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The American eel, *Anguilla rostrata*, is a catadromous species which enters freshwater or estuarine habitats as a glass eel after metamorphosing from the planktonic leptocephalus larval stage. The glass eel phase is followed by the yellow phase, which is the primary feeding and growth stage. The yellow phase is maintained until a critical length is obtained, at which time the eel undergoes another metamorphosis to the silver stage and begins a return migration to the Sargasso Sea (Frost, 1945; Helfman et al., 1987). The age at which the eel reaches the size necessary for the silver metamorphosis is an important factor in understanding the growth of the eel in specific habitats.

Age determination of anguillid eels is restricted to otolith analysis (Tesch, 1977). Numerous otolith examination techniques exist (summarized by Panfili et al., 1990). The common problems with present methods are the subjective nature of interpreting annuli and the difficulty in distinguishing false rings (Deelder, 1976, 1981; Michaud et al., 1988). These problems can be reduced by validating the periodicity of otolith ring formation. Once the presence of true annuli is established, false (supernumerary) rings can be more easily discerned.

Several annulus validation methods have been reported. These have used eels of known age (*Anguilla rostrata*: Liew, 1974), tagged eels in the field (*A. anguilla*: Berg, 1985), and tetracycline- or calcein-treated eels in captive ponds (*A. anguilla*: Panfili et al., 1991; Mounaix et al., 1992; Dekker[1]). Chisnall and Kalish (1993) used tetracycline-treated otoliths to confirm the periodicity of ring formation in wild populations of *Anguilla dieffenbachii* and *A. australis*. No validation study has been done for any wild population of American eels. This study validates annular ring formation and describes supernumerary zones for tetracycline-injected American eels from a Rhode Island river.

Materials and methods

Eels were collected from the Annaquatucket River, North Kingstown, RI (lat. 41°30'N , long. 71° 22'W), a tributary of Narragansett Bay. Collections were made from seven locations along a 5.5-km section of the river (Fig. 1).

Yellow-phase American eels, *Anguilla rostrata*, were collected in August 1990 and September 1990. Eels were collected with a Smith-Root model 12 Electrofisher. Captured eels were anesthetized in MS-222, measured (total length, TL), and individually marked with liquid nitrogen-cooled brands. Four hundred eels less than 30 cm TL were given an intraperitoneal injection of 75 mg tetracycline/kg body weight (after Dekker[1]). Eels larger than 30 cm were not injected owing to the high probability of migration out of the river in the fall. Most eels (more than 95%) undergo the silver-phase metamorphosis and begin their seaward migration out of the Annaquatucket River at total lengths between 30 and 40 cm (Oliveira, unpubl. data). Upon recovering from anesthesia, all eels were released at the location from which they were captured.

All stations were resampled bimonthly from August 1990 to September 1992. Marked, tetracycline-injected eels recaptured from April through September (1991 and 1992) were kept for otolith examination. Some marked eels recaptured prior to the 1992 interval were anesthetized, identified, and measured prior to being released again; nine of these eels were eventually recaptured and kept for otolith examination.

Both sagittal otoliths were removed from each eel, cleaned of extraneous tissue, and stored dry in glass vials. Otoliths were prepared for examination according to Secor et al. (1992) with the following modifications. One of each pair was embedded in epoxy, and a 0.18 mm section that transected the nucleus was cut with an Isomet low-speed saw. The sections were mounted on glass slides with Crystal Bond 509 thermoplastic adhesive and polished with 5-μm and 3-μm metallurgical lapping film. Polished sections were viewed under

[1] Dekker, W. 1986. Age reading of eels using tetracycline marked otoliths. ICES Council Meeting 1986; Copenhagen, Denmark, 14 p.

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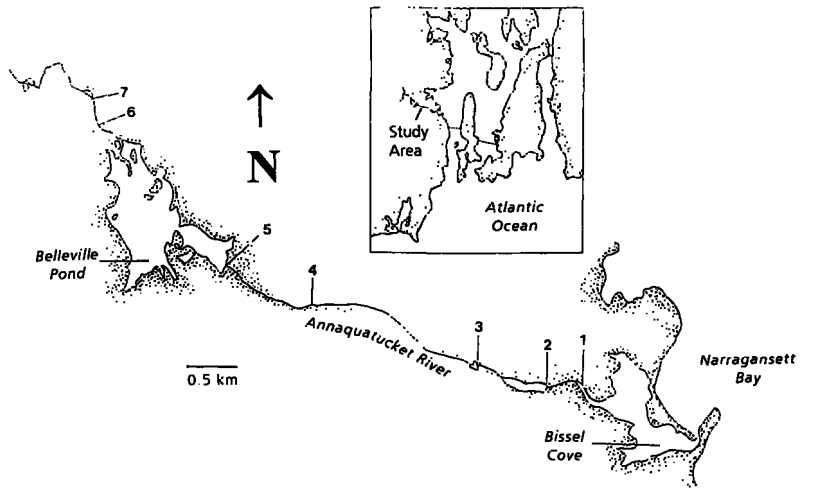

Figure 1

Map of collection sites in the Annaquatucket River, a tributary of the Narragansett Bay, Rhode Island. The inset shows the location of the river to the Bay as well as the Atlantic Ocean. Yellow-phase American eels, *Anguilla rostrata*, were electrofished from stations 1–7 in August and September 1990.

fluorescent light with a compound microscope at 40× magnification. When a fluorescent ring was visible, a preliminary count of the number of rings after the tetracycline mark was made. The sections were then etched for one minute with a 5% ethylenediaminetetraacetic acid (EDTA) solution and stained for two minutes with 0.01% toluidine blue. This procedure enhanced all otolith rings and enabled the differentiation of supernumerary zones. A 3-minute treatment with both EDTA and toluidine blue produced greater resolution of rings but decreased the intensity of the fluorescent mark. The otoliths were also observed under both transmitted light and reflected UV light so that the tetracycline mark and ring formations were visible simultaneously. All opaque ring formations (complete and incomplete) described were observed with transmitted light.

Results

Nine tetracycline-injected eels were recaptured after the first winter and 11 after the second winter at liberty. Examination under UV-light revealed a fluorescent mark on all otoliths. All otoliths showed a distinctive, complete opaque ring aligned with the external edge of the tetracycline mark (Fig. 2). This ring (false complete) was distinguishable from other complete rings because of its association with the tetracycline mark and its atypical spacing compared with other complete rings (Fig. 2). The association of these rings and the tetracycline marks was the same for all eels, whether tagged in early August or late September; therefore no seasonal effect was apparent.

Eels were recaptured from all locations except station 5: 3 from station 1, 3 from station 2, 1 from station 3, 4 from station 4, 2 from station 6, and 7 from station 7. Nine eels were recaptured more than once (4 twice, 3 three times, and 2 four times), but only one false complete ring was observed on each otolith. These nine eels were all recaptured twice prior to the first winter.

After the fluorescent mark and its associated false ring, one complete ring (an opaque zone which completely encircled the otolith, as seen with transmitted light) for each winter was visible on all otoliths (Fig. 2). Otoliths from all locations had a similar tetracycline mark and false ring arrangement as well as only one new ring for each winter.

Many otoliths had numerous incomplete rings made of narrow opaque zones which did not extend completely around the otolith. These incomplete rings were located throughout the translucent zone bounded by complete rings (Fig. 2).

Discussion

All otoliths showed a single complete ring (annulus) for each winter after the tetracycline mark. Two complete rings beyond the tetracycline mark were

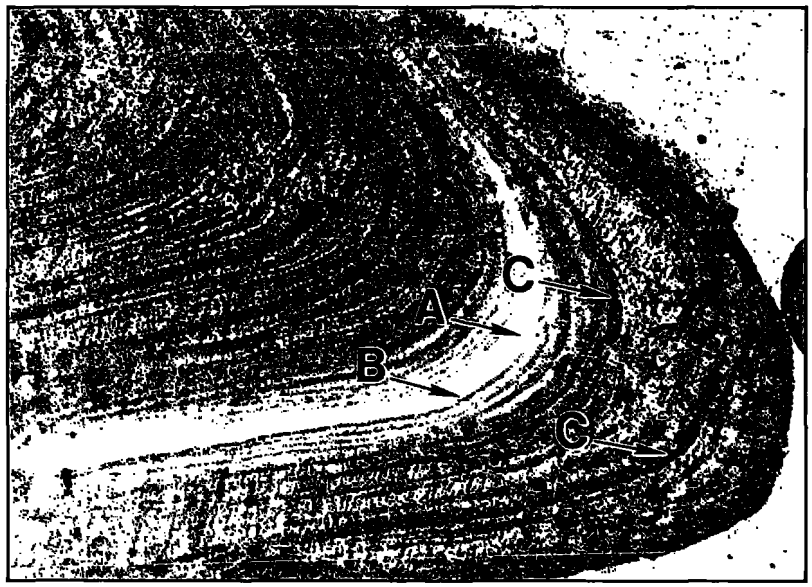

Figure 2

Transverse section from a tetracycline-marked sagittal otolith taken from an American eel, *Anguilla rostrata*, at liberty for two winters after marking. The section was etched with 5% EDTA and stained with 0.01% toluidine blue. Photographed with both transmitted light and reflected UV light. A = tetracycline mark; B = false complete ring; and C = complete ring (annulus). The first complete ring after the tetracycline mark was not fully enhanced owing to the dissolution of the tetracycline mark with extensive EDTA treatment.

present only in otoliths from eels recaptured after the second winter at liberty, indicating that normal complete ring formation takes place during winter months. For this population of *Anguilla rostrata*, it may be concluded that complete rings are produced annually. This is in agreement with Liew (1974) and with reports for *A. anguilla* (Frost, 1945; Berg, 1985; Panfili et al., 1991; Mounaix, 1992).

Two forms of false annuli were present: complete and incomplete. The false rings most similar to true annuli (false complete) were found only adjacent to the fluorescent mark and resulted from one or a combination of several stressful events: anesthetizing, tagging, and tetracycline injection. Tagging has been reported to cause growth discontinuities in the otoliths of *Anguilla anguilla* (Berg, 1985). In the present study, eels recaptured more than once, but receiving no further branding or injection, did not produce additional false complete rings. The lack of additional false complete rings after the tetracycline mark in these eels suggests that the stimulus for their production was absent. Natural stimuli necessary for formation of false complete rings appear to be missing. Therefore, ages of eels reported for this river are not overestimated by misconstrued false complete rings.

Incomplete rings may result from environmental stresses associated with a freshwater habitat (Mounaix, 1992). Deelder (1981) and Liew (1974) suggested that temperature extremes were a source of supernumerary zone formation. The Annaquatucket River is a shallow freshwater system subject to extreme temperature fluctuations in the summer months (Oliveira, unpubl. data). The presence of incomplete rings throughout the interval between annuli indicates that incomplete rings form throughout the year. The similarity in ring formation among otoliths collected from different locations indicates that the factors influencing ring formation are homogeneous in the Annaquatucket River.

The validation of the formation of true annuli and the clarification of false rings in this study facilitate the use of otoliths for ageing Annaquatucket River eels. Extension of these conclusions to eels from other locations requires further study.

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