29'75	7.1	RNA/DNA

Staging and measuring. Stages of larvae were noted according to the terminology of Ryland (1966), but stages 2a and 2a', 2b and 2b', 3a and 3a' and 3b and 3b' were taken together as 2a, 2b, 3a and 3b respectively. Standard length and the height of the myotomal musculature at the anus were measured under a dissecting microscope with an ocular micrometer. No allowances were made for shrinkage or loss of weight after fixation, and shrinkage was assumed to be the same for alcohol and formaldehyde fixation. The amount of shrinkage after preservation in liquid nitrogen was not known.

Otolith preparation and examination. Sagittal otoliths were removed under a dissecting microscope, mounted in clear nail polish (sulcus side down) and photographed on high contrast black-and-white film using transmitted light at magnifications of ca 300 to 1600 ×. All measurements were taken from projections of the negatives. Otolith increments were counted and the positions of the first clearly visible increment and each subsequent 10th increment relative to the otolith nucleus were measured. The radius along the transect of reading and the minimum and maximum diameter were also measured. The transect of reading was chosen where the increments were most clearly visible. Three counts were made and if counts differed by more than 3 (absolutely) or 10 % of the mean count the otoliths were excluded from further analysis. The otoliths were not always completely circular, so the mean diameter was calculated from the minimum and maximum diameter. Furthermore the nucleus was often located asymmetrically, and so all measured distances were standardized to the mean otolith radius. Average increment widths were calculated for Increments 1 to 10, 11 to 20, 21 to 30 and 31 to 40. To obtain an impression of the most recent otolith growth, the sum of the width of the last 5 complete increments was measured (in 1988 and 1989).

RNA/DNA ratios. RNA/DNA ratios were measured as described by Clemmesen (1988). All chemicals used were analytical grade. Calf thymus DNA and yeast RNA were used for calibration. The concentration of

RNA and DNA together was determined fluorometrically after enhancement of fluorescence with ethydium bromide. The concentration of DNA was determined in the same way using bisbenzimidazol (Hoechst 33258), after which the concentration of RNA was calculated. Fluorescence was assessed on a Perkin Elmer fluorescence spectro-photometer. A 360 nm filter was used for excitation, and emission was set at 448 nm (DNA) or 590 (RNA). A modification made to the Clemmesen method was that 1200 µl of trisbuffer was added before the last step in the analysis instead of 200 µl, to obtain adequate sample volumes.

Data analysis. Estimates of larval growth were calculated using 3 different methods: (1) size and age data, (2) otolith growth and (3) RNA/DNA ratios.

Size and age: The sizes of the larvae were plotted against the estimated age of the larvae, and exponential growth curves were fitted using linearly transformed data. Growth curves were compared by applying ANCOVA procedures on the linearized model. The product of standard length and squared body height ($L \times H^2$) is linearly related to dry weight (Hovenkamp 1990). The relations between $L \times H^2$ and dry weight and between $L \times H^2$ and otolith diameter were subject to much less variation than those between length, dry weight and otolith diameter, so $L \times H^2$ was taken as a measure of larval size. $L \times H^2$ can be converted to dry weight by dwt (10^{-5} g) = 18.81 + 1.395 $L \times H^2$ (Hovenkamp 1990). Length and height were not corrected for shrinkage; this was not necessary as the relation between size and weight was also established without correcting for shrinkage. Otolith increment deposition was demonstrated to be daily (Karakiri & Westernhagen 1989), but increments formed during the yolk-sac stage are often not visible by light microscopical techniques, and the number of increments is thus an underestimate of the actual age. At hatching otoliths are circular with a diameter of ca 20 µm, and it was found in the laboratory that increment size during the yolk-sac stage was ca 0.6 µm d^{-1}. This is well above the resolution of the light microscope, but these increments are optically very vague, or indistinct. Assuming this rate of deposition, the age was estimated from the size of the otolith at the first visible increment and the number of increments thereafter as:

$$\text{Est. age (d)} = ((R_1 - R_0)/0.6) + \text{No. of increments}$$

in which R_1 = radius of the first increment (µm); R_0 = radius at hatching; and 0.6 = estimated growth per day.

Differences in size or age per stage between years were tested with a 2-factor analysis of variance, using years and stages as factors.

Otolith growth: Increment widths cannot be compared directly. A significant linear relationship was observed between increment width and increment radius, i.e. the distance from the increment to the otolith nucleus. This means that when increments which were formed at different radii are compared, the increment at the largest radius will on average be wider. However, the relative growth of this increment may be less than that of the increment at the smaller radius. Therefore the average increment width of Increments 1 to 10, 11 to 20 etc. was regressed on the radius of Increment 1, 10, etc. A logarithmic transformation of both variables was applied to make variances independent of the mean. The regression residuals were taken as an index of otolith growth. When relative otolith growth has been more than average, the otolith growth indices will be positive, and when relative growth has been less than average, they will be negative. The same procedure was followed in calculating recent growth indices from the width of the 5 marginal increments. There were no trends in the relationships between growth indices and increment diameter.

The day at which an increment was formed was calculated by subtracting the number of increments from Increment 1, 11, 21, or 31 to the margin of the otolith from the year-day at which the larvae were sampled. The data are averaged over periods of 10 d (Period 1 is year-days 1 to 10, 2 is 11 to 20, etc.), i.e. all larvae were taken together when their first or subsequent 10th increment had been deposited in the same 10 d period.

RNA/DNA ratios: RNA/DNA ratios were converted to daily protein growth rates (DPGR) according to Buckley (1984):

$$\text{DPGR} = 0.93\ \text{Temperature} + 4.75\ \text{RNA/DNA} + 18.18$$

RESULTS

Larval sizes were plotted against estimated ages for each year and each cruise (Figs. 2, 3 & 4). Data for 1987

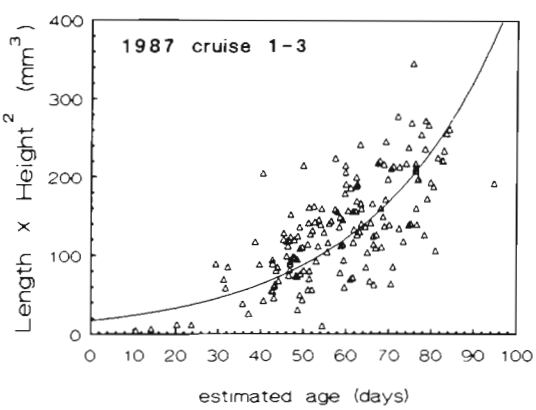

Fig. 2. *Pleuronectes platessa*. Growth of larval plaice, 1987. Exponential growth curves fitted. For growth model parameters see Table 2

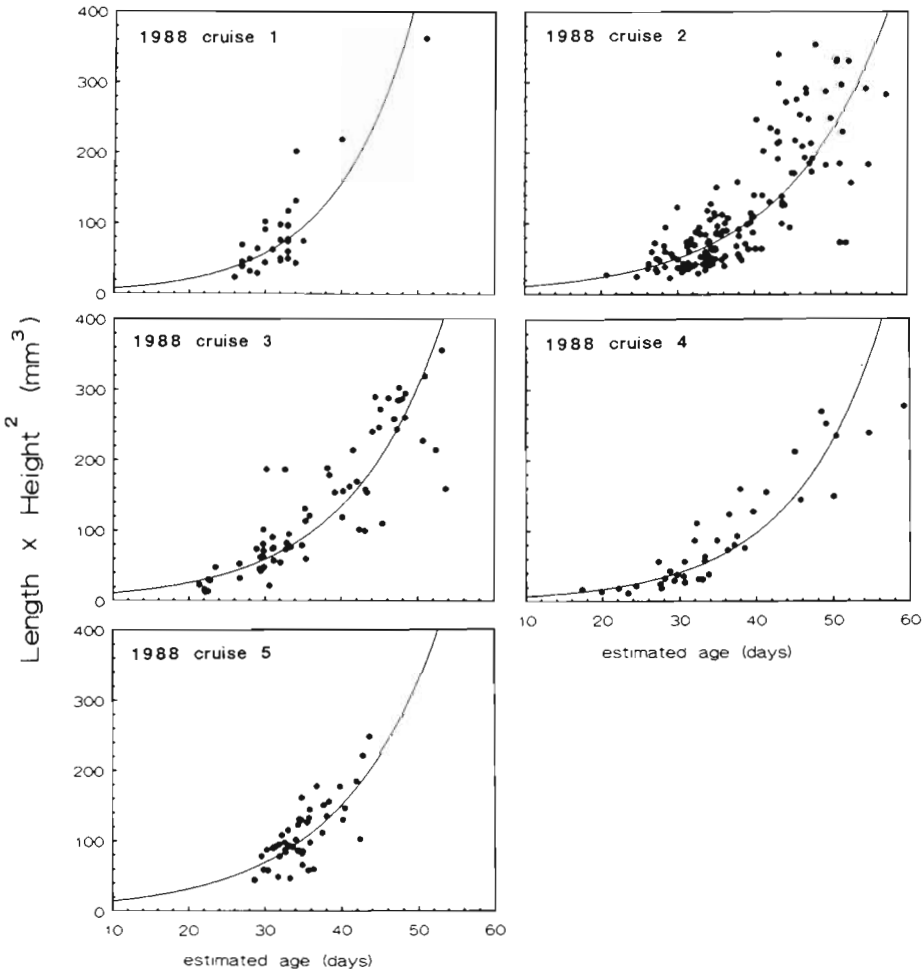

Fig. 3. *Pleuronectes plaessa*. Growth of larval plaice, 1988. Exponential growth curves fitted. For growth model parameters see Table 2

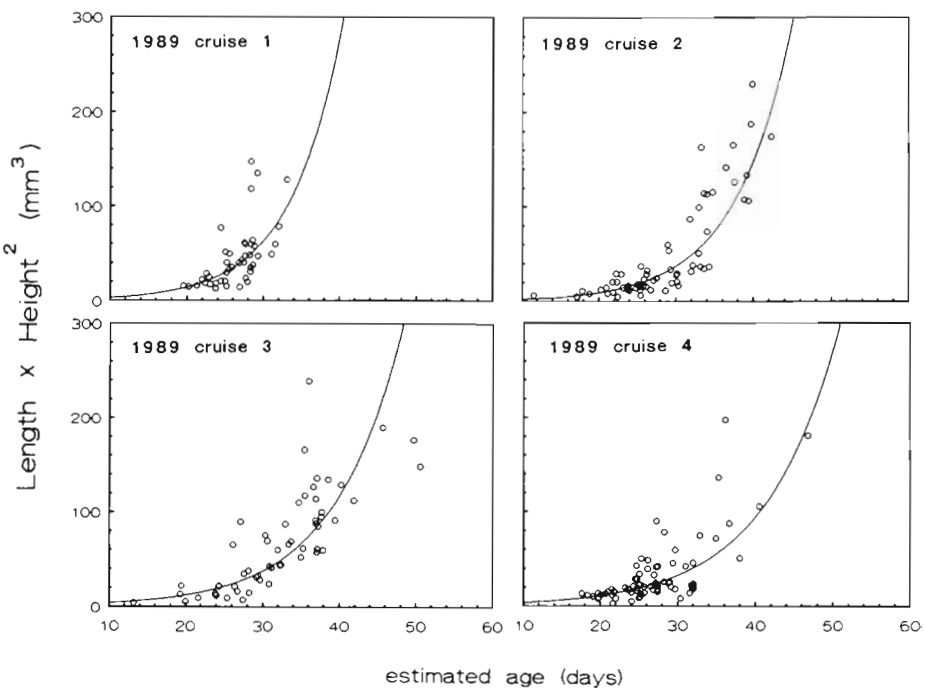

Fig. 4. *Pleuronectes platessa*. Growth of larval plaice, 1989. Exponential growth curves fitted. For growth model parameters see Table 2

were pooled for all cruises, because during some of the cruises only larvae stages 4 and 5 were collected, yielding severe underestimates of growth rate for these cruises. Growth rates were significantly different between years (ANCOVA, $p < 0.0005$), and between cruises within years ($p < 0.0005$). Growth rates (Table 2) were plotted against the average seawater temperature (Fig. 5). The relationship between growth rate and

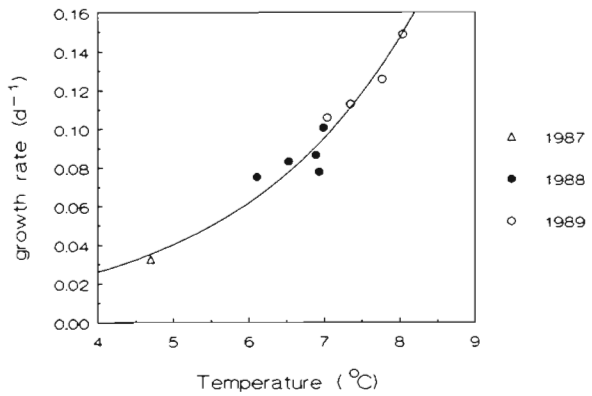

Fig. 5. *Pleuronectes platessa*. Relation between growth rates (G), from size-at-age data, versus seawater temperature (T). $G = 0.00464\ e^{0.432T}$, $R^2 = 0.95$, N = 9

temperature was best described by an exponential function:

gr. rate = 0.00464 exp (temperature 0.4319),
r = 0.974, N = 10

Relation between larval size and otolith diameter

Larval size was linearly related to otolith diameter in each year (Fig. 6). The slopes of the regression lines were significantly different among years (ANCOVA, $p < 0.005$). The otoliths were largest relative to body size in 1987 and smallest in 1988.

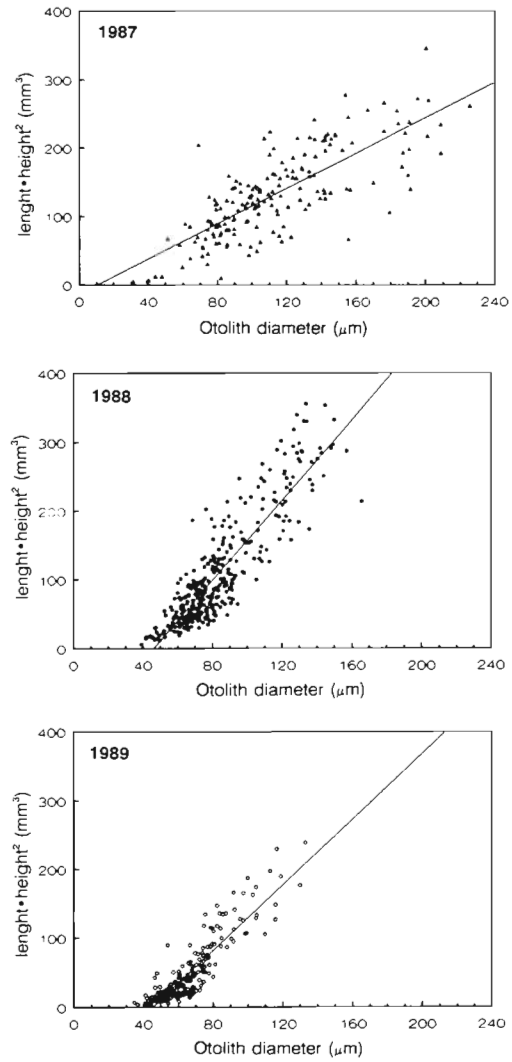

Fig. 6. *Pleuronectes platessa*. Relation between larval size ($L \times H^2$) and otolith diameter (OD) for 1987, 1988 and 1989.
1987: $L \times H^2 = -13.3 + 1.290$ OD, r = 0.78, N = 176
1988: $L \times H^2 = -134.2 + 2.916$ OD, r = 0.89, N = 340
1989: $L \times H^2 = -107.4 + 2.378$ OD, r = 0.90, N = 240

Table 2. *Pleuronectes platessa*. Estimates of exponential growth model parameters. Growth rate, size at hatching ($L \times H^2_0$), coefficient of determination (R^2), average seawater temperatures (T) and no. of larvae used (N), for each cruise

Year	Cruise	Model parameters				
		Gr. rate	$L \times H^2_0$	R^2	T	N
1987	1–3	0.0326	17.2	0.51	4.7	166
1988	1	0.1006	2.82	0.50	7.0	32
	2	0.0751	5.33	0.66	6.1	146
	3	0.0823	4.96	0.77	6.5	65
	4	0.0865	3.03	0.83	6.9	39
	5	0.0778	6.64	0.51	6.9	48
1989	1	0.1487	0.72	0.50	8.0	44
	2	0.1257	0.82	0.75	7.8	66
	3	0.1128	1.36	0.72	7.3	56
	4	0.1058	1.36	0.51	7.0	74

Relations between stage, age and size

The estimated ages for each stage (Table 3; stage 4 was split into stages 4a and 4b) were significantly different among years (ANOVA, $p < 0.0005$), with age at each stage in general declining with increasing temperature. Size at each stage (Table 4) was different among years (ANOVA, $p < 0.001$) but here the relation with temperature was not clear.

Otolith growth indices

Otolith growth seemed to decline in the beginning of each season (January), and to increase in the end of the season (March–April) (Fig. 7). Growth of all increments was slowest in 1987. Growth of Increments 1 to 10, but not 11 to 20, was faster in 1988 than in 1989. Not enough data were available to calculate growth indices of Increments 21 to 30 in 1989 or Increments 31 to 40 in 1988 and 1989. Mean values of recent otolith growth indices (Table 5) were significantly different among years (*t*-test, $p < 0.0001$). The relationship between recent otolith growth index and growth rates from size-at-age data (Fig. 8) was not significant ($r = 0.654$, $p = 0.056$).

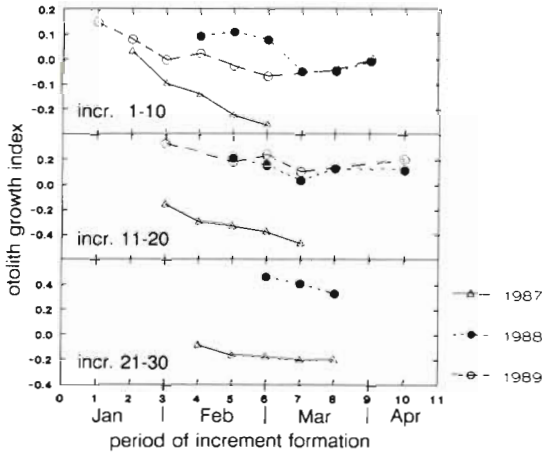

Fig. 7: *Pleuronectes platessa*. Otolith growth indices for Increments 1 to 10, 11 to 20 and 21 to 30 per 10 d period for 1987, 1988 and 1989. Period 1 = year-days 1 to 10, etc.

RNA/DNA ratios

Average RNA/DNA ratios and calculated protein growth rates are given for each cruise in Table 5. Protein growth rates were plotted against growth rates from size and age data (Fig. 9) and against recent growth indices (Fig. 10). Individual RNA/DNA ratios ranged from 0.72 to 4.81 in 1988, resulting in protein growth rates from −9.1 to 10.25 % d^{-1}. In 1989 RNA/DNA ratios ranged from 2.98 to 10.68, yielding protein growth rates from 2.69 to 40.19 % d^{-1}. RNA/DNA ratios and protein growth rates were significantly correlated with growth rates from size-at-age data (Table 6). The correlations with recent otolith growth index were not significant ($p \approx 0.10$). All growth rates and indices were significantly correlated with temperature (Table 6).

DISCUSSION AND CONCLUSIONS

Growth rates

The winters of 1987 to 1989 were quite different in temperature, providing the opportunity to study growth

Table 3. *Pleuronectes platessa*. Estimated age per stage for each year. Means, standard deviations and no. of larvae used

Stage	1987			1988			1989		
	Est. age	SD	N	Est. age	SD	N	Est. age	SD	N
2	17.2	5.1	4	22.0	4.5	6	23.0	3.8	38
3	44.0	9.5	14	30.0	4.0	105	26.3	3.4	99
4a	48.5	5.9	21	36.0	5.1	91	29.9	4.9	48
4b + 4b′	59.0	9.9	96	41.5	6.9	131	35.6	5.2	54

Table 4. *Pleuronectes platessa*. Size ($L \times H^2$) per stage for each year. Means, standard deviations and no. of larvae used

Stage	1987			1988			1989		
	$L \times H^2$	SD	N	$L \times H^2$	SD	N	$L \times H^2$	SD	N
2	8.5	3.2	5	9.4	4.1	30	3.5	1.6	16
3	55.8	21.7	15	40.9	23.4	538	25.6	14.7	138
4a	95.9	30.6	22	107.9	51.6	305	55.1	31.4	50
4b, 4b′	134.3	47.4	102	181.6	75.3	469	107.7	49.4	55

Table 5. *Pleuronectes platessa*. RNA/DNA ratios, protein growth rates, and recent otolith growth indices for each cruise. Means, standard deviations and no. of larvae used

Year	Cruise	RNA/DNA	SD	Prot. gr.	SD	N	Otol. gr.	SD	N
1988	1	3.03	0.607	2.71	2.88	23	0.1588	0.131	32
	2	2.03	0.644	–2.82	3.06	26	–0.1498	0.188	133
	3						–0.0692	0.195	57
	4	3.00	0.259	2.30	1.23	11	–0.0848	0.171	34
	5	2.74	0.653	0.24	3.10	13	0.0346	0.128	47
1989	1	4.96	1.838	13.02	8.73	12	0.124	0.231	43
	2	4.99	1.386	12.81	6.58	16	0.044	0.245	64
	3	4.59	0.772	10.32	3.67	9	0.056	0.203	54
	4	4.77	1.208	10.97	5.73	13	0.121	0.205	74

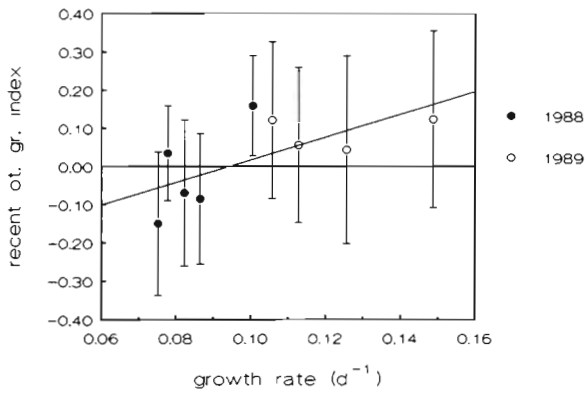

Fig. 8. *Pleuronectes platessa*. Relation between recent otolith growth index (ROGI) and growth rate (G) from size-at-age data. ROGI = −2.622 + 2.823 G, r = 0.65, N = 9

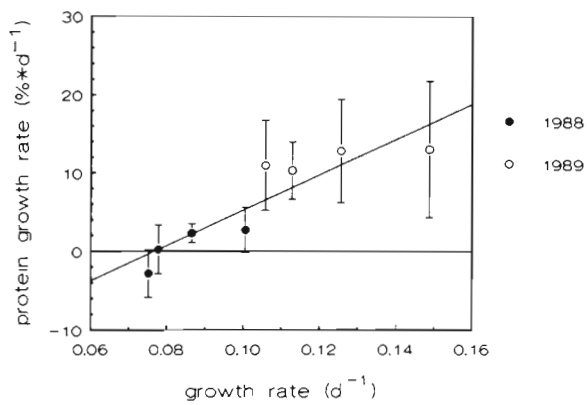

Fig. 9. *Pleuronectes platessa*. Relation between protein growth rate (PGR) and growth rate (G) from size-at-age data. PGR = −16.3 + 217.5 G, r = 0.89, N = 8

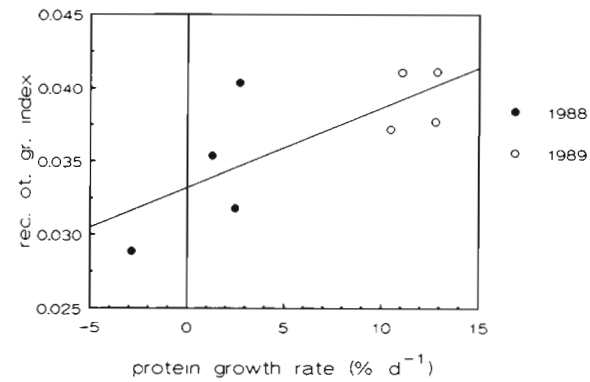

Fig. 10. *Pleuronectes platessa*. Relation between recent otolith growth index (ROGI) and protein growth rate (PGR). ROGI = −0.032 + 0.0109 PGR, r = 0.62, N = 8

Table 6. *Pleuronectes platessa*. Correlation coefficients between growth rate, protein growth rate (PGR), RNA/DNA ratios, recent otolith growth index (ROGI) and temperature. N = 9 or 10

	Growth rate	RNA/DNA	PGR	ROGI
Growth rate	–	–	–	–
RNA/DNA		–	–	–
PGR	0.890••	–	–	–
ROGI	0.654	0.613	0.650	–
Temperature	0.954•••	0.861••	0.882••	0.684•

• $p < 0.05$, •• $p < 0.01$, ••• $p < 0.001$

in the field under different circumstances. Until recently, growth rates of larval plaice were only known from laboratory studies. Laboratory growth rates however cannot be extrapolated into the field. Riley (1966) found that laboratory reared plaice of 3 mo were much smaller than wild counterparts grown at approximately the same temperature. Karakiri & Westernhagen (1989) remarked that growth in the laboratory was stunted below 5 °C. Hovenkamp (1990) found that larvae reared in the laboratory at 9 °C were only growing as fast as wild larvae at ca 4.7 °C (Hovenkamp 1990).

Growth rates calculated from size-at-age data appear to be closely related to water temperature. An exponential curve was fitted between growth rates and temperature, because a linear relation would lead to

zero growth at a temperature of ca 4 °C. This is clearly not realistic, as the 1963 year-class was one of the largest of the century, with seawater temperatures (February) as low as 1.5 °C. The effect of temperature on larval growth is very strong, and the formerly assumed Q_{10} of 2.5 (Hovenkamp 1990) was probably too low for this range of temperatures. Different food levels or an effect of temperature on foraging activity may also have had an effect in this study, so a more accurate Q_{10} cannot be estimated from these data.

Some error will undoubtedly have occurred in the determination of the initial age, i.e. the age at which the first (light microscopical) visible increment is observed. The initial age ranged from −1 to 30 d, with an average of ca 15 d in 1987 and 1988, and an average of ca 10 d in 1989. Karakiri & Westernhagen (1989) reported the first increments to be ca 0.4 µm, which was also the smallest increment width observed in the present study. The average width of the first increments was ca 0.8 µm in all years. An average width of 0.6 µm seems therefore not unreasonable. The initial age also complies with the age of stage 1 larvae as found by Ryland (1966) and in our own laboratory (Hovenkamp 1990). The average age observed for stage 1 larvae was ca 15 d. The maximum age was 31 d, which is close to the ca 28 d in which Ryland (1966) reported to have obtained an 'established feeding stock'. Scanning electron microscopical (SEM) techniques would probably have yielded more accurate results (Karakiri & Westernhagen 1989), but otolith preparation for SEM analysis is much more time consuming, and would have very much limited the scope of research.

Apart from difficulties in accurately estimating the age of the larvae, growth rates calculated from size-age relationships are long-term growth rates, and they may not be very informative about short-term events.

Otolith growth

In plaice, direct back-calculation of larval size from otolith size is seriously hindered by the dependence of the relationship between otolith size and body size on growth. Consequently, only otolith growth indices were studied here. Otolith growth is conservative in relation to somatic growth (Gutiérrez & Morales-Nin 1986), so small changes in somatic growth may go undetected. When changes in otolith growth are observed, however, a real change in somatic growth must have taken place.

The relationship between otolith size and body size showed an unexpected result. According to the literature (Templeman & Squires 1956, Marshall & Parker 1982, Mosegaard & Titus 1987, Mosegaard et al. 1988, Secor & Dean 1989, Hovenkamp 1990) the otoliths are thought to be smaller relative to body size in fast-growing larvae. The otoliths of the 1988 larvae were indeed relatively small, but the otoliths in 1989 were larger relative to body size than in 1988, although the 1989 larvae showed the highest growth rates. A closer look at otolith growth showed that otolith growth indices for Increments 1 to 10 were lower in 1989 than in 1988, however, while temperatures in 1989 were higher. A possible explanation is that high temperatures had an adverse effect on growth during the youngest stages. A negative effect of high temperatures on growth during only the youngest stages would result in a steeper size-at-age curve, with a lower intercept (i.e. a lower initial weight) and a higher exponent. The intercept in 1989 was indeed lower then in 1988 (ANCOVA, $p < 0.05$). During an initial period of slow growth the otoliths will grow to a relatively large size. This difference will persist during the later stages, and may explain relatively large otoliths in apparently faster-growing larvae.

The effect of temperature on plaice pro-larvae has been investigated by Ryland & Nichols (1967) and Ryland et al. (1975). They found that plaice pro-larvae showed optimal growth and yolksac utilization at temperatures between 6.5 and 8 °C, and that the length of larvae hatched from eggs incubated at high temperatures (10.45 °C) was reduced. The optimum temperature for growth increases as the larvae grow older. Juvenile plaice will grow well between ca 15 and 28 °C (Fonds 1979), a temperature range certainly lethal to eggs and larvae. Buckley (1982) suggested "... that growth and survival of winter flounder (*Pseudopleuronectes americanus*) was optimized by a gradual increase in water temperature during the embryonic and larval periods, thereby balancing the higher yolk conversion efficiency at lower temperatures with the increased growth potential of older larvae at the higher temperatures ..."

The highest temperature encountered during sampling was ca 8 °C, in January 1989. Long-term temperature series (Table 7) (Hoeven 1984) show that temperatures during the months December to March are ca 1.5 °C higher in the southern part of the Southern Bight than in the northern part. During the development of the eggs and early larvae the temperature therefore

Table 7. Mean seawater temperatures (1891 to 1910, 1921 to 1940, 1951 to 1970) in the southern and northern part of the Southern Bight of the North Sea (°C)

Location		Month			
Latitude	Longitude	Dec	Jan	Feb	Mar
51°39′	2°34′	9.2	7.3	6.2	6.2
53°01′	4°22′	7.8	5.6	4.6	4.9

was probably higher, and the eggs were probably incubated and hatched at temperatures above their optimum. Such a temperature effect on growth implies that when growth is to be predicted from temperature data (see Campana & Hurley 1989) a 3-dimensional growth model would be needed, with temperature and age as independent variables.

However, during this study no estimates were obtained on the amount of food available for the larvae, so the possibility cannot be excluded that the observed differences were due to nutritional circumstances, instead of temperature, and further research will be needed.

Growth and metamorphosis

There was no clear relation between the size at metamorphosis (here taken as stage 4b and 4b') and temperature. The fast-growing 1989 larvae were of approximately the same size at metamorphosis as the slow-growing 1987 larvae. The 1988 larvae, with an intermediate growth rate, were largest. The age at metamorphosis declined with temperature, indicating that development is more related to temperature than to size.

The rate of development can be defined as the reciprocal of age at metamorphosis (Chambers & Leggett 1987), or at any other clearly defined developmental stage. When development is related to temperature, a relationship may exist between developmental stage and the cumulative temperature (day-degrees). Day-degrees for each stage were calculated using the mean temperature during the period of sampling. Fig. 11 shows that the number of day-degrees for each stage was similar in all years, except for stage 2, which may have been due to the small sample size, and to the fact that age estimates are least reliable for the youngest stages because of the error made in estimating the initial age. Metamorphosis is an important event in the

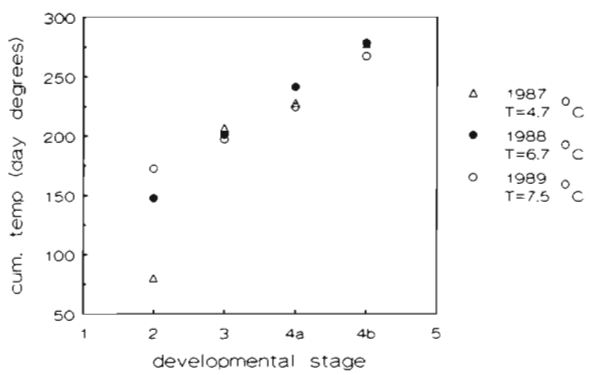

Fig. 11. Relation between cumulative temperature (day-degrees) and developmental stage. Temperature values were averaged over all cruises for each year

early life of fishes, and associated with considerable ecological changes. Size and age at metamorphosis have been reviewed by Chambers & Leggett (1987). They remarked that '. . . any generalizations are conditional until more data are collected on these characters, but length at metamorphosis is consistently less variable than age at metamorphosis . . .'. Riley (1966) conducted experiments with plaice larvae at different feeding regimes. He found that larvae reared at the lowest food levels were very small, but that they metamorphosed only a short time later than well-fed larvae, and concluded that metamorphosis was controlled by temperature and age, rather than size. A similar conclusion was reached, although tentatively, by Seikai et al. (1986) working with Japanese flounder *Paralichthys olivaceus*.

A relationship between age and developmental stage was used by Harding et al. (1978) to estimate the age of the larvae and the corresponding daily mortality rates. His estimates of ages of larvae stage 4 ranged from ca 68 to 93 d. According to the present results, the ages of larvae stage 4 more likely range from 37 to 59 d. Formerly the larval ages may have been overestimated considerably, and daily larval mortality rates were probably underestimated accordingly.

RNA/DNA ratios

The method of RNA/DNA analysis used is capable of measuring quantities of nucleic acids as small as 0.2 µg, and can be used for determining RNA/DNA ratios of individual larvae. However, the accuracy of the method should not be overestimated. The amount of RNA is calculated from the amount of DNA and the total amount of nucleic acids, so any error in the numerator of the RNA/DNA ratio will show up in the denominator as well, but in the reverse way, thus increasing the total error. Some of the experimental error stems from inaccuracy of the fluorescence assay. The magnitude of this error was estimated from multiple readings of standard DNA or RNA solutions, but proved to be of minor significance.

There was a marked difference between the protein growth rates in 1988 and 1989, but the differences within both years were small, compared to differences in growth rates. Recent otolith growth rates should theoretically be better related to protein growth rates than to overall growth rate, because they both give an indication of short-term growth. The relationship between recent otolith growth rates and protein growth rate was weak, which may partly have been due to the fact that they were estimated from different subsamples within the same period. For practical purposes the otoliths are easier to analyze, and may be more useful for assessing differences in growth under normal cir-

cumstances. Under conditions of starvation the RNA/DNA method may be more useful. In case of different larval size-otolith size relationships, as presented here, RNA/DNA ratios might be used to relate otolith growth to somatic growth, but then a more accurate relationship between these 2 factors would be needed.

Implications for recruitment processes

Increased recruitment of plaice has been related to temperature, with good year-classes resulting from cold winters (Harding et al. 1978, Zijlstra & Witte 1985). The causal mechanisms are not clear, although decreased predation during the early juvenile stage has been suggested as one of the factors (Bergman et al. 1988). Egg mortality has been shown to be positively related to temperature (Zijlstra & Witte 1985), which may be due to increased predation at high temperatures. It is not known whether a relationship exists between larval mortality and temperature. Estimating predation on fish larvae in the field is difficult, but from laboratory results it has been shown that size and condition are important to successfully escaping predators (Bailey & Batty 1983, 1984, Bailey 1984, Rice et al. 1987). Swimming speeds of plaice larvae are positively correlated to the size of the larvae (Ryland 1963). Adverse effects of high temperatures during egg development and consequently the early larval stages, when the larvae are most vulnerable to predation, might be a factor causing warm years to yield suboptimal year-classes.

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