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Factors modulating herbivory patterns in *Cymodocea nodosa* meadows

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Abstract

In coastal marine food webs, seagrass-grazer interactions play a fundamental role in ecological processes by regulating the structure and functioning of plant communities. Therefore, assessing the strength of these seagrass-grazer links and identifying the mechanisms that regulate these relationships are crucial to increasing our understanding of community and ecosystem structure and dynamics. Herbivory on the temperate seagrass *Cymodocea nodosa* was evaluated in four locations with contrasting abiotic and biotic factors (i.e., depth; meadow size; seagrass shoot density, productivity, and leaf traits; and herbivore presence) using cross-transplantation experiments carried out once per season to account for seasonal variation. Patterns of feeding rates on *C. nodosa* meadows showed high temporal variability, with peaks occurring in spring and summer. Results indicate that between 46.59% and 74.08% of the annual leaf production is lost to herbivory at the locations sampled in this study. Herbivory rates increased with higher nitrogen content and shoot density for all types of consumers (mesograzers, fishes, and sea urchins). This work highlights the need to integrate the abiotic and biotic factors modulating herbivory patterns, particularly in a foundation species like *C. nodosa*, for which changes in plant traits and grazer abundance may trigger trophic cascades with far-reaching consequences for associated species.

Current understanding of the drivers of marine community structure is based on the concepts of top-down and bottom-up control (Carpenter et al. 1985). These opposing but complementary concepts may be particularly useful in understanding the complex interactions between macrophytes and herbivores. Although the top-down/bottom-up debate has at times been highly polarizing (White 1978), general consensus is that both processes are crucial to the functioning of ecosystems (Hunter and Price 1992). The dilemma for marine ecologists arises from how different ecosystems can have significant differences in the relative importance of these two controls (Valiela 1995). One of the premises of the herbivory process is to understand the relative dominance of these two controls on the functioning of marine ecosystems. Many researchers understand that both controls can act together, determining the structure and functioning of coastal ecosystems (Lotze 2006). However, it is necessary to delve more deeply into theory and research to better understand whether and how both are responsible for the changes occurring in communities. Addressing several factors associated with top-down and bottom-up effects, this study focuses on seagrass-grazers interactions and the factors potentially involved in seagrass consumption.

Seagrasses are considered to be foundation species that host communities with high biodiversity in coastal areas (Heck et al. 2003). Seagrass-associated herbivores feed heavily on both seagrass leaves and their epiphytic communities, influencing seagrass productivity, population structure (Heck and Valentine 2006) and ecosystem services (Scott et al. 2018). A wide variety of herbivores, from mesograzers to large vertebrates, feed directly on seagrass leaves (*see* Heck and Valentine 2006 and references therein). Most of them exhibit selective feeding, according to leaf nutritional quality (i.e., nitrogen content; McGlathery 1995; Cebrián and Lartigue 2004) leaf structural composition and design (i.e., carbon and fiber contents, biomechanical properties; De los Santos et al. 2013), and the presence of chemical deterrents (i.e., phenolic compounds; Vergés et al. 2007, Prado and Heck 2011). Such leaf properties determine tissue palatability, which can be defined as plant susceptibility to consumption by herbivores (Heady and Child 1994). Leaf palatability can be modified by several abiotic factors, such as nutrient content in seawater (Tomas et al. 2011), temperature, and/or acidification events (Poore et al. 2013), which modify consumption. In addition to the aforementioned factors, hydrodynamics such as waves and current may also modify seagrass palatability, since seagrasses can adjust their mechanical design to

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Additional Supporting Information may be found in the online version of this article.

counterbalance hydrodynamic forces to protect plants from structural failure (De los Santos et al. 2013). Herbivory in seagrasses is highly variable over space and time, and different herbivores present different feeding patterns (Steele et al. 2014). This variability has been partially attributed to changes in the abundance and distribution of herbivores as a consequence of interactions among factors, such as migration events (Lodge et al. 1998), predation effects (McClanahan et al. 1994) or overfishing (Klumpp et al. 1993), and so on. Meadow size can also play an important role in macrophyte-herbivore interactions (Prado et al. 2008). Moreover, higher shoot density can also act as a key factor on the presence of herbivores since this indirectly increases recruitment and reduces hydrodynamics, thus decreasing the degree of physical stress, which in turn can modify the feeding and foraging behavior of grazers (Schanz et al. 2002; Jiménez-Ramos et al. 2019).

In general, consumption rates in seagrasses depend on abiotic and biotic factors that interact in a complex way, and that influence the behavior of herbivores as well as plant properties. While these behaviors and patterns have been frequently explored in terrestrial ecosystems, they are not well known for aquatic ecosystems. In this study, we seek to understand how biotic and abiotic local factors drive seagrass consumption by affecting herbivory processes at different levels. Specifically, our goals include the following: (1) to determine herbivore composition within the seagrass community, (2) to determine herbivore activity, and (3) to modify leaf palatability and therefore herbivore consumption rates of seagrass leaves. This study was conducted in shallow meadows of *Cymodocea nodosa* using in situ seasonal manipulative cross-transplantation experiments across a gradient of locations ranging from sheltered to open waters in Cádiz bay, which were also inhabited by different dominant herbivore species (mesograzers, fish, and sea urchins).

Material and methods

Study site

Cádiz Bay (southern Spain) is an excellent outdoor laboratory at which to undertake this study, since the seagrass *C. nodosa* forms perennial populations at several locations with different abiotic and biotic conditions: Caño de Cortadura (CC), Santibáñez (ST), Bajo de la Cabezuela (BC), and El Chato (CH) (Fig. 1). Cádiz area is characterized by the typical seasonal trend for temperate climates, with maximal values of solar radiation and temperature in summer and minimal in winter (Egea et al. 2019). The tidal regime in Cádiz Bay and surroundings is mesotidal and semidiurnal, with a mean tidal range of 2.3 m and mean spring tidal range of 3.7 m (del Río et al. 2012), with the amplitude of the tide increasing from the mouth of the outer bay towards its interior (Álvarez et al. 1999). Westerly and easterly winds are predominant in the study area. Due to coastline orientation (approximately NNW–SSE), westerly winds yield both sea and swell waves, giving rise to a prevailing longshore current toward the E and SE (del Río et al. 2012). Previous works (e.g., De los Santos et al. 2013) and the experience of the researchers in the area show that there are differences in hydrodynamic conditions in the studied locations. However, hydrodynamic was only used in this work from a qualitative point of view, as background information, since this work did not provide in situ hydrodynamic measurements during the study period. Locations have contrasting wave exposure (since they have different spatial orientation, thus different fetch) which is strongly correlated with currents (Zarzuelo et al. 2015). Therefore, as previously indicated by De los Santos et al. (2013), *C. nodosa* meadows from each location maybe exposed to different mechanical loads, with a gradient of exposure to waves and currents ranging from Caño de Cortadura, Santibáñez (low exposure), Bajo de la Cabezuela (medium exposure), to El Chato (high exposure). On the other hand, scientific data on the diversity and abundance of pelagic and benthic fauna in each of these areas are limited, and the data available are based on only a few published articles (e.g., López De La Rosa et al. 2006) or master's and Ph.D. theses (e.g., González-Ortiz 2009, 2014) and the data are based mainly on personal observations. For this reason, we have collected data on the seasonal diversity and abundance of benthic and pelagic fauna in *C. nodosa* meadows, classified into functional feeding groups (*see* Supporting Information Results, Table S1).

Manipulative field experiments were conducted during different seasons throughout the year to encompass the seasonal variation of consumers in the four study areas (Fig. 1; Table 1). The Caño de Cortadura (CC: 36°52′60″N, 06°21′77″W) is a small and shallow lagoon located near the Rio San Pedro, which is connected to the river through a small artificial channel. This artificial opening ensures that this area is subject to very low levels of hydrodynamic forces (data not available), that is, without waves and influenced only by semidiurnal tidal oscillations. The most common consumers of *C. nodosa* plants at this location are the amphipods *Gammarus* sp., and the isopods *Idotea baltica* and *Lekanesphaera hookeri* (*see* Supporting Information Table S1).

Santibáñez saltmarsh (ST, 36°28′09.08″N, 06°15′04.64″W), located at the southern point of the inner bay, is a shallow tidal lagoon (3 m mean depth with respect to mean low water level) with wide tidal flats and a complex system of tidal creeks. The typical waves in Cádiz Bay are short-period wind waves (mean significant wave height of 0.6 m; De los Santos et al. 2013); however, the inner bay, and specially the site of collection, is protected from the action of large waves (Fig. 1), so that the wave height may be below the average value. The mean velocity profile during a tidal cycle ranges from 0.015 to 0.08 m s^{-1} (Lara et al. 2012; data obtained in *Zostera noltei* meadows, approximately 15 m far from the study site), and this is expected to increase during strong easterly winds and during winter (Kagan et al. 2003). *C. nodosa* populations are

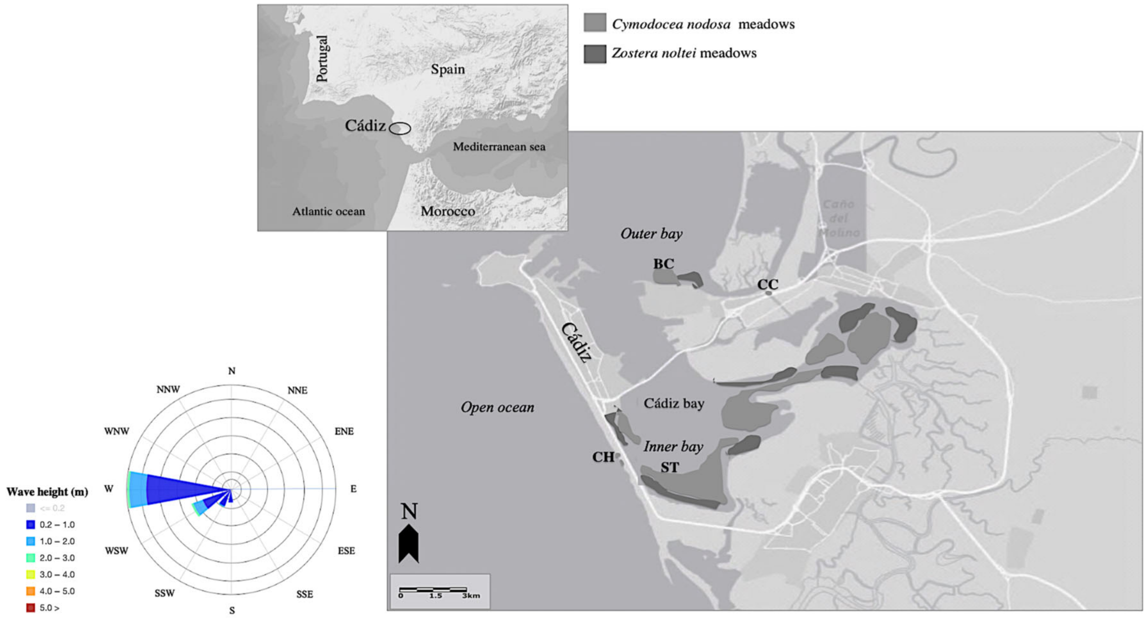

Fig 1. Map of the study area (south of Spain) showing the location of *C. nodosa* meadows at each study location (from low to high exposure, CC: Caño Cortadura; ST: Santibáñez; BC: Bajo de la Cabezuela; CH: El Chato). Annual compass card of wave height (m) along experimental period measured in Cádiz bay (September 2015–September 2016).

Table 1. Summary of environmental context in each experimental location.

	CC	ST	BC	CH
Latitude	36°52′60″N	36°28′09.08″N	36°31′42.52″N	36°28′38.16″N
Longitude	06°21′77″W	06°15′04.64″W	06°14′32.16″W	06°15′49.21″W
Depth (m)	0.25	0.5	0.5	0.25
Experimental meadow area (ha)	0.36	0.8	0.77	0.74

found in the shallow southwestern areas, both at the intertidal fringe and as continuous monospecific meadows at the subtidal zone at 0.4 and −0.5 m above the chart datum (lowest astronomical tide), respectively (Egea et al. 2019). *C. nodosa* populations in Santibáñez exhibit a unimodal seasonal pattern of growth, with maximum growth rates and productivity in summer (Egea et al. 2019). Shoot density and biomass in this area vary with tidal position (De los Santos et al. 2013). The decapods *Hyppolite* sp. and the amphipods *Gammarus* sp. are the main mesograzers at this location, and maximum abundance occurs at the beginning of summer (López De La Rosa et al. 2006).

Bajo de la Cabezuela (BC: 36°31′42.52″N, 06°14′32.16″W) is a shallow inlet located at the Rio San Pedro saltmarsh and comprises a tidal sound with a wider mouth open to outer Cadiz Bay and other artificially closed systems upstream. This sandflat is characterized by semidiurnal mesotides with the tidal current flowing from the bay along the inlet. Plants were collected at the mouth of the inlet, a location which is subject to strong tidal currents and short-period wind-generated waves (which increase during the winter), and recurrent episodes of sediment erosion and accretion (Kagan et al. 2003). The average velocity near the mouth (about 20 m far from the study area) varies between 0.05 and 0.1 $m\ s^{-1}$, even reaching maximum values of 0.7 $m\ s^{-1}$ (González et al. 2010; data obtained over bare sediments). At the last two locations (Santibánez and Bajo de la Cabezuela), the fish *Sarpa salpa* is commonly observed feeding at depths between 1 and 10 m, particularly during the warm period (i.e., late June to late September; Crespo and Ponce 2003). Therefore, the present study was undertaken within the depth range of maximum herbivore density (i.e., 1–5 m depth at high tide).

El Chato (CH: 36°28′38.16″N, 06°15′49.21″W) is a rocky outcrop parallel to the shoreline at Cortadura beach, facing the open ocean with a NNW–SSE orientation. This rocky platform has a maximum width of 100–200 m (1–1.5 m above chart datum) and several sandy pools are located there (Velasco 2006). In these pools, *C. nodosa* meadows occur at low shoot densities. The hydrodynamic conditions of this location are very different from the others as it is the only location exposed to the open ocean, and thus may be subject to strong currents (data not available) and large waves reaching and breaking on the rocks (mean annual significant wave height 2.96 m and maximum height of 3.94 m, Puertos del Estado 2016) (mean annual significant wave height 2.96 m and maximum height of 3.94 m, Puertos del Estado 2016). The main potential consumer at this location is the sea urchin *Paracentrotus lividus* (Supporting Information Table S1).

Leaf trait analyses

At each location, samples (400 cm^2; $n = 5$) were haphazardly collected within seagrass beds at different seasons to determine shoot density. Morphological (length, width, and thickness), nutritional (nitrogen content), structural (carbon and fiber content), and biomechanical properties (force-to-cut) of leaves were examined in 10 independent shoots of *C. nodosa* for each combination of location and season. Prior to measurement, seagrass leaves were carefully cleansed of epiphytes using a piece of soft paper. Total leaf length was measured with a ruler (accuracy ± 1.0 mm), and leaf width and thickness with a digital caliper and a dial thickness gauge, respectively (accuracy ± 0.01 mm, Mitutoyo®). The water content of the leaf samples was calculated as the difference between fresh and dried biomass after 48 h in an oven (60°C). Samples were freeze-dried and pulverized in a ball-grinder to determine leaf carbon (C), nitrogen (N), and fiber content (NDF). Elemental analyses were performed in a Perkin-Elmer 2400 elemental analyser, while fiber content was estimated using the method of Van Soest et al. (1991) modified by De los Santos et al. (2012).

The biomechanical properties of *C. nodosa* leaves were measured with an Instron testing machine (model 5542) and BlueHill® software (v.2.18). We sampled the first outermost fully developed leaf from the selected shoots (normally the second oldest leaf), and a portion of the leaf blade was cut at 4–5 cm above the ligule for testing. Five replicates were measured at each location. The leaves were measured within 1 d of collection and the specimens were tested in the same sequence as they had been collected so that the time of storage was similar among samples and treatments. Leaf-fracture properties were evaluated by cutting tests. The cutting test measures the force required for foliar breakage (Aranwela et al. 1999). During the test, a force to displacement curve was monitored. Since the whole leaf was cut transversally during the test, the force exerted to cut the lamina also included the leaf veins (De los Santos et al. 2012). Results were expressed at two levels: (1) total quantity of force needed to cut or tear a single leaf blade, which depends on the leaf size and its mechanical properties at the material level (F_{TA}, N), and (2) material biomechanical traits, generally referred to as "material properties," which are inherent properties of the material (F_{TS}, N mm^{-2}).

Epifauna collection and processing

Each season, triplicate samples of benthos and epifauna were collected from the four locations, using a 20 cm diameter × 50 cm long acrylic coring tube. A 1 mm mesh bag was attached to one end of the tube, and the other end was slipped over the seagrass patch and its attached sediment. Seagrass leaves were cut off at the base with scissors, and the content (leaves and epifauna) was collected in the mesh bag (*see* Douglass et al. 2010). Epifaunal samples were frozen at −20°C until they were sorted. During sorting, seagrass leaves were separated from fauna, and benthic/epifauna were identified to the lowest taxonomic level possible and subsequently sorted into feeding functional groups.

Sea urchin abundance was measured in the field using a 1 m^2 quadrat at 10 different randomly selected areas in the *C. nodosa* beds at El Chato. Abundance of *S. salpa* was not measured in this study, but has been previously measured to be at its highest between spring and summer (Crespo and Ponce 2003).

Cymodocea nodosa herbivory and productivity

Tethering experiment

A seasonal field tethering assay (Kirsch et al. 2002; Prado et al. 2007) was conducted to directly measure herbivory rates in *C. nodosa*. At each location, we collected *C. nodosa* shoots haphazardly from similar depths (0.5 ± 0.25 m at low tide). Care was taken to collect shoots with intact vertical rhizomes to minimize changes in leaf properties and in order to allow the safeguarding of leaves throughout the experiment. Once transported to the laboratory and prior to any measurement, 40 healthy shoots (no necrosis or bites) from each location were carefully selected and epiphytes were carefully scraped off to avoid their potentially confounding role in feeding preference (Jiménez-Ramos et al. 2018*b*).

In order to optimize retrieval of samples, individual shoots were attached by their vertical rhizome to a line (referred to as uprooted shoots hereafter) by a bowline knot, and two labeled pickets (at both ends) were used to anchor the lines to the sediment. Previously, the length and number of leaves were measured for each experimental shoot, as baselines to quantify leaf loss and breaking leaves during the field assay. In order to determine whether *C. nodosa* consumption is mediated by location-dependent factors (i.e., differences in herbivore composition and/or effects of hydrodynamics on herbivore activity) or by leaf properties (i.e., palatability), four uprooted shoots separated from each other by approximately 0.25 m (i.e., each line holds four shoots) were attached to a line. Within each line, these four shoots were collected from the four locations described earlier, each from a different location (arrangement was random within the line) (Fig. 2). Therefore, each line contained a local uprooted shoot along with three transplanted shoots from the other experimental locations (*see* Table 2 for shoot type definition). Ten lines were set up in a meadow at each location at the same depth of collection

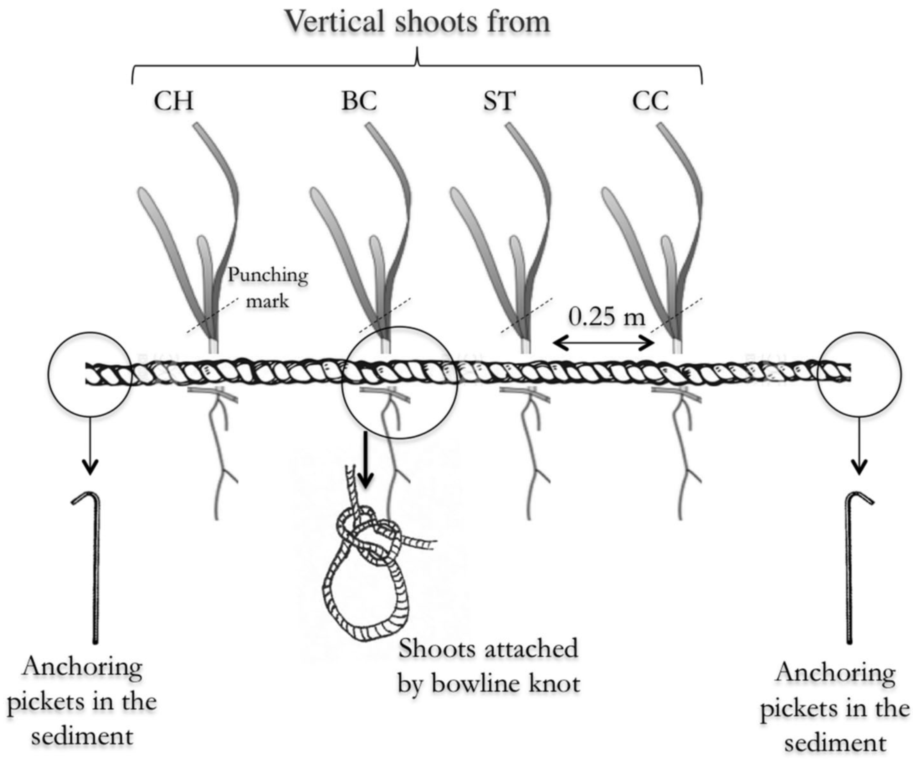

Fig 2. Drawing of experimental setup for tethered method to measure herbivory rates over *C. nodosa* shoots.

Table 2. Shoot type definition and measurements.

Type of shoot	Definition	Measurements
Native uprooted shoots	Collected shoots and attached by their vertical rhizome to lines in tethering test (i.e., tethered shoots). Anchored in the same location where collected	Herbivory rate over *C. nodosa* transplanted in the same location where collected
Non-native uprooted shoots	Collected shoots and attached by their vertical rhizome to lines in tethering test (i.e., tethered shoots). Anchored in different locations where collected	Herbivory rate over *C. nodosa* transplanted in different locations where collected
Native non-uprooted shoots	Non-manipulated shoots inhabiting each location	*C. nodosa* population production

(0.5 ± 0.25 m approximately at low tide). Lines were separated by 3 m from each other and, thus, each line was considered to be an independent sample. The base of the leaves attached to each line was also marked using a modified Zieman method (Zieman 1974; Peralta et al. 2000) to measure leaf growth during the experiment. In short, this method involves placing a staple at a convenient reference point, such as the junction between the leaf and the leaf sheath. Then, shoots are left for 15 d (or another experimental period) and the incremental growth between the leaf base and the staple is measured upon harvest. In our study, this experimental period was sufficient to obtain significant results in herbivory rates but reducing the probability of shoots loss. During transport, and laboratory and field manipulation, plants were kept underwater to avoid stress. Moreover, when inserting pickets into the sediment to tether uprooted shoots, extreme care was taken to ensure that leaves were neither above nor below the average height of the leaf canopy.

After 15 d, all experimental lines from all locations (Caño de Cortadura, Santibáñez, Bajo de la Cabezuela and El Chato) were carefully collected and transported under cold conditions. Once in the laboratory, we recorded leaf length and width, the number of leaves per shoot that had been lost, and those that were still intact or had been attacked by grazers.

We used a modified punching method to estimate leaf elongation rate (cm^2 $shoot^{-1}$ d^{-1}) of tethered shoots, which was calculated by dividing the area of new tissue produced by the number of days elapsed since marking (i.e., 15 d). Leaf elongation rates allowed us to correctly estimate herbivory rates.

Seagrass consumption was estimated by subtracting the leaf area remaining from each initial shoot measurement and then adding the growth area of the tethered shoots obtained after 15 d, and consumption was expressed in cm^2 shoot^{-1} d^{-1} (Kirsch et al. 2002). Leaves that had been lost during this period were not included in the analyses, which may have led to underestimation of the total herbivory rates.

Leaf bite mark identification

Herbivore bite marks on leaves were divided into three identifiable shapes with respect to the physiology of the oral apparatus: bites from mesograzers (not identifiable at species level), bites from fishes (semicircular shape), and sea urchins (irregularly slashed scars and only present in El Chato) (*see* Fig. 3).

Local shoot production at the four sites

In addition to the aforementioned measurements, leaf elongation rates of local shoots (*see* Table 2 for shoot type definition) of *C. nodosa* at the four study locations were measured each season using the modified punching method (Peralta et al. 2000) for 10 independent, non-uprooted shoots. Leaf elongation rate was expressed in terms of cm^2 shoot^{-1} d^{-1}.

Statistical analyses

Data are presented as means and associated standard error (SE). To determine the effect of location in each season for each response variable, we used generalized linear models (GLMs). We selected the gamma distribution model with log link function to fulfill the assumptions of linearity, homogeneity of variances and no overdispersion, and these assumptions were checked through visual inspection of residuals and Q-Q plots (Harrison et al. 2018). The GLM *F*-test was used to assess the effects of location on each response variable. Pairwise comparisons were tested using estimated marginal means with a Bonferroni correction ("emmeans" R package, Lenth et al. 2019).

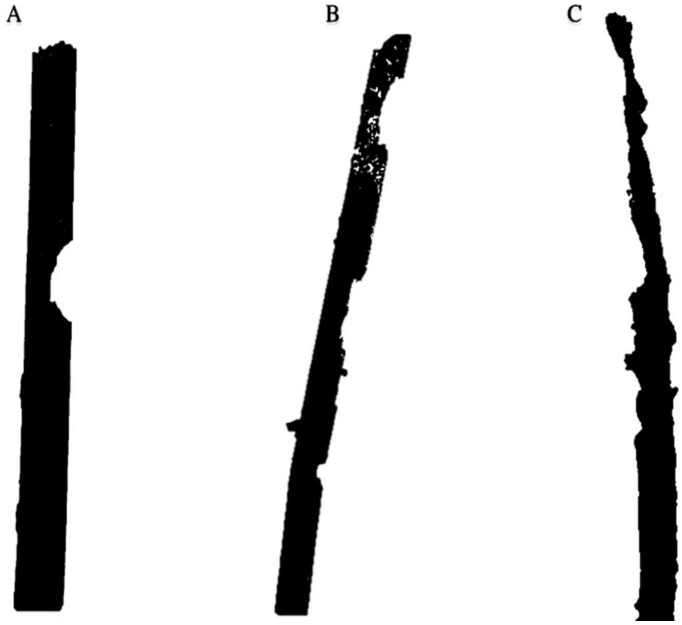

Fig 3. Images of *C. nodosa* leaves with morphological distinctive bite marks of: (**A**) *S. salpa*, (**B**) Mesograzers, and (**C**) *Paracentrotus lividus*.

To partition the relative contribution of environmental factors (depth and meadow area), leaf traits (length, width, thickness, leaf nitrogen, carbon and fiber content, absolute and specific force-to-cut), and shoot density at each location on herbivory rates for all uprooted shoots, GLMs were used. Gaussian distributions with an "identity" link function were selected to fulfill the assumptions of linearity and homogeneity of variances, which were checked through visual inspection of residuals and Q-Q plots (Harrison et al. 2018). Variable collinearity was checked through using the variation inflation factor (VIF) of the preliminary full-factor GLMs (variables with VIF < 5 were removed from data matrix). Cross-correlated and noninformative variables were removed from GLMs using backward selection to retain only the predictor variables with significant effects on the response variable (i.e., herbivory rate). We generated models with all possible combinations of predictor variables using the MuMIn package (Barton 2019), and selected the best fitted model using Akaike's information criterion (AIC). Finally, we used GLMs to quantify how the predictor variables from the best fitted models interacted with the response variable.

In addition, for each location, we computed Cohen's *d* "effect sizes" between local uprooted shoots vs. cross-transplanted plants (transplanted uprooted shoots) on *C. nodosa* herbivory averaged over a year to visualize similarities in leaf consumption according to the origin of tethered shoots (Fritz et al. 2012). In order to estimate annual production and herbivory rates at the four locations independently, production (in situ measurements by punching method in local non-uprooted shoots) and herbivory rates (uprooted shoots from tethering test) from local shoots at each whole location were estimated as the mean of the sampling time points throughout the study period (i.e., autumn 2015–autumn 2016). Sampling time points corresponding to the same seasonal period in the two consecutive years were averaged prior to analysis. The significance level (α) was set at 0.05 in all tests. Statistical analyses were computed with R statistical software 4.0.2 (R Development Core Team 2020).

Results

Spatial and temporal differences in seagrass leaf traits, biomass, and density

On an annual basis, the highest shoot densities in *C. nodosa* meadows were recorded at ST, with the maximum values occurring during summer (630.12 ± 29.12 shoot m^{-2}). Meadows from El Chato had the lowest shoot densities, with minimum values occurring during winter (25.0 ± 7.69 shoot m^{-2}) (Tables 3, 4).

Table 3. Mean ± SE of above and belowground biomasses (g DW m^{-2}) and shoot density (shoot m^{-2}) of *C. nodosa* meadows from the four experimental locations (CC, Caño Cortadura; ST, Santibáñez; BC, Bajo de la Cabezuela; CH, El Chato) at each sampling time. Letters indicate significant differences among locations at *p* value < 0.05. Data are showed as mean ± SE.

	Aboveground biomass (g DW m^{-2})	Belowground biomass (g DW m^{-2})	Density (shoot m^{-2})
Autumn 2015			
CC	65.76 ± 14.64^{a}	82.16 ± 21.12^{a}	161.53 ± 22.67^{a}
ST	109.40 ± 10.59^{b}	195.16 ± 50.51^{b}	410.25 ± 63.61^{b}
BC	77.60 ± 11.60^{a}	328.93 ± 18.69^{c}	107.34 ± 20.56^{c}
CH	20.90 ± 7.18^{c}	46.23 ± 10.36^{d}	48.07 ± 26.43^{d}
Winter 2016			
CC	66.23 ± 10.83^{a}	89.00 ± 10.88^{a}	140.38 ± 23.39^{a}
ST	78.20 ± 10.54^{a}	188.10 ± 11.45^{b}	280.12 ± 17.44^{b}
BC	55.26 ± 6.55^{b}	321.03 ± 22.51^{c}	80.12 ± 28.93^{c}
CH	12.71 ± 4.06^{c}	26.13 ± 5.08^{d}	25.00 ± 7.69^{d}
Spring 2016			
CC	141.23 ± 16.41^{a}	99.33 ± 10.81^{a}	351.28 ± 54.53^{a}
ST	148.53 ± 15.90^{a}	144.30 ± 9.20^{b}	577.56 ± 30.84^{b}
BC	93.73 ± 15.27^{b}	185.06 ± 53.69^{c}	183.33 ± 11.59^{c}
CH	33.73 ± 6.69^{c}	45.46 ± 9.15^{d}	125.64 ± 14.93^{d}
Summer 2016			
CC	98.22 ± 8.95^{a}	111.63 ± 4.99^{a}	316.66 ± 87.90^{a}
ST	97.90 ± 9.41^{a}	83.86 ± 23.62^{ab}	630.12 ± 29.12^{b}
BC	68.46 ± 14.68^{b}	71.87 ± 20.83^{b}	219.87 ± 94.06^{c}
CH	49.20 ± 13.57^{b}	44.10 ± 3.65^{c}	108.97 ± 35.78^{d}
Autumn 2016			
CC	64.60 ± 12.2^{a}	71.78 ± 12.10^{a}	187.17 ± 21.87^{a}
ST	104.36 ± 3.8b^{b}	158.18 ± 46.73^{b}	351.28 ± 25.09^{b}
BC	61.83 ± 13.68^{a}	257.36 ± 50.29^{c}	118.58 ± 8.67^{c}
CH	31.13 ± 7.44^{c}	48.53 ± 18.25^{a}	96.15 ± 39.45^{d}

Leaf traits showed spatial and temporal variability, with higher nutritional properties (i.e., high nitrogen content) found in plants collected from Caño de Cortadura and Santibáñez, while stronger structural properties (i.e., high carbon and fiber contents) were recorded at Bajo de la Cabezuela and El Chato. Overall, the thinnest and shortest leaves were found at El Chato, which also had leaves with the highest structural properties (leaf carbon and fiber content, specific force-to-cut). Plants from Bajo de la Cabezuela had the most resistant leaves (i.e., higher absolute and specific force-to-cut), and these were found in spring (Fig. 4; Table 5).

Fauna samples

There were different types of consumers at each site (Table 4). A total of seven taxa were found at all sampled locations (including Arthropoda, Equinodermata, Mollusca, and Annelida) (Supporting Information Table S1). The most abundant seagrass consumers at Caño de Cortadura were the decapod *Hyppolyte* sp., the amphipod *Gammarus* sp., and the isopods *Idotea* sp. and *L. hookeri*, with higher abundances found in spring and summer. A high abundance of *Hyppolyte* sp. was also found at Santibáñez, especially in summer (3769 ± 1543 ind m^{-3}), and a lower abundance was found at Bajo de la Cabezuela (805 ± 326 ind m^{-3}). The equinoderm *Paracentrotus lividus* was only found at El Chato with high numbers especially in spring (11 ± 6 ind m^{-2}). We did not directly estimate *S. salpa* numbers, but it is commonly observed in the Santibáñez and Bajo de la Cabezuela areas, especially during the summer (Crespo and Ponce 2003).

Seagrass herbivory in tethered uprooted shoots

The location with the highest herbivory rates for both types of uprooted shoots (i.e., local and transplanted) was Santibáñez, where fishes were mainly responsible for *C. nodosa* consumption (Fig. 5). Mesograzers were mainly responsible for *C. nodosa* herbivory at the Caño de Cortadura, while sea urchins were the main consumers at El Chato, for all types of uprooted shoots (i.e., local and transplanted) averaged per year. Herbivory rates were near zero during winter in local and transplanted shoots at both Bajo de la Cabezuela and El Chato (Fig. 5). The highest herbivory rates for uprooted local and transplanted shoots were found in summer for all locations

Table 4. Main species of grazers found in each location (CC, Caño Cortadura; ST, Santibáñez; BC, Bajo de la Cabezuela; CH, El Chato). For species abundance in each season, see Supporting Information Table S1.

	Category of grazers		
	Mesograzers	Fish	Sea urchin
CC	*Hyppolite* sp., *Gammarus* sp., *Idotea baltica*, *Idotea chelipes*, *Lekanesphaera hookeri*	—	—
ST	*Hyppolite* sp., *Gammarus* sp., *Idotea baltica*, *Idotea chelipes*, *Lekanesphaera hookeri*, *Palaemon adspersus*	*S. salpa*	—
BC	*Hyppolite* sp., *Gammarus* sp., *Palaemon adspersus*	*S. salpa*	—
CH	*Palaemon serratus* and *Palaemon adspersus*	—	*Paracentrotus lividus*

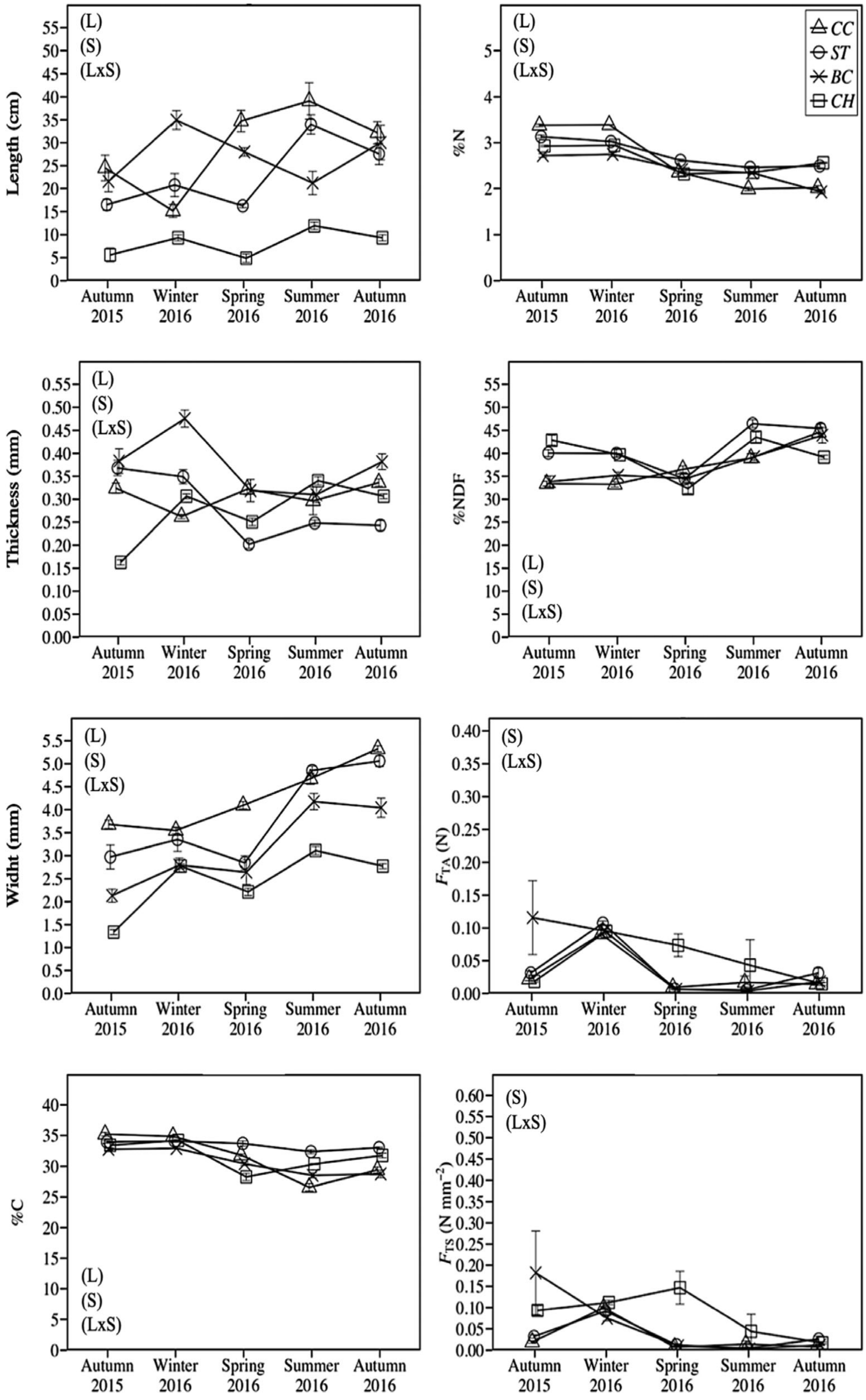

Fig 4. Legend on next page.

Table 5. (A) Model selection using ΔAICc scores of GLMs testing for size, density, length, N, NDF, F_{TA}, and grazers groups (i.e., fish, mesograzers, sea urchins) on herbivory rate. The model with the most support (lowest AICc, delta < 1.0) is indicated in bold. (B) Summary output of factors from the best fit model. N, nitrogen; NDF, fiber content. Symbols indicate significant differences at $\alpha < 0.05$ (*), $\alpha < 0.01$ (**), $\alpha < 0.001$ (***).

(A)

Model	Factors	df	Deviance	AICc	Delta	Weight
1	**Density, N, fiber, grazer**	**8**	**26.24**	**35.72**	**0.00**	**0.40**
2	Density, N, grazer	7	24.55	34.52	1.20	0.22
3	Density, grazer	6	23.31	34.19	1.53	0.19
4	Density, NDF, grazer	7	24.18	33.77	1.95	0.15
5	N, grazer	6	20.87	29.30	6.42	0.02
6	N, NDF, grazer	7	21.65	28.72	7.00	0.01
7	Grazer	5	18.92	27.54	8.18	0.01

(B)

Factors	Estimate	SE	*z* value	Pr(> |*z*|)	VIF
(Intercept)	0.9730	0.2210	4.391	$p < 0.001$***	
Density	0.0002	0.0001	2.474	0.013*	1.325
NDF	−0.0029	0.0035	0.830	0.406	1.205
Grazer groups	−0.369	0.0764	4.806	$p < 0.001$***	1.476
N	−0.0450	0.0457	0.995	0.319	1.465

except for Santibáñez, where the highest value was found in spring (Fig. 5).

After removing collinear and noninformative variables, fitted GLMs included size, density, length, leaf nitrogen and fiber content, absolute force-to-cut, and species of grazers as predictors of herbivory rate. The most parsimonious model for explaining herbivory rate included density, leaf nitrogen and fiber content, and grazer type (Table 5, column A). Herbivory rate was significantly positively associated with density and also significantly associated with herbivore feeding group (i.e., fish, sea urchin, and mesograzer) (Table 5, column B).

The effect size analysis is used to visualize differences in herbivory rates between local uprooted shoots vs. cross-transplanted plants (transplanted uprooted shoots). Results showed very few significant differences in herbivory among shoots within each location. There were significantly lower herbivory rates only for transplanted uprooted shoots in Caño de Cortadura in spring and summer, and shoots transplanted from El Chato into Santibáñez during spring (Fig. 6).

Estimation of annual herbivory rates in Cádiz Bay

Production differed among locations and seasons (Table 6) with Caño de Cortadura and Santibáñez showing higher *C. nodosa* production rates. Annual herbivory patterns also varied among locations with maximum values found at Caño de Cortadura, where only mesograzers were recorded as potential herbivores (*see* Supporting Information Table S1). Meanwhile, El Chato showed the lowest value of annual herbivory rates (46.59%) and leaf production, with sea urchins as the main consumers (Table 4).

Discussion

Palatability patterns in *Cymodocea nodosa* leaves

C. nodosa showed different leaf traits (morphological, structural, nutritional, and biomechanical) depending on season and location. This plasticity is in agreement with previous findings by De los Santos et al. (2013), who observed this capacity to thrive under a range of hydrodynamic forces through adaptation of leaf traits. Furthermore, this study also demonstrates that this plasticity modifies leaf palatability and, consequently, the consumption rates by herbivores (Fig. 4). Overall, the different abiotic conditions affected leaf traits of *C. nodosa*, since plants from locations El Chato and Bajo de la Cabezuela showed stronger structural traits (i.e., high leaf carbon and fiber content and thickness but low %N) and thinner

FIG 4. Spatiotemporal differences in the morphological, nutritional, structural, and biomechanical properties of *C. nodosa* leaves from the four experimental sites (CC: Caño Cortadura; ST: Santibáñez; BC: Bajo de la Cabezuela; CH: El Chato) at each sampling time. Length (cm); thickness (mm); width (mm); nitrogen content (%N), carbon content (%C) and fiber content (%NDF) in leaves; absolute force-to-cut (F_{TA}, N) and specific force-to-cut (F_{TS}, N mm^{-2}). Significant differences resulting from GLM test among factors "location" (L) and "season" (S) and their interactions (LxS) are shown in brackets. Absence of brackets indicates no significant differences. See Supporting Information Table S2 for statistics values.

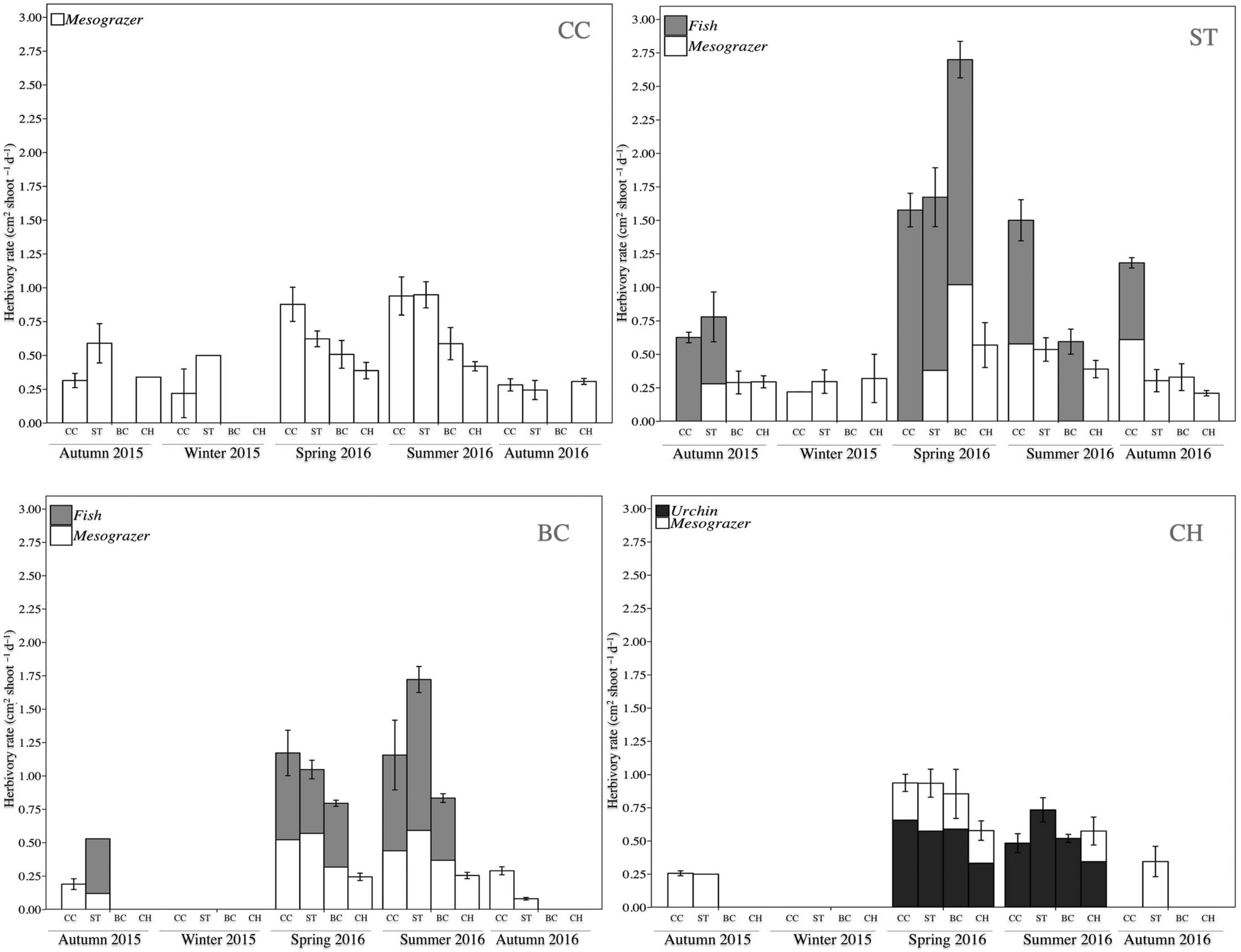

Fig 5. Spatial–temporal differences in herbivory rates (cm^2 shoot^{-1} d^{-1}) sorted by consumer type (mesograzer, fish, and sea-urchin) in *C. nodosa* meadows from the four experimental locations in each type of tethered shoot (CC: Caño Cortadura; ST: Santibáñez; BC: Bajo de la Cabezuela; CH: El Chato). Data are showed as mean ± SE. Lack of data in the graph indicates negligible consumption.

and/or shorter leaves (De los Santos et al. 2013), which can also yield low palatability in leaves. Meanwhile, plants from Caño de Cortadura and Santibáñez showed higher nutritional quality (i.e., higher leaf nitrogen content), lower strength (i.e., low absolute and specific force-to-cut), and weaker structural properties (i.e., low leaf carbon and fiber content and thickness), which can make tissues more palatable for consumers (Heck and Valentine 2006).

The aforementioned differences in palatability induced by abiotic conditions had a direct impact on the herbivory rates in *C. nodosa*. Substantial *C. nodosa* consumption was recorded in the meadows at different locations, reaching a gross annual estimate between 46.59% and 74.08% of annual leaf production (Table 6). The highest rate of annual herbivory was recorded at the sheltered location (i.e., Caño de Cortadura), where leaves were more palatable, with a downward trend in herbivory toward the most exposed location (i.e., El Chato, Table 6). Some seagrass herbivores have been documented to discriminate key properties when consuming seagrass leaves (Cebrián and Lartigue 2004; Heck and Valentine 2006; Prado and Heck 2011), such as nutritional quality (McGlathery 1995; Cebrián and Lartigue 2004), structural properties, and the presence of chemical deterrents (Vergés et al. 2007). These key elements control leaf palatability and, thus, foraging decisions. In general, El Chato and Bajo de la Cabezuela plants produced shorter and tougher leaves, being less palatable, which could be due to an adaptation to the prevailing environmental conditions in the areas (De los Santos et al. 2013). Consequently, these plants obtained low rates of herbivory. This was particularly notable in leaves coming

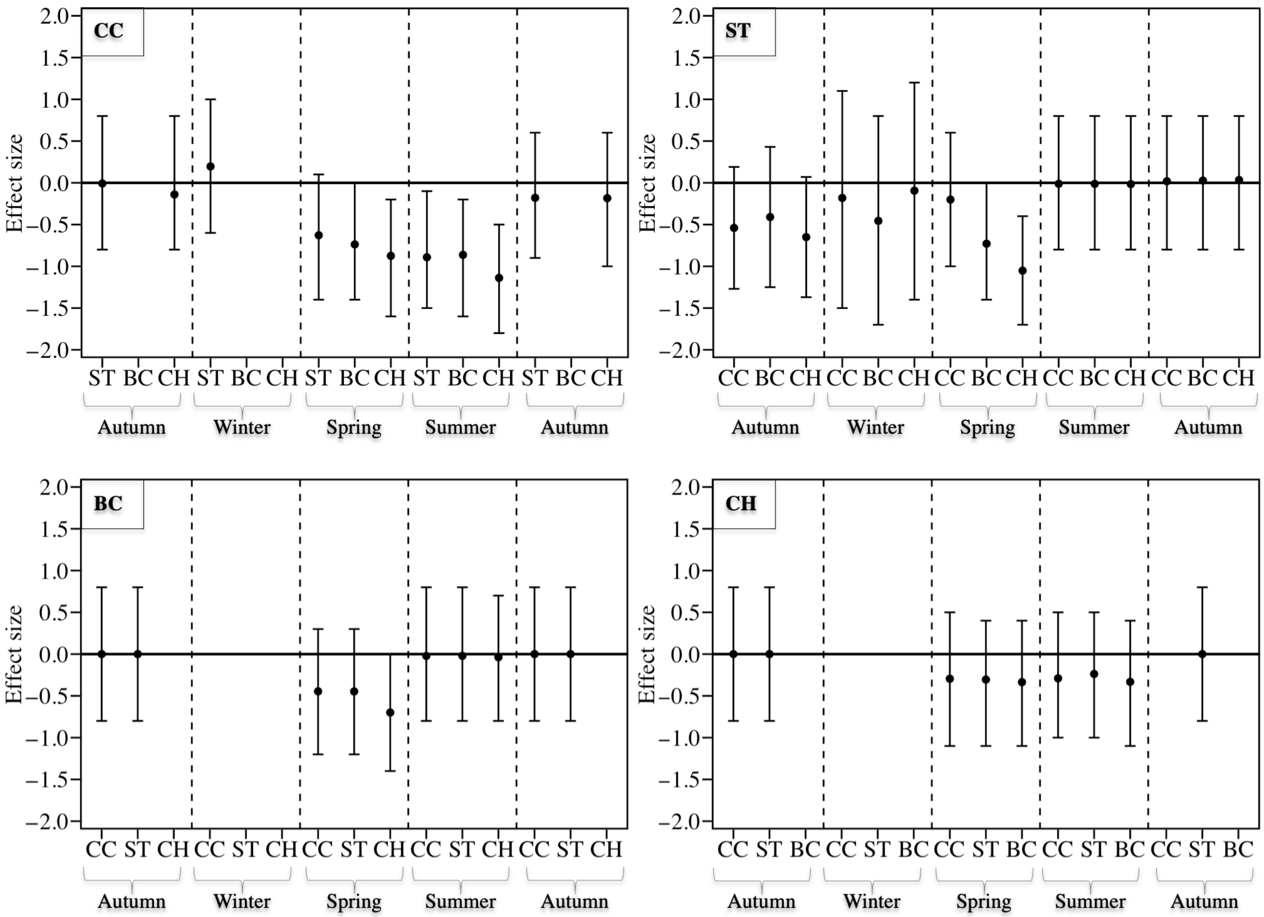

Fig 6. Effect sizes ($n = 10$) of herbivory rates over *C. nodosa* in each sampling site for non-native uprooted shoots with respect to the native uprooted shoots. CC, Caño Cortadura; ST, Santibáñez; BC, Bajo de la Cabezuela; CH, El Chato.

from El Chato, even during the spring and summer, which correspond to the periods of greatest abundance of consumers. Hence, as indicated by previous works in the studied locations (De los Santos et al. 2013) and other studies (e.g., La Nafie et al. 2012), hydrodynamic conditions can modulate the palatability of the seagrass leaves by modifying their morphological, structural, and biomechanical properties. Although our results seem to indicate this outcome, it is necessary to measure the hydrodynamic conditions in situ at these locations in order to confirm such results. On the other hand, it should be noted that in our study, we did not discriminate between genotypic and phenotypic (environmental acclimation) differences in plant traits. Thus, the differences recorded in the studied plant variables can be the sum of both environmental

Table 6. Annual production (cm^2 shoot^{-1} yr^{-1}) from native non-uprooted shoots and annual herbivory rate (i.e., including all types of consumers, cm^2 shoot^{-1} yr^{-1}) from native uprooted shoots of *C. nodosa* meadows at each sampling location. Annual herbivory data only consider tethered shoots (i.e., uprooted shoots) from their original site (native uprooted shoots). BC, Bajo de la Cabezuela; CC, Caño Cortadura; CH, El Chato; ST, Santibáñez. Data are showed as mean ± SE. Letters indicate significant differences among locations at p value < 0.05 (ANOVA one-way test).

	Annual leaf production (cm^2 shoot^{-1} yr^{-1})	**Annual herbivory rate (cm^2 shoot^{-1} yr^{-1})**	**Annual production consumed (%)**
CC	298.57 ± 12.41^{a}	221.19 ± 120.45^{ab}	74.08
ST	325.94 ± 15.33^{b}	220.09 ± 124.10^{ab}	67.52
BC	246.74 ± 65.70^{ac}	141.98 ± 93.44^{bc}	57.54
CH	224.84 ± 43.80^{c}	104.75 ± 95.26^{c}	46.59

acclimation and genetic differences (Martínez-Crego et al. 2016). Several studies on terrestrial and marine plants have reported different herbivory patterns between different genotypes, which were driven by differences in plant traits (Tomas et al. 2011). In this way, past studies have shown that *C. nodosa* populations in Cadiz bay (Alberto et al. 2005) bear a high genetic variability, while others have also demonstrated that environmental differences may modify traits related to palatability (de los Santos et al. 2013), also including the synthesis of natural products (Manck et al. 2017). Therefore, both genetic variability and the acclimation to such different environmental conditions may explain the differences in traits that we observed between plants from the inner and outer bay, and thus both may contribute to the differences in the recorded consumption rates.

Feeding activity and diversity of grazers

The population density of mesograzers was variable throughout the year at all locations (pelagic fauna, Supporting Information Table S3), with higher densities found in spring and summer (in accordance with López De La Rosa et al. 2006). The summer peak in grazing coincided with the greatest abundance of mesograzers at all locations (Supporting Information Table S1) and the highest density of *C. nodosa* shoots (Table 3 and Supporting Information Table S2). The persistence of abundant summer mesograzers in Cádiz Bay and other temperate seagrass communities (e.g., Best and Stachowicz 2012) may be due to a trade-off between invertebrate recruitment and predator avoidance. Seagrass canopies create refuges for small invertebrates against predation, thus increasing recruitment and growth of invertebrates (Connolly 1995). Moreover, Jiménez-Ramos et al. (2019) demonstrated that this habitat complexity created by seagrass meadows (i.e., shoot density) affected mesograzer feeding, with higher feeding rates being recorded at higher shoot densities under low and moderate hydrodynamic flows. In our study, higher herbivory rates in *C. nodosa* meadows were found in populations with high shoot density and areas exposed to milder hydrodynamic conditions (i.e., Caño de Cortadura and Santibáñez), which is in agreement with this previous study (Jiménez-Ramos et al. 2019). In areas subjected to rough hydrodynamic conditions (Bajo de la Cabezuela and El Chato), the highest rates of annual herbivory also occurred under high shoot densities (spring and summer), which reduces hydrodynamic stress and allows mesograzers to invest more in feeding and swimming, which in turn leads to higher rates of biomass consumption from the canopy.

Mesograzers (including isopods, decapods, and amphipods) accounted for the remaining 40.28% of annual seagrass consumption estimated over all locations. The highest values of herbivory were obtained in Caño de Cortadura where all consumers were invertebrates. In temperate areas (with a strong focus on northern Europe), invertebrate mesograzers (i.e., crustaceans and gastropods) mainly feed on ephemeral and epiphytic algae and rarely consume seagrasses directly (e.g., Moksnes et al. 2008). Our study is restricted to a single location in the Atlantic Ocean, but still demonstrates the potential importance of mesograzers on seagrass herbivory, especially in sheltered areas. In Caño de Cortadura, the size effect analysis revealed differential herbivory rates by mesograzers between transplanted and local leaves, with the local ones being found more palatable (i.e., higher leaf nitrogen content and lower leaf carbon and fiber content and thickness) and therefore supporting higher rates of herbivory. In summary, the influence of environmental factors and meadow characteristics (e.g., density, size) on seagrass herbivory is not only determined by its effects on leaf palatability, but also by its influence on consumer feeding, even may have consequences in their swimming behavior, as recently was described by Jiménez-Ramos et al. (2019) in a flume study.

Sarpa salpa is a major consumer of seagrasses in the Mediterranean Sea, contributing up to 75% of the total herbivorous consumption of *Posidonia oceanica* (Prado et al. 2007). In the case of *C. nodosa* consumption by *S. salpa*, Goldenberg and Erzini (2014) estimated *S. salpa* adults feeding of approximately 32 g kg fish^{-1} d^{-1} whereas Marco-Méndez et al. (2017) observed the highest average feeding in summer at approximately 12.75 ± 3.43 mg WW d^{-1}. Herbivory by fish (*S. salpa*, personal observation) was only recorded at the Santibáñez and Bajo de la Cabezuela locations with higher values recorded during spring and summer (ca. 0.83 ± 0.06 cm^2 shoot^{-1} d^{-1} averaged over both locations). This seasonal effect probably occurs because fish need to accumulate reserves for the winter period, when they feed less and adults prepare for reproduction (Peirano et al. 2001). The average feeding rates on *C. nodosa* leaves obtained in this study area are within the same order of magnitude as those reported for *C. nodosa* at other areas (Cebrián et al. 1996). Notwithstanding the experimental limitations of our study (temporal and accounting of individuals in situ), our results suggest that small-scale changes in feeding activity by *S. salpa* may have important implications for the overall intensity of herbivory on *C. nodosa* in Cádiz Bay, especially in spring and summer. The spatial and temporal differences observed in Cádiz Bay are most probably caused by the seasonal migratory and feeding behavior of fish species (Cebrián et al. 1996). Herbivory rates clearly differed among sampling times (seasonal contribution to total herbivory ranged from 62.14% in spring to null consumption in winter) and were higher in more palatable leaves from Caño de Cortadura or Santibáñez (higher leaf nitrogen content and lower structural leaves, Fig. 5). This also confirms that higher nutritional values (i.e., higher leaf nitrogen content and lower structural traits) in seagrasses can mediate fish herbivore preferences and consumption rates (Marco-Méndez et al. 2017). It is important to note that herbivory estimations for fishes may be slightly overestimated due to the fact that lateral bites taken by large individuals may enhance leaf breakage through wave action; however, these cases were rare and only affected two local samples placed in Bajo de la Cabezuela during spring.

Sea urchins accounted for 90.05% of the total annual losses to herbivores in El Chato (the most exposed location to hydrodynamics) and herbivory rates by sea urchins differed among sampling times, being null in autumn and winter. These differences can be attributed to the influence of seasonal variables on feeding activity, such as temperature and the nature and abundance of other trophic factors, such as presence of predators (Régis 1979). Furthermore, like fish, sea urchins preferentially fed on transplanted shoots with higher nutritional content coming from Caño de Cortadura and Santibáñez, suggesting a selective behavior toward more palatable plants, as shown by others (Tomas et al. 2015; Jiménez-Ramos et al. 2018*a*).

Interacting grazer loops

Our study showed how mesograzers are key herbivores in removing photosynthetic material. Locations like Caño de Cortadura, where mesograzers were only found in shallow conditions, the consumption of the annual production of *C. nodosa* was up to 74.08% (the highest value of the four locations studied). The presence or absence of mesograzers can also modulate the presence of epiphytes. Direct consumption of epiphytes attached to seagrass leaves is usually by mesograzers and can have positive consequences on leaf growth and indirectly, for herbivores that consume seagrass (Borowitzka et al. 2006). On the other hand, the removal of epiphytes might also negatively affect herbivores such as fish or sea urchins, many of which gain nutrition from the epiphytic algae, in addition to seagrass itself (Marco-Méndez et al. 2017) or could be also attracted by them (Jiménez-Ramos et al. 2018*b*).

In Santibáñez and Bajo de la Cabezuela, mesograzers lived together with fish, but consumption rates of annual production were not as high as in Caño de Cortadura. The fish removed greater amounts than the mesograzers, as occurred in El Chato with the sea urchins. The coexistence of different herbivores can, therefore, affect the herbivory rates of each taxonomic group. This may be due to the fact that these herbivores may affect plant communities in many ways, since bite grazing can lead to changes in the complexity of the seagrass habitat, which may affect where grazers choose to feed (Unsworth et al. 2007). For example, this may be the case of fishes, who remove photosynthetic material and can reduce the density and length of leaves. This effect could have a consequence in the predation of other herbivores such as sea urchins, making them more visible to predators (Pagès et al. 2012). In addition, fishes may unintentionally consume mesograzers while feeding on the seagrass they live among (Marsh et al. 2011) and then, modify direct leaf consumption. Thus, the interaction among the different functional groups of grazers and their relationship with the habitat complexity may be also key factors performing direct seagrass consumption.

Ecological implications

Seagrasses often have intraspecific differences in their leaf traits and meadow structure (i.e., density) that reflect phenotypic plasticity (King et al. 2017). These changes indirectly affect the palatability of seagrass tissues, a factor that has been widely demonstrated to directly influence the consumption of seagrass leaves by consumers (i.e., Heck and Valentine 2006; Prado and Heck 2011; Jiménez-Ramos et al. 2018*a*). Moreover, herbivore distribution and feeding behavior patterns are greatly influenced by both biotic and abiotic factors (Best and Stachowicz 2012). Our results indicate that seagrasses act as foundation species, having a positive biological effect on mesograzers through facilitation (e.g., increasing structural complexity, reducing hydrodynamics and hidden from predators mesograzers) of the direct consumption of leaves, and also as a food resource for other consumers, such as fishes and sea urchins. Furthermore, direct grazing of seagrass leaves has been highlighted as a complex process. Although many studies have focused on how the palatability of seagrass leaves influences consumption rates (Valentine and Duffy 2006; Tomas et al. 2011; Martínez-Crego et al. 2016) or how abiotic factors and habitat structure affect seagrass-grazer interactions (Schanz et al. 2002; González-Ortiz et al. 2014; Jiménez-Ramos et al. 2019), these factors have been addressed in an integrated manner in this study. We also show how diverse drivers like type of grazer, habitat complexity (i.e., density), and leaf traits (leaf nitrogen and fiber content) directly and indirectly affect herbivory rates by modifying leaf palatability, consumer abundance, composition, and activity of different grazers. Grazing intensity and groups of grazers (e.g., mesograzers, fishes, or sea urchins) structure seagrass meadows and influence the level and type of ecosystem services provided (Scott et al. 2018). Our results suggest that plant-herbivore interactions depend on season, habitat complexity, seagrass properties (i.e., significant predictors, leaf nitrogen, and fiber content) and on the community composition of herbivores. Knowledge of the complex interactions among herbivores, seagrasses, and the delivery of ecosystem services is required to achieve balanced systems or other desired management outcomes and the consequences of these.

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Conflict of Interest

None declared.

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