

Research Article

Genetic identity, allometric traits, and gastro-somatic index of *Chromis viridis* in Agatti Island, Lakshadweep: Implications for conservation

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Chromis viridis, a key live bait fish, being used for the pole-and-line tuna fishery of Lakshadweep, plays a vital role in the sustainability of the said traditional fishing practice. Given its ecological and economic importance, understanding the species' biological and genetic characteristics is essential for effective management and conservation. This study presents the first gender-specific analysis of Length-Weight Relationship (LWR), condition factors, and Gastro-Somatic Index (GaSI) of *C. viridis*, alongside genetic characterisation using mitochondrial cytochrome c oxidase subunit 1 (CO1) and 16S rRNA gene markers. Specimens were sampled monthly from March 2022 to February 2023. Results revealed distinct seasonal and sex-specific growth patterns. Males exhibited isometric growth ($b \approx 3$) in February, April, October, November, and December, while females showed similar growth in March, April, September, and November. Negative allometric growth ($b < 3$) observed for both sexes indicate fluctuations in energy allocation potentially linked to spawning cycles and environmental variability. The condition factors: Fulton condition factor (K), allometric condition factor (K_A), and relative condition factor (K_R) varied seasonally in both sexes. GaSI values fluctuated throughout the year, reflecting seasonal changes in feeding intensity. Both sexes showed peak GaSI in April, May, and October. Notably, females consistently exhibited higher GaSI values than males, suggesting more energy investment in feeding, which is likely to support oogenesis. These findings provide critical insights into the growth dynamics and physiological responses of *C. viridis*, contributing valuable data for species-specific, sex-oriented conservation and fisheries management strategies.

[**Keywords:** *Chromis viridis*, Condition factor, Gastro somatic index, Genetic characterisation, Length-weight relationship]

Introduction

Chromis viridis, commonly known as the Blue green chromis or Blue green puller, is a prominent species in the coral reef ecosystem of the Lakshadweep islands¹. This species is distinguished by its vibrant blue-green colour and small size. It commonly forms aggregations over *Acropora* corals². It is vital for pole and line fishing, a traditional method in Lakshadweep, serving as a key live bait to lure larger pelagic species such as yellow-fin tuna, trevally, and groupers. However, common practices such as harvesting live baits during the spawning season, night-time fishing with torchlight, and overharvesting have increased fishing pressure on the wild stock, leading to a decline in the live bait population³. Current exploitation rates for most live bait fishes are reported to be above the Maximum Sustainable Yield (MSY)⁴, necessitating urgent monitoring of live bait populations³. The scarcity of live bait is often reported as a limiting factor for the expansion of the tuna fishery in Lakshadweep. Beyond its role as baitfish, *Chromis viridis* holds global significance in the aquarium trade due to its captivating

colouration and calm demeanour. Balancing the needs of the local fishery with global trade becomes imperative for ensuring the sustainability of *C. viridis*.

Two species of blue-green damselfishes are found in the Indo-Pacific region and can be distinguished by a black pectoral fin axil, which is present only in *C. tripterygion*⁵⁻⁸. *Chromis tripterygion* has a black area at the pectoral fin base, while *C. viridis* has black dots forming a dusky area at the upper pectoral fin base⁹. The similarity in colouration and overlapping number of pectoral rays makes it difficult to differentiate these sibling species, highlighting the need for genetic characterisation studies to accurately identify species in Lakshadweep.

Systematic studies investigating length-weight relationships, condition factors, and gastro-somatic index throughout the annual cycle are essential for gaining insights into the growth patterns, reproductive cycles, and overall population health of *C. viridis*. The examination of Length-Weight Relationships (LWRs) holds significance in fisheries science and population dynamics, as it allows for the estimation of weights

from easily measurable lengths, particularly when direct weight measurements are time-consuming¹⁰⁻¹⁴. Understanding the correlation between length and weight in a specific geographical area, such as Lakshadweep waters, is valuable for computing weight-at-age, evaluating fish conditions, and comparing the life history of fish across regions¹⁵. The establishment of LWR creates a mathematical relationship between these variables, facilitating the calculation of weight variations for individual or grouped lengths¹⁶. This not only provides crucial insights into fish growth but also contributes to assessments of body condition. Such information is essential for a comprehensive understanding of population dynamics and for evaluating potential impacts of anthropogenic activities on *C. viridis* in the studied region.

The condition factor (K) is a crucial biological parameter that reflects the growth status and overall health of a fish species and its community¹⁷. It serves as a key indicator for assessing the holistic health, productivity, and physiological well-being of the fish population, with regular calculations performed for this purpose¹⁸. This metric is intricately influenced by both biotic and abiotic environmental factors, making it a valuable index for evaluating the state of the aquatic ecosystem where the fish reside¹⁹. Organisms display diverse dietary behaviours influenced by changes

in ontogeny, prey abundance, and environmental conditions²⁰. The Gastro-Somatic Index (GaSI) serves as a vital indicator of feeding intensity in fish^{21,22}. Fluctuations in monthly GaSI values signify changes in feeding intensity, highlighting the dynamic nature of feeding behaviour. Therefore, the study of GaSI is essential for both scientific comprehension and the practical aspects of fisheries.

In the present investigation, *Chromis viridis* specimens were genetically characterised using mitochondrial genes. Additionally, monthly variations in the LWR, condition factor (K), and gastro-somatic index (GaSI) were examined for both male and female *C. viridis* to determine potential fluctuations in these parameters across different months. Regular monitoring of these metrics is crucial for detecting trends, identifying potential threats, and implementing effective conservation and management strategies. This approach ensures the sustainability of fish populations and the health of aquatic environments, particularly reef systems.

Materials and Methods

Fish collection and identification

The fish specimens were collected from the lagoons of Agatti Island, Lakshadweep (10°49'30.2" N, 72°09'45.2" E; Fig. 1) monthly from March 2022 to

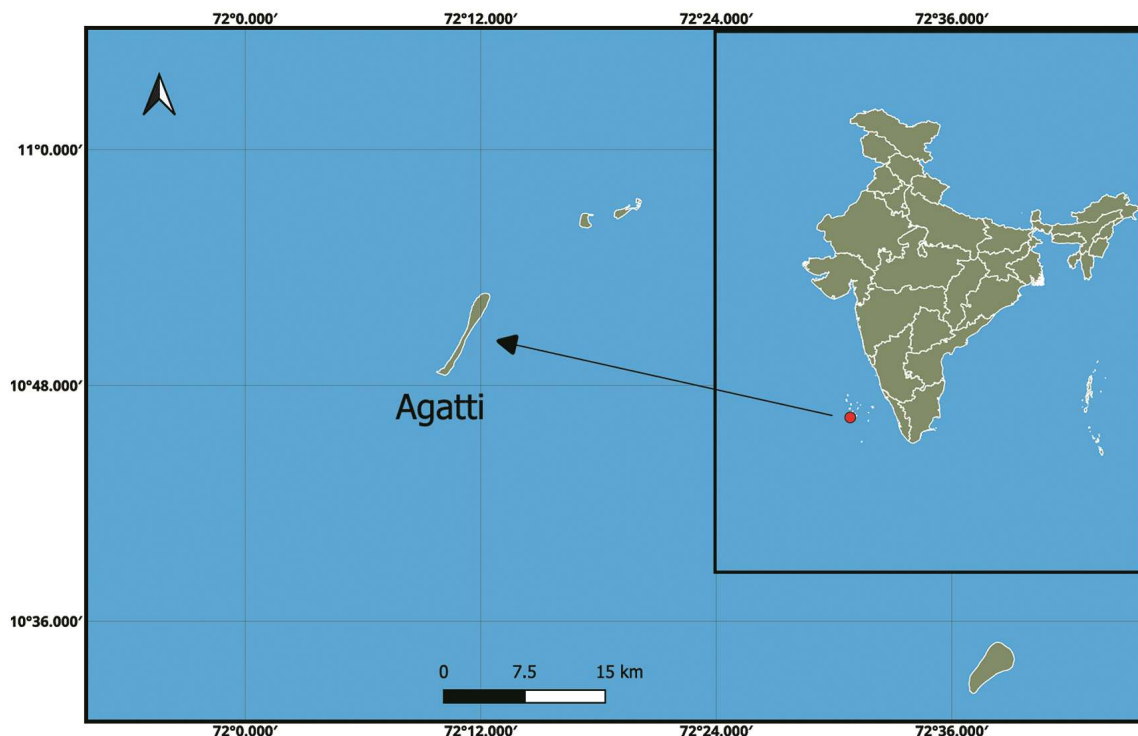


Fig. 1 — Layout of the sampling site, Agatti Island, Lakshadweep

February 2023 using lift nets. The fishes could not be collected in June and July due to the challenging collection conditions caused by rough seas during the monsoon season. Fish specimens were identified using standard identification keys and published documents^{5,23}, and the scientific names were validated by consulting Eschmeyer's Catalogue of Fishes²⁴.

Molecular identification and characterisation

Chromis viridis specimens (n = 5) were characterised using universal primer sets to amplify the 16S rRNA and CO1 region of mitochondrial DNA. The DNA was extracted from fin tissues using a standard phenol/chloroform extraction protocol. PCR amplification was conducted with Emerald Amp MAX PCR Master Mix (Takara, Japan) in a 25 µl reaction mixture containing 12.5 µl of 2X PCR Master Mix, 1 µM of each primer, 100 ng of template DNA, and nuclease-free water. The 16S rRNA gene was amplified using the universal primers 16sbr-H (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and 16sar-L (5'-CGC CTG TTT ATC AAA AAC AT-3')²⁵. The PCR protocol included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 54 °C for 30 sec, and extension at 72 °C for 30 sec, with a final extension at 72 °C for 5 min. The universal primers of Co1FF2d (5'-TTCTCCACCAACCACAA-RGAYATYGG-3') and Co1FR1d (5'-CACCTC-AGGGTGTCCGAARAAYCARAA-3')²⁶ were used to amplify cytochrome c oxidase subunit 1 (CO1) gene. The PCR protocol is as follows: Initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 40 sec, extension at 72 °C for 40 sec followed by a final extension at 72 °C for 5 min. The PCR products were visualised on a 1.5 % agarose gel and sequenced using the Big Dye Terminator 3.1 Cycle Sequencing Kit on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems) at Genespec Inc. The raw sequences were trimmed and aligned using the ClustalW algorithm²⁷ in BioEdit v. 7.7.1^(ref. 28).

Reference 16S rRNA sequences for *C. viridis* (FJ616433, MW630404), *C. atripectoralis* (JF457369), *C. multilineata* (EF489727), *C. fumea* (AY365121), *C. xanthura* (JF457405), and *C. notata* (KC767732) were obtained from the NCBI GenBank database. Accordingly, the reference CO1 sequences for *C. viridis* (KJ967980, KJ967992), *C. ternatensis* (OR114069), *C. weberi* (OQ690650), *C. bowesi* (MH170479), *C. cadenati* (GQ341589), *C. alta* (MW630786) and

C. degruyi (EU358590) were also obtained from the NCBI GenBank database. *Acanthochromis polyacanthus* (FJ616407) was selected as the outgroup. Genetic distances were calculated in MEGA 11^(ref. 29) using the Kimura two-parameter model (K2P)³⁰ with 1000 bootstrap replications. Phylogenetic trees were generated with the maximum likelihood method using the Kimura 2 parameter with 5000 randomised bootstrap iterations.

Length-Weight Relationship (LWR)

The sex of the fish was determined based on the morphology of the urogenital papillae. In males, the papillae are conical and pointed, while in females, they are blunt and round³¹. However, this characteristic is underdeveloped in juveniles. To determine the length-weight relationship of male, female, and unsexed individuals, accurate recordings of fish length, measured to the closest millimeter (mm), and total weight (W), measured to the nearest gram (g), were recorded. Length measurements were conducted using a Vernier calliper (Mitutoyo, Japan) with a precision of 0.01 mm, and weight measurements were taken with a digital balance (Sartorius, Germany) with a precision of 0.1 mg. Total Length (TL) and weight in millimetres (mm) and grams (g) were used for computing the LWR. The equation, $\text{Log}W = \text{Log} a + b \text{Log} L$, representing the linear form $W = aL^b$, was employed for LWR estimation¹⁵. The inclusion of either the isometric range (b value close to 3) or the allometric range (negative allometric; $b < 3$ or positive allometric; $b > 3$) was determined based on the comparison results.

Condition factor

Fulton's condition factor (K) was computed using the formula $K = 100 \times (W/L^3)$, where 'W' is the total weight and 'L' is the length in centimetres (cm)³². The allometric condition factor (K_A) was calculated using the formula $K_A = W/L^b$, considering 'W' as the total weight, 'L' as the length in cm, and 'b' as the parameter of the LWR^(ref. 33). The relative condition factor (K_R) was determined using the equation $K_R = W/(a \times L^b)$, where 'W' is the total weight, 'L' is the length in cm, and 'a' and 'b' are parameters of LWR^(ref. 15).

Gastro-Somatic Index (GaSI)

Gastro-Somatic Index (GaSI) was calculated as the $\text{Weight of gut} / \text{Weight of fish} \times 100$ ^(ref. 34). All statistical analyses were conducted in MS Excel 2010, and a significance level of 5 % ($p < 0.05$) was considered for the evaluations.

Table 1 — Morpho-meristic measurements of *Chromis viridis* from the Indian Ocean (Agatti Island, Lakshadweep)

Measurements	Mean ± SD	TL%	Mean ± SD	TL%
	Males (n = 19)		Females (n = 22)	
Gender (sample size)				
Total length (mm)	68.16±7.31	—	69.62±7.22	—
Fork length (mm)	58.63±6.81	86.03	61.57±6.35	88.43
Standard length (mm)	50.32±5.91	73.82	54.04±4.58	77.61
Weight (g)	5.69±1.64	8.36	6.72±1.35	9.65
Body depth (mm)	22.63±2.91	33.19	23.21±1.77	33.34
Head length (mm)	14.23±1.65	20.88	14.53±2.71	20.87
Post orbital length (mm)	6.43±0.92	9.42	6.75±1.22	9.83
Eye diameter (mm)	4.81±0.36	7.06	5.14±0.52	7.38
Predorsal fin distance (mm)	19.51±1.86	28.62	20.82±1.21	29.90
Dorsal fin base length (mm)	25.79±3.97	37.84	27.76±2.39	39.88
Preanal fin distance (mm)	35.41±4.06	51.94	36.89±2.49	52.98
Anal fin base length (mm)	8.93±1.18	13.09	9.69±1.05	13.91
Minimal caudal peduncle depth (mm)	6.89±0.62	10.12	6.81±0.44	9.78
Pectoral fin length (mm)	13.15±1.68	19.29	14.11±1.67	20.27
Ventral fin length (mm)	13.52±1.69	19.84	13.15±1.53	18.88
Meristic characters				
Dorsal fin			XII/9-10	
Anal fin			II/9-10	
Pectoral fin			15-17	
Ventral fin			I/5	

Results

Morpho-meristic measurements of *Chromis viridis*

Morphological data from males (n = 19) and females (n = 22) were analysed. For males, the average length was 68.16±7.31 mm (minimum – 56.04 mm, maximum – 75.96 mm), and the average weight was 5.69±1.64 g (minimum – 3.4 g, maximum – 8.58 g). In females, the average length was 69.62±7.22 mm (minimum – 60.65 mm, maximum – 79.14 mm), and the average weight was 6.72±1.35 g (minimum – 4.78 g, maximum – 8.73 g). The values were expressed as mean ± standard deviation. Detailed morphometric characteristics and measurements are provided in Table 1. The Standard Length to Total Length ratio (SL/TL) was 73.82 % in males and 77.61 % in females, whereas the body depth to total length ratio was nearly identical, which is 33.19 % in males and 33.34 % in females.

Molecular identification and characterisation

DNA extracted from each specimen was successfully amplified, sequenced, and analysed. Generated 16S rRNA and CO1 sequences were submitted to the GenBank database with the following accession numbers: 16S rRNA - PP843543, PP843544, PP843545, PP843546, PP843547 and CO1 - PP936053, PP936054, PP936055, PP936056, PP936057. The length of sequences of partial 16S rRNA and CO1 genes were 580 bp and 643 bp, respectively.

BLAST analysis revealed a species match of higher than 99 % sequence similarity for the 16S rRNA and CO1 genes. A maximum likelihood tree, generated based on the 16S rRNA (Fig. 2) and CO1 sequences (Fig. 3), produced well-defined clusters with available reference sequences. When compared to other *Chromis* species, the analysis of 580 bp of 16S rRNA sequences revealed a total of 33 parsimony-informative sites, 547 conserved sites, 75 variable sites, and 41 singleton sites. Similarly, analysis of 643 bp of CO1 sequences identified 115 parsimony-informative sites, 516 conserved sites, 164 variable sites, and 49 singleton sites. The genetic distance analysis based on the 16S rRNA gene indicated that the specimens used in the present study are closely related to *C. viridis* found in Fiji, with a divergence value of less than 0.002. In contrast, the CO1 gene analysis showed the closest relationship with *C. viridis* sampled from French Polynesia, with a low genetic distance (d = 0.005) (Table 2).

Length frequency distribution

A total of 1,278 individuals of *C. viridis* were collected from Agatti Island for the study, with lengths ranging from 5.4 to 10.1 cm TL and weights between 2.4 and 14.8 g (Table 3 and Fig. 4). Analysis of the length frequencies of *C. viridis* specimens revealed no differentiation between sexes below 5 cm. These fish reach maturity only after surpassing a length of 5 cm. Within the 5 to 10 cm size range,

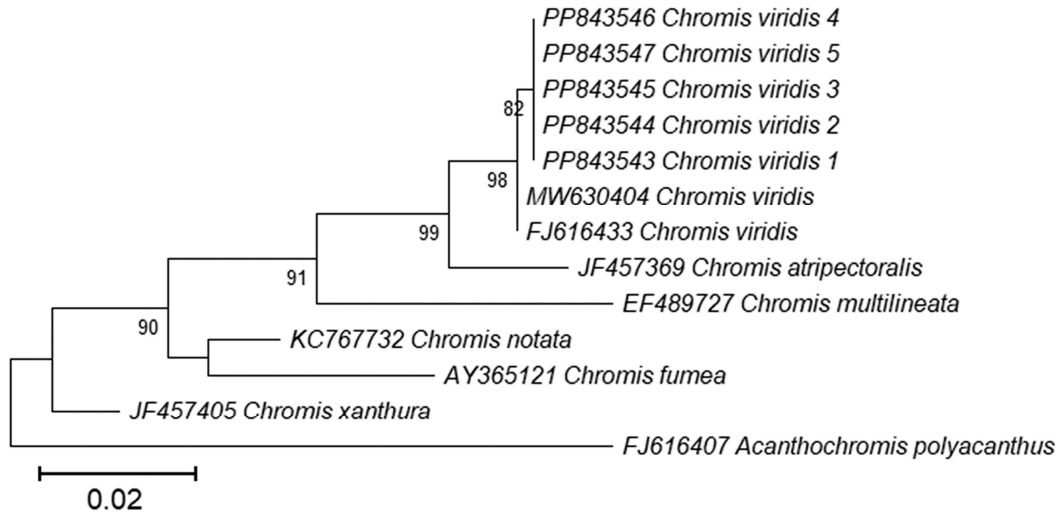


Fig. 2 — Maximum likelihood tree constructed by the 16S rRNA sequences of *C. viridis* with reference sequences of specimens belonging to genus *Chromis* and outgroups

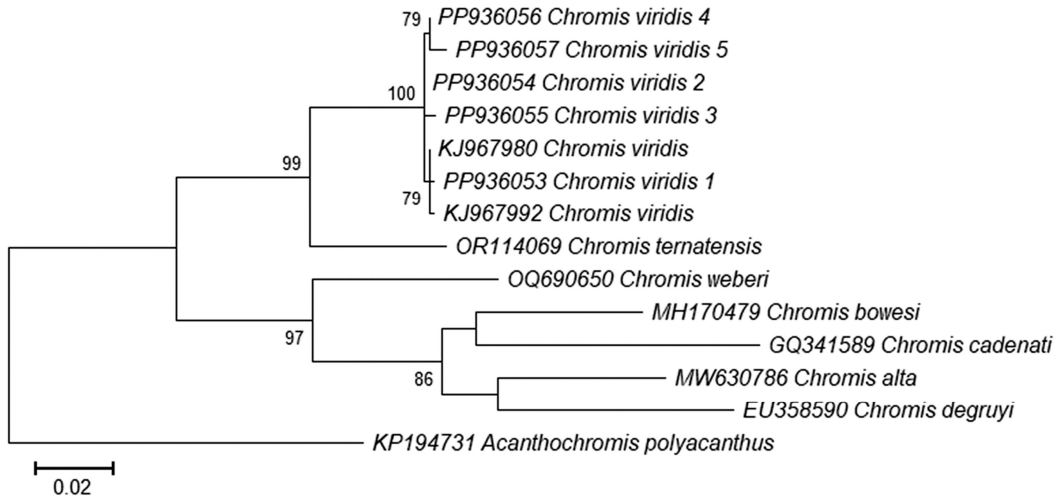


Fig. 3 — Maximum likelihood tree constructed by the COI sequences of *C. viridis* with reference sequences of specimens belonging to genus *Chromis* and outgroups

males, females, and unsexed individuals comprised 35.42, 38.78, and 25.79 % of the recorded samples, respectively (Fig. 5).

Length-Weight Relationship (LWR)

The LWR parameters, including the coefficient of determination (r^2), intercept (a), growth coefficient (b), t -value, and type of growth for male and female *C. viridis*, are detailed in Table 4. This investigation documents monthly variation in LWR for *C. viridis*.

For males, isometric growth (b value close to 3) was observed in February, April, October, November, and December ($b = 2.856 - 3.061$; $p < 0.00001$) with a highly significant length-weight regression

relationship. Negative allometric growth ($b < 3$) was recorded in *C. viridis* during January ($b = 2.36$), March ($b = 2.76$), September ($b = 2.60$), August ($b = 2.40$), May ($b = 2.71$), and October ($b = 2.46$), with all regressions showing high significance ($p < 0.00001$; Fig. 6).

For females, isometric growth ($b \approx 3$) was observed in November ($b = 2.82$), September ($b = 2.89$), May ($b = 3.13$), April ($b = 3.03$), and March ($b = 2.97$), while negative allometric growth ($b < 3$) was observed in February ($b = 2.68$), January ($b = 2.75$), December ($b = 2.60$), October ($b = 2.46$), and August ($b = 2.46$), with all regressions highly significant ($p < 0.00001$; Fig. 7).

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

Sl. No.	Sequence ID _ Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	PP936053_ <i>Chromis viridis</i> 1		0.002	0.003	0.003	0.004	0.002	0.002	0.012	0.015	0.018	0.018	0.017	0.017	0.019
2	PP936054_ <i>Chromis viridis</i> 2	0.003		0.002	0.001	0.003	0.002	0.002	0.012	0.015	0.018	0.018	0.017	0.017	0.019
3	PP936055_ <i>Chromis viridis</i> 3	0.006	0.003		0.003	0.004	0.003	0.003	0.012	0.015	0.018	0.017	0.017	0.017	0.019
4	PP936056_ <i>Chromis viridis</i> 4	0.005	0.002	0.005		0.003	0.002	0.003	0.012	0.015	0.018	0.018	0.017	0.017	0.019
5	PP936057_ <i>Chromis viridis</i> 5	0.009	0.006	0.009	0.005		0.004	0.004	0.013	0.015	0.019	0.018	0.017	0.018	0.018
6	KJ967980_ <i>Chromis viridis</i>	0.002	0.002	0.005	0.003	0.008		0.002	0.012	0.015	0.018	0.018	0.016	0.017	0.019
7	KJ967992_ <i>Chromis viridis</i>	0.003	0.003	0.006	0.005	0.010	0.002		0.012	0.015	0.018	0.017	0.017	0.017	0.018
8	OR114069_ <i>Chromis ternatensis</i>	0.070	0.066	0.070	0.068	0.074	0.069	0.071		0.016	0.019	0.019	0.018	0.018	0.019
9	OQ690650_ <i>Chromis weberi</i>	0.137	0.133	0.137	0.135	0.139	0.133	0.135	0.137		0.015	0.017	0.015	0.017	0.021
10	MH170479_ <i>Chromis bowesi</i>	0.155	0.155	0.155	0.157	0.162	0.156	0.158	0.153	0.115		0.014	0.014	0.015	0.021
11	GQ341589_ <i>Chromis cadenati</i>	0.159	0.157	0.157	0.159	0.162	0.154	0.152	0.167	0.141	0.110		0.015	0.015	0.020
12	MW630786_ <i>Chromis alta</i>	0.145	0.141	0.141	0.143	0.143	0.141	0.143	0.152	0.114	0.105	0.114		0.013	0.020
13	EU358590_ <i>Chromis degruyi</i>	0.159	0.159	0.159	0.161	0.165	0.156	0.158	0.151	0.154	0.115	0.127	0.106		0.019
14	KP194731_ <i>Acanthochromis polyacanthus</i>	0.183	0.178	0.178	0.176	0.174	0.180	0.178	0.183	0.203	0.210	0.195	0.206	0.204	

Table 3 — Total number of *Chromis viridis* specimens collected from Agatti, Lakshadweep

Month	No. of females	No. of males	Juveniles	Total
Mar-22	179	67	26	272
Apr-22	55	42	36	133
May-22	30	23	13	66
Jun-22	0	0	0	0
Jul-22	0	0	0	0
Aug-22	28	29	16	73
Sep-22	71	60	20	151
Oct-22	31	55	15	101
Nov-22	91	65	19	175
Dec-22	47	33	0	80
Jan-23	27	61	6	94
Feb-23	51	78	4	133

For male *C. viridis*, the coefficient of determination (r^2) values indicated a well-fitted growth model, with maximum r^2 values in October (0.972), April (0.965), November (0.931), March, and September (0.925). Slightly lower r^2 values were observed in August (0.914) and May (0.890), indicating a proper fit, whereas January (0.849) and December (0.807) exhibited the lowest r^2 values.

For female *C. viridis*, the coefficient of determination (r^2) values indicated a well-fitted growth model, with maximum r^2 values in November (0.961), August (0.945), October (0.941), April (0.921), September, and January (0.905). Slightly lower r^2 values were observed in March (0.885), February (0.872), and December (0.825), indicating a proper fit, whereas May (0.804) exhibited the lowest r^2 value.

Condition factor

The trends of three condition factors Fulton condition factor (K), allometric condition factor (K_A),

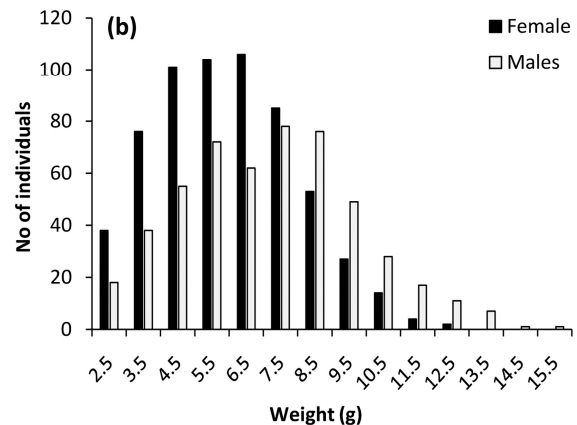
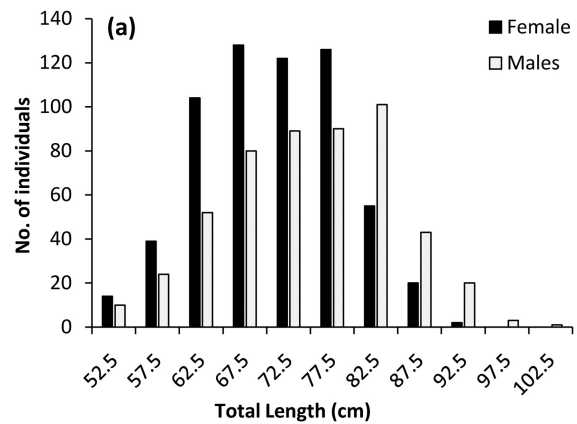


Fig. 4 — (a) Length, and (b) weight frequency distribution of male and female *Chromis viridis* from Agatti island, Lakshadweep

and relative condition factor (K_R) for male and female *C. viridis* from the Agatti Lagoon of the Lakshadweep Islands, spanning from March 2022 to February 2023, are highlighted in Table 5 and Figure 8.

In males, lower Fulton condition factor (K) values were observed in April (1.36 ± 0.094), December (1.55 ± 0.154), and September (1.59 ± 0.167), with higher values in May (1.91 ± 0.167). The highest allometric condition factor (K_A) was recorded in January 2023 (5.632 ± 0.439), while the lowest value occurred in April (1.36 ± 0.035). The relative condition factor (K_R) exhibited the smallest values in January (0.911 ± 0.071) and August (0.995 ± 0.065), and the highest in March (1.009 ± 0.126).

For females, lower Fulton condition factor (K) values were observed in April (1.51 ± 0.146) and September (1.608 ± 0.171), with higher values in February (1.83 ± 0.209) and May (1.81 ± 0.235). The highest allometric condition factor (K_A) was recorded in August 2023 (4.759 ± 0.389), while the lowest value was noted in May (1.41 ± 0.183). The relative condition factor (K_R) exhibited the smallest values in October (1.001 ± 0.075) and November (1.003 ± 0.037), with the highest value in February (1.53 ± 0.165).

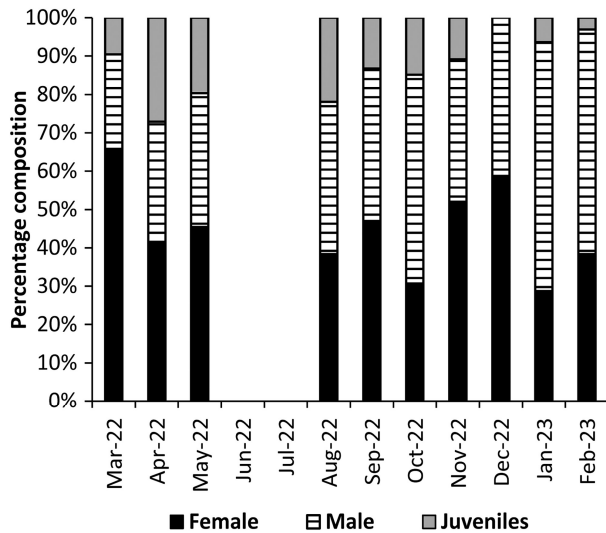


Fig. 5 — Monthly percentage composition of female, male, and juvenile *Chromis viridis* from Agatti island, Lakshadweep

Gastro-Somatic Index (GaSI)

The gastrosomatic index of *C. viridis* exhibited varying values across different months (Fig. 9). In males, the peak values were observed in October 2022 (4.23 ± 0.56) and April 2022 (3.73 ± 1.09), indicating the highest recorded GaSI levels for males. May (3.30 ± 0.28) and August (3.08 ± 0.31) also showed relatively high values, but with lower variability. The lowest values were recorded in January 2023 (1.39 ± 0.67) and December 2022 (1.98 ± 0.49), followed by February 2023 (1.59 ± 0.96). Moderate values were observed in March 2022 (2.54 ± 0.52), September 2022 (2.60 ± 0.52), and November 2022 (2.78 ± 1.48).

The female GaSI values exhibited distinct peaks, low points, and moderate variations across different months. The first peak occurred in April 2022 (4.99 ± 1.71), marking the highest recorded value, followed by a slight decline in May (4.52 ± 0.45). Another notable peak was observed in November 2022 (5.39 ± 2.37).

Discussion

The genus *Chromis* is a prominent member of the family Pomacentridae, encompassing over 424 species with well-documented systematic and genetic diversity^{35,36}. This study provides first-hand information on the genetic characterisation of *Chromis viridis* from Agatti Island, Lakshadweep, focusing on its length-weight relationship, biometric properties, and genetic makeup. This finding is notable as it highlights the genetic continuity of *C. viridis* across oceans. Furthermore, this study provides the first 16S rRNA and CO1 sequence data for *C. viridis* from this region, offering a valuable resource for future research on population dynamics and phylogenetic relationships^{37,38}. Despite the close morphological similarity between *Chromis viridis* and its sister species, *C. atripectoralis*, which are primarily distinguished by the presence

Table 4 — Estimated parameters of LWR ($W = aL^b$) of male and female *Chromis viridis* in the Agatti lagoon of Lakshadweep

Species	Males						Females					
	Month	n	a	b	r ²	Growth	Month	n	a	b	r ²	Growth
<i>Chromis viridis</i>	Mar-22	67	0.0284	2.756	0.925	A-	Mar-22	179	0.0168	2.974	0.885	A
	Apr-22	42	0.0136	3.061	0.965	A	Apr-22	55	0.0143	3.028	0.921	A
	May-22	23	0.0332	2.707	0.890	A-	May-22	30	0.014	3.13	0.804	A
	Aug-22	29	0.0494	2.396	0.914	A-	Aug-22	28	0.0474	2.461	0.945	A-
	Sep-22	60	0.0352	2.596	0.925	A-	Sep-22	71	0.0196	2.894	0.905	A
	Oct-22	55	0.0192	2.925	0.972	A	Oct-22	31	0.0511	2.456	0.941	A-
	Nov-22	65	0.0215	2.878	0.931	A	Nov-22	91	0.0237	2.821	0.961	A
	Dec-22	33	0.0195	2.883	0.807	A	Dec-22	47	0.0356	2.597	0.825	A-
	Jan-23	61	0.0667	2.363	0.849	A-	Jan-23	27	0.0278	2.751	0.905	A-
	Feb-23	78	0.0237	2.856	0.924	A	Feb-23	51	0.0339	2.680	0.872	A-

a = Intercept; b = Slope; r² = Coefficient of determination; Growth type A = Isometric; A- = Negative allometric

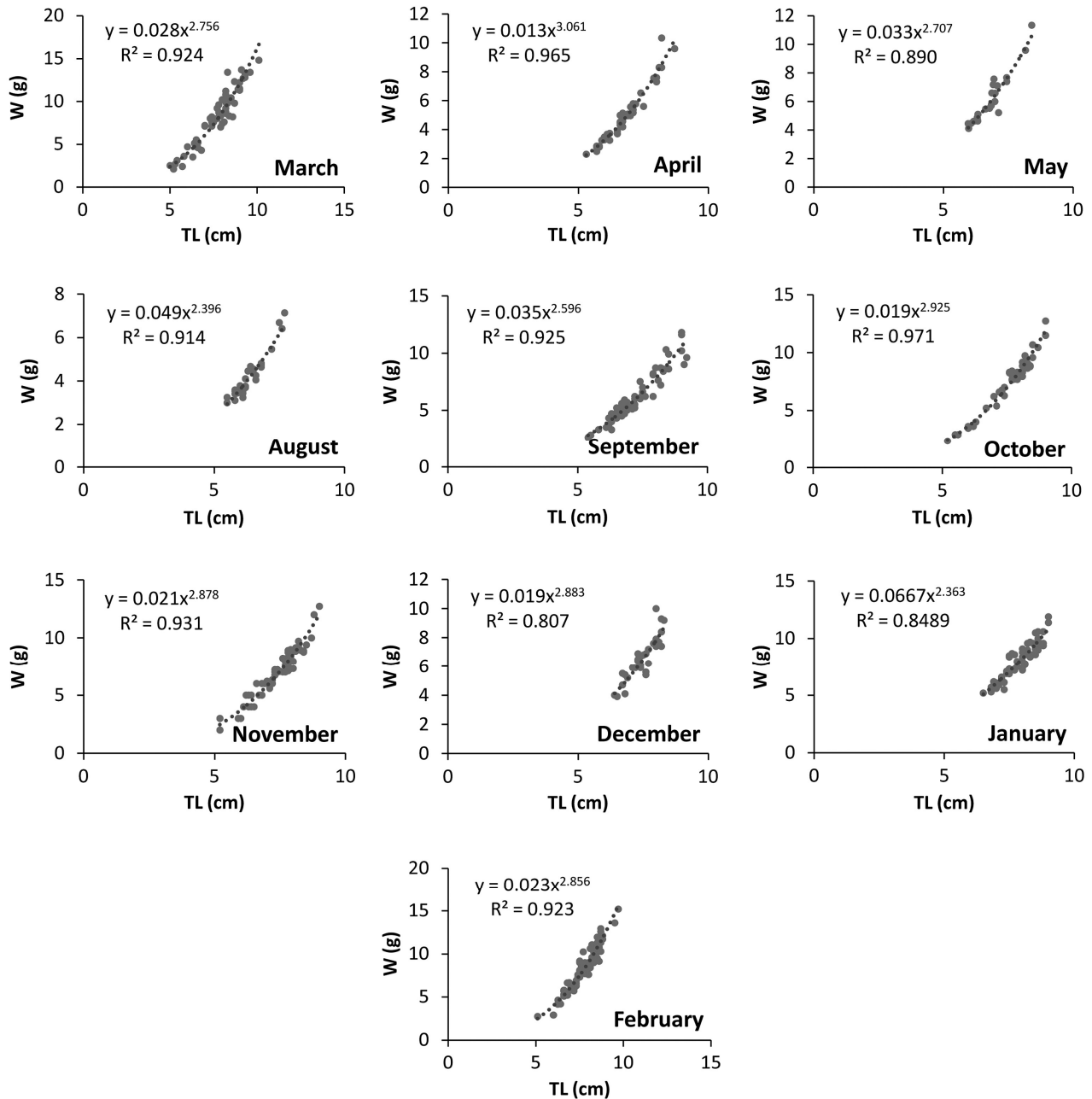


Fig. 6 — Scatter plot of LWRs of male *Chromis viridis* depicting monthly variations

of a black spot at the axil of the pectoral fin in *C. atripectoralis*. The molecular analysis of this study confirms that all specimens sampled from Agatti Island of Lakshadweep are *C. viridis*. No genetic or morphological evidence of *C. atripectoralis* was found, indicating that this species is not present in the region. The results underscore the significance of integrating genetic and biometric data to enhance understanding of the species' diversity and evolutionary history.

The current study's investigation of the length-weight relationship, condition factor, and gastro-somatic index of *C. viridis* by gender represents a novel approach, offering fresh insights into the species' growth and physiological responses. The observed gender-specific differences in growth patterns and condition factors underscore the need for tailored conservation strategies that consider sex-specific requirements and responses. In males, isometric growth ($b \approx 3$) was observed during

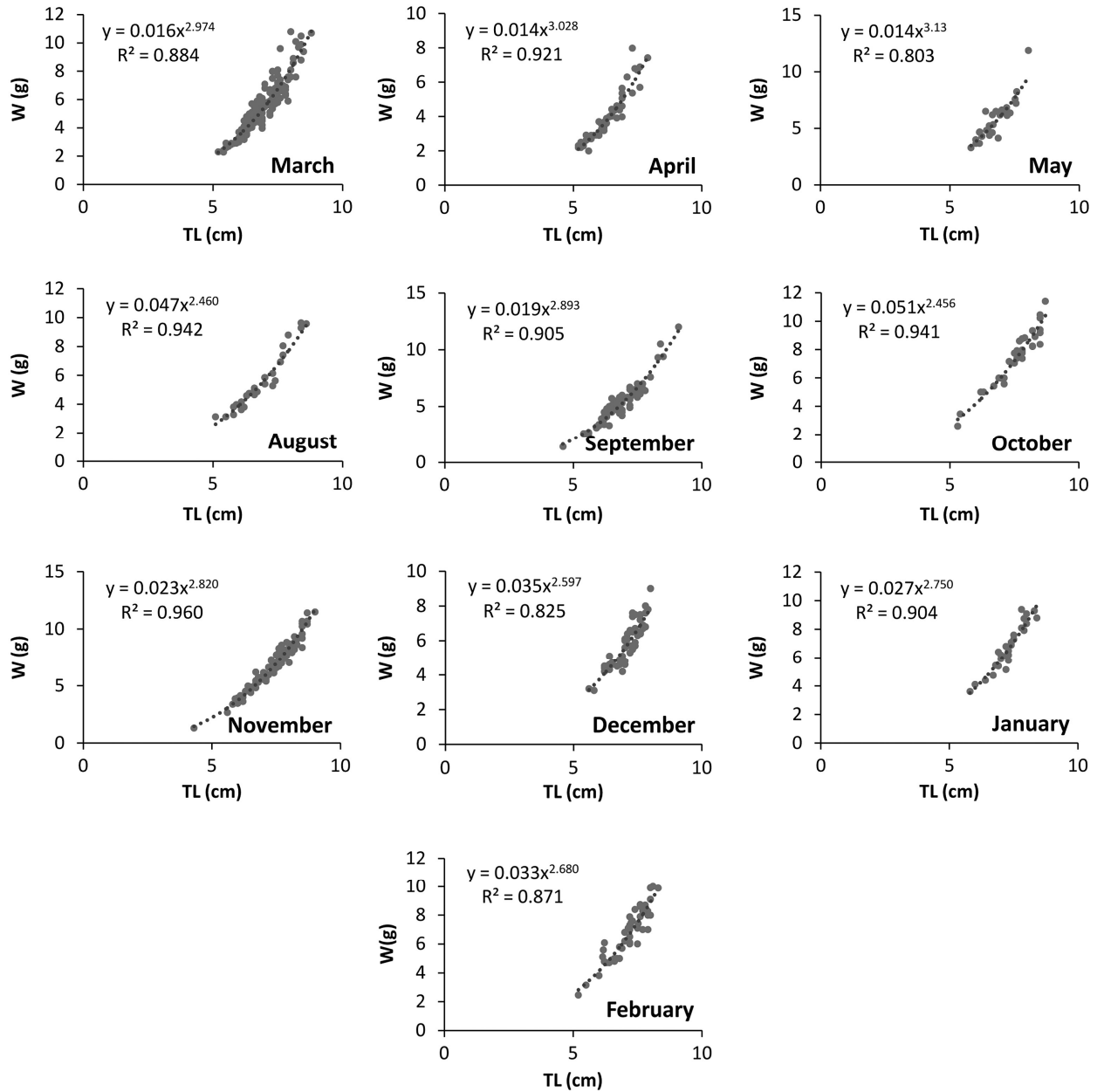


Fig. 7 — Scatter plot of LWRs of female *Chromis viridis* depicting monthly variations

February, April, October, November, and December, suggesting a proportional increase in weight relative to length indicative of optimal growth conditions or balanced energy used for somatic maintenance and reproductive activity. Conversely, negative allometric growth ($b < 3$) occurred in January, March, May, August, and September, where weight gained lagged with length increment. This pattern likely reflects periods of reduced feeding, post-spawning recovery, or energetic trade-offs favouring reproduction over somatic growth. These findings are consistent with previously reported

b -values for various reef fishes, such as *Abudefduf vaigiensis* ($b \approx 3.0$)³⁹ and *Centropomus viridis* ($b = 2.958$)⁴⁰, and support broader ecological observations, where b -values typically fall between 2.5 and 3.5, depending on species, sex, habitat quality, and seasonal conditions⁴¹⁻⁴³.

In females, the growth pattern showed a different temporal distribution. Isometric growth was noted in March, April, September, and November, while negative allometric growth was recorded in January, February, October, and December. The tendency

Table 5 — Fulton's Condition factor (K), Allometric condition factor (K_A) and Relative condition factor (K_R) for male and female *Chromis viridis* from Agatti, Lakshadweep

Species	Month	Condition factors	Males (Mean \pm SD)	Females (Mean \pm SD)
<i>Chromis viridis</i>	Mar-22	K	1.75 \pm 0.232	1.61 \pm 0.197
		K_A	2.86 \pm 0.358	1.69 \pm 0.207
		K_R	1.01 \pm 0.126	1.01 \pm 0.123
	Apr-22	K	1.36 \pm 0.094	1.51 \pm 0.146
		K_A	1.36 \pm 0.035	1.44 \pm 0.138
		K_R	1.00 \pm 0.068	1.01 \pm 0.097
	May22	K	1.91 \pm 0.167	1.81 \pm 0.235
		K_A	3.34 \pm 0.281	1.41 \pm 0.183
		K_R	1.01 \pm 0.084	1.10 \pm 0.131
	Aug-22	K	1.63 \pm 0.141	1.71 \pm 0.194
		K_A	4.92 \pm 0.323	4.76 \pm 0.389
		K_R	0.99 \pm 0.065	1.00 \pm 0.082
	Sep-22	K	1.59 \pm 0.167	1.61 \pm 0.171
		K_A	3.53 \pm 0.329	1.97 \pm 0.209
		K_R	1.00 \pm 0.093	1.01 \pm 0.106
	Oct-22	K	1.68 \pm 0.100	1.72 \pm 0.179
		K_A	1.93 \pm 0.115	4.30 \pm 0.385
		K_R	1.00 \pm 0.059	1.00 \pm 0.075
	Nov-22	K	1.69 \pm 0.161	1.66 \pm 0.124
		K_A	2.16 \pm 0.200	2.38 \pm 0.169
		K_R	1.00 \pm 0.093	1.00 \pm 0.037
	Dec-22	K	1.55 \pm 0.154	1.63 \pm 0.169
		K_A	1.96 \pm 0.194	3.58 \pm 0.352
		K_R	1.00 \pm 0.09	1.00 \pm 0.099
	Jan-23	K	1.63 \pm 0.155	1.70 \pm 0.139
		K_A	5.63 \pm 0.439	2.79 \pm 0.219
		K_R	0.91 \pm 0.071	1.00 \pm 0.078
	Feb-23	K	1.77 \pm 0.170	1.83 \pm 0.209
		K_A	2.38 \pm 0.223	3.41 \pm 0.368
		K_R	1.00 \pm 0.094	1.53 \pm 0.165

towards lower b -values during certain months may reflect reproductive energy allocation, particularly during oogenesis and spawning. This phenomenon is well documented in fish biology, where reproductive investment during spawning periods causes a decline in relative weight, leading to b -values lower than 3. It is therefore plausible that these months coincide with the breeding season of *Chromis viridis* in the Lakshadweep Islands. Supporting this pattern, a study on *Parachanna obscura* revealed that males had a b -value of 3.133, while females had a lower value of 2.674, reflecting reduced weight gain due to reproductive energy investment⁴⁴. Likewise, *Parachaeturichthys ocellatus* showed b -values of 2.907 in males and 2.722 in females, with juveniles exhibiting near-isometric growth ($b = 3.008$), highlighting ontogenetic and sex-based variability in growth dynamics⁴⁵.

These observations suggested that female *C. viridis* are more likely to exhibit negative allometric growth

during peak reproductive periods, likely due to physiological shifts that prioritise reproductive investment over weight gain. Males, while also subject to seasonal variability, maintain more consistent isometric growth, potentially reflecting different reproductive roles and energy demands between the sexes. The correlation between length and weight is critical for estimating weight-at-age, evaluating fish condition, and comparing life history patterns across regions, thus providing valuable insights into fish life history within fisheries science⁴⁶. The recorded r^2 values of 0.889^(ref. 47) and 0.869^(ref. 48) from New Caledonia, and 0.889 from Lakshadweep lagoons⁴⁹, indicate moderate to high levels of correlation. In the Lakshadweep region, growth patterns in marine ornamental fish, including *C. viridis*, exhibit variability influenced by habitat conditions and resource variability^{49,50}.

Fulton's Condition Factor (K) assumes isometric growth ($b = 3$) and is widely used due to its

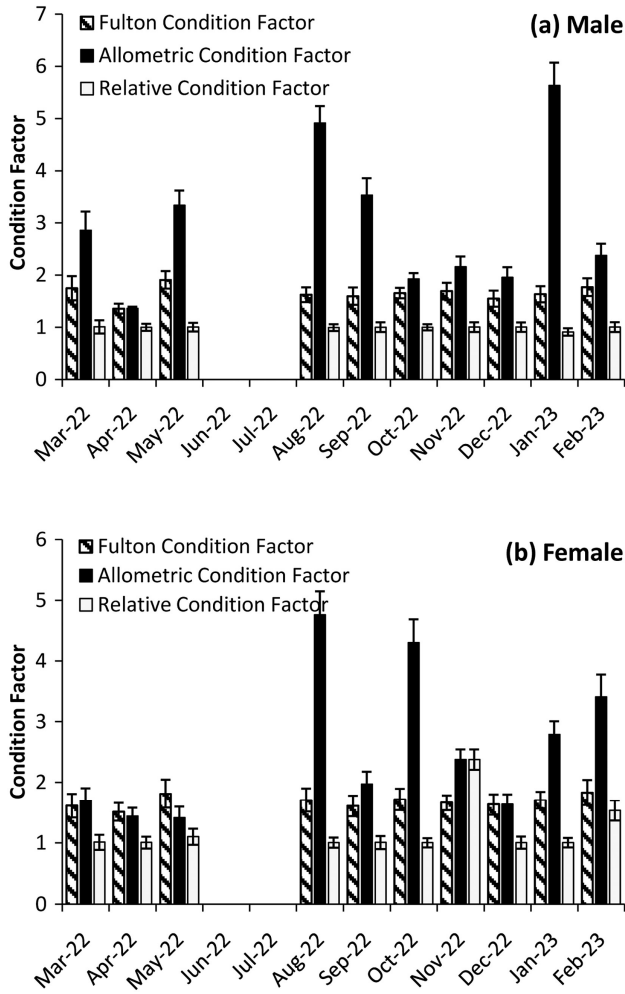


Fig. 8 — Monthly variations in condition factors of (a) male, and (b) female *Chromis viridis* from Agatti island, Lakshadweep

simplicity. However, it can lead to biased results when applied to species that exhibit allometric growth, as it does not account for deviations in the length-weight exponent¹³. To address this limitation, the allometric condition factor (K_A), incorporates the species-specific growth coefficient (b), making it more accurate for assessing condition in species with known allometric growth patterns¹⁵. On the other hand, the relative condition factor (K_R) compares the observed weight to the expected weight from the length-weight relationship and is particularly useful for evaluating individual health in relation to population norms⁵¹.

In males, low Fulton’s condition factor (K) values observed during April, September, and December, suggested periods of nutritional stress or post-spawning recovery, where energy is redirected away from somatic growth. Conversely, the highest

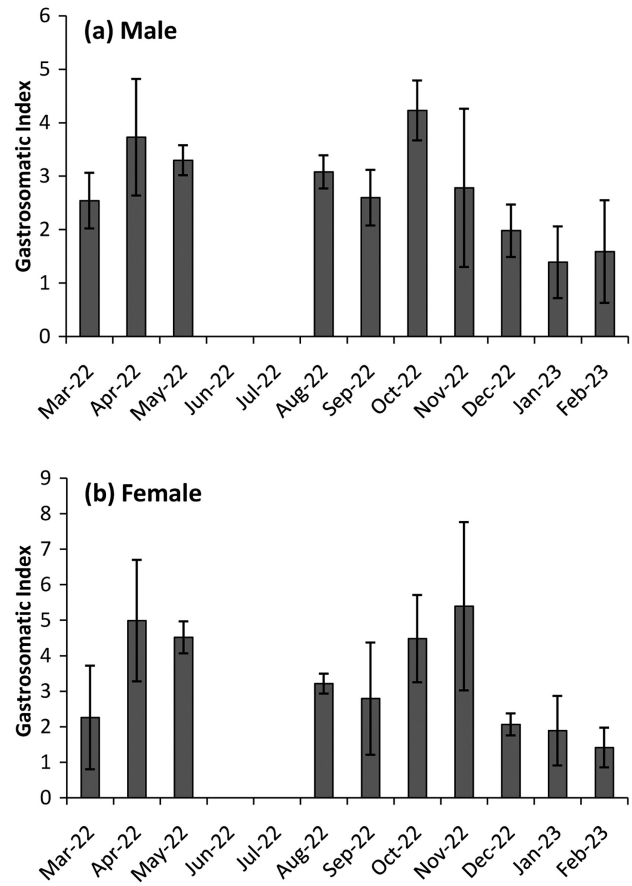


Fig. 9 — Monthly variations in Gastro somatic index of (a) male, and (b) female *Chromis viridis* from Agatti island, Lakshadweep

K value in May (1.91 ± 0.167) reflects improved body condition, likely associated with increased feeding or recovery following reproductive activity. The K_A peaked in January, indicating better overall condition, but dropped significantly in April, highlighting a seasonal shift, where energy may be diverted toward reproductive investment. Similarly, the K_R was lowest in January and August and peaked in March, suggesting fluctuations in weight relative to body length that correspond with the fish’s reproductive and foraging cycles.

In females, similar seasonal trends were observed, but with notable differences in timing and intensity. Lower K values in April and September indicate a shift in energy allocation towards gonadal development, reducing somatic condition during these months. In contrast, higher K values in February and May suggested improved nutritional status, likely during non-spawning or recovery phases. The K_A reached its peak in August, aligning with enhanced somatic growth, while its lowest value in May

(1.41 ± 0.183) reflects a strong reproductive energy investment. The K_R pattern in females diverged slightly from that of males, with the lowest values in October and November and a peak in February, indicating different timing in the physiological trade-offs between maintenance and reproduction.

While both sexes exhibit cyclical changes in condition factors tied to seasonal and reproductive rhythms, females showed more pronounced fluctuations, likely due to the higher energy demands of oocyte production and spawning. These findings are consistent with previous research on related damselfishes. For example, *Chromis chromis* in the Mediterranean exhibits reduced condition factors in females during spawning periods, attributed to reproductive energy depletion, while post-spawning recovery leads to increased body condition later in the year⁵². Similar patterns were documented in *Serranus cabrilla*, where condition improved during energy accumulation phases before spawning and declined thereafter⁵³. Moreover, growth and condition factors are influenced not only by reproductive cycles, but also by environmental conditions and seasonal changes⁵⁴⁻⁵⁷.

These sex-specific patterns in condition factors emphasise the need for differentiated management strategies that consider the distinct reproductive energetics and ecological roles of males and females. Furthermore, the observed variations underline the broader influence of environmental conditions such as food availability and temperature on the physiological condition of reef fishes, which may become increasingly important under changing climate scenarios.

The comparison of monthly Gastro-Somatic Index (GaSI) values between male and female *C. viridis* reveals clear sex-specific seasonal trends, shedding light on the differential physiological and reproductive dynamics of each gender. Both sexes exhibit peak GaSI values during similar periods particularly in April, May, and October indicating heightened feeding activity likely associated with gonadal development and preparatory phases for reproduction. However, females consistently displayed higher GaSI values than males across most months, suggesting a greater energy investment in feeding, potentially to support higher reproductive demands such as oogenesis^{52,58}.

For males, the highest GaSI was recorded in October (4.23 ± 0.56), followed by April (3.73 ± 1.09) and May (3.30 ± 0.28). These peaks may coincide with

periods of gonadal maturation when energy intake is ramped up to support reproductive readiness. In contrast, the lowest GaSI values in January (1.39 ± 0.67), February (1.59 ± 0.96), and December (1.98 ± 0.49) suggest reduced feeding, possibly due to colder temperatures, post-spawning recovery, or decreased food availability.

Female GaSI values mirrored some of these seasonal trends but were generally more pronounced. The highest value was in November (5.39 ± 2.37), with other peaks in April (4.99 ± 1.71), May (4.52 ± 0.45), and October (4.48 ± 1.23). These elevated levels likely reflect increased metabolic demands during vitellogenesis or pre-spawning energy accumulation. The lowest female GaSI values were recorded in February (1.41 ± 0.56), January (1.89 ± 0.98), and December (2.07 ± 0.31), consistent with reduced feeding activity and possible spawning season.

These patterns align with broader findings in reef fishes, where feeding intensity and digestive gland indices are often synchronised with reproductive timing. For instance, seasonal increases in GaSI have been observed in damselfish such as *Chromis chromis*, where higher values correspond with pre- or post-spawning seasons and energy storage^{52,58}. The higher GaSI values in females may also reflect sex-specific dietary or metabolic adaptations necessary for egg production, a common trend in marine teleosts⁵⁹. The high variability in standard deviations during peak months, particularly in November and April, indicates individual differences in physiological state, possibly due to asynchronous reproductive development. Overall, the presence of multiple peaks and relatively lower GaSI values across several months suggests an extended reproductive season for *C. viridis*.

These findings are consistent with existing literature on gastrosomatic indices and feeding behaviour, which link variations in GaSI to seasonal and environmental influences on diet composition, feeding intensity, and gut fullness^{60,61}. GaSI reflects not only dietary shifts but also physiological responses to environmental conditions and reproductive cycles^{62,63}. The significant monthly differences observed in both sexes emphasise the role of biological rhythms and external factors in shaping feeding patterns, as reported in other fish species⁶⁴⁻⁶⁶. These insights will enhance understanding on *C. viridis* feeding ecology and underscore the need to consider seasonal and environmental factors in fisheries management and ecological assessments.

This study provides an essential foundation for developing conservation and management strategies tailored to the biology and ecology of *Chromis viridis*, especially in the context of its widespread use as a live bait fish in the Lakshadweep Islands. Identifying distinct periods of somatic stress such as reduced feeding and body condition during certain months can inform the timing of fishing restrictions to protect vulnerable populations during key life stages. Moreover, the documentation of both isometric and allometric growth patterns across sexes and seasons underscores the dynamic nature of this species' biology, which must be considered in any sustainable exploitation framework.

By integrating physiological indicators such as length-weight relationships, condition factors, and gastro-somatic index across sexes and seasons, it captures nuanced patterns of reproductive investment and energy allocation. These patterns highlight critical windows of biological vulnerability, offering a science-based framework for implementing seasonal harvest regulations. Consistent monitoring of indicators such as condition factor, growth indices, and feeding activity allows for the detection of temporal patterns and stress signals that may be linked to habitat degradation or ecological imbalance. Given the ecological and economic significance of *C. viridis*, regular monitoring of biological parameters is vital. Furthermore, the inclusion of mitochondrial genetic markers (16S rRNA and CO1) data from the Lakshadweep region contributes valuable baseline data for assessing cryptic diversity, a key element for identifying management units and preserving evolutionary potential. It enables the development of sustainable harvesting practices, ensures population viability, and helps maintain the ecological balance of coral reef habitats, where this species plays a key role.

Conclusion

The integrated analysis of length-weight relationships, condition factors, and gastro-somatic index in male and female *C. viridis* reveals distinct yet overlapping seasonal physiological trends shaped by environmental and reproductive dynamics. Both sexes exhibit alternating patterns of isometric and negative allometric growth across months, indicating shifts in energy allocation between somatic growth and reproductive investment. Condition factor indices (K , K_A , K_R) similarly show seasonal variation in body

condition, with lower values aligning with potential spawning or post-spawning periods, and higher values during times of increased feeding and energy storage. GaSI trends in both sexes mirror these cycles, peaking in April, May, and October – November, which likely represent pre-spawning or spawning preparation phases. The lowest GaSI and condition factor values observed in January, February, and December suggest reduced feeding activity, possibly due to post-spawning recovery or environmental limitations. Collectively, these findings suggest a probable reproductive season for *C. viridis* spanning over multiple months. Importantly, this study also provides the first 16S rRNA and CO1 sequence data for *C. viridis* from the Lakshadweep region, offering a valuable reference point for future studies on population dynamics, connectivity, and phylogenetic relationships. This finding is notable as it highlights the genetic continuity of *C. viridis* across ocean basins, enriching our understanding of the species' evolutionary history and biogeographic patterns.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

Recently deceased fish specimens were collected directly from traditional fishermen on Agatti Island, Lakshadweep. No animals were stressed or killed for this research, which adhered to India's current animal welfare laws. The studied fish species are not included in the schedules of the Government of India's Wildlife Protection Act of 1972, so its provisions do not apply.

Author Contributions

MUR: Design and implementation of the research, analysis of the results, and writing of the manuscript. TH, SB, and SA: Specimen collection, measurements and data collection. TTAK: Revised of the manuscript; and UKS: Planning and supervising of the work.

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