Drastic decadal decline of the seagrass *Cymodocea nodosa* at Gran Canaria (eastern Atlantic): interactions with the green algae *Caulerpa prolifera*

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Abstract

The shoot density, leaf length and biomass of the seagrass *Cymodocea nodosa* (Ucria) Ascherson were found to severely decline in the last 17 years in the oceanic island of Gran Canaria (central Eastern Atlantic). Five seagrass meadows were sampled in summer and winter of 1994-1995 and in winter and summer 2011. The decrease in *C. nodosa* correlated with a 3-fold increase in the biomass of the green rhizophytic algae *Caulerpa prolifera* (Forsskål) J.V. Lamoroux over the same time period, although this increase varied notably among meadows. We also documented a negative correlation between the biomass of *C. nodosa* and *C. prolifera* at the island-scale, sampling 16 meadows in 2011. Experimental evidence demonstrated that *C. prolifera* can cause significant negative impacts on *C. nodosa*: plots with total (100%) removals of *C. prolifera* had ca. 2.5 more shoots and 3.5 times more biomass of *C. nodosa*, after 8 months, compared to plots with 50% removals and untouched control plots. Interference by *C. prolifera* appears to partially explain the decay in the abundance of *C. nodosa* populations in Gran Canaria. This study, however, did not identify potential underlying processes and/or environmental alterations that may have facilitated the disappearance of *C. nodosa*.

Keywords: seagrass, decline, competition, decadal trends, *Caulerpa*, Canary Islands.

1. Introduction

On shallow subtidal soft bottoms, seagrasses are the main foundation species from tropical to temperate oceans. The mechanisms behind their influence on community structure are multifaceted, but key ecological functions include modifying local environmental conditions and the provision of food and habitat for a wide range of organisms (Constanza et al., 1997). Conservation of these valuable habitats is therefore important, particularly since seagrass meadows are declining worldwide, mainly in areas of intense human activities (Duarte et al., 2008; Hughes et al., 2009; Waycott et al., 2009). There is, however, no information on seagrass distribution and abundance patterns from most coasts of the world over the last decades, and so many losses are unknown (Duarte et al., 2008).

Cymodocea nodosa (Ucria) Ascherson is a seagrass distributed across the Mediterranean Sea and the adjacent eastern Atlantic coasts, including the Macaronesian oceanic archipelagos of Madeira and the Canaries (Alberto et al., 2006; Mascaró et al., 2009). Meadows constituted by *C. nodosa* are the dominant vegetated communities in shallow soft substrates throughout the Canaries (Pavón-Salas et al., 2000; Barberá et al., 2005), where they provide food and shelter for diverse invertebrate and fish assemblages (Tuya et al., 2001; Tuya et al., 2006; Espino et al., 2011). These meadows are generally located along the eastern and southern coasts of the islands, sheltered from the dominant swells from the north and north-west. *C. nodosa* forms extensive, but often fragmented, subtidal meadows (Reyes et al., 1995a; Pavón-Salas et al., 2000; Espino et al., 2003; Barberá et al., 2005). In this region, *C. nodosa* shows a clear seasonal pattern, with a summer peak in shoot density and biomass (Reyes et al., 1995a, 1995b; Tuya et al., 2006), similar to what has been observed in the Mediterrean (e.g. Terrados and Ros, 1993).

In the Mediterranean, widespread decline of seagrass meadow has often resulted in the replacement by green algae such as *Caulerpa* (Ceccherelli and Cinelli, 1997; Lloret et al., 2005), though the time scale can affect interactions between *C. nodosa* and *Caulerpa*. For example, on the long-term, effects of *Caulerpa* on *C. nodosa* may not be so severe (Ceccherelli and Sechi, 2002). The ecological mechanisms behind these shifts in seagrass abundance (cover) vary between studies. For example, human-induced increases in nutrient loading and suspended sediments in the water column, involving a reduction in water transparency, can locally facilitate the replacement of *C. nodosa* by *Caulerpa prolifera* (Forsskål) J.V. Lamoroux (Lloret et al., 2005; Morris et al., 2009). In other circumstances, competition for nutrients in the sediment has been pointed out as the main ecological mechanism explaining the regression of *C. nodosa* and the concurrent expansion of *Caulerpa* beds (Ceccherelli and Cinelli, 1997).

In this study, we (i) compared the shoot density, leaf length and biomass of the seagrass *C. nodosa* in 5 seagrass meadows at the oceanic island of Gran Canaria (Canary Islands, eastern Atlantic) between 1994-1995 and 2011. Since we detected a sharp decrease in the abundance (shoot density and biomass) of *C. nodosa* that was partially matched with an increase in the biomass of the green, rhizophytic, native algae *C. prolifera*, we additionally (ii) tested whether the biomass of *C. nodosa* and *C. prolifera* are currently negatively correlated across the island, and in a manipulative field experiment (iii) tested if *C. prolifera* can have a negative effect on *C. nodosa*, i.e. whether removal of *C. prolifera* would increase the abundance of *C. nodosa*.

2. Materials and methods

2.1. Historical comparison

Five seagrass meadows (Table 1) were selected across the entire distribution area of *C. nodosa* in Gran Canaria. Each meadow was between 0.5-10 km apart from the adjacent studied meadow to encompass a range of conditions across the island. Each meadow was sampled in 4 occasions (Table 1), including a winter and summer season in 1994-1995, and winter and summer of 2011. On each sampling time, three cores (20 cm of inner diameter) were pushed into the sediment by a SCUBA diver. All material was then transferred to labelled bags and frozen (-20ºC) until being processed in the lab. For each sample, we counted the number of shoots (shoot density), as well as measured the length of 30 randomly selected leaves. The biomass was separated into leaves and rhizomes and subsequently oven-dried (24h at 70ºC) to obtain dry-weight biomass measurements. The dry biomass of all accompanying macroalgae, mainly the green algae *Caulerpa prolifera*, was also obtained. All measurements were standardized to m^{-2} to facilitate comparisons with other studies, and followed standardized procedures (Bortone, 2000). Temporal differences between years (hereafter mid-1990s *vs*. 2011), seasons (winter *vs*. summer) and sites (=meadows) for all demographic descriptors were tested by a 3-way permutation-based ANOVA, including the factors: 'Year' (fixed factor), 'Season' (fixed factor and orthogonal to 'Year') and 'Site' (random factor orthogonal to both 'Year' and 'Site'). Pairwise comparisons using permutations (Anderson, 2001) resolved differences between years for each site (significant 'Year x Site' interactions). Permutational Analysis of Variance uses permutations to calculate *P*-values. This was preferable because the data were over-dispersed and contained many zeros. In this sense, the Cochran's test was used to check for homogeneity of variances of each variable. However, no transformation rendered homogeneous variances for the biomass of *C. nodosa* and *C. prolifera* (Cochran's test, $p < 0.05$, for all type of transformations). The ANOVAs were then carried out on untransformed data, as it is

robust to heterogeneity of variances for large balanced experiments (Underwood, 1997). To avoid an increase in a type I error rate, α values were then established at a conservative value of 0.01 (Underwood, 1997). Similarly, the significance of pairwise comparisons was fixed at the α = 0.01 level. The test statistic (*pseudo-F*) is a multivariate analogue of the univariate Fisher's *F* ratio, and in the univariate context the two are identical when using Euclidean distance as the dissimilarity measure (Anderson, 2001).

2.2. Interaction between C. nodosa *and* C. prolifera*: comparative field analysis*

We sampled another 11 meadows (for a total of 16 meadows) in winter and summer of 2011 along the entire perimeter of Gran Canaria (Table 1), following the same criteria outlined previously. All these seagrass meadows were included in the shallow-water marine qualitative seagrass cartography of the island produced in 2002 and 2003 (Espino et al., 2003). A linear regression model tested whether the total biomass of *C. nodosa* and *C. prolifera* were significantly correlated at the island scale, i.e. including all 16 meadows, separately for winter and summer 2011, since both *C. nodosa* and *C. prolifera* show larger biomasses in summer than in winter in the Canary Islands (Reyes, 1993).

2.3 Interaction between C. nodosa *and* C. prolifera*: experimental approach*

We set up twelve 2 x 2 m plots on a mixed *C. nodosa* and *C. prolifera* meadow ('*Gando Castillo*', Table 1); adjacent plots were 2 m apart. Four plots were randomly assigned to each of 3 treatments: total (100%) removal of *C. prolifera* (ca. 140 ± 21 g

DW m^{-2}), partial removal (50%, ca.70 ± 9 g DW m^{-2}) and no removal (0%, procedural control) of *C. prolifera*. Removal was performed by SCUBA divers that carefully hand-picked stolons and blades of *C. prolifera*. An analogue disturbance, through flipping fins, but without actually removing *C. prolifera*, was conducted in control plots to avoid confounding results with manipulation artifacts. The experiment started on early March 2011 and lasted for 8 months; plots were visited every 5-6 weeks to maintain experimental treatments. At the end of the experimental period, 2 cores (20 cm of inner diameter) were collected, as previously described, from the center of each experimental plot, to avoid edge effects. All material was transferred to labelled bags and frozen (-20ºC) until processed in the lab, following the same routines outlined before. Differences in shoot density, leaf length and total biomass were tested with 1-way ANOVAs, including the factor 'Treatment' (as fixed) with 3 levels: 100, 50 and 0%). Pairwise comparisons resolved differences among treatments. ANOVAs were carried out on untransformed data, since all variables showed homogeneous variances among groups (Cochran's test, $p > 0.05$).

3. Results

3.1. Historical comparison

Shoot density, leaf length and total biomass of *C. nodosa* suffered a reduction from the mid-1990s to 2011 (Table 2) that resulted in significant differences between years (Table 3, 3-way ANOVA: 'Year', $p < 0.05$ in all cases). This reduction over time, however, varied in magnitude, but not in direction, from site to site (Table 3, 3-way ANOVA: 'Year x Site', $p < 0.01$ in all cases): pairwise comparisons showed a significant decrease, from the mid-1990s to 2011, for all descriptors, except leaf length,

at the 5 sites. At one site ('Arinaga', Table 2), the entire meadow had disappeared. Over the same time period, the total biomass of *C. prolifera* overall increased ca. 3-fold, although this trend varied notably among sites (Table 2) and resulted in an inconsistency in the differences between years (Table 3, 3-way ANOVA: 'Year x Site', $p < 0.01$). Post-hoc pairwise comparisons showed that only two meadows experienced a significant increase in the biomass of *C. prolifera*.

3.2. Interaction between C. nodosa *and* C. prolifera

In the island-scale comparison, biomass of *C. nodosa* and *C. prolifera* correlated negatively (Fig. 1) in the growing season (summer), but not in winter.

At the end of the experimental period, shoot density and total biomass of *C. nodosa* experienced a significant (Table 4, 1-way ANOVA: 'Treatment', $p < 0.0001$) increase in total *C. prolifera* removal plots (100%) relative to plots where *C. prolifera* was partially removed (50%) or left untouched (0%) (Figs. 2a and 2b, respectively). However, the leaf length of *C. nodosa* did not differ significantly between treatments (Fig. 2c, Table 4, 1-way ANOVA: 'Treatment', $p > 0.05$).

4. Discussion

We observed a severe decline in the abundance, i.e. shoot density and biomass, of the seagrass *Cymodocea nodosa* at Gran Canaria between the mid-1990s and 2011, which is consistent with other studies reporting declines in seagrass abundance over the last decades (Hall et al., 1999; Hemminga and Duarte, 2000; Hughes et al., 2002; Waycott et al., 2009). Similarly, a recent study has also documented an overall decrease

in the cover of *C. nodosa* meadows at Gran Canaria in the last 3 decades (Martínez-Samper, 2011).

The exact reasons for the loss of *C. nodosa* in Gran Canaria are not clear, but we here discuss possible patterns. Four of the five studied sites are far away from any well-defined, visible, sources of human-induced disturbance, such as sewage and brine outlets, fish farms and ports. The only exception is 'Arinaga', which could have been seriously affected by the construction of an industrial port, only 500 m away, 10 years ago (Martínez-Samper, 2011). This is the only site with a complete disappearance of *C. nodosa* and *C. prolifera* during the study period (Table 2). Therefore, it could well be that processes occurring at the island-scale, rather than punctual stressors have caused the seagrass deterioration at Gran Canaria.

Interactions with the green algae *C. prolifera* may partially contribute to the reduction in the abundance of *C. nodosa*, as we have empirically demonstrated here using both observational and experimental evidence. *Caulerpa prolifera* is a nitrophilic alga with clonal modular morphology (Collado-Vides, 2002). Contrary to many seaweeds, rhizoids of *C. prolifera* can take up nutrients from the sediment porewater, which may cover the total N requirement (Williams, 1984; Chisholm et al., 1996). In the Mediterranean, *C. prolifera* has been shown to overgrow the rhizomes of *Posidonia oceanica* and *C. nodosa* (Ceccherelli et al., 2000); therefore, species interactions in these systems may occur both in the below and above ground compartments, interfering respectively with nutrient acquisition and light availability. In the Mediterranean, the growth, as well as the germination, of *C. nodosa* has been shown to be nutrient limited (Pérez et al., 1991; Terrados and Ros, 1993; Balestri et al., 2010). This is likely pertinent for the Canary Islands, since adult leaves are considerably N-limited in the field (e.g. ~ 1% in N content, F. Tuya, unpublished data). Hence, it is plausible that *C.*

prolifera could limit nutrient uptake by *C. nodosa* from the sediment, in particular since *Caulerpa* species have a high N requirements to support their large production of secondary metabolites. In this sense, Malta et al. (2005) estimated the minimum and critical N levels of *C. prolifera* at 1.7 and 5.2 %, respectively. The genus *Caulerpa* typically holds high levels of terpenes, including caulerpenyne, as herbivore deterrents and natural antibiotics (Pesando et al., 1996; Jung et al., 2002), yet terpenes do not contain N molecules and contradicting data have been reported on proteins (Malta et al., 2005). It has been shown that allelochemicals can be involved in inter-specific competition among marine macrophytes. For example, caulerpenyne can be relevant in the interference of *Caulerpa* and *C. nodosa*, by decreasing photosynthetic yield (Raniello et al., 2007). In addition, *C. prolifera* may alter the biogeochemical conditions of the substrate by increasing organic matter pools, microbial activity and sulfide pools of the sediments which can negatively affect the performance of seagrasses, as has been described for meadows constituted by *Posidonia oceanica* in the Mediterranean (Holmer et al., 2009). Both *C. prolifera* and *C. nodosa* are native species in the study region, co-occurring on these sites for times much longer than the recently observed decline in *C. nodosa*. Apparently, some variable(s) has changed, altering the coexistence equilibrium between the species in favour of *C. prolifera*.

In the Mediterranean and the adjacent Atlantic coasts, competitive interactions between *C. nodosa* and *C. prolifera* have been suggested to explain the local regression of *C. nodosa* meadows (Lloret et al., 2005; Morris et al., 2009, respectively). In both studies, however, local changes in environmental conditions, e.g. turbidity and water column nutrient concentrations, were a source of disturbance that facilitated the replacement of *C. nodosa* by *C. prolifera*. These two studies (Lloret et al., 2005; Morris et al., 2009) come from sheltered estuarine/coastal lagoon systems quite different

relative to Gran Canaria: an ˈopenˈ oceanic island exposed to oceanic currents and large turbulence induced by waves. Oceanic swells, particularly in winter, are a source of physical disturbance that may remove large quantities of *C. nodosa* along the southern parts of Gran Canaria, accumulating as ˈwrackˈ on adjacent beaches (Portillo, 2008). As a result, it is plausible that *C. prolifera* may have taken advantage, after frequent storms that rip off seagrasses or other disturbances (e.g. abrupt sedimentation and light reduction events), to colonize open soft substrates that cannot be re-colonized by *C. nodosa* afterwards. This mechanism, however, remains untested and deserves attention in future studies. Moreover, meadows of *C. nodosa* are most likely susceptible to nutrient enrichment, as a result of the low nutrient concentrations in the water column (ca. 0.01 mg l^{-1} for nitrates and 0.1 mg l^{-1} for phosphates, F. Tuya unpublished data). Although we lack historical data for the study region on nutrient concentrations in the water column, oceanic nutrient concentrations have raised worldwide, even in nearly pristine environments (Halpern et al., 2008).

C. nodosa is a dioecous species, a trait that could lead to a higher vulnerability to population bottlenecks (Cunha et al., 2011). *C. nodosa* has a limited long-distance dispersal potential through seeds as a result of their negative buoyancy (Alberto et al., 2001), limiting successful meadow establishment to sporadic events (Alberto et al., 2005). Therefore, recovery from large disturbance events (e.g. storms, abrupt sedimentation, light reduction, etc.) may take long time, suggesting a low resilience of *C. nodosa* to disturbances in the study area.

References

Alberto, F., Mata, L., Santos, R., 2001. Genetic homogeneity in the seagrass

Cymodocea nodosa at is northern Atlantic limit revealed through RAPD. Mar. Ecol.

Prog. Ser. 221, 299–301.

Alberto, F., Arnaud-Haond, S., Duarte, C.M., Serrao, E.A., 2006. Genetic diversity of a

clonal angiosperm near its range limit: the case of *Cymodocea nodosa* at the Canary

Islands. Mar. Ecol. Prog. Ser. 309, 117-129.

Anderson, M.J., 2001. Permutation tests for univariate and multivariate analysis of

variance and regression. Can. J. Fish. Aquat. Sci. 58, 626-639.

Barberá, C., Tuya, F., Boyra, A., Sánchez-Jerez, P., Blanch, I., Haroun, R.J., 2005.

Spatial variation in the structural parameters of *Cymodocea nodosa* seagrass meadows

in the Canary Islands: a multiscaled approach. Bot. Mar. 48, 122-126.

Balestri, E., Vallerini, F., Lardicci, C., 2010. Effect of seed density and sediment

nutrient heterogeneity on recruitment and early patch growth in the seagrass *Cymodocea

nodosa.* Mar. Ecol. Prog. Ser. 417, 63–72.

Bortone, S.A., 2000. Seagrasses: monitoring, ecology, physiology and management. CRC Press, Boca Ratón, Florida.

Ceccherelli, G., Cinelli, F., 1997. Short-term effects of nutrient enrichment of the sediment and interactions between the seagrass *Cymodocea nodosa* and the introduced green alga *Caulerpa taxifolia* in a Mediterranean bay. J. Exp. Mar. Biol. Ecol. 217, 165-177.

Ceccherelli, G., Campo, D., 2002. Different effects of *Caulerpa racemosa* on two co-occurring seagrasses in the Mediterranean. Bot. Mar. 45, 71-76.

Ceccherelli, G., Sechi, N., 2002. Nutrient availability in the sediment and the reciprocal effects between the native seagrass *Cymodocea nodosa* and the introduced rhizophytic alga *Caulerpa taxifolia*. Hydrobiologia 474, 57–66.

Chisholm, J.R.M., Dauga, C., Ageron, E., Grimont, P.A.D., Jaubert, J.M., 1996. 'Roots' in mixotrophic algae. Nature 381, 382.

Collado-Vides, L., 2002. Morphological plasticity of *Caulerpa prolifera* (Caulerpales, Chlorophyta) in relation to growth form in a coral reef lagoon. Bot. Mar. 45, 123–129.

Constanza, R., D'arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., Van den Belt, M.,

1997. The value of the world's ecosystem services and natural capital. Nature 387: 253–260.

Cunha, A.H., Assis, J.F., Serrao, E.A., 2011. Seagrass in Portugal: a most endangered marine habitat. Aquat. Bot. (in press).

Duarte, C.M., Borum, J., Short, F., Walker, D., 2008. Seagrass ecosystems: their global status and prospects. In: Polunin, N. (Ed.), Aquatic Ecosystems. Cambridge University Press, Foundation for Environmental Conservation, pp. 281–294.

Espino, F., Garrido, M., Herrera, R., Tavío, O., 2003. Seguimiento de Poblaciones de Especies Amenazadas: *Cymodocea nodosa*, Gran Canaria. Informe Técnico de la Viceconsejería de Medio Ambiente, Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, Las Palmas.

Espino, E., Tuya, F., Brito, A., Haroun, R.J., 2011. Ichthyofauna associated with *Cymodocea nodosa* meadows in the Canarian Archipelago (central eastern Atlantic): Community structure and nursery role. Cien. Mar. 37, 157–174.

Hall, M.O., Durako, M.J., Fourqurean, J.W., Zieman, J.C., 1999. Decadal changes in seagrass distribution and abundance in Florida Bay. Estuaries 22, 445–459.

Halpern, B.S., Walbridge, S., Selko, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinermann, D., Lenihan,

H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. Science 319, 948-952.

Hemminga, M.A., Duarte, C.M., 2000. Seagrass Ecology. Cambridge Univ. Press, London.

Holmer, M., Marbá, N., Lamote, M., Duarte, C.M., 2009. Deterioration of sediment quality in seagrass meadows (*Posidonia oceanica*) invaded by macroalgae (*Caulerpa* sp.). Estuar. Coast. 32, 456–466.

Hughes, A.R., Williams, S.L., Duarte, C.M., Heck, K.L., Waycott, M., 2009. Associations of concern: Declining seagrasses and threatened dependent species. Front. Ecol. Environ. 7, 242–246.

Jung, V., Thibaut, T., Meinesz, A., Pohnert, G., 2002. Comparison of the wound-activated transformation of caulerpenyne by invasive and non-invasive *Caulerpa* species of the Mediterranean. J. Chem. Ecol. 28, 2091–2105.

Kenworthy, W.J. Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. USA 106, 12377–12381.

Lloret, J., Marin, A., Marin-Guirao, L., Velasco, J., 2005. Changes in macrophytes distribution in a hypersaline coastal lagoon associated with the development of intensively irrigated agriculture. Ocean Coast. Manage. 48, 828-842.

Malta, E.J., Ferreira, D.G., Vergara J.J., Pérez-Lloréns J.L. 2005. Nitrogen load and irradiance affect morphology, photosynthesis and growth of *Caulerpa prolifera* (Bryopsidales : Chlorophyta). Mar. Ecol. Prog. Ser. 298, 101-114.

Martínez-Samper, J., 2011. Análisis espacio-temporal de las praderas de *Cymodocea nodosa* (Ucria) Ascherson en la isla de Gran Canaria. Master Thesis, Universidad de Las Palmas de G.C.

Mascaró, O., Oliva, S., Pérez, M., Romero, J., 2009. Spatial variability in ecological attributes of the seagrass *Cymodocea nodosa*. Bot. Mar. 52, 5-429.

Morris, E.P., Peralta, G., Benavente, J., Freitas, R., Rodrigues, A.M., Quintino, V., Álvarez, O., Valcárcel-Pérez, N. Vergara, J.J., Hernández, I., Pérez-Lloréns, J.L., 2009. *Caulerpa prolifera* stable isotope ratios reveal anthropogenic nutrients within a tidal lagoon. Mar. Ecol. Prog. Ser. 390, 117–128.

Pavón-Salas, N., Herrera, R., Hernández-Guerra, A., Haroun, R., 2000. Distributional pattern of seagrasses in the Canary Islands (Central-East Atlantic Ocean). J. Coastal Res. 16, 329-335.

Pérez, M., Romero, J., Duarte, C., Sand-Jensen, K., 1991. Phosphorus limitation of *Cymodocea nodosa* growth. Mar. Biol. 109, 129-133.

Pesando, D., Leme´e, R., Ferrua, C., Amade, P., Girard, J.P., 1996. Effects of caulerpenyne, the major toxin from *Caulerpa taxifolia* on mechanisms related to sea urchin egg cleavage. Aquat. Toxicol. 35, 139–155.

Portillo, E., 2008. Arribazones de algas y plantas marinas en Gran Canaria: características, gestión y posibles usos. Instituto Tecnológico de Canarias, Las Palmas.

Raniello, R., Mollo, E., Lorenti, M., Gavagnin, M., Buia, M.C., 2009. Phytotoxic activity of caulerpenyne from the Mediterranean invasive variety of *Caulerpa racemosa*: a potential allelochemicals. Biol. Invas. 9: 361–368.

Reyes, J., 1993. Estudio de las praderas marinas de *Cymodocea nodosa* Cymodoceaceae, Magnoliophyta) y su comunidad de epifitos, en El Médano (Tenerife, Islas Canarias). PhD Thesis, Universidad de La Laguna, Tenerife.

Reyes, J., Sansón, M., Afonso-Carrillo, J., 1995a. Distribution and reproductive phenology of the seagrass *Cymodocea nodosa* (Ucria) Ascherson in the Canary Islands. Aquat. Bot., 50: 171-180.

Reyes, J., Sansón, M., Afonso-Carrillo, J., 1995b. Leaf phenology, growth and production of the seagrass *Cymodocea nodosa* at El Médano (south of Tenerife, Canary Islands). Bot. Mar. 38, 457-465.

Terrados, J., Ros, J.D., 1993. Limitación por nutrientes del crecimiento de *Cymodocea nodosa* (Ucria) Ascherson en sedimentos carbonatados en el Mar Menor, Murcia, SE de España. Boletín del Instituto Español de Oceanografía 11, 9-14.

Tuya, F., Pérez, J., Medina, L., Luque, A., 2001. Variaciones estacionales de la macrofauna invertebrada de tres praderas marina de *Cymodocea nodosa* en Gran Canaria (Centro-Este del océano Atlántico). Cien. Mar. 27, 223-234.

Tuya, F., Martín, J.A., Luque, A., 2006. Seasonal cycle of a *Cymodocea nodosa* seagrass meadow and of the associated ichthyofauna at Playa Dorada (Lanzarote, Canary Islands, eastern Atlantic). Cien. Mar. 32, 695-704.

Underwood, A.J., 1997. Experiments in Ecology: their logical design and interpretation using Analysis of Variance. Cambridge University Press, Cambridge.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, C.W, Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Willians, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. USA 106, 12377-12381.

Williams, S.L., 1984. Uptake of sediment ammonium and translocation in a marine green macroalga *Caulerpa cupressoides*. Limnol. Oceanogr. 29, 374–379.

Table 1. Geographical description of sites (= seagrass meadows, from north to south) sampled for *Cymodocea nodosa* and *Caulerpa prolifera* across the island of Gran Canaria; dates of sampling are included. Sites in bold are those considered for the historical comparison (sampled in both the mid-1990s and 2011), while the rest were only sampled in 2011.

				Mid-1990s		2011	
Site	**UTM X**	**UTM Y**	**Depth (m)**	**Winter**	**Summer**	**Winter**	**Summer**
Gando	463434	3089688	4	Jan-95	May-95	Feb	Aug
Gando-piscina	464252	3089224	15			Feb	Aug
Gando Castillo	463105	3089320	10			Feb	Aug
Gando-boya	463760	3089133	19			Feb	Aug
Roque de Arinaga	462589	3081567	14	Jan-95	Aug-94	Feb	Sep
Risco Verde	462095	3081237	10	Mar-95	Jul-94	Feb	Sep
Arinaga	460946	3081019	5	Jan-95	Jul-94	Feb	Sep
Arinaga-muelle viejo	461108	3080464	9			Feb	Sep
Arinaga-2	460863	3080742	7			Feb	Sep
Pasito Blanco Playa	439077	3069016	8			Feb	Aug
Pasito Blanco	439109	3068979	8	Dec-94	Aug-95	Feb	Aug
Pasito Blanco-fuera	439142	3068942	9			Feb	Aug
Centro-Com	439175	3068905	10			Feb	Aug
Meloneras-1	439224	3068923	5			Feb	Aug
Meloneras-2	439386	3068553	11			Feb	Aug
Faro Maspalomas	439550	3068460	12			Feb	Aug

Table 2. Shoot density (shoots m^{-2}), leaf length (cm), total biomass (g DW m^{-2}) of *Cymodocea nodosa* and total biomass (g DW m^{-2}) of *Caulerpa prolifera* at each site on each sampling occasion. Values are means ± SD (n=3).

			Risco Verde	Roque Arinaga	Arinaga	Gando	Pasito Blanco
		Winter	1866 ± 76	777 ± 581	1586 ± 441	1980 ± 299	629 ± 268
	Mid-1990s	Summer	1732 ± 120	1294 ± 101	3078 ± 1349	1650 ± 424	856 ± 263
		Winter	76 ± 19	164 ± 18	0	28 ± 21	57 ± 55
Shoot density	2011	Summer	66 ± 60	108 ± 36	0	42 ± 7	81 ± 33
		Winter	12.5 ± 2.9	17 ± 2.4	19.6 ± 3.9	12.1 ± 1.7	23 ± 4.3
	Mid-1990s	Summer	24.1 ± 1.2	28.6 ± 1.5	18.6 ± 2.1	17.6 ± 2.9	21.2 ± 2.2
		Winter	12.1 ± 1.4	8.4 ± 1.1	-	3.6 ± 1.9	12.1 ± 3
Leaf length	2011	Summer	24.1 ± 2.9	18.6 ± 2.7	-	6.4 ± 1.1	13.7 ± 1
		Winter	262.5 ± 0.5	334 ± 255	404.2 ± 79.1	333.3 ± 54.3	128.7 ± 20
	Mid-1990s	Summer	439 ± 78	942 ± 2	612.5 ± 335	366.7 ± 130.2	93.8 ± 84.2
		Winter	19.9 ± 1.9	56 ± 12	0	5.6 ± 5.7	17.2 ± 18
***C. nodosa* biomass**	2011	Summer	25.1 ± 12.6	45 ± 17.1	0	29.4 ± 27.8	54.5 ± 39.9
		Winter	0	34.4 ± 28.5	18 ± 5.3	10.3 ± 17.9	19.8 ± 33.1
	Mid-1990s	Summer	5 ± 5	108.5 ± 77.5	80.83 ± 91.1	18.6 ± 18.2	74.7 ± 54.8
		Winter	0	22.4 ± 20	0	0,7 ± 0.8	0,5 ± 0,9
***C. prolifera* biomass**	2011	Summer	4.8 ± 8.3	24.8 ± 28.9	0	0.5 ± 0.7	0

Table 3. Results of 3-way ANOVAs testing for differences in shoot density, leaf length and total biomass of *Cymodocea nodosa* and total biomass of *Caulerpa prolifera* between years (fixed factor, mid-1990s *vs.* 2011), seasons (fixed factor, winter *vs.* summer) and among sites (random factor).

Source of variation	df	Shoot density			Leaf length			*C. nodosa* total biomass			*C. prolifera* total biomass		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Year	1	2983467.34	23.57	0.0072	1388.88	10.38	0.0318	2013538.98	17.17	0.0132	4509.74	1.18	0.3568
Season	1	457111.28	1.21	0.3446	399.76	3.99	0.1166	164361.28	4.04	0.1158	325.77	0.06	0.8128
Site	4	1107301.13	7.88	0.0004	248.16	46.30	0.0002	119448.70	10.84	0.0002	1270.34	1.00	0.4062
Year x Season	1	486351.33	1.25	0.3304	0.33	0.09	0.7782	131414.64	2.45	0.1952	12780.93	22.39	0.0874
Year x Site	4	1399306.62	9.96	0.0002	133.74	24.96	0.0002	117234.94	10.64	0.0002	3807.91	2.99	0.0426
Season x Site	4	377483	2.69	0.0410	100.16	18.69	0.0002	40676.78	3.69	0.0118	5335.82	4.18	0.0128
Year x Season x Site	4	389980.17	2.78	0.0444	3.65	0.68	0.6	53626.35	4.87	0.0026	570.83	0.45	0.718
Residual	40	140423.20			5.36			11015.32			1275.14		

Table 4. Results of 1-way ANOVAs testing for differences in shoot density, leaf length and total biomass of *Cymodocea nodosa* between experimental treatments (fixed factor) at the end of the experimental period.

Source of variation	df	Shoot density			Leaf length			Total biomass		
		MS	F	P	MS	F	P	MS	F	P
Treatment	2	1030.16	20.75	0.0000	9.56	0.83	0.4489	26.62	17.22	0.0000
Residual	21	49.64			11.49			1.54		

Figure legends

Figure 1. Relationship between the total biomass of *Cymodocea nodosa* and *Caulerpa prolifera* at 16 sites throughout the island of Gran Canaria on winter and summer 2011. Linear regression models tested the significance of this relation separately for winter and summer 2011. Only the linear regression for summer is shown, as the winter regression was non-significant (p= 0.08).

Figure 2. Differences in (a) shoot density, (b) total biomass, and (c) leaf lenght of *Cymodocea nodosa* between plots where complete (100%), partial (50%) and no (0%, controls) removals of *Caulerpa prolifera* were carried out. Different letters above bars denote significant differences.

Contributions of each author:

Conceived and designed the study: FT, FE, RJH. Financially managed the study: FE, RJH. Performed the study in the field: FT, HH, FE. Performed the study in the lab: FT, HH. Analyzed the data: FT. Wrote the paper: FT. Commented on the paper: HH, FE, RJH. All authors have approved the final version of article.