Note on otolith growth in elvers, *Anguilla anguilla* (L.), and the relative otolith size during somatic growth

Kerstin Holmgren, Håkan Wickström

Abstract. Newly pigmented glass-eels were kept in a cold water quarantine, before being exposed to increased water temperature and weaned with cod roe and commercial dry feed. The otoliths started to grow before fish growth was observed. As a consequence, the relative otolith size increased. This increase was higher at higher temperature. During the first period with significant eel growth, no further change in the otolith/fish size ratio was observed. Initially, the otolith size was not correlated to fish size, but a positive correlation occurred in growing elvers. The results are discussed with reference to variation in relative otolith size of larger eels. As otoliths have frequently been used in back-calculation of previous eel size, further studies on factors determining otolith growth are suggested.

Keywords: eel elvers, otolith growth, relative otolith size

Introduction

The otoliths (sagittae) are the calcified structures most commonly used for age determination of eels (Liew 1974, Vollestad et al. 1988). Additionally, a linear relationship between otolith length (or the length of a particular radius) and total fish length has been taken as a criterion justifying back-calculation of previous eel length (e.g. Penaz

and Tesch 1970, Rossi and Villani 1980, Moriarty 1983, Vollestad and Jonsson 1986, Paulovitz and Biro 1986, Fernandez-Delgado et al. 1989, Nagićě and Bahnsawy 1990).

The research on otolith growth patterns of larvae and juvenile fish was intensified by the discovery of daily growth increments (Pannella 1971). Campana and Nielson (1985) critically reviewed how well otolith growth reflects fish age and growth at the daily level of precison. An uncoupling of the otolith-fish size relationship may occur under suboptimal conditions. At low temperature the otolith growth appears to cease, although body growth continues at a reduced rate (Marshall and Parker 1982). On the other hand, the otoliths continue to grow through periods of starvation and cessation of somatic growth (Brothers 1981, Campana 1983). Mosegaard et al. (1988) suggested that some metabolic activity, and thus an increasing function of fish size and temperature, is responsible for the otolith growth rate. Modified models have later been proposed (Secor and Dean 1989, Wright et al. 1990).

A daily formation of otolith increments is validated in Japanese elvers, *Anguilla japonica* T. and S. (Tsukamoto 1989), but the increment deposition is disrupted by low temperature as well as food deprivation (Umezawa and Tsukamoto 1991).

This study is based on European elvers, *Anguilla anguilla* (L.), reared at different temperatures in a long-term experiment on the sex differentiation of

K. Holmgren [✉], H. Wickström
Institute of Freshwater Research, S-178 93 Drottningholm, Sweden

eels. The eels were not size graded during their first eight months of culture due to a long period with high mortality and low growth rates. Otolith samples were collected on several occasions in order to get indications whether the relative size of otoliths could reflect metabolic state and/or recent growth history. The results are discussed with reference to observed residuals in otolith length versus fish length regressions from earlier studies on larger eels.

Material and Methods

Experimental fish and rearing conditions

Glass eels, caught in the English River Severn (Bristol Channel Fisheries Ltd.), were imported by a Swedish eel farm (Scandinavian Silver Eel AB, Helsingborg) in April 1991. About 38 800 specimens were kept in a cold water quarantine (3-5°C) for eight weeks, before being transported to the Institute of Freshwater Research, Drottningholm. During transport the water temperature accidently increased to 10-13°C.

At arrival all surviving eels were moved to one of the tanks in a recirculating freshwater system (Holmgren et al. 1992). During the first five days the water temperature in the tank was gradually raised from 9.3 to 17.0°C. Initially, the elvers were given frosen and subsequently thawn cod roe. Later on, the feed was served as a paste of cod roe and an increasing proportion of commercial dry feed (as powder and in granulated form). Manual feeding was performed 4-11 times per 24 hours. Finally, dry feed was given as hourly rations from a feeder.

After five weeks, the eels were randomly grouped and distributed into six tanks (about 1300 eels/tank). In two tanks the temperature was retained at 17°C. In another two tanks it was gradually raised to 20°C, and the final temperature in the last two tanks was 26°C. At daily basis, the feed rations were given in excess at all temperatures

according to Seymour (1989). This particular study is based on samples of small eels, taken during the first eight months of culture, i. e. before the first grading.

General sampling of surviving and dying eels

The abundance and size distributions of surviving and dying eels were monitored by the sampling scheme listed in Table 1. Alive eels were anaesthesized using 0.12 g benzocaine/L of culturing water. Total length was measured to the nearest mm. Excess water was shaken off, and total weight recorded with the accuracy of 0.01 g. The pigmentation stage was defined according to Strubberg (1913) and Elie et al. (1982).

Special sampling of otoliths

On six occasions (Table 1), the sagittal otoliths were collected in random samples of eels, with individual data on total length, total weight and pigmentation stage. The right sagitta was mounted in clear nail polish on an objective slide. By use of an ocular micrometer, otolith length and width was measured with the accuracy of 0.01 mm. Otolith length was defined as the maximum length parallel to the sulcus, and otolith width as the maximum length perpendicular to the defined otolith length. Relative otolith length was computed as the otolith length in percent of total fish length.

Statistical analyses

The program package „SPSS/PC+", V4.0 (Norusis 1990) was used in the computations. Parametric t-tests, analyses of variance (ONEWAY) and correlation tests were used, as Kolmogorov-Smirnov Goodness of Fit tests and plotted histograms indicated no or only minor deviations from assumed normal distributions and equal variances.

Table 1
Numbers of living and observed dying eels in chronological order, and numbers of eels in samples for total length (TL), total weight (TW), pigmentation stage (PIGM) and otolith size (OTOL)

Occasion or period, 1991	Number of weeks in Sweden	Water temp. (°C)	Total number (% of initial)	Samples of living eels			Observed dying eels				Notes
				TL, TW	PIGM	OTOL	Counted	TL	PIGM	OTOL	
Helsingborg, 4 April	0	-	38,795 (100)	164	80	30					
Quarantine	0-8	3-5					18,358	0	0		1)
Drottningholm, 28 May	8	10-13	20,437 (52,7)	293	138	17					
Weaning in one tank	8-9	9-17					11,627	3,690	0		2)
Sampling, 9 June	9	17		63	63	30	129	72	30		
Continued weaning	9-14	17					743	all	47		2) 3)
Distribution to six tanks, 10-11 July	14	17	7,875 (20,3)	284	30	30					
		17					264		5		
Experimental culture	14-19	20					177	all	2		3)
		26					530		13		
		17		349	20	20					
Sampling, 13-14 August	19	20		327	20	20					
		26		318	20	20					
		17					348				
Continued culture	19-29	20					263	all	0		
		26					508				
		17	1,876	390							
Sampling, 21-22 October	29	20	1,644	423	0	0					
		26	1,247	382							
Special sampling, 30 October	30	20		30	30	30					
		26		28	28	28					

Notes: 1) = at least daily number registered

2) = at least daily number and mean weight registered

3) = otolith samples as yet not measured

Results

Total length and pigmentation stage of surviving elvers

The total length of the imported elvers varied between 62-80 mm (mean 71.0 mm). There was no difference in total length between samples taken on 4 April, 28 May, 9 June and 10-11 July (Fig. 1), i. e. the elvers did not grow during eight weeks in the cold water quarantine or during six weeks of weaning at 17°C. Mean total weight decreased from 0.33 to 0.23 g (t-test, P=0.001). As only fresh weights were taken, these data were considered to be of minor value in the further discussions. In August some growing individuals (up to 100 mm) were observed at 20 and 26°C, but this did hardly affect the mean value. Ten weeks later, the majority of surviving elvers had total length above the initial mean value and a considerable number were larger than the initial maximum.

When the elvers arrived from England in April, they were already more or less pigmented (Table 2), with a mode in stage VIA_2. After eight weeks in quarantine, 52.7% of initial numbers arrived to the laboratory in Drottningholm. At this point, 95% of surviving eels had reached the last elver stage, VIA_4. During the following weeks, some eels became fully pigmented, stage VIB, but

Table 2
Pigmentation stage in samples of living elvers, from arrival to Sweden (Helsingborg) 4 April 1991 and during the following months until October (see specifications and sample sizes in Table 1). Listed figures are the relative freqeuncies (%) per class

Number of weeks in Sweden		VB	VIA_0	VIA_1	VIA_2	VIA_3	VIA_4	VIB
0		8	11	19	36	22	4	0
8		0	0	1	1	4	95	0
9		0	0	0	0	11	84	5
14		0	0	0	0	10	83	7
19	17°C	0	0	0	0	15	45	40
	20°C	0	0	0	0	10	60	30
	26°C	0	0	0	0	5	45	50
30	20°C	0	0	0	0	7	43	50
	26°C	0	0	0	0	11	57	32

eels in earlier stages, VIA_3 and VIA_4, still occurred in October.

Otolith length of surviving elvers

The smaller subsamples taken for otolith measurements (Table 1) were considered representative, as estimates of mean total length and standard deviation were comparable to that of the larger samples
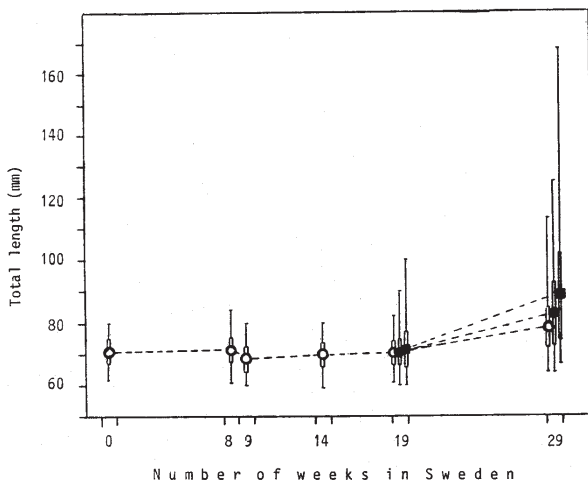

Figure 1. Total length of elvers during the first 29 weeks in Sweden. Central symbols are mean values, surrounded by standard deviation and total range. Open circles represent initial samples and the sample from 17°C in week number 19. Field circles and boxes are used for samples from 20 and 26°C, respectively. Sample size are given in Table 1.

(Fig. 2a and Fig. 1, t-tests, P-values 0.05). There was no difference in otolith length between samples taken 0, 8 or 9 weeks after arrival to Sweden (Fig. 2b, ONEWAY, P=0.251). The pooled mean value was 0.349 mm, with a coefficient of variation of 6.5%. In these initial samples there was no difference in otolith length between stages of pigmentation (ONEWAY, P=0.314). In July, after another five weeks at 17°C, the otolith length had increased compared to the pooled material of earlier samples (t-test, p.001), and in August virtually all otoliths were larger than the initial maximum. Additionally, the otoliths were significantly larger at 26°C than at the lower temperatures (ONEWAY, P=0.0003).

Otolith length in relation to Total fish length

The small subsamples of living eels taken on 4 April, 28 May and 9 June, and the subsample of dying eels on 9 June, were all homogeneous with respect to total length and otolith length. In these initial samples, there was no correlation between otolith length and total length (Fig. 3). The relative otolith length varied between 0.42-0.60%, with a mean value of 0.50%. From June to August, when the otoliths increased in length in spite of no change in
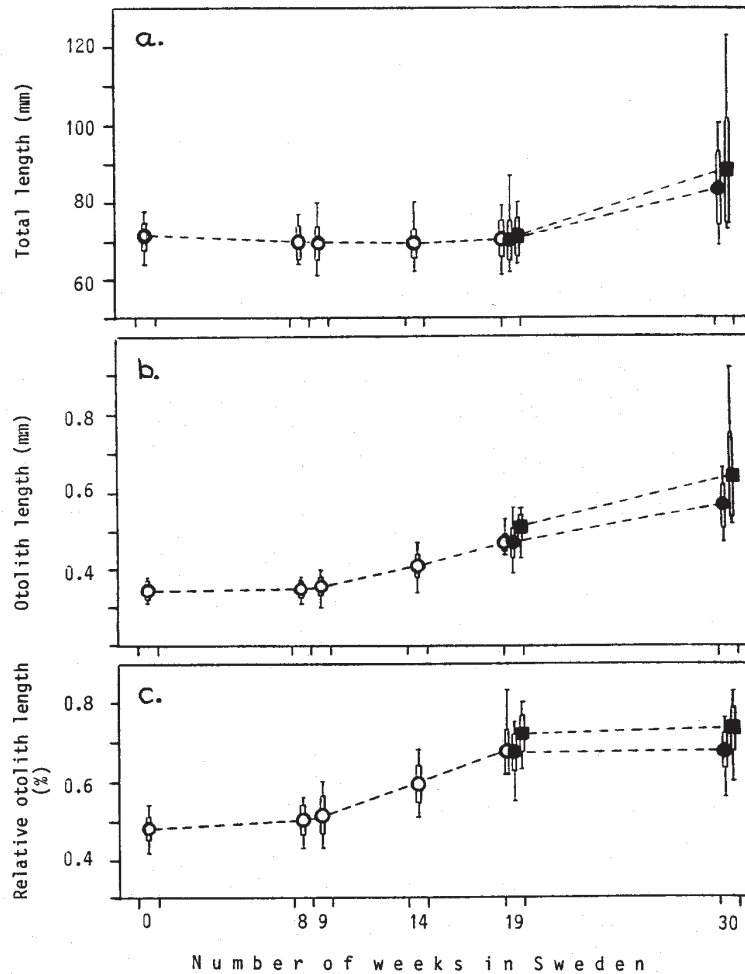
Figure 2. Total length, otolith length and relative otolith length of elvers in the smaller subsamples for the otolith study (see Table 1). Symbols for mean values, standard deviations and total ranges are used as in Figure 1.

total length, there was a linear increase in relative otolith length (Fig. 2c). During the following weeks until October, there was no further change in relative otolith length at 20 or at 26°C (t-tests, P-values 0.756 and 0.430, respectively), indicating an isometric relation between the length increases of otoliths and somatic tissue. This was also expressed as positive correlations between otolith length and total length, at both temperature regimes (Fig. 4).

Discussion

With a set of data indicating no increase in total length of glass eels/elvers during at least four

months, one might be lost in efforts to explain the growth failure. Such a discussion could include aspects on keeping already pigmented elvers in cold water, on stress related to transport and on infections with fungi and bacteria during the weaning period. That is definitely not the object of this study.

In this experiment, not more than 12.4% of initial numbers survived from April to October. Neither total length nor otolith length was repeatedly measured on individual elvers. Nevertheless, the insignificant change in total length distributions until August, and the fact that most otoliths in August were larger than the largest otolith before and after eight weeks in the cold water quarantine, together indicate uncoupling between otolith and somatic growth. Umezawa and
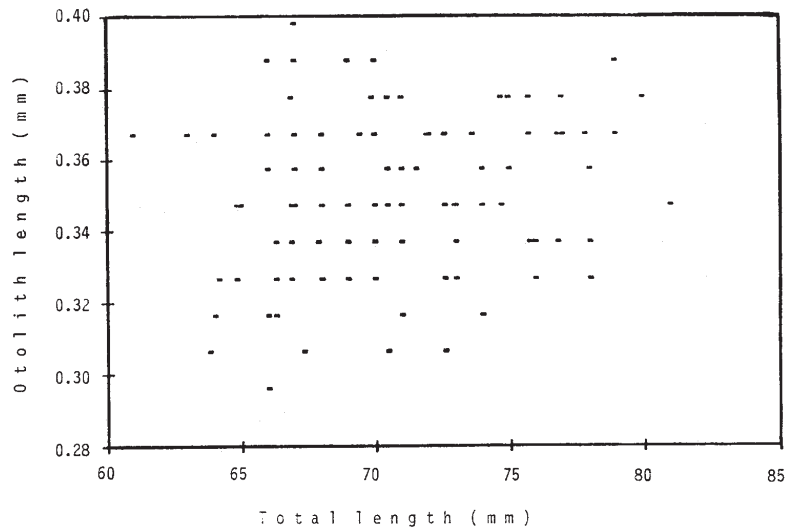
Figure 3. Otolith length in relation to total length in initial samples of elvers. Data are pooled of subsamples taken after 0, 8 and 9 weeks in Sweden (N=107, r=0.186, not significant correlation).
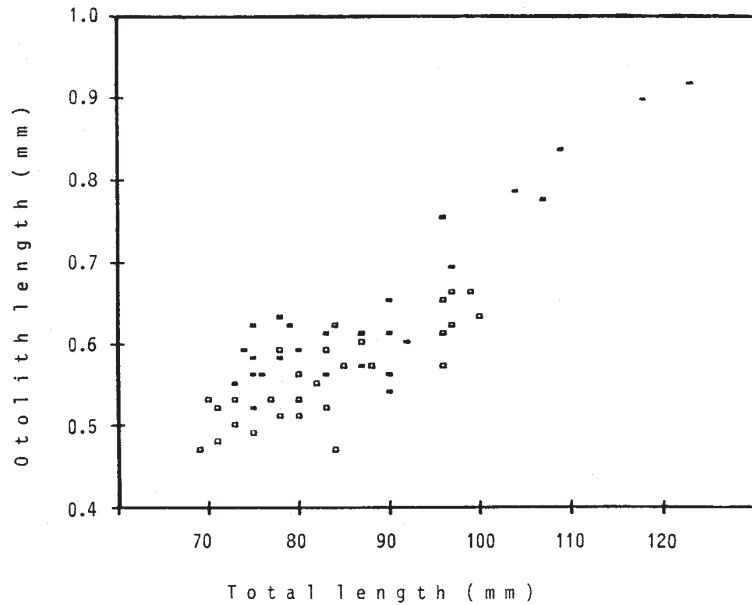

Figure 4. Otolith length in relation to total length of elvers after 30 weeks in Sweden. Eels from 20°C are assigned with open boxes (N=30, r=0.817), and filled boxes represent eels from 26°C (N=28, r=0.900).

Tsukamoto (1991) studied otolith increment deposition of Japanese elvers during 50 days. In unfed groups and/or at low temperature (12°C) total length remained unchanged and otolith growth was sharply reduced compared to the other groups. In the present study, feed was provided in excess throughout the period of weaning and culturing in heated water. Surviving elvers were probably feeding, although initially at insufficient rates for somatic growth. The growth of their otoliths might be seen as an index of metabolic activity (Mosegaard et al. 1988, Wright 1991). The observation of largest relative otolith length at the highest temperature (26°C) in August is an accordance with the highest expected metabolic rate.

In the Loire estuary, mean total length decreased and otolith radius increased from the glass eel stages (VA/VB) to the late elver stages (VIA$_3$/VIA$_4$)

(Lecomte-Finiger 1992). Within each pigmentation stage the coefficient of variation (CV) was about 5% in total length and about 10% in maximum otolith radius. In the initial samples from the present study, the mean otolith length was 0.349 mm with a CV of 6.5%, which was not explained by variation in total length or pigmentation stage. The hypothesis that otolith growth reflects metabolic scope has been adopted in studies of salmonids, where early fry size was poorly predicted by otolith size. Selection for fish larger otoliths at emergence was observed in brown trouts which later established territories (Titus and Mosegaard 1991).

Furthermore, otolith size and dominance status at first feeding were good predictors of subsequent growth of Atlantic salmon (Metcalfe et al. 1992). The otolith size of glass eels can not as simply as in salmonid alevins be explained by standard metabolic rate. The glass eels have precedingly been feeding and growing as leptocephali.

When the cultured elvers began to increase in total length, there was at least initially no further increase in relative otolith length. In October this isometric relationship between otolith and somatic growth was expressed as positive correlations between otolith and total length at both 26°C (r=0.900) and 20°C (r=0.817). Such levels of correlations have earlier been taken as acceptable criteria for back-calculations of previous eel size (Rossi and Villani 1980, Vollestad and Jonsson 1986, Nagíčě and Bahnsawy 1990). In other fish species, it has been shown that slow growing fish have larger otoliths than fast growing individuals of the same size (Secor and Dean 1989, Reznick et al. 1989). Growth back-calculations usually assume that the relationship between fish and otolith length is linear through time, at least within individuals. Campana (1990) reduced back-calculation bias by defining individual fish trajectories and a biological determined intercept.

The present study on elvers is a part of a long term experiment. As preliminary results it can be mentioned that the eels which first attained total weights above 150 g had relative otolith lengths within the same range as glass eels (Holmgren, unpublished results). This might indicate that the otolith growth in relation to fish growth can switch repeatedly within an individuals life time. Further research on the subject is suggested. For the time being, it is recommended to consider uncoupling between otolith and somatic growth as a possible bias when interpreting data from back-calculations.

References

Brothers E.B. 1981 – What can otolith microstructures tell us about daily and subdaily events in the early life history of fish? – Rapp. P.-v. Reun. Cons. int. Explor. Mer. 178: 393-394.

Campana S.S. 1983 – Feeding periodicity and the production of daily growth increments in the otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*) – Can. J. Zool. 61: 1591-1597.

Campana S.E. 1990 – How reliable are growth back-calculations based on otoliths? – Can. J. Fish. Aquat. Sci. 47: 2219-2227.

Campana S.E., Neilson J.D. 1985 – Microstructure of fish otoliths – Can. J. Fish. Aquat. Sci. 42: 1014-1032.

Elie P., Lecomte-Finiger R., Cantrelle I., Charlon N. 1982 – Definition des limites des differents stades pigmentaires durant la phase civelle d'*Anguilla anguilla* L. (Poisson teleosteen anguilliforme) – Vie Milieu, 32: 149-157.

Fernandez-Delgado C., Hernando J.A., Herrera M., Bellido M. 1989 – Age and growth of yellow eels, *Anguilla anguilla*, in the estuary of the Guadalquivir river (South-west Spain) – J. Fish. Biol. 34: 561-570.

Holmgren K., Wickström H., Fredga K. 1992 – Growth of eel in a mesoscale experiment – Irish Fisheries Investigations Series A, No. 36: 90-103.

Lecomte-Finiger R. 1992 – Growth history and age at recruitment of European glass eels (*Anguilla anguilla*) as revealed by otolith microstructure – Mar. Biol. 114: 205-210.

Liew P.K.L. 1974 – Age determination of American eel based on the structure of their otoliths – In: The ageing of fish - (T.B. Bagenal, ed.) pp. 124-136, Old Working, Surrey, England.

Marshall S.L., Parker S.S. 1982 – Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths – Can. J. Fish. Aquat. Sci. 39: 542-547.

Metcalfe N.B., Wright P.J., Thorpe J.E. 1992 – Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*) – J. Anim. Ecol. 61: 585-589.

Moriarty C. 1983 – Age determination and growth rate of eels, *Anguilla anguilla* (L.) – J. Fish Biol. 23: 257-264.

Mosegaard H., Svedäng H., Taberman K. 1988 – Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response – Can. J. Fish. Aquat. Sci. 45: 1514-1524.

Nagięć M., Bahnsawy M.H. 1990 – Age and growth of female eels, *Anguilla anguilla* L. in a Polish lake, Jeziorak Lake, Mazurian Lake District, Poland – Aquacult. Fish. Mgment. 21: 459-470.

Norusis M.J. 1990 – SPSS/PC+ V.4.0. Base manual for the IBM PC/XT/AT and PS/2. SPSS inc., Chicago, USA.

Pannella G. 1971 – Fish otoliths: daily growth layers and periodical patterns – Science 173: 1124-1127.

Paulowitz G., Biro P. 1986 – Age determination and growth of eel, *Anguilla anguilla* (L.), in Lake Fertö, Hungary – Fish. Res. 4: 101-110.

Penaz M., Tesch W. 1970 – Geschlechtsverhältnis und Wachstum beim Aal (*Anguilla anguilla*) an verschiedenen Lokalitäten von Nordsee und Elbe – Ber. dtsch. Komm. Meereforsch. 21: 290-310.

Reznick D., Lindbeck E., Bryga H. 1989 – Slower growth results in larger otoliths: an experimental test guppies (*Poecilia reticulata*) – Can. J. Fish. Aquat. Sci. 46: 408-112.

Rossi R., Villani P. 1980 – A biological analysis of eel catches, *Anguilla anguilla* L., from the lagoons of Lesina and Varano, Italy – J. Fish. Biol. 16: 413-423.

Secor D.H., Dean J.M. 1989 – Somatic growth effects on the otolith-fish size relationship in young pond-reared striped bass, *Morone saxatilis* – Can. J. Fish. Aquat. Sci. 46: 113-121.

Seymour E.A. 1989 – Devising optimum feeding regimes and temperatures for the warm water culture of eel, *Anguilla anguilla* L. – Aquacult. Fish. Mgment. 20: 129-142.

Strubberg A. 1913 – The metamorphosis of elvers as influenced by outdoor conditions, some experiments – Medd. fra Komm. for Havundersogelser. Serie: Fiskeri. Bind IV (3), 11 p.

Titus R.G., Mosegaard H. 1991 – Selection for growth potential among migratory brown trout (*Salmo trutta*) fry competing for territories: evidence from otoliths – Can. J. Fish. Aquat. Sci. 48: 19-27.

Tsukamoto K. 1989 – Otolith daily increments in the Japanese eel – Nippon Suisan Gakkaishi 55: 1017-1021.

Umezawa A., Tsukamoto K. 1991 – Factors influencing otolith increment formation in Japanese eel, *Anguilla japonica* T. and S., elvers – J. Fish. Biol. 39: 211-223.

Vollestad L.A., Jonsson B. 1986 – Life-history characteristics of the European eel *Anguilla anguilla* in the Imsa River, Norway – Trans. Amer. Fish. Soc. 115: 864-871.

Vollestad L.A., Lecomte-Finiger R., Steinmetz B. 1988 – Age determination of *Anguilla anguilla* and related species – EIFAC Occasional Paper 21: 1-28.

Wright P.J. 1991 – The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. – J. Fish. Biol. 38: 929-933.

Wright P.J., Metcalfe N.B., Thorpe J.E. 1990 – Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L.: evidence against coupling – J. Fish. Biol. 36: 241-249.