



# Communication Plant and Meadow Structure Characterisation of Posidonia oceanica in Its Westernmost Distribution Range

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Abstract: Posidonia oceanica is an endemic seagrass species from the Mediterranean Sea that provides critical ecological services to coastal environments. This species is distributed from the Turkish to the Spanish coast, where its westernmost record was documented in Punta Chullera, Malaga (36°18'36.45" N, 5°14'54.31" W). Nevertheless, previous studies suggested that its distribution was even further west, although these populations were never described. In this study, we documented and characterised the only known P. oceanica population on the coast of Cadiz, in Cala Sardina (36°18'38.80" N, 5°15'15.13" W). The newly documented population of P. oceanica presented a fragmented structure, consisting of nine patches found in a rocky shallow area surrounded by the invasive algae Rugulopteryx okamurae, with a total size of 61.14 m<sup>2</sup>. Shoots had a relatively small size ( $21.0 \pm 2.9$  cm) in comparison with centrally-distributed populations. The relatively small size of the plants, alongside the observed low shoot density ( $437 \pm 42$  shoots m<sup>-2</sup>) and leaf area index  $(4.8 \pm 0.7 \text{ m}^2 \text{ m}^{-2})$ , may indicate that this meadow could be exposed to sub-optimal environmental conditions for plant development. By contrast, the meadow showed relatively high production rates ( $0.03 \pm 0.01$  leaf day<sup>-1</sup> shoot<sup>-1</sup>) in comparison with other Mediterranean populations. The percentage of carbon in plant leaves was  $38.73 \pm 1.38\%$ , while the nitrogen and C/N were  $1.38 \pm 0.37\%$ and  $29.93 \pm 6.57$ , respectively. The documentation of this meadow extends the distribution of this species to the Mediterranean coast of Cadiz, making this region the place with the highest seagrass biodiversity (four species) in the Iberian Peninsula, and potentially in Europe. This exploratory study provides a baseline to examine the potential effects of climate change, anthropogenic disturbances or the presence of invasive species.

Keywords: new record; Cadiz region; baseline; morphology; population structure; biochemistry; production; seagrass; Rugulopteryx okamurae

# 1. Introduction

Seagrasses are marine flowering plants that provide a wide variety of ecosystem services, such as their capacity to act as a carbon sink capturing great amounts of atmospheric  $CO_2$  and burying it into sediments, the reduction of pathogens in the environment or the enhancement of biodiversity [1,2]. Posidonia oceanica is a large-sized seagrass species endemic to the Mediterranean Sea that grows from the shallows to depths of up to 40 m in clear waters (e.g., Liguria) in sandy and rocky substrates [3]. It is distributed from the Turkish coast (eastern Mediterranean) to the southern Spanish coast (western Mediterranean), covering an extension of approximately 12,247 km<sup>2</sup> [4]. However, the high level of endemism, together with slow growth rates, makes this species highly vulnerable to anthropogenic pressures and climate change effects [5,6]. In the last few decades, several



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*P. oceanica* meadows have disappeared due to anthropogenic causes (e.g., illegal trawling or pollutants), estimating a reduction of approximately 10 to 30% of its extension over the last 50 years [4,7].

*Posidonia oceanica* is one of the most abundant seagrass species along the Mediterranean Iberian Peninsula coast. It is distributed from PortBou, Girona, Catalonia (north-eastern Spanish coast;  $42^{\circ}25'47.08''$  N,  $3^{\circ}9'58.64''$  E) to Punta Chullera, Malaga, Andalusia (southern Spanish coast;  $36^{\circ}18'36.45''$  N,  $5^{\circ}14'54.31''$  W) [8,9]. Extensive and well-established meadows are found in Cabo de Gata-Nijar Natural Park, Almeria, Andalusia (south-eastern Spanish coast). However, its distribution decreases towards the west, being to date very scarce in Malaga, where they have experienced a dramatic regression in the last few decades [9]. Although Malaga has been historically described as the westernmost area of distribution of this species [9,10], some chronicles have suggested its presence in the inner bay of Algeciras (eastern coast of Cadiz region) [11], but these populations were never documented [12]. Shaw (1993) also suggested the presence of *P. oceanica* specimens in the port of Gibraltar ( $36^{\circ}8'38.73''$  N,  $5^{\circ}21'48.63''$  W), at approximately 20 km from the westernmost *P. oceanica* population registered to date [13]. Recently, an attempt to verify the presence of this population in the port of Gibraltar was made by Bull et al. (2010); however, no *P. oceanica* specimens or remaining dead mattes were found [14].

Seagrass populations inhabiting the edges of their geographical distribution range are often exposed to less favourable environmental conditions for living in comparison with centrally distributed ones [15–17]. For instance, it could be expected that Andalusian *P. oceanica* populations, influenced by Atlantic water masses, are exposed to colder seawater temperature and lower salinity levels in comparison with populations from other Mediterranean regions [9]. Therefore, the study of these populations is of high relevance to examine the potential effects of global change alongside the shifts in their habitat range [18]. Seagrass traits, such as morphology, population structure, production or biochemistry have been widely used in seagrass ecosystems to assess changes in their health status due to their fast responsiveness to environmental changes [19–21].

With these considerations in mind, the main objective of this study was to document and characterise undescribed *P. oceanica* populations at their westernmost distribution limit by conducting a seagrass survey on the eastern coast of the Cadiz region, where previous chronicles and spatial models have suggested the presence of this species [11,13,22]. We hypothesise that the newly documented population exhibits relatively smaller aboveground sizes and displays lower growth rates than centrally and warmer adapted plants due to the less favourable environmental conditions of the study area.

#### 2. Materials and Methods

#### 2.1. Study Area

Based on the existing literature, using satellite-derived images, and the contribution of local divers, we identified Cala Sardina (Algeciras, Cadiz, Andalusia, Spain; 36°18′38.80″ N, 5°15′15.13″ W; Figure 1) as a potential area for the presence of *P. oceanica* meadows. A snorkelling-based survey was then conducted in August 2021 to cross-over the potential presence of the target species. The confirmed patchy meadow was located in a shallow (2–4 m depth) rocky area surrounded by the non-native algae *Rugulopteryx okamurae* (Figure 2). This meadow was located 530 meters to the west of the previously west-ernmost documented meadow at Punta Chullera, Malaga [8,9]. Moreover, a small patch of *Cymodocea nodosa* was found near to the *P. oceanica* patches at a depth of 1–2 m.

Cala Sardina is located in the western Alboran Sea, bordering with the Strait of Gibraltar, where the colder and fresher Atlantic water flowing eastward interacts with the deeper, warmer and saline Mediterranean water flowing westward. Moreover, the influence of strong winds favours an upwelling mechanism of subsurface waters, increasing the nutrient input to surface waters, especially in coastal areas. As a result of these interactions, a more productive, cold (Winter: 15 °C; Summer: 22 °C) and less saline (37 PSS) water mass is generated [23,24].



**Figure 1.** (**A**) Map of satellite-derived data of mean annual sea surface temperature (SST [°C]; https://www.bio-oracle.org/ (accessed on 9 May 2022)) in the Spanish Mediterranean coast and (**B**) map of the northwestern coast of the Alboran Sea showing the known locations of *P. oceanica* populations (brown circles) and the newly reported population (red circle). Records of known *P. oceanica* populations were derived from Chefaoui et al. (2017) [25]. (**C**) Map of the eastern coast of Cadiz region showing the locations of the newly documented *P. oceanica* meadow (red circle), the Punta Chullera's meadow (yellow circle; Record obtained from Ruiz et al. (2015) [9]), the port of Gibraltar (purple circle) and the bay of Algeciras (green circle). (**D**) Satellite image of Cala Sardina, Cadiz (36°18′38.80″ N, 5°15′15.13″ W; Google Earth Pro (https://earth.google.com (accessed on 9 May 2022)) showing the newly discovered patches of *P. oceanica*. (**E**) A photograph of one of the new documented *P. oceanica* patches.



**Figure 2.** Photographs of the newly documented *P. oceanica* population surrounded by the invasive algae *R. okamurae* at Cala Sardina (36°18′38.80″ N, 5°15′15.13″ W).

### 2.2. Morphometric Analyses

To evaluate morphometric descriptors, we randomly collected three vertical (orthotropic) shoots in each of the five largest patches (n = 5). Collected shoots were transported to the laboratory within zip-lock bags filled with seawater and stored in a cooler container. Once in the laboratory, each shoot was carefully cleaned of epiphytes and sediment. For each shoot, all leaves were numbered from the oldest (outermost leaf) to the youngest (innermost leaf). Plant analysis included measurements of leaf lengths (cm), leaf widths (cm), leaf thickness (mm) and number of leaves per shoot (leaves shoot<sup>-1</sup>). To obtain the leaf biomass (mg DW), leaves were cut at the height of the base and then the blades without sheaths were dried at 60 °C for 48 hours and then weighed. Finally, shoot leaf biomass (mg DW shoot<sup>-1</sup>) was estimated by summing the weights of each leaf within a single plant.

#### 2.3. Population Analyses

In each patch, *x* and *y* axes were measured and an elliptic shape was used to estimate patch area (m<sup>2</sup>). Population descriptors were also studied on the five largest patches. Shoot density (shoots m<sup>-2</sup>) was calculated by counting the number of shoots present in three  $20 \times 20$  cm quadrants randomly placed within each patch (*n* = 3). Data were then normalised per m<sup>2</sup>. To avoid damaging the meadow, aboveground (AG) biomass (kg DW m<sup>-2</sup>) was estimated by the product of shoot leaf biomass (weight of the leaves without sheath) and shoot density. Finally, the leaf area index (LAI; m<sup>2</sup> m<sup>-2</sup>) was assessed by multiplying the total leaf area per shoot (m<sup>2</sup>) of the patches by the shoot density (shoots m<sup>-2</sup>). In

addition, a comparative study of the extension of the meadow from 2008 to 2022 was conducted by analysing satellite-derived images taken from Google Earth Pro every 4 years (Figure S1). Analyses of the extension of the patches were calculated by measuring the longitudinal extension of the marked patches in meters using Google Earth Pro (https://earth.google.com (accessed on 9 May 2022)).

# 2.4. Production Analyses

Productivity descriptors were assessed by implementing a modified version adapted to *P. oceanica* of the punching method described by Peralta et al. (2000) [26]. A plastic tie and a small floater were allocated to the base of marked shoots to facilitate its recognition (August 2021). After 44 days (September 2021), marked shoots were collected and transported to the laboratory within zip-lock bags filled with seawater and stored in a cooler container. Once in the laboratory, each shoot was carefully cleaned of epiphytes and sediment, and then leaf growth rate (cm day<sup>-1</sup> shoot<sup>-1</sup>), leaf biomass production (mg DW day<sup>-1</sup> shoot<sup>-1</sup>) and leaf production (leaf day<sup>-1</sup> shoot<sup>-1</sup>) were assessed by implementing the following equations [27].

$$GR = \frac{\sum_{i=1}^{n} [(LL_{if} - LL_{i0}) > 0]}{t_f - t_0}$$
(1)

$$BP = \frac{\sum_{i=1}^{n} [(LL_{if} - LL_{i0}) > 0]}{t_f - t_0} * \frac{DW}{LL}$$
(2)

$$LP = \frac{\sum NL}{t_f - t_0} \tag{3}$$

where *GR* is leaf growth rate (cm day<sup>-1</sup> shoot<sup>-1</sup>); *BP* is leaf biomass production (mg *DW* day<sup>-1</sup> shoot<sup>-1</sup>); *LP* is leaf production (leaf day<sup>-1</sup> shoot<sup>-1</sup>); *LL* is the leaf length (cm), subscript *i* refers to the *i*<sup>th</sup> leaf of the shoot, subscript *f* refers to the final moment and subscript 0 refers to the initial moment;  $t_f - t_0$  refers to the marked period in days;  $\frac{DW}{LL}$  refers to the dry weight-length ratio (mg DW cm<sup>-1</sup> leaf<sup>-1</sup>); *NL* refers to the number of new (unmarked) leaves (new leaves shoot<sup>-1</sup>).

#### 2.5. Biochemical Analyses

Intact green leaves without signs of necrosis (second youngest leaf) were randomly collected from the four largest patches for biochemical analysis (n = 4). Leaves were frozen at -80 °C for 48 h and then freeze-dried. Carbon and nitrogen contents were analysed by using a high temperature catalytic oxidation with an elemental analyser Perkin Elmer 2400.

# 3. Results

The studied morphometric descriptors of the newly described population, including leaf length, width and thickness, showed average values of 21.0  $\pm$  2.9 cm, 1.0  $\pm$  0.03 cm and  $0.31\pm0.03$  mm, respectively, while the average of maximum shoot length was 29.9  $\pm$  4.2 cm (Table 1). The number of leaves was  $6 \pm 1$  leaves shoot<sup>-1</sup>, where the majority of the oldest leaves showed signs of herbivory. Shoot biomass was  $633.0 \pm 77.1$  mg DW shoot<sup>-1</sup>. The newly documented P. oceanica population was formed by 9 patches separated approximately 2-7 m from each other with a total extension of 61.14 m<sup>2</sup>. Shoot density showed an average value of  $437 \pm 42$  shoots m<sup>-2</sup> and an AG biomass of  $0.28 \pm 0.03$  kg DW m<sup>-2</sup> (Table 2). Leaf area index (LAI) had a mean value of  $4.8 \pm 0.7$  m<sup>2</sup> m<sup>-2</sup>. In relation to the productivity parameters, only four labelled shoots were found after 44 days. The leaf growth rate, leaf biomass production and leaf production of the four shoots reported mean values of  $0.7 \pm 0.2$  cm day<sup>-1</sup> shoot<sup>-1</sup>,  $4.3 \pm 1.2$  mg DW day<sup>-1</sup> shoot<sup>-1</sup> and  $0.03 \pm 0.01$  leaf day<sup>-1</sup> shoot<sup>-1</sup>, respectively. Based on our results, the studied meadow yielded an annual leaf biomass production of  $0.68 \pm 0.18$  kg DW m<sup>-2</sup> year<sup>-1</sup>, which could correspond to an annual carbon fixation of  $0.98 \pm 0.26$  kg CO<sub>2</sub> m<sup>-2</sup> year<sup>-1</sup>. Carbon content in seagrass leaves was  $38.73 \pm 1.38\%$ , while nitrogen was 1.38  $\pm$  0.37%, and C/N was 29.93  $\pm$  6.57. Lastly, satellite-derived

images showed that the extension of the patchy meadow remained constant over the last 14 years.

**Table 1.** Morphometric descriptors: leaf (L) length (cm), leaf width (cm), leaf thickness (mm), leaf biomass (mg DW), shoot leaf biomass (mg DW shoot<sup>-1</sup>), number of leaves (leaves shoot<sup>-1</sup>) and production descriptors: leaf growth rate (cm day<sup>-1</sup> shoot<sup>-1</sup>), leaf biomass production (mg DW day<sup>-1</sup> shoot<sup>-1</sup>), leaf production (leaf day<sup>-1</sup> shoot<sup>-1</sup>) of shoots from the newly reported *P. oceanica* population (36°18′38.80″ N, 5°15′15.13″ W) studied during August 2021. Data represents mean ± standard deviation. (-): no data available. \* Youngest leaves (<5 cm) were not taken into account for the calculation of the mean value.

	Leaf (L)						
Descriptors	L-1	L-2	L-3	L-4	L-5	L-6	Leaf Average
Leaf length (cm) Leaf width (cm) Leaf thickness (mm)	$\begin{array}{c} 18.2 \pm 5.1 \\ 1.1 \pm 0.02 \\ 0.51 \pm 0.06 \end{array}$	$\begin{array}{c} 23.5 \pm 6.4 \\ 1.1 \pm 0.1 \\ 0.38 \pm 0.05 \end{array}$	$\begin{array}{c} 27.6 \pm 5.2 \\ 1.0 \pm 0.1 \\ 0.32 \pm 0.05 \end{array}$	$\begin{array}{c} 21.5 \pm 3.7 \\ 1.0 \pm 0.1 \\ 0.23 \pm 0.03 \end{array}$	$\begin{array}{c} 13.7 \pm 4.0 \\ 1.0 \pm 0.1 \\ 0.18 \pm 0.01 \end{array}$	$\begin{array}{c} 4.3 \pm 1.9 \\ 1.0 \pm 0.0 \\ 0.15 \pm 0.01 \end{array}$	$\begin{array}{c} 21.0 \pm 2.9 \ ^* \\ 1.0 \pm 0.03 \\ 0.31 \pm 0.03 \end{array}$
Leaf biomass (mg DW)	$109.0\pm31.9$	$141.9\pm40.2$	$167.9\pm32.6$	$129.7\pm23.1$	$80.6\pm25.1$	$22.1\pm12.0$	$108.5\pm47.2~^{*}$
Shoot leaf biomass (mg DW shoot <sup>-1</sup> )	-	-	-	-	-	-	$633.0\pm77.1$
Shoot leaves (leaves shoot $^{-1}$ )	-	-	-	-	-	-	$6\pm1$
Growth rate $(\text{cm day}^{-1} \text{ shoot}^{-1})$	-	-	-	-	-	-	$0.7\pm0.2$
$\begin{array}{c} \text{Biomass production} \\ (\text{mg DW day}^{-1} \\ \text{shoot}^{-1}) \end{array}$	-	-	-	-	-	-	$4.3\pm1.2$
Leaf production (leaf day $^{-1}$ shoot $^{-1}$ )	-	-	-	-	-	-	$0.03\pm0.01$

**Table 2.** Population descriptors: shoot density (shoots m<sup>-2</sup>), aboveground (AG) biomass (kg DW m<sup>-2</sup>) and leaf area index (LAI; m<sup>2</sup> m<sup>-2</sup>) of the newly reported *P. oceanica* population (36°18′38.80″ N, 5°15′15.13″ W) studied during August 2021. Data are represented as mean  $\pm$  standard deviation. (-): no data available. \* Patches with a size of <1 m<sup>2</sup> were excluded from the calculation of the mean.

	Patch Size	Shoot Density	AG Biomass	LAI
Patch	(m <sup>2</sup> )	(Shoots m <sup>-2</sup> )	(kg DW m <sup><math>-2</math></sup> )	$(m^2 m^{-2})$
1	10.0	$408\pm42$	$0.26\pm0.03$	$4.4\pm1.2$
2	17.6	$475\pm20$	$0.30\pm0.01$	$5.8\pm0.8$
3	20.2	$425\pm20$	$0.27\pm0.01$	$5.3 \pm 1.8$
4	5.2	$408\pm31$	$0.26\pm0.02$	$3.8\pm0.5$
5	5.7	$467\pm31$	$0.30\pm0.02$	$4.8\pm1.1$
6	1.0	-	-	-
7	0.5	-	-	-
8	0.5	-	-	-
9	0.5	-	-	-
Mean	$9.8\pm6.5$ *	$437\pm42$	$0.28\pm0.03$	$4.8\pm0.7$

#### 4. Discussion

In this study, as a result of the coastal survey conducted in Cala Sardina (36°18'38.80" N, 5°15'15.13" W) during August 2021, we documented and characterised the only known *P. oceanica* population along the coast of Cadiz, potentially resulting in the westernmost described population to date in the literature. Furthermore, the documentation of this population increases the number of seagrass species in Cadiz coasts up to four (*Zostera marina*, *Z. noltei*, *C. nodosa* and *P. oceanica*), turning Cadiz into the region with the highest seagrass biodiversity of the Iberian Peninsula, and potentially of Europe.

Most of the morphometric descriptors of the newly documented population, including leaf width, thickness and number of leaves, were within the expected ranges for this species [28–30]. Shoot size was relatively small compared with shoots lengths (20 to 100 cm) from warmer and centrally adapted populations [31–33], but it was in agreement with those reported in populations from the southern Iberian Peninsula (16 to 33 cm), exposed to similar environmental settings [31,34]. Small plant sizes (12 to 36 cm) were also observed in disturbed and cold-adapted populations from Liguria [35,36].

The newly described population presented a fragmented patchy structure. In addition, based on satellite-derived images (Figure S1), we estimated that the number and extension of the meadow has remained relatively constant over the past 14 years. However, since only the largest five of the nine patches were identified by using satellite-derived images, our estimation should be considered as exploratory. Patches smaller than 1 m<sup>2</sup> were not ascertained by using satellite images. The observed densities of the population are especially low compared with meadows from the southern Iberian Peninsula (800 shoots  $m^{-2}$ ) and western-distributed shallow populations from Catalonia (>700 shoots  $m^{-2}$ ) [10,37]. However, our results are more similar to those observed in anthropogenically impacted meadows [38], as for example in Murcia or Liguria, where densities lower than 400 shoots  $m^{-2}$  have been reported [36,39,40]. The LAI of the meadows was also low in comparison with centrally and warmer distributed populations. For instance, shallow populations from the Adriatic Sea or from Greece showed a LAI ranging from 8 to  $12 \text{ m}^2 \text{ m}^{-2}$  [41,42]. The lower plant size, densities and LAI observed in the patchy population from Cadiz could be attributed to sub-optimal seawater temperatures for plant development, associated with the influence of cold Atlantic waters [31,43,44], thus partially confirming our stated hypothesis. Notably, other reasons may also explain the observed morphological and population characteristics of the newly documented meadows: (i) the shallow location of the population (2-4 m)could limit plant development due its exposure to high hydrodynamic forces [45,46], (ii) plants may suffer photo-inhibition due to high irradiances [47], and (iii) plants growing in rocky substrates usually do not develop large aboveground structures [48].

Surprisingly, leaf production of the newly documented population was relatively high ( $10.37 \pm 3.59$  leaves per year) compared with centrally distributed populations along the Mediterranean Sea, such as France, eastern Spain, Italy or Turkey, which produce between 6 to 8 leaves per year [49–51]. Carbon content and C/N on leaves of the studied population also appeared to be high compared with warm-adapted populations, while leaf nitrogen content was likely similar [52–54]. Noteworthy, it is important to note that our measurements were taken only one time during the season of maximum production, therefore our results should be considered as exploratory.

Seagrass distribution is mainly controlled by abiotic factors (e.g., temperature, salinity, light conditions and nutrient levels), but also by biotic factors (e.g., invasive species, grazing) [55]. In the context of climate change, predicted increases in SST would threaten the survival of *P. oceanica* in most of the Mediterranean Sea [5,22]. However, despite this dramatic projection, it is also expected that *P. oceanica* may find refuge in the western Mediterranean Sea, where SST will not exceed the thermal threshold of this species [25]. Moreover, the survival of the newly documented population could be endangered by the high abundance of *R. okamurae*. This invasive algae species showed an exceptional capacity to colonize new ecosystems due to diverse ecological and environmental acclimations (e.g., toxic natural compounds), and it is currently endangering the biodiversity of the Strait of Gibraltar [56,57]. However, the are no studies to date assessing the biological interaction of *P. oceanica* and *R. okamurae*. Notably, genetic studies will be of interest to determine the genetic connection of this population with southern meadows and from other Mediterranean regions.

Lastly, the creation of a baseline for this species at its western limit of distribution in the Mediterranean Sea is of great ecological and conservation value for several reasons: (i) to increase the ecological knowledge in areas under-studied, where meadows have reported a progressive habitat loss in the last few decades, (ii) to assess whether seagrass meadows acclimate to less favourable environmental conditions, and also (iii) to examine the long-term effects of global change.

# 5. Conclusions

This study describes and characterises the only documented *P. oceanica* population in the region of Cadiz, increasing knowledge of the ecology of this species in its western geographical limit. Our results indicate that the newly described population may be exposed to suboptimal climate conditions for plant and meadow development. Furthermore, it could be threatened by the presence of the invasive algae *R. okamurae*. To conclude, more research should be carried out to assess the effects of global change in order to implement effective science-based conservation initiatives in this region.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/d15010101/s1, Figure S1: Satellite images of the newly reported *P. oceanica* meadow at Cala Sardina (36°18'38.80" N, 5°15'15.13" W) taken from Google Earth Pro (https://earth. google.com (accessed on 9 May 2022)) every 4 years, from 2008 to 2020.

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