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# PREY PREFERENCE OF THE LONG-SNOUTED SEAHORSE (*Hippocampus guttulatus* Cuvier, 1829) AT THE ROMANIAN BLACK SEA COAST

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***Abstract***

*The long-snouted seahorse (Hippocampus guttulatus Cuvier, 1829) is a representative species of the Romanian coast, due to its charismatic appearance and extraordinary biology. Although it is not a commercial fish in Romania, it is subjected to harvesting to be sold as curio or for the aquarium business, and many times is by-caught in trawl or pound net fishery. The current research aimed at the examination of the gut content of wild seahorse specimens, in order to determine the prey preferences of the species along the Romanian Black Sea coast. In the wild, large prey items (Amphipoda, Balanus larvae) were identified as the preferred prey of adult specimens, indicating that size and availability are important factors in prey selection. Adult seahorses appear to prefer larger prey both in wild and controlled environments, as previous research has indicated.*

***Key words***: *feeding behavior, gut contents, long-snouted seahorse, prey availability, zooplankton.*

## INTRODUCTION

The long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829) is a representative species of the Romanian coast, due to its charismatic appearance and extraordinary biology (Figure 1). Although it is not a commercial fish in Romania, it is subjected to harvesting to be sold as curio or for the aquarium business (Vincent et al., 2011).

Figure 1. *H. guttulatus* individual in its natural habitat (southern Romanian Black Sea coast) (Photo: Nenciu)

Moreover, everywhere in the world, seahorses are often fished with non-selective gear (trawls) (Caldwell and Vincent, 2012) and are vulnerable to the degradation of habitats they inhabit (Woodall, 2012). There is an urgent need for concrete guidelines and initiatives to ensure the conservation of seahorse populations, with emphasis on their biology and ecology. It is also extremely important to understand also the socio-economic aspects of the fishery, which can significantly affect the populations of these species, as well as the way in which anthropogenic activities and their consequences on the marine environment can affect seahorse populations (Nenciu et al., 2013).

The genus *Hippocampus* is included in Annex II of CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) since November 2002. The presence of 3 species, namely *Hippocampus guttulatus* (Cuvier, 1829), *Hippocampus hippocampus* (Linnaeus, 1758) and *Hippocampus fuscus* (Rüppel, 1838) is reported in the Romanian Black Sea waters by bibliographic sources (Lourie et al., 2004; Foster and Vincent, 2004). In more recent works, only one species (Radu and Radu, 2008) is reported at the Romanian coast, although several species are described by previous sources (Banarescu, 1964). However, only a genetic study can confirm the presence of these three different species, which have not been done so far in the Black Sea and is currently ongoing (Nenciu et al., 2015b; Taflan et al., 2017).

Seahorses are carnivorous fish that hide in strategic places along the edges of reefs or seagrass beds waiting for prey to come within striking reach (Van Wassenbergh et al., 2011). Seahorses capture highly evasive prey such as small shrimp or larval fishes (Kendrick and Hyndes, 2005). To do so, they make use of a two-phase prey-capture mechanism that is commonly referred to as pivot feeding: a rapid upward rotation of the head is followed by suction to draw the prey into the seahorse's snout (Roos et al., 2009).

Although voracious, seahorses choose to be opportunistic instead of dynamic predators. Seahorses have no teeth and so they stalk their prey, waiting for them to get close enough before sucking them in through their tubular snout. Moreover, seahorses have no stomach, thus digestion occurs very rapidly, consequently they need to constantly hunt and consume prey.

In Romania, the first experiments conducted on the breeding and rearing in captivity of seahorses were carried out by the National Institute for Marine Research and Development (NIMRD) „Grigore Antipa" Constanta, in 2008. The results of the experiments conducted have shown that the breeding and subsequent rearing of these fish in captivity is feasible (Figure 2).

Figure 2. *H. guttulatus* male in controlled environment (captive breeding experiment) (Photo: Nenciu)

However, the major drawback in rearing *H. guttulatus* was supplying the most appropriate diet for the fry, as many individuals died of starvation before reaching maturity due to the lack of a small-sized food alternative (Nenciu et al., 2015a).

An experiment previously conducted by NIMRD experts indicated that a combined diet of the rotifer *Brachionus plicatilis* and the brine shrimp *Artemia salina* is the most recommended for rearing seahorses in captivity, due to the advantages that the two invertebrates have separately. On the one hand, rotifers develop greater densities and have a higher protein content (reflected in the protein content of the batches analyzed), while brine shrimps have a higher lipid content and are easier to hunt, being larger and more visible in the tank and always selected as preferred prey (Nenciu et al., 2015a).

Under these circumstances, the current research aimed at the examination of the gut content of wild seahorse specimens, in order to determine the prey preferences of the species along the Romanian Black Sea coast.

## MATERIALS AND METHODS

For the gut content analysis of wild specimens, 30 individuals were collected from the Romanian coast, from by-catches in commercial trawls and pound nets (3 sampling Stations, Edighiol, Agigea and 2 Mai, 10 individuals each, August 2017) (Figure 3).

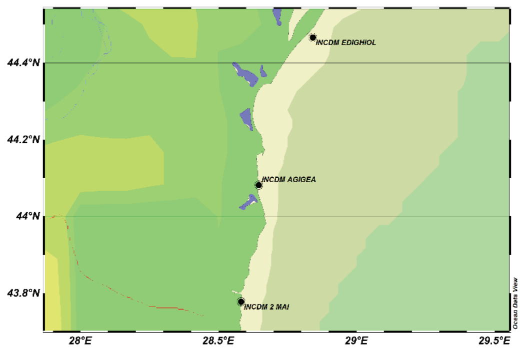


Figure 3. Sampling stations for wild *H. guttulatus* specimens

Specimens were dissected and guts were removed (Figure 4).

Figure 4. Dissection and gut content analysis (Photo: Nenciu M.I.)

The number of empty and full guts of the specimens was recorded. Gut contents were recovered and homogenized in Petri dishes and examined using a binocular stereo microscope.

The prey items were identified to the lowest possible taxonomic level and assigned to different prey categories such as Amphipoda, Isopoda, Copepoda etc.

Two types of methods were used, namely qualitative and quantitative methods (Totoiu et al., 2014). The qualitative analysis consisted in the identification of the food components found in the fish's gut. The quantitative method consisted in numerical analysis (frequency of occurrence - FO% - and numerical abundance - N - of the analyzed stomachs where a specific prey group was identified) (Hyslop, 1980; Hansson, 1998).

The frequencies of occurrence (FO%), as numerical percentages of prey items, were calculated to characterize the gut contents (Hyslop, 1980; Hansson, 1998). The frequency of occurrence calculates the percentage of the total number of guts in which the specific prey species occurs:

$$FO\% = FOi/FOt \times 100,$$

where: FOi is the number of guts in which the species "I" occurs, and FOt is the total number of full guts. The Index of Relative Importance (IRI) was impossible to calculate due to the very small size of the prey and guts, which prevented weighing.

## RESULTS AND DISCUSSIONS

The three sampling stations were not selected randomly (Figure 3), but so as to cover the northern, central and southern parts of the Romanian coast, with different habitat types. The 30 individuals investigated, all adults, 10 per each station, were equally divided between males and females (5 males and 5 females per each station). No significant differences between males and females were identified in any of the sampling station.

In the northern station, Edighiol, the main food group identified were amphipods (FO% 66.66), two thirds of the prey items in the gut contents being represented by this group. The next group as frequency was meroplankton, namely *Balanus* cypris larvae (FO% 44.44). The other three items, Isopoda, Copepoda and other (non-identified semi-digested items) held equal shares (FO% 22.22) (Table 1, Figure 5).

Table 1. Prey groups identified in *H. guttulatus* gut contents (Station Edighiol)

| Group | N | FO% |
|---|---|---|
| Crustacea: Amphipoda | 6 | 66.66 |
| Crustacea: Isopoda | 2 | 22.22 |
| Crustacea: Copepoda | 2 | 22.22 |
| Meroplankton: *Balanus* cypris | 4 | 44.44 |
| Other (non-identified) | 2 | 22.22 |

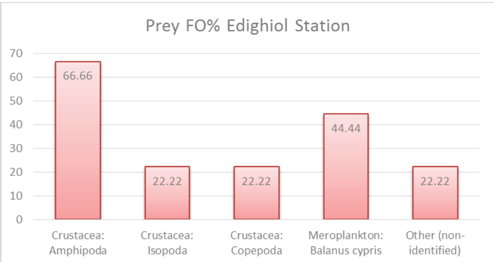


Figure 5. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station Edighiol)

In the central station (Agigea), however, the largest share was held by meroplankton - Balanus cypris larvae (FO% 77.77), followed by Amphipoda (FO% 55.55), isopod crustaceans (FO% 33.33), with the smallest share held by copepods and other non-identified items (FO% 22.22) (Table 2, Figure 6).

Table 2. Prey groups identified in *H. guttulatus* gut contents (Station Agigea)

| Group | N | FO% |
|---|---|---|
| Crustacea: Amphipoda | 5 | 55.55 |
| Crustacea: Isopoda | 3 | 33.33 |
| Crustacea: Copepoda | 2 | 22.22 |
| Meroplankton: *Balanus* cypris | 7 | 77.77 |
| Other (non-identified) | 2 | 22.22 |

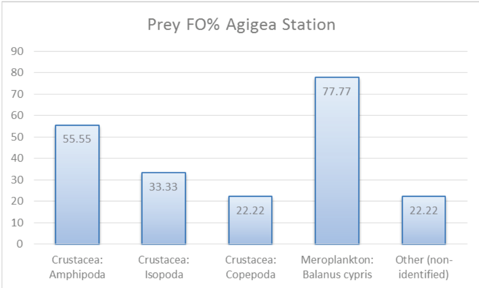


Figure 6. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station Agigea)

The southern most station (2 Mai) also recorded a clear dominance of *Balanus* cypris larvae (meroplankton), which were identified in all seahorse guts analyzed (FO% 100), followed by amphipods (FO% 50), copepods (FO% 40), other non-identified items (FO% 30), the last group as frequency being isopods (FO% 20) (Table 3, Figure 7).

Table 3. Prey groups identified in *H. guttulatus* gut contents (Station 2 Mai)

| Group | N | FO% |
|---|---|---|
| Crustacea: Amphipoda | 5 | 50 |
| Crustacea: Isopoda | 2 | 20 |
| Crustacea: Copepoda | 4 | 40 |
| Meroplankton: *Balanus* cypris | 10 | 100 |
| Other (non-identified) | 3 | 30 |

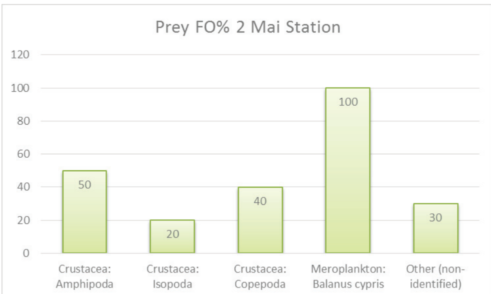


Figure 7. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station 2 Mai)

Overall, for all stations investigated, the dominant prey group was represented by *Balanus* cypris larvae (meroplankton), with FO% 70, followed by amphipods (FO% 57.14) Isopoda and Copepoda recorded close values, FO% 25 and FO% 21.42, respectively (Table 4, Figure 8).
Out of the total guts investigated, only two were lacking any prey, while all other contained, in different shares, all the major groups identified.

Table 4. Prey groups identified in *H. guttulatus* gut contents (TOTAL)

| Group | N | FO% |
|---|---|---|
| Crustacea: Amphipoda | 11 | 57.14 |
| Crustacea: Isopoda | 7 | 25 |
| Crustacea: Copepoda | 8 | 21.42 |
| Meroplankton: *Balanus* **cypris** | 21 | 75 |
| Other (non-identified) | 7 | 25 |

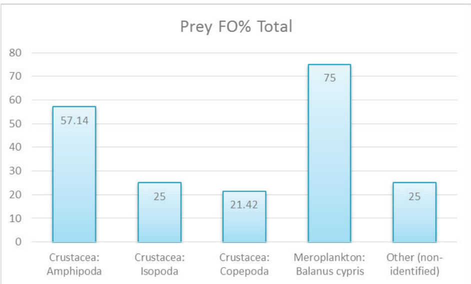


Figure 8. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (TOTAL)

*Amphibalanus improvisus* (Darwin, 1854) (*Balanus*) is a small sessile crustacean, typical for the shallow fringe of sea (less than 10 m deep), occurring in marine and brackish environments. *A. improvisus* has been dispersed by shipment outside its natural distribution area, which is considered to be the western Atlantic. It was first recorded as invasive in the Black Sea in 1844 (Skolka and Gomoiu, 2004). Since then, it has rapidly developed on rocky substrate, on man-made structures, buoys, ships' hulls, the shells of crabs and mollusks (mussels), and certain seaweeds. Its larval stages have become a significant dietary item in the food chain of coastal ecosystems, especially for small fish such as Syngnathids (seahorses and pipefish). The analyses performed on the gut contents of seahorses at the Romanian Black Sea coast revealed that *Balanus* larvae were identified in 21 of the 28 full guts (FO% 75), probably due to its high availability at the time sampling was performed (August) (Figure 9). The distribution map points out its highest abundance in the southern part of the Romanian coast, not surprisingly given the rocky habitat type and the existence of numerous hydro-technical constructions (Figure 10).

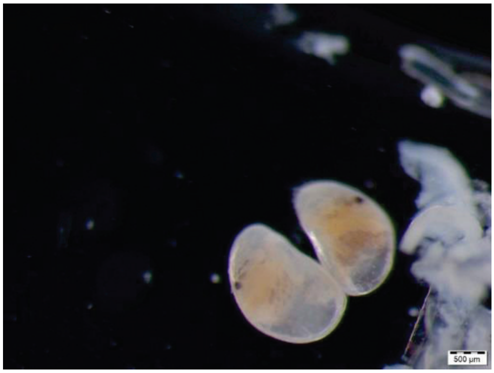


Figure 9. *Balanus* cypris larvae in seahorse gut (Photo: Harcota G.E.)

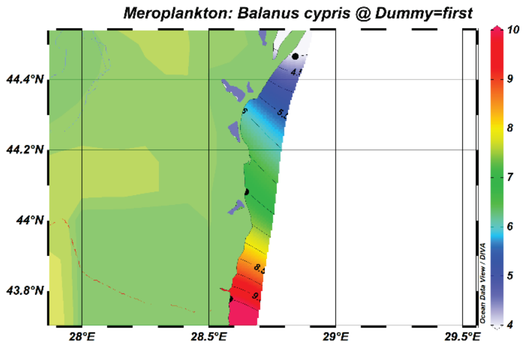


Figure 10. Distribution map of *Balanus* larvae identified in the gut content of *H. guttulatus*

The second dietary group identified were amphipod crustaceans (FO% 57.14). Amphipods are utilizers of primary and partly secondary production in marine ecosystems. Moreover, they transform sediment composition by enriching it with organic matter. These small crustaceans are dietary items for many of coastal fishes, seahorses included (Figure 11). Relatively large in size, amphipods are easily visible and provide a high nutritional value for the voracious seahorses.
The distribution map shows a higher abundance of amphipods in the northern part of the coast (Figure 12).


Figure 11. Amphipods identified in seahorse gut content (Photo: Harcota G.E.)

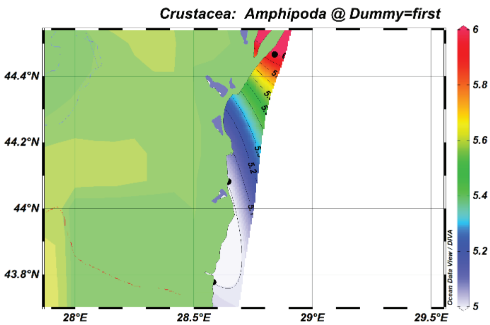


Figure 12. Distribution map of Amphipoda identified in the gut content of *H. guttulatus*

Copepods (FO% 21.42) were not so frequent in the gut contents of the investigated specimens, most likely due to the fact that they were all adults, thus preferably selecting larger prey items (Figure 13). Nevertheless, copepods are the biggest source of protein in the marine environment and are an important prey, especially for small forage fish or juveniles. The distribution map (Figure 14) also shows a dominance of copepods in the southern part of the coast.

Figure 13. Copepods identified in seahorse gut content (Photo: Bisinicu E.)

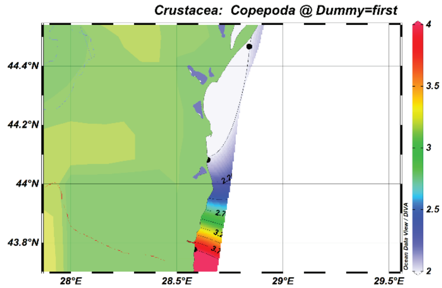


Figure 14. Distribution map of Copepoda identified in the gut content of *H. guttulatus*

Similarly to copepods, isopod crustaceans did not occur in extremely high frequencies (FO% 25). However, they are often dietary components of coastal fish, due to their large size and availability.

The distribution map (Figure 15) revealed a concentration of isopod crustaceans in the central part of the coast.

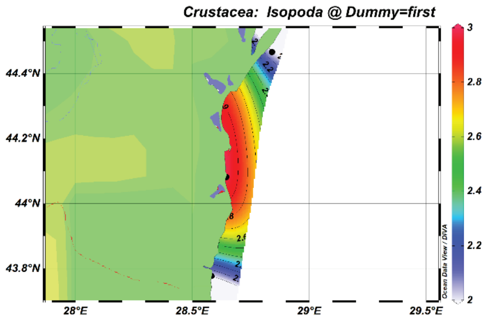


Figure 15. Distribution map of Isopoda identified in the gut content of *H. guttulatus*

The group generically called „other non-identified items" includes semi-digested prey fragments, impossible to identify (Figure 16).

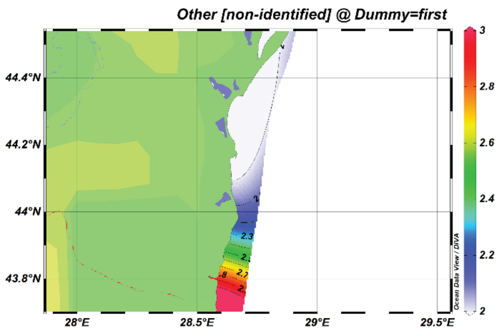


Figure 16. Distribution map of other preys (non-identified) in the gut content of *H. guttulatus*

To date, there are no other studies on the gut contents of *H. guttulatus* in the Black Sea, which makes it difficult to infer which other prey items are likely to be found in seahorses' diet.

Yet, similar investigations performed on the same species in the Aegean Sea revealed that Mysidaceae (42.59%) and Decapod crustacean larvae (22.22%) constituted the most important food source of *H. guttulatus*. (Gurkan et al., 2011). Amphipods and isopods were also present. Based on the number and frequency, the dominant preys of *H. guttulatus* in the Aegean are Decapod crustacean larvae, Mysidaceae and Amphipoda and unidentified prey. These results are consistent with previously published data (Kitsos et al., 2008).

## CONCLUSIONS

Summing up, the dominant prey group identified in the gut content of *H. guttulatus* along the Romanian Black Sea coast was represented by *Balanus* cypris larvae (meroplankton) (FO% 70), followed by amphipods (FO% 57.14) Isopoda (FO% 25) and Copepoda (FO% 21.42). The other non-identified prey items could be represented by Mysid crustaceans or Decapod crustacean larvae.

No significant differences between the gut contents of males and females were identified.

Out of the total guts investigated, only two were lacking any prey, while all other contained, in different shares, all the major groups identified.

It is important to point out the fact that the dominance of one group or the other is closely related to its availability in the environment at a certain moment.

Thus, seahorses, as opportunistic predators, will preserve energy by selecting the largest and most available prey items.

This is why it is not by chance that *Balanus* larvae, which are abundant in areas with hard substrate, were identified in large numbers in the guts of seahorses sampled in the southern part of the Romanian coast.

The preference for larger prey (amphipods, for example) was also demonstrated by captive rearing experiments, where adult seahorse always selected the larger brine shrimps *Artemia salina* compared to the rotifer *Brachionus plicatilis*.

Further investigations will be performed on a larger number of specimens, in order to reveal more correlations between prey preference, habitat type and prey availability in the long-snouted seahorse *H. guttulatus* at the Romanian Black Sea coast.

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