

Trophic ecology and potential predation impact of *Carybdea marsupialis* (Cnidaria: Cubozoa) in the NW Mediterranean

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Summary

We have studied the diet and the feeding behaviour of *Carybdea marsupialis* using different approaches. Gut contents analyses revealed copepods, mysids, and gammarids, as the main prey items, followed by polychaete, and larvae and juveniles of fish. Diet composition and proportion of empty/non empty stomachs, and prey selectivity index varied between day and night. We also present, and discuss, data on carbon and nitrogen stable isotope analysis to provide more information about the trophic role of this species. Finally, we estimated the potential predation impacts on zooplankton and fish larvae of this blooming organism by combining the results of our experiments with quantitative data on the abundance of *C. marsupialis* and their prey.

1. Introduction

The species *Carybdea marsupialis* is the only cubozoan known to inhabit the Mediterranean (Linnaei 1758). *C. marsupialis* was never considered a blooming species in the western Mediterranean; however, since 2008, blooms of this species have been detected along the Spanish coast, reaching unusual very high densities in some beaches (Bordehore *et al.* 2011 for Denia; LIFE+ CUBOMED project, unpublished data for other localities).

Box jellyfish (Cubomedusae) are entangling predators, capturing a variety of both invertebrate and vertebrate prey. Their major preys are planktonic crustaceans, some species being capable of capturing and swallowing relatively large fish. It is particularly important to determine whether high densities of *C. marsupialis* in the NW Mediterranean will alter ecosystem function and/or biodiversity, as well as to understand the main causes and factors that are currently enhancing this native species to outbreak.

2. Materials and methods

The abundance of *C. marsupialis* medusae was measured in three coastal transects of 20 meters length at six different beaches at Denia (Spain). Four samplings were conducted both during day (from 8:00 a.m. to 13:00 p.m.) and night (from 21:00 p.m. to 1:00 a.m.). Three kinds of hand nets were used on each transect (200 µm, 500 µm and 4 mm mesh size) to include the whole size range of the species as well as mesozooplankton associated with the captures. A flow meter was placed in the mouth of each net to estimate the volume of water filtered. Some of the captured cubomedusae were preserved in sea

water-formaldehyde solution (4%) for the later dissection and gastric content analysis under the stereomicroscope. The rest of the captured cubomedusae were transported to the laboratory to conduct feeding experiments.

We also analysed the C and N stable isotopes of *C. marsupialis* of different sizes and their prey. Samples were dried at 60°C and ground to a fine powder. All of the samples were weighed into tin cups, combusted at 1000°C, and analysed in a FlashEA1112 (ThermoFinnigan) elemental analyser. Total %C and %N of the dry weight were measured and the stable isotope abundances were expressed in δ notation.

We conducted incubations with different concentrations of natural zooplankton, *Acartia grani*, and *Artemia salina* to investigate prey selectivity and to calculate clearance and ingestion rates. Individual observations of gut contents over time were also registered to calculate digestion times of different kinds of prey.

3. Results and Discussion

The gut content analysis revealed differences between feeding habits of the species during day and night. The captures of cubomedusae and the number of individuals with prey in the stomach were higher during the night than during the day (GLM Poisson $z=8.92$; $p < 0.001$). Distinct day/night diet composition was also obtained from the comparison (Permanova: Pseudo-F: 9.19; $p = 0.0001$). Copepods were the main prey in the diurnal stomachs analysed and gammarids were the most important item at night. The diet was more diverse in the nocturnal samples, because the mesoplankton available were also more diverse, probably due to the upward diel migration from the *Posidonia oceanica* meadows. This fact is also reflected in the prey selectivity index (Chesson's α index). Moreover, we observed a progressive shift in the preferred dietary composition as the cubomedusae grew, similar to our laboratory observations (Acevedo *et al.* 2013), and those for other cubozoan species (Nogueira and Haddad 2008). Stable isotopes C and N ratios also showed these differences between cubomedusae stages. This was especially evident in $\delta^{15}\text{N}$, which was more variable in the < 5 mm diagonal bell width (DBW) individuals (mean \pm SD; 5.6 ± 5.5 mm) than in larger juveniles (5-15 mm DBW; 9.6 ± 1.2 mm) and adults (> 15 mm DBW; 10 ± 0.6 mm), indicating that smaller stages are more opportunistic than larger ones. Consistent with these results, the maximum potential predation impact of the species varied depending on the cubomedusa stage and the type of prey considered.

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