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Transactions of the American Fisheries Society 113:365–369, 1984

Comparison of Age Determination Methods for the Starry Flounder

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Abstract

Sagittal otoliths from 808 starry flounders *Platichthys stellatus* from Bellingham Bay, Washington were examined for annular structures by two techniques: traditional external readings of whole otoliths and internal readings of "cracked and burnt" otoliths. The annular nature of rings observed by both techniques was confirmed by seasonal progression of the width of opaque otolith margins in fish 1–3 years old. Annular rings were also confirmed in internally read otoliths with 4–10 rings. More annuli could be identified in sexually mature fish when read internally (maximum, 24) than externally (maximum, 11). Anteroposterior growth of starry flounder sagittae was attenuated with age, although the sagittae continued to thicken; this accounts in large measure for the relative success of the two techniques. Despite the likelihood of geographical variation, it appears that starry flounders have a longer lifetime than was previously suspected.

Received November 17, 1983 Accepted April 8, 1984

The starry flounder *Platichthys stellatus* is an ubiquitous inshore species of little commercial value in the north Pacific Ocean. Although there have been reports on the fish's feeding behaviour (Miller 1967; Campana, in press) and general life history (Orcutt 1950; Campana 1983), little is known of its population dynamics. Orcutt (1950) used scales and otoliths to derive an age structure for a California population. Since the time of Orcutt's work, however, the value of scales for ageing long-lived species has been questioned (Bagenal and Tesch 1978); otolith examination, though of greater value, also can be misleading. In this paper, I demonstrate the latter point for starry flounders.

Traditional methods for otolith ageing, involving external examination of whole structures, do not take into account the asymmetric deposition of material that may occur in old fish (Beamish 1979). The unstated assumption is that otolith growth along the long (anteroposterior) axis continues throughout the fish's lifetime; however, otolith deposition may act only to thicken the structure after a certain age (Beamish 1979; Bennett et al. 1982). In such a situation, only transverse sections would reveal recently formed growth structures. This method of otolith preparation has been applied infrequently, and age validations of old age groups are rare (Beamish and McFarlane 1983). As a result, many of the previously published age distributions of long-lived species now appear to be incorrect (Chilton and Beamish 1982).

The objective of this study was to determine the relative validity of external otolith examinations and "internal" readings of cracked and burnt structures for a Washington population of starry flounders. The best age estimator then was applied in an examination of the population age structure.

Methods

Starry flounders were collected between 1978 and 1982 from Bellingham Bay, Washington, with a shrimp try net (2.5-m lead line) towed at 1–2 m/second (details in Campana 1983). The collection gear appeared to sample the males and all but the largest females representatively, because the length-frequency distribution of commercial otter trawl samples caught in Bellingham Bay was similar (Smith 1936). The age of young-of-the-year fish was confirmed through length-frequency analysis and the absence of hyaline zones in the sagittae. Standard length, weight, and ancillary data were recorded for 808 fish age 1 and older (Campana 1983). Sagittae were mounted on microscope slides in Permount, blind-labelled and measured with an ocular micrometer at magnifications of 25–50×.

Two techniques were used to "read" otolith annuli. External counts (the traditional method) were made along the long axis of cleared sagittae under reflected light and against a black background. The same otoliths were reread independently 1 year later by the "crack and burn" method of Christensen (1964). Briefly,

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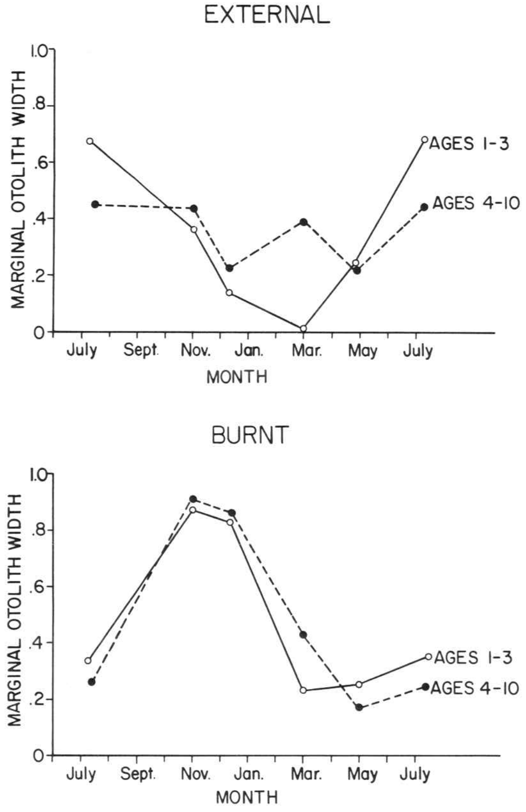

FIGURE 1.—*Yearly cycle of marginal otolith growth since the last-formed hyaline zone in starry flounders. External (N = 145) and internal (cracked and burnt) (N = 133) marginal widths were recorded as proportional measurements relative to the width of the previous inter-hyaline growth zone.*

TABLE 1.—*"Annulus" counts derived from external and internal readings of starry flounder sagittae.*

Internal count	External count 1	2	3	4	5	6	7	8	9	10	11
1	448	6									
2	10	191	1			1					
3	1	10	17	1	1						
4		4	8	8	3						
5		3	4	11	9	5					
6		2	4	6	4	5	2	1			
7		2	1		2	2	3				
8				2	1	4	2	1			
9				2	1	1		1	1		
10				1				1			
11			1			2			1		
12										1	1
13					1	1	1				
14			1								
15						1					
17									1		
18										1	
24											1

this involves breaking the otolith along a transverse plane through the nucleus and charring the protein exposed at the broken surface. After application of a light coat of oil, the dark rings were counted just lateral to the sulcus under reflected light. Rings were most visible in this region of continuous otolith growth (Chilton and Beamish 1982). Under both methodologies, otoliths collected at intervals throughout the year were assessed for marginal growth past the last-formed hyaline zone. In order to standardize observations from fish of different growth rates and ages, marginal otolith growth was recorded as a proportion of the last full year's growth. Otolith thickness was measured across the surface of cracked otoliths, from the proximal to the distal surfaces just lateral to the sulcus. Reading precision for both techniques was calculated by recounting a random sample of otoliths. The coefficient of variation (SD/mean) of the second set of counts for those otoliths originally observed to have two or four rings (N = 20 for each) was then determined.

Results and Discussion

The distance of the last-formed ring from the otolith periphery should cycle with a 1-year frequency if the rings are true annuli. Such a cycle was confirmed in whole otoliths with 1–3 hyaline zones (N = 88), but not in otoliths with 4–10 hyaline zones (N = 57) (Fig. 1).

Confidence in the validity of counts derived from external otolith readings decreased with the estimated age of the fish. The first two or three rings were generally clear and well spaced; later hyaline zones often were crowded into a radial distance amounting to less than 10% of the total otolith length. As a result of this increased ring proximity, the coefficient of variation for repeated counts increased from 0.143 for otoliths with two rings to 0.199 for those with four.

A yearly cycle of hyaline zone formation was confirmed in burnt otolith sections, both in the 1–3 (N = 66) and the 4–10 (N = 67) ring groups (Fig. 1). In addition, annulus counts derived from burnt otolith sections were more precise than were the external readings. Coefficients of variation for repeated observations decreased from

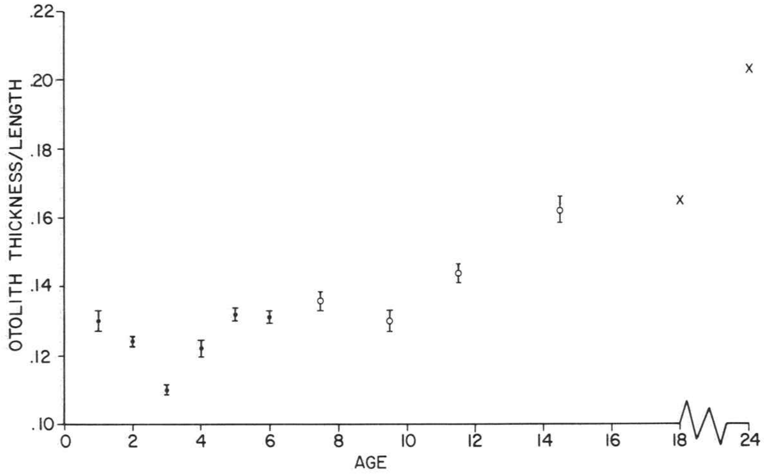

FIGURE 2.—*Otolith size (in terms of the thickness : length ratio) as a function of age in male starry flounders. Age was estimated from burnt transverse sections (internal). Closed circle = 8 < N < 20; open circle = 2 adjacent ages pooled; × = single observation; bar = 95% confidence interval; N = 78.*

0.143 at age II to 0.098 at age IV and to still less for the the older fish. In general, the only questionable hyaline zone occurred right at the otolith periphery. Therefore, I conclude that internally observed otolith rings accurately reflect the age of starry flounders up until at least age X; hyaline zones visible externally are useful as yearly indicators to a much lesser age.

"Annuli" counted in burnt otoliths generally exceeded those counted externally when more than two rings were present (Table 1). As a result, 8.2% (versus 5.0%) of the fish were estimated to be over 5 years old. Maximum observed fish age was 24 years (versus 11 years derived externally). Count discrepancies among young fish probably were due to the differing times of hyaline-zone formation observed externally and internally. Whereas formation of the external hyaline zone occurred from November through April, internally observed rings were more spatially restricted, and could not be differentiated from the otolith periphery until later in the year (Fig. 1).

Ages derived externally appeared to reach an asymptote around age X; no such asymptote was observed in readings of burnt sagittae. Annuli should be apparent in both longitudinal (external) and transverse (burnt) observations if otoliths grow concentrically. Because the former consistently underestimated the latter in old fish, otolith growth may be asymmetric. This hypothesis was tested by monitoring the increase in otolith length and thickness with age in male starry flounders. The ratio of otolith thickness: length would remain constant with increased age if otolith growth was isometric. With the exception of a decrease around the age of sexual maturity, isometric otolith growth was observed until approximately age X; after that age, the ratio increased sharply, denoting reduced growth in otolith length relative to thickness (Fig. 2). Cessation of anteroposterior sagitta growth through periods of continued transverse growth has been documented in other species (Beamish 1979). This age-dependent asymmetry in otolith growth is consistent with the difference in results between age-determination methodologies reported in this study.

Deviations in counts between two ageing methodologies cannot be used to assess accuracy. However, annuli were validated in otoliths with more than four hyaline zones only when the otoliths were cracked and burnt (sagittae with less than three rings were validated by both techniques). In addition, internal readings were both more precise and more consistent with the otolith growth measurements. Therefore, it appears that internal ring counts accurately reflect starry flounder age, at least until age X, and probably beyond. Total confidence in the annulus readings of older fish requires validation of the oldest age classes. Nevertheless, both ra-

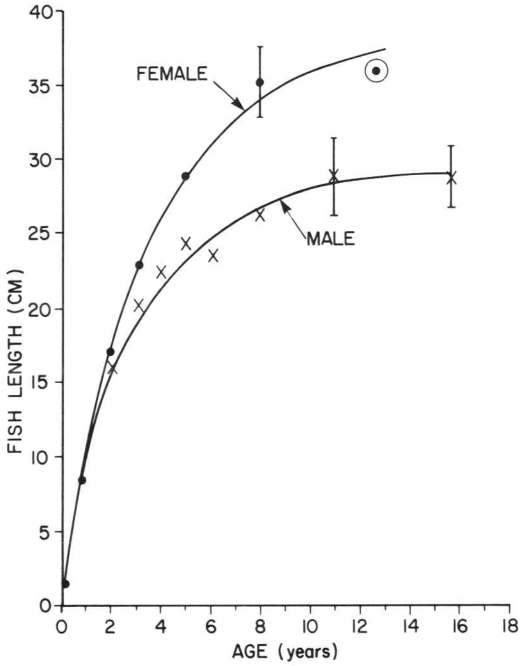

FIGURE 3.—*Age-structured growth curve for male and female starry flounders in Bellingham Bay, Washington.* N = *259; maximum* N *per data point = 25. Points for fish older than 7 years represent a weighted mean for adjacent years. The 95% confidence interval (bar) increased with age, and was less than 2.0 for all but the indicated points.* N = *3 for circled point.*

dioisotope decay rates (Bennett et al. 1982) and tetracycline marking of wild fish (Chilton and Beamish 1982) have confirmed the accuracy of cracked and burnt annuli in other species (as well as the inaccuracy of external readings). Because the appearance of such annuli differs little among species or age groups, there is no reason to doubt the age determinations reported here.

From the internal age readings reported here, an age-structured growth curve can be developed for the Bellingham population of starry flounders (Fig. 3). Males and females were plotted separately to emphasize the differences in their growth rates. Although females were the largest, the longest-lived individuals were male. The growth rate of males dropped sharply after age II, and that of females decreased after age III. Orcutt (1950) used scales and externally read otoliths to determine size at age of starry flounders in California. His data suggest a relatively rapid growth rate and only a slight difference between the sexes. Maximum fish age was reported to be 5 years (standard length = 39 cm) and 7 years (standard length = 51 cm) for males and females, respectively. My results demonstrate that the growth of Washington starry flounders slows after sexual maturation, particularly in the males. Males matured at age II and females at age III (Campana, unpublished). In addition, maximum observed ages of 24 and 17 years were recorded in males and females respectively. The age-structured growth rates of flounders in California and Washington may differ. However, recent improvements in ageing methodology probably contribute to apparent differences in growth rate between the areas.

Acknowledgments

I thank Paul Bentzen, Mark Ridgway, and Doug Walton for field assistance. Jim Simon and Randy Stone provided technical assistance. Jacques Gagné and Kees Zwanenburg made useful comments on an earlier version of the manuscript. This study was funded through a Natural Sciences and Engineering Research Council of Canada grant to Norman Wilimovsky (flounder collections) and operating funds of the Marine Fish Division.

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