



Photosynthetic animals and where to find them: abundance and size of a solar-powered sea slug in different light conditions

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Abstract

Three populations of the solar-powered sea slug *Elysia crispata* on reefs from the southern Gulf of Mexico and the Caribbean were analyzed. The aim was to describe and compare the changes in abundance and size of this species in different localities, as a function of depth and time-of-day. We hypothesized that differences in abundance would be related to locality, time of the day and depth, and differences in size would be related to locality and time of the day. Using snorkeling and SCUBA diving, all individuals within quadrats were counted and measured. A total of 680 organisms were recorded at Verde, Arcas and Puerto Morelos (PM) reefs at five times of the day (sunrise, morning, zenith, evening, and night) and depths of 0–13 m. Zero inflated negative binomial (ZINB) regressions adjusted to abundance data showed that *E. crispata* in Arcas and Verde reefs is expected to be more abundant (> 50) in shallow depths (< 2 m) at any time of the day except sunrise, whereas a low abundance (≤ 1 organism) is predicted in PM regardless of depth and time-of-day. According to linear models, size was not related to depth, but was related to locality and time-of-day, with sea slugs from Arcas and Verde having similar size, and both larger than those in PM. This information suggests that this sea slug is capable of moving within the reefs and helps to understand the unique biological phenomena of kleptoplasty.

Keywords Kleptoplasty · Photosymbiosis · *Elysia crispata* · Mollusca · Gulf of Mexico · Caribbean · Coral reefs

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Introduction

Light conditions in the ocean depend on the amount of radiation reaching different areas, due to variations in the sun's angle throughout the day, as well as depth, turbidity and other parameters (Kirk 2011). Light is a resource for autotroph organisms because it represents the source of energy to maintain metabolic functions, but it also represents a limiting condition. In mobile animals with photosynthetic capacity, it can determine physiological and behavioral aspects, such as activity peaks (circadian rhythms) or be the cue to initiate certain displays, such as food searching or predator avoidance (Begon et al. 2006). Light changes, thus, define the occurrence and distribution of all photosynthetic organisms (Lalli and Parsons 1997), and animals with photosymbioses are no exception (Melo Clavijo et al. 2018).

Sacoglossan sea slugs (Mollusca: Gastropoda) are the only metazoan group that can retain and keep functional for months the chloroplasts from the algae they feed on (Händler et al. 2009). As these mollusks can benefit from the maintenance of the foreign organelles through the obtention of energetic products from their photosynthesis (Laetz

et al. 2017; Cruz et al. 2020; Cartaxana et al. 2021), they have been named “solar-powered” sea slugs (Rumpho et al. 2000). The retention time of this process (also known as kleptoplasty) depends on the slug species, the algal source of the chloroplasts (kleptoplasts) and the light conditions (Cruz et al. 2013; de Vries et al. 2014). Light intensities mediate the photosynthesis within the animal cells, thereby affecting its fitness as well as kleptoplast survival (Vieira et al. 2009; Cruz et al. 2015). For instance, moderate irradiance in the habitat of *Plakobranthus ianthobaptus* can increase its fitness through kleptoplast survival (Donohoo et al. 2020), while high light intensities can decrease photosynthetic performance in *Elysia timida* and *Elysia viridis* (Vieira et al. 2009; Cruz et al. 2015; Cartaxana et al. 2018, 2019).

Elysia crispata is the most conspicuous solar-powered sea slug in the Gulf of Mexico and the Caribbean. Because of its large size (can reach 150 mm in length), it can be easier to find than other slugs. It is common on a broad variety of substrata all year long (e.g., dead coral, sea grass, algae and under rocks), inhabiting borrow pits and mangrove lagoons, as well as coral reefs throughout the Caribbean at up to 25 m depth (Sanvicente-Añorve et al. 2012; Camacho-García et al. 2014; Krug et al. 2016). Clark (1994) suggested the existence of two subspecies of *E. crispata*, one inhabiting mangrove areas and another living in coral reefs, and Pierce et al. (2006) later proposed a new species named *Elysia clarki* for the mangrove slugs. Krug et al. (2016) recently analyzed the populations and confirmed that *E. crispata* was a single species. Nonetheless, the latter study recognized two ecotypes coinciding with the proposed separation by Clark (1994): *clarki* animals of a more consistently green coloration, that inhabit shallow, low energy waters, with less light incidence; and *crispata* animals of more variable body coloration that are present in deeper, high energy waters with more light (Pierce et al. 2006; Krug et al. 2016). This species can keep functional kleptoplasts for up to 4 months, hence it is considered a long-term retention species (Curtis et al. 2010). These characteristics make *E. crispata* an ideal model to study the ecology of photosynthetic animals.

Field studies regarding sacoglossan sea slugs are difficult to perform, as they are small sized animals, cryptic in the substrate (host algae) they are usually associated to and a high effort is required to obtain data, challenging the studies of their populations (Clark 1994; Jensen 1994). Despite these adversities, some species have been studied in situ. For instance, Baumgartner and Toth (2014) found that size and abundance of *E. viridis* vary among seasons and depend on the host algae: larger but fewer individuals were found on *Cladophora rupestris* in autumn, whereas smaller ones were more abundant on *Cladophora sericea* in summer. These authors hypothesized that predation might explain the differences in abundance and size of *E. viridis* between algal hosts and time of year.

Research on *E. crispata* has focused on describing biochemical and biomolecular components (Gavagnin et al. 1996, 1997; Middlebrooks et al. 2012; Vital et al. 2021), photosynthetic activity (Curtis et al. 2006; Christa et al. 2015), physiology related to kleptoplasty (Curtis et al. 2006, 2007, 2010; Middlebrooks et al. 2019) and even microbiota (Mahadevan and Middlebrooks 2020). In general, this species has been found to have a long-term retention of chloroplasts from different algae, with a good physiological condition after months of starvation. Population studies of *E. crispata* have only been conducted in Florida in mangrove swamps and pits, and most of them focused on the *clarki* ecotype (Clark 1994; Middlebrooks et al. 2014, 2020). Equivalent studies of this species in coral reefs in their western distribution are still lacking and would provide relevant information of this interesting biological model.

Light conditions influence the transfer of carbon and nitrogen from kleptoplasts to sea slugs (Cruz et al. 2020; Cartaxana et al. 2021). While ecological research efforts have mainly focused on the relation between light and *Elysia*'s physiology and behavior (Schmitt and Wägele 2011; Miyamoto et al. 2015; Cartaxana et al. 2018), studies on its natural distribution are scarce. There is evidence that kleptoplast photosynthesis minimizes weight loss and size reduction, and increases survival in *E. viridis*, *E. timida* and *E. chlorotica* under starving conditions (Giménez-Casaldueiro and Muniain 2008; Pelletreau et al. 2014; Cartaxana et al. 2017). In addition, the growth rate of *E. viridis* relative to the rate in which it consumes algae (i.e., efficient growth) has been correlated with exposure to regular light and increased photosynthesis (Baumgartner et al. 2015). Thus, it is reasonable to expect that light conditions influence the abundance and size of *E. crispata* by limiting its access to photosynthetic resources.

Solar-powered sea slugs will thrive in habitats where optimum light conditions are met. Light conditions vary both between localities and with depth due to the vertical attenuation of light caused by absorption, scattering and diffraction (Kirk 2011), and other factors related to it (e.g., turbidity). In addition, individuals at any site will experience variations in the quality and quantity of light throughout a 24-h period. Circadian rhythms in sea slugs have been documented to be present in swimming and crawling behaviors (*Melibe leonina*, Newcomb et al. 2014) and the opening and closing of the parapodia (*E. timida*, Monselise and Rahat 1980). However, the need of light by photosynthetic sacoglossans makes them vulnerable to photodamage and predation, especially at higher light intensities, where the probability of location by visual predators is increased (Weaver and Clark 1981). If sea slugs can move within a small spatial scale between places with varying light quality (e.g. from the top of a dead coral to a crevice nearby), then individuals of different size would occur in different numbers throughout

the day. Information on changes in size and abundance of *E. crispata* throughout a 24-h period will help understand the patterns and time scales of sea slug activity and mobility within the reef. Therefore, the aim of the present study was to describe and compare changes in size and abundance of three populations of *E. crispata* in Southern Gulf of Mexico and the Mexican Caribbean, as a function of time of the day and depth. We hypothesized that differences in abundance would be related to locality, time of the day and depth, and differences in size would be related to locality and time of the day.

Materials and methods

Study area

Field studies in three coral reefs of the Gulf of Mexico and Caribbean were conducted: Verde ($19^{\circ}12'09''$ N, $96^{\circ}03'58''$ W) in August 2018 and June 2019, Cayo Arcas ($20^{\circ}13'12''$ N, $91^{\circ}58'22''$ W) in April 2018 and Puerto Morelos ($20^{\circ}50'59''$ N, $86^{\circ}52'23''$ W; Fig. 1) in

March and July–August 2019. Verde, located 5 km off the coast, is part of the reef system “Sistema Arrecifal Veracruzano-SAV” in Southern Gulf of Mexico, and has a marked seasonal variation in salinity, temperature, and turbidity. These variations are due to the influence of three major rivers flowing into the Gulf of Mexico and winter winds locally known as “nortes” (Salas-Perez and Granados-Barba 2008; Mateos-Jasso et al. 2012). Arcas is a remote reef located 128 km off the coast of Campeche in the Yucatan and Campeche Bank-YCB (Tunnell 2010). Puerto Morelos (PM) is part of the Mesoamerican reef in the Mexican Caribbean-CAR and is located at an approximate distance ranging from 500 m to less than 3 km parallel to the coast. The continental platform in the Yucatan Peninsula is karstic with little or no transport of suspended sediments to the sea; thus, the coastal influence on the reef is minimal and waters are usually clear (SEMARNAP 2000). These coral reefs were selected because variations in the abundance and distribution of *E. crispata* between them are probably related to the diverse biological, geological, physical and chemical processes occurring within each region (SAV, YCB, CAR) (Carrillo et al. 2007).

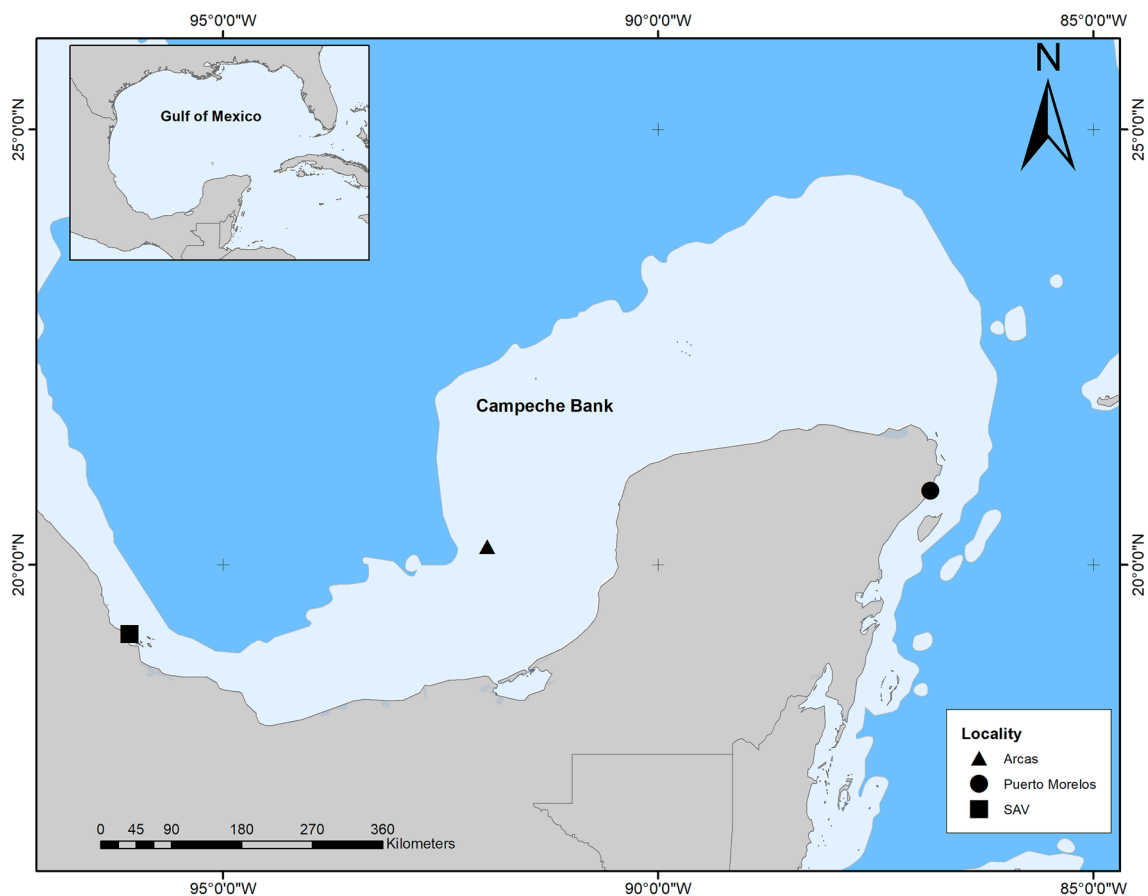


Fig. 1 Localities where populations of *Elysia crispata* were studied in Southern Gulf of Mexico and Caribbean: Arcas (YCB), Verde (SAV) and Puerto Morelos (CAR). Map created by authors in ArcGIS

Sampling design and fieldwork

Quadrats (1 and 25 m²) were haphazardly sampled in different sites of the three localities at five different times of day: sunrise (57), morning (48), zenith (78), evening (86) and night (55). Sampled quadrats were located at either side of different transects (20–40 m) which were used as guides to facilitate the identification of the area that had been searched and assure it was not sampled repeatedly. Searching times ranged between 0620 and 0745 h (sunrise), 0930 and 1110 h (morning), 1200 and 1340 h (zenith), 1700 and 1930 h (evening) and 2040 and 2300 h (night). By means of snorkeling and SCUBA diving, all individuals of *E. crispata* in each quadrat were counted and measured (head–tail length) with a Vernier caliper (± 0.1 mm). Sea slugs on all types of substrata occurring within each quadrat were counted and measured but were never collected. As sacoglossan sea slugs are cryptic, we reduced searching bias by only using experienced divers in sampling trips and dive lights were used during night searches. Depth and light intensity were measured with a dive computer (Hollis DG03) and a light logger (HOBO® Pendant MX2202, Onset Computer Pocasset, MA, USA, with a precision of ± 1 –40 lx), respectively. Measurements were recorded every time that slugs were found and averaged per sampling unit. Light units were converted to $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ following Thimijan and Heins (1983) to facilitate comparison with other studies.

Statistical analyses

To model the number of sea slugs as a function of reef (Verde, Arcas and PM), time-of-day (sunrise, morning, zenith, evening, night) and depth (from 0 to 13 m), a zero-inflated negative binomial (ZINB) generalized linear model with a log link function was used. The log link function ensures positive fitted values, while the negative binomial distribution is typically used for count data with many zero observations and strong overdispersion (Zuur et al. 2009). The ZINB model has two components to estimate the expected values of abundance as a function of a set of explanatory variables: a logit (zero inflated) component to assess the probability of finding false zeros and a negative binomial (count) component to predict the number of individuals.

Absence of organisms (zeros in counts) could be the result of (1) sampling in habitats that are unsuitable for the species, (2) poor experimental design or sampling practices, (3) the lack of experience or otherwise ability to identify specimens by the observer, (4) sampling in habitats that are suitable, but contain unexploited sites or (5) sampling outside the distribution range of the species, among others (Zuur et al. 2009). Thus, true zeros refer to the real absence of organisms in that site, while false zeros refer to

the inability to record organisms where they are most likely to be found. When the probability of finding a false zero is low, then, a recorded zero will truly reflect the absence of an organism (i.e., it is likely to be a true zero). By contrast, when the probability of finding a false zero is high, then, the study might not have been able to record true zeros (for instance, individuals could have been at the sampling site, but passed unnoticed by the observer). In ZINB models, if you find organisms in any of the conditions studied, their abundance will be predicted by the NB component of the model, hence the expected abundance would be consistent with having recorded them.

The terms in the ZINB model were *reef* (categorical factor with three levels), *time-of-day* (categorical factor with five levels), and *depth* (continuous). The interaction term was *depth* \times *time-of-day* in the logit component:

$$EC_i \sim \text{ZINB}_{(\mu_i, k, \pi_i)}$$

$$E(EC_i) = \mu_i \times (1 - \pi_i) \text{ and } \text{var}(EC_i) \\ = (1 - \pi_i) \times \mu_i \times \left(1 + \pi_i \times \mu_i + \frac{\mu_i^2}{k} \right)$$

$$\log(\mu_i) = \alpha_1 + \text{Reef} + \text{Depth}$$

$$\text{logit}(\mu_i) = \alpha_2 + \text{Time of Day} + \text{Depth} + \text{Time of Day} \times \text{Depth}.$$

EC_i represents the abundance (counts) of *E. crispata* in observation i , which follow a negative binomial distribution with mean μ_i , k as the dispersion parameter and π_i as the probability that observation EC_i is a false zero. We simplified the notation for the predictor function by omitting the regression parameters that are usually provided before the variable names (Zuur et al. 2009).

The procedure to select the optimal ZINB model consisted of dropping terms one by one in a systematic order and deciding whether they were likely to pertain to the model. To assist each decision the Akaike Information Criterion (AIC) and likelihood ratio tests were used (Zuur et al. 2009). Since all three reefs had suitable habitats for this species, we considered that origin of false zeros were most likely explained by differences in *time-of-day* (*TOD*) or *depth*, but not by differences between localities. Thus, the initial full model in the count component included *reef*, *TOD*, *depth*, and the interactions of *depth* with *reef* and *time*, whereas *TOD*, *depth* and their interaction were included only in the logit component.

In addition, a linear model was adjusted to the size data using *depth*, *reef*, *TOD* and the interaction of *reef* and *TOD* as explanatory variables. A model selection sequence based on F tests similar to the one described

previously, was used to find the best combination of variables explaining changes in sea slug size. This procedure showed that the model including *reef* and *TOD* and their interaction was the optimal model. Tukey's tests for unequal *N* samples were then performed to assess significant differences in size between *reef* and *TOD* once these terms resulted statistically significant (Zar 2009). As a low number of organisms were found in PM reef ($N = 7$), size data from this reef were only described and not considered in the hypothesis testing procedures.

Models were validated by visual analysis of the residuals using plots of Pearson residuals versus fitted values and each explanatory variable (Zuur et al. 2010). The best ZINB model derived from these procedures included *reef* and *depth* for the count data, while the best model for the binomial data included *TOD*, *depth*, and the interaction between them. All statistical analyses and graphs were performed in R v. 3.5.3 (R Core Team 2019), using *stats* v3.5.3 (R Core Team 2019), *pscl* v.1.5.1 (Jackman et al. 2017), *lmtest* v. 0.9–37 (Hothorn et al. 2019) and *ggplot2* v3.3.5 (Wickham et al. 2021) packages. Differences were considered significant at $p < 0.05$ (Zar 2009).

Results

A total of 680 organisms of *E. crispata* was recorded in Verde, Arcas and PM reefs at five moments of the day (sunrise, morning, zenith, evening, night) and depths ranging from 0 to 13 m. Sampling covered a similar area of ~ 1.5 km in PM and Verde, but was reduced to 0.44 km² in Arcas. Despite differences in sampling effort, Arcas and Verde had a more similar sea slug density compared to PM, where only seven individuals of *E. crispata* were recorded (Table 1).

Arcas and Verde slugs resembled the description of the *crispata* ecotype in possessing a completely white foot, whereas sea slugs from PM had a darker green coloration and presented green diverticula in the foot, similar to the *clarki* ecotype (Fig. 2). Sea slugs were usually found exposed on top of hard substrates, such as coral fragments and coral pavement, yet some individuals were spotted on algae of the genus *Halimeda* and *Padina*. In general, few macroalgae species consistent with their potential food sources were observed near the sea slugs. An unidentified filamentous green alga was frequently found on the rocky substrates where sea slugs were common.

Measured at zenith (1200–1340 h), the overall mean light intensity was the highest at Arcas ($309.78 \pm 282.12 \mu\text{mol}$

Table 1 Number of sites sampled, depth range and total sampled area at each locality

Locality	Number of sites	Depth (m)	Sampled area (m ²)	Number of individuals	Density (individuals m ⁻²)
Arcas	11	0.5–11	440	152	0.35
Puerto Morelos	9	1.5–10.5	1450	7	0.005
Verde	18	1–13	1546	521	0.63

The number and average density of *E. crispata* individuals recorded at each locality is also included

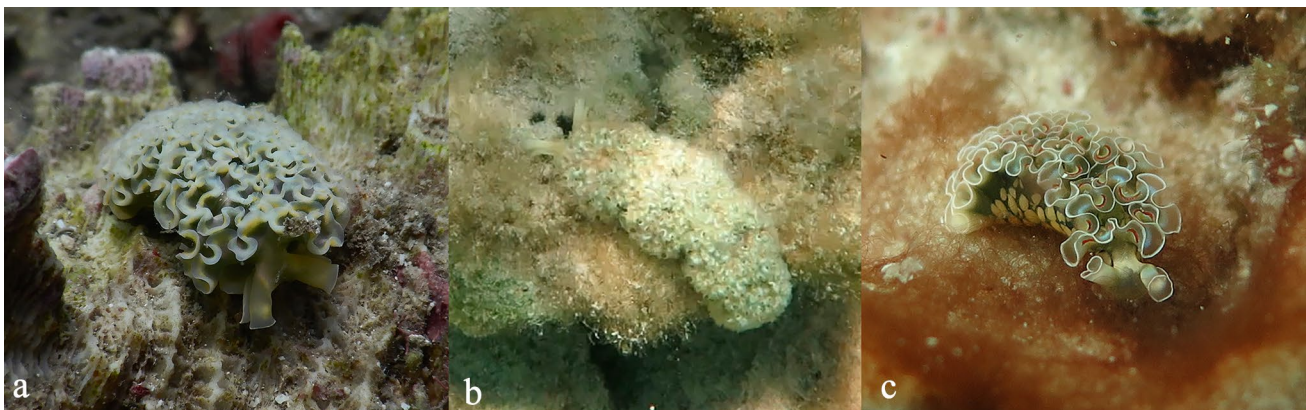


Fig. 2 *Elysia crispata* found in Verde (a), Arcas (b) and Puerto Morelos (c). Sea slugs from Verde and Arcas resembled the description of the *crispata* ecotype, whereas slugs from PM had some charac-

teristics of the *clarki* ecotype (darker green coloration and presented green diverticula in the foot)

photons $\text{m}^{-2} \text{s}^{-1}$), followed by PM ($289.68 \pm 195.22 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), and Verde with the lowest mean light intensity ($148.52 \pm 187.30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Light intensity also varied with depth (Fig. 3). In Arcas and Verde, light intensity decreased as depth increased, with mean values of $258.76 \pm 188.4 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at $< 5 \text{ m}$ and $131.73 \pm 224 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at $> 5 \text{ m}$ deep. In PM, however, abnormally high values of light intensity were recorded at 10.5 m deep (Fig. 3).

Abundance

The highest numbers of slugs were found in Verde (521), followed by Arcas (152), and the lowest number of individuals was found in PM (7). When the times of day were considered, the highest number of organisms was found during night (203), followed by zenith (184) and evening (157), while the lowest during morning (58) and sunrise

(78). Despite having a lower sampling effort in Arcas (i.e., less area sampled), the data was analyzed because it provides information of a very isolated population of sea slugs.

The zero-inflated component of the ZINB model describing the absence of sea slugs showed that the interaction between *TOD* and *depth* was statistically significant (Chi-square test, $\chi^2 = 32.898$, $P < 0.001$), indicating that the probability of finding *E. crispata* changes with depth and these changes vary depending on the time-of-day (Table 2). During sunrise, the likelihood of false zeros decreased with depth, while in the morning, zenith, evening, and night, the likelihood of finding false zeros increased with depth (Fig. 4a). Therefore, the model suggests that the true absence of sea slugs at any time-of-day, except sunrise, is likely to decrease with depth.

Once the condition of finding slugs was met, the negative binomial component of the model predicted the number of individuals that would be found. This logistic

Fig. 3 Light intensity as a function of depth measured in quadrats where *Elysia crispata* were sampled at zenith (1200–1340 h) in Arcas ($n = 10$), Verde ($n = 54$) and Puerto Morelos ($n = 14$)

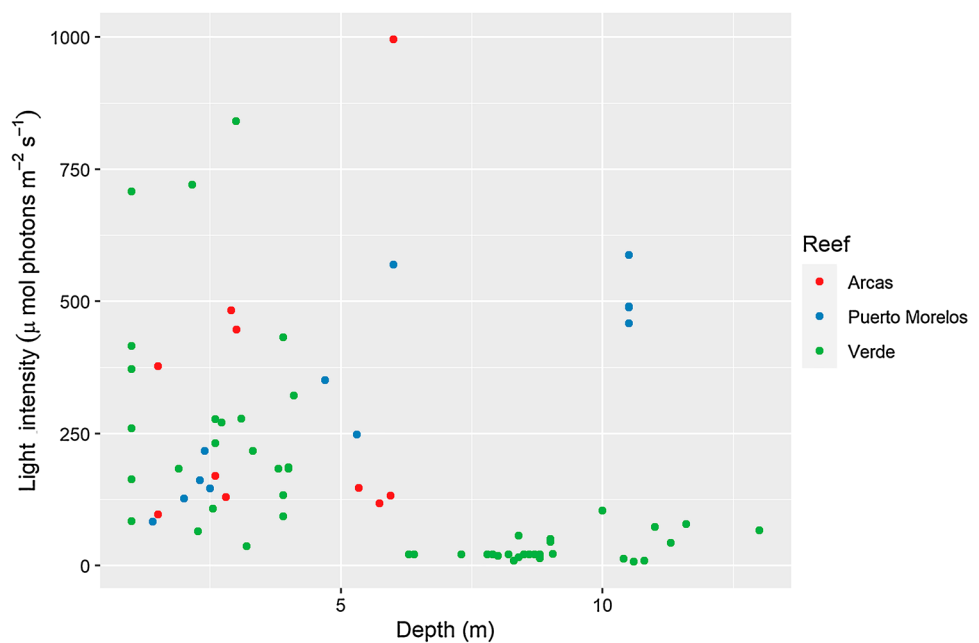


Table 2 Results of the model selection procedure applied to the NB segment

Terms in the model	<i>df</i>	AIC	Term dropped	χ^2	<i>p</i>
R + TOD + D + R:D + TOD:D	25	967.70	None		
R + TOD + D + R:D	21	964.55	TOD:D	4.85	0.303
R + TOD + D + TOD:D	23	989.13	R:D	25.08	<0.001
R + D + R:D	17	962.83	TOD	11.01	0.201
R + D	15	980.66	R:D	32.89	<0.001

The terms in the model are the combination of explanatory variables included in each model tested, together with the corresponding degrees of freedom (*df*) and Akaike Information Criteria (AIC). At each step, a model containing all but the term dropped was taken as the full model and the term's contribution assessed by means of a likelihood test using χ^2 ; *p* is the probability of the observed χ^2 under the null hypothesis of no contribution

D depth, *R* reef, *TOD* time-of-day

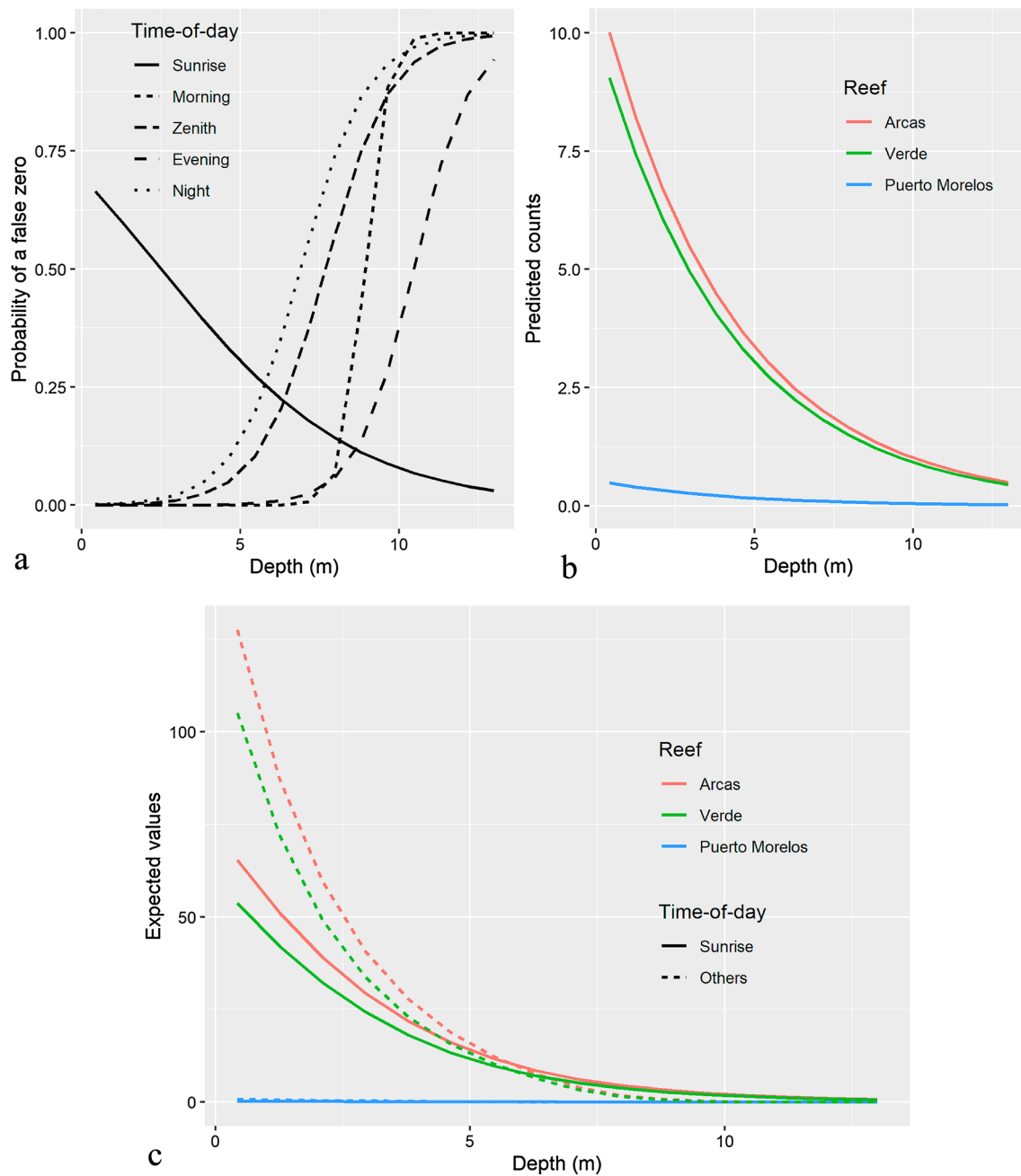


Fig. 4 Zero inflated negative binomial (ZINB) regressions adjusted to abundance data of *Elysia crispata* in the three localities studied (reefs: Arcas, Verde and Puerto Morelos) at five times of the day (sunrise, morning, zenith, evening, and night). a) Probability of finding false zeros of *E. crispata* in different times of day and depths;

b) predicted counts of *E. crispata* in the three localities and depths studied; c) expected values of *E. crispata* considering the explanatory variables included in both components of the ZINB model; “others” represent all times of day, except sunrise

component predicts a decrease in the number of slugs with increasing depth in all three reefs. Nevertheless, the overall predicted counts in Arcas and Verde are much higher at lower depths compared to PM (Fig. 4b). In summary, the ZINB model showed that *E. crispata* in Arcas and Verde is expected to be more abundant (> 50 individuals) in shallow depths (< 2 m) at any time of the day, except

sunrise, when true zeros are most likely. By contrast, low abundance (≤ 1 organism) is estimated in PM at all depths considered (Fig. 4c). The model, for example, implies that if there was an unfruitful search for this species in waters deeper than 6 m during the night in Verde and Arcas, sea slugs were probably there, but we failed to find them. In

the event of finding them, the abundance of slugs would nonetheless have very low numbers (<2 individuals).

Size

The smallest recorded individual (9 mm) was found in Arcas during zenith, whereas the three largest (70 mm) were found in Verde in the evening. Only small organisms were observed in shallower waters, and no sea slugs smaller than 20 mm were found deeper than 7 m. Despite these contrasting numbers, the observed changes in size with depth did not show statistical significance ($F(1,663) = 0.365$, $P = 0.545$; Online Resource 1 and 2).

The size of *E. crispata*, however, was statistically related to reef and TOD, as showed by the significant interaction term ($F(4,666) = 8.66$, $P < 0.001$; Online Resource 2). Pair-wise comparisons indicated that sea slugs sampled during the morning, zenith and evening in Verde were larger than those sampled at the same TOD in Arcas ($P < 0.05$). Nonetheless, sea slugs sampled at sunrise and night were similar in size ($P > 0.05$; Fig. 5). Sea slugs from PM ranged from 10 to 25 mm in length and their mean size was 18.42 ± 4.64 mm (\pm SD).

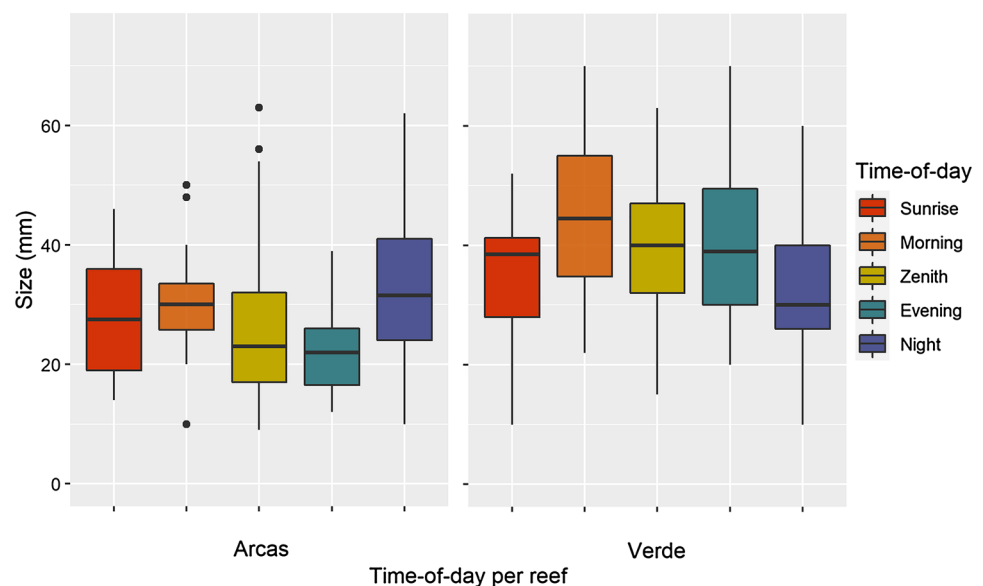
Discussion

Decades of research in the laboratory have addressed the role of kleptoplasts in the fitness and survival of solar-powered sea slugs (see review by Wägele and Martin 2014). Still, the aspects of their abundance and distribution in their natural environments are unknown for most of the species. Even though *E. crispata* is a widely distributed and common species in the Caribbean, there are few records in the three

localities studied herein (Gavagnin et al. 1997; Zamora-Silva and Ortigosa 2012; Ortigosa and Simões 2019). In preliminary field studies in the remote coral reef islands of Alacranes and the submerged shallow reef (10 m) at Bajos del Norte, both located in the Southern Gulf of Mexico, we found 95 and 26 organisms of *E. crispata*, respectively. However, in coastal reefs 23 km off the coast of Sisal, Yucatan (Madagascar and Bajo de 10), no organisms of this species were found, confirming previous reports (Ortigosa et al. 2013) as well as potential distribution predictions (Jiménez et al. 2021). It appears that not only the abundance of *E. crispata* is markedly variable, but its distribution is patchy with high numbers in some localities (e.g., Verde and Arcas) compared to very low occurrence in others (e.g., PM). Such heterogeneity poses a challenge in terms of describing and predicting the occurrence of *E. crispata* in the natural environment. Together with the sea slug's cryptic nature, these irregular patterns allow for debate on the causes that explain its absence in places where they could be plausibly expected. To attend this difficulty, the model used in our study (ZINB) enables to assess the probability of the true absence of sea slugs at different combinations of time-of-day and depth (in contrast with a false absence associated to a reduced ability of detection). Our results suggest that an effective detection of *E. crispata* depends on the light conditions found at certain depths and times of day.

Jiménez et al. (2021) found that distance to the coast was the best predictor with the highest contribution in the niche modeling of *Elysia* (including *E. crispata*) from the Caribbean. The authors associated this result to the fact that areas closer to the coast usually have more light availability because they are shallower, warmer and more productive. The characteristics of shallow waters can be found in reefs far from the coast, where large numbers of these organisms

Fig. 5 Size of *Elysia crispata* at different times of day, sunrise, morning, zenith, evening and night in two localities, Arcas and Verde reefs. The line represents the median, top and bottom of the box are the 25th and 75th percentiles, the whiskers represent the maximum and minimum values, and dots are outliers



can be expected. Despite Arcas is noticeably more distant from the coast than Verde and PM, it has three islands that fulfill the description of the niche modeled for *Elysia* (Jiménez et al. 2021): high light availability due to shallow, warm, and productive waters. Accordingly, less favorable conditions for sea slugs should include turbidity, and excess of sediment. These conditions are present in Verde (at least in certain seasons; Mateos-Jasso et al. 2012; Avendaño et al. 2019), yet this reef had a high abundance of sea slugs. In addition, mangrove lagoons and borrow pits are characteristic of another recognized habitat of the *clarki* ecotype in Florida (Middlebrooks et al. 2014). Taken as a whole, these results suggest that this species can inhabit a wide range of environmental conditions.

Studies in laboratory have shown that sacoglossan sea slugs regulate the potentially harmful excess of light through behavioral and physiological mechanisms (Jesus et al. 2010; Cartaxana et al. 2018). The differences in abundance of *E. crispata* found between Arcas, Verde and PM might be related to different light conditions (Fig. 3), particularly if turbidity is acting as a light regulator through the absorption by suspended particles (Carruthers et al. 2001). Verde is reported to have a high turbidity as the result of substantial discharge of Jamapa, La Antigua and Papaloapan rivers (Avendaño et al. 2019; Liaño-Carrera et al. 2019), while Arcas and Puerto Morelos usually present clear waters (SEMARNAP 2000; Chávez et al. 2007). Despite the contrasting conditions of Arcas and Verde, *E. crispata* were found in similar abundances, signaling that other factors such as food accessibility, larval availability or conditions at a microhabitat scale might be determinant in the distribution of this species.

While sampling in Arcas, Verde and PM took place from March to August, it is unlikely that seasonal variability in environmental factors are responsible for the differences in sea slug abundance found between the reefs. Differences in temperature and photoperiod, which have been signaled as important factors regulating sea slug temperate populations (Clark 1975; Mondy and Pierce 2005), are less extreme in tropical than in temperate waters, and *E. crispata* occurs almost all year around in coral reefs of the Southern Gulf of Mexico and Mexican Caribbean (Gavagnin et al. 1997; Sanvicente-Añorve et al. 2012; Zamora-Silva and Ortigosa 2012; Ortigosa and Simões 2019). Moreover, the most contrasting abundances found in this study were between Puerto Morelos and both, Arcas and Verde, yet PM and Verde were both sampled in the same months. During this study, massive sargassum arrivals affected the area near PM. Sargassum so vastly accumulated can decrease light illuminance almost 75% (van Tussenbroek et al. 2017; Hendy et al. 2021) and such events can also increase temperature and decrease pH and oxygen concentrations (van Tussenbroek et al. 2017;

Hendy et al. 2021). These factors are known to affect both the normal development and kleptoplasty of *E. crispata*, through bleaching and causing body deformities (Dionísio et al. 2017, 2018). As there is no previous data on the occurrence of *E. crispata* in PM, we can only suggest that sargassum arrivals could have been a cause for the low density of individuals found in this study.

Microhabitat availability is distinct between localities (Withers and Tunnell 2007), and could be providing different types of refuge for the slugs to take cover at different times of the day. As previously reported (Weaver and Clark 1981; Middlebrooks et al. 2014; Krug et al. 2016), most individuals in the present study were found on top of hard substrates without algae. Weaver and Clark (1981) suggest that this could be a tactic to increase photosynthesis; however, in Bahamas it is common to find *E. crispata* on the underside of rocks in < 2 m depth (Redfern 2013). It is possible that the occurrence of sea slugs on substrate depends on microhabitat conditions at a smaller spatial scale, which in turn, vary with depth (Chávez et al. 2007). In Verde, for example, crevices are common at most of the depths, but they are less frequent both in shallow waters and close to the reef lagoon. By contrast, the reef lagoon has larger rocks and sea grass patches that provide shade in these otherwise luminous shallow waters. The great variety of microhabitats found in these coral reefs can be expected to serve as shelter for slugs to avoid highly irradiated waters, a condition that could be physiologically limiting (Vieira et al. 2009).

The low abundance of *E. crispata* with increasing depth could be related to the reduction of resources at deeper waters. Macroalgae abundance and diversity can change with increasing depth in the reef system (SAV) where Verde is located (Horta-Puga et al. 2020). Adults from this species apparently consume a wide variety of macroalgae (Vital et al. 2021) but we rarely observed sea slugs associated to any macroalgae in the present study. Middlebrooks et al. (2014) also found low occurrence of *E. crispata* associated to their algal food in a completely different habitat in Florida. Adults might be temporarily staying at lower depths to lay eggs in algae and, moving back to deeper waters, as it has been suggested in other sea slugs (Willan 1979). While juvenile *E. crispata* have a narrower food range compared to adults (Curtis et al. 2007), the induction of metamorphosis in the larvae does not depend on the presence of a particular food source (Krug 2009). It is possible that juveniles were consuming filamentous green algae other than *Bryopsis plumosa* or *Derbesia tenuissima* (Curtis et al. 2007), such as the one we observed near the rocky substrates where organisms were frequently found. Overall, these results suggest that the presence of food sources is unlikely to be the main factor determining the occurrence of this species within its geographical distribution, but further research should consider the feeding ecology of larval and juvenile stages.

A weak association of *E. crispata* to its food sources would allow sea slugs to explore deeper waters under conditions of low abundance or even absence of food. Within aquatic ecosystems irradiance decreases and the spectral composition of light changes with depth, thereby influencing photosynthetic activity (Hill 1996). Long (red) wavelengths are absorbed at the first few meters and short (blue) wavelengths are the last to be absorbed as the depth increases (Kirk 2011). Moving deeper could be an advantageous strategy for sea slugs exploring lower light intensities, although limits to such advantages are surely imposed by the pigments in their chloroplasts and how these respond to the varying wavelengths. *Elysia crispata* has a wide variety of pigments in its chloroplasts and its composition and concentration do not seem to be related to depth (Vital et al. 2021), which might represent an advantage to them. The relationship between pigment concentration and light preferences by sea slugs, hence, constitutes an interesting area of research yet to be investigated.

In the present study we confirmed the significant effect of TOD as a relevant factor predicting both sacoglossan occurrence and size, and suggest that they have circadian rhythms as do other sea slug groups (Newcomb et al. 2014). A daily pattern with a lower number of organisms of *E. crispata* at sunrise was statistically identified in Arcas and Verde, suggesting that sea slugs are capable of moving within the reef. Light wavelengths vary with the angle of the sun as it changes throughout the day. If sea slugs respond to daily variations in light by moving, its quality and quantity could be determining sea slug presence in these reefs. Research on circadian rhythms in sea slugs is very scarce, but studies on *E. timida* in the laboratory and their habitat suggested the presence of a biological clock that partially controls its parapodial behavior (Rahat and Monselise 1979; Monselise and Rahat 1980). During daylight, *Plakobranthus ocellatus* seems to take cover on the underside of rocks or by burrowing in the sand (Tanamura and Hirose 2016); likewise, *E. crispata* might move to crevices in the reef or under rocks. While only experienced observers participated in the present study, sampling bias cannot be completely overruled. The use of lights during night dives might have increased the detection of organisms by focusing the observer's attention to a better illuminated, yet reduced field. It is unsure to what extent does *E. crispata* present nocturnal activity but results herein suggest mobility during that period of the day.

One of the main features of day light is that it synchronizes physiological mechanisms of living organisms to a period that allows the recognition of 24-h cycles. Changes in ambient light constitute an external signal to initiate certain activities (Takahashi 1991). The fact that *E. crispata* of varying size were found in certain times of day and that these differed between Arcas and Verde leads to suggest that sea slugs are capable of a relatively wide range of mobility

within 0 and 13 m deep, and that this is influenced, at least partially by day light. Such light-dependent movement could be the result of one or more of the following explanations: (1) light stress avoidance, (2) circadian rhythms, and/or (3) predation avoidance.

Avoiding light stress to maintain functional chloroplasts has been supported by laboratory experiments in other species, such as *E. timida* (Jesus et al. 2010). Some of the algae used as food by slugs have photoprotection mechanisms through changes in pigments or by acclimating to depth and time of the day (Raniello et al. 2006). However, not all *E. crispata* present these pigments (Vital et al. 2021), and the macroalgae consumed most frequently by this species (e.g., *Bryopsis*, *Penicillus* and *Halimeda*) might not have them (Middlebrooks et al. 2019; Giossi et al. 2021). Other potential mechanisms for the protection of excessive light in *E. crispata* are mucus excretion, which has been speculated to be used as a sunscreen (Ireland and Scheuer 1979; Gavagnin et al. 1996; Havurinne et al. 2021), and behavioral strategies, such as closing the parapodia (Cartaxana et al. 2018, 2019) or moving towards areas in the reef that provide light protection.

Dial changes of light could also trigger movement of sea slugs within the reef. While the solar elevation changes throughout the day, there is no simple relation between this and the spectral distribution (i.e., the proportion of different wavelengths) or total irradiance (i.e., light intensity/photon flux). When the solar elevation is reduced at sunrise, the ratio of short to long wavelength light in the direct solar beam decreases. This is due to a removal of short wavelength (blue) light in the atmospheric path caused by scattering (Kirk 2011). It might be that *E. crispata* detects changes of the spectral distribution at certain moments of the day and modifies its position in the reef by moving towards more sheltered areas. For this behavior to be displayed, photoreceptors detecting different wavelengths must exist. The eyes of *E. timida* perceive light at 540 nm and the presence of extraocular receptors has also been considered (Rahat and Monselise 1979). While *E. crispata* has eyes, the extent to which they can detect quality of light and display avoidance, or preference accordingly is still unknown. Either confirming light selective behaviors or finding other type of photoreceptors in *E. crispata* would constitute an additional element supporting the idea of behavioral mechanisms of photoprotection in this species and may help to explain some of the patterns in which they naturally occur.

Crypsis and secondary metabolites have been mentioned as mechanisms used by sacoglossans to avoid predation (Gavagnin et al. 1997, 2000). Predators of this sea slug group include crustaceans, fish, other sea slugs and even corals (Trowbridge 1994; Mehrotra et al. 2019). While we witnessed sea slug attacks by different fish species during our field work, no consumption of *E. crispata*

was observed. Predation avoidance could be considered the most unlikely explanation of the patterns described herein, but it should not be fully discarded until further studies assess the consumption of *Elysia* by visual predators.

Our research provided novel population information, showing that the abundance and size of *E. crispata* in coral reefs of Southern Gulf of Mexico and Mexican Caribbean Sea depend on locality, depth, and time of the day. Such information suggests that this sea slug is capable of moving a few meters within the reefs, and its mobility may be triggered by light quality and intensity. Further research is needed to better understand the unique biological phenomena of solar-powered sea slugs as they interact with their natural habitat and use it in management and conservation initiatives.

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Data availability The datasets analyzed during the current study are available in the Zenodo repository and can be found at <https://doi.org/10.5281/zenodo.7686727>.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This is an observational study. The study was conducted according to all applicable international, national and/or institutional guidelines for the care and use of animals.

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