A Preliminary Estimate of Age and Growth of Two Populations of Dasyatid Stingray *Urogymnus polylepis* in Thailand

Pisit Phomikong[1], Suwimon Seehirunwong[1] and Tuantong Juatagate[2*]

ABSTRACT

Age and growth of the stingray *Urogymnus polylepis* were determined, for the first time, from bands in the vertebrae. Samples were obtained from naturally dying specimens in the two major rivers in central Thailand. Eighteen stingrays with disc length (DL) ranging between 30 and 187 cm were collected from the Chao Phraya River, while 53 fish ranging between 30 and 250 cm were collected from the Mae Klong River. The von Bertalanffy parameters were calculated for the Chao Phraya and the Mae Klong rivers as DL_∞ = 195.6 and 301.3 cm, K = 0.26 and 0.11 year^{-1} and DL_0 = 28.1 and 27.2 cm, respectively. The likelihood ratio test showed significant difference in growth between the two populations.

Keywords: Ageing, *Urogymnus polylepis*, Vertebra, Von Bertalanffy growth parameter

INTRODUCTION

Declines in populations and increases in exploitation of elasmobranchs have been substantial worldwide, despite attempts at the conservation and management of these cartilaginous fishes (SEAFDEC, 2017). To sustain the populations of these fishes, an understanding of their age and growth is needed for constructing age-structured population dynamic models, which can be further used for fisheries management (Cailliet, 2015; Harry, 2018). This type of study is important and urgent in Southeast Asia, which is rich in elasmobranchs, but where the status of their stocks is still largely unknown (SEAFDEC, 2017).

The freshwater elasmobranchs are more vulnerable than their marine relatives, either by their biology, as having strongly K-selected life histories and feeding at high trophic levels, or by their habitat constraints, i.e., more limited water volume and more physicochemical variability than the ocean (Martin, 2005). Although this group of species is more vulnerable, few studies have examined their age and growth, which are among the most fundamental aspects needed for fisheries management. The threatened Dasyatid stingray, the giant freshwater stingray (*Urogymnus polylepis*, syn. *Himantura chaophraya*), is one of these freshwater elasmobranchs, and is the focus of this study. The members of the stingrays in the Dasyatidae family include species that are marine-living or euryhaline, as well as species that are restricted to freshwater environments (Otake *et al*., 2005). It is these freshwater species that have never been described in terms of their age and growth rates. This lack of knowledge limits the ability to assess their stock status and develop the appropriate management strategies (Martin, 2005; Cailliet, 2015).

The giant freshwater stingray is distributed throughout South and Southeast Asia, from India to eastern Indonesia, and is native to the Mekong

[1] Inland Fisheries Research and Development Division, Department of Fisheries, Thailand
[2] Fisheries Program, Faculty of Agriculture, Ubon Ratchathani University, Thailand
* Corresponding author. E-mail address: tuantong.j@ubu.ac.th
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and Chao Phraya river basins (Vidthayanon *et al*., 2013). This stingray lives mostly in freshwater habitats, but is also found in estuarine areas, and can grow up to 600 kg, with a 2-meter disc diameter (Iqbal and Yustian, 2016). The IUCN Red List classifies this stingray as endangered (Vidthayanon *et al*., 2013). In both river basins, the *U. polylepis* population is presently recognized as degraded due to human stressors, in particular infrastructure development and hydrological changes (Gray *et al*., 2017). These main stressors have not only threatened *U. polylepis* but also several other large fish species such as *Pangasianodon gigas*, *Pangasius sanitwongsei* and *Catlocarpio siamensis*. These species are more vulnerable due to their low resilience and capacity for adaptation, compared to small- and medium-sized riverine fish (Jutagate *et al*., 2016; Gray *et al*., 2017). Gray *et al*. (2017) recently reported that *U. polylepis* is perceived by fishers around the Siphandone waterscape in the Mekong mainstream, Lao PDR as the third most sharply declining species, after *P. gigas* and *P. sanitwongsei*.

Many techniques have been applied to the study of age and growth in elasmobranchs, based on growth patterns of hard parts, e.g., vertebral and dorsal fin spines (Cailliet and Goldman, 2004; Cailliet, 2015). Due to the lack of understanding of age and growth of the vulnerable *U. polylepis*, the objectives of our study were to estimate age by using cross-sections of vertebrae and to formulate the von Bertalanffy growth function. The study was conducted in two populations from the Chao Phraya and the Mae Klong rivers. The difference in growth between the two populations was also tested.

MATERIALS AND METHODS

Field sampling and laboratory work

Samples were taken from the Chao Phraya and Mae Klong river, which flow through central Thailand to the Gulf of Thailand (Figure 1). Both rivers are reported to have a high diversity of teleost fishes; more than 300 species have been listed in FishBase from the Chao Phraya River (Jutagate *et al*., 2016) and almost 100 species have been recorded from the Mae Klong River (Tongnunui *et al*., 2016). Since fishing for *U. polylepis* is prohibited in Thailand, the samples from both rivers were collected by officers of the Department of Fisheries of Thailand from fish that died naturally, i.e., by pollution or senescence, during the years 2012 to 2017. After the carcasses were labeled, they were measured in situ for disc-length (DL) and disc-width (DW) to the nearest 1 cm, following the standard procedures for elasmobranchs (SEAFDEC, 2017). Two or three anterior vertebrae at the caudal position of each specimen were removed for further laboratory study.

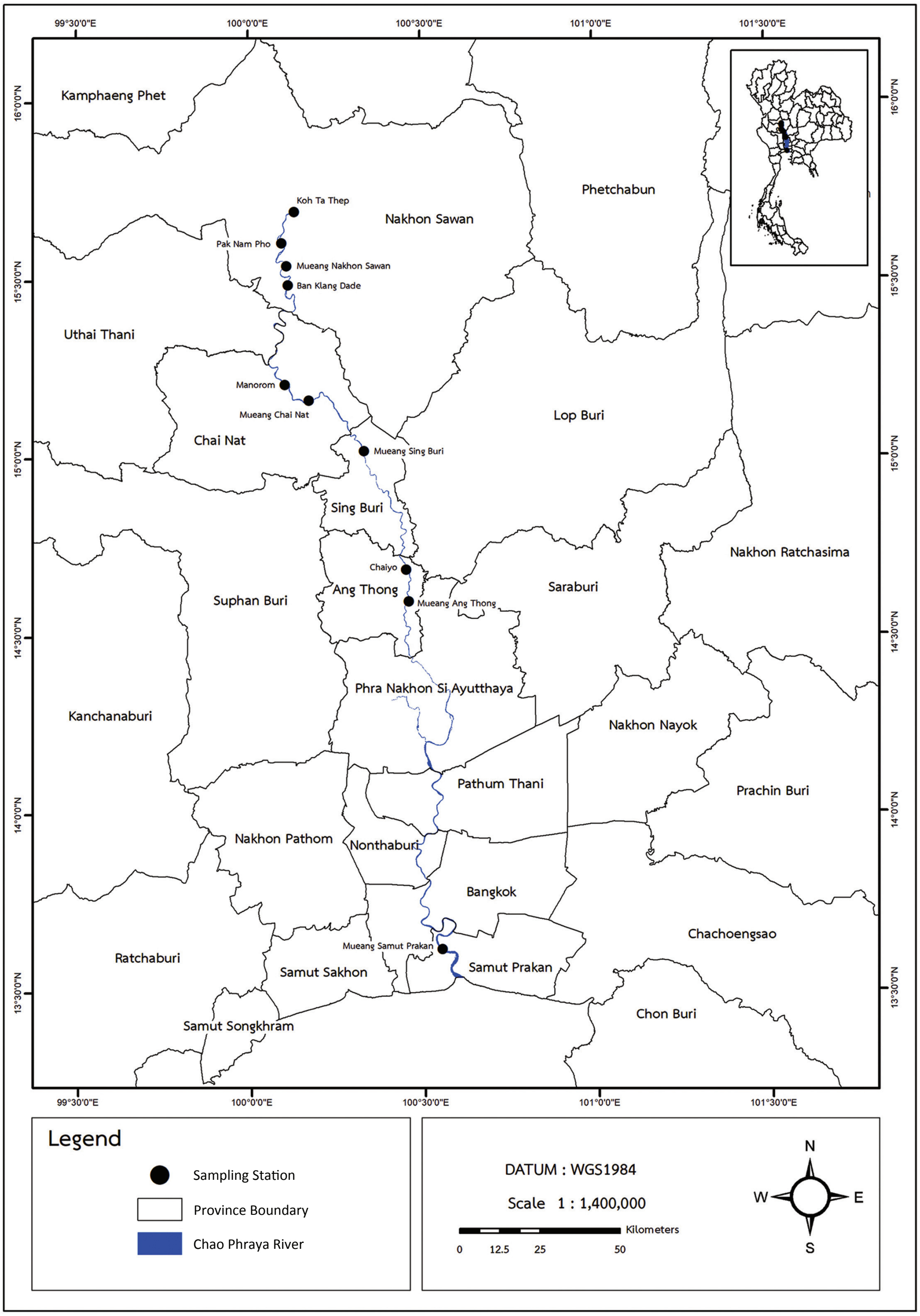
The vertebrae were prepared by removing any remaining connective tissue, soaking in 5% sodium-hypochlorite for 30 minutes, rinsing with distilled water, then air-drying and storage in vials for ageing. The dried vertebrae were measured for vertebral radius (VR), then sectioned by low-speed diamond saw (South Bay Technology, Inc., model 650). Each vertebra was sectioned longitudinally, to reveal the “bowtie” perspective with no embedding, and ground with wet fine-grit sand paper to a thickness of 0.3-0.5 mm. (Campana, 2014). The number of annual rings in the vertebrae of each sample was counted under light microscope by three readers, who had no information about the size of the sampled stingrays.

Data analysis

Difference between size ranges of the two populations was examined by the Kolmogorov-Smirnov two sample test (Smirnov, 1939). The relationship between DW and DL was examined by simple regression analysis. The relative precision of age estimates among the three readers, or verification (Campana, 2014; Ogle, 2016), was tested by both average percent error (APE, Equation 1) and coefficient of variation (CV, Equation 3).

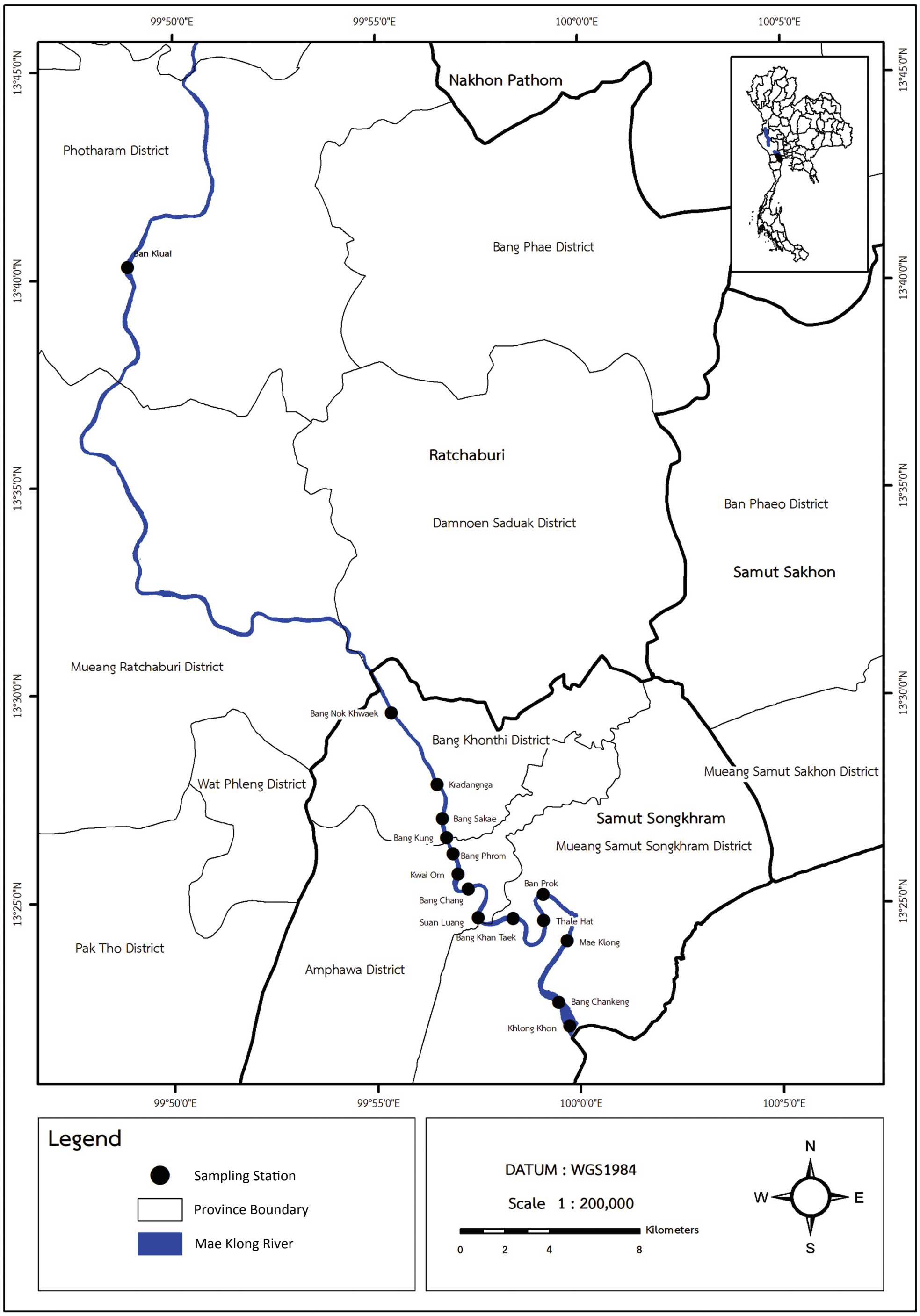
$$APE = \frac{\sum^{n}_{j=1} APE_j}{n} \qquad \text{--------- (1)}$$

where APEj is the average percent error for the j^{th} fish and calculated as



(a)

Figure 1. Sampling locations of giant freshwater stingray, *Urogymnus polylepis*, from the Chao Phraya River (a) and the Mae Klong River (b).



(b)

Figure 1. (cont.)

$$APE_j = 100 \times \sum_{i=1}^{r} \frac{\frac{|x_{ij} - \bar{x}_j|}{\bar{x}_j}}{n} \qquad \text{--------- (2)}$$

where x_{ij} = the ith age estimate on the j^{th} fish; $\bar{x}_j$= the mean age estimate for the jth fish; r = the number of times that each fish was aged; and n = the number of aged fish in the sample.

$$CV_j = 100\ \% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(x_{ij} - x_j)^2}{R-1}}}{x_j} \qquad \text{--------- (3)}$$

where CV_j is the age precision estimate for the j^{th} fish; x_{ij} is the ith age determination of the jth fish, x_j is the mean age estimate of the j^{th} fish, and R is the number of times each fish is aged.

The relationship between VR and DL was calculated to confirm that this hard part grew proportionally to the length of the stingray. The obtained DL at age data were further used to construct the modified von Bertalanffy growth function (modified VBGF, Equation 4; Fabens, 1965), which incorporates the size at birth to ensure that the curve passes through the length at birth (L_0) when age equals 0 (Kwangkhang, 2016):

$$DL_t = DL_0 + (DL_\infty - DL_0)\,(1 - e^{-Kt}) \qquad \text{--------- (4)}$$

where, DL_t is the (disc) length at time t, DL_∞ is the asymptotic (disc) length, and K is the growth coefficient. Appropriate starting values of the parameters for VBGF were obtained by the Ford-Walford Method (Equation 5; Walford, 1946) and Beverton and Holt Method (Equation 6; Beverton and Holt, 1957).

$$L_{t+1} = L_\infty\,(1 - e^{-K})\,) + e^{-K}\,L_t \qquad \text{--------- (5)}$$

$$ln\,(L_\infty - Lt) = (lnL_\infty + Kt_0) - Kt \qquad \text{--------- (6)}$$

where L_t and L_{t+1} pertain to (disc) lengths separated by a constant time interval. Then, the estimated parameters, L_∞ and K, were used as inputs for fitting the non-linear least squares regression with the obtained length at age data. After the modified VBGF curves of the two populations (Chao Phraya and Mae Klong) were constructed, they were compared for growth difference by a likelihood ratio test using chi-square (χ^2) statistics (Kimura, 1980). Data analyses were conducted under Package FSA (Ogle, 2016) within the Program R (R Development Core Team, 2016).

RESULTS

The sample sizes of the stingrays used in this study were 18 and 53 from the Chao Phraya and Mae Klong rivers, respectively. The mean DL (±SD) of the population from the Chao Phraya River was 115.0±56.6 cm from specimens ranging from 30 to 187 cm, while mean DL (±SD) was 143.4± 54.8 cm from specimens ranging from 30 to 250 cm from the Mae Klong River (Figure 2). There was no significant difference in DL between the two populations (Kolmogorov-Smirnov two sample test, D = 0.232, p-value = 0.467). High correlation was found between DL and DW (Figure 3), with the slope coefficient close to 1, implying the similar extent of these two dimensions.

There were four samples from the Chao Phraya population and three from the Mae Klong population for which the annual periodicity of banding, i.e., annual rings, were not clearly identified, and so were excluded from the analysis. The sizes of these samples were around 30 cm DL, which is similar to the size at birth reported by Last and Stevens (1994). The number of counted annual rings (Figure 4) of the *U. polylepis* samples ranged from 0 to 9 bands for Chao Phraya samples, and from 0 to 12 bands for Mae Klong samples. The percentage of fish for which age estimates agreed among all three readers was 69.23 %, with average APE for all samples of 2.2 %, and CV of 2.9. The levels of precision were thus considered acceptable.

The vertebral radius and DL were highly correlated for both populations, i.e., $R^2 = 0.93$ and 0.96, for the populations of the Chao Phraya River and Mae Klong River, respectively (Figure 5). From the length at age data (Figure 6), the estimated starting values for VBGF were calculated and are presented in Table 1. The scatter plots of the counted rings (age) versus DL, incorporated with the modified VBGF curves of the two *U. polylepis* populations are shown in Figure 6. The results from the likelihood ratio test showed that there was significant difference in growth between the two *U. polylepis* populations (χ^2-value = 12.4; P-value < 0.01); the modified VBGF of the two populations are as follows:

$$DL_t = 28.1 + (195.6–28.1)\ (1 - e^{-0.26t}) \quad \text{---- (7)}$$

for Chao Phraya River

$$DL_t = 27.2 + (301.3 - 27.2)\ (1 - e^{-0.11t}) \quad \text{---- (8)}$$

for Mae Klong River

Table 1. Starting values for VBGF of *U. polylepis* from Chao Phraya and Mae Klong rivers.

Parameters	Chao Phraya River	Mae Klong River
Age range of samples (year)	0-9	0-12
Size, *DL*, ranges (cm)	30-187	30-250
DL_∞ (cm)	195.6	301.3
K (year^{-1})	0.26	0.11
DL_O (cm)	28.1	27.2

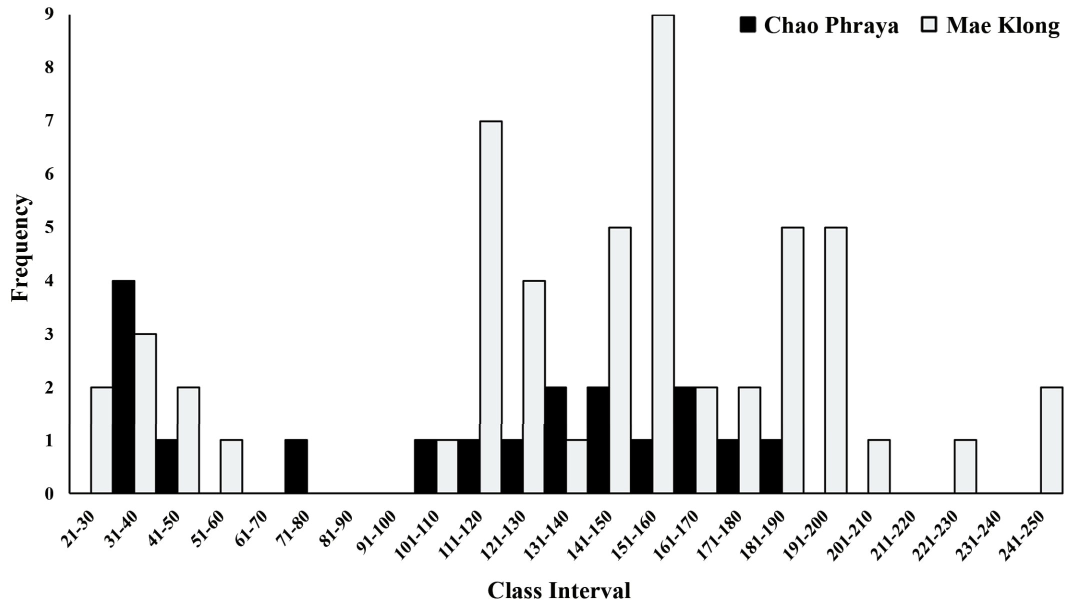


Figure 2. Size distribution of *U. polylepis* sampled from the Chao Phraya River and the Mae Klong River.

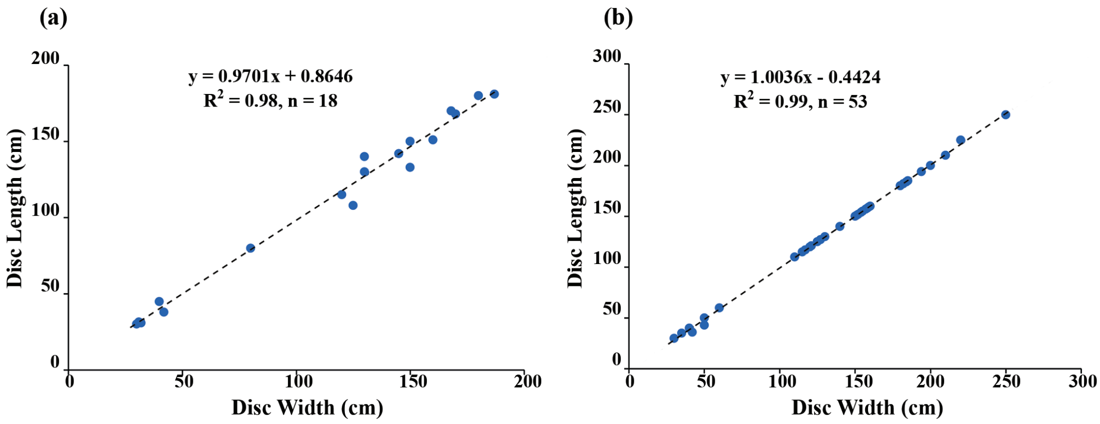


Figure 3. Scatter plots and regression lines between DW and DL of *U. polylepis* in the Chao Phraya River (a) and the Mae Klong River (b).

Figure 4. Sectioned vertebra of 4+ year-old *U. polylepis*.
Note: Black dots indicate the location of annual rings.

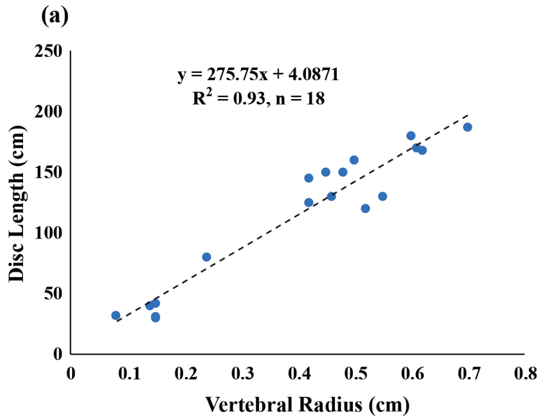


Figure 5. Scatter plots and regression lines between VR and DL of *U. polylepis* from the Chao Phraya River (a) and the Mae Klong River (b).

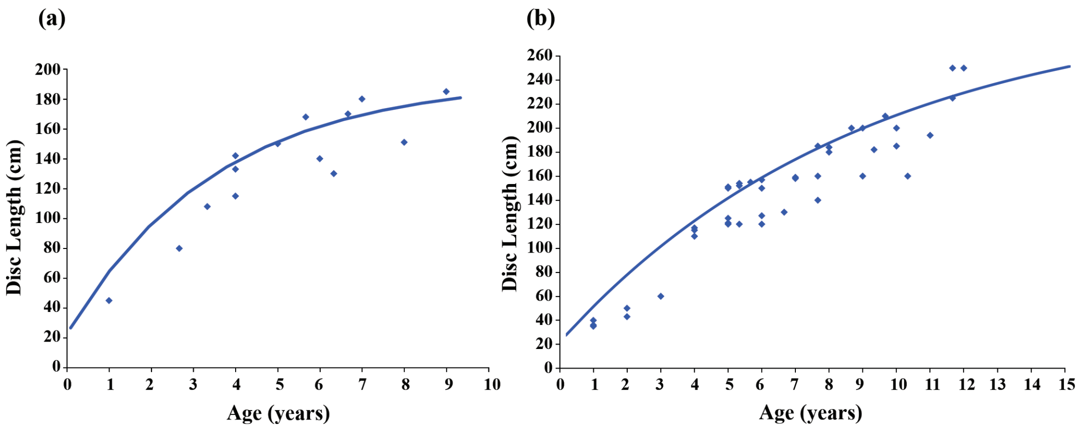


Figure 6. VBGF fit to disc length-at-age estimates of *U. polylepis* from the Chao Phraya River (a) and the Mae Klong River (b).

DISCUSSION

Concerns over the status and conservation of elasmobranch populations have increased worldwide, particularly in Southeast Asia, where information on their stock status is limited (Martin, 2005; SEAFDEC, 2017). This study presents results for the first time on age and growth of *U. polylepis* as a baseline for further productivity calculations and stock assessment. Although the number of samples was relatively small due to the limited availability of carcasses from natural deaths, the variation in size and reading age made it possible to construct the VBGF for this stingray. There are also a few other studies that managed to age and estimate the growth of fishes with a small sample size. Campana *et al*. (2006) used about ten specimens for ageing and age validation of dogfish (*Squalus acanthias*) from the Atlantic and Pacific, and O'Shea *et al*. (2013) estimated growth of *Himanutra uarnak* from the north-western coast of Western Australia from 19 specimens. The regression line between DL and DW of *U. polylepis* was almost straight, similar to other Dasyatid stingrays, i.e., *Dasyatis pastinaca* and *Dasyatis marmorata*, for which the correlation coefficients were 0.975 and 0.985, respectively (Yeldan and Gundogdu, 2018).

The precision estimates, i.e., verification by both APE and CV, provided relatively low values (only 2 %), which implied a close agreement among the three readers. The high correlation between the VR and DL implies that this calcified structure grows proportionally to the length of stingrays and can be used for ageing (Natanson and Kohler, 1996; Cailliet, 2015). Although validation of band formation was not conducted in this study, the explicit results on centrum edge analysis and marginal increment analysis on the diamond stingray (*Dasyatis dipterura*) should confirm the periodic band formation on the vertebra of any Dasyatid stingrays (Smith *et al*., 2007).

Estimated size at birth (L_0) was about 28 cm for both populations and close to that found by Last and Stevens (1994), who reported that pups of *U. polylepis* are born at about 30 cm DW. Although there was no difference in size distribution between the two populations, significant differences in growth rate (K) and average size at maximum age (L_∞) were observed. Differences in growth characteristics between two populations of the same species could be a result of techniques used, reader accuracy and precision, individual variation of the sample, goodness-of-fit of the selected growth model, sample size, or bias (Natanson and Kohler, 1996). In addition, the integrity of the river basins should be taken into account in this study. The Chao Phraya River is relatively polluted, particularly in the location where the samples were collected (Avakul and Jutagate, 2012), compared to the Mae Klong River, where the water quality is generally clean to slightly polluted (Kullasoot *et al*., 2017). Moreover, genetic variation between stocks is suspected, and could also account for differences in growth (Khudamrongsawat *et al*., 2017). It also appeared that individuals in the Mae Klong population grow more slowly than the population in Chao Phraya, i.e., lower K value, particularly during the first five years of age, which could be ascribed to density-dependence (Cailliet, 2015). Although there has been no actual census of the stingray population in the Maeklong River, the mass mortality of at least 45 stingrays in September 2016 due to a sudden deterioration of water quality from pollution, implied that a large number of *U. polylepis* inhabit this river (Khudamrongsawat *et al*., 2017).

The growth performance index ($Ø' = log_{10} K + 2log_{10} L_\infty$) is applied to validate the growth parameters (Pauly and Munro, 1984). This value should be constant between populations of the same species and close among species within the same family (Pauly and Munro, 1984). The $Ø'$ values of the two *U. polylepis* populations were 3.98 and 3.99, in Chao Phraya and Maeklong rivers, respectively, and close to other large Dasyatid stingrays that have a maximum size of over 100 cm, for which $ø'$ is around 3.5 (Table 2). However, for those Dasyatid stingrays with maximum size of less than 100 cm, the $ø'$ values were around 0.27 (Smith *et al*., 2007; Jacobsen and Bennett, 2011; O'Shea *et al*., 2013). Most elasmobranchs have relatively slow growth and a late age of maturity. Size at first maturity of *U. polylepis* is about 110 cm (Froese and Pauly, 2019), which corresponds to an age of over 3 years, according to the VBGF models in this study. These growth characteristics, incorporated

Table 2. Growth performance index ($Ø'$) of Dasyatid stingrays.

Species	**Location**	**$Ø'$**	**Source**
Dasyatis pastinaca	Northeastern Mediterranean Sea	3.402	Yeldan and Gundogdu, 2018
D. pastinaca	Eastern Mediterranean Sea	3.119	Ismen, 2003
D. pastinaca	North Aegean Sea	3.364 (male)	Yigin and Ismen, 2012
		3.093 (female)	
Dasyatis lata	d Oahu, Hawai'	3.344	Dale and Holland, 2012
Himantura uarnak	North-western coast of Western Australia	3.504	O'Shea *et al*., 2013
Pastinachus atrus	North-western coast of Western Australia	3.525	O'Shea *et al*., 2013

with low birth rate, make this stingray have low resilience, i.e., more than 14 years (Froese and Pauly, 2019). Therefore, prohibition of fishing, including for recreation, as well as establishment of a monitoring program for this stingray should be considered for effective conservation management (Martin, 2005; Gray *et al*., 2017).

It is a concern that comparisons and interpretations of growth, either intra- or inter-species are restricted by sample size, size range of specimens in the study, validation and verification, as well as model fitting techniques (Cailliet and Goldman, 2004; Morioka *et al*., 2019). Therefore, other alternative functions may be applied to examine whether they provide better descriptions of fish growth (Smith *et al*., 2007; O'Shea *et al*., 2013). Furthermore, alternative techniques for validation, as well as awareness of bias in age reading, in particular feasibility on systemic age underestimation (Campana, 2014; Harry, 2018), should be taken into consideration for better understanding of age and growth of *U. polylepis*.

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