

## B. Semi-direct validation

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One of the most used validation methods, semi-direct validation, consists of observing the evolution of CS marginal zones over time. This is a powerful method which requires regular observations throughout the time period concerned, according to the cycle of growth increments (daily, seasonal or other) under study, and the observation of a large number of individuals. The method consists of observing the edges of the CS, selecting a given mark, and following its formation through time in a single population. The result is an average and/or percentage of observations at population level. The selected mark must be sufficiently precise to permit the detection of its formation at the extreme edge of the CS, which may be difficult. Semi-direct validation is usually used to validate seasonal deposition, and two types of studies are possible, one of which uses qualitative data and the other, quantitative data.

### 1. Qualitative and/or quantitative data?

#### 1.1. Qualitative data

This process consists of evaluating the presence or absence of the mark at the edge of the CS, and expressing the results in percentages for the population under study. The evolution of this percentage through time is then studied: figure IV.B.1 shows the result for one growth cycle per year (fig. IV.B.1a) and two cycles per year (fig. IV.B.1b). The second case is less common in a temperate environment but it has been observed for tropical fishes (Yosef & Casselman, 1995). In North America, a system of edge codes, first presented in Casselman (1987) and elaborated in Casselman (1996), is now widely used.

#### 1.2. Quantitative data

This method consists of measuring the distances separating the latest marks at the edge of the CS. The axis of measurement and the description of the marks being utilised must be rigorously standardised. The absolute marginal distance (AMD) is the distance that separates the last mark from the edge (fig. IV.B.2). The relative marginal distance (RMD) is the ratio of the absolute marginal distance to the distance separating the two last marks ( $D_{i,i-1}$ ) (fig. IV.B.2):

$$(VI.B.1) \quad RMD = \frac{AMD}{D_{i,i-1}}$$

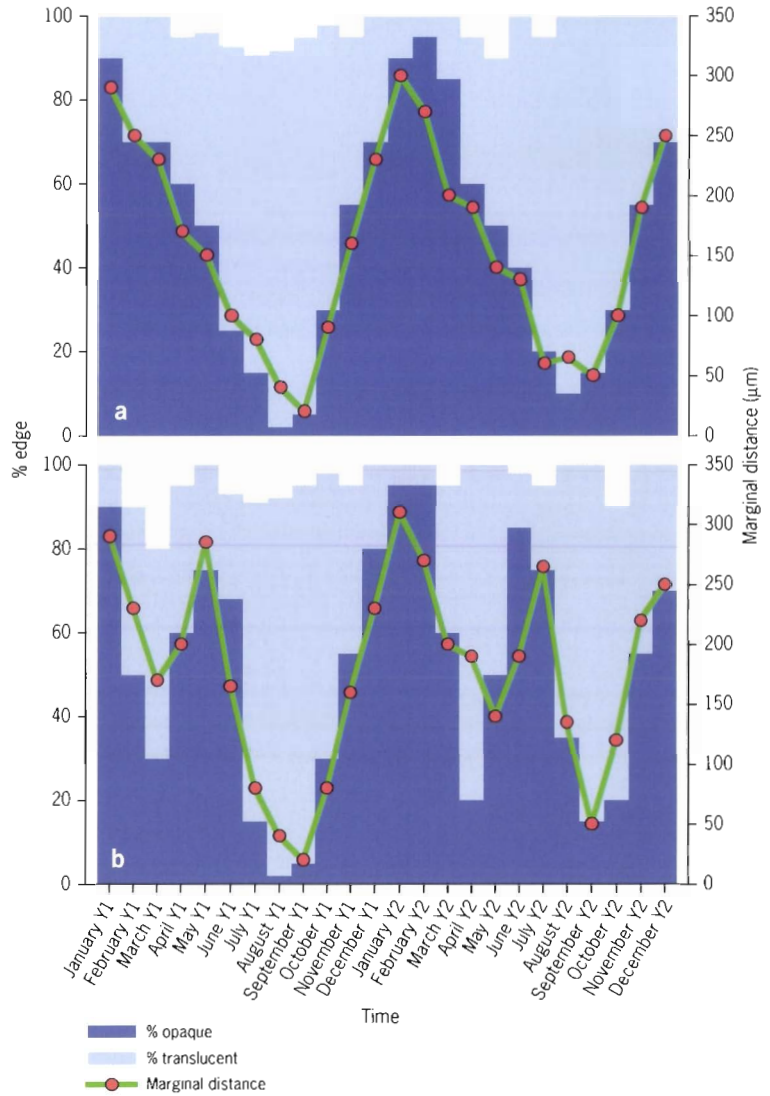


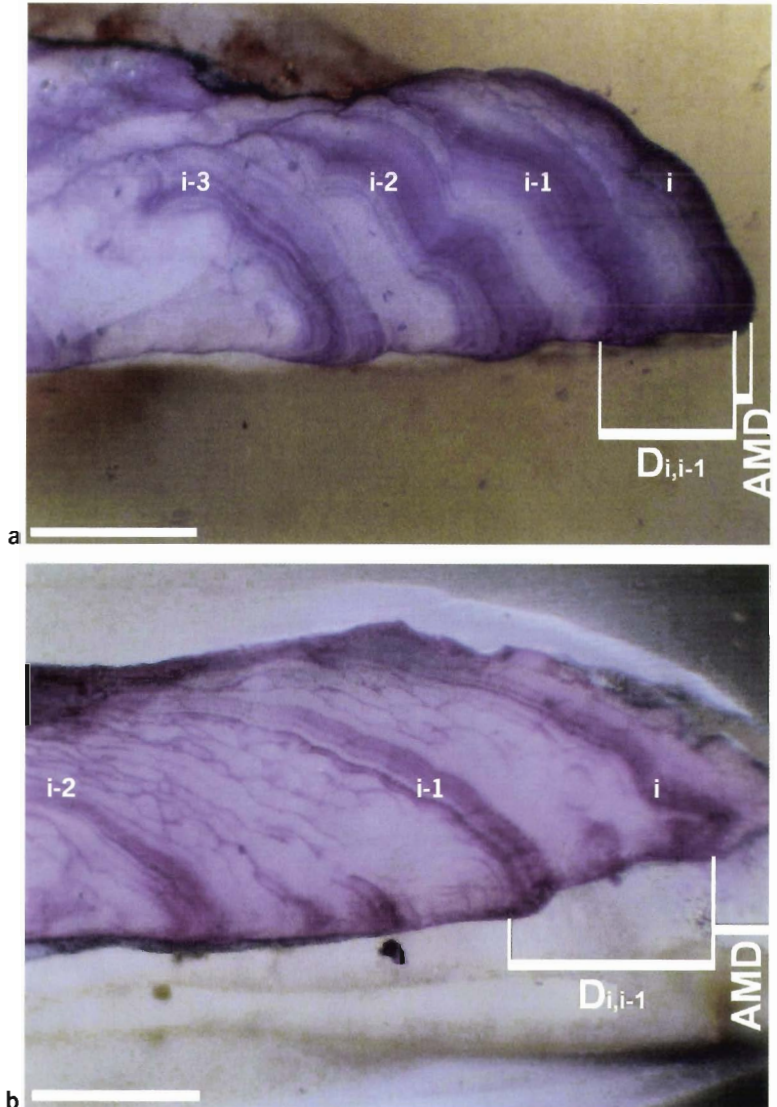
Figure IV.B.1 - Theoretical graph of the evolution over time (two consecutive years) of the percentage of opaque edges, the percentage of translucent edges and the margin width on a CS. These examples are totally artificial and they are not taken from observations in the field. The nature of the edge, opaque or translucent, is determined macroscopically. The margin width corresponds to the distance between the beginning of the last opaque zone and the edge.

a) Theoretical graph of the appearance of one cycle per year. The opaque zone is laid down at the end of each year (maximum percentage) while the translucent zone is completely deposited in August. For some CS, the evaluation of the nature of the edge is not possible and the sum of percentages differs from 100% (e.g. July Y1). The margin width follows the evolution of percentages over time.

b) Theoretical graph of the appearance of two cycles per year. The opaque zones are laid down around May and at the end of each year (maximum percentage) while the translucent zones are deposited in April and August every year. An inter-annual variation is observed with a slight shift of the zone deposition every year: the first opaque zone is totally deposited in March in year 1 and in April in year 2. For some CS, the evaluation of the nature of the edge is not possible and the sum of percentages differs from 100% (e.g. March Y1). The margin width follows the evolution of percentages over time.

The use of AMD is recommended when working separately on different age (or increment) classes. It takes directly into account the differences in growth rates between individuals. The RMD, which is sometimes expressed as a percentage, compensates for the effects of the reduction in growth with age: it is less sensitive to the variations in growth rate since measurements are relative (fig. IV.B.3). It is often more difficult to follow a given cycle with the AMD than the RMD. In general, quantitative data regarding the margin cannot be measured for 0-year-old individuals (without increments), unless there is a

Figure IV.B.2  
Edge aspect of transverse stained sections of otoliths of *Colossoma macropomum* (Serrasalimidae). AMD, absolute marginal distance;  $D_{i,i-1}$ , distance between the last zone  $i$  and the previous one  $i-1$ . The relative marginal distance is then  $RMD = AMD / D_{i,i-1}$ . Scale bar = 500  $\mu\text{m}$  (photos J. Panfili).  
a) Stained zone on the edge of a 455 mm (SL) individual caught in September in Bolivian waters.  
b) Unstained zone on the edge of a 457 mm (SL) individual caught in March in Bolivian waters.

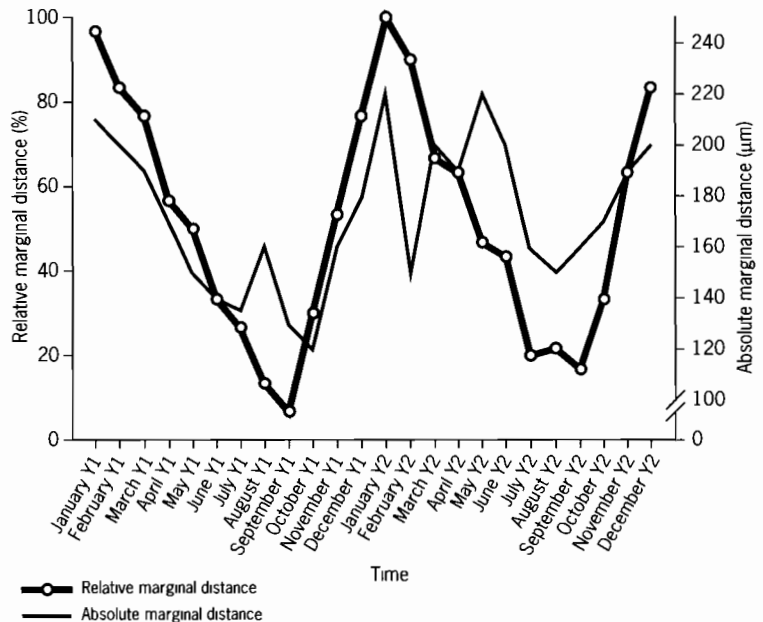


demonstration of equivalence between the period of birth and that of the formation of the considered increment. Otherwise, the quantitative method is only used with fish more than one year old.

### 1.3. Examples of semi-direct validation studies

We give here an example of the observation of margin of the transverse stained section of the otolith of *Colossoma macropomum* (Serrasalmidae) from the Mamoré basin (Bolivian Amazonia). For qualitative data, the otolith edge is either stained (fig. IV.B.2a) or left unstained (fig. IV.B.2b). It can be difficult to interpret the edge because of the accumulation of stain at the resin-otolith interface. For quantitative data, the measurement axis and where to measure the increment (start, middle, end) need to be clearly defined. In the example, the beginning of the stained zone is taken as the starting point. The dimensions of the stained zones may vary because a stained zone consists of several chromophilic bands (fig. IV.B.2a). The semi-direct validation applied to the *C. macropomum* otolith has shown that stained zones are laid down annually and correspond to the dry season in Bolivian Amazonian freshwaters (Loubens & Panfili, 1997).

Figure IV.B.3  
Theoretical graph of the evolution over time (two consecutive years) of the margin width of the last growth zone on a calcified structure. The relative margin width in percentage is the division of the distance between the beginning of the last zone and the edge (AMD) by the distance of the last complete zone ( $D_{i-1}$ ). The zone is fully formed at the end of each year (maximum width). The signal is clearer for the relative distance than the absolute one because in the last case all age classes are mixed and the reduction of growth with age is not taken into account.



The marginal increment analysis has been used mainly to determine the timing of annual increment formation for all types of CS (scales, otoliths, vertebrae, spines) in temperate Teleost species (Beall & Davaine, 1988; Robert & Vianet, 1988; Crawford *et al.*, 1989;

Schramm, 1989; Vianet *et al.*, 1989; Mann & Beaumont, 1990; Fletcher, 1991; Hales & Hurley, 1991; Hyndes *et al.*, 1992; Tserpes & Tsimenides, 1995; Pearson, 1996; Machias *et al.*, 1998; Vilizzi & Walker, 1999, *inter alia*), tropical Teleosts (Loubens, 1978; Cayré & Diouf, 1983; Davis & Kirkwood, 1984; Sosa-Nishizaki *et al.*, 1989; Loubens & Panfili, 1992, 1997, 2000; Crabtree *et al.*, 1995; Yosef & Casselman, 1995; Fabre & Saint-Paul, 1998; Chung & Woo, 1999; Morales-Nin *et al.*, 1999), deep water species (Massutí *et al.*, 1994; Gordon & Swan, 1996; Morales-Nin *et al.*, 1996b) and sharks (Lessa *et al.*, 1999).

The semi-direct validation of daily increments has been much less developed but this method has also been employed (Tanaka *et al.*, 1981; Ré *et al.*, 1985; Geffen, 1987; Gartner, 1991). It has not often been used in this way because it is technically much more complicated, involving complex otolith preparation techniques (e.g. thin slices, SEM). Because of the low power of resolution of the compound microscope the SEM must often be used.

## **2. Problems of edge interpretation of otoliths**

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All semi-direct validation studies have mentioned the difficulty of evaluating the nature of the otolith edge: opaque v. translucent, stained v. unstained, etc. There may also remain a certain number of “uninterpretable” otoliths when we examine the edge. Three explanatory hypotheses can be put forward for the observation of whole otoliths:

- The otolith is thicker in the central area than at its edge, where the marks are less clear. Under transmitted light the edge can be highly refringent (fig. IV.B.4a). If this is the case, the whole otolith should be prepared (e.g. thick section) to enhance the observation of the edge.
- The interpretation of the nature of the edge refers to the observation of the previous marks (e.g. opaque and translucent) and the density of these marks varies according to the observed otolith area.
- The growth rate differs in the various otolith faces (e.g. anterior, posterior, dorsal, ventral), leading to thicker increments on the preferential axis of growth (fig. IV.B.4b). The observation is thus different regarding the otolith area, making it necessary to define interpretation criteria (increment continuity around the otolith, presence or absence on a particular area). In older fish, growth is often limited to the internal and dorso-ventral sides of the otolith and marginal increments are only visible in these areas.

These remarks also concern observations of otolith microincrements, as those deposited near the edge tend to be less visible (fig. IV.B.4c).

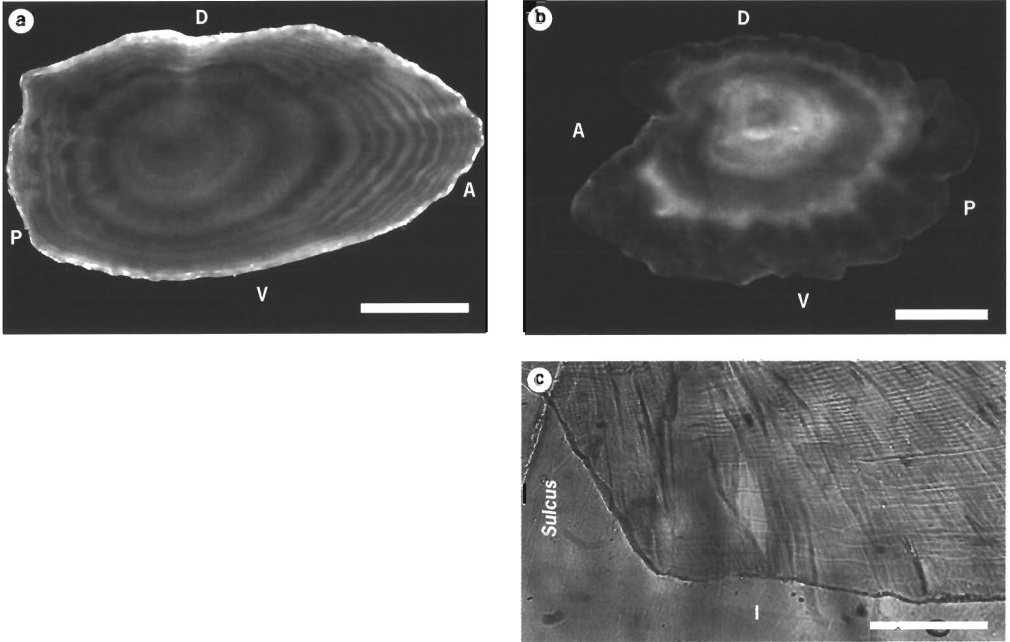


Figure IV.B.4 - Difficulties in interpreting the edge aspect of otoliths. A, anterior; D, dorsal; I, internal; P, posterior; V, ventral (photos J. Panfili).

a) Whole otolith of plaice (*Pleuronectes platessa*) under transmitted light. The edge of the otolith is particularly refringent and it is difficult to interpret it. Scale bar = 2 mm.

b) Whole otolith of the European eel (*Anguilla anguilla*) under reflected light. The aspect of the edge is opaque on the ventral and anterior face and more translucent on the posterior and dorsal face. This is due to two factors: (1) the reduced thickness of the otolith on the edge and (2) the differences of growth rates in each face. Scale bar = 500  $\mu$ m.

c) Thin transverse section of the otolith of the oxeye scad (*Selar boops*) under transmitted light. Edge interpretation is also difficult when looking at the microincrements which lose contrast near the edge. Scale bar = 20  $\mu$ m.

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