

The determination of sexual size dimorphism in long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829) from the coast of Aegean sea

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The study investigates the sexual dimorphism in 205 samples (♀:98; ♂:107) consisting of newly deceased fishes seen in the fishnets of the commercial fishers and long-snouted seahorses (*Hippocampus guttulatus* Cuvier, 1829) obtained in two research projects, in the coasts of Aegean Sea. All samples were divided by their standard-length groups (I: 60-89 mm; II: 90-120 mm; III: > 121 mm) of morphometric measurements. The sexes of the species differed in terms of tail length (TaL), dorsal fin base length (DL), trunk depth (TD9-10), and trunk length (TrL) ($P < 0.05$). Accordingly, it was determined that the positively allometric growth TD9-10, DL, and TaL values in male seahorses were higher than those of the female seahorses. The females were longer than the males in terms of TrL. However, sexual differences according to the length groups in male seahorses were significant starting from the length group II. Sexual dimorphism seen in the body parts is more likely effective in the development of eggs that the males took from the females related with the sexual selection pressure, while the longer tails and dorsal fins suggested that male seahorses were more advantageous in balancing their body weight during mating behaviors (tail grabbing and courtship etc.). In this research, as *H. guttulatus* distributed in the coasts of the Aegean Sea showed differentiation starting from length group II, it is understood that, there was a sexual dimorphism in terms of TrL and TaL which determine the body size in mate selection.

[**Keywords:** Body proportion, Morphometry, Sex differences, Sexual difference]

Introduction

Seahorse is a rare bony fish species with distinct morphological characteristics along with their rare reproductive biology in which parental care is provided by male seahorse¹. Albeit their mostly monogamous behavior², sexual dimorphism is also observed³. Sexual dimorphism in general is a systematic difference between the sexes of the same species and may result from three important forces: food competition⁴, developing different fecundity models between the sexes⁵, and mate competition⁴. Although sexual dimorphism is closely related to the polygamy, it is not uncommon in seahorses⁶. Monogamous mating system in many seahorses allows female seahorse to transfer all the egg reserves and size-assortative mating, while, for males, it helps finding the female seahorse that can produce eggs matching to the male's pouch size³. Therefore, the effect of the sexual dimorphism in this whole algorithm on mate competition in the seahorses show that sexual dimorphism is intensely seen in some species belonging to Syngnathidae family^{3,4,6-9}.

Morphological characteristics are accepted as the basis of species identification. The shape of the heads

of seahorses and pipefishes is an important morphometric feature. Snout length, snout depth, head length, head depth, and pectoral fin length are among the most important morphometric characteristics¹⁰. According to Goffredo *et al.*,¹¹ the only way to identify *H. guttulatus* is the presence or absence of filament structure on the top of the heads, whereas other researchers regard the presence of filaments as an indicator of sexual maturity in *H. guttulatus*¹². Accordingly, seahorses with standard lengths longer than 110 mm are accepted to be sexually mature in the Southern Portuguese Coasts, whereas, in *H. hippocampus*, the thin filament structure is not accepted as an indicator of length, sex, or sexual maturity¹³. Therefore, the multiple morphological characteristics proposed by Louire *et al.*,¹⁴ are used in the identification of European seahorses (*H. guttulatus* and *H. hippocampus*). According to Jones and Avise¹⁵, in syngnathid species, secondary sexual characteristics are important for the development of the theories on sexual selection and sexual dimorphism. Although this feature of the sexual dimorphism seen in body ratios are accepted to be rare in monogamous species, it is evaluated with

the sexual selection pressure^{3,7}. Accordingly, it is generally accepted that female seahorses are brighter and larger in size compared to the males¹⁶. In the literature, studies on sexual dimorphism, monomorphism, morphometric, and meristic characteristics mostly start from 2000s^{6,7}, while biological, distribution, nutrition, and reproduction studies on seahorse species are numerous^{3,6,7,9,10,17}.

In seahorse species in which monogamy is evident, recent studies on length, weight, length-weight relationship, the presence and the effect of sexual dimorphism are very limited^{3,9}. Although there are biological studies conducted in the coastal regions of Turkey reporting an allometric growth in seahorse species¹⁸⁻²¹, there are no studies reporting sexual dimorphism in length groups that were evaluated by morphometric measurements.

The main objective of this research was to investigate sexual dimorphism characteristics of *Hippocampus guttulatus*, which is listed as Data Deficient (DD) worldwide and as Near Threatened (NT) for the Mediterranean Basin in the Redlist⁴⁰ in the coasts of the Aegean Sea.

Materials and Methods

As the material of the study, the long-snouted seahorse (*Hippocampus guttulatus* Cuvier 1829) samples consisted of the dead seahorses captured in the Aegean Sea by commercial fishers with fishnets in 2000-2006 (Fig. 1). The samples obtained in the research projects No 2001 SUF 003 and No. 2006 SUF 017 and kept in 10 % formalin solution. Accordingly, 205 seahorses (107 males; 98 females) were included in the study. The sex determination in the seahorses was made macroscopically based on the presence or absence of the pouch. In this study, a total of 13 morphometric measurements were evaluated, 5 in the head region and 8 in the trunk region. The standard length (SL) measurement (the sum of head length, trunk length and tail length) and the other measurements for the body parts were performed according to the standards determined⁴¹. These morphometric measurements include head length (HL), snout Length (SnL), snout Depth (SD), coronet height (CH), pectoral fin length (PL), trunk length (TrL), tail length (TaL), trunk depth between 4th and 5th trunk rings (TD4-5), trunk depth between 9th and 10th trunk rings (TD9-10), and dorsal fin base length (DL) using a digital caliper with a 0.01 mm precision (Fig. 2). Body weights (W, g) were measured using a scale with a precision of 0.01 g. All calculations were

converted to total length percentage (SL %). Since age is not taken into account for the species, three length groups were established to determine sexual differentiations morphometrically (I: 60-89 mm; II: 90-120 mm; III: > 121 mm).

The length values of the morphological characteristics were not evaluated according to the

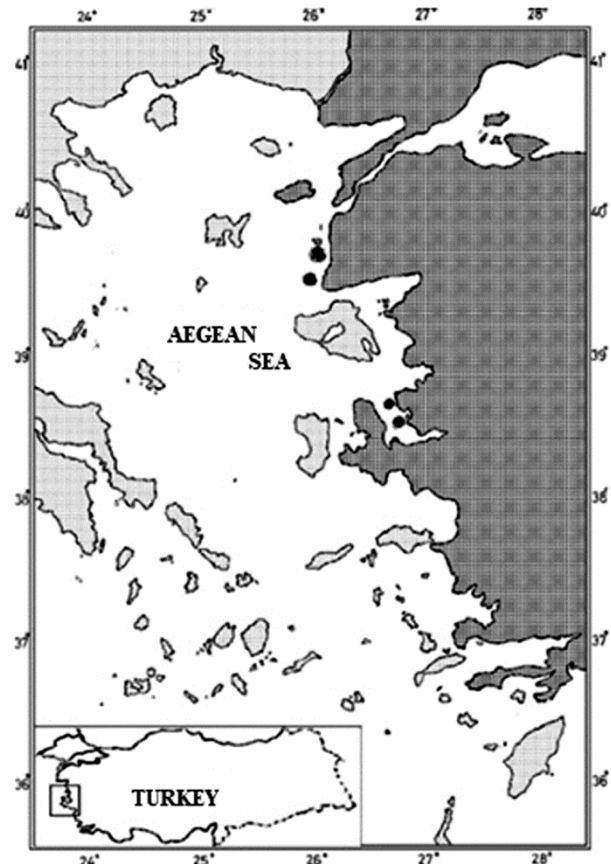


Fig. 1 — Research area (● the sampling areas for the seahorse collected).

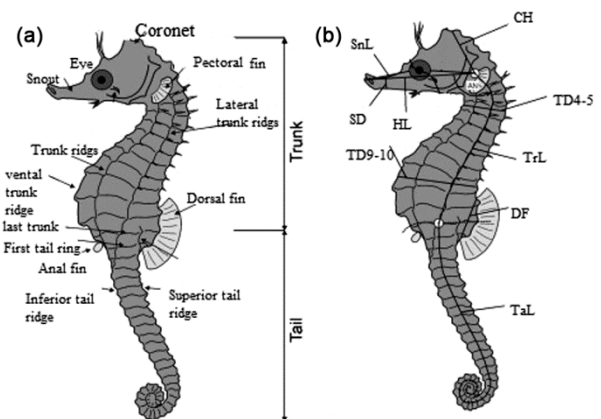


Fig. 2 — Morphometric measurements for seahorses²².

standard length (SL). Accordingly, $b = 1$ refers to isometric growth, $b > 1$ refers to allometric growth, and $b < 1$ refers to negative allometric growth. Linear regression values are shown in equation $\log y = \log a + b \log x$ by providing logarithmic transformation of the independent variables²².

Student's *t*-test was used for the mean morphometric values between the sexes. Sexual dimorphism is used to determine the morphometric strength in sexes in terms of standard length (L) and weight (W) relationships²³. In addition, the regression relation (r^2) of the morphometric characteristics and the length conversion equations was determined²⁴. The *t*-test was used to compare morphometric relationships with respect to sex and length groups. The < 0.05 significance test used in STATISTICA 11 software was taken into account for statistical differences.

Total length-weight relationship of the samples was evaluated according to Ricker²⁵. To evaluate the length-weight relationship, logarithmic conversions of the data ($\log W = \log a + b \times \log L$) was carried out and the values were used in equation $W = a \times L^{b23}$. *W* refers to the total weight, *L* refers to standard length, *a* refers to slope, and *b* refers to the regression coefficient. The *b*-values different from 3 (positively allometric $b > 3$, negatively allometric $b < 3$, isometric $b = 3$)⁴³ were determined and determination coefficient (R^2) was calculated for the degree of length-weight relationship.

Results

The mean morphometric values of *Hippocampus guttulatus* with respect to sexes are given in Table 1.

The average standard length value for all seahorse samples was 108.43 ± 11.61 mm. With 109 ± 9.89 mm, the samples with the highest average length was determined in males. Therefore, no sexual difference was determined in terms of standard length values ($P > 0.05$) (Table 1).

The mean TrL was 33 % of the SL % and the mean value of the samples was 35.40 ± 4.34 mm. The female samples had longer TrLs than the male samples. Hence, there was a statistical difference between the sexes in terms of TrL (t_{cal} : 4.162, p : 0.00005, $P < 0.05$). The mean TaL was 73.21 ± 7.98 mm for all seahorse samples and was approximately 68 % of the SL. The female samples had smaller tail length values than the male samples. According to the Student's *t*-test, the difference between the sexes in terms of the tail lengths was significant (TaL) (t_{cal} :-3.687, P : 0.0002, $P < 0.05$). There are two different cases for trunk ring depths. The mean values in all samples for trunk depth (TD 4-5) were 9.84 ± 1.61 mm and was approximately 9 % of the SL. Although the body width values of females were higher than those of the males, the difference between sexes was not significant ($P > 0.05$). On the other hand, TD9-10 value, which was 13 % of the SL, was higher in the females and the difference was statistically significant (t : -2.440 $P < 0.05$). The mean value for TD in all seahorses was 5.15 ± 1.77 mm and was approximately 5 % of the SL. Although this structure appears to be larger in male seahorses, the difference was not statistically significant ($P > 0.05$). The mean DL value was 11.58 ± 1.57 mm for all seahorses and was 11 % of the SL. The male seahorses had longer fin

Table 1 — Morphometric features of *H. guttulatus* in Aegean Sea

Morphometric features	Male	Female	Total	t-value	Mean (SL %)	Significance
Standard Length	109.81± 9.89	107±1.352	108.43±11.61	-1.778	-	NS
Trunk Length	34.57±2.98	36.28±5.30	35.40±4.34	-	32.65	S
Tail Length	75.24±7.55	71.03±7.84	73.21±7.98	-3.687	67.52	S
Head Length	24.20±2.17	24.70±2.20	24.41±2.19	1.805	22.51	NS
Snout Length	8.92±1.03	9.22±1.03	9.05±1.03	0.988	8.35	NS
Snout Depth	3.19±0.55	3.13±0.61	3.16±0.57	-0.349	2.91	NS
Coronet Height	7.98±1.29	7.60±1.05	7.82±1.20	-1.115	7.21	NS
Head depth	11.90±1.58	11.83±1.40	11.87±1.49	-0.143	10.95	NS
Trunk Width	4.11±0.80	4.43±1.10	4.25±0.94	1.191	3.92	NS
Trunk depth	5.75±1.64	4.20±1.57	5.15±1.77	-	4.75	NS
Dorsal fin base length	11.99±1.51	11.03±1.51	4.44±0.65	-2.218	10.68	S
TD4-5	9.82± 1.20	7.83±1.05	9.14±1.61	0.685	9.15	NS
TD9-10	15.75± 1.86	13.22±1.85	13.99±1.84	-2.440	14	S
Pectoral fin base length	4.48±0.59	4.40±0.73	11.58±1.57	-3.398	4.09	NS

Note: If Prob <0.05 then significant at 5% level

NS: Not significant
S: Significant

length compared with the females and the difference was statistically significant ($t: -2.218, P < 0.05$).

The evaluation of the five morphometric measurements at the head region of the species showed that HL and SnL values were higher in the females, while SD, CH and HD values were higher in males (Table 1). According to the Students t -test results, the differences between the sexes in the morphometric measurements for the head region of the species was not statistically significant ($P > 0.05$). As a result, sexual dimorphism was detected only in TaL, TrL, DL, and TD9-10 ($t_{\text{TaL}}: -3.687, t_{\text{TrL}}: -2.355, t_{\text{DL}}: -2.218, t_{\text{TD9-10}}: -2.240, P < 0.05$) body parts in the *H. guttulatus* species in the Aegean Sea.

The differences between the sexes according to the length groups determined in the TaL, DL, TD9-10, and TrL body parts are given in Table 2.

There were differences between the TaL values according to the length groups between the sexes ($P < 0.05$). The t -test revealed that tail length differences was statistically different between the sexes starting from length group II (90-120 mm) (length group II; $P: 0.000009$, length group III: $P: 0.0351, P < 0.05$) and the tail length of the males was longer than that of the females (Table 2). The examination of the (DL) showed that there was only a difference in the length

group II ($P: 0.0024, P < 0.05$) and the male seahorses had longer dorsal fins. Similarly, in TD9-10 measurement, there was a difference in length group II ($P: 0.006, P < 0.05$) and this difference was in favor of the female seahorses. In terms of trunk lengths, there was a difference between the sexes in the length group II ($P: 0.079, P < 0.05$), and the female seahorses had longer trunk in this length group.

In the study, the male seahorses had higher length (SL: 109.81 ± 9.89 mm) and weight (W: 6.68 ± 1.81 g) values, whereas the female seahorses had relatively lower length (SL: 107 ± 1.35 mm) and weight (W: 6.32 ± 1.67 g) values and there were statistically significant differences between the sexes in terms of both the SL and W values ($t = 137.87$ df:201 $P < 0.05$). Positive allometric growth was determined in both sexes and seahorses ($\text{♀}; W = 0.0008L^{3.41}, r = 0.79; \text{♂}; W = 0.0003L^{3.92}, r = 0.83; \text{♀+♂}; W = 0.0012L^{3.17}, r = 0.78$) (Fig. 3A). Allometric growth model of the TaL, DL, TD9-10, and TrL measurements of the sexes is given in Table 3.

As revealed by Table 3, TD9-10 increases in both sexes depending on the length ($b = 1.47$). TaL showed a positive allometric growth in male seahorses ($b = 1.83$), while it indicated isometric growth in females (Figs. 3 B-C). The increase in TrL with the body

Table 2 — The results of the body parts with sexual dimorphism in sexes according to body lengths

Body parts		III. length size (M ± SD)	III. length size (M ± SD)	III. length size (M ± SD)
TaL	Female	57.40±7.35	70.66±5.63	83.55±4.62
	Male	57.79±1.97	74.61±5.65	88.12±4.35
	Sign.	NS	S	S
DL	Female	9.12±2.62	11.05±1.24	12.19±1.42
	Male	9.55±1.29	12.07±1.42	12.96±0.72
	Sign.	NS	S	NS
TD9-10	Female	11.21±2.67	11.66±1.46	15.57±1.79
	Male	11.39±1.82	15.81±1.79	16.32±2.14
	Sign.	NS	S	NS
TrL	Female	25.28±10.6	36.76±2.77	41.25±3.31
	Male	27.51±1.43	34.36±2.29	38.86 ±2.29
	Sign.	NS	S	NS

Note: If Prob <0.05 then significant at 5% level

NS: Not significant
S: Significant

Table 3 — Allometric growth models of *H. guttulatus* according to the seahorses in $L = aX^b$ equation

Male	Female			All sexes					
	a	b	r	a	b	r			
TaL	0.81	1.83	1.83	0.92	0.98	0.9	1.28	0.86	0.79
DL	0.37	1.52	1.52	0.26	0.81	0.8	0.21	1.24	0.11
TD9-10	0.09	2.70	2.70	0.43	1.39	0.8	0.41	1.47	0.69
TrL	0.71	1.08	1.08	0.70	1.11	0.4	0.73	1.05	0.79

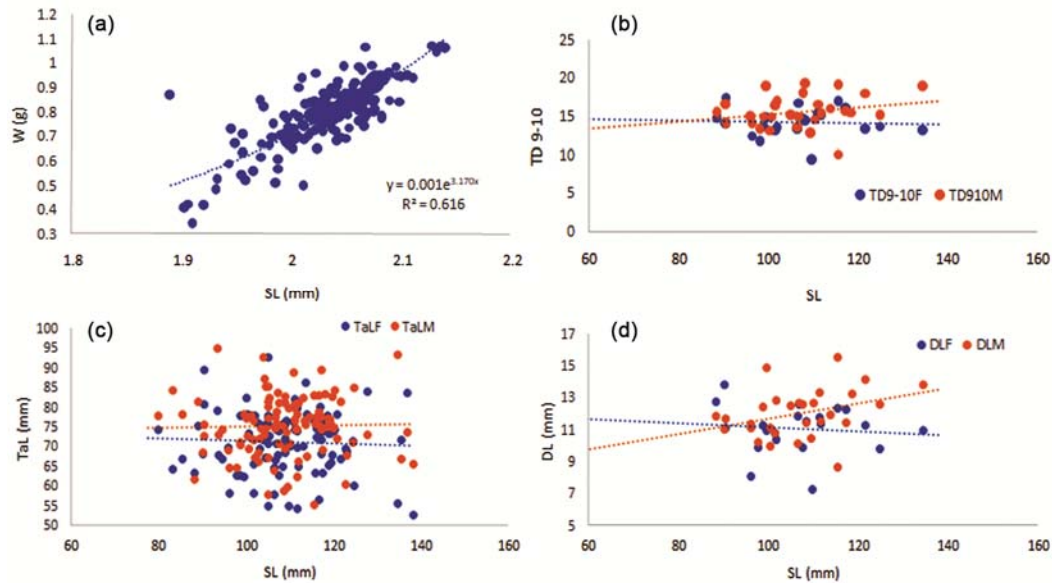


Fig. 3 — Allometric relationships in *Hippocampus guttulatus* (A) L-W relationship for all seahorses, (B) Allometric growth relationship between SL and TD9-10 for all seahorses, (C) Allometric growth relationship between SL and TaL for all seahorses, and (D) Allometric growth relationship between SL and DL for all seahorses (F:Female, M:Male).

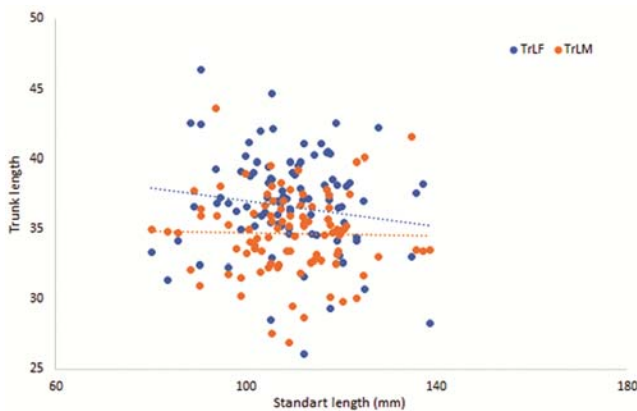


Fig. 4 — Regression relationship between SL and TrL.

length indicates an isometric growth ($r = 1.05$). DL indicates positive allometric growth in males ($b = 1.52$), while it shows that it is shorter ($b = 0.81$) depending on the length (Figs. 3D & 4).

Discussion

The present study investigates the presence of sexual size dimorphism in terms of length groups in the Long-Snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829) species distributing in the Aegean Sea. Body length is an important trait and a reason for preference when choosing a mate in most animal species^{26,27}, since larger animals have advantages in terms of reproduction area and food competition²⁶. The results of the study showed that,

compared with female seahorses, male seahorses had higher length and body weight values and these results agree with the results of other studies conducted in the Aegean Sea, Black Sea coasts^{21,28,29}, Portuguese coasts (Ria Formosa lagoon)³⁰ and Central Gulf coasts of Florida³. However, a sexual difference between the length values of the female and male populations was determined in the coasts of the Northeastern Brazil, Macaronesian islands, and the Mediterranean and Black Seas^{31,32}. Woodle *et al.*,³² reported that sexual dimorphism, in the form of a shorter standard length of males, was observed in *H. guttulatus* and *H. hippocampus*.

In the study, there was no dimorphism in terms of the length-weight relationship but there was an allometric growth. The difference between the sexes in the length-weight relationship was also supported by other studies^{3,21,28,29,33}. The allometric weight increase suggests progressive investment in the development of body gonads³. However, the differences between the L-W relationships obtained in the said studies are associated with many factors including season, habitat, sampling method, hydrographical conditions, the number of samples, feeding, and reproduction period and sexes^{23,39}.

Studies have shown that, in some seahorse species, there were no sexual differences in terms of standard length values^{3,9,17,27}. In other words, similar length groups or longer groups are preferred in mating³⁰. The differences between the standard lengths of the sexes

help them to optimize their reproduction potentials in mating depending on the length^{26,27}. According to Naud *et al.*,³⁴ the secret of the reproduction success of *H. guttulatus* is very closely associated with the SL of male seahorses and the fact that seahorses with larger size are chosen for mating for both sexes. Contrary to this view, there are also cases in which the choice of mate according to length or body weight is not seen in *H. abdominalis* either for female or male seahorses²⁶. It has reported that, in *H. guttulatus*, mates in similar standard length groups are also selected as well as the ones with large pouch sizes²⁷. In our study, it was found that there were no differences between the sexes in terms of SL indicating SL had no effect on sexual selection.

According to Foster and Vincent⁶, sexual dimorphism in the lengths of seahorses may be related to the species-specific motility ratios. Considering the tail lengths, long tail structure seen in male seahorses was helpful in supporting the wide pouch or tail grabbing during mate competition^{2,3,17}. The longer TaL obtained in male seahorses in our study support this theory. However, trunk length is regarded to be important for some species in terms of sexual dimorphism. It has been reported that longer trunks (TrL) in females was a common feature for seahorse species⁶ however, it was shown as a morphometric characteristic for sexual dimorphism for the *H. trimaculatus* species distributed in the coasts of India¹⁷. Large trunk sizes in female seahorses obtained in our study reflect the importance of this structure and indicates sexual dimorphism.

It is known that male seahorses generally prefer the females with larger sizes due to their high fecundity²⁶. It has been reported that the larger abdominal area in the females suggest that the eggs in the ovaries are hydrated³⁵ while the oval shape of the trunk hints that it can transfer its eggs to the male pouch by genital papilla^{26,42}. During this study, sexual dimorphism was not detected in the high TD4-5 values measured in the females, while differences were determined between the sexes in the TD9-10 values. This difference indicates that females are ready to transfer their eggs while the large sized partners in *H. guttulatus* provide reciprocal selection in reproduction investing³⁴.

In the study, in terms of the DL, fast positive allometric growth in the trunk region in longer and heavier males supported the allometric growth rhythm in the dorsal fin. The increase in the length provides an advantage in maintaining the balance of the body and creates an evident sexual dimorphism in terms of

DL value. It has reported that the relationship between the DL and TrL may be associated with the growth rhythm of different parameters³⁶. A similar rhythm was observed in the head profile of fry *H. reidi* in different positions in the pouch during the ontogenetic process. Accordingly, the head profile of the juvenile expelled from the brood pouch goes through a gradual change¹⁰.

In our research, regarding the head region, HD, SD and CH values were higher in the male seahorses, while the HL and SnL values were higher in the females. Determined differences in seahorses and pipefishes following ontogeny in SL, SH, HD, and PL measurements and associated the differences with different prey capture kinematics (long snouted small headed versus short snouted large headed) and vertical trunk-sloped head posture¹⁰. In a study conducted in the Aegean Sea, although *H. guttulatus* had a richer food composition, there were no sexual differences in terms of food selection²⁸. In this case, the differentiation in the snout length affects the water absorption performance of the buccal structure and, therefore, the time to reach the prey^{37,38}.

On the other hand, it has stated that the differences in SnL and structure was also important for competition¹⁷, which is regarded as a behavioral characteristic. Therefore, in males, in addition to feeding differences, the presence of a higher structure in terms of head and mouth morphology can be associated with competition behavior.

In this study, as *H. guttulatus* distributed in the coasts of the Aegean Sea showed differentiation starting from length group II, it is understood that, there was a sexual dimorphism in terms of trunk length and tail length which determine the body size in mate selection.

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