



## Morphologic and radiographic analyses of *Lethrinus erythropterus* (Lethrinidae) from the Spermonde Archipelago, Indonesia

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**Abstract.** The aims of the study were to identify the morphometric and meristic characteristics, radiography, hydroxyapatite, elements, particle sizes of hydroxyapatite, and to determine the differences or kinship relationships on both straight and curved spine of *Lethrinus erythropterus* caught from the Spermonde archipelago, Indonesia. A total of 20 fish samples (straight body = 10, curved body = 10) were measured using a digital caliper to examine 7 meristic characters and 25 morphometric characters. Soft-X-ray analysis was performed to observe the skeletal forms of the examined fishes. X-ray Diffraction (XRD) analysis of bone material was also carried out to identify the hydroxyapatite spectrum and their elemental composition. Measurement of hydroxyapatite particles was conducted using the Scherrer method. The values of standardized morphometric and meristic characteristics were analyzed using discriminant factorial analysis from Microsoft Excel and SPSS software (16.0). The results of the discriminant factorial analysis showed a significant difference ( $p < 0.05$ ) on 6 morphological characters out of 32 characters measured. Radiographs analysis using soft-X-ray showed a curved backbone structure located between vertebrae 15 and 19. The hydroxyapatite content in the bone of the straight skeletal fish was about 1.5% lesser with smaller crystal size than those of the curved skeletal fish. The elemental compositions of both straight and curved skeletal fishes were dominated by calcium (Ca) and phosphorus (P) but there were no significant differences in the elemental percentages between these two types of fishes.

**Key Words:** morphometric, meristic, hydroxyapatite, bone mineral, particle size.

**Introduction.** Coral reef fishes of the family Lethrinidae, the emperors, are widely distributed throughout the tropical and subtropical Indo-Pacific, where they are primary targets of important commercial and non-commercial fisheries (Carpenter 2001; Kimura & Matsuura 2003; Sato 1986). The Lethrinidae include 39 species in 5 genera, with 29 species in the most common genus *Lethrinus* (Carpenter & Allen 1989). In terms morphology, Allen (1985) used body shape, body color, stripes, spines and the rays of the dorsal fin as primary distinguishing characteristics.

During our review studies of the genus *Lethrinus*, we found several examples that have similarity to the common *Lethrinus erythropterus* from the Spermonde archipelago, Indonesia. In general, the fishes appear to have same characteristics, i.e. no scales on cheek, inner surface of pectoral fin base scaled, teeth on lateral part of jaws rounded, molar-like in adult, a red stripe from eye to tip of snout, often 2 pale vertical bars on caudal peduncle and a red spot at pectoral fin base. However, the species from the Spermonde archipelago has a curved back shape. Several researchers (Chatain 1994; Cobcroft & Battaglione 2009; Davies et al 1976; Dedi et al 1997; Haga et al 2003; Kihara et al 2002; Madsen & Dalsgaard 1999; Sharber & Carothers 1988) state that scoliosis, kyphosis, lordosis, jaw malformation and branchial artery deformities are several forms of the common bone abnormalities.

Nutrition, environment, and genetics are factors that influence the development of bone formation (Fernández et al 2008). Mineral content also affects the strength and stiffness of fish bones. Research on the curvature of the spine was mostly conducted

using treatment methods and only a few were conducted in nature. Therefore, it is important to conduct a further research by performing morphometric, meristic and radiographic characteristic comparisons between the straight and curved skeletal fishes.

Morphological differences, especially the curved skeletal part can become a marker of genetic differences or kinship relationships between fish population. The morphometric analysis is one of the methods that can be performed to determine the kinship relationship by comparing the morphology of fish (Moyle & Cech 1982).

The aims of the present study were to identify the morphometric and meristic characteristics, radiography, and the elements on the straight and curved *L. erythropterus* bones so that the kinship relationship of both type fishes caught from the Spermonde Archipelago waters can be determined.

## Material and Method

**Morphometric and meristic characteristics.** Measurement of morphometric and meristic characters followed Hubbs & Lagler (1958), Bookstein et al (1985) and Burhanuddin et al (2002). Twenty-five morphometric characters and seven meristic characters were identified from 20 fish samples (straight body = 10, curved body = 10). Measurements were made using a digital caliper with 0.11 mm level of accuracy (Figure 1, Table 1).

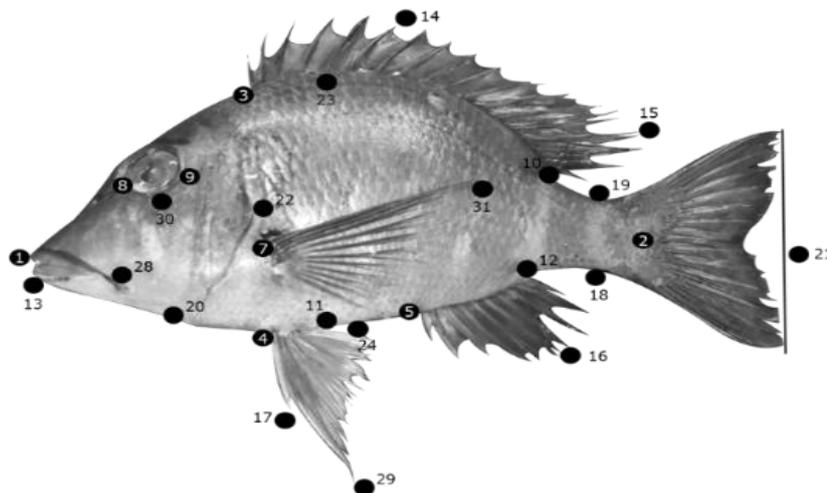


Figure 1. Measurement of morphometric characters based on box-truss protocol (original).

To standardize the morphometric data of the specimens, the model was generated using regression analysis (Elliott et al 1995). This step was also to homogenize the variances (Jolicoeur 1963). The regression of Elliott was calculated for each characteristics following the equation:

$$M_s = M_o \left( \frac{L_s}{L_t} \right)^b$$

Where:

$M_s$  = standardized measurement,  $M_o$  = measured character length (mm),  $L_s$  = overall mean standard length from all samples (mm),  $L_t$  = standard length of the specimen (mm), and  $b$  was estimated for each character from the observed data by the allometric growth equation  $M = aL^b$ . Parameter "b" was estimated as the slope of the regression  $\log M_o$  on  $\log L_t$  using all fish samples (Ballesteros et al 2016).

Table 1

Morphometric and meristic measurement based on the box-truss protocol

<i>Code</i>	<i>Morphometric character</i>
M1-2	Standard Length
M23-24	Body depth at fifth dorsal spine
M7-25	Body width
M1-22	Head length
M8	Snout length
M8-9	Orbit diameter
M26-27	Interorbital width
M1-28	Upper-jaw length
M13-28	Lower-jaw length
M1-3	Predorsal-fin length
M13-5	Preanal-fin length
M13-4	Prepelvic-fin length
M3-10	Dorsal-fin base length
M4	Longest dorsal-fin spine length (6th)
M15	Longest dorsal-fin ray length (5th)
M16	Longest anal-fin ray (1st)
M7-31	Longest pectoral-fin ray length
M4-17	Pelvic-fin spine length
M29	Longest pelvic-fin ray length (1st)
M2-12	Caudal-peduncle length
M18-19	Caudal-peduncle depth
M2-21	Caudal-fin length
M20-30	Suborbital width
M1-21	Total length
M7-9	Pectoral-fin origin to posterior margin of orbit
	<b>Meristic character</b>
	Dorsal-fin rays
	Anal-fin-rays
	Pectoral-fin-rays
	Pelvic-fin-rays
	Lateral-line scales
	Scales above/below lateral line
	Gill rakers

**Radiography.** All samples collected from the Fish Market Place (TPI) Paotere, Makassar were cleaned and stretched on the fin by formalin. The dry samples were then tested via soft X-Ray (General Electric 500T; voltage 35 KV; automatic exposition cell 5 Ma.s), the results were printed using a 11x14 mm Fuji's X-ray medical film. X-ray images were used to compare between the straight and curved spine fishes. The spine was measured from the fishtail bones (Burhanuddin & Iwatsuki 2003).

**Hydroxyapatite.** Samples were boiled for 30 minutes to separate the bones from meat (Lokapuspita et al 2012). The fish bones were then dried in an oven at 100°C for an hour to remove the remaining boiled water. The fish bones were burned in a furnace at 600°C with a fixed burning time of three hours. The crystals produced were crushed and sieved

through a 200 mesh size prior to analyze the hydroxyapatite content and elements in bones using Shimadzu X-Ray Diffraction (XRD) 7000 (Wilson et al 1999; Grew et al 2007). The Scherrer method was performed to compare the particle sizes between the straight and curved fishes.

**Statistical analysis.** Standardized morphometric and meristic values were analyzed using discriminant factorial analysis of Microsoft Excel and SPSS software (16.0), while the contents of hydroxyapatite and bone mineral were analyzed using Match!2 software.

**Results.** Based on discriminant factorial analysis of 20 fish samples having straight and curved body part, only 27 out of 32 morphologic characters were analyzed. There were six morphological characters that have significant differences ( $p < 0.05$ ) between straight and curved fishes, which were: body width (0.015), interorbital width (0.028), preanal-fin length (0.028), longest anal-fin ray (third) (0.057), caudal-peduncle length (0.003), and pectoral-fin origin to posterior margin of orbit (0.019) (Table 2).

Table 2

The results of factorial discriminant analysis of 27 morphology characters of straight and curved fishes

<i>Character</i>	<i>Wilks' Lambda</i>	<i>F</i>	<i>Sig.</i>
Standard Length	0.959	0.771	0.392
Body depth at fifth dorsal spine	0.844	3.331	0.085
Body width	0.715	7.189	0.015
Head length	0.969	0.573	0.459
Snout length	0.821	3.928	0.063
Orbit diameter	0.848	3.235	0.089
Interorbital width	0.759	5.707	0.028
Upper-jaw length	0.968	0.587	0.453
Lower-jaw length	0.842	3.388	0.082
Predorsal-fin length	1	0.003	0.955
Preanal-fin length	0.76	5.689	0.028
Prepelvic-fin length	0.998	0.041	0.842
Dorsal-fin base length	0.987	0.245	0.627
Longest dorsal-fin spine length (6th)	0.999	0.026	0.874
Longest dorsal-fin ray length (5th)	0.965	0.652	0.43
Longest anal-fin ray (3rd)	0.813	4.135	0.057
Longest pectoral-fin ray length	0.928	1.39	0.254
Pelvic-fin spine length	1	0.001	0.974
Longest pelvic-fin ray length (1st)	0.974	0.485	0.495
Caudal-peduncle length	0.596	12.183	0.003
Caudal-peduncle depth	0.981	0.348	0.563
Caudal-fin length	0.863	2.865	0.108
Suborbital width	0.911	1.755	0.202
Total length	0.964	0.663	0.426
Pectoral-fin origin to posterior margin of orbit	0.731	6.628	0.019
Lateral-line scales	1	0.004	0.95
Gill rakers	0.949	0.958	0.341

Through a transformation of 27 morphological characters, the discriminant analysis produced a major constituent axis that represented 100% of data diversity. The axis illustrated 100% of the temporal data diversity, which was produced through the extraction of the 27 components of morphological characters measured. Canonic axis was influenced by the diameter orbital variables ( $Y = 1.055$ ), preanal-fin length ( $Y = -1.530$ ), and caudal peduncle length ( $Y = 0.912$ ).

**Radiography.** Morphologically, there was a difference on the backs between the two examined fishes (Figure 2). The fish at figure A had a straight body part compared to the more curved fish (Figure 2B). Both fishes had similar morphological characteristics but it was still difficult to distinguish the differences in the spinal cord, therefore soft X-ray analysis was conducted.

Soft X-ray analysis was performed to examine the differences in the spinal cord between the fishes that have straight and curved body parts. The results indicated that there were differences on the spinal cord that have a total of 24 vertebrae between the two type of examined fishes, where the curved body fishes were characterized by a more curved and misaligned vertebrae (Figure 2D) compared with the straight body part fishes (Figure 2C). The results of soft X-ray analysis of 20 samples (straight = 10, curved = 10) indicated that the curved spines were generally located between vertebrae 15 and 19 (the abdominal region between the dorsal and pelvic fin).

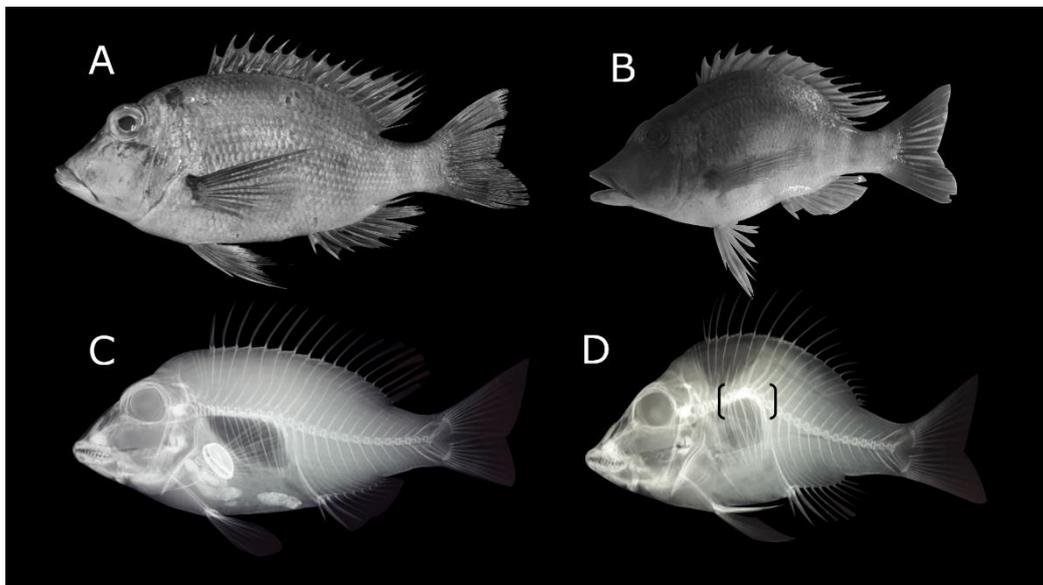


Figure 2. *Lethrinus erythropterus*. (A) morphology of the straight body part fish (TL=216.64 mm), (B) morphology of the curved body part fish (TL=222.13 mm), (C) soft X-ray of the straight vertebral segment (D), soft X-ray of the curved vertebrae (original).

**Hydroxyapatite spectrum.** The XRD analysis was performed on the fish bones of both straight and curved body parts fishes that had been given heat treatment of 600°C for 3 hours. The XRD results indicated their conformities with the JCPDS 96-900-2216. The results showed that the straight fish bone contained approximately 83.1% of hydroxyapatite and about 16.9% of other elements, while the curved fish bone contained about 81.6% of hydroxyapatite and 19.4% of the other elements (Figure 3).

The results illustrated that there were three highest peak intensities showed on the bones of both straight and curved spine fishes. The straight spine fishes has the first highest peak intensity located at 457 cps, the second highest peak intensity occurred at 261 cps, and the third highest intensity peak located at 163 cps. Similar results obtained in the curved spine fishes, where the highest peak intensity occurred at 1146 cps, the second highest peak intensity located at 773 cps, and the third highest peak intensity occurred at 367 cps.

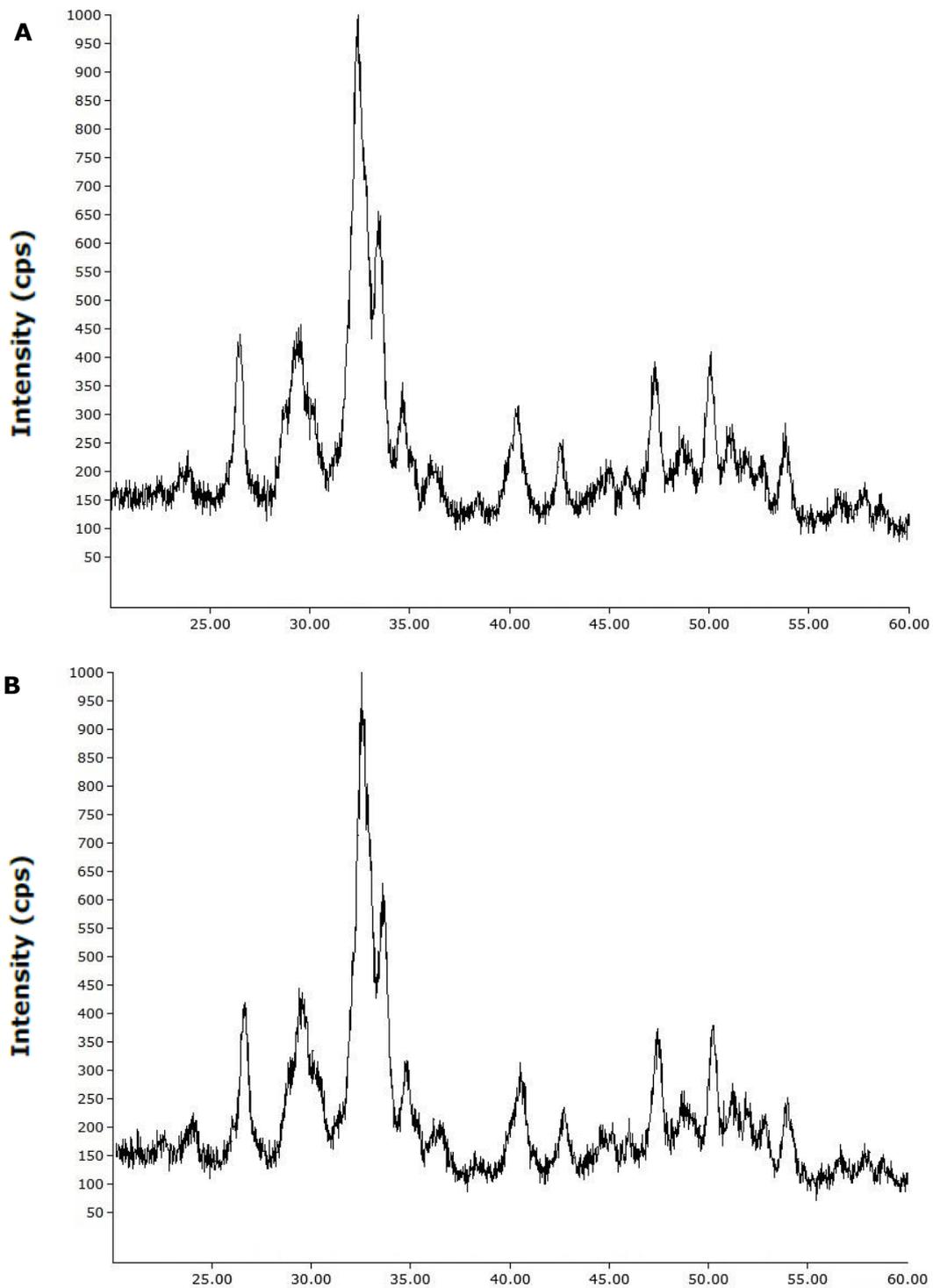


Figure 3. Comparison of the synthesized nanoparticles hydroxyapatite X-ray. Diffraction pattern of between the straight (A) and curved (B) spine fishes.

**Hydroxyapatite particle sizes.** The Scherrer test results showed that the difference of median particle sizes between straight and curved skeletal fishes was 5.76 nm. The median values of particle sizes of straight and curved fishes were about 11.33 nm and 17.09 nm, respectively. While the median values of micro-strain in the straight and curved skeletal fishes were 0.01035 and 0.007, respectively (Table 3).

Table 3

Comparison of hydroxyapatite particle sizes using the Scherrer measurement method

Sample	Matched Phase/Formula	[hkl]	$D_{Scherrer}$ (nano-meter)	Micro-Strain only	Median
Straight	Hydroxylapatite/ $Ca_5H_2O_{13}P_3$	200	11.33	0.01620	11.33 nm 0.01035
		200	13.68	0.01206	
		112	8.23	0.01638	
		221	10.59	0.01035	
		202	14.81	0.00703	
		400	11.00	0.00900	
		230	8.40	0.01089	
		232	15.34	0.00520	
		330	22.53	0.00342	
Curved	Hydroxylapatite/ $Ca_5H_2O_{13}P_3$	200	19.5	0.01040	17.09 nm 0.007
		111	10.5	0.01777	
		300	16.1	0.00825	
		202	17.09	0.00753	
		13	16.76	0.00661	
		230	11.02	0.00837	
		231	18.3	0.00480	
		140	19.7	0.00442	
		232	19.8	0.00406	
501	21.8	0.00356			

**Bone minerals.** Elemental analysis of fish bone samples using X-ray diffraction method showed the presence of several minerals as shown in Table 4.

Table 4

Results of elemental analysis in fish bones using X-Ray Diffraction

Element	Straight spine (m/m%)	Curved spine (m/m%)
Calcium (Ca)	74.95	75.02
Phosphor (P)	23.62	23.71
Potassium (K)	0.85	0.71
Strontium (Sr)	0.464	0.478
Niobium (Nb)	0.0298	0.0284
Molybdenum (Mo)	0.0199	0.0193
Titanium (Ti)	0.0162	0.0107
Stibium (Sb)	0.012	0.0089
Indium (In)	0.0109	0.0086
Stannum (Sn)	0.0101	0.0075
Ruthenium (Ru)	0.0088	0.0054
Tellurium (Te)	0.0076	0

The elements existed in both skeletal type of fishes were Ca, P, K, Sr, Nb, Mo, Ti, Sb, In, Sn, Ru, and Te, which were dominated by Ca and P. The Ca content in the straight and

curved fish bones were 74.95 m/m% and 75.02 m/m%, respectively. The phosphorus content in the straight and curved fish bones were 23.62 and 23.71, respectively. The results showed that the curved fish bones contained a higher proportion of calcium and phosphorus than those in the straight fish bones. However, Te did not exist in the curved fish bones. Nevertheless, there was no significant difference in the amount of mineral contents between the straight and curved fish bones.

**Discussion.** Morphometric and meristic methods are important methods in describing fish species, this method consists of several standard measurements and calculations applied in fishes (Burhanuddin 2003). Morphometric and meristic analysis on the straight and curved body parts of *L. erythropterus* showed significant differences on 6 out of 32 measured characters. This condition may be a form of adaptation or response to environmental changes. Hossain et al (2010) state that fishes are very sensitive to environmental changes, therefore, they quickly adapt by changing the necessary morphometric characters. Juveniles are commonly found in seagrass meadows, mangroves, and sand areas, while adults are commonly live solitary and seek for deeper waters. *L. erythropterus* is a bottom-feeder carnivore that eats echinoderms, mollusks, crustaceans and small fishes (Carpenter & Allen 1989).

Changes in the spinal cord of *L. erythropterus* located between vertebrae 15 and 19, which formed a curved spine, could be due to various reasons. The curved spine can be caused by intensive muscle pressures, which occurs especially when swimming (Chatain 1994). This phenomenon also occurs in other types of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) (Chatain 1994).

The development of bone deformity is also associated with collagen metabolism disorders produced by certain pesticides, which can trigger muscle contraction, which leads to bone deformities (Mehrlé et al 1981). Naturally, bone consists of 70% inorganic minerals, 20% organic matter, and 10% water. The organic material is mostly made of type I collagen, while the mineral consists of carbonated hydroxyapatite (White & Best 2007; Toppe et al 2007). Hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) is a crystalline phosphate of calcium which has a hydroxyl ion which is a primary mineral contained in bones, which is about 43% by weight of bones (Wang et al 2009). The relationship between the collagen and hydroxyapatite is very important in bone toughness and stiffness. Although the hydroxyapatite contents between the straight and curved spine differ only slightly (1.5%), it was sufficient enough to affect the bone strength.

The crystal sizes of hydroxyapatite in the bones of the straight spine fishes were smaller than the curved spine fishes. The grain sizes also decreased the strength of the hydroxyapatite material by affecting the bond amongst the grains. Pores that are located irregularly and are not interconnected/sticked each other can weaken the strength of hydroxyapatite materials (Smith 1996).

Spinal disorders can also be influenced by the mineral imbalance in fish bone through the mobilization of excessive calcium and phosphorus content (Mehrlé et al 1981). The main mineral contents in fish bones include calcium, phosphate and carbonate and a number of other small minerals, magnesium, sodium, strontium, lead, citrate, fluoride, hydroxide, and sulfate (Lall 2002). Lack of calcium and phosphorus can disrupt the development of soft bones and bone deformities (Baeverfjord et al 1998). Skeleton abnormalities commonly appear in the early stages of fish development (Cahu et al 2003; Lall & Lewis-McCrea 2007), and worsen in the growth stage toward adulthood (Witten et al 2006). There were no significant differences on the percentages of mineral contents in fish bones between the straight and curved spine fishes, which suggested that the difference in the shape of the fish bones was not due to the bone mineralization.

**Conclusions.** There was a slight difference in morphometric and meristic characters between the straight and curved *L. erythropterus* (22.22% of the 32 characters measured). In general, the curvature of the spinal was generally located between vertebrae 15-19. The hydroxyapatite content and crystal size of hydroxyapatite in the curved spine were smaller than those in the straight spine fishes. There was no significant difference the mineral contents between straight and curved spine fishes. The

differences in morphological characters, spinal deformities, content, and size of hydroxyapatite may be influenced by other environmental parameters or genetic factors.

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