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Genetic identity and length-weight relationships of Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy and Gaimard, 1825) from the south-east coast of India

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ABSTRACT

Ambiguity regarding the identification of Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy and Gaimard, 1825) was sought out through classical taxonomic approach along with molecular confirmation. DNA barcoding of 650 bp region of the Cytochrome C oxidase 1 gene revealed the confirmation of taxonomical identity of the species from the region. Length-weight relationships and relative condition factor in *A. vaigiensis* were investigated on the samples collected from the south-east coast of India, Gulf of Mannar, to assess the significance of allometric factor and the wellbeing. A total of 360 fishes were collected for the study and categorised into four groups namely males, females, unsexed (fishes with morphologically undifferentiated gonads) and overall pooled samples. Among 360 fishes, 154 were males and 88 were females and the remaining 118 were categorised as unsexed. The analysis was based on fish samples with total length (TL) ranging from 83 to 150 mm and weighing 12.5 to 62.5 g. Length-weight equation and regression coefficient obtained for males, females, unsexed and pooled fishes were $W = 0.06\ L^{2.55}$ ($R^2 = 0.82$); $W = 0.02\ L^{2.94}$ ($R^2 = 0.88$); $W = 0.04\ L^{2.68}$ ($R^2 = 0.96$) and $W = 0.04\ L^{2.67}$ ($R^2 = 0.92$) respectively. The regression parameter 'b' obtained in all the cases were below 3, indicating negative allometric relationship of length and weight. Relative condition factor (K) among pooled fishes showed a value above 1, which indicates better condition of fish. The results of the present study showed that the existing hydrobiological conditions of the Gulf of Mannar area were conducive for food availability, free movement, feeding and better growth of the fish. The present study is also the first report on the molecular confirmation of the species from Indian waters and its morphometry from south-east coast of India

Keywords: COI sequencing, Indo-Pacific sergeant major, Length-weight relationship, Relative condition factor, South-east coast of India

Introduction

Fish accounts for the highest number of species kept in captivity among vertebrates (Miller, 2009). Among the aquarium fishes, marine ornamental fishes are immensely popular for aquarium hobbyists and is a key component of multibillion dollar industry. The marine ornamental fishes are associated with coral reefs, which also supports other fishes, invertebrates, reef building corals and sponges. The proportion of marine ornamental fish produced in captivity and traded accounts only 1-2% of the volume of marine ornamental trade (Wabnitz *et al.,* 2003; Bruckner, 2005). Overexploitation of these fishes from their natural environment to meet the high international demand causes further destruction of coral reefs and killing of non-targeted fishes, invertebrates, corals and sponges. It is estimated that the world has effectively lost 19% of the original area of coral reefs, 15% are seriously threatened with loss within the next 10-20 years and 20% are under threat of loss in 20-40 years (Wilkinson, 2008).

The marine ornamental fishes in the family Pomacentridae are diverse, primarily inhabiting coral reefs in most parts of the world, with over 412 species belonging to 29 genera. These fishes are found throughout the world in tropical and warm temperate waters, with majority of species occurring in the Indo-west and Central Pacific region (Thresher, 1984; Nelson, 1994; Helfman *et al.,* 1997; Allen, 1998). These small and brilliantly coloured fishes represents majority of marine ornamental fishes reared in captivity (Gopakumar, 2007). The genus *Abudefduf* belonging to Pomacentridae family (Allen and Woods, 1980; Allen 1991; Bessa *et al.* 2007; Feitosa *et al*., 2012; Litsios *et al*., 2012; Cowman *et al*., 2013; Froese and Pauly, 2015) is represented by 20 species of which 9 species are found in India (Joshi *et al*., 2016). The south-east coast of India is also reported to have all the 9 species of the genus *viz, Abudefduf bengalensis*, *A. biocellatus*, *A. notatus*, *A. septemfasciatus*, *A. saxatilis*, *A. sexfasciatus*, *A. sordidus*, *A. uniocellatus* and *A. vaigiensis* (Joshi *et al*., 2016). The members of the genus are among the world's top ten most traded marine ornamental fishes (Wabnitz *et al*., 2003).

As the trade of marine ornamental fishes mainly depend on wild collection and since there is no hatchery technology for the species belonging to the genus, it is important to conduct biometric studies, to obtain information for an estimated assessment of their biomass (Zargar *et al*., 2012). Length-weight relationships (LWRs) of commercially important fishes are highly significant for management and conservation of populations in natural water bodies. LWRs in fish provides information with respect to the growth pattern, general health, habitat conditions, life history, fish fatness and condition, along with morphological characteristics (Schneider *et al*., 2000, Froese, 2006). At present, there is no report on the LWRs and growth patterns of Indo-Pacific sergeant, *A. vaigiensis* (Quoy and Gaimard, 1825) from the south-east coast of India. *A. vaigiensis* is often misidentified and confused (Allen, 1991) due to their morphological similarity with other species (Sen *et al*., 2021). The marine ornamental fish *A. vaigiensis* is a potential candidate for ornamental trade and thus the confirmation of the species and the basic knowledge about the morphometric characteristics of the species is essential. The present work aims to confirm the taxonomical position, derive baseline data on the LWRs and relative condition factor of the species. Investigations were also carried out for combining conventional taxonomy with mitochondrial COI gene sequencing to confirm the occurrence and distinguish *A. vaigiensis*.

Materials and methods

Fish collection and identification

The specimen collection was restricted to Gulf of Mannar, south-east coast of India, which covers approximately an area of 10,500 km^2 along 8°54' N-9°25'N and 78°08'E-79°30'E. Fish samples were collected by encircling nets from 4 stations *viz,* Vedalai, Marikkar Pattinam, Mandapam and Pamban in Gulf of Mannar (Fig. 1). For determination of LWRs, fish specimens were collected monthly from June 2019 to May 2020 from the selected stations. The moribund specimens were shifted to the laboratory for further analyses. *A. vaigiensis* was identified and distinguished according to Allen (1991) and Randall (2007).

Molecular identification

Tissues collected from representative samples (4 nos.) were preserved in 95% ethanol. DNA extraction was carried out following a standard phenol/chloroform extraction protocol. A 650 bp region of the Cytochrome C oxidase 1 was amplified using the universal primer (Folmer *et al*., 1994). PCR amplifications were carried out in 25 µl reaction mixture consisting of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each oligonucleotide, 1 unit of Taq DNA polymerase and 50 ng of template DNA. The amplifications were carried out in Biorad T100 thermocycler (Biorad, USA) with the following PCR conditions; initial denaturation at 94°C for 4 min followed by 33 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, extension at 72°C for 40 s and a final extension at 72°C for 7 min. The PCR products were purified using Qiagen PCR purification kit and subsequent sequencing was carried out with LCO1490 and HC02198 (Folmer *et al*., 1994) primers using the Big Dye Terminator Sequencing Ready Reaction v 3.0 kit (Applied Biosystems). The sequences of cytochrome c oxidase 1 were aligned with sequences retrieved from GenBank of other valid species of *Abudefduf* using Clustal W in MEGA6. A phylogenetic tree was constructed using

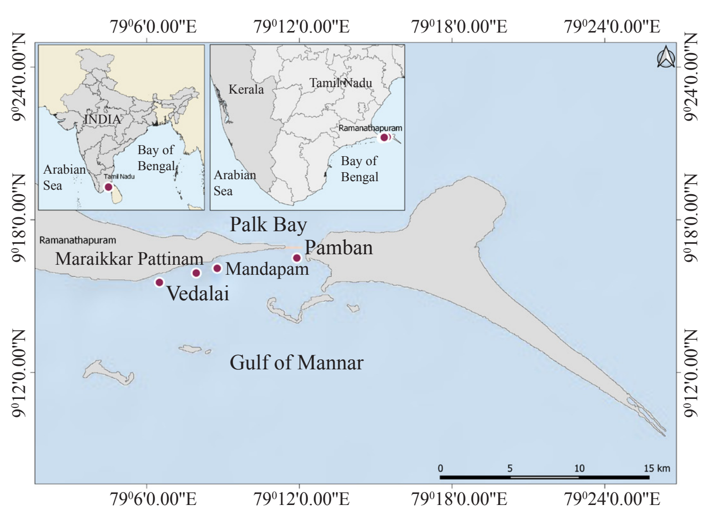

Fig. 1. Map showing the sampling stations

maximum likelihood method. The tree was then rooted with CO1 sequence of *Nemipterus japonicus* retrieved from NCBI, GenBank. Kimura-2-parameter (K2P) distance between *Abudefduf* species was also analysed using MEGA6.

Analysis of LWRs

Total length (TL) of the fish was measured in fresh condition using a wooden scale with 1 mm accuracy. Individual fish were weighed using an electronic balance (Denver Instruments, USA) of accuracy up to 0.1 g. The length-weight relationship was estimated using the formula $W = a L^b$ (Le Cren, 1951) as Log W = log a + b log L, where, W = weight of the fish (g), L = length of the fish (cm) and 'a' and 'b' are constants. The values of 'a' and 'b' in the equation were estimated using the method of least squares. The t-test was employed to test the level of significance of weight on length ($p<0.05$). The regression equation was fitted separately for each group and the slopes (b values) were tested to find out significant variation among them, if any, with ANOVA. Statistical analysis was carried out with the help of the statistical software SPSS version 16.

Sex ratio

The proportion of the two sexes relative to one another among the 360 specimens collected from Gulf of Mannar region was used to calculate the sex ratio. The percentage of different sexes was also determined as the percentage of male, female or unsexed fish in the total sample.

Relative condition factor (Kn)

The relative condition factor, Kn can be used to compare the general well-being (Thomas, 1969) and also an index of feeding intensity (Manojkumar and Kurup, 2010). The formula used to express relative condition factor was, $Kn = W/\hat{W}$, where W is the weight of the fish under consideration and $\hat{W}$ the estimated weight derived from the established length-weight relationship (Le Cren, 1951).

Results and discussion

Taxonomical ambiguity was reported from the Pomacentridae family which is having a common feature of vertical bands on the body surface in fishes such as *A. vaigiensis* (Wibowo *et al.,* 2017) and *A. saxatilis* (Vella *et al.,* 2016). The major morphological features such as colouration and bands (Fig. 2) indicated that the species belongs to Pomacentridae. Detailed taxonomical evaluation was carried out using the collected specimens. The morphometry (Table 1) and meristic characters (Table 2) of the specimens almost matched the previous reports (Vella *et al*., 2016; Sen *et al*., 2021) confirming the species status. The prominent identification features include bluish green and silvery dorsal to whitish ventral body colour and forked caudal fin without bands (Randall, 1995; Allen and Erdmann, 2012). Five broad vertical bluish black bars on the body (De Beaufort, 1940; Randall, 1995) and the fourth band starts at the base of

Fig. 2. Representative specimen of *A. vaigiensis*

Table 1. Morphometry of *A. vaigiensis* samples collected for identification

Parameters	Present study		Previous report (Sen *et al.,* 2021)
	Measurement	Proportion	
Total length, TL (cm)	10.8-13.2		8-13.6
Standard length, SL (cm)	7.8-9.5	71.5-7.2% TL	6-9.9
Body depth (cm)	3.8-5.2	47.5-54.7% SL	2.91-5.02
Head length, HL (cm)	2.3-2.9	27.4-30.5% SL	1.8-3.1
Caudal fin height (cm)	3-4.1	34.0-43.1% SL	1.7-3.7
Pre-dorsal length (cm)	2.4-3.9	28.5-40.3% SL	2-4.05
Pre-pectoral length (cm)	2.5-3	29.7-31.9% SL	1.9-3.25
Pre-pelvic length (cm)	2.5-3.8	32.0-40.0% SL	2.54-4.45
Inter-orbital length (cm)	0.8-0.9	9.5-10.2% SL	0.5-1.2
Upper jaw length (cm)	0.5-0.6	6.6-6.9% SL	0.39-0.7
Eye diameter (cm)	0.7-0.9	8.9-9.5% SL	0.6-0.9
Snout length (cm)	0.4-0.8	4.8-9.0% SL	0.5-1
Caudal peduncle length (cm)	0.9-1.2	11.5-12.6% SL	0.7-1.2
Pre-anal length (cm)	5-6.8	57.5-71.6% SL	4.3-7.2

Table 2. Meristic counts of *A. vaigiensis* samples collected for identification

Present study		Previous reports	
Parameters	Counts	Sen *et al*. (2021)	Vella *et al*. (2016)
Dorsal fin spines	XIII	XIII	XIII
Dorsal fin soft rays	13	13	13
Ventral fin spines	I	I	I
Ventral fin soft rays	5	5	4-5
Anal fin spines	II	II	II
Anal fin soft rays	12	12	11-13
Pectoral fin soft rays	16	16	16-18
Gill rakers	25	-	25-28
Lateral line scales	21	-	21-22

the dorsal soft rays (Saad *et al*., 2020) well behind the last dorsal spine (Allen, 1991) and fifth bar is restricted to caudal peduncle (Vella *et al*., 2016; Dragicevic *et al*., 2021), which had no black spots (Tsadok *et al*., 2015) as seen in *A. saxatilis* (Allen, 1991).

The morphological measurements as well as meristic counts match with earlier reports of the same species (Vella *et al*., 2016; Saad *et al*., 2020; Mai and Tsai, 2021; Sen *et al*., 2021). The use of molecular analysis in combination with morphological studies can be used to clarify the morphological characters used for identification. Molecular tools have been employed for identification of species in Pomacentridae (Pradipunt, 2017) and has also been used in the genus *Abudefduf* with a complex evolutionary history (Bariche *et al*., 2015; Tsadok *et al*., 2015; Vella *et al*., 2016; Bertrand *et al*., 2017). The most commonly used gene as a marker for the barcode is the mitochondrial cytochrome c oxidase I (COI) (Hebert *et al*., 2003; Hebert *et al*., 2004). All the partial sequences of *A. vaigiensis* derived from the study was submitted to NCBI, GenBank (www.ncbi.nlm.nih.gov) with the accession numbers MN057943, MN057944, MN057945 and MN057946. Distinct clustering was evident between species in the maximum likelihood tree constructed using COI sequences of *Abudefduf* sp. (Fig. 3) indicating the distinct species status of *A. vaigiensis* specimens collected from the sampling stations. K2P (Table 3) distance between species indicated that *A. sexfasciatus* is closely related to *A. vaigiensis* (K2P; 0.7%) followed *by A. conformis* (1.9%).

LWR is of paramount importance in fishery science as it assists in understanding the general wellbeing and growth patterns in a fish population. About 360 moribund samples were collected, sexed and grouped into categories such as male, female and unsexed. The sex identification was done by dissecting out individual specimens and by physical examination of the gonad since sexual dimorphism does not exist among males and females. The LWRs of the fishes collected is depicted in Fig. 4. The results of ANOVA on length-weight regressions were found to be highly significant ($p<0.05$) in the case of all sets of data. The value of 'b' (regression coefficient) in all the equations were less than 3 which indicated negative allometric growth. Sudhakar and Shameem (2009) also reported negative allometric growth in male and female *A. vaigiensis* from Vishakhapatnam coast. Similar to the present study, Gumanao *et al*. (2016) reported negative allometric growth in *A. vaigiensis* from Philippines while Macieira and Joyeux (2008) have also reported negative allometric growth in *A. saxatilis* from Brazil. Ideally a fish

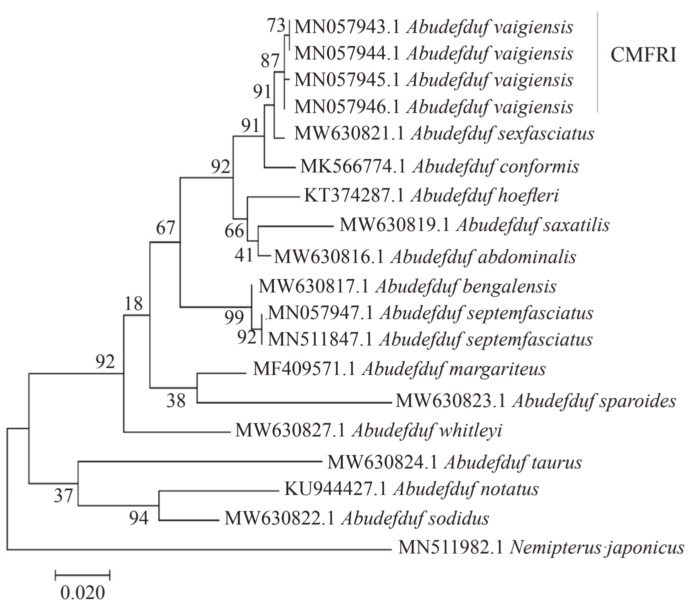

Fig. 3. Maximum likelihood tree constructed using COI sequences of *Abudefduf* spp.

Table 3. Kimura-2-parameter distance values based on COI sequences and standard error values between different species of *Abudefduf* (K2P values on the left side of the diagonal and Standard error on the right side of the diagonal)

	A. vaigiensis	*A. septemfasciatus*	*A. saxatilis*	*A. abdominalis*	*A. hoefleri*	*A. whitleyi*	*A. sexfasciatus*	*A. bengalensis*	*A. margariteus*	*A. sparoides*	*A. notatus*	*A. conformis*	*A. sordidus*	*A. taurus*
A. vaigiensis		0.011	0.009	0.007	0.009	0.012	0.003	0.010	0.011	0.015	0.015	0.006	0.014	0.016
A. septemfasciatus	0.061		0.012	0.010	0.011	0.011	0.010	0.002	0.012	0.015	0.015	0.011	0.015	0.016
A. saxatilis	0.050	0.084		0.008	0.009	0.013	0.009	0.012	0.013	0.015	0.016	0.009	0.016	0.016
A. abdominalis	0.032	0.061	0.032		0.007	0.012	0.008	0.010	0.012	0.015	0.015	0.007	0.014	0.016
A. hoefleri	0.044	0.071	0.044	0.027		0.013	0.010	0.011	0.011	0.014	0.016	0.010	0.015	0.016
A. whitleyi	0.086	0.073	0.095	0.075	0.097		0.012	0.011	0.012	0.014	0.015	0.013	0.014	0.016
A. sexfasciatus	0.007	0.056	0.050	0.032	0.044	0.086		0.009	0.011	0.015	0.015	0.005	0.014	0.016
A. bengalensis	0.056	0.004	0.079	0.057	0.067	0.073	0.052		0.011	0.015	0.015	0.010	0.014	0.016
A. margariteus	0.075	0.075	0.092	0.071	0.067	0.086	0.077	0.071		0.013	0.014	0.012	0.012	0.015
A. sparoides	0.119	0.114	0.128	0.112	0.114	0.110	0.121	0.110	0.090		0.017	0.015	0.016	0.015
A. notatus	0.136	0.150	0.153	0.141	0.155	0.136	0.143	0.150	0.127	0.159		0.016	0.011	0.016
A. conformis	0.019	0.069	0.050	0.029	0.048	0.086	0.019	0.065	0.086	0.126	0.145		0.015	0.016
A. sordidus	0.115	0.132	0.138	0.127	0.132	0.125	0.118	0.127	0.102	0.157	0.067	0.120		0.016
A. taurus	0.154	0.166	0.156	0.151	0.156	0.159	0.154	0.166	0.142	0.154	0.140	0.149	0.131	

with 'b' value 3 is very difficult to observe in the natural environment (Allen, 1938) and in majority of fishes, 'b' value is either less than or greater than 3. The change in 'b' value mostly reflects the change in the body form, when the weight of the fish gets affected by environmental factors like temperature, food supply, spawning conditions and other factors like sex, age, fishing time and area and fishing vessels (Le Cren, 1951; Ricker, 1973; Bagenal and Tesch, 1978) and influenced by geographical and ecological changes (Mommsen, 1998).

The macroscopic study of gonads for evaluating the sex ratio showed that, there were 88 (24.44%) females, 154 (42.78%) males and 118 (32.78%) unsexed individuals. The sex ratio in the present work has been evaluated considering only the sex differentiated animals. A total of 88 females and 154 males were obtained for which the male to female ratio is 1.75: 1. The sex ratio in the present work indicates a male dominated population of *A.vaigiensis* available in Gulf of Mannar region. Bessa *et al.* (2007) also reported a male dominant population in *A. saxtilis* from Brazil. On contrary, Sudhakar and Shameem (2009) reported a female dominant population of the same species off Vishakapatanam. Pillai and Mohan (1990) reported a sex ratio of 1:1.4 in *A. glaucus* from Lakshadweep waters. Mohan *et al.* (1986) also reported a female dominant population in pomacentrid blue puller Chromis caeruleus. Based on the sex ratio, the nature of sexual dimorphism can be predicted. In the damsel, Neopomacentrus cyanamos, higher female to male ratio (4.6:1) indicates that the species is protogynus (Sreeraj and Gopakumar, 2004). The near equal sex ratio in the present study indicates that *A. vaigiensis* is a gonochoristic fish.

Relative condition factor (Kn) is preferred over condition factor (K) as the latter is highly influenced by many environmental and biological factors (Le Cren, 1951) such as onset of maturity (Hoda, 1987), spawning (De-Silva and Silva, 1979; Al-Dham and Wahab, 1991), sex and maturity (Gowda *et al*., 1987; Doddamani and Shanbouge, 2001) biotic and abiotic environmental conditions (Baby *et al*., 2011), feeding intensity (Wheatherley, 1972) and pollution (Bakhoum, 1999; Devi *et al*., 2008). Kn is used to compare general well-being (Thomas, 1969) and reveals information on food availability (Le Cren, 1951). Kn for *A. vaigiensis* was estimated as 1.0036 for males, 1.0051 for females, 1.0031 for unsexed and 1.033 for pooled samples. In the present study, estimated Kn value above 1.00 in all the groups indicates that fish of a specific length weighs more than the expected weight and shows that the sites from which fishes were collected provided conducive environment for growth of the species. The values of Kn equal or close to 1 indicates overall fitness of fish species (Jisr *et al*., 2018).

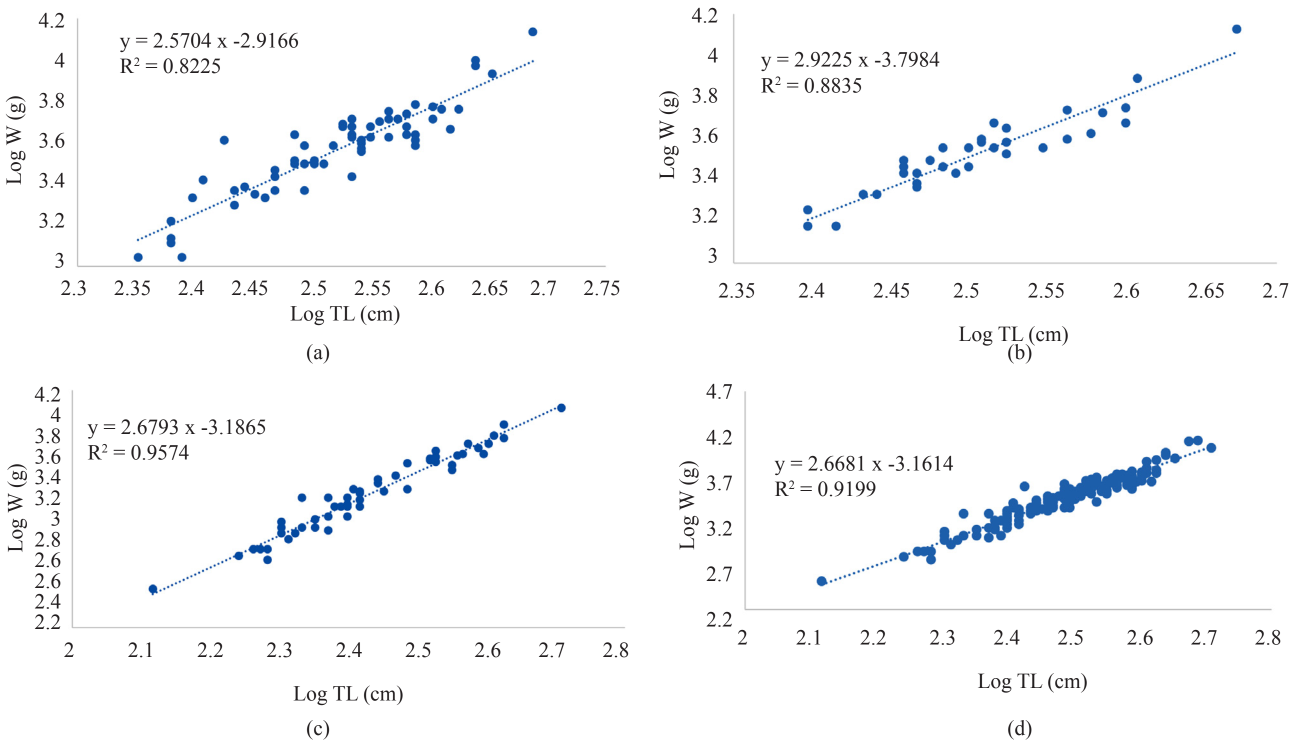

Fig. 4. Length- weight relationship of *A. vaigiensis*: (a) Male; (b) Female; (c) Unsexed; (d) Pooled

In conclusion, the results of the present study indicate that *A. vaigiensis* in south-east coast of India follow a negative allometric growth pattern with 'b' values less than 3 with a male dominant population. The study also adds basic information of this species, which may be useful for fishery biologists and conservation agencies to impose adequate regulations for sustainable fishery management and conservation in Indian waters.

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