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OCEAN AND COASTAL RESEARCH

Spatio–temporal dynamics of the nudibranch *Doris kyolis* living on the sponge *Halichondria melanadocia*

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Along with other invertebrates, hawksbill turtles, and fishes, nudibranchs are among the main sponge predators. These organisms are able to feed on sponges that typically contain a high level of secondary metabolites, which they incorporate into their body and can be stored in glands (mantle dermal formations [MDFs]) to be used for their own defense (Rogers and Paul, 1991; Wägele et al., 2006). These feeding and defensive benefits entail that many nudibranch species specialize in just one prey species or taxonomic group, suggesting an important role in the food web of benthic ecosystems (Becerro et al., 1998; Rudman and Bergquist, 2007). However, despite the important progress in understanding these interspecific relationships, there is almost no information about these species' population dynamics in the localities they inhabit (Rogers and Paul, 1991; Knowlton and Highsmith, 2000; Page et al., 2011). This is important, especially because these predators (at high densities) may play a role in regulating the populations of their sponge prey and influence its spatial distribution (Dayton et al., 1974; Knowlton and Highsmith, 2000; Page et al., 2011).

Doris kyolis (Ev. Marcus and Er. Marcus, 1967) (Nudibranchia, Dorididae) has been reported in tropical and subtropical locations of the western Atlantic, from Florida, USA, to Rio de Janeiro, Brazil (Belmonte et al., 2015; Caballer-Gutiérrez et al., 2015). Throughout its distribution range, it has been frequently observed in interaction with sponges (Belmonte et al., 2015; Ortea et al., 2017) but there is no data of its density on the sponges from which it feeds. A recent study conducted in a southern Gulf of Mexico estuary (Laguna de Términos, Mexico) revealed that this nudibranch species is part of the associated macrofaunal assemblage with the sponge *Halichondria* (*Halichondria*) *melanadocia* Laubenfels, 1936 in seagrass meadows and red mangrove prop roots habitats (Ávila and Briceño-Vera, 2018). However, although *D. kyolis* was common in the samples of this sponge, basic aspects of its population dynamics, such as the small-scale spatial and temporal variation in its frequency of occurrence and density, have not been investigated. Therefore, the aim of the present study was to determine whether the frequency of occurrence and density of *D. kyolis* in association with *H.* (*H.*) *melanadocia* varies (i) between seagrass meadows and mangrove prop root habitats and (ii) throughout the year as a function of the sponge prey size and/or environmental factors, such as water temperature and salinity.

Samplings of *H.* (*H.*) *melanadocia* were carried out in the Laguna de Términos (Campeche, Mexico) in the southern Gulf of Mexico. Within this tropical estuarine system, two sampling sites (at a distance of 4.5 km from each other) were chosen: a red mangrove prop root habitat (*Rhizophora mangle* Linnaeus, 1753) (18°40'23"N–91°41'48"W) and a seagrass meadow (*Thalassia testudinum* Banks ex König, 1805 mixed with *Halodule wrightii* Ascherson, 1868) (18°44'29"N–91°32'05"W). In both sites, ten individuals of *H.* (*H.*) *melanadocia* were collected randomly (between 10:00 am and 12:00 pm and at depths of 0.5 to 0.8 m) at monthly intervals from March 2014 to March 2015. No samples were obtained in January 2015. Each sponge collected was first covered with

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a plastic bag and detached from the substrate with a knife, and then the bag was closed immediately to prevent the loss of the associated nudibranch *D. kyolis*. During field samplings, observations were also made in order to make a brief description of the interaction.

The water temperature (°C) was recorded daily at 12:00 h using a temperature sensor (HOBO Water Temp Pro v2 U22-001 data logger) tied in a stake at 20 cm from the bottom. Salinity of the bottom was measured on each sampling date by using a multiparameter sensor (YSI-63, Yellow Springs, OH, USA).

In the laboratory, nudibranchs were separated from the sponge by using dissecting forceps. Also, the seawater in the bags was filtered through a sieve (mesh size of 2 mm) to recover those nudibranchs that could have detached from the sponge during transport (Ribeiro et al., 2003). Based on the presence of *D. kyolis* on the sponges examined, its frequency of occurrence (hereafter frequency) was calculated as the percentage of sponges that contained this nudibranch species at each site monthly. Its density was expressed as the average number of individuals per sponge and as the number of individuals per sponge volume. The volume (mL) of each sponge was measured by using the fluid displacement method (Rützler, 1978). Also, the size (total body length) of each *D. kyolis* was measured with a Vernier (mm). After this, nudibranchs were returned to their original field sites.

Additionally, to verify if this nudibranch feeds on *H.* (*H.*) *melanadocia*, ten specimens of *D. kyolis* were kept (individually) for four or five hours in Petri dishes with water from the site until they deposited their feces. Then, their feces were analyzed with an optical microscope to search for *H.* (*H.*) *melanadocia* spicules.

To determine whether the frequency and density of *D. kyolis* and the volume of *H.* (*H.*) *melanadocia* significantly vary throughout the study period (12 months) and between habitats (seagrass meadow and mangrove roots), the non-parametric analysis of variance of Kruskal–Wallis was used, with a posteriori Mann–Whitney *U*-test for comparisons between groups. The Shapiro-Wilk's and Levene's tests were used to check normality of the distribution and variance homogeneity of the data (frequency of occurrence and density of *D. kyolis*), respectively (Zar, 1984). To evaluate relationships between the frequency and density (individuals/sponge volume) of *D. kyolis* and the temporal variations of water temperature, salinity, and sponge volume, Spearman´s rank correlation coefficient (r_s) was used. Multiple regression analyses were also performed to investigate whether monthly data of temperature, salinity and sponge volume could significantly predict the density and frequency of occurrence of *D. kyolis* on the sponge.

Individuals of *D. kyolis* (size range from 6.7 to 17.4 mm, average ± SE = 10.3±0.58 mm, n=158) as well as its ribbon eggs (of yellow color and spiral–like form) were always found between branch bases of *H.* (*H.*) *melanadocia* (Figure 1A, C). In this interaction, *D. kyolis* has a color (dark gray, almost black) and texture similar to its sponge prey (mimicry strategy). Unlike other nudibranchs with aposematic coloration, this species has a cryptic coloration that makes it difficult to distinguish from the sponge (Figure 1A).

This nudibranch was present in 30% of the total sponge individuals examined (n=240), with an overall density ranging from 1 to 13 individuals/sponge and from 0.004 to 0.097 individuals/mL of sponge. Its frequency and density in *H.* (*H.*) *melanadocia* varied significantly between habitats (KW tests, $p<0.01$, respectively), being significantly higher (Mann–Whitney *U*-tests, $p<0.05$ and $p<0.01$, respectively) in the seagrass habitat (mean frequency = 39±5.2%; mean density = 0.9±0.2 individuals/sponge) than in those from mangrove roots (21±5.0% and 0.3±0.1 individuals/sponge) (Figure 2B, D). Both the frequency and density of *D. kyolis* did not vary significantly (KW tests, $p>0.05$, in both cases) throughout the year (Figure 2A, C). In the same way, the volume of the sponges (50–900 mL) varied significantly between habitats (KW test, $p<0.05$) but not throughout the year (KW test, $p>0.05$). Individuals from the seagrass habitat (377±21 mL) had a volume significantly higher (Mann–Whitney *U*-tests, $p<0.05$) than those from the mangrove roots (304±18 mL). There were also no significant relationships between these population descriptors and the sponge volume, water temperature (25°C–31.2°C), and salinity (25–36.6). Regarding the multiple regression analyses, the results indicated that the models were not a significant predictor of the intra-annual variability of frequency (adjusted-R^2 = -0.03, ANOVA: $F_{[3, 8]} = 0.88$, $p>0.05$) and density (adjusted-R^2 = 0.07, ANOVA: $F_{[3,8]} = 1.31$, $p>0.05$) of *D. kyolis*.

After examining the feces of *D. kyolis*, only spicules (oxeas fusiform with acerate sharp point: 109 – [245] –540 µm length x 4 – [8.4] –15 µm wide) were found, which corresponded to those of *H.* (*H.*) *melanadocia* (Figure 1B). This finding confirms its predatory activity

Figure 1. a) The nudibranch *D. kyolis* feeding on the sponge *H.* (*H.*) *melanadocia*. b) Spicules (oxeas) and tissue remnants of *H.* (*H.*) *melanadocia* found in the feces of *D. kyolis*. c) The arrow indicates egg ribbons of *D. kyolis* in the base of the sponge.

and likely specificity for this sponge species in the study area. Despite this, it was not possible to detect extensive damage in the sponge individuals where *D. kyolis* was found. Only superficial marks were observed in its ectosome.

This is the first formal record of *D. kyolis* as predator of the sponge *H.* (*H.*) *melanadocia* and is reported for the first time from the southern Gulf of Mexico. Although this nudibranch species has been reported in different tropical and subtropical regions of the western Atlantic, the sponge *H.* (*H.*) *melanadocia* had not been reported as its prey. Some of the characteristics of this nudibranch such as the mimicry coloration, agree with previous reports of this species from other locations, where it has been mentioned that its color depend on the sponges on which it feeds (Ortea et al., 2017). Also, its specific location in the

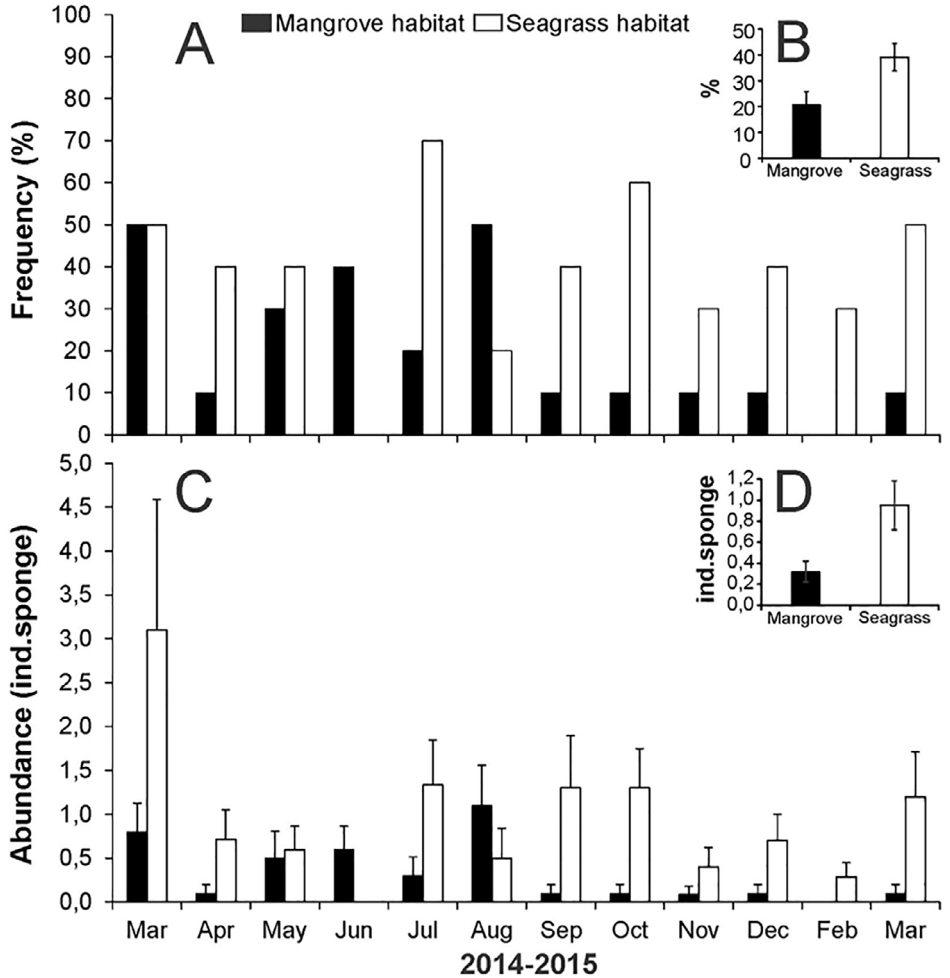

Figure 2. a) Intra-annual variability of the frequency of *D. kyolis* in the *H.* (*H.*) *melanadocia* samples from the seagrass meadow and mangrove prop roots and b) overall average frequency per habitat. c) Intra-annual variability of the average density (± standard error) of *D. kyolis* in the *H.* (*H.*) *melanadocia* samples from the seagrass meadow and mangrove prop roots and d) overall average density per habitat.

sponge (between branch bases) may suggests an active site selection on the part of this nudibranch as has been documented in other sponge-feeding opisthobranchs (Becerro et al., 2003). These characteristics could help *D. kyolis* to hide from possible predators.

Regarding its density on *H.* (*H.*) *melanadocia*, it seems to be moderate in comparison with that reported in other sponge-eating opisthobranchs. For example, in *Glossodoris pallida* (Rüppell and Leuckart, 1830), which feeds on the sponge *Hyrtios erecta* (Keller, 1889) from Guam, a mean density of 1–3.4 individuals/sponge was reported (Rogers and Paul, 1991). Also, in the case of *Hoplodoris nodulosa* (Angas, 1864) (from Pelorus Sound, New Zealand) densities of up to 2,983 individuals per 100g wet weight of the sponge *Mycale hentscheli* (Bergquist and Fromont, 1988) were reported (Page et al., 2011). Although the density of *D. kyolis* does not seem to be so high and that does not cause significant lesions in the sponge, it is known that these small predators (in high densities) may play a role in regulating the populations of their sponge prey (Dayton et al., 1974), especially in species under culture conditions (Page et al., 2011), even influencing its small-scale spatial distribution (Knowlton and Highsmith, 2000).

Notwithstanding that *H.* (*H.*) *melanadocia* inhabits in both seagrass meadows and mangrove root environments in the study area, the frequency of occurrence and density of *D. kyolis* in this sponge varied significantly between these two habitats. In this

regard, some studies in nudibranchs have suggested that its spatial distribution can be a result of both habitat preference (in relation to a food source) and aggregation behaviors (in order to facilitate the reproductive process) (Darumas et al., 2007; Hubner, 2011). However, given the closeness of these habitats in the study area (less than 20 m in some sites) and that the dietary species is present in both, it is also possible that the small-scale spatial distribution of this nudibranch is linked to other factors such as predation, i.e. the structural complexity of the seagrass meadow could offer greater protection against potential *D. kyolis* predators than the mangrove root network. This small-scale variation has also been recorded in other members of the macrofauna associated with *H.* (*H.*) *melanadocia* in the same study area (Ávila and Ortega-Bastida, 2015). That study documented the spatial variability (between habitats) in the composition and overall density of associated macroinvertebrates with this sponge and it was mentioned that it may be due to a set of factors such as variations in the sponge morphology (related with its phenotypic plasticity), environmental conditions (e.g. sedimentation rate and light intensity), substrate orientation and the fauna inhabiting the surrounding area (Ávila and Ortega-Bastida, 2015).

Moreover, the frequency and density of *D. kyolis* did not vary significantly throughout the year and were not related with the sponge volume, temperature and salinity. These findings agree with those of previous studies where the temporal variability of nudibranchs has been associated with the presence of the food source rather than with variations in environmental conditions such as water temperature (Aerts, 1994). This relative stability in the population of *D. kyolis* appears to be explained by the abundant and perennial food source that *H.* (*H.*) *melanadocia* represents in the study area (Ávila et al., 2015). Another study also documented that temporal variations in the abundance and species diversity of intertidal nudibranchs were correlated with wave action, i.e. lower values being recorded during times of storms (Nybakken, 1978). Although the region where this study was conducted has a well-defined storm season (June-October), there was no significant change in frequency and density of this nudibranch during that period.

There was also some evidence that *D. kyolis* could have specificity for *H.* (*H.*) *melanadocia* in the study area, since in addition to finding only remains of this sponge in its feces, it was not observed preying on other sponge species, as has been documented in other dorid species (Penney, 2013). However, this result contrasts with that of Belmonte et al. (2015) who reported to *D. kyolis* as a polyphagous species in a region outside the distribution range of *H.* (*H.*) *melanadocia* (Rio de Janeiro, Brazil). There, *D. kyolis* preys on sponges of different taxonomic groups such as *Dysidea etheria*, *Haliclona* sp., *Lissodendoryx* (*Lissodendoryx*) *isodictyalis* (Carter, 1882) and *Plakina* sp. Schulze, 1880 (Belmonte et al., 2015). In this sense, it has been documented that some dorid nudibranch species can have a plastic polyphagous diet (e.g. *Platydoris argo*), i.e., they can feed on other sponges when the main prey species is unavailable or even the preferred prey can change from one habitat to another (Megina et al., 2001). Thus, to determine whether *D. kyolis* is a stenophagous or polyphagous feeder further studies are required.

In summary, this study contributes to knowledge of the relationship between the nudibranch *D. kyolis* and the sponge *H.* (*H.*) *melanadocia*, mainly on basic aspects of its population dynamics such as the frequency of occurrence and density of the nudibranch on the sponge and its spatio-temporal variability. However, it would be advisable for future studies to determine whether this predator has the ability to choose between different sponge preys as food source and whether it acquires secondary metabolites from the sponge for its own defense.

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AUTHOR CONTRIBUTIONS

E.A.: Conceptualization; Writing – original draft; Writing – review & editing; Funding acquisition.

Y.C.M.: Investigation; Writing – review & editing.

M.A.R.S.: Methodology; Writing – review & editing.

M.B.: Supervision; Validation; Writing – review & editing.

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