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Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier, 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea

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The diet composition of the European seahorses, *Hippocampus guttulatus* and *Hippocampus hippocampus* was determined based on the analysis of 279 and 19 specimens, respectively, collected in the Aegean Sea. The diet of both species was mainly based on Crustacea, with Amphipoda, Anomura Decapoda and Mysidacea being the dominant prey categories. ANOSIM analyses, however, indicated statistically significant differences in the diet of the two species as well as differences in the diet composition of non-brooding males, brooding males and females within each species. In *H. guttulatus*, stomach fullness percentages and vacuity coefficient values indicated that female individuals seem to have a higher feeding activity in relation to males.

Key words: diet composition; *Hippocampus guttulatus*; *Hippocampus hippocampus*; seahorse.

INTRODUCTION

The study of the natural diets of fish species is a very useful approach for understanding aspects of the species biology and ecology, towards a more sustainable management of their stocks and the development of conservation measures (Pedersen, 1999; Watanabe *et al.*, 2006; La Mesa *et al.*, 2007; Sara & Sara, 2007).

In the past, seahorses have attracted attention due to their heavy exploitation for traditional medicines, aquarium pets and curios (Vincent, 1996). Although these species are of interest to fisheries and have raised concern about their conservation status (IUCN, 2006), many studies have indicated the lack of information on their biology and ecology (Colson *et al.*, 1998; Foster & Vincent, 2004; Curtis & Vincent, 2005; Curtis & Vincent, 2006). In this context, there are also few studies dealing with the feeding habits and diet of *Hippocampus*

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species in the wild (Tipton & Bell, 1988; Teixeira & Musick, 2001; Woods, 2002; Kendrick & Hyndes, 2005).

The long-snouted seahorse *Hippocampus guttulatus* Cuvier, 1829 and the short-snouted seahorse *Hippocampus hippocampus* (L., 1758) are characteristic species associated with seagrass assemblages (Foster & Vincent, 2004; Curtis & Vincent, 2005), while *H. hippocampus* is also able to exploit habitats with lower complexity, such as sparsely vegetated areas (Curtis & Vincent, 2005). Both species occur in the Mediterranean Sea and the north-eastern Atlantic Ocean (Whitehead *et al.*, 1996).

The aim of this study was to give, for the first time, information on the diet composition of *H. guttulatus* and *H. hippocampus* and to compare their diets.

MATERIALS AND METHODS

Samplings were carried out in March 2004. Specimens of *H. guttulatus* and *H. hippocampus* were collected with an otter trawl (mesh size 60 mm) deployed at one station over a depth range of 12–15 m. The station was located north-east of Rhodos Island (Aegean Sea; Fig. 1) and meadows of *Posidonia oceanica* existed on the bottom. Five consecutive hauls were carried out and the duration of each haul was *c.* 1 h. All samplings were carried out during night-time, between midnight and early morning.

After collection, all individuals of *H. guttulatus* and *H. hippocampus* were immediately and rapidly sacrificed with an excess of anaesthetic (2-phenoxyethanol solution, 0·40 mg l^{-1}). Individuals were placed in the anaesthetic solution for at least 20 min, and removed not less than 10 min after ventilation stopped. All individuals were then placed in 10% formalin.

Following transfer from the field back to the laboratory, seahorse standard length (L_S; head + trunk + tail length) was recorded. Measurements were taken as straight lines between the reference points rather than following the curvature of the trunk and tail.

The abdomen of each seahorse specimen was opened with a ventral incision along the keel and the gut removed. Gut fullness was estimated on a five point percentage

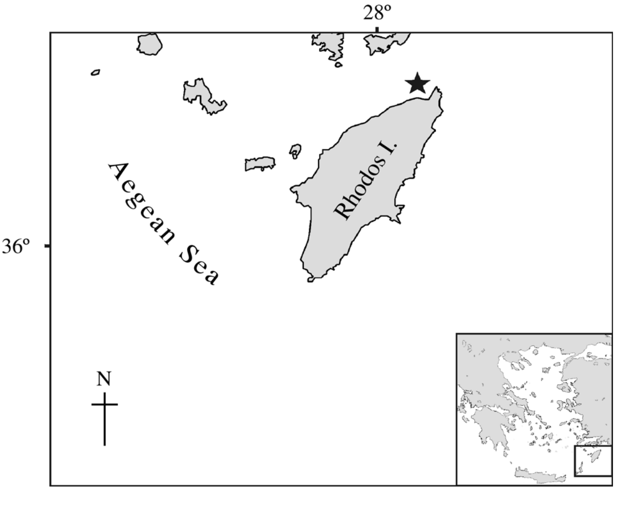

FIG. 1. Map showing the location of the sampling station (★).

scale: empty (0%), moderately full (25%), half full (50%), full (75%) and very full (100%). A lengthwise incision along the gut was then made and the contents were washed through a 125 μm sieve. All prey items were identified to the lowest possible taxonomic level and counted.

In order to analyse the data that resulted from the gut-content analysis, the methods described by Hyslop (1980), Williams (1981) and Kelleher *et al.* (2000) were used, and the following indices were calculated: frequency of occurrence ($\%F_o$): $\%F_o = n100N_s^{-1}$ and percentage of prey ($\%N$): $\%N = n'100N_p^{-1}$, where n = the number of guts containing a certain prey, N_s = the total number of guts examined, n' = the total number of individuals of a certain prey and N_p = the total number of prey items.

Based on the number of empty guts, the vacuity coefficient index (I_v) was calculated from: $I_v = E_v100N_s^{-1}$, where E_v = the number of empty guts.

In order to investigate similarities and differences in the diet of *H. guttulatus* and *H. hippocampus*, a matrix of similarities between each pair of all individuals was calculated using the Bray–Curtis similarity coefficient (data transformed to presence or absence). The one-way ANOSIM analysis was used to assess possible statistically significant differences in the diet composition between the predefined groups of *H. guttulatus* and *H. hippocampus* individuals while, the two-way nested ANOSIM was employed to test for differences among non-brooding males, brooding males and females within each species studied (Clarke, 1993; Clarke & Warwick, 1994). Non-metric multidimensional scaling (MDS) was used as an ordination technique for graphical representation of the diet similarities or dissimilarities of the two species. All the above analyses were carried out using the PRIMER 5.1 statistical package for Windows (Clarke & Warwick, 1994).

RESULTS

In all, 279 specimens (137 females, 62 non-brooding males and 80 brooding males) of *H. guttulatus* and 19 (five females, eight non-brooding males and six brooding males) of *H. hippocampus* were collected. In the female specimens of *H. guttulatus*, L_S ranged from 78 to 134 mm (mean ± S.E. $L_S = 108 \pm 1$ mm) and in males from 78 to 226 mm (mean ± S.E. $L_S = 108 \pm 1$ mm), while in *H. hippocampus*, L_S in females ranged from 86 to 98 mm (mean ± S.E. $L_S = 93 \pm 2$ mm) and in males from 69 to 104 mm (mean ± S.E. $L_S = 92 \pm 3$ mm).

Prey categories found in the guts of the specimens examined are given in Table I. In *H. guttulatus* a total of 720 prey items were identified belonging to 15 prey categories, while in *H. hippocampus* the corresponding examination yielded 32 prey items belonging to 10 prey categories.

The $\%F_o$ and $\%N$ values for the different prey categories found in *H. guttulatus* are given in Fig. 2(a) and Table I. The most dominant prey categories were Amphipoda ($\%F_o$ = 88·53, $\%N$ = 34·31), Anomura Decapoda ($\%F_o$ = 62·72, $\%N$ = 24·31), Mysidacea ($\%F_o$ = 42·29, $\%N$ = 16·39) and algae ($\%F_o$ = 53·76, $\%N$ = 20·83). These four categories accounted cumulatively for *c.* 96% of the species diet. In terms of higher taxonomic groups, Crustacea was the most dominant prey category ($\%F_o$ = 94·26, $\%N$ = 76·68).

The $\%F_o$ and $\%N$ values for *H. hippocampus* are given in Fig. 2(b) and Table I. Amphipoda ($\%F_o$ = 73·68, $\%N$ = 43·75) was the most abundant prey category followed by Mysidacea ($\%F_o$ = 26·32, $\%N$ = 15·63), Anomura Decapoda ($\%F_o$ = 21·05, $\%N$ = 12·50) and algae ($\%F_o$ = 15·79, $\%N$ = 9·38). These three categories accounted cumulatively for *c.* 80% of the species diet. In terms of higher taxonomic groups Crustacea ($\%F_o$ = 84·21, $\%N$ = 81·27) was the most abundant and frequent prey category.

TABLE I. Prey categories found in the gut of *Hippocampus guttulatus* and *Hippocampus hippocampus*

	Hippocampus guttulatus				*Hippocampus hippocampus*			
	n'	n	$\%F_o$	$\%N$	n'	n	$\%F_o$	$\%N$
Foraminiferea								
Unidentified Foraminiferea	2	2	0·72	0·28	—	—	—	—
Platyhelminthes								
Turbellaria								
Unidentified Turbellaria	1	1	0·36	0·14	—	—	—	—
Mollusca								
Gastropoda								
Unidentified Gastropoda	1	1	0·36	0·14	—	—	—	—
Annelida								
Polychaeta								
Unidentified Polychaeta	—	—	—	—	1	1	5·26	3·13
Oligochaeta								
Unidentified Oligochaeta	2	2	0·72	0·28	—	—	—	—
Crustacea								
Maxillopoda								
Unidentified Ostracoda	4	4	1·43	0·56	1	1	5·26	3·13
Amphipoda								
Unidentified Amphipoda	247	247	88·53	34·31	14	14	73·68	43·75
Cumacea								
Unidentified Cumacea	—	—	—	—	1	1	5·26	3·13
Copepoda								
Unidentified Copepoda	1	1	0·36	0·14	—	—	—	—
Isopoda								
Unidentified Isopoda	1	1	0·36	0·14	—	—	—	—
Decapoda								
Unidentified Natantia	5	5	1·79	0·69	—	—	—	—
Unidentified Anomura	175	175	62·72	24·31	4	4	21·05	12·50
Unidentified Brachyura	—	—	—	—	1	1	5·26	3·13
Mysidacea								
Unidentified Mysidacea	118	118	42·29	16·39	5	5	26·32	15·63
Tanaidacea								
Unidentified Tanaidacea	1	1	0·36	0·14	—	—	—	—
Chordata								
Pisces								
Unidentified Pisces	2	2	0·72	0·28	1	1	5·26	3·13
Hippocampus spp. ova (with embryos)	10	10	3·58	1·39	1	1	5·26	3·13
Algae								
Unidentified algae	150	150	53·76	20·83	3	3	15·79	9·38

n', total number of individuals of a certain prey; n, number of gut containing a certain prey; $\%F_o$, frequency of occurrence index; $\%N$, percentage of prey index.

The I_v value for *H. guttulatus* is given in Fig. 2(c) while percentage gut fullness of males and females of this species is given in Fig. 3(a). A total of 82 guts were found empty giving a I_v of 29·39% [Fig. 2(c)]. Percentage gut fullness

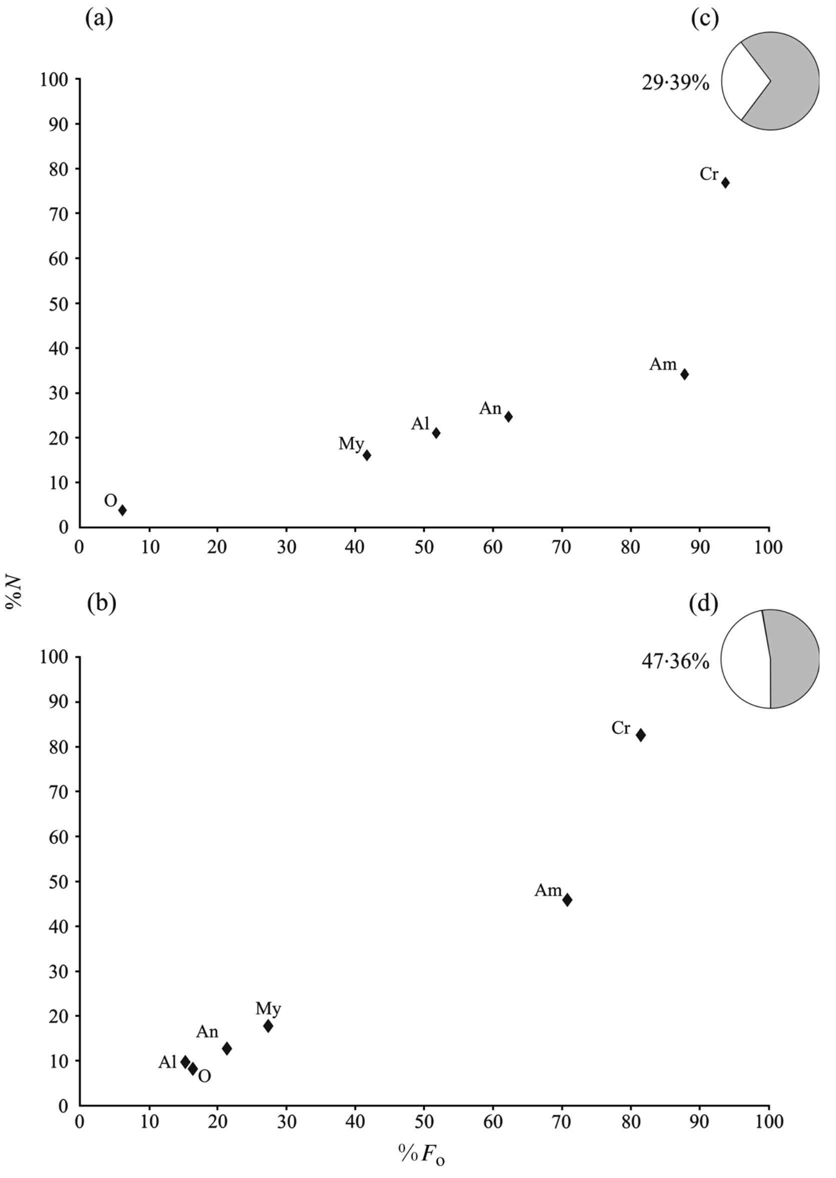

FIG. 2. Percentage frequency of occurrence index (%F_o) and percentage of prey index (%N) values for the various prey categories found in: (a) *Hippocampus guttulatus* and (b) *Hippocampus hippocampus*. Cr, Crustacea; Am, Amphipoda; Al, algae; An, Decapoda Anomura; My, Mysidacea and O, others. Vacuity coefficient (I_v) values are given for (c) *H. guttulatus* and (d) *H. hippocampus*.

among male and female specimens of *H. guttulatus* was significantly different (Pearson χ^2, d.f. = 4, $P < 0.01$). Hence, 13·14% of the female specimens had an empty gut and 23·36% had a full or very full one, while in males, 45·07% had an empty gut and 8·45% were full or very full [Fig. 3(a)]. Moreover, no significant differences were detected among males and brooding males of this species (Pearson χ^2, d.f. = 4, $P > 0.05$).

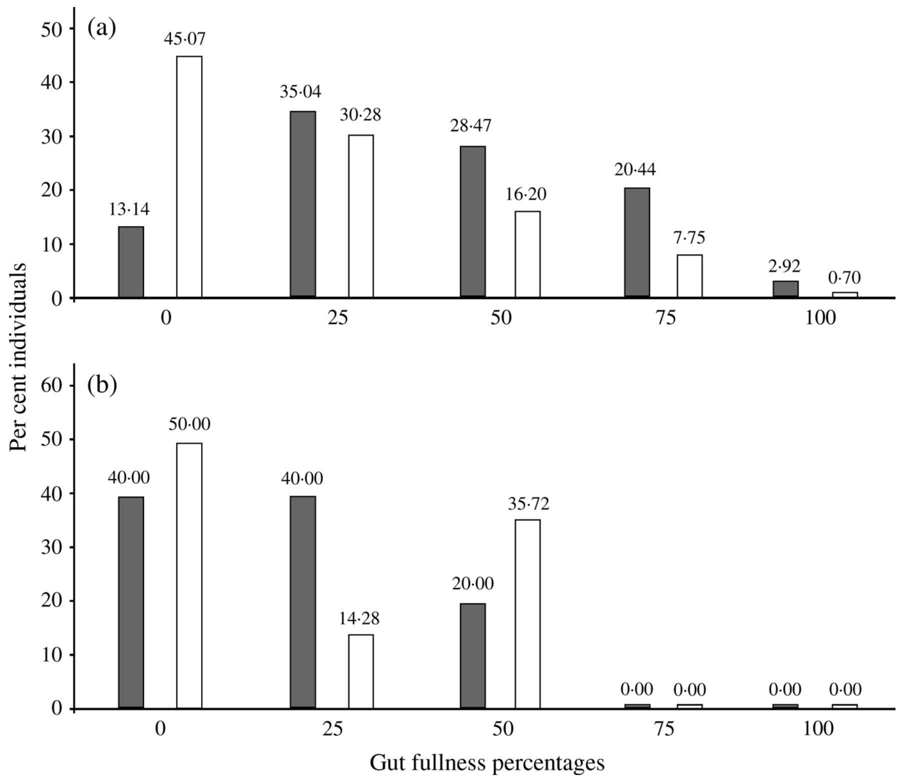

FIG. 3. Gut fullness percentages of female (■) and male (□) individuals for: (a) *Hippocampus guttulatus* and (b) *Hippocampus hippocampus*.

For *H. hippocampus* out of the 19 individuals collected, nine were found with an empty gut, giving a I_v value of 47·36% [Fig. 2(d)]. No males or females were found with a full or very full gut [Fig. 3(b)].

The results of the one-way ANOSIM analysis indicated statistically significant differences in the diet composition of the two *Hippocampus* species (one-way ANOSIM, global R = 0·317, $P < 0{\cdot}01$). Furthermore, the two-way nested ANOSIM analysis indicated statistically significant differences in the diet composition among non-brooding males, brooding males and females within each one of the two species studied (two-way ANOSIM, global R = 0·089, $P < 0{\cdot}01$).

DISCUSSION

The results of the present study show that the diet of *H. guttulatus* is diverse. This could be attributed to the fact that *H. guttulatus* is a visual ambush predator (Curtis & Vincent, 2005) foraging mainly during daytime, when the diversity of the macrobenthic fauna within the *P. oceanica* beds is higher in relation to night-time (Pérès, 1982). Only four prey categories, however, dominated in terms of abundance and frequency of occurrence: Amphipoda, Anomura Decapoda, Mysidacea and algae.

Previous studies have documented the importance of Amphipoda and Mysidacea in the diet of other *Hippocampus* species, while algae has been considered, so far, as a non-important dietary component (Teixeira & Musick, 2001; Woods, 2002; Kendrick & Hyndes, 2005). No relevant information exists for the diet and feeding habits of *H. guttulatus*. Curtis & Vincent (2005), however, noted that *H. guttulatus* is a relatively sedentary species that ambushes planktonic prey more frequently than *H. hippocampus*.

The diet of *H. hippocampus* is also dominated by Amphipoda, Anomura Decapoda and Mysidacea. There is also no information concerning the diet of this species, except from the note of Bell & Harmelin-Vivien (1983) who found amphipods in two specimens of *H. hippocampus* collected in the Gulf of Marseille (Mediterranean Sea).

The female individuals of *H. guttulatus* had higher percentages of full guts and lower percentages of empty ones in relation to males, a fact which suggests that female individuals possibly have a higher feeding activity in relation to males. This is possibly due to the need of females to feed more intensively in order to obtain more energy for their reproduction. In *H. hippocampus* the small number of individuals studied does not permit reliable conclusions on the feeding activity of this species.

In the present study, the diets of *H. guttulatus* and *H. hippocampus* demonstrated five common prey categories, three of which (Amphipoda, Anomura Decapoda and Mysidacea) were the dominant ones in the diet of each species. It should be noted, however, that the taxonomic resolution of the prey item identifications was rather low, which at the same time, was the highest possible due to the constraints in the identification of digested prey. Furthermore, significant differences in the diet of *H. guttulatus* and *H. hippocampus* could not be detected based on the present data.

It has been well documented that the meadows of *P. oceanica* are suffering a reduction in their density and spatial distribution mainly due to anthropogenic disturbances such as mooring, dumping and coastal constructions (Francour *et al.*, 1999; Marcos-Diego *et al.*, 2000). As noted by Curtis & Vincent (2005) habitat loss and fragmentation in seagrass beds might clearly affect the two species of European seahorses. Habitat degradation in the seagrass beds also results in a reduction of the diversity of the associated fauna (Frost *et al.*, 1999) which, as demonstrated in the present study, constitutes the main food resource of these two species.

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