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Weight-length relationship, morphometric and meristic characters of the yellow seahorse, *Hippocampus kuda* (Syngnathiformes: Syngnathidae) from central west coast of India

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Abstract

Eighteen morphometric and five meristic characters of the yellow seahorse, *Hippocampus kuda* inhabiting the coastal waters of Ratnagiri, central west coast of India, were studied. Morphometric/numerical analysis confirmed the identity of the species from Ratnagiri coastal waters as *H. kuda*. A negative allometric relationship was observed in both genders of *H. kuda*. The estimated weight-length relationships for male and female specimens, respectively were, W=0.0034 SL^{2.8533} and W=0.0078 SL^{2.5253} (r^2 >0.95). The biological information on morphometric and meristic characters, WLR and LLR provided in this study would be helpful for conservation and management of this vulnerable seahorse species.

Keywords: *Hippocampus kuda*, Length-length relationship, Meristic, Morphometric, Weight-length relationship, Yellow seahorse.

Seahorses, pipefishes, pipehorses and seadragons are members of the family Syngnathidae (Syngnathiformes), which include 320 species under 56 genera (Kuiter, 2009). Seahorses, representing the subfamily Hippocampinae, are characterized by a fully enclosed brood pouch with a small opening for the transfer of eggs, vestigial caudal fin and a prehensile tail (Lourie et al., 1999; Lourie et al., 2004). All seahorses have been placed under a single genus, Hippocampus. Till date 41 species of seahorses are reported to be valid around the world (Lourie et al., 2016). The yellow seahorse, Hippocampus kuda (Bleeker, 1852) is considered as one of the most problematic seahorse species in respect of taxonomy, because the name H. kuda, earlier has been used for all non-spiny seahorses in the Indo-Pacific region (Lourie et al., 1999). Distinct morphometric features of H. kuda consist of single eye and cheek spine, rounded and backward curled coronet and deeper head and rounded or less developed body spines (Lourie et al., 1999; 2004). An exhaustive taxonomic work by Lourie et al. (1999) suggested that at least 15 names for apparent species were merely the synonyms for H. kuda. Species such as Barbour's seahorse H. barbouri, the Reunion seahorse H. borboniensis, the tiger tail seahorse H. comes, Fisher's seahorse H. fisheri, the sea pony H. fuscus and the great seahorse H. kelloggi, hitherto included under H. kuda species complex, have now been isolated as separate species. . Based on recent extensive morphometric and molecular studies by Lourie et al. (2016), it has been now confirmed that H. borboniensis and H. fuscus are also synonyms of H. kuda due to lack of distinguishable morphological, genetic and/or geographic differences from H. kuda. The escalating trade and worldwide decline in seahorse populations reiterate the need to resolve seahorse taxonomy (Bijukumar et al., 2008). Precise species identification will help to modify fishing practices appropriately, design protective marine reserves, assess captive breeding potential for seahorses and develop protective legislations (Sreepada et al., 2002; Bijukumar et al., 2008; CITES, 2018).

Weight-length relationship (WLR) has been widely employed in fisheries research because it allows the conversion of growth-in-length equations to growth-in-weight, stock assessment models (Pauly, 1993; Petrakis and Stergiou, 1995) as well as in estimation of biomass from length observations. Furthermore, often WLRs are useful in estimating the condition indices and life history stages, thus allowing morphological comparison of populations from different regions (LeCren, 1951; Petrakis and Stergiou, 1995). Relationships between different types of lengths (length–length relationships, LLR), are also very important for comparative growth studies (Kara and Bayhan, 2008). Unfortunately, such information is scanty for *H. kuda* - one of the highly valued seahorse species popularly traded for both, traditional Chinese medicine (TCM) and aquaria (Vincent, 1996). Moreover, *H. kuda* has been listed as 'vulnerable' in the IUCN Red List of Threatened Species

(Aylesworth, 2014). Also, seahorses are first amongst the marine fishes of commercial importance listed in CITES with regard to regulation of its international trade (CITES, 2018).

Based on molecular studies, Teske et al. (2005) found that H. kuda population inhabiting east and west coasts of India are genetically distinct and H. kuda population from India were the oldest one. Therefore, the present study was undertaken to update the knowledge on morphological features which could be useful for comparing Indian populations of H. kuda with that from the rest of the world. Specimens of H. kuda were collected from Mirya creek, Ratnagiri, (Lat 17°02'52"N and Long 73°16′08″E), from incidental catches by local fishermen operating gill nets (mesh size, 2.5 to 4 cm) at water depth of 2-10 m. The habitat and bottom topography mostly comprises of oyster beds with small rock pebbles, rocky bottom with brown seaweed, Sargassum sp. and sandy bottom. Fishes were sexed based on the presence or absence of a brood pouch. Morphometric measurements were taken on wet seahorses (60 male and 58 females). Morphometric characters such as height (Ht), standard length (SL), head length (HL), trunk length (TrL), tail length (TaL), snout length (SnL), snout depth (SnD), head depth (HD), spine width (SW), pectoral fin base length (PL) and dorsal fin base length (DL) were taken with the help of a caliper with 0.1 mm precision (Lourie et al., 1999; Lourie, 2003; Lourie et al., 2004). Coronet height (CH) was measured as vertical distance between tip of the coronet and first spine of cleithral ring. Trunk depth on 4th trunk ring (TD4) was recorded as narrowest distance between the superior and inferior trunk ridges on 4th trunk ring whereas, trunk depth on 9th trunk ring (TD9) were recorded as narrowest distance between the superior and inferior trunk ridges on 9th trunk ring. Meristic counts included the number of trunk rings (TrR), tail rings (TaR), dorsal fin rays (DF), pectoral fin rays (PF) and anal fin rays (AF) which were counted as described by Lourie et al. (1999) under stereo zoom microscope (Olympus SZX7, Japan). For WLR studies, weight measurements of wet individuals were taken on an analytical balance to the nearest 0.01 mg.

Mann-Whitney U test (Siegel, 1956) was used to test significant difference between meristic counts of male and female specimens. The WLRs in *H. kuda* was established for males and females, using the formula $W = aSL^b$ and linear regression analysis, $Log W = a + b \log SL$, where 'a' is the intercept of the regression curve and 'b' is the regression coefficient (slope). The degree of association between the variables was computed by the determination coefficient, r^2 . A Student *t*-test was applied to determine whether the regression coefficients (b) obtained from the linear regression differed significantly from the isomeric value of 3. Significant difference was tested at a 5% level, represented as p<0.05.

The entire body colour of freshly landed wet specimens was observed to be bright yellow, orange with small reddish spots and brownish black. The shape of the coronet was observed to be round, medium size and overhanging towards the back with a cup like depression on the top. In freshly landed fishes, prominent round bumps with white patch were observed on 1-, 4-, 7- and 11- TrR, whereas bumps and patches were observed on 5-, 9-, 13-, 17-, 20-, 23-, 26-, 29-, 32- and 34- or 35- TaR on the dorsal side. Dorsal fin originated between 9th and 10th TrR and terminated between 1st and 2nd TaR.

The minimum, maximum and mean values of measured morphometric characters (±SD) of male and female specimens of *H. kuda* collected from Ratnagiri are presented in Table 1. No gender-wise significant difference in measured morphometric characters (SL, Ht, TrL, PFH, DFH, AFH, PL, DL, AL, TD4, TD9 and SW) was discernible (p>0.05).

Morphometric	Male (<i>n</i> = 60)				Mean as		
Character	Min	Max	Mean \pm SD	Min	Max	$Mean \pm SD$	% of SL
Standard Length	12.90	20.50	17.18 ± 1.89	13.30	20.40	17.50 ± 1.55	-
Height	12.10	18.80	15.63 ± 1.64	12.50	18.50	15.85 ± 1.29	90.77
Head Length	2.25	3.30	$2.82 \pm 0.24*$	2.60	3.20	$2.97\pm0.13^*$	16.71
Snout length	0.85	1.40	$1.12\pm0.15*$	1.00	1.40	$1.24\pm0.08*$	6.80
Snout Depth	0.30	0.50	$0.41\pm0.05*$	0.30	0.50	$0.44\pm0.05*$	2.44
Head Depth	1.25	1.80	$1.46\pm0.12*$	1.35	1.70	$1.54\pm0.10^{\ast}$	8.67
Trunk Length	3.90	5.30	4.54 ± 0.37	4.20	5.40	4.98 ± 0.36	27.44
Tail length	7.60	11.50	$9.84 \pm 1.03*$	7.60	10.90	$9.59\pm0.91*$	56.04
Coronet Height	0.70	1.10	$0.85\pm0.07*$	0.60	0.95	$0.78\pm0.09*$	4.69
Pectoral fin height	0.35	0.65	0.53 ± 0.07	0.40	0.50	0.53 ± 0.05	3.04
Dorsal fin height	0.45	0.70	0.56 ± 0.06	0.40	0.65	0.52 ± 0.06	3.11
Anal fin Height	0.50	0.80	0.42 ± 0.05	0.30	0.60	0.42 ± 0.06	2.48
Pectoral fin base length	0.50	0.80	0.63 ± 0.07	0.45	0.80	0.61 ± 0.08	3.58
Dorsal fin base length	1.15	1.70	1.48 ± 0.15	1.25	1.70	1.48 ± 0.13	8.52
Anal fin base length	0.10	0.35	0.22 ± 0.06	0.20	2.0	0.46 ± 0.4	1.97
TD4	0.85	1.35	1.06 ± 0.11	0.80	1.40	1.10 ± 0.13	6.23
TD9	1.50	2.20	1.79 ± 0.17	1.30	2.05	1.79 ± 0.18	1.32
Spine width	0.35	0.80	0.59 ± 0.12	0.45	0.95	0.63 ± 0.10	3.53

Table 1. Minimum, maximum, mean and standard deviation (cm) of morphometric characters ofHippocampus kuda inhabiting the coastal waters of Ratnagiri, central west coast of India.

*indicate significant difference between two genders (p<0.05).

However, gender-wise significant differences (p<0.05) in morphometric characters such as HL, HD, SnD, SnL, TaL and CH were observed. Meristic counts recorded on seahorses are presented in Table 2. The number of TrR was 11 in both male and female *H. kuda*, whereas, in males the median

number of TaR was 35 (32–37), while in females it was 33 (33–36). Results of Mann–Whitney U test showed that the number of TaR, PF and DF varied significantly between the genders (p<0.05). Morphometric and meristic characters recorded in specimens of H. kuda collected from Ratnagiri coast were similar to those described earlier by Lourie et al. (1999; 2004), Choo and Liew (2004) and Thangaraj and Lipton (2011). From a comparative assessment of the measured morphometric and meristic characters with those described by Lourie et al. (1999), the taxonomic identity of seahorse species inhabiting coastal waters of Ratnagiri, was confirmed as H. kuda. Due to obvious difficulties in differentiation, H. fuscus is often misidentified as H. kuda (Thangaraj and Lipton, 2011). But very recently, studies by Lourie et al. (2016) have confirmed that H. fuscus could be a synonym for H. kuda due to lack of prominent differences in morphological characters, geographical distribution and genetics between these two species. Maximum height of seahorse species is a quite useful parameter for taxonomical differentiation, as it allows elimination of some seahorse species. For example, a smaller-sized seahorse species like Japanese seahorse, H. mohnikei (max height, 8 cm) cannot be a larger-sized seahorse species like H. kuda (max height, 17 cm) (Lourie et al., 2004). The recorded maximum height of freshly caught male (18.8 cm) and female (18.5 cm) specimens of *H. kuda* in the present study were relatively higher than previously reported for same species (17 cm) by Lourie et al. (2004). Maximum height recorded in male and female specimens of H. kuda in the study region might be attributable to absence of commercial exploitation (target fishing and seahorse trade) and food availability.

Meristic character	Male $(n = 60)$					Fema	U		
	Min	Max	Mean	Median	Min	Max	Mean	Median	U
Trunk rings	11	11	11	11	11	11	11.00	11	P<0.05
Tail rings	32	37	35.03	35	33	36	33.74	33	P>0.05
Dorsal fin rays	17	19	17.84	17	17	19	17.33	17	P>0.05
Pectoral fin rays	16	17	16.35	16	15	17	16.14	16	P>0.05
Anal fin rays	4	5	4.06	4	4	4	4	4	P<0.05

Table 2. Meristic counts of male and female specimens of *Hippocampus kuda* collected from the coastal waters of Ratnagiri, central west coast of India.

Different types of length-length relationships (LLR) determined separately for two genders and pooled data of *H. kuda* are presented in Table 3. All LLRs showed significant positive correlation between different length measurements. The LLR of fish species under various environmental conditions will allow more reliable comparative assessment between populations (Kara and Bayhan, 2008). Furthermore, knowledge on LLR is of great importance for comparative growth studies (Moutopoulos and Stergiou, 2002). Considering the scanty information on LLR in syngnathid fishes, the LLR reported for *H. kuda* during the present study, might therefore be useful for comparison of different populations of *H. kuda* from various geographical regions.

The estimated values of WLR parameters are presented in Table. 4. In the present study, the calculated 'b' values for males (2.8533) and females (2.5253) are indicative of negative allometric growth (<3.0). A significant difference in b values of male and female H. kuda (p<0.05) was also observed. The estimated WLR for male specimens was calculated as $W = 0.0034 \text{ SL}^{2.8533}$ whereas, for the female $W = 0.0078 \text{ SL}^{2.5253}$.

A similar negative allometric growth pattern has been observed in laboratory reared *H. kuda* juveniles (b = 2.76) by Choo and Liew (2006) and in wild caught males (b = 2.42) and females (b = 1.72) by Vaitheeswaran and Venkataramani (2012) from Thoothukudi, southeast coast of India. Negative allometric growth pattern has also been observed in few other seahorse species as well.

LLR		Male $(n = 60)$		Female $(n = 58)$	Pooled (<i>n</i> = 118)		
LLK	r	Regression equation	r	Regression equation	r	Regression equation	
Ht on SL	0.9643	Ht =1.2737 + 0.8358SL	0.9816	Ht =1.5869 + 0.8149SL	0.9708	Ht =1.4516 + 0.8261SL	
TrL on SL	0.8666	TrL = 1.6649 + 0.1676SL	0.8897	TrL = 1.2865 + 0.2109SL	0.7908	TrL =1.3738 + 0.1952SL	
TaL on SL	0.9747	TL =0.7426 + 0.5293SL	0.9326	TL =0.0909 + 0.05425SL	0.9316	TL =0.6535 + 0.5227SL	
SnL on HL	0.9014	SnL =0.3767 + 0.5283HL	0.7363	SnL =0.0446 + 0.4332HL	0.8731	SnL =-0.4183 + 0.5510HL	
HD on HL	0.8798	HD =0.2250 + 0.4386HL	0.8290	HD =-0.4039 + 0.6545HL	0.8626	HD =0.0778 + 0.4918HL	
SW on TD9	0.8142	SW =-0.4080 + 0.5600TD9	0.8594	SW =0.0699 + 0.3875TD9	0.8072	SW =0.2427 + 0.4754TD9	
TD4 on TrL	0.7649	TD4 =0.0308 + 0.2257TrL	0.8187	TD4 =-0.3112 + 0.2845TrL	0.7689	TD4 =0.0489 + 0.2168TrL	
TD9 on TrL	0.8467	TD9 =-0.0695 + 0.4083TrL	0.8891	TD9 =-0.3512 + 0.4304TrL	0.7525	TD9 =0.3065 + 0.3115TrL	
DL on TrL	0.8293	DL =0.0212 + 0.2930TrL	0.7650	DL =0.0599 + 0.3116TrL	0.6869	DL =0.4098 + 0.2245TrL	
PL on TrL	0.8387	PL =-0.3760 + 0.1974TrL	0.7101	PL =-0.0155 + 0.1428TrL	0.6869	PL =0.4098 + 0.2245TrL	

Table 3. Length-length relationships (LLR) of Hippocampus kuda collected from the coastal waters of Ratnagiri, central west coast of India.

Gender <i>n</i>			ndard h (cm)	Weig	Weight (g)		b	<i>b</i> 95% C.I.	r^2
		Min	Max	Min	Max				
Male	60	12.9	20.50	5.0129	18.8993	0.0034	2.8533	2.6341-3.0739	0.9605
Female	58	13.3	20.40	5.6778	17.2145	0.0079	2.5253	2.3115-2.7398	0.9542

Table 4. Weight-length relationship (WLR) of *Hippocampus kuda* collected from the coastal waters of Ratnagiri, central west coast of India.

Gurkan and Taskavak (2007) observed a 'b' value of 2.47 in the long snouted seahorse, H. guttulatus collected from Aegean Sea, Turkey. Vieira et al. (2014) observed negative allometric growth (b = 2.95) in the short snouted seahorse, *H. hippocampus* inhabiting Ria Formosa, SW Iberian coast. Similarly, Basusta et al. (2014) observed negative allometric growth pattern in *H. hippocampus* (b = 2.41) and *H. guttulatus* (b = 2.84) inhabiting eastern Black Sea, and Balasubramanian and Murugan (2017) observed negative allometric growth (males b = 2.7; females b = 2.5) in *H. kelloggi* off Coromandel Coast, southeast coast of India. Many authors have hypothesized that the WLR depends upon abiotic (temperature, salinity), biotic and biological factors (season, food availability, habitats, gonad development, health) and anthropogenic activities (habitat alteration/destruction, pollution and fishing pressure) (Gurkan and Taskavak, 2007; Vieira et al., 2014; Balasubramanian and Murugan, 2017). However, in the absence of targeted fishing and seahorse trade, bottom trawling and ample availability of live prey organisms over oyster and Sargassum beds (pers. observ.) at the collection sites do not support either of the above contentions. Therefore, the most plausible explanation for negative allometric growth observed in different seahorse species seems to be linked more to their body structure rather than to other abiotic, biotic and anthropogenic factors. When compared to other bony fishes, seahorses differ morphologically. For example, seahorses have a layer of skin stretched over a series of bony plates in lieu of scales (Ginsburg, 1937; Harris et al., 2007). Furthermore, they lack fleshy muscles inside their body and much of muscle tissues are confined to the prehensile tail, dorsal fin muscle support and superior trunk region (Thomson, 1993; Hale, 1996). Presence of scales, muscles, heavy jaws and well developed fins (especially caudal fin) which

contribute to the weight in other fin fishes (more towards isometric or positive allometric WLR) are completely lacking in seahorses. Hence, a significant difference in weight and length proportions in seahorses might explain the negative allometric growth.

In conclusion, a detailed analysis of morphometric and meristic characters presented here would be helpful in identification of *H. kuda*. WLR derived in the present study might be useful to estimate weight parameter when only length data is known. Future studies with larger samples size would provide greater insights into the population characters of *H. kuda* and will aid conservation and management of *H. kuda* inhabiting the study region.

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