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Assessing diet composition of seahorses in the wild using a non destructive method: *Hippocampus reidi* (Teleostei: Syngnathidae) as a study-case

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This paper presents the results of the first analysis of the natural diet of *Hippocampus reidi*, one of the most sought after seahorse species in the international aquarium trade. Its main goals were to investigate food items and prey categories consumed by the species, and to discuss feeding strategy and inter and intra-individual components of niche breadth. Data were gathered from October 2005 to September 2006 at the Mamanguape estuary, State of Paraíba, NE Brazil. Food items from seahorses anaesthetized with clove oil were obtained by using a modified version of the flushing method, and were counted and identified to the lowest possible taxonomic level. Specimens were marked and had their height, sex, life and reproductive stage recorded, and then returned to the same place where they were found for the further assessment of anaesthetization/gut flushing on seahorses. Food items were analyzed using frequency of occurrence, relative abundance, index of preponderance and prey-specific abundance using the points method. The graphic method of Amundsen *et al*. (1996) was used to interpret the feeding strategy and contribution to niche breadth. Nematodes and crustaceans were the most important items found, the latter item usually being the most commonly found in the gut contents of syngnathids. No significant differences in diet composition were found between reproductive stages, however, a higher proportion of large items were consumed by the larger seahorses. The feeding strategy and niche breadth analysis suggests that *H. reidi* has a generalist feeding strategy, with high variation between phenotypes. Our results suggest that the anaesthetization-flushing technique has the potential to be a useful tool in seahorse research.

Este artigo apresenta os resultados do primeiro estudo sobre a alimentação natural de *Hippocampus reidi*, uma das espécies de cavalos-marinhos mais intensamente comercializadas pelo mercado aquarista. Seus principais objetivos foram descrever os itens alimentares e categorias de presas consumidos pela espécie e fornecer informações acerca da estratégia alimentar e dos componentes inter e intra-individuais para a largura de nicho. As amostragens foram realizadas mensalmente por meio de busca intensiva, entre outubro de 2005 e setembro de 2006, no estuário do rio Mamanguape, Paraíba, onde os cavalos marinhos foram marcados, anestesiados com óleo de cravo e submetidos à técnica da lavagem estomacal. Para cada cavalo-marinho registrou-se a altura, sexo, estágio de vida e estado reprodutivo. Após a coleta de dados, todos os cavalos-marinhos foram devolvidos ao mesmo local onde haviam sido encontrados e o material obtido através da lavagem foi identificado, em laboratório, até o menor nível taxonômico possível, com o auxílio de especialistas. Os itens alimentares foram descritos através de freqüência de ocorrência, abundância relativa, índice de preponderância e abundância específica; já a estratégia alimentar e contribuição para a largura de nicho foram descritos através do método de Amundsen. Os principais itens encontrados foram nematodos e crustáceos, não tendo sido encontradas diferenças entre os itens consumidos e o estado reprodutivo dos cavalos marinhos. Entretanto, verificou-se que exemplares de maior porte consumiram uma maior proporção de itens grandes. Os resultados sugerem que *H. reidi* apresenta uma estratégia alimentar generalista e com alta variação entre os fenótipos. Nossos resultados sugerem que a técnica da lavagem estomacal pode vir a representar uma ferramenta útil em estudos acerca de cavalos-marinhos.

Key words: Natural diet, Husbandry, Conservation.

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Introduction

Seahorses (*Hippocampus* spp.) are among the many genera whose life histories might render them vulnerable to overfishing or other disruptions such as habitat damage (Foster & Vincent, 2004). Different species of *Hippocampus* are harvested on a large-scale, and traded by at least 77 nations in high volumes and various product forms (McPherson & Vincent, 2004). Seemingly unsustainable exploitation has focused attention on the large gaps in our knowledge of wild seahorse biology and ecology (Woods, 2002), and has highlighted the need for further research to assist both *in situ* and *ex situ* conservation initiatives.

The most commonly encountered problems in attempts to keep and/or culture seahorses relate to the provision of adequate food and the treatment of diseases (Wilson & Vincent, 1998) which, in some cases result from inadequate feeding and malnutrition. Understanding how seahorses use food resources in the wild is also crucial for identifying factors that affect their distribution, choice of habitat and abundance, as well as for improving husbandry techniques.

Published data on the diet composition of seahorses from field-based studies, however, are limited to a few species (Tipton & Bell, 1988; Kanou & Kohno, 2001; Texeira & Musick, 2001; Woods, 2002; Kendrick & Hyndes, 2005), and typically based on destructive sampling.

In this study, we used a non-destructive approach to investigate (a) diet composition, (b) feeding strategy and (c) inter and intra-individual components of niche breadth of *Hippocampus reidi* Ginsburg, 1933, commonly known as the longsnout seahorse.

Hippocampus reidi is one the most widely held species of *Hippocampus* in public aquaria (Bull, 2002), and also one of the most sought after seahorse species in the international aquarium trade (Rosa *et al*., 2006). The species is currently listed as Data Deficient by the IUCN (2007).

Through this work we hope to increase our understanding of seahorse feeding ecology, and to stimulate the development of sampling approaches that can help to balance the need to use specimens in research and the conservation needs.

Material and Methods

Monthly samplings were carried out at the Mamanguape estuary, State of Paraíba, in northeastern Brazil, from October 2005 to September 2006, totaling 40 days of fieldwork. The Mamanguape estuary is 24 km long from east to west, and is part of a protected area (06°43'- 06°51'S, 35°07'- 34°54'W) that includes ca. 6.000 ha of mangrove forest (main species: *Rhizophora mangle, Avicennia germinans, A. schaueriana, Laguncularia racemosa,* and *Conocarpus erectus*).

Specimens of *H. reidi* were carefully hand-picked at shallow water areas (0.01-2.0 m) and immediately transferred to plastic containers (2 L) containing filtered (40 mm mesh size) local seawater mixed with clove-oil (0.05%), a natural anesthetic (Cunha & Rosa, 2006). Each sampled seahorse was marked with a cotton marker around the base of the tail (see Felício *et al*., 2006), and released at the same place where they were originally found (locations of initial collections were recorded using GPS).

Food items were obtained from the gut of the previously anaesthetized seahorses through an adaptation of the flushing method (see Kamler & Pope, 2001), as follows: a needleless plastic syringe filled with 3 ml of freshwater was connected to a catheter (4 mm in diameter), which was gently introduced into the snout of each examined specimen. The freshwater was slowly flushed down the snout of seahorses and provoked regurgitation of food items. Following regurgitation, all items were filtered and preserved in 4% formaline.

Seahorses were then transferred to plastic containers with continuously aerated seawater, until their full recovery. Their height (following Lourie *et al*., 1999), sex, life stage and reproductive state were then recorded (adapted from Perante *et al*., 2002), as follows: Male seahorses were identified by the presence of a brood pouch, while sexual maturity was inferred by full development of the pouch. Females were considered as sexually mature based on their size being equivalent to the mature males (Foster & Vincent, 2004), and a bulging on their ovipositor region. All juvenile and subadult individuals were considered as immature. To avoid the risk of classifying young males (where brood pouches are absent) as females, all juvenile individuals were placed in the category "undetermined sex". The height of the smallest male exhibiting a pouch (10.0 cm) was used to nominally define the transition from juvenile to subadult, while the smallest male exhibiting a fully developed brood pouch (12.5 cm) marked the transition between subadult and adult.

In order to assess the potential effects of the methods on the specimens, we attempted to relocate marked specimens through visual censuses (transect and random search) to record their survival, life stage and reproductive status.

In the laboratory, preserved food items were counted and identified to the lowest possible taxonomic level, with the aid of taxonomy specialists. Where identification of items was not possible, these were classified as "digested matter" and "unidentifiable fragments" and were not included in the statistical analyses.

Times to induction of, and recovery from, clove oil anaesthetization for each seahorse were submitted to t tests and one-way analysis of variance (ANOVA) to evaluate the effectiveness of clove oil per sex, life stage and reproductive state for *H. reidi*. Honestly Significant Difference (HSD) Tukey post-hoc test was used to determine significantly different means. Where appropriate, logarithmic transformations [$y = \log(x + 1)$] were performed to meet the prerequisites of normality and homogeneity (Zar, 1999). Due to the low sample size ($n = 3$) juveniles were excluded from the statistical analyses. Recapture data were analyzed through t and χ^2 tests (Zar, 1999).

Food items were analyzed using the following methods: percentage frequency of occurrence (%O) (Hyslop, 1980) and relative abundance (%A) (Marshal & Elliott, 1997) using the points method (Vendel & Chaves, 1998), which accounts for gut fullness (Hyslop, 1980).

The values of "%O" and "%A" were combined through the index of preponderance, which produces a single value for each attribute based on frequency of occurrence (%O) and relative abundance (%A), using the equation:

$$IP = \frac{\%Ai \cdot \%Oi}{\sum(\%Ai \cdot \%Oi)} \times 100$$

where %A and %O are % abundance and occurrence of prey i (Marshall & Elliott 1997).

To assess possible variations in diet composition among sexes, life and reproductive stages, the IP values for each consumed item were calculated month by month, and then used in a similarity matrix constructed using the Bray–Curtis similarity coefficient (Clarke & Warwick, 2001). Additionally, a non-parametric one-way analysis of similarities (ANOSIM) and a similarity of percentages (SIMPER) were carried out using PRIMER (Clarke & Warwick, 2001).

The graphical analysis proposed by Amundsen *et al*. (1996) was used to interpret the stomach contents data. This method is a modification of the traditional method of Costello (1990) and allows prey importance, feeding strategy, and inter- and intra-individual components of niche breadth to be analyzed by a two-dimensional representation of prey abundance and frequency of occurrence.

Results

Throughout the study, 170 seahorses were sampled for diet, from which 65 were subsequently re-sighted *in situ* (n = 110 re-sightings). The maximum period between the first and last sighting was 219 days (mean = 80.35 ± 59.54 days), with no difference between sexes (t = -0.29; d.f. = 163; p = 0.77) or in the number of males and females re-sighted (χ^2 = 0.27; d.f. = 1; p = 0.61). Of the 65 individuals re-sighted, 41 changed from a non-reproductive to a reproductive state (two gave birth to young).

For all seahorses, induction of anaesthesia occurred more rapidly than recovery (induction: 89 ± 62 sec; recovery: 119 ± 78 sec; t = -5.72; d.f .= 232; p < 0.001). No significant differences were found in the induction and recovery times between sexes (induction: t = -0.13; d.f. = 238; p = 0.89; recovery: t =1.72; d.f. = 234; p = 0.09) or life stages (induction: t = 0.29; d.f. = 238; p = 0.77; recovery: t = -0.37; d.f. = 234; p = 0.71). Although we did not find significant differences in the induction times (F(5,234) = 1.08; p = 0.37) among the different reproductive states, mature females and non-brooding males exhibited significantly different times for recovery (F(5,230) = 2.52; p = 0.03) (Fig. 1). During sampling, we recorded the mortality of three individuals (two before being anaesthetized, and one during the induction period), which have been deposited in the Fish Collection of the Universidade Federal da Paraíba (UFPB 6031, UFPB 6032).

The diet analysis suggests that *H. reidi* has a generalist feeding strategy, with high variation between the phenotypes

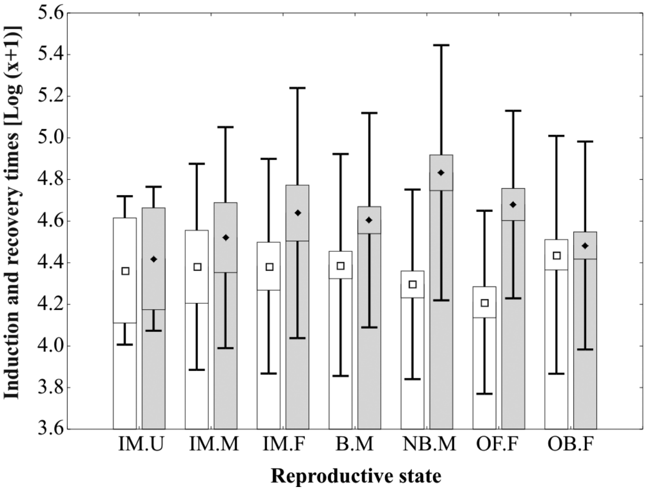

Fig. 1. Mean values of induction (square) and recovery (lozenge) times of *Hippocampus reidi* (n = 242) in seconds (box = standard error; whisker = standard deviation). Reproductive state: IM = immature, OF = ovipositor region flat, OB = ovipositor region bulging, B = brooding, NB = non-brooding. Sex: U = undetermined, F = female, M = male.

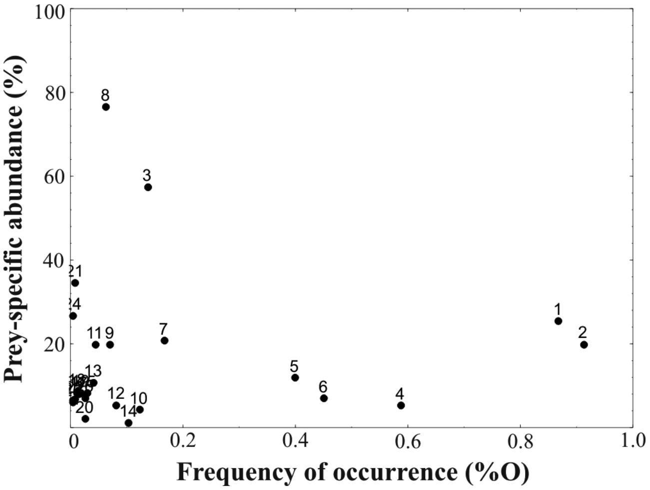

Fig. 2. Feeding strategy diagram. Prey-specific abundance plotted against frequency of occurrence of prey items in the diet of the seahorse *Hippocampus reidi* (n = 280). Prey items: 1. Nematoda, 2. Copepoda, (Harpacticoida), 3. Caridae, 4. Copepoda (nauplii), 5. Copepoda (Calanoida), 6. Copepoda (Cyclopoida), 7. Caridae (chelipods), 8. Teleostei (Gobiidae), 9. Insecta (Hymenoptera), 10. Amphipoda (Gammaridae), 11. Teleostei (scales), 12. Polichaeta (larvae), 13. Amphipoda (Caprellidae), 14. Ostracoda, 15. Eggs (possibly of mollusks or crustaceans), 16. Polichaeta (Nereididae), 17. Brachyura (nauplii), 18. Insecta (Chironomidae), 19. Crustacea (larvae), 20. Gastropoda (larvae), 21. Bivalvia (larvae), 22. Caridae (zoea), 23. Isopoda, 24. Oligochaeta, 25. Foraminifera.

(Fig. 2; Tables 1-4). Generally, nematodes (IP = 42.6778) and harpacticoid copepods (IP = 34.9798) were the main prey categories consumed by *H. reidi* (n = 280). Hymenoptera (IP = 18.90) and eggs (possibly of mollusks or crustaceans) (IP = 4.73) were important categories to specimens smaller than 10 cm. Gobiid fishes and cyclopoid copepods were consumed by seahorses larger than 10 cm in height, while caridean shrimps were increasingly consumed by seahorses larger than 12 cm. (Table 1). Significant differences where found when comparing the diet according to sex and life stage simultaneously (R = 0.115; p = 0.014), particularly between subadult and adult males, and subadult males and females (Tables 2-3) – differences related to a greater consumption of gobiid fishes and a comparatively lower consumption of harpacticoid copepods by the subadult males (Table 4).

As shown in Table 3, ANOSIM permutations with juveniles showed the biggest R values, related to their comparatively greater consumption of calanoid and cyclopoid copepods and Hymenoptera insects (mean IP = 23.31, 9.75, 17.01, respectively) (Table 4). Adult females, on the other hand, separated from the remaing groups due to their large consumption of caridean shrimps (mean IP = 8.41) (Table 4).

Discussion

Although there are no published data on induction and recovery times for seahorses, anaesthetic substances such as MS-222 or AQUI-S™ have been used in seahorse research (Woods, 2000, 2002; Bull, 2002; Woods & Martin-Smith, 2004; Koldewey, 2005; Morgan & Bull, 2005). Among anaesthetics, clove-oil (85-95% eugenol) has been considered as the most adequate product for anaesthetizing fishes, due to its low cost, reduced mortality, safety of use, low induction and recovery times, but sufficient to identify the fishes and record biological information (Griffiths, 2000). Our initial assessment of the use of clove oil in seahorse research suggests that it was an effective substance, as *H. reidi* individuals exhibited short induction (< 90 sec) and recovery times (< 120 sec). We also highlight the low mortality rate observed throughout the study period.

While crustaceans have been considered one of the main food items consumed by syngnathids (see Foster & Vincent, 2004 and Kendrick & Hyndes, 2005), the large consumption of nematodes found in our study has not been previously recorded to that group, exceptions being small amounts found in the gut

Table 1. Frequency, abundances and index of preponderance by height (IP) of prey taxa for 10 size classes of *Hippocampus reidi* (n = 280) in the wild. Number of stomachs examined is given in parentheses for each size class.

Prey Items	Frequency of occurrence (%O)	Relative abundance (%A)	Index of preponderance										
			General	<10 (n = 3)	10 (n = 8)	11 (n = 17)	12 (n = 27)	13 (n = 28)	14 (n = 38)	15 (n = 51)	16 (n = 62)	17 (n = 27)	≥18 (n = 19)
Nematoda	0.8672	0.2360	42.6776	23.4405	55.2463	49.4783	49.6058	44.5866	33.9497	45.8488	34.8725	36.4851	42.3756
Copepoda (Harpacticoida)	0.9114	0.1841	34.9797	43.1002	32.0771	42.1899	28.4461	34.5713	41.5275	29.0932	42.8817	31.5014	17.6463
Caridea (shrimps)	0.1365	0.1845	5.2522	0	0	0.2407	2.3384	0.8578	2.8602	4.8618	4.9357	13.8703	21.5793
Copepoda (Nauplii)	0.5867	0.0337	4.1174	2.4575	2.1842	2.5165	4.4593	6.3695	7.0041	2.8516	4.2086	3.0735	2.0269
Copepoda (Calanoida)	0.3985	0.0484	4.0249	5.1985	6.1099	2.8311	2.3742	8.1710	6.8058	4.2943	2.1503	2.4366	3.8491
Copepoda (Cyclopoida)	0.4502	0.0423	3.9734	2.1739	0.3426	1.3511	0.8841	0.6380	1.3262	5.3430	8.4020	5.5171	7.5528
Caridea (shrimp parts)	0.1661	0.0678	2.3466	0	0	0.4813	2.0178	0.3789	0.9437	5.8259	1.0252	5.7303	3.7825
Teleostei (Gobiidae)	0.0627	0.1514	1.9796	0	3.4261	0.4125	8.5195	3.1991	4.6520	1.5121	0.9627	0.3036	0.5221
Insecta (Hymenoptera)	0.0701	0.0169	0.2469	18.9036	0	0.2750	0.6345	0.0894	0.6153	0.0060	0.1378	0.6073	0.0307
Amphipoda (Gammaridae)	0.1218	0.0055	0.1389	0	0	0.0172	0.1465	0.0447	0.0337	0.1028	0.2205	0.1987	0.4197
Teleostei (fish scales)	0.0443	0.0088	0.0814	0	0	0.0688	0.0927	0.5987	0.1081	0.1072	0.0404	0	0
Polychaeta (larvae)	0.0812	0.0039	0.0653	0	0.1428	0.0172	0.2242	0.1635	0.0499	0.0119	0.0514	0.1242	0.0287
Amphipoda (Caprellidae)	0.0406	0.0046	0.0386	0	0.4283	0.0516	0.1943	0.0357	0.0782	0.0187	0.0254	0	0.0348
Ostracoda	0.1033	0.0016	0.0354	0	0.0428	0.0344	0.0404	0.0456	0.0166	0.0333	0.0331	0.0290	0.0706
Eggs (mollusks or crustaceans)	0.0295	0.0031	0.0192	4.7259	0	0	0.0075	0.1608	0	0.0099	0.0015	0.0863	0
Polychaeta (Nereididae)	0.0258	0.0014	0.0075	0	0	0	0.0112	0	0	0.0214	0.0276	0.0069	0
Brachyura (nauplii)	0.0148	0.0012	0.0038	0	0	0.0344	0	0	0	0	0.0092	0	0.0758
Insecta (Chironomidae)	0.0111	0.0014	0.0033	0	0	0	0	0	0.0208	0.0099	0	0.0276	0
Crustacea (zoea larvae)	0.0185	0.0008	0.0030	0	0	0	0	0.0894	0	0.0020	0.0055	0	0
Gastropoda (larvae)	0.0258	0.0004	0.0024	0	0	0	0.0037	0	0.0062	0.0010	0.0037	0.0021	0.0051
Bivalvia (larvae)	0.0074	0.0012	0.0018	0	0	0	0	0	0	0.0198	0.0026	0	0
Caridae (zoea larvae)	0.0074	0.0005	0.0008	0	0	0	0	0	0	0.0198	0	0	0
Isopoda	0.0037	0.0003	0.0002	0	0	0	0	0	0	0.0056	0	0	0
Oligochaeta	0.0037	0.0002	0.0001	0	0	0	0	0	0	0	0.0028	0	0
Foraminifera	0.0037	0.0001	0.0000	0	0	0	0	0	0.0021	0	0	0	0

Table 2. Global tests of the analyses of similarities (ANOSIM) of the natural diet of *Hippocampus reidi* (n = 280), between life stages (1), sexes (2), sexes and life stage simultaneously (3) and reproductive states (4). Life stage: J = juveniles, SA = subadults, AD = adults. Sex: U = undetermined, F = females, M = males. Reproductive state: IM = immature, OF = ovipositor region flat, OB = ovipositor region bulging, B = brooding, NB = non-brooding.

Global tests	Sample statistic (Global R)	P	Number of permutations	Number of permuted statistics greater than or equal to global R
(1) J x SA x AD	0.126	0.076	1000	76
(2) U x F x M	0.084	0.103	1000	103
(3) J.U x SA.F x SA.M x AD.F x AD.M	0.115	0.014	1000	14
(4) IM.J x IM.F x IM.M x OF.F x NB.M x OB.F x B.M	0.039	0.139	1000	139

Table 3. Pairwise tests of the analyses of similarities (ANOSIM) of the natural diet of *Hippocampus reidi* (n = 280) between sexes and life stages simultaneously. Life stage: J = juveniles, SA = subadults, AD = adults. Sex: U = undetermined, F = females, M = males.

Pairwise T tests (sex and life stage simultaneously)	Sample statistic (Global R)	p	Number of permutations	Number of permuted statistics greater than or equal to global R
AD.F x AD.M	-0.006	0.493	1000	493
AD.F x SA.F	-0.055	0.766	1000	766
AD.F x SA.M	0.211	0.036	1000	36
AD.F x J.U	0.335	0.055	91	5
AD.M x SA.F	0.094	0.096	1000	96
AD.M x SA.M	0.281	0.028	1000	28
AD.M x J.U	0.458	0.066	91	6
SA.F x SA.M	0.207	0.049	1000	49
SA.F x J.U	0.297	0.127	55	7
SA.M x J.U	-0.052	0.464	28	13

contents of *Syngnathus taenionotus*, *S. abaster* (Franzoi *et al*., 1993) and *S. schlegeli* (Huh & Kwak, 1997). The abundance of nematodes may be a result of prey accessibility rather than preference, as they constitute the most abundant meiofaunal group in estuarine environments, representing 60-90% of the total fauna, followed by Harpacticoid copepods with 10-40% (Coull, 1999). The occasional consumption of insects (ants) observed in this study is unique, and is possibly related to the massive presence of mangrove trees along the margins of the estuary.

While Harpacticoida was the principal item consumed by *Hippocampus zosterae* (Tipton & Bell, 1988), *H. subelongatus* (Kendrick & Hyndes, 2005) and *H. reidi* (this study), the other studied seahorse species mostly consumed Cyclopoida copepods, amphipods and caridean shrimps (see Kanou & Kohno, 2001; Kendrick & Hyndes, 2005; Teixeira & Musick, 2001; Burchmore *et al*., 1984; Woods, 2002). These results highlight the importance of crustaceans as a prey category to seahorses.

The occasional consumption of larger prey like caridean shrimp and fishes by *H. reidi* - a situation also observed in *S. typhle* (Oliveira *et al*., 2007) and in the females of *S. folletti* by Garcia *et al*. (2005) - could result in a larger energy intake by seahorses than preying upon smaller prey such as copepods; a desirable mechanism for a species that mainly uses a sit-and-wait strategy. Furthermore, Garcia *et al*. (2005) have suggested that, in the case of *S. folletti*, that feeding behavior may also determine higher between-phenotype variability than previously suggested.

The consumption of epibenthic prey (*e.g.* harpacticoid copepods, nematodes, polychaetes) by *H. reidi* has also been recorded for *H. whitei, H. zosterae, H. abdominalis, H. breviceps,* and *H. subelongatus*, which mainly consumed epibenthic invertebrates (Burchmore *et al*., 1984; Tipton & Bell, 1988; Woods, 2002; Kendrick & Hyndes, 2005), and possibly reflect the sedentary behaviour of seahorses, rather than a gape limitation.

The feeding strategy and niche breadth analyses conducted in this study suggested that *H. reidi* has a generalist feeding strategy, with high variation between phenotypes. Nevertheless, similar to what has been recorded for *S. typhle* (Oliveira *et al*., 2007), our findings suggest that *H. reidi* may have a specialist strategy in early life stages, consuming only small planktonic prey, and then becoming a moderately generalized benthonic predator. According to Foster & Vincent (2004), seahorses may change their diet ontogenetically, or as they grow, which may be a reflection of changes in feeding capabilities (*e.g.*, change in mouth gape) and/or habitat shifts (Ryer & Orth, 1987; Tipton & Bell, 1988; Woods, 2002).

Syngnathids in general exhibit a high degree of trophic specialization compared with many other epibenthic or demersal marine teleosts, and such differences can be explained, at least in part, by differences in their snout morphologies and feeding behaviors, and also, in the case of seahorses, by their relatively restricted swimming ability (Kendrick & Hyndes, 2005). With swimming capabilities more suited to maneuverability than speed, and with a strongly prehensile tail (Hale, 1996; Consi *et al*., 2001), the seahorse body form is adapted to inhabiting relatively complex habitats and these fishes rarely venture into open water when feeding (Flynn & Ritz, 1999).

In our study we found that seahorses smaller than 10 cm consumed a larger proportion of Calanoida and Cyclopoida copepods, Hymenoptera (ants) and eggs than the larger individuals, which consumed more larger prey items, such as fish and caridean shrimps. A similar result was found by Woods (2002), where smaller individuals of *H. abdominalis* (< 13.75 cm) consumed a larger proportion of total gut volume of amphipods than larger seahorses (> 13.8 cm), which mostly consumed caridean shrimps. Ontogenetic dietary shifts were also recorded to *H. erectus*, as smaller fish (<60 mm TL) fed mainly on amphipods, with copepods secondarily important,

Table 4. Similarities of percentages (SIMPER) indicating which prey items contributed simultaneously to the dissimilarity between sexes and life stages in the natural diet of *Hippocampus reidi* (n = 280). Life stage: J = juveniles, SA = subadults, AD = adults. Sex: U = undetermined, F = females, M = males. **Significant values according to ANOSIM.

Prey items	Mean index of preponderance					Mean dissimilarity									
	J.U	SA.F	SA.M	AD.F	AD.M	J.U x SA.F	J.U x SA.M	J.U x AD.F	J.U x AD.M	SA.F x SA.M	SA.F x AD.F	SA.F x AD.M	SA.M x AD.F	SA.M x AD.M	AD.F x AD.M
						43.95	52.54	47.89	46.49	47.25**	39.55	39.54	47.93**	46.98**	39.35
Nematoda	20.71	43.51	37.05	33.64	32.89	2.84	4.17	2.48	2.79	4.29	2.53	2.87	4.14	4.52	2.85
Copepoda (Harpacticoida)	22.77	36.03	13.84	31.47	36.95	3.30	4.23	3.02	3.21	5.48	2.57	2.24	4.70	5.17	2.51
Copepoda (nauplii)	2.21	3.95	3.88	5.95	6.54	4.01	4.09	4.28	4.28	4.57	4.30	4.25	4.75	4.95	3.68
Caridea (shrimps)	0.00	1.34	0.00	8.41	3.81	1.54	0.00	4.23	3.22	1.62	4.53	3.58	4.42	3.35	4.39
Teleostei (Gobiidae)	0.00	1.57	27.98	7.18	1.97	1.58	6.25	4.05	1.76	6.92	4.38	2.67	7.12	6.45	4.12
Copepoda (Cyclopoida)	9.75	2.18	1.54	3.24	3.91	5.98	6.14	5.48	5.58	3.57	3.67	3.61	3.54	3.44	3.43
Copepoda (Calanoida)	23.31	1.16	4.86	2.88	4.93	7.70	7.79	7.04	7.03	4.27	3.07	4.26	3.98	4.03	3.91
Caridea (shrimp parts)	0.00	3.07	5.36	2.59	3.83	1.89	2.79	2.28	3.62	3.87	3.28	4.16	3.81	4.49	3.69
Insecta (Hymenoptera)	17.01	3.77	0.00	1.70	0.07	6.79	6.86	6.25	6.23	2.97	3.56	2.79	2.16	0.22	1.99
Amphipoda (Gammaridae)	0.00	0.17	0.17	0.55	1.11	0.37	0.35	1.13	1.00	0.68	1.30	1.26	1.30	1.25	1.64
Polichaeta (larvae)	0.00	0.08	3.85	0.50	1.12	0.22	3.26	0.99	1.06	3.43	1.08	1.19	3.42	3.51	1.49
Teleostei (fish scales)	0.00	1.79	0.50	1.45	0.07	1.77	0.71	1.63	0.19	2.25	2.68	1.78	2.07	0.82	1.60
Ostracoda	0.00	0.02	0.64	0.07	0.05	0.06	0.99	0.22	0.14	1.03	0.24	0.17	1.04	1.00	0.28
Gastropoda (larvae)	0.00	0.00	0.00	0.02	1.54	0.00	0.00	0.06	0.87	0.00	0.06	0.88	0.06	0.90	0.86
Polichaeta (Nereididae)	0.00	0.95	0.00	0.00	0.06	0.85	0.00	0.01	0.17	0.89	0.81	0.96	0.01	0.18	0.17
Eggs (mollusks or crustaceans)	4.25	0.00	0.00	0.01	0.31	4.25	4.35	3.92	3.97	0.00	0.05	0.72	0.05	0.74	0.39

while mid size and larger specimens fed almost exclusively on amphipods (Teixeira & Musick, 2001). Additionally, Tipton & Bell (1988) found an increase in the consumption of amphipods by larger specimens of *H. zosterae*, despite the dominance of harpacticoid copepods in all size classes, and Kanou & Kohno (2001) reported that juveniles *H. mohnikei* exhibited a division of prey items between size classes, with the smaller seahorses feeding mainly on cyclopoid copepods smaller than those consumed by larger specimens.

Some authors state that the reproductive system of Syngnathidae may result in differential reproductive costs, which may be reflected in different prey selection by males and females (Svensson, 1988; Steffe *et al*., 1989; Lyons & Dunne, 2003; Garcia *et al*., 2005). In seahorses it is known that the period of pregnancy represents a high energetic cost for the males (Teixeira & Musick, 2001). In *H. zosterae*, for example, male metabolic rates increases from 10 to 52% over pregravid levels (Masonjones, 2001), as these animals are responsible by osmotic regulation and oxygen and perhaps provide nutrition to the embryos (Strawn, 1958; Masonjones, 2001; Foster & Vincent, 2004). According to Lyons & Dunne (2004), the male seahorse pregnancy may produce a behavioral response that limits the movements of males, reducing energetic expenditure on chasing larger vagile prey (Ryer, 1988; Lyons & Dunne, 2004) and risk of capture by predators (Lyons & Dunne, 2004; Oliveira *et al*., 2007). Thus, it is possible that the greater consumption of harpacticoid copepods and reduced consumption of caridean shrimps and gobiid fishes by the adult males in the present study is related to reproduction, as found in another species of Syngnathidae (Ryer, 1988; Lyons & Dunne, 2004; Oliveira *et al*., 2007).

The flushing method, or gastric lavage, is the most simple and efficient method used to safely remove food items without sacrificing the fish (Hyslop, 1980; Hartleb & Moring, 1995; Hakala & Johnson, 2004), and has been used safely and efficiently in studies of food habits of various salmonids and sturgeon species (see Kamler & Pope, 2001; Brosse *et al*., 2002).

However, because the great variability in the partitioning and length of the digestive tracts of different species of fish, it is not possible to predict which species will be amenable to the procedure unless the alimentary tract morphology is known (Baker & Fraser, 1976). Some associated risks with the procedure may include a prolonged manipulation time and stress and some injuries to the flash tissues of esophagus, stomach or gut (Haley, 1998; Wanner, 2006).

Our results suggest that the flushing technique has the potential to replace destructive sampling procedures in seahorse research. Also, this technique may reduce research pressure on vulnerable wild seahorse stocks. Furthermore, the re-sighting of individuals in this study, particularly of those which had changed reproductive state or were sighted releasing young, suggests that the clove oil anaesthetization followed by the flushing procedure was non-destructive to the sampled individuals. Nevertheless, caution should be taken while manipulating the specimens to avoid injuries and to minimize stress.

Although encouraging, these first results are by no means conclusive, and we strongly recommend further research to better quantify the efficacy of the flushing method on seahorses and better evaluate potential impacts of the procedures on the behavior and growth of the anaesthetized specimens.

The results of the dietary analysis presented in this study provide essential ecological data that may help both in in situ and ex situ conservation actions directed to seahorses, particularly to *Hippocampus reidi*.

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