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Communication

Ageing of shortspine spurdog in the Andaman Sea of Thailand

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Abstract: Shortspine spurdogs (*Squalus mitsukurii*) were sampled from the Andaman Sea of Thai waters with bottom vertical longline and deep sea traps. The total length (TL) of the individual sample was measured and the second dorsal spine was removed. The number of growth bands on the external surface of the spine were counted using a dissecting microscope. Sixty-five *S. mitsukurii* (20 males and 45 females) were used in this study. TL of the fish ranged from 43.4–72.1 cm for females and 44.9–50.5 cm for males. The estimated ages were 4–12 years old. The relatively low coefficient of variation (CV) values at 22.90% implies the consistency of age-reading between the two readers and make merit to the estimated ages. The relationship between the enamel base diameter (EBD) and TL is positively linear as EBD = 10.66 + 7.58TL ($R^2 = 0.751$, $S_{y.x} = 0.07$). The result from ANCOVA indicates that there is no significant difference in the lengths at any given age between males and females ($F = 0.9966$, $p = 0.3221$). The EBD from the second dorsal spine of *S. mitsukurii* can be used to determine its age. The ageing method by sectioning the spine can also be employed to verify the estimated age from the external surface of the spine. The age reading taken from the external surface compared to the cross-sectioned spine shows no significant difference ($\chi^2 = 0.848$,$p = 1.000$). The age reading for *S. mitsukurii* is therefore easily determined from the external band of the second dorsal spine.

Keywords: age determination, shortspine spurdog, *Squalus mitsukurii*, second dorsal spine

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INTRODUCTION

The shortspine spurdog (*Squalus mitsukurii,* Jordan & Snyder, 1903) is an elasmobranch fish (Figure 1) which is commonly found in cold to temperate tropical waters near or at the bottom of the continental shelf mostly at a depth of between 100–700 m. This species has a wide distribution in the Atlantic, Pacific and Indian Oceans [1]. Its distribution in Thai waters is at depths from the surface to deeper than 150 m [2], and in the Andaman continental shelf it is present at up to a depth of 90 m [3]. The conservation status of the species is now considered as 'data deficient' by the International Union for the Conservation of Nature and Nature Resources (IUCN) red list of threatened species [4].

Figure 1. *Squalus mitsukurii* Jordan & Snyder, 1903

Similar to other shark populations elsewhere in the world, fisheries and market demand are the major causes of stock depletion. In addition, the slow growth rate, long gestation and low fecundity are all factors that make the populations of *S. mitsukurii* decline globally [2]. For example, it was found that catches of *S. mitsukurii* in Australian coastal waters were down from an average of 44.8 kg per hour during 1976–1977 to 1.2 kg per hour from 1996–1997 [4].

The ageing of fish in a population is of fundamental importance in fishery biology, and an understanding of the age structure and growth of fish populations is crucial for effective conservation and management. The band counting technique (i.e. from vertebrae, dorsal fin spine, caudal thorn and neural arch) is a common technique used for age determination in elasmobrach fish because of a lack of hard parts [5]. Dogfish species (Family Squalidae) normally have spines with a banding pattern on the external surface and these are commonly used for age determination [6-8]. An annulus is usually defined as the winter band. For example, the age and growth of *S. blainvillei* and *Chimera monstrosa* were studied using the external and transverse section of the whole spine [5]. For *S. mitsukurii* in Thai waters, information is limited and more study on the life history in relation to age and growth is needed to define the population status for management plans.

This study is aimed at finding an appropriate ageing technique for dorsal fin spines of *S. mitsukurii* from the Andaman Sea. In addition, the consistency of age estimates between external and internal bands are also compared. We hope that the technique will be useful for age determination in other *Squalus* species. Moreover, it will be the input parameters for fitting the growth model and bridge the gap of information on the life history of *S. mitsukurii* in Thailand.

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MATERIALS AND METHODS

Sample Collection and Spine Preparation

Sixty-five *S. mitsukurii* (20 males and 45 females) were taken from the continental shelf of the Andaman Exclusive Economic Zone (EEZ) with bottom vertical longline and deep sea traps in March 2007. The sex was determined, the total length (TL) was measured to the nearest centimetre and the body weight taken to the nearest gram. The second dorsal spine was removed by cutting horizontally above the notochord, ensuring the spine base and stem was intact [9–12]. The spines were cleaned by thawing and dipping in boiling water. The tissue was removed by scalpel, washed under tap water and air-dried for at least 24 hr. The spines were then kept in zip-lock plastic bags for their age determination.

The spines were measured in terms of last readable point (LRP), enamel base diameter (EBD), spine base diameter (SBD), base length (BL) and spine total length (STL) using vernier callipers (±0.01 mm) [8] (Figure 2).

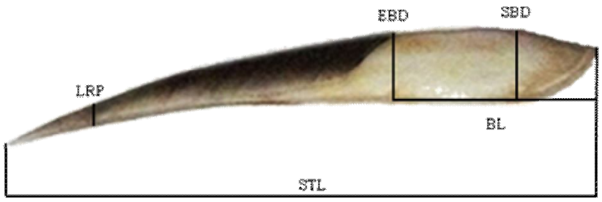

Figure 2. Lateral view of the second dorsal spine showing spine measurements

Age Determination

There are two types of band formed in a year. An opaque band from a faster growth usually occurs in summer, producing a wider band, and a translucent band from a slower growth usually occurs during winter/spring, producing a narrower band; the annuli is composed of one translucent and one opaque band. For this ageing study each spine was read at the external surface band and the cross-sectioned band under a dissecting microscope. After the external band examination was completed, the spine was marked at the heights of 0.5 mm and 1 mm from SBD and embedded in an epoxy resin mould. The spine was cross-sectioned at the marked position by a low-speed diamond wheel saw with a thickness of about 500 µm. The sections were affixed onto a slide glass with crystal bond and polished with a fine-grade wet sandpaper (P 2000, P 2200) until the thickness was 250–350 µm. The age was read at the inner dentine layer at the clearest banding pattern. Both readings were 'blind-check' counted by two readers. All samples were re-examined two weeks after the first readings.

Statistical Analysis

The coefficient of variation (CV) was calculated to test the precision of age reading between the two readers [13]. The CV was expressed as follows:

$$CV = 100 * \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where R = the number of times each fish is aged
X_{ij} = the i^{th} age estimation of the j^{th} fish
X_j = the average age calculated for the j^{th} fish

The age determination was estimated as follows: 1) the age reading from the external surface of the spine compared to EBD cross-sectioned spine was tested by chi-square; 2) the relationship between enamel base diameter (EBD) and spine total length (STL) was analysed by linear regression; and 3) the difference in length at any given age between male and female sharks was checked by analysis of covariance (ANCOVA). All statistical analyses were done under R statistical program [14].

RESULTS AND DISCUSSION

The second dorsal spine of *S. mitsukurii* is of a 'non-worn' type because the diameter at LRP is less than 2.45 mm [8]. The TL of fish ranged between 44.9–50.5 cm for males and 43.4–72.1 cm for females. The estimated ages were 4–8 years for males and 4–12 years for females. The precision (mean CV) of age reading between the two readers was 22.90%. In general, the fact that the CV is less than 30% implies a consistency in age reading of the readers [8]. This and the estimated ages provide merit for further analysis, although the age determination of *S. mitsukurii* in this study is less precise than that for the other dogfish sharks; for example, the CV for *S. acanthias* was 19% [15].

Because the spines were too fragile, only twelve spines were successfully cross-sectioned and were further used for age determination. Meanwhile, the enamel surface of the whole spine was observed from the enamel base to the tip. For the sectioned spine, the growth band was most distinguishable at the inner dentine layer. The growth bands that appeared in both areas gave the same banding numbers (Figures 3 and 4).

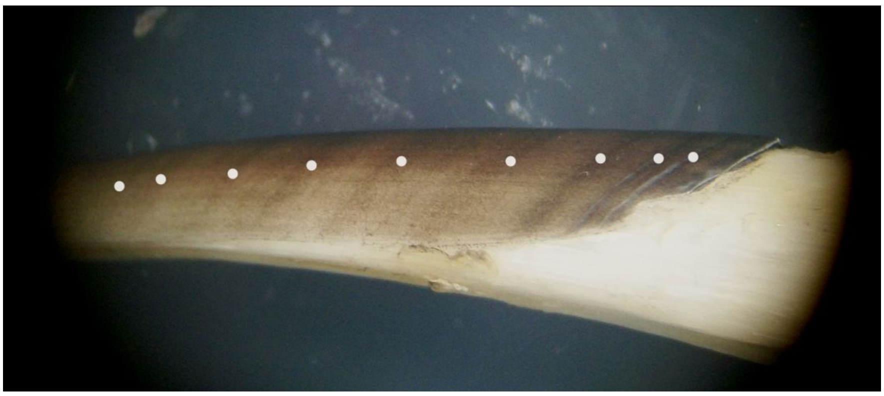

Figure 3. Second dorsal spine showing nine growth bands on the external enamel surface

Age reading of *S. mitsukurii* from the external surface and cross-sectioned spine ranged between 7–11 years and the mean CV between both methods was 12.58%. The age determinations from external surface and cross-sectioned spine were tested by chi-square (χ^2). The results showed a non-significant difference ($\chi^2 = 0.84$, $p = 1.00$). Thus, the age reading from the external surface of spine could be used effectively as reported by Irvine et al [16]. The relationship between EBD and TL was positively linear as EBD = 10.66 + 7.58TL ($R^2 = 0.751$, $S_{y.x}=0.07$) (Figure 5).

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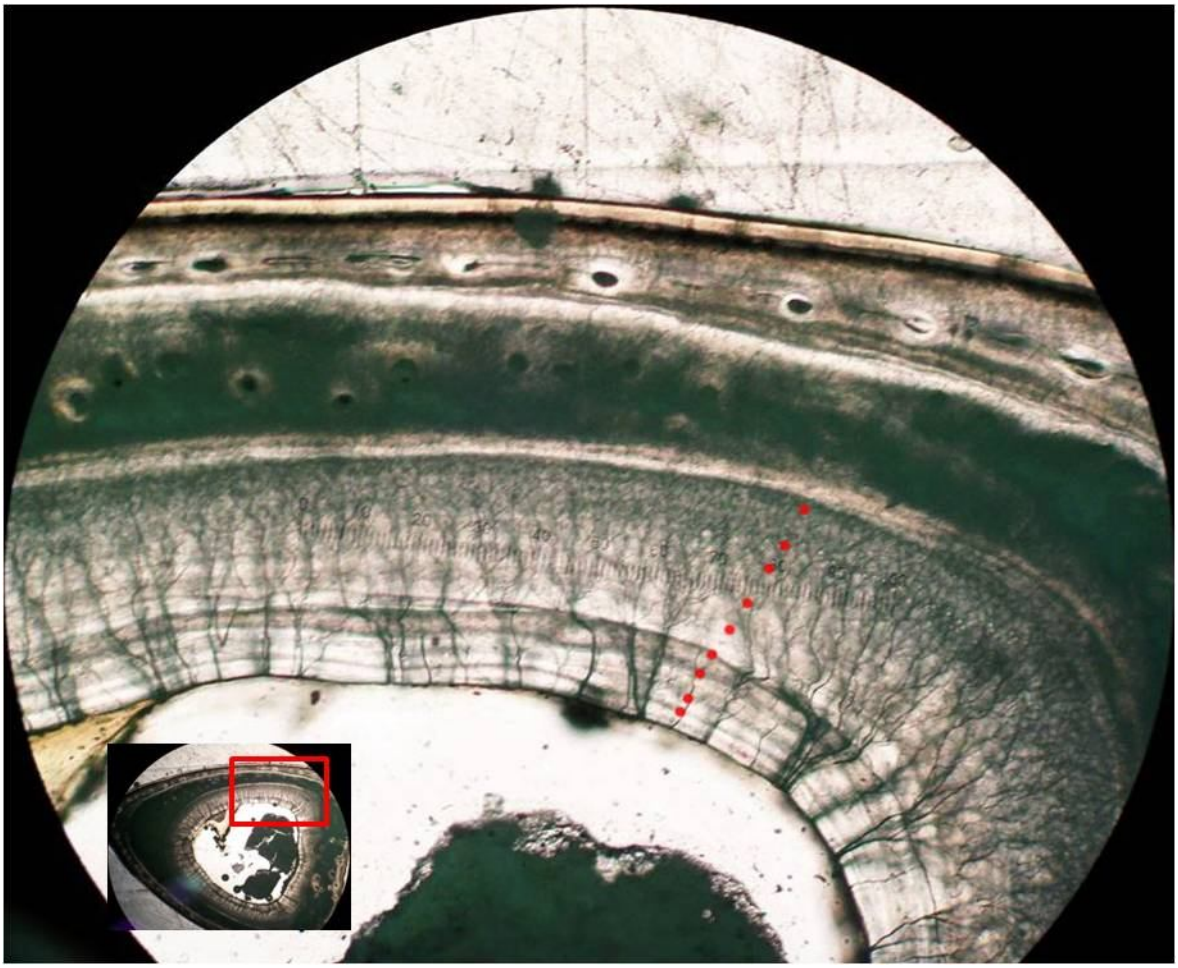

Figure 4. Inner dentine layer of the second dorsal spine of the same *S. mitsukurii*, also showing nine growth bands

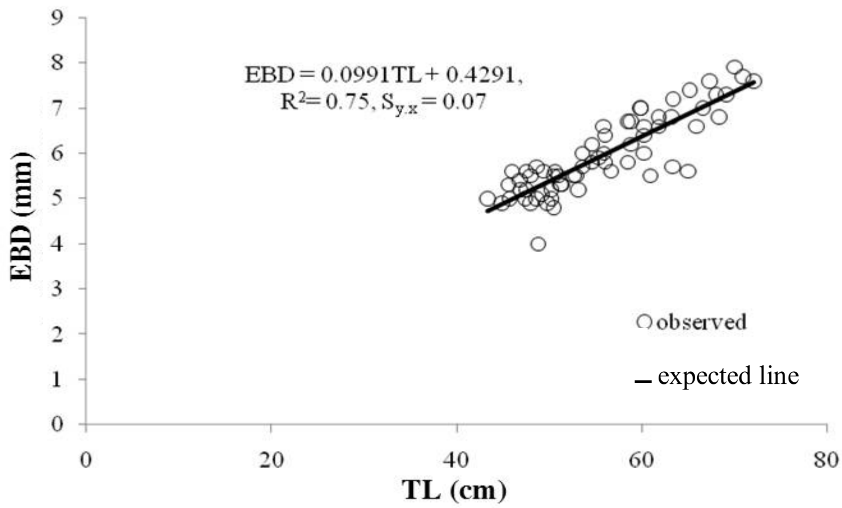

Figure 5. Relationship between TL and EBD of *S. mitsukurii*

From the relationship between TL and age, there was no significant difference between males and females (ANCOVA, F = 0.9966, p = 0.3221) (Figure 6). Thus, for Thai stock of *S.*

mitsukurii, there is no different growth rate between males and females, which differs from some other studies which showed that that the female mostly grows faster than male [17-19].

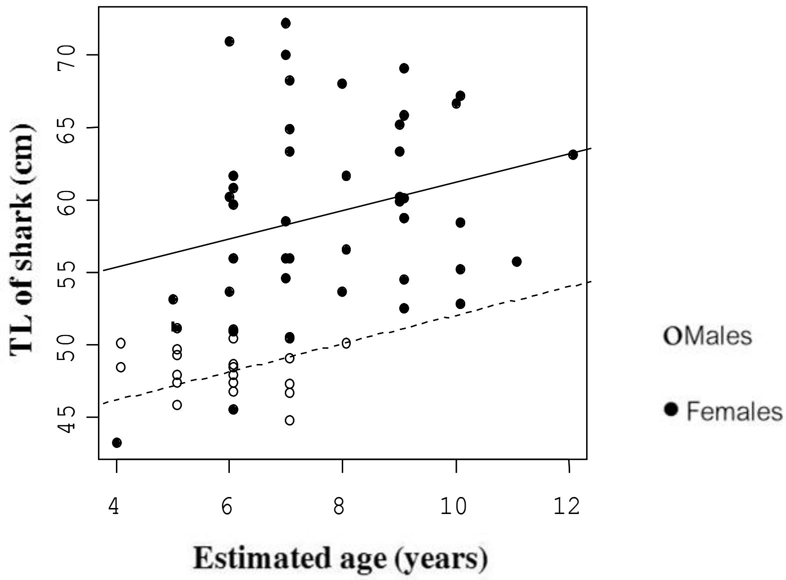

Figure 6. Relationship between age and TL of male (open circle) and female (closed circle) *S. mitsukurii*

CONCLUSIONS

Age analysis of elasmobranchs is generally difficult due to their placoid scales and lack of otholits. Although we may use calcified yearly rings in the vertebrae of many groups of elasmobranchs as well as length-frequency analysis, such methods are complicated and time-consuming and some of them are even unsuccessful. For the group of Squalidae, the most appropriate hard part for age determination is the dorsal spine, which is easily recognised from the external surface. We may also use the enamel base diameter from a cross section of the dorsal spine for determining the growth band if we have the necessary specific apparatus such as a low-speed diamond wheel saw. In this study, the ages of *Squalus mitsukurii* (4–12 years old), with the total length of 43.4–72.1 cm for females and 44.9–50.5 cm for males, have been successfully estimated by annuli reading from the external surface band of the second dorsal spine. The success of this study may also provide an age-reading technique for other spurdog species.

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