

## LARVAL OTOLITH MICROSTRUCTURE STUDIES IN PORTUGAL (Pedro Ré)

Growth rings in scales, otoliths, opercular bones and vertebrae have been used to age fish for a long time. The finding of G. Pannella in the early 1970s that many teleost fish deposit otolith growth increments with a 24h periodicity, was a major step forward in assessing age and growth with greater accuracy and precision. Deposition of daily growth increments appears to be a universal phenomenon under all but the most severe conditions. The field of otolith microstructure research is now an accepted, and in most cases, a preferred tool for the study of fish. Applications of information derived from otolith microstructure are numerous and include: (i) age determination; (ii) daily growth rate estimations [population (integrated) and individual growth]; (iii) life history events; (iv) recruitment variability and (v) stock identification among others (Stevenson & Campana, 1992). The quantification of some of these life history parameters is essential for the evaluation of the causes underlying recruitment variability, especially as described by Hjort's (1914) critical period concept, Lasker's (1981) stable ocean hypothesis, Sinclair's (1988) member/vagrant hypothesis and Bakun's (1996) ocean triad hypothesis. Understanding the processes affecting recruitment is a fundamental objective of fisheries biology (Bakun, 1996). It is commonly assumed that annual recruitment is determined during the early life history stages, particularly the larval and juvenile stages. Studies of larval otolith microstructure can provide valuable insights into the daily growth rate variability both on the individual and population levels. Otolith microstructure has been shown to be sensitive to both environmental change and ontogenic transitions during the first developmental stages. In this context it can be used as a kind of "black box" to infer the dynamics of larval growth, particularly the identification of stressful periods, and is considered to reflect important past events during the early life history (Ré, 1986, Ré & Gonçalves, 1993).

For most fishes the formation of daily growth rings starts at the end of the yolk sac stage or at the time of eyes become pigmented. The exact time of formation of the first daily growth increment varies among species. The width of daily increments can be influenced by a number of factors such as food uptake, temperature and other environmental conditions. The distance between increments expresses the daily growth of the individual, while the number indicates its age in days (Secor *et al.* 1995, Fossum *et al.* 2000, Panfili *et al.*, 2002).

In Portugal a number of larval otolith microstructure studies were performed mainly with two small pelagic fishes: sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*). These studies deal with otolith microstructure, growth and detection of life history events in sardine (Ré, 1983a, 1983b, 1984a, 1984b, 1986a, 1986b) and anchovy (Ré, 1986b, 1987a, 1994, 1996, Ribeiro *et al.*, 1996). A few validation and otolith microstructure studies were also performed under controlled laboratory conditions (*Tilapia*, Rosa & Ré, 1985, *Dicentrarchus*, Ré *et al.*, 1985, 1986, *Solea*, Ré *et al.*, 1988, *Engraulis*, *Sparus*, *Diplodus*, Ré, unpublished data). Ré (1987b) emphasised that otolith microstructure can be a very powerful tool in aquaculture studies namely in: (i) the determination of daily growth rates during the rearing period; (ii) the detection of important events during the ontogeny; (iii) the evaluation of the efficiency of diets in relation to growth and survival; (iv) the detection of pathologies during the rearing period.

### MICROSTRUCTURE

Otolith microstructure increments are deposited on a daily basis in sardine larvae (Ré, 1983a, 1983b, 1984a, 1984b, 1986a, 1986b) and anchovy larvae (Ré, 1986b, 1987a, 1994, 1996, Ribeiro *et al.*, 1996).

## *Sardine*

The *sagittae* exhibit a clear nucleus, which corresponds to the yolk-sac stage. The first increment appears after yolk-sac absorption when the mouth and the digestive tract become functional. On a given otolith the width of the daily increments can vary significantly. Near the nucleus, the first 8 to 10 growth units are comparatively narrower and less intense (Figure 1). Microgrowth units become wider from ring 13 onwards.

The first transition in the microstructure of the otolith is related to the onset of exogenous feeding. The wider rings are related to the onset of a diel rhythm of swim bladder inflation and also with the existence of more pronounced rhythms of feeding and vertical migrations (Table 1).

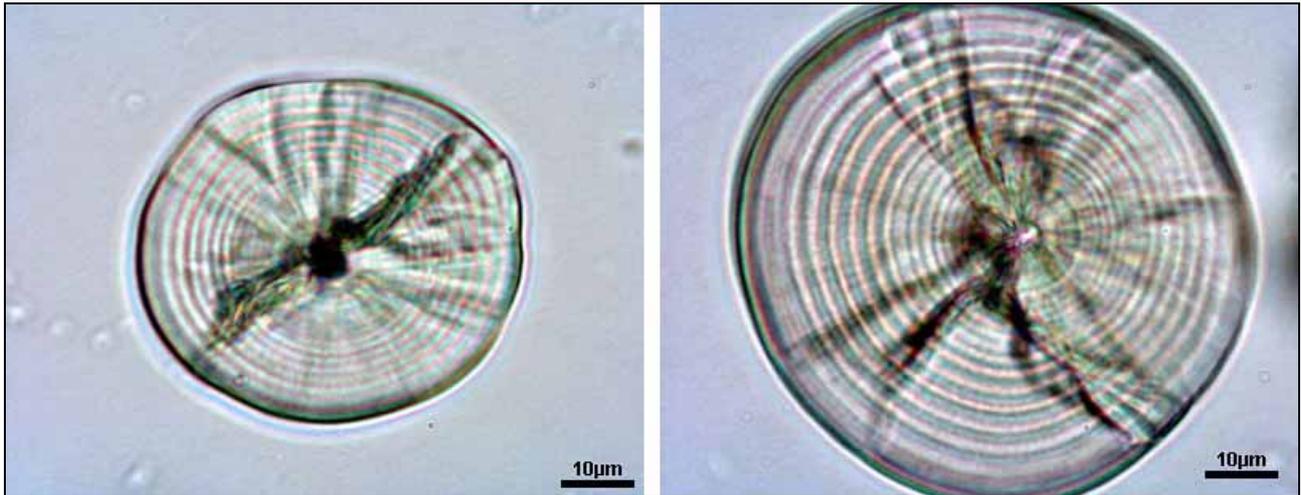


Figure 1 – Sardine larval otolith.

## *Anchovy*

*Sagittae* are similar to those of sardine. A clear nucleus is apparent and daily microgrowth increments vary also in width and intensity being wider and sharper after a certain age (Figure 2). The nucleus corresponds to the yolk-sac period and the first daily growth increment is deposited after the onset of exogenous feeding. The existence of daily rhythms of swim bladder inflation in conjunction with other diel rhythms of activity (mainly feeding and vertical migration) explains the marked transition in the otolith microstructure (Table 2).

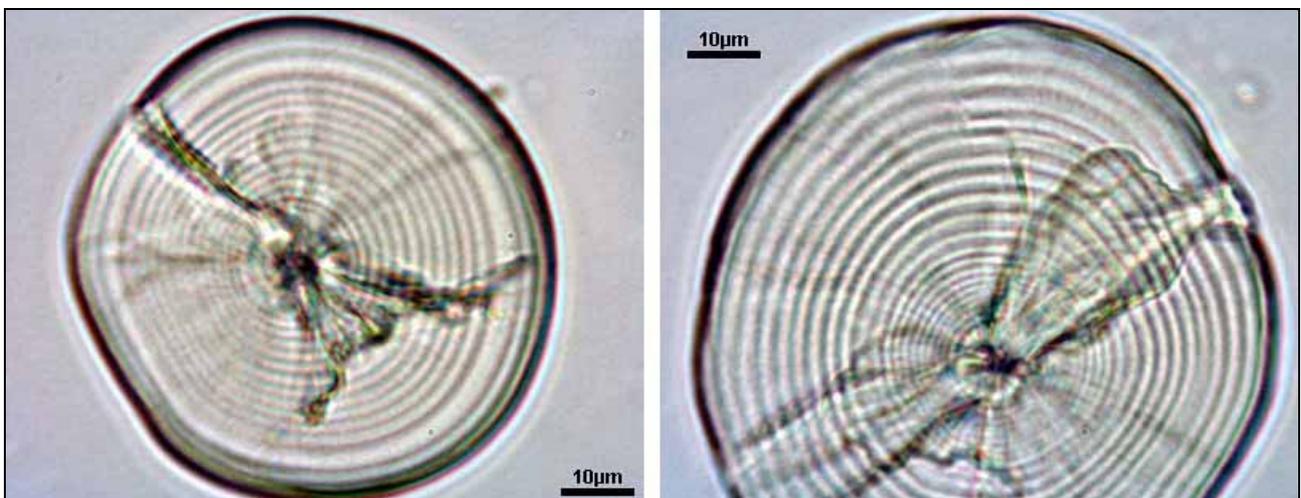


Figure 2 – Anchovy larval otolith.

Table 1- Main ontogenetic events in sardine larvae.

Age (days)	Length (mm)	Main ontogenetic events
1	4	Eclosion, newly hatched larva (3.3 – 4 mm)
4	5	Yolk completely absorbed, larva (4.0 - 5.5 mm)
5	6	Typical larval pigmentation
7	7	
9	8	Beginning of development of the dorsal fin (7.5 mm)
10	9	
12	10	Swim-bladder formation
14	11	Flexion of notochord (11 – 12.5 mm)
16	12	
17	13	Diel rhythms of swim-bladder inflation (12.5 mm). More pronounced rhythms of feeding and vertical migration
19	14	
21	15	
23	16	
24	17	
26	18	
28	19	
30	20	First appearance of pelvic fins (level with pylorus)
31	21	
33	22	
35	23	
36	24	
38	25	
40	26	Dorsal fin with complete number of fin rays
	27	
	28	Anal fin with complete number of fin rays
	(...)	
	40	Beginning of metamorphosis

Table 2- Main ontogenetic events in anchovy larvae.

Age (days)	Length (mm)	Main ontogenetic events
1	3	Eclosion, newly hatched larva (3.0 – 4 mm)
5	4	Yolk completely absorbed, larva (4.0 - 5.0 mm)
9	5	Typical larval pigmentation
11	6	Beginning of development of the dorsal fin
13	7	Swim-bladder formation
15	8	
18	9	Flexion of notochord (9 – 10 mm)
20	10	Diel rhythms of swim-bladder inflation
22	11	Dorsal and anal fin rays developed
24	12	
26	13	
28	14	
30	15	Pelvic fins appears on level with pylorus
32	16	
	(...)	
	35	Beginning of metamorphosis (35 – 40 mm)

## GROWTH PARAMETERS

### Sardine

Integrated growth of sardine larvae can be estimated from the relationship between body size and the number of daily growth increments enumerated from the otoliths. Sardine larval growth vary from 0.41 to 0.57 mm/day (Ré, 1984). Sardine larval growth parameters are presented in Figure 3.

### Anchovy

Growth of anchovy larvae can also be adequately described, up to an age of about 30 days, using linear regression analysis. Integrated daily growth rates vary from 0.25 to 0.41 mm/day (Ré, 1994, 1996). Anchovy larval growth parameters are presented in Figure 4.

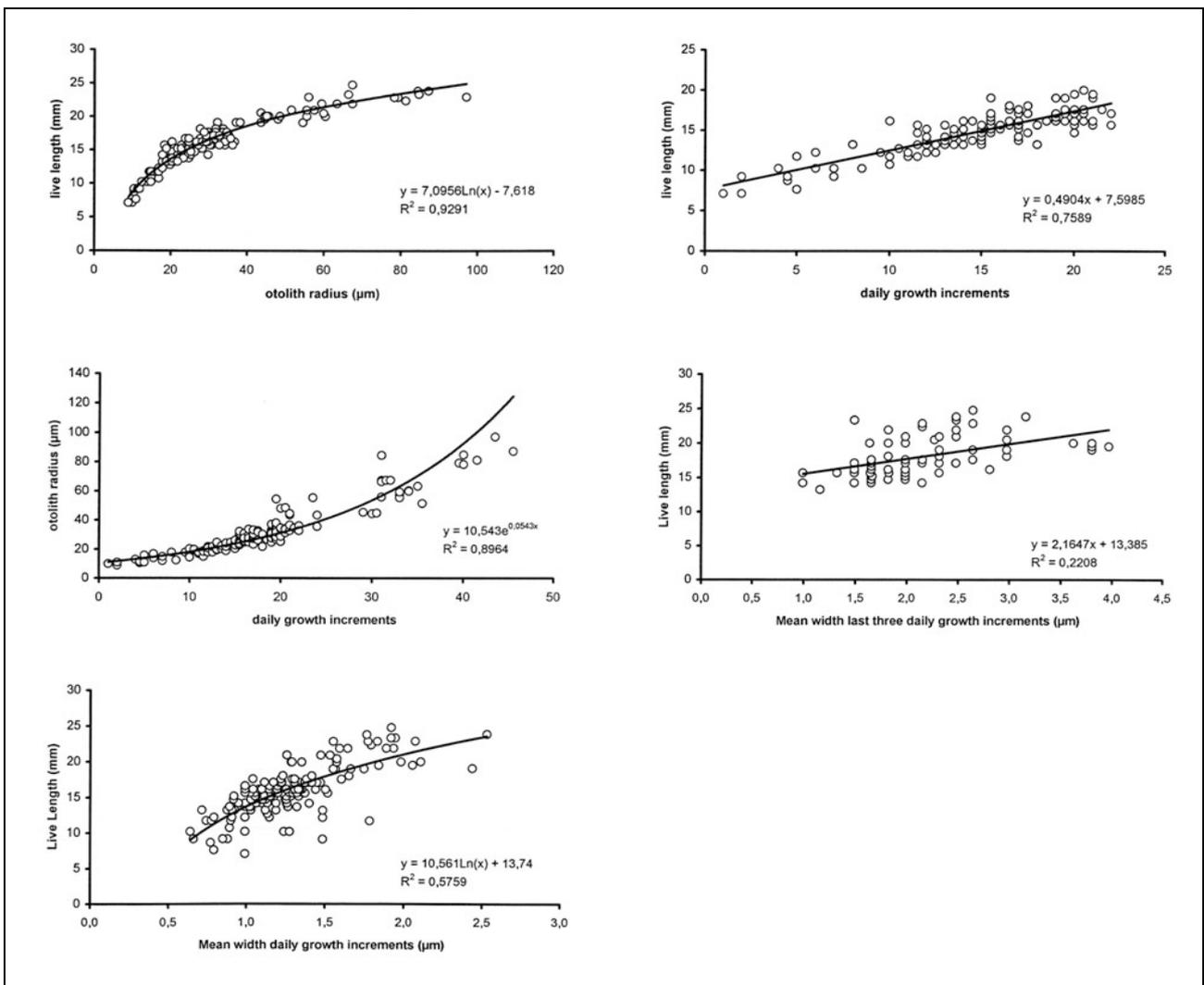


Figure 3- Sardine larval growth parameters

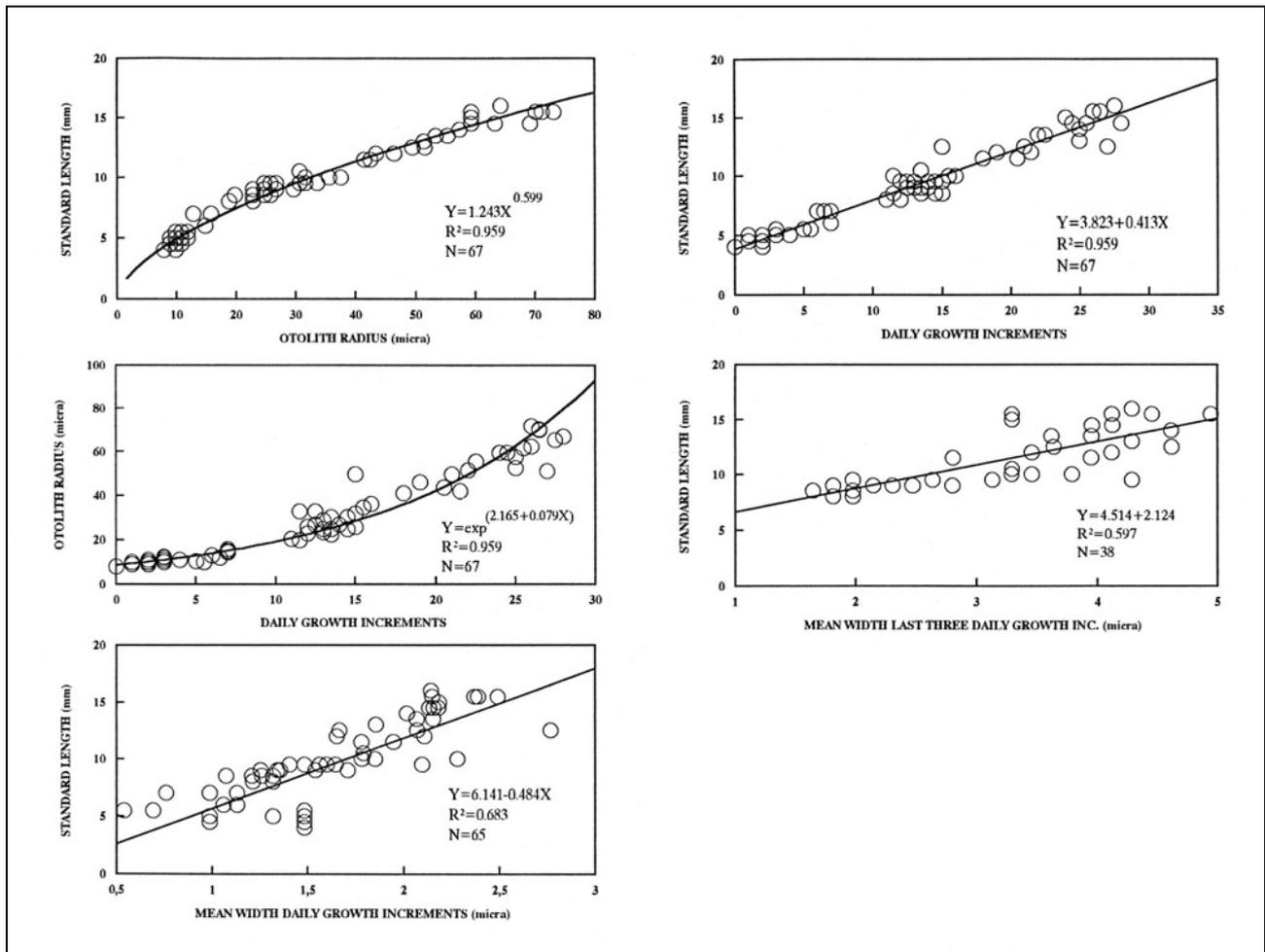


Figure 4- Anchovy larval growth parameters

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