Great Lakes Fishery Commission
Project Completion Report *

TITLE: Validation of Age Determination by Statoliths in the Sea Lamprey, *Petromyzon marinus*

SUBMITTED TO: The Great Lakes Fishery Commission

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Introduction

Discrimination of age in larval lamprey has relied on an analysis of length frequency distributions from large numbers of animals. This technique appears satisfactory for the young age classes, but slower and more variable growth rates in older fish result in greater overlapping of length distributions corresponding to year classes (Potter 1980).

Hard tissues including, scales, fin rays and otoliths are typically used to age teleost fishes. Recognition of annual patterns requires the understanding of how these patterns relate to the annual growth cycles in fishes. In temperate latitudes, extremes in growth imposed by sharp seasonal changes in temperature are manifested in hard tissue by the formation of annuli (Beamish and McFarlane 1983).

Volk and Brothers (1980) described a positive relationship between statolith dark band number and length of larval sea lamprey collected from a tributary of Cayuga Lake, New York. The circumstances under which dark and opaque bands are formed have not been demonstrated for sea lamprey statoliths. However, a similarity in banding patterns of sea lamprey statoliths and hard tissues in teleost fishes which are used for aging suggests that each dark band represents one annulus.

Oxytetracycline is an *in vivo* bone-tissue marker and is detectable in undecalcified bone tissue by fluorescence microscopy (Milch et al. 1957). Oxytetracycline injected into the body cavity of fishes is deposited in calcium-containing tissues (Jensen and Cumming 1967; Campana and Neilson 1982; Campana 1983). Oxytetracycline was detected in the statoliths of Northern Brook lamprey, (*Ichthyomyzon greeleyi*) within two weeks after injection and persisted in the statolith for at least one year (Medland and Beamish 1987).

The current study evaluated statolith banding patterns as an aging technique for slow and fast growing larval sea lamprey populations.

Materials and Methods

Larval sea lamprey were electrofished from Great Chazey River, a tributary to Lake Champlain in eastern New York State, in October of 1985. After capture, lampreys were anesthetized in methane tricaine sulfonate (Sandoz), measured to the nearest millimetre and frozen for later aging. Seventy live lamprey ranging in length from 52 to 167 mm were returned to the laboratory. In, November 12th, 1985 they were anesthetized, measured to the nearest millimetre, weighed to the nearest milligram, and given intraperitoneal injections of oxytetracycline at 35 mg oxytetracycline/kg live body weight. Lamprey were placed in an outdoor holding tank (200 l), supplied with a suitable substrate for burrowing, and a continuous flow of aerated, non-chlorinated ground water and fed brewers yeast (300 g dry yeast per 70 lamprey) once weekly. Water flow to the tank was stopped for 24 h following feeding. Water temperature was cycled to mimic seasonal temperatures in the lamprey's natal stream. Half of the surface area of the tank lid was covered to simulated shaded areas in a stream. Lampreys were sampled from the holding tank six weeks, nine months, and 12 months after injection with oxytetracycline, anesthetized and measured ($\pm$ 1 mm) and their statoliths removed.

A second population of sea lamprey larvae were electrofished from Big Garlic River on May 28th, 1985 by Sea Lamprey Control personnel from Marquette, Michigan. Seventy lamprey ranging in length from 63 to 119 mm were anesthetized, measured to the nearest millimetre, weighed to the nearest milligram, given intraperitoneal injections of oxytetracycline at 35 mg oxytetracycline/kg live body weight and returned to the stream in each of three holding cages. All lamprey were sampled, from one cage on each of 3 occasions, 4, 6 and 12 months after the injection of oxytetracycline, anesthetized, measured ($\pm$ 1 mm) and their statoliths removed.

All statoliths collected over the course of the study were examined under a dissecting microscope for the presence and persistence of oxytetracycline, as well as banding patterns. Statoliths from frozen animals (n = 130) were also removed, stored in immersion oil for 30 days, and the number of bands counted with a dissecting microscope, as described by Volk (1986). The precision of this technique was calculated using the following index of average error

$$\frac{1}{N} \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|Xij - Xj|}{Xj} \right] \times 100,$$

where N is the number of lamprey aged, R, the number of times each is aged, Xij, the ith age determination of the jth lamrpey and Xj, the average age calculated for the jth lamprey (Chilton and Beamish 1982).

Sex of each larva was determined by visual internal inspection of the gonad under a dissection microscope. Histological examinations were periodically conducted on gonads of animals to confirm identifications made by visual inspection.

Results and Discussion

In ectothermic animals, growth is a manifestation of biotic and abiotic conditions (Beamish et al. 1975; Brett and Groves 1979). In temperate climates, growth is particularly responsive to sharp seasonal changes in ambient temperature. Fluctuations in biotic and abiotic factors other than temperature may also influence statolith growth but presumably not the number of annuli.

Larvae injected with oxytetracycline burrowed in the substrate of the holding tank or cage within 24 h after injection. Statoliths examined six weeks after injection of oxytetracycline fluoresced along their outer margins (Fig. 1a) whereas no fluorescence was observed in lampreys not injected. Oxytetracycline persisted in the statoliths throughout the subsequent eleven months. Six and nine months after injection a zone of growth was present on the margin of the statolith beyond the area marked with oxytetracycline (Fig. 1b). Twelve months after injection a complete annulus had formed on the statolith immediately beyond the area marked with oxytetracycline (Fig. 1c).

When a statolith is oriented properly to transmit incident light, bands appear as layers within the statolith (Fig. 2). Growth of larval *P. marinus* is most rapid during the spring and summer (Manion and McLain, 1971). This period of rapid growth is manifested in the statolith as an opaque zone laid down after the narrow darker band produced in the winter. This narrow dark band represents one annulus. Approximate age is equivalent to the number of annuli. Actual age can be calculated from information on the number of annuli, capture date, and the date lamprey are known to hatch in the study stream.

This growth pattern of alternating dark and opaque zones on the

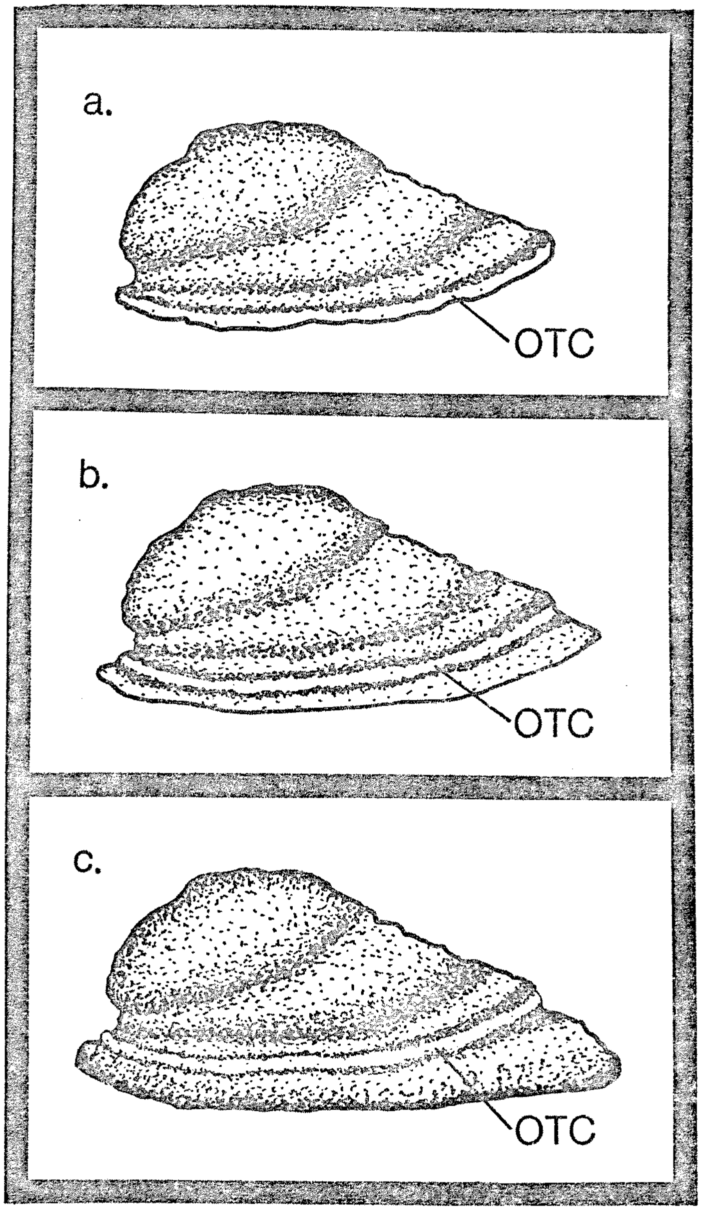
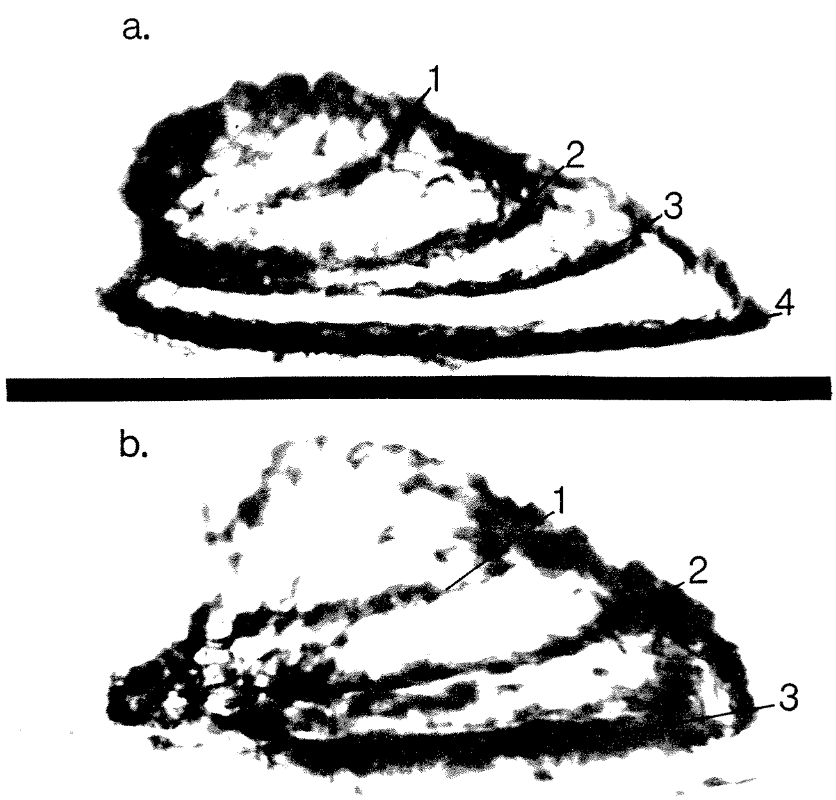

a.
OTC
b.
OTC
c.
OTC

FIG. 2. Lateral view of statoliths of P. marinus from Great Chazey River.

(a) Statolith sampled in January showing four annuli.

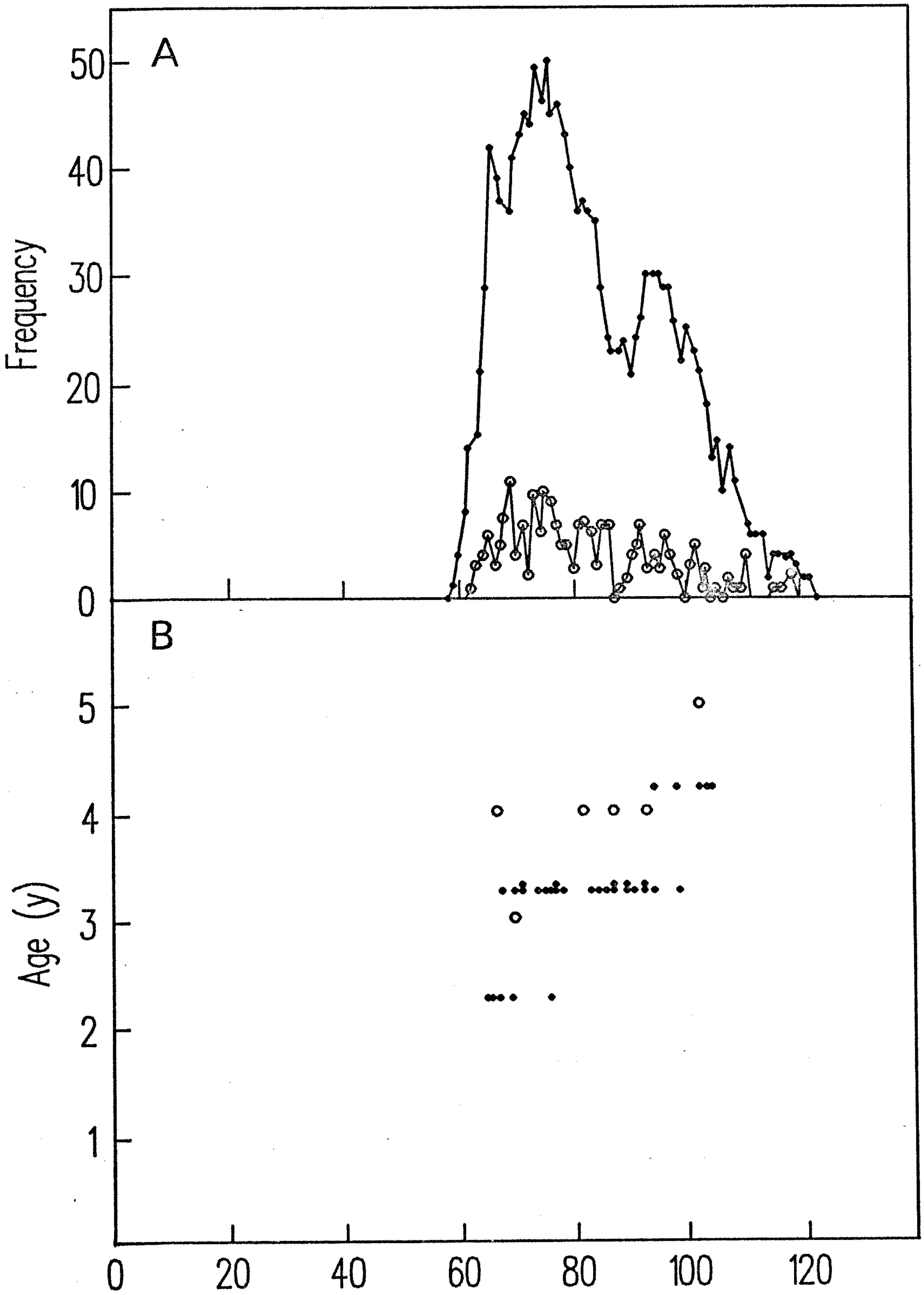
(b) Statolith sampled in June showing three annuli followed by an opaque zone of growth.


a.
1
2
3
4
b.
1
2
3

statolith was observed in both study populations. Although measurements were not taken, both the dark and opaque bands of the slower growing Big Garlic River population were consistently narrower than those of the Great Chazey River population.

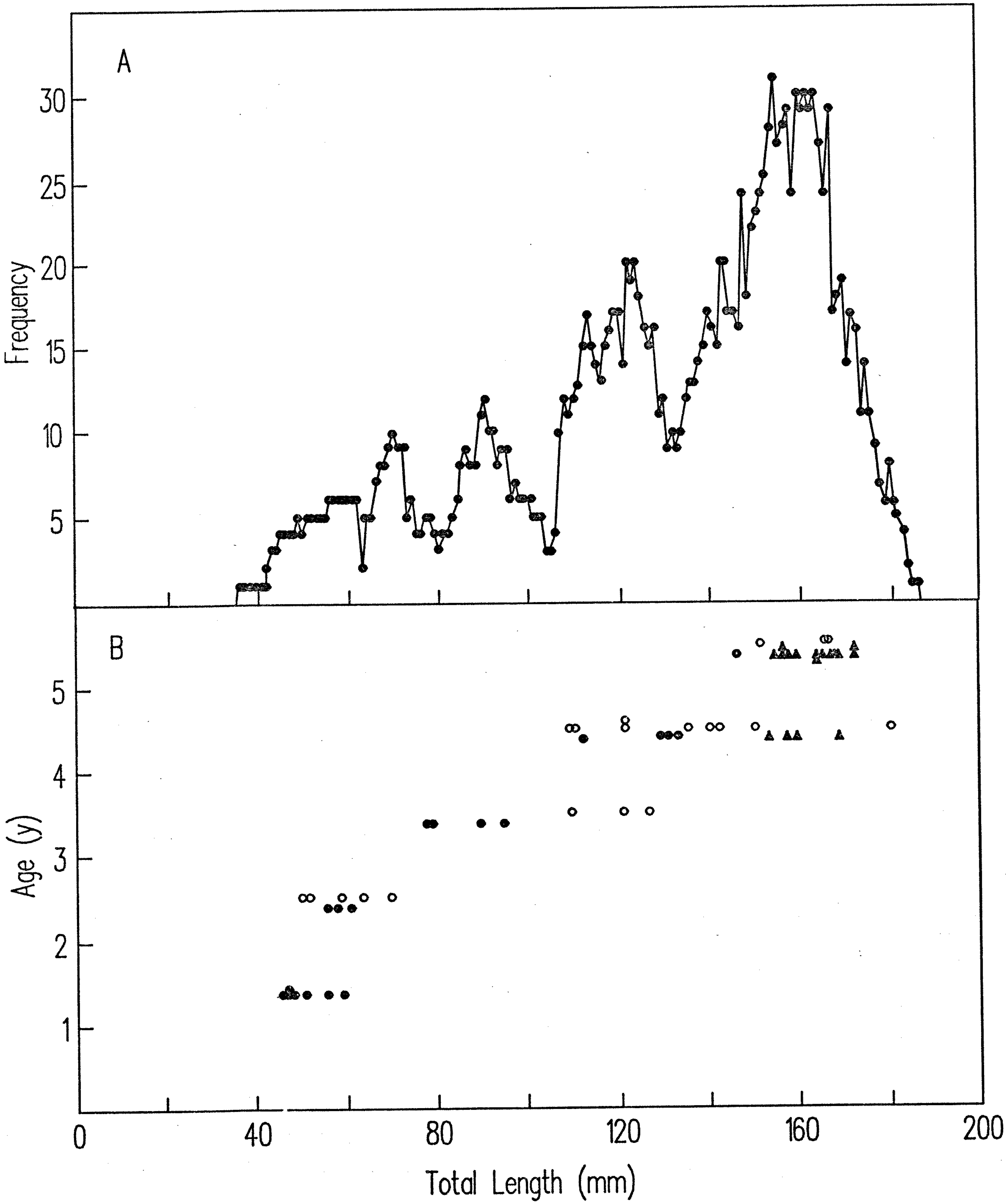
Age structure of the Big Garlic River lamprey population could not be determined from the length frequency distribution of the 210 larvae used in the study (Fig. 3a). When a moving average of seven (Ricker 1975) was applied to the data three age classes of larvae were distinguished (Fig. 3a). Specific age could not be assigned to these age classes because a full spectrum of larval lengths was not available from the sample. Statoliths from 32 of these larvae were examined in September, 1985. Based on the number of annuli, 3 age classes were identified, 2.3, 3.3 and 4.3 years. At the completion of the Big Garlic River study in June 1986 only 6 larvae remained alive. All six of these larvae had one complete annulus formed on their statoliths after the mark made by oxytetracycline. The total number of annuli present on the statoliths ranged from 3 to 5. Since these larvae were sampled in June and hatching occurs between mid and late May in the Big Garlic River (Manion and McLain, 1971), actual age of these lamprey ranged between 3 and 5 years.

Age structure of the sea lamprey population of the Great Chazey River could not be determined by examination of length-frequency distribution from 180 larvae and 60 metamorphosing lamprey collected in October 1985. When a moving average of seven (Ricker 1975) was applied approximately five age classes were distinguished (Fig. 4a). Examination of statoliths from lamprey collected and frozen in October 1985 (n = 19 ammocoetes, and 17 metamorphosing lamprey) yielded statoliths containing 1 to 5 annuli. Larvae hatch between early and



A
B
Frequency
Age (y)
Total Length (mm)
0
10
20
30
40
50
1
2
3
4
5
0
20
40
60
80
100
120

FIG. 4. (a) Length frequency distribution, adjusted by a moving average of seven (Ricker 1975), of *Petromyzon marinus* (n = 240) from Great Chazey River, Champlain, New York sampled on Oct. 4th, 1985. (b) Age length distribution of 19 larvae (closed circles) and 17 metamorphosing lamprey (closed triangles) aged on Oct. 4th, 1985, and 20 larvae (open circles) aged on Nov. 15th, 1986 using statolith banding patterns, from the above (a) sample population.


A
B
Frequency
Age (y)
Total Length (mm)
5
10
15
20
25
30
1
2
3
4
5
0
40
80
120
160
200

mid May in Great Chazey River (Gersmehl, personal communication). Actual age of larvae ranged from 1.4 to 5.4 years (Fig. 4b). Metamorphosing animals ranged from 4.4 to 5.4 years (Fig. 4b). Sex ratio of metamorphosing animals was 1 ♂ : 3 ♀ but the average age of the animals did not vary significantly ($p = 0.05$) with sex.

The findings of this study suggest statolith banding patterns provide a valid procedure for assigning age to both relatively fast and slow growing sea lamprey populations. Quantification of age from statoliths removes the uncertainties inherent in estimating the age structure of lamprey populations from length frequency distributions. This is particularly important in distinguishing age of long-lived species where overlapping of length distributions corresponding to year classes is a common occurrence. This technique would also be useful in determining the age structure of smaller native populations of lamprey where it is often impossible to collect a large enough sample for a length frequency distribution.

References

Beamish, R.J. and G.A. McFarlane, 1983. The forgotten requirement for age validation in fisheries biology. Trans. Amer. Fish. Soc. 112 (6): 735-745.

Beamish, F.W.H., A.J. Niimi and P.F.K.P. Lett. 1975. Bioenergetics of teleost fishes: Environmental influences. In: L. Bolis, H.P. Maddrell and K. Schmidt-Nielsen (Editors), Comparative Physiology - Functional Aspects of Structural Materials. North-Holland Publishing Company, Amsterdam, pp. 187-209.

Brett, J.R. and T.D.D. Graves. 1979. Physiological energetics. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. 8. Academic Press, New York, pp. 279-352.

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

Campana, S.E. and J.D. Neilson. 1982. Daily growth increments in otoliths of starry flounder (Platichthys stellatus) and the influence of some environmental variables in their production. Can. J. Fish. Aquat. Sci. 39: 937-942.

Chilton, D.E. and R.J. Beamish. 1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Can. Spec. Publ. Aquat. Sci. 60: 120 p.

Jensen, A.C. and K.M. Cumming. 1967. Use of lead compounds and tetracycline to mark scales and otoliths of marine fishes. Progressive Fish-Culturist 29: 166-167.

Manion, P.J. and A.L. McLain. 1971. Biology of larval sea lamprey

(*Petromyzon marinus*) of the 1960 year class isolated in the Big Garlic River Michigan, 1960-1965. Great Lakes Fishery Commission Technical Report. No. 16.

Medland, T.E. and F.W.H. Beamish. 1987. Age validation for the mountain brook lamprey, *Ichthyomyzon greeleyi*. J. Fish. Aquat. Sci. 00:00.

Milch, R.A., D.P. Rall and J.E. Tobie. 1957. Bone localization of the tetracycline. Journal of the National Cancer Institute 19: 87-93.

Potter, I.C. 1980. Ecology of larval and metamorphosing lampreys. Can. J. Fish. Aquat. Sci. 37: 1641-1657.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Environment Canada, Fisheries and Marine Service, Bulletin 191.

Volk, E.C. 1986. Use of calcareous otic elements (statoliths to determine age of sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. 43: 718-722.

Volk, E.C. and E.B. Brothers. 1982. Morphology and growth of calcareous otic elements in sea lamprey (*Petromyzon marinus*). (ms).