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Selecting the optimal zone for the coupling of age and LA-ICPMS data : case study on brown trout, *Salmo trutta* L., in the Kerguelen Islands

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Introduction

In the Kerguelen Island (49° S, 70° E) little knowledge exists on the growth of salmonid otoliths and annulus formation. At this latitude, only few studies used otoliths as a tool to estimate brown trout age. The validation of age and annuli position and the characterisation of an optimal zone where to make laser ablation (LA-ICPMS) appear as a fundamental preliminary stage before developing ageing and life history studies.



The aim of this study is to defining a zone of the otolith sagittal plane which optimizes the probability to make reliable and repeatable measurements and which also minimize the inter-individual variability of these measurements. This zone will help to select the axis from the core to the edge of the otolith where LA-ICPMS transects have the maximum probability to cross annuli, with the aim of **linking age and microchemical data**.

Methods

Field collection: Fish were captured ($n = 220$; mean FL : 14.9 ± 7.8 cm.) in the La Ferme river and were marked using Alizarin red S (100 mg.L^{-1} ; 8°C) before releasing. Fish were recaptured later in the same summer and in the next two summer ($n=53$).

Data sampling & measurement procedure: Sagittae were grounded to expose the core. Images were taken in transmitted and fluorescent light. to obtain measurements on the dorsal part the following procedure was followed 1) a longitudinal axis was defined (core \rightarrow rostrum) and 37 radii were plotted every 5 degrees (0° to 180°) 2) the measurements were taken between the core and the intersection of each radius with annuli / ARS / margin (Fig 1). In some areas the intersections were not identifiable which unable to make measurements.

Age validation and selection of the optimal otolith zone: counting the number of visible annuli beyond the ARS mark and its correspondence with the number of winters following the marking event validated the annual formation of annuli. We calculated the probability (P) to place a crossing point (i.e. to make a measurement along radius 1 to 37). To select the optimal zone of the sagittae where the inter-individual variability of the measured distances (SD, the standard deviation of the measure) was minimum and the probability (P) to realize a measurement was maximum, we created a composite index (I_i , Formula 1).

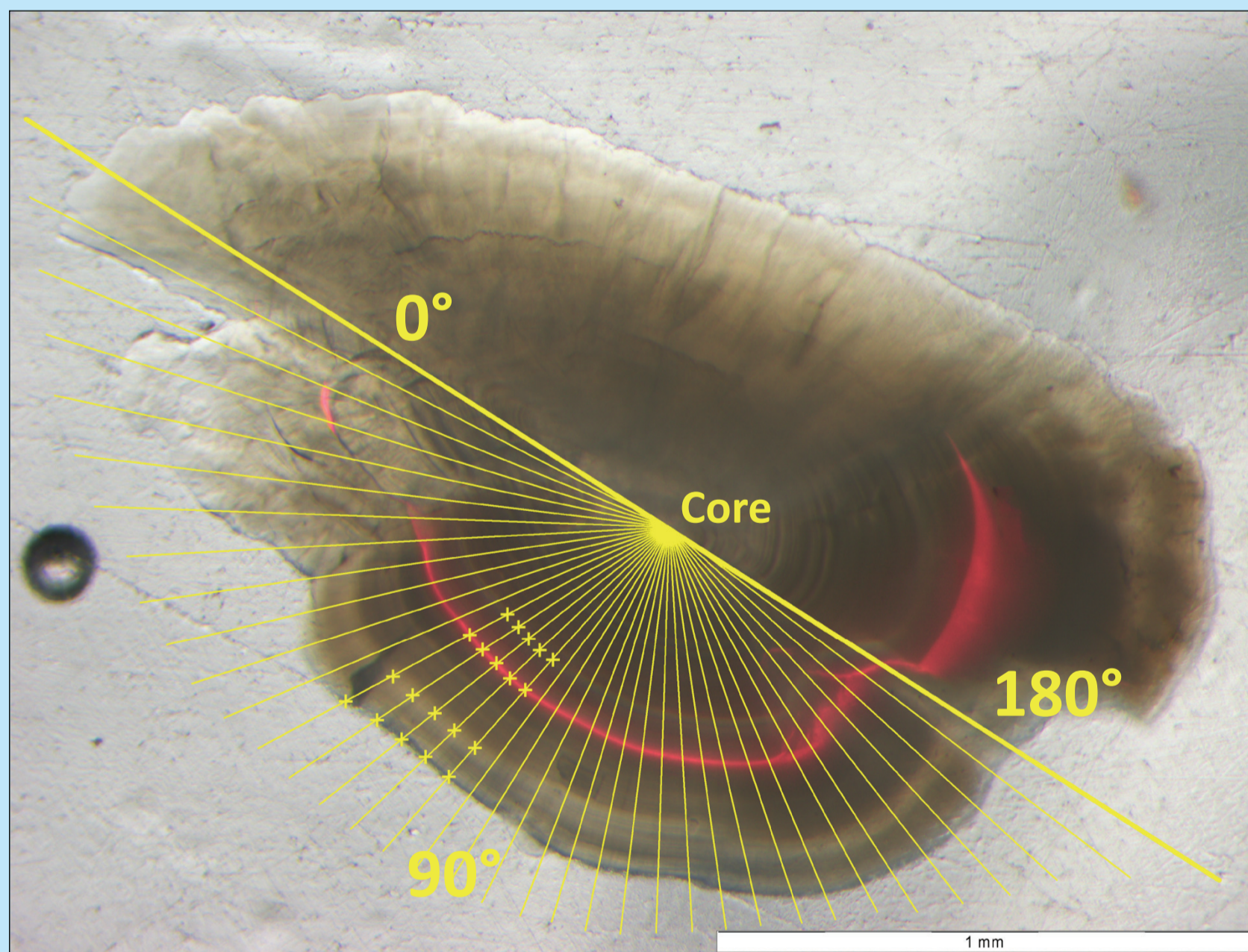


Fig 1 : Two-year-old Kerguelen Brown trout otolith marked with Alizarin red S. Yellow lines : 37 radii and longitudinal axis (0° to 180°). Yellow crosses indicate the intersection of the radii with the annuli, the ARS mark and the edge of the otolith (five examples are drawn for illustrative purpose, from 60° to 80°).

$$I_i = \text{stand} \left[\alpha \sum_j \text{stand} [P_i] + (1 - \alpha) \sum_j \text{stand} [SD_i] \right]$$

Formula 1 : Formula of the composite index I_i . with: P_i = the probability [0-1] to place a point on the crossing between the radius i (1 to 37) and the j annulus ; SD_i = the standard deviation of the distance between the core and the positioned points; stand = standardized value [0-1]. α = weighting factor.

Results

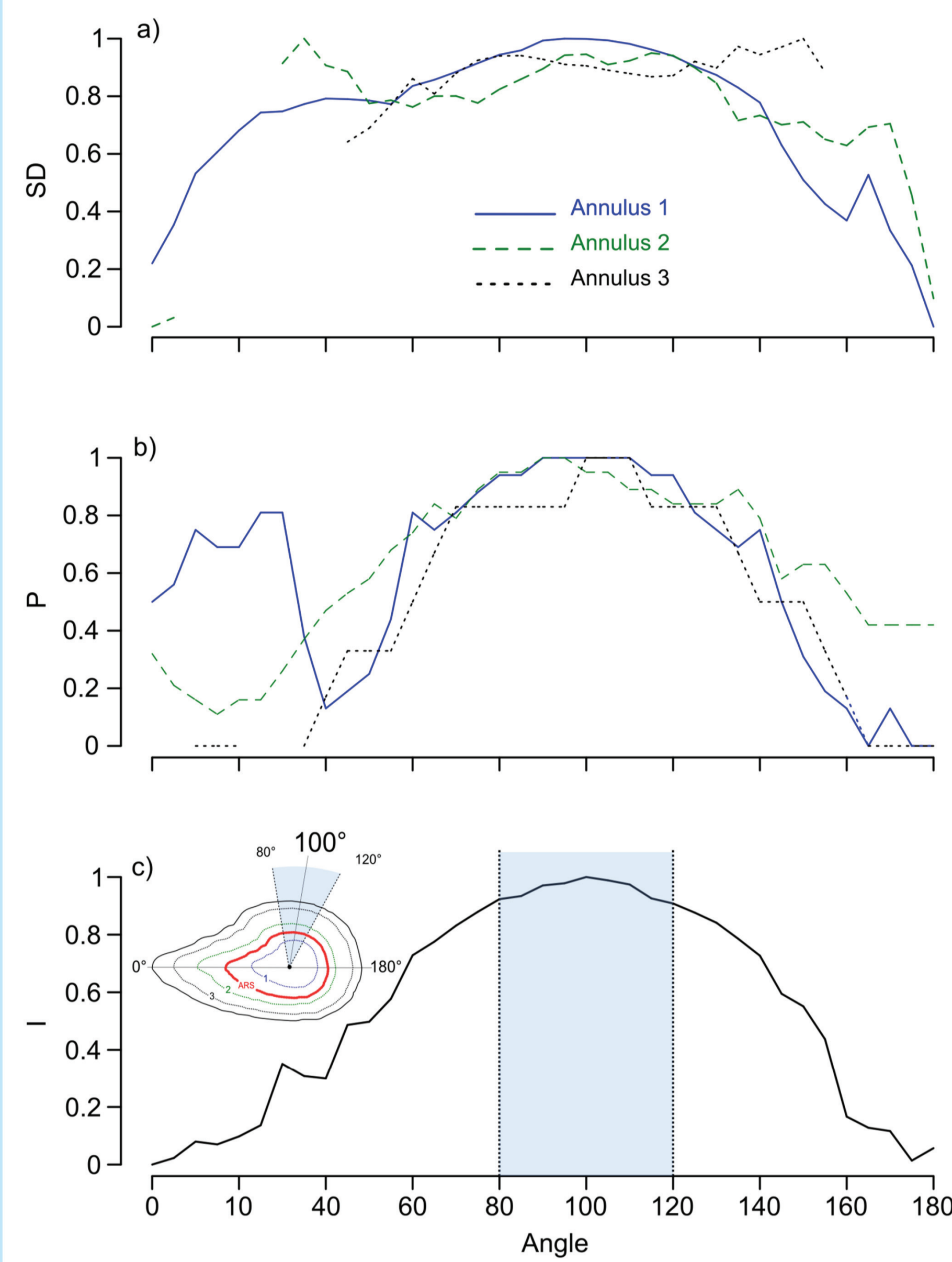


Fig 2: Variation of SD_i (a), P_i (b) and Index (c) according to the angle selected on the dorsal part of the otolith. Blue area indicates the optimal zone.

The I index calculated thanks to P_i and SD_i was superior to 0.9 only in a **zone localized between 80° to 120°** . The index was maximum at 100° , indicating both a high probability to observe annuli and a restricted variation in the annulus position (Fig 2).

Discussion/conclusion

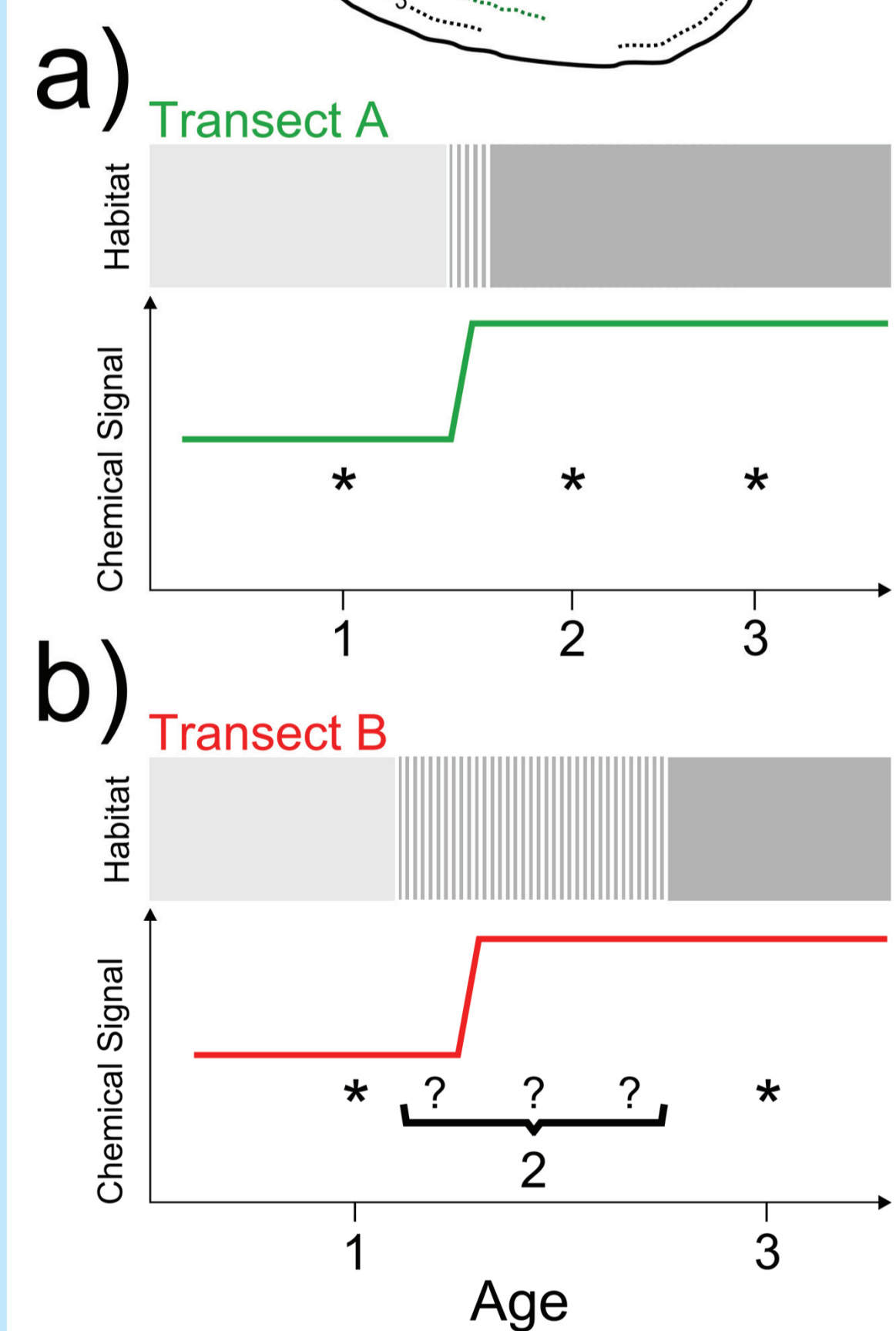
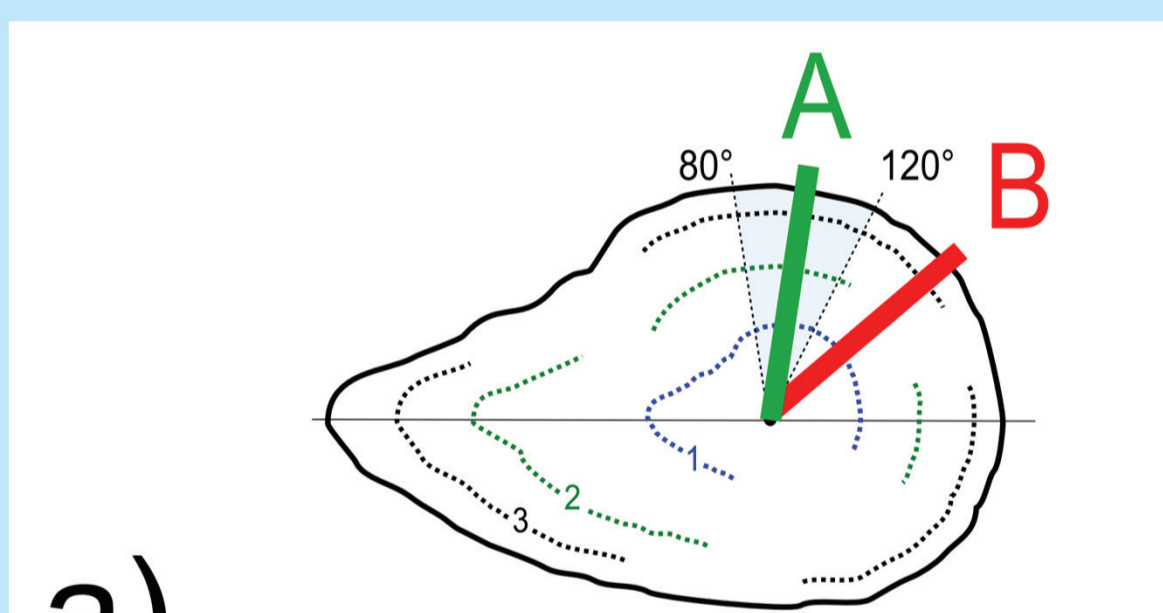


Fig 3: Simulation of a chemical transect in the optimal area (a) and outside (b). Transect B : habitat uncertainty at a given age or uncertain age for a given event. * = annulus.

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