

# Validation of the dorsal spine method of age determination for spiny dogfish

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**GORDON A. McFARLANE AND  
RICHARD J. BEAMISH**

DEPARTMENT OF FISHERIES AND OCEANS  
FISHERIES RESEARCH BRANCH  
PACIFIC BIOLOGICAL STATION  
NANAIMO, BRITISH COLUMBIA V9R 5K6  
CANADA

## ABSTRACT

The annuli that formed on the spines of 68 spiny dogfish Squalus acanthias, at liberty for 2 years or more after marking with oxytetracycline, corresponded to the years at liberty. Because the recovered fish ranged in age from 17 to 70 years we consider that this age determination method is valid. The grouping of marks or the exclusion of ages because annuli were difficult to interpret has led to a misunderstanding of life history parameters, particularly the over-estimation of growth rate. A revised method of age determination based on the validation study is proposed.

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Spiny dogfish Squalus acanthias have been exploited for their oil since the 1870s. By the early 1940s, dogfish became the single most important groundfish species in the commercial fishery off the west coast of Canada (Ketchen, 1986). This fishery collapsed in the late 1940s as a consequence of declining stocks and decreasing demand. The stocks recovered more quickly than anticipated and by the late 1950s spiny dogfish were considered a nuisance. The relatively rapid increase in stock size resulted from the recruitment of the unexploited juvenile biomass (Wood et al. 1979).

At present there is a small commercial fishery for dogfish that does not reduce its nuisance to other fisheries. Effective management of this species must consider the value of a commercial fishery, as well as the nuisance caused by incidental catches and the importance of this predator in the ecosystem. The basis for these management decisions requires accurate estimates of age.

Ketchen (1975) estimated age using growth zones on the second dorsal spine, accepting only very clear zones, and

excluding ages that differed  $\pm 2$  yr. Similarly, Holden and Meadows (1962) rejected all readings from spines which appeared to be worn down below the first annual ring. Bonham et al. (1949) selected spines for which there was little disagreement. The consequences of accepting or rejecting ages in these studies were not evaluated.

The present study validated the method of age determination using the second dorsal spine. This resulted in a modification of the method previously described (Bonham et al. 1949; Ketchen 1975). This paper also provides the detailed methodology on the age validation studies that were not included in a previous paper (Beamish and McFarlane 1985) on the histology and growth of the spine in relation to annulus formation.

#### METHODS

Dogfish were tagged with a modified Petersen disc in the Strait of Georgia (Fig. 1) between 1980 and 1983 (McFarlane and Beamish 1986). Approximately 52% of the fish tagged received either an intermuscular or intraperitoneal injection of 25 mg/kg of oxytetracycline (OTC) (Table 1). The dosage was similar to that recommended by Beamish et al. (1983) for sablefish *Anoplopoma fimbria*; laboratory tests found this dosage to produce a strong mark in the spine without causing mortality.

All dogfish receiving OTC injections were caught on a longline anchored to the bottom at depths from 20 m to 220 m (79%), or in a bottom trawl (21%), and held in a 3000 L holding tank which received a constant flow of seawater. Fish to be tagged and injected were transferred into a smaller (150 L) fiberglass tank and anaesthetized using tricaine methane sulfonate (MS222). Each fish was measured from the tip of the snout to the tip of the upper lobe of the caudal fin when depressed in a line horizontal with the body. After length and sex were determined, fish were tagged and injected with OTC. Some fish were held in recovery tanks prior to release if they had not recovered from the anaesthetic by the end of the tagging operation.

Table 1. Number of spiny dogfish tagged, tagged and injected, and released from 1980-1983, and number of fish recovered by year as of December 31, 1984.

Release year	Number released total	Number <sup>a</sup> released injected	Number of injected fish recovered by year (spine recovered)					Total
			1980	1981	1982	1983	1984	
1980	7482(2721) <sup>b</sup>	1460	3(2)	9( 5)	6( 4)	7( 5)	6(27)	31( 18)
1981	6968(3574) <sup>b</sup>	3572	-	13( 8)	83( 54)	82(57)	47(29)	225(148)
1982 <sup>c</sup>	10502(10502) <sup>b</sup>	7981	-	-	67( 50)	92(70)	79(58)	238(178)
1983	1613(1613) <sup>b</sup>	812	-	-	-	7( 6)	7( 4)	14( 10)
Total	26565(18410)	13825	3(2)	22(13)	156(108)	188(138)	129(93)	508(354)

<sup>a</sup>Both intermuscular and intraperitoneal.

<sup>b</sup>Number released from cruises where fish were injected.

<sup>c</sup>3965 (2938 injected) were trawl caught, all others longline caught.

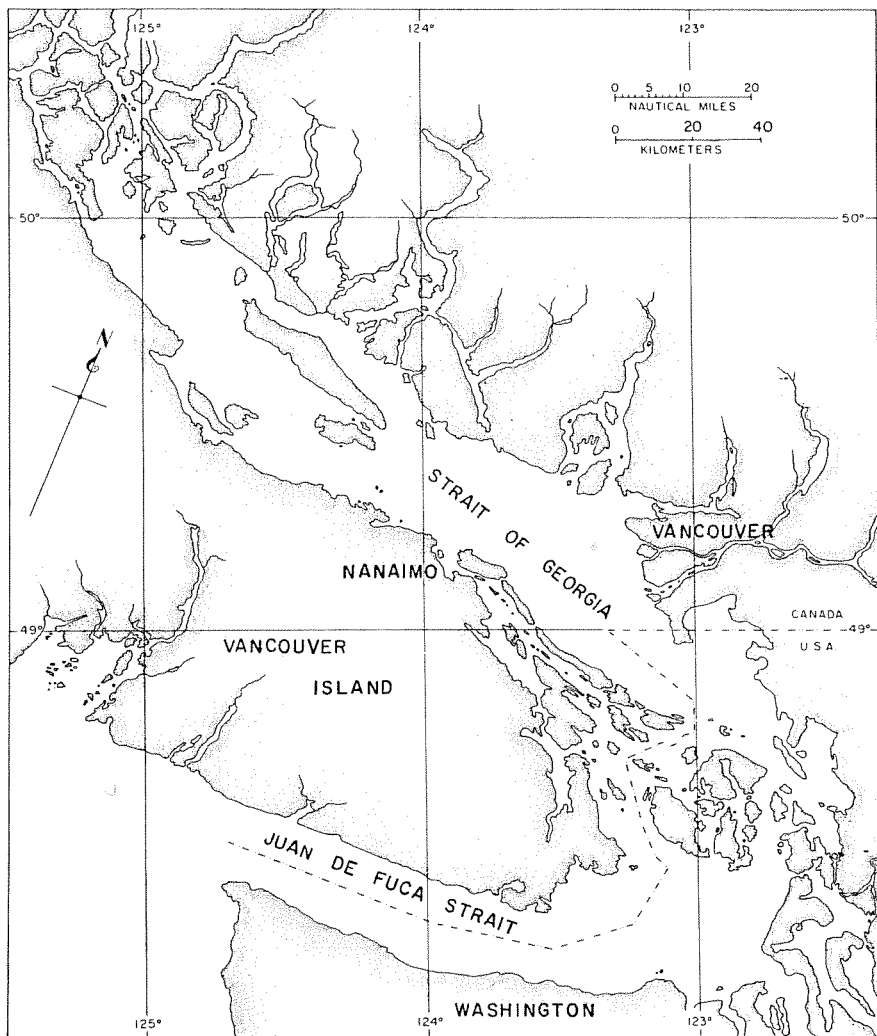


Figure 1. Study area, Strait of Georgia, for spiny dogfish tagged and injected with OTC, 1980-1983.

Recaptured fish were measured and the second dorsal spine (Fig. 2) removed by cutting horizontally just above the notochord to ensure that the spine base and stem were intact. These spines were frozen prior to processing according to the procedure outlined by Chilton and Beamish (1982). Three readers identified the annuli on each spine using UV light and a dissecting microscope. The distance from the OTC mark on the

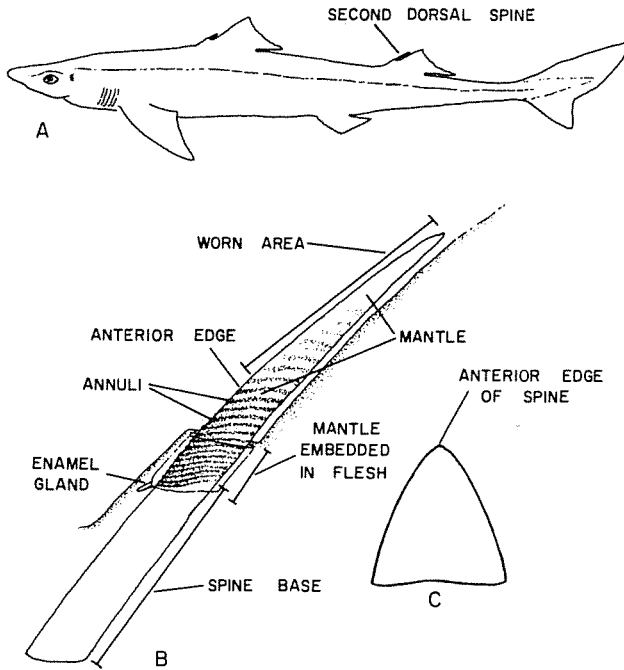


Figure 2. (A) Diagram of a spiny dogfish showing position of second dorsal spine. (B) Second dorsal spine with structural components identified. (C) Cross section of second dorsal spine.

leading edge of the spine to the base of the enamel was measured. Using this measurement the position of the OTC mark was identified under reflected light and the number of annuli that formed after the mark were counted. The annulus was defined according to Beamish and McFarlane (1985) as a darkened band or ridge or both, present on the enamelled portion of the spine.

Estimates of growth in length of spiny dogfish were obtained using the von Bertalanffy growth equation. The median length at 50% maturity for female dogfish was identified using probit analyses (Leslie et al. 1945), and the inflection of the growth curve.

## RESULTS

A total of 26,565 spiny dogfish were tagged and released between 1980 and 1983, of which 13,825 received an injection of OTC (Table 1). Of these, 11,211 were injected intermuscularly and 2616 intraperitoneally. Recoveries as of December 31, 1984, were used in this report. The recovery percentage for fish

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injected intermuscularly (3.7%) was not significantly different (t-test  $P \geq 0.05$ ) for fish which received an intraperitoneal injection (3.6%). Thus recovery results of injected fish have been pooled.

The recovery percent (6.3%) of fish tagged and injected with OTC in the fall (November 1981) was significantly greater (t-test  $P < 0.05$ ) than for fish tagged and injected in the spring (March-July, 1980-1983) (2.8%) (Table 2). The higher recovery percentage from fish released in the fall was probably a result of tagging larger fish which would be more susceptible to capture in the commercial fishery. The percentage of fish (83%) which had an identifiable OTC mark on the spine was also greater for fish tagged and injected in the fall than in the spring (53%).

Table 2. Recovery percentage of spiny dogfish from fall (1981) and spring (1980-1983) releases and percentage of dorsal spines recovered which show an OTC mark.

	Fall release (Nov 1981)	Spring release (1980-1983)
No. released	3573	10254
No. recaptures	225	283
Percent recaptured	6.3	2.8
No. spines recovered	148	206
Percent spines with OTC mark	83	53

In the spring cruises, where approximately 75% of the fish received OTC injections, the recovery rate of injected (2.8%) and uninjected (2.9%) fish was similar, indicating that there was no mortality introduced with the injection of OTC.

A total of 508 fish have been recaptured and spines were recovered from 354 of these fish. Sixty-six percent (234) of the recovered spines had an OTC mark. Approximately 20% of the spines were not used in the validation study because they were damaged or the wrong spine was collected.

### Validation

Preliminary results are summarized in Beamish and McFarlane (1985) and have been updated for this report (Table 3). In the present study we used fish that had been recovered after being at liberty for two years or more; those at liberty less than two years were excluded because the slow growth of the spine made it difficult to separate the OTC mark from the annulus. Of the fish examined that had an OTC mark, 41 fish had been at liberty for two years, 23 for three years, 2 for four years, and 2 for five years (Table 3). Fish at liberty for two, four, and five years developed 2, 4, and 5 annuli, respectively, after the OTC mark formed (Fig. 3). Of the 23 fish at liberty for three years, 20 had developed 3 annuli, and in 3 spines only 2 annuli could be identified.

Table 3. Expected number of annuli that formed on spines from fish tagged and injected with oxytetracycline that had been at liberty for 2 or more years.

Expected no. of annuli (yr) beyond OTC mark <sup>a</sup>	n	Estimated no. of annuli (n)	Age range (yr) at time of recapture (n) <sup>b</sup>
2	41	2(41)	17-58(39)
3	23	3(20), 2(3)	19-70(17)
4	2	4(2)	26-43(2)
5	2	5(2)	40(1)

<sup>a</sup>Annulus formation was assumed to occur from November to April. A fish that was expected to form 2 or more annuli had to be at liberty for at least two consecutive November to April periods.

<sup>b</sup>Ages could not be estimated on all spines because portions of the spine were damaged.

The OTC mark frequently appeared as a double line (Fig. 3B). Because spine growth is slow and OTC is incorporated into the tissue over a short time (Milch et al. 1958), the 2 OTC marks must have formed simultaneously. This means that one mark was deposited in the enamel and the other in the mantle dentine (Beamish and McFarlane 1985).

In most samples the annulus was easily identified as a ridge that was darker than the areas above and below it (Fig. 4A). In some older fish, annuli that formed after the OTC mark were quite closely spaced (Fig. 4B), but under reflected light they were very distinct, forming prominent darkened ridges. In others, annuli could be identified only on the anterior edge of the spine (Fig. 3A). In younger or faster-growing fish the annuli were more widely spaced and ridges were less prominent (Fig. 4C). Spines showing abnormal enamel deposition (Fig. 4D) or extreme wear (Fig. 4E) were common, however, annuli were still readily identified on the enamelled portion of the mantle. Spines displaying variable growth zones (Fig. 4F) were quite common and easily aged using our definition of an annulus. No checks were observed in any of the tagged and injected mature fish examined.

Fish that had been at liberty for 2 or more years ranged in age from 17 to 70 years. All spines showed some wear at the tip. The number of missing annuli were estimated using the procedure of Ketchen (1975) and added to the annuli count to produce an estimated age. For example, the youngest fish recaptured in this study (17 yr) had an estimated 4 annuli worn from the tip of the spine.

#### Growth and Maturity

The Strait of Georgia growth curve developed using the validated age determination techniques indicated slower growth than reported previously (Bonham et al. 1949; Ketchen 1975)

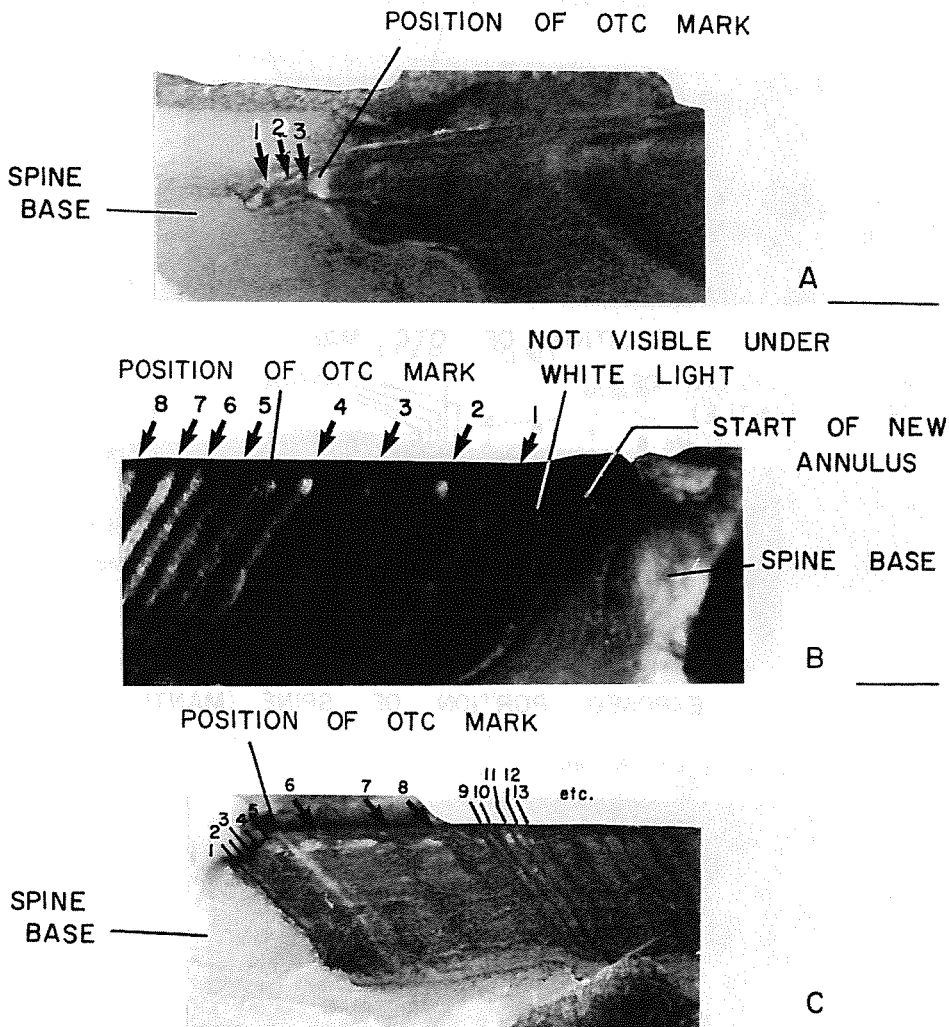


Figure 3. Dorsal spines from recaptured tagged and injected spiny dogfish. All spines photographed using ultraviolet light. (A) Spine from 87 cm male recaptured in April 1984 after 3 yr at liberty. Note that annuli formed after the mark appeared on the anterior edge of the spine only. (B) Spine from 75 cm male, recaptured in late 1984 after 4 yr at liberty. (C) Spine from 91 cm female, recaptured in January 1985 after 5 yr at liberty, showing variable spacing between annuli and the potential for grouping annuli. Annuli are numbered beginning with the most recent year. (Scale bar = 2mm).

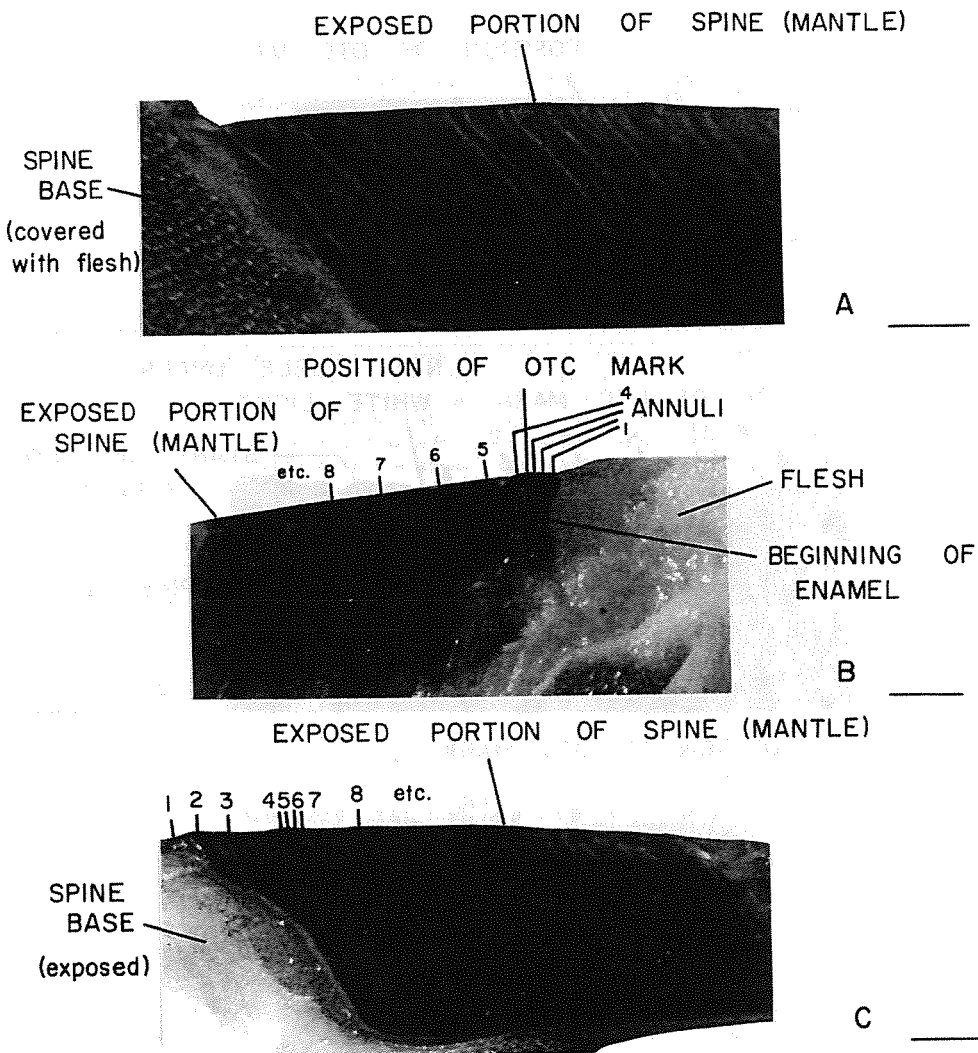


Figure 4. Examples of dorsal spines from recaptured tagged and injected spiny dogfish. All spines photographed using white light. (A) Annulus easily identified as a ridge that is darker than areas above or below it. (B) Spine from a slow-growing fish at liberty 3 yr showing crowded ridge-like annuli. Position of OTC mark is indicated. (C) Spine from a faster-growing fish. Note annuli are wider spaced and ridges less prominent than A and B (above). (D) Spine showing abnormal enamel deposition in recent yr. (E) Spine showing extreme wear. (F) Spine displaying variable growth zones.



AREA OF ABNORMAL ENAMEL DEPOSITION

SPINE BASE



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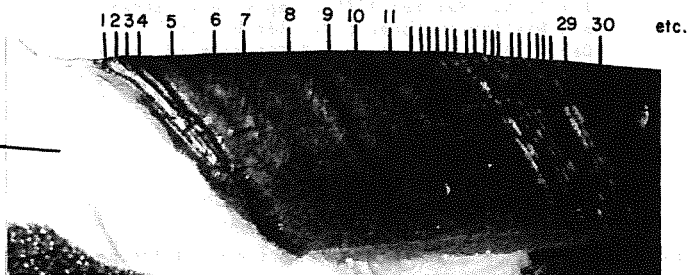
NO WEAR POINT

WORN PORTION OF SPINE (MANTLE)



E

SPINE BASE



F

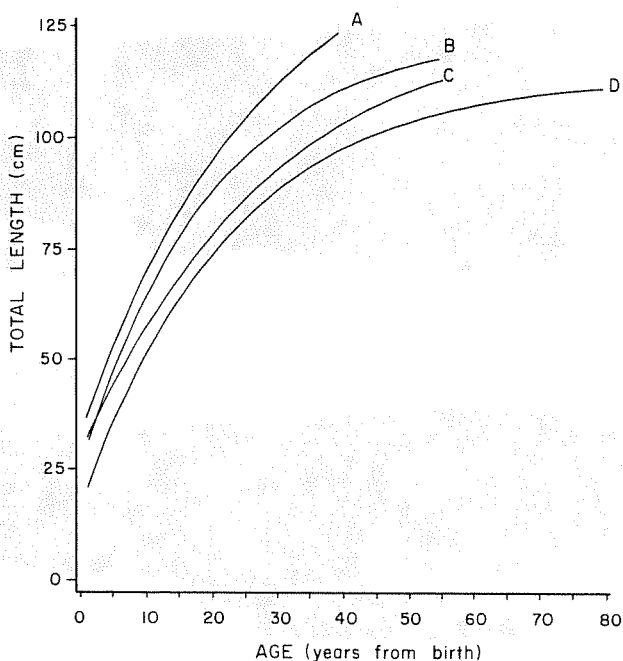


Figure 5. Comparison of von Bertalanffy growth curves for female spiny dogfish from the northeast Pacific Ocean. A. Bonham et al. 1949 (Washington coast from Ketchen 1975); B. Ketchen 1975 (composite of Strait of Georgia and Washington coast); C. Ketchen 1975 (Strait of Georgia); D. Present study (Strait of Georgia). (Data from Ketchen 1975 reproduced with permission of the author and editors of the *Journal of the Fisheries Research Board*, publisher.)

(Fig. 5). In addition, maximum ages obtained in this study (80+) were twice that reported by Bonham (as reported in Ketchen 1975) and 45% greater than those reported by Ketchen (1975).

Previous estimates of the age at 50% maturity for female spiny dogfish in the northeast Pacific Ocean range from 20 yr (west coast of Washington) to 31 yr (Strait of Georgia) (Ketchen 1975). Ketchen (1975) concluded that the age that best accommodates the data from Bonham et al. (1949) and Ketchen (1975) is 23 yr. In our study the age at 50% maturity for female dogfish for the Strait of Georgia is 35 yr (Fig. 6), similar to the maximum value reported by Ketchen (1975). Despite the difference in time and location of these previous studies, there can be little doubt that the difference in reported age at maturity is attributable, to a large degree, to the rejection of difficult-to-age spines and the grouping of annuli.

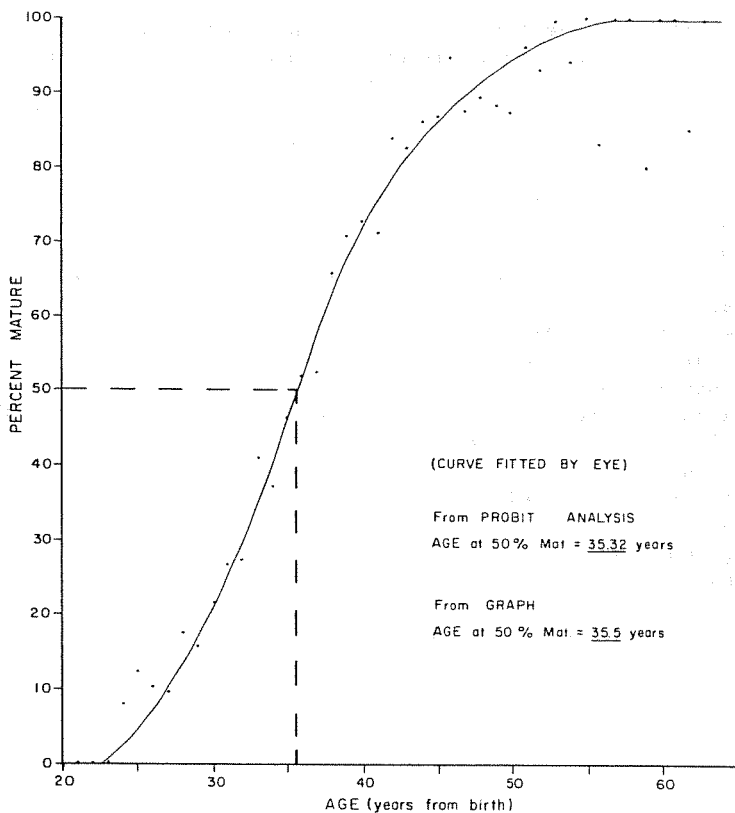


Figure 6. Age at 50% maturity for female spiny dogfish captured in the Strait of Georgia, fall of 1980 and 1981.

#### DISCUSSION

We can only assume that injected and tagged fish behaved in the same manner as untagged fish. This is a standard assumption made in tagging studies that is almost impossible to test. Accepting these assumptions, our study demonstrates that the method of ageing spiny dogfish using marks on the spine (Bonham et al. 1949; Holden and Meadows 1962; Ketchen 1975) does produce valid estimates of age, provided an annulus is defined as a darkened band or ridge or both. Darkened bands or ridges that are closely spaced are distinct annuli and must not be grouped. Once it is known that these zones must be counted the number of spines rejected as being difficult to age is greatly reduced.

Other marks or zones form annually in the spine (Beamish and McFarlane 1985) and these marks have been used by others to estimate age (Holden and Meadows 1962; Soldat 1982). A mark

forms in the stem dentine that is probably annual, but it is difficult to detect and requires the careful removal of enamel, pigment, and mantle dentine in order to identify marks in the exposed area of the spine (Beamish and McFarlane 1985). An annual mark also appears in cross section which results from the growth of the stem dentine. Once again the mark (zone) is indistinct, and because of the upward growth of the spine it is not possible to produce sections for older fish that have all the annuli. Beamish and McFarlane (1985) indicated that the annulus on the mantle is much clearer than the other two areas and that it be used exclusively.

Using our definition of the annulus we conclude that spiny dogfish are older, slower growing, and later maturing than previously thought. Previous investigations have tended to group annuli or reject difficult-to-read or worn spines (Bonham et al. 1949; Holden and Meadows 1962; Ketchen 1975). While it is not possible to compare directly the methodologies of these other investigations with this study, it is probable that these procedures did result in the exclusion of the older and slower-growing component of the population, which can affect understanding of the biology of this species.

As a result of the present study, we recommend that the method for ageing spiny dogfish be modified as follows:

1. Remove spine by cutting close to the notochord; the spine is then air dried and cleaned.
2. Count annuli using reflected light and a dissecting microscope using sufficient magnification (120-250x).
3. All zones that occur on the enamelled surfaces that are darkened bands, ridges, or both must be counted; spacing should not be an overriding factor in the decision to count an annulus.
4. If the tip of the spine is worn then the number of missing annuli can be estimated. The "no wear point" occurs at the first complete annulus below the worn spine. The diameter of the spine is measured at this point, and the number of missing annuli are estimated using the procedure of Ketchen (1975) and added to the number of zones counted on the spine. We used Ketchen's original data to estimate the 95% confidence interval (Fig. 7). The interval increases with the spine base diameter (number of missing annuli). For example, an estimate of 20 missing annuli can range from 10 to 30 annuli.

#### ACKNOWLEDGMENTS

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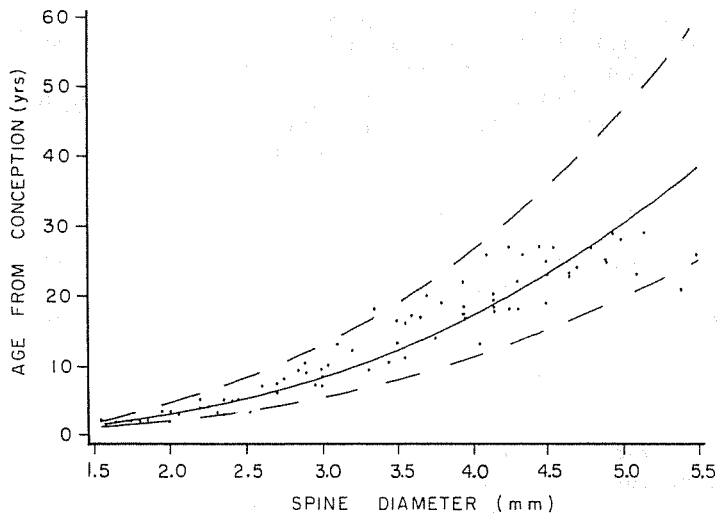


Figure 7. Relationship of age of spiny dogfish to the diameter of the base of the second dorsal spine,  $y = 0.5097x + 2.5469$  (Ketchen 1975). (Original data from Ketchen 1975 used with permission of the author.)

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