

## Marine Environmental Research

# Reconstructed life history metrics of the iconic seagrass *Posidonia oceanica* (L.) detect localized anthropogenic disturbance signatures

--Manuscript Draft--

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<b>Abstract:</b>	Substantial losses of the seagrass <i>Posidonia oceanica</i> have initiated investigations into localized resilience declines related to anthropogenic disturbances. In this study, we determined reconstructed shoot age and interannual growth metrics can detect anthropogenic impact effects on <i>P. oceanica</i> production. Interannual vertical growth, leaf production, and demographics of shoots collected from sewage and trawling impacted areas were examined using mixed effects modeling. Detected impact effects were specific to the type of impact, manifesting as an older-skewed age distribution of sewage outfall shoots and reduced vertical growth and reduced leaf production of trawling site shoots. A stress event period was also detected for all shoots, with trawling impacted shoots indicating little recovery. Reconstructed age and growth metrics are simple to measure, incorporate multiple years of in situ shoot development, and are advantageous for identification of declining <i>P. oceanica</i> resilience prior to catastrophic losses.
<b>Suggested Reviewers:</b>	<p>Juan Manuel Ruiz Fernandez Spanish Institute of Oceanography juanm.ruiz@ieo.csic.es Strong familiarity with <i>Posidonia oceanica</i> and the influence of anthropogenic stressors on seagrass meadows</p> <p>Xavier Buñuel Center for Advanced Studies of Blanes xbunuel@ceab.csic.es Familiarity with climate change related impacts on <i>Posidonia oceanica</i> interactions and habitat</p> <p>Candela Marco Mendez Center for Advanced Studies of Blanes c.marco@ceab.csic.es Strong familiarity with <i>Posidonia oceanica</i> and other seagrass systems, as well as anthropogenic and climate related stressor effects on these systems</p> <p>Matthew Fraser The University of Western Australia matthew.fraser@uwa.edu.au Strong familiarity with stressor effects on seagrass ecosystems</p>
<b>Response to Reviewers:</b>	



Institute of Food and Agricultural Sciences  
Nature Coast Biological Station

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Dear Marine Environmental Research Editor(s),

My coauthors and I are pleased to resubmit our manuscript titled “Reconstructive life history metrics of the iconic seagrass *Posidonia oceanica* (L.) detect localized anthropogenic disturbance signatures” to be considered for publication in Marine Environmental Research. After consideration of the helpful reviewer comments that we received on our original submission, we have made changes that we believe address the intended points of each comment/suggestion raised. We thank the editor and anonymous reviewer for their time and effort in providing supportive feedback.

With regards on behalf of myself and coauthors,

A handwritten signature in black ink, appearing to read 'Ashley McDonald', with a large, stylized flourish at the end.

Ashley McDonald, Research Scientist, UF | IFAS Nature Coast Biological Station.

AM McDonald and coauthors' response to reviewers for manuscript "Reconstructed life history metrics of the iconic seagrass *Posidonia oceanica* (L.) detect localized anthropogenic disturbance signatures."

Thank you to our editor and helpful reviewer for your comments, we were happy to make the suggested changes to produce a more well-rounded manuscript.

Reviewer Comments:

- Introduction: other local should be at least cited in intro, namely: boat anchoring and coastal infrastructure building.
  - We intended the terminology of "coastal development" to be a catch-all term that would include any coastal construction activities, such as infrastructure building, but we did add the term "recreational boating" to our list (LL 37) to include both boat anchoring and prop-scarring.
- LL169: Here, and elsewhere in the ms, please precise it is rhizome growth rate.
  - Rhizome vertical growth was clarified here and in the following places: LL18; LL108; LL166; LL201; LL227; LL350; LL378; LL402.
- I am also a little confused by the term "leaf production" which could be understood as leaf primary production which is not the case. I suggest to define the used terms in your mat met.
  - Response- Wording was clarified in the following places: subheading title (LL189) and defined as number of leaves produced in materials and methods (LL 202).
- How do you explain the persistence of trawling effect eight years after cessation? Is it related to loss of seagrass bed and resuspension of sediment?
  - Persistence of trawling effects after cessation was not addressed in our manuscript, due to lack of environmental measurements, but the original authors of the manuscript we consulted to compare our results (Gonzalez-Correa et al., 2005) suppose it is linked to instability of sediments caused by prior trawling efforts that allows for easier resuspension, and this point was added to text to clarify (LL 406). In our manuscript, it has been noted that illegal trawling was still occurring at trawl impact sites, which could explain ongoing sediment resuspension occurring in proximity to where shoots were collected.
- I remember that 2006-2007 was a very particular year everywhere in Mediterranean not specially in term of heat wave but in term of low wind and low winter water mixing and consequently low nutrient input in shallow areas. Globally, my comment here is that heat waves is not the only "natural" process explaining *P. oceanica* production annual variability. I suggest to read papers of Champenois and Borges (Limnology and Oceanography) or Mateo et al on long term variability.
  - We thank the reviewer for suggesting this literature, and agree that nutrient limitation caused by water column stratification is a realistic potential to be a largescale stressor that limits *P. oceanica* production. The following changes/additions have been made to address this point:
  - Changed the following text when first addressing "mid-2000s Stress event" in mat met (LL232): "Limitations must be set due to the impossibility of retroactively determining a definitive cause to this decline, however we intended to use modeling techniques to

investigate any potential effects of this unconfirmed but broad-reaching event on rhizome growth and leaf production recovery from RT 2007 onward."

- In the section of Discussion that addresses the "mid-2000s stress event" (5th paragraph, Lines 411-434), the following text was altered to reduce the certainty of a "heat wave event" and include other potential large scale environmental stressors that may have occurred: (Sentences at LL 414-420) "The Mediterranean region has been designated a "hot spot" of climate change intensification (Diffenbaugh et al., 2007), increasing the frequency of marine heat wave events (Oliver et al., 2018) that have resulted in mass mortalities of *P. oceanica* and sessile benthic invertebrates (Marbà and Duarte, 2010; Crisci et al., 2011; Rivetti et al., 2014). .Also, a climate change-related increase in stratification events can severely reduce benthic community production due to water column stability and exhaustion of surface layer nutrients (Coma et al., 2009)."
- (Sentence at LL 425-429) "Largescale climate events can also exacerbate more frequent natural occurrences, such as mild winter seasons where a lack of winter storms leads to a reduction in *P. oceanica* community net primary production due to a buildup of *P. oceanica* leaf litter that blocks light to benthic algae and increases respiration (Champenois and Borges, 2012; Champenois and Borges, 2021)."
- (Sentence at LL 431-434) "Therefore, although the identity of the large-scale stressor that caused synchronous shoot growth declines can only be speculated on, our findings reiterate how interactions of climate change, anthropogenic alterations, and naturally occurring conditions can negatively influence production of *P. oceanica*."

### Highlights:

- Localized stressors lower *Posidonia oceanica* resilience to larger-scale disturbances.
- We examined stress impacts through reconstruction of *P. oceanica* life history.
- Sewage and trawling impacts were elucidated using reconstruction methods.
- The way *P. oceanica* life history was influenced differed by stressor type.
- Trawl impacted seagrass showed evidence of low recovery from large-scale stressor.

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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1 Reconstructed life history metrics of the iconic seagrass *Posidonia oceanica* (L.)  
2 detect localized anthropogenic disturbance signatures

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14 Abstract

15 Substantial losses of the seagrass *Posidonia oceanica* have initiated investigations into  
16 localized resilience declines related to anthropogenic disturbances. In this study, we determined  
17 reconstructed shoot age and interannual growth metrics can detect anthropogenic impact effects  
18 on *P. oceanica* production. Interannual rhizome vertical growth, leaf production, and  
19 demographics of shoots collected from sewage and trawling impacted areas were examined using  
20 mixed effects modeling. Detected impact effects were specific to the type of impact, manifesting  
21 as an older-skewed age distribution of sewage outfall shoots and reduced vertical growth and  
22 reduced leaf production of trawling site shoots. A stress event period was also detected for all  
23 shoots >5 years old, with trawling impacted shoots indicating little recovery. Reconstructed age  
24 and growth metrics are simple to measure, incorporate multiple years of in situ shoot  
25 development, and are advantageous for identification of declining *P. oceanica* resilience prior to  
26 catastrophic losses.

27 Keywords: Mediterranean Sea, sewage, trawling, seagrass, life history, lepidochronology, leaf  
28 production, shoot age, vertical growth, resilience

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30 1. Introduction

31 Anthropogenic activities are a considerable driving force influencing the production and  
32 subsequent function of coastal vegetated habitats. A peak in global human population near the  
33 coast (Bengtsson et al., 2006) has vastly increased reliance on coastal and marine resources,  
34 resulting in alteration of the functionality and resilience of saltmarshes, mangrove forests, kelp  
35 forests, and seagrass meadows that fringe the globe’s highly populated temperate, subtropical,  
36 and tropical coastlines (Boudouresque et al., 2009; Feist and Levin, 2016). Localized human  
37 alterations linked to coastal development, recreational boating, aquaculture, fisheries, and other  
38 natural resource extractions (e.g. oil and gas exploration) can be substantial sources of stress to  
39 coastal habitats due to nutrient enrichment (Malone and Newton, 2020), sedimentation (Serrano  
40 et al., 2016), trophic disruption (Heck et al., 2000; Östman et al., 2016), and habitat  
41 fragmentation (Turner, 1997; Gilby et al., 2021), despite occurring on relatively small spatial  
42 scales (i.e. tens of kilometers or fewer). Furthermore, these small-scale stressors can indirectly  
43 lead to substantial habitat losses by lessening the resilience of habitat-forming species to  
44 stochastic large-scale environmental perturbations, such as coastal flooding (Hanley et al., 2020)  
45 or anomalous temperature events (Wernberg et al., 2016). To inform the search for more  
46 effective protections of these coastal habitat-forming plant communities, it is essential to  
47 determine how localized anthropogenic stressors erode resilience features and how the loss of  
48 such features may be quantified prior to catastrophic habitat loss.

49 In the Mediterranean Sea, localized anthropogenic pressures have a high impact on the  
50 extensive seagrass meadows that are formed primarily by the endemic Neptune grass (*Posidonia*  
51 *oceanica*). This single submerged habitat-forming species provides an estimated monetary value  
52 of €190 million year<sup>-1</sup> as habitat for targeted fisheries species (Boudouresque et al., 2006;

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4 53 Jackson et al., 2015), is part of the trophically important submerged benthic community that  
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6 54 contributes substantially to marine production (Cebrián et al., 1996; Prado et al., 2007), and  
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9 55 serves as a substantial sediment and carbon sink (Boudouresque et al., 2009; Monnier et al.,  
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11 56 2021). This long lived, slow growing species has shown widespread regression at an estimated  
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14 57 13-38% of total areal coverage throughout the western Mediterranean during the last five  
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16 58 decades (Telesca et al., 2015; Blanco-Murillo et al., 2022), and extensive meadow fragmentation  
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19 59 has occurred in the last two decades (Marbà et al., 2014). Nutrient enrichment, land use change,  
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21 60 coastal development, and other factors associated with anthropogenic activities have been  
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24 61 implicated in meadow regressions (Boudouresque et al, 2009; Tamburello et al., 2012), with  
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26 62 losses becoming further exacerbated by increasingly frequent extreme climate events (Marbà and  
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29 63 Duarte, 2010; Guerrero-Meseguer et al., 2020; Helber et al., 2021). International protections of  
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31 64 Mediterranean seafloor habitats (EU Habitat Directive 92/43/CEE, Bern and Barcelona  
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34 65 Conventions, Marine Strategy Framework Directive 2008/56/EC, among others) have been  
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36 66 established to monitor and conserve *P. oceanica* meadows, however this species' extreme slow  
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39 67 growth and limited reproduction make natural reestablishment in degraded areas irrecoverable on  
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41 68 the timescales of human generations (González-Correa et al., 2005; Marbà, 2009). Thus, in some  
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44 69 Mediterranean countries such as Spain, the current objective of *P. oceanica* conservation efforts  
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46 70 is to slow or prevent further losses through identification, assessment, and mitigation of threats to  
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49 71 seagrass resilience (LIFE, 2014).

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51 72 The province of Alicante, on the Mediterranean coast in eastern Spain, is the location of  
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53 73 an estimated 212 km<sup>2</sup> of *P. oceanica* meadows (Sánchez-Lizaso et al., 2002). In this region, *P.*  
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55 74 *oceanica* meadow declines over the last 20 years were driven primarily by localized  
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58 75 anthropogenic disturbances (Blanco-Murillo et al., 2022). Wastewater outflow and illegal  
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4 76 benthic trawling are priority environmental issues of concern regarding negative effects on water  
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6 77 quality and *P. oceanica* meadow health (European Environment Agency, 2006). Two wastewater  
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8 78 treatment plants service the province of Alicante, with a resident population of over 1.8 million  
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10 79 (Instituto Nacional de Estadística, 2017). These treatment plants are only two of the approximate  
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12 80 1,681 wastewater treatment plants that release effluent into the Mediterranean Basin, with  
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14 81 treatment processes throughout the Basin ranging from untreated to tertiary treatment for  
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16 82 lowering biological oxygen demand (UNEP, 2011) Wastewater discharges are equivalent to  
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18 83 riverine inputs of nitrogen and phosphorus to the Mediterranean Sea, substantially contributing  
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20 84 to nutrient loading of the coastal waters (Powley et al., 2016). Benthic trawling is the second  
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22 85 most destructive localized anthropogenic practice in this area and is banned in waters down to 50  
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24 86 m depth for all EU member states, including Spain, to restrict the practice from occurring in *P.*  
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26 87 *oceanica* meadow depths (European Union, 1994). However, frequent trawling in restricted *P.*  
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28 88 *oceanica* meadows at depths shallower than 50 m still takes place illegally (Díaz-Almela and  
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30 89 Duarte, 2008).

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38 90       Spatially explicit, direct impacts such as organic material deposition from sewage outfall  
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40 91 plumes and illegal benthic trawling induce stress effects on *P. oceanica* shoot production directly  
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42 92 (e.g. burial, mechanical destruction of shoots) and indirectly (e.g. light limitation, nutrient  
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44 93 enrichment) (Sánchez-Lizaso et al., 2002; Balestri et al., 2004; Boudouresque et al., 2009).  
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46 94 Evidence from laboratory experiments and field monitoring studies suggest that production  
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48 95 declines caused by anthropogenic stressors also reduce seagrass resilience (Gera et al., 2013;  
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50 96 Ontoria et al., 2019; Helber et al., 2021) and that these shoots are more likely to succumb to the  
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52 97 stress of an extreme event (Pazzaglia et al., 2020), although the strength of this effect is possibly  
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54 98 species specific (see Connolly et al., 2018) and long-term studies are still needed. *P. oceanica*  
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99 meadows are particularly vulnerable to extreme heat wave events (Marbà and Duarte, 2010) that  
100 have also caused mass mortalities of sessile benthic invertebrates in the Mediterranean (Rivetti et  
101 al., 2014). These mass mortalities are likely the result of the benthic communities' extended  
102 exposure time to thermal anomalies (Crisci et al., 2011), rather than a single temperature  
103 threshold point, suggesting an erosion of the organisms' resilience features over the exposure  
104 period. The Mediterranean region is currently designated as a "hot spot" of climate change  
105 related heat stress intensification (Diffenbaugh et al., 2007) with a high likelihood of increasing  
106 frequency of these extended heat stress periods for sessile benthic organisms (Oliver et al.,  
107 2018).

108         In this study, we examine the appropriateness of using the *P. oceanica* life history metrics  
109 of orthotropic shoot age structure, rhizome vertical growth patterns, and leaf production to  
110 indicate effects of the localized, direct anthropogenic impacts of illegal trawling and sewage  
111 outfall. Previous studies have shown that these metrics, which represent production over multiple  
112 growth seasons over the entire lifetime of a shoot, have high potential to characterize the growth  
113 environment of meadows afflicted with both natural and anthropogenic stressors (Duarte et al.,  
114 1994; Guidetti, 2001; Olesen et al., 2002; Balestri et al., 2004; González-Correa et al., 2005;  
115 Marbà et al., 2006). A secondary aim of this study is to determine the usefulness of these life  
116 history metrics as proxies for the likelihood of reduced *P. oceanica* resilience from sewage  
117 outfall and trawling effects. Determination of reliable indicators would benefit the designation of  
118 high risk meadows for the prioritization of conservation actions (Giakoumi et al., 2015) and  
119 management of local stressors (Brown et al., 2013).

120 2. Methods

121 2.1 Sample collection sites

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122 To determine responses of *P. oceanica* to these ubiquitous anthropogenic stressors,  
123 orthotropic shoots were sampled from two trawling affected sites and two sewage outfall  
124 affected sites in the Alicante province. Shoot production parameters were examined in  
125 comparison to shoots from suitable reference sites of the same depth, but without direct  
126 anthropogenic impacts. Sample collection at all sites took place during August 2011.

127 2.1.1 Sewage outfall sites

128 By the time of its release, the effluent from Alicante's two wastewater treatment plants  
129 has undergone biological treatment that is intended to lower both the biological and chemical  
130 oxygen demands but does not alleviate high nutrient loads (Table 1). Effluent is released from  
131 two different outfall pipes at 9 and 6 m depth (de la Ossa-Carretero et al., 2016) and particulates  
132 from these effluent plumes deposit onto the *P. oceanica* meadow at approximately 17 m depth  
133 (coauthor J.L. Sánchez-Lizaso, pers. comm.). Samples were collected at two 17 m depth  
134 deposition sites for each wastewater treatment plant plumes, named Orgegia (38.34N, 0.47W;  
135 Figure 1) and Rincon de Leon (38.31N, 0.49W; Figure 1).

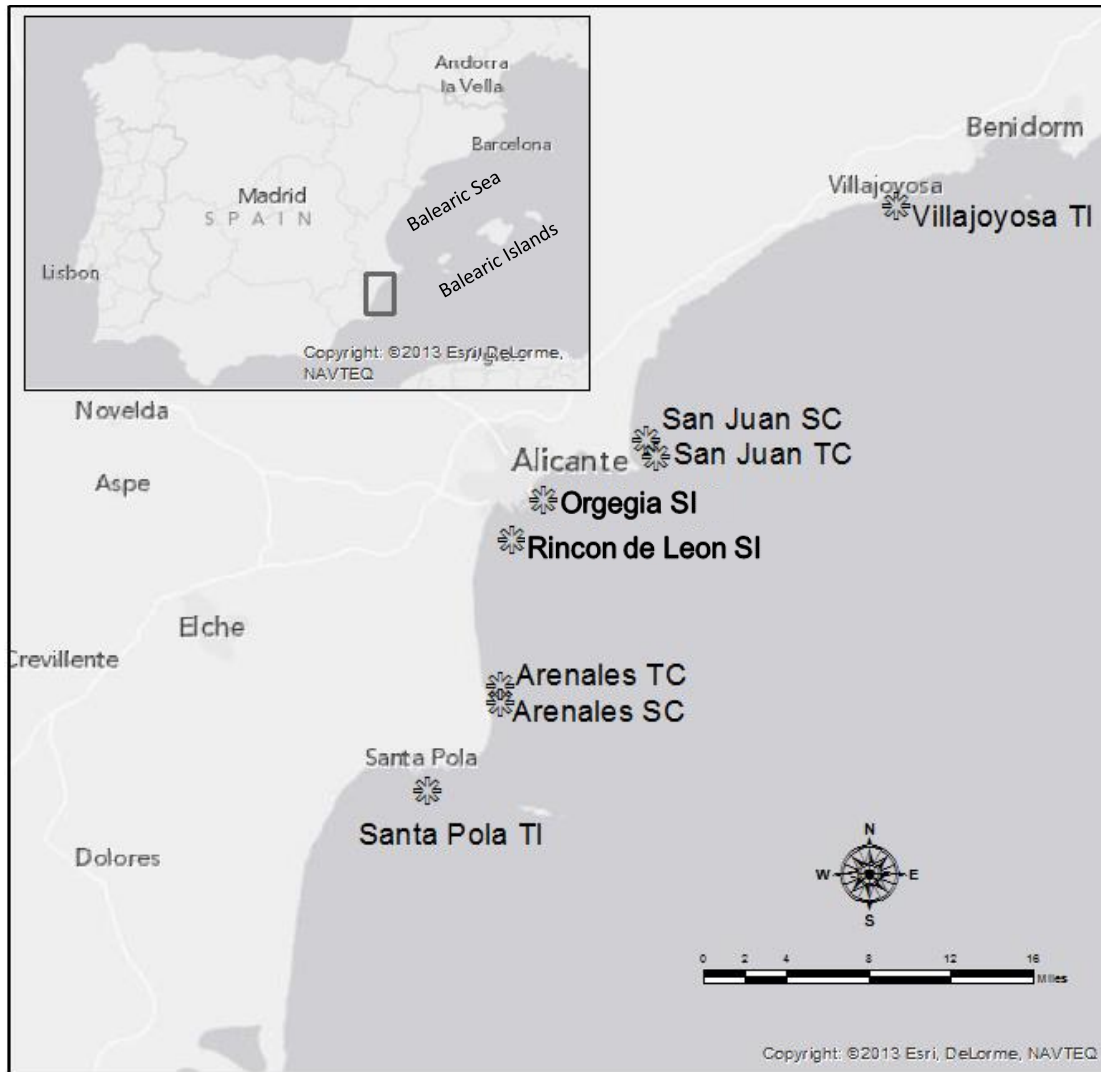


Figure 1. Sampling locations within the Alicante province of Spain (sewage impact (SI) and sewage control (SC) are 17m depth sewage impacted and unimpacted collection sites, respectively; trawling impact (TI) and trawling control (TC) are 20m depth trawling impacted and unimpacted collection sites, respectively).

### 2.1.2 Trawling sites

Approximately 20% of the meadow surrounding the Alicante province is directly affected by the mechanical drag of illegal trawlers (Sánchez-Lizaso et al., 2002), with highest areas of stress occurring around the fishing harbors of Santa Pola (38.18N, 0.55W; Figure 1) and

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145 Villajoyosa (38.5N, 0.22W; Figure 1), where trawling impacted shoots were collected. These  
146 two sites were sampled at 20 m depth, which is the shallowest depth fished by smaller trawling  
147 vessels in the Alicante province (Instituto Español de Oceanografía, 2001).

### 148 2.1.3 Reference sites

149 Shoots collected to serve as references were from the areas of San Juan (38.37N, 0.4W;  
150 Figure 1) and Arenales (38.22N, 0.5W; Figure 1) which are not subjected to sewage outfall and  
151 near artificial reefs that discourage trawler activity. Shoots at both sites were collected at 17 m  
152 depth for comparison with sewage outfall impacted shoots and at 20 m depth for comparison  
153 with trawling impacted shoots to maintain depth-related consistencies (Peirano et al., 2011).

### 154 2.2 Shoot measurements

155 For *P. oceanica* production characteristics pertinent to the sites affected by the direct  
156 anthropogenic impacts, only orthotropic rhizomes were examined to determine stressor effects  
157 on interannual growth, demography, and biomass production. Within each site, a minimum of 50  
158 shoots were collected for processing and analysis. Shoots collected at all sites were processed at  
159 the CIMAR field research station in Santa Pola, Spain.

#### 160 2.2.1 Lepidochronological measurements

161 A minimum of ten shoots from each site were lepidochronologically processed, a  
162 standard ageing method where cyclical thicknesses of remaining leaf sheaths (i.e. scales) are  
163 used as a proxy for seasons (Pergent, 1990; Pergent-Martini et al., 1994). Scale thickness  
164 extremes vary from 200µm at seasonal growth minima to 1200µm at peak seasonal growth and  
165 are therefore stark enough to be determined tactilely, a standard technique for accurately  
166 determining shoot ages, interannual rhizome vertical growth, and annual leaf production  
167 (Pergent, 1990; Guidetti et al., 2000). Processing consisted of detaching the orthotropic rhizome,

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168 then carefully removing non-decomposed scales while marking the placement and order on the  
169 rhizome. Lepidochronology is thus performed by determining the number of scales (and by  
170 proxy, leaves) produced from scale minima to minima and measuring the distance of rhizome  
171 vertical growth, which provides the information of annual growth rate ( $\text{cm y}^{-1}$ ) and number of  
172 leaves produced annually.

### 173 2.2.2 Demography

174 The average number of leaves produced annually for all shoots examined  
175 lepidochronologically from anthropogenic impact and reference sites at both depths was then  
176 used to determine shoot ages. For all shoots from each site, we counted the total number of leaf  
177 scars on the shoot and divided this by the average number of leaves produced per year for that  
178 site, a standard procedure for estimating shoot ages (Duarte, et al., 1994; McDonald et al., 2016).  
179 Histograms of shoot ages were then constructed for each site using the distribution function in  
180 JMP 13.0 (SAS© 2016) with a continuous fit model applied to each site distribution. Appropriate  
181 distribution fit models were selected by choosing the lowest AICc and -2loglikelihood values of  
182 all possible distribution fits. To examine the effects of each anthropogenic impact on shoot age  
183 demographics, shoots were separated by depth, ordered by age, then distributed into 0.2 quantile  
184 age-bins of equivalent shoot number. By doing so, each of the five age-bin ranges was  
185 determined by the demographics of randomly collected shoot samples. A chi-square analysis was  
186 then performed on age-bins between anthropogenic impact sites and corresponding grouped  
187 reference sites for each depth. For all demographic comparisons, significance was accepted at the  
188 0.05  $\alpha$ -level and analyses were computed using RStudio v.1.0.136 (©2009-2016).

### 189 2.2.3 Interannual shoot growth and leaf number production



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4 190 Interannual growth lengths of shoots were recorded during the process of  
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7 191 lepidochronological examination of a subset of shoots within impact and reference sites. These  
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9 192 annual lengths were assigned rhizomal timestamps (RT) according to the order measured on the  
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11 193 orthotropic rhizome, beginning with the year at time of collection (2011) and retroceding until  
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13 194 the rhizome end was met. For analysis, total growth length for each annual cycle only included  
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15 195 complete years, excluding the RT representing year of collection. Additional analysis was done  
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17 196 using a more conservative estimate of average internodal growth calculated from dividing total  
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19 197 interannual growth by the number of leaves produced for that annual cycle (as represented by  
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21 198 scale number). For this measurement, the incomplete annual cycle was included in the analysis  
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23 199 and represented by the final length of collection year RT divided by number of leaves produced  
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25 200 up to collection point.

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31 201 Interannual comparisons of complete rhizome vertical growth, vertical growth per leaf,  
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33 202 and number of leaves produced (from here, termed leaf production) were analyzed for sewage  
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35 203 outfall effects, trawling effect, and depth effect separately using mixed model repeated measures  
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37 204 procedures to investigate evidence of effects on each metric over time. Full mixed effects models  
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39 205 consisted of response variables (Production), the fixed effects: anthropogenic impact presence  
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41 206 designation or depth (Impact/Depth), RT (Time), and the interaction (Interaction), and random  
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43 207 effects: random intercept and slope across sites. Nesting of shoots within site (Site) was modeled  
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45 208 with a compound symmetry correlation structure to fit the dependent nature of the repeated  
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47 209 measure of growth per annual cycle of a given rhizome. These model parameters are represented  
48  
49 210 by Equation 1.:

$$\begin{aligned} 54 \\ 55 \text{ 211 } & Production_{ij} = (\beta_0 + Site_{0j}) + (\beta_{I/D} + a_{I/Dj}) * Impact/Depth_{ij} + (\beta_T + a_{Tj}) * (Time_{ij}) \\ 56 \\ 57 \\ 58 \text{ 212 } & + (\beta_{int} + a_{intj}) * Interaction_{ij} + e_{ij} \\ 59 \\ 60 \\ 61 \\ 62 \\ 63 \\ 64 \\ 65 \end{aligned}$$

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213 where:

214  $\beta_0 + Site_{0j}$  are the global intercept and random intercept

215  $\beta_{I/D} + a_{I/Dj}, \beta_T + a_{Tj}, \beta_{Int} + a_{Intj}$  are the coefficient and random slope for fixed factors:

216 Impact/Depth, Time, and Interaction, respectively

217  $e_{ij}$  is the subject residual

218 Akaike's information criterion (AIC) values and p-values were obtained with a maximum  
219 likelihood ratio test of full models versus reduced models without the effect in question.

220 Analyses were run with the nlme package (Pinheiro et al., 2017) of RStudio v.1.0.136(©2009-  
221 2016) to determine the most parsimonious model fit for fixed factors and random structure. The  
222 nlme package was also used to perform the parsimonious linear mixed model with restricted  
223 maximum likelihood estimation for variance components.

### 224 2.3 Detection and analysis of "mid-2000s stress event"

225 Visual examination of interannual growth parameters prompted a post hoc investigation  
226 following the observation of a shared growth trend of all shoots > 5 years old, regardless of  
227 origin site. A consistent trend of declining rhizome vertical growth and leaf production leading  
228 up to the growth period that corresponds with RT 2007 was highly compelling because it was  
229 detected in the older shoots from all sites, and therefore became a point for a secondary  
230 investigation in our study. This period of reductions in production metrics will henceforth be  
231 referred to as the mid-2000s stress event, as this period likely represents a large-scale stress  
232 event. Limitations must be set due to the impossibility of retroactively determining a definitive  
233 cause to this decline, however we intended to use modeling techniques to investigate any  
234 potential effects of this unconfirmed but broad-reaching event on rhizome growth and leaf  
235 production recovery from RT 2007 onward.

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236 To elucidate distinct growth patterns prior to and following RT 2007, interannual growth  
237 and annual leaf production were modeled using a piecewise growth analysis that split slopes  
238 prior to and post RT 2007. Split slope models were then compared to linear models, constructed  
239 as described above, to determine which model behavior provided a more parsimonious fit for  
240 shoot growth and annual leaf production. In the event that the split slope models were a better fit  
241 for the response data, linear mixed effects model analyses were repeated after separating the  
242 Time factor into two time periods. To fully capture the impact of the 2007 event on annual  
243 growth and leaf production rate shifts, time phase 1 (stress event period) represents  
244 measurements for RT 2003 through 2007 and time phase 2 (recovery period) represents  
245 production measurements from RT 2007 to 2010 or 2011, depending on the measurement.

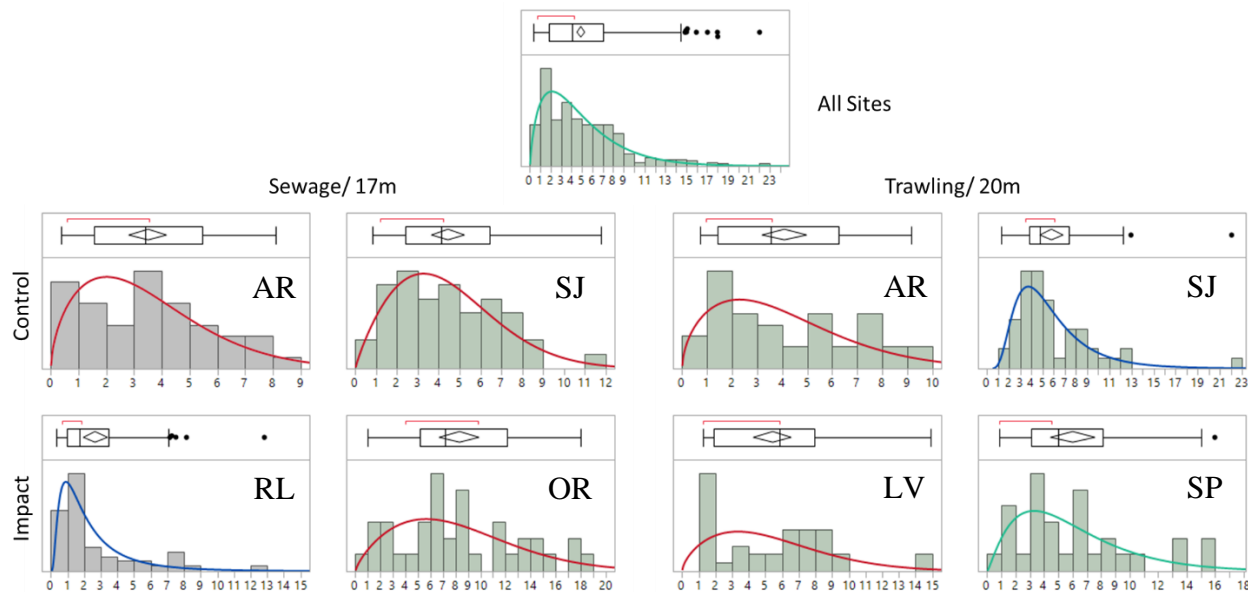
### 246 3. Results

#### 247 3.1 Demography

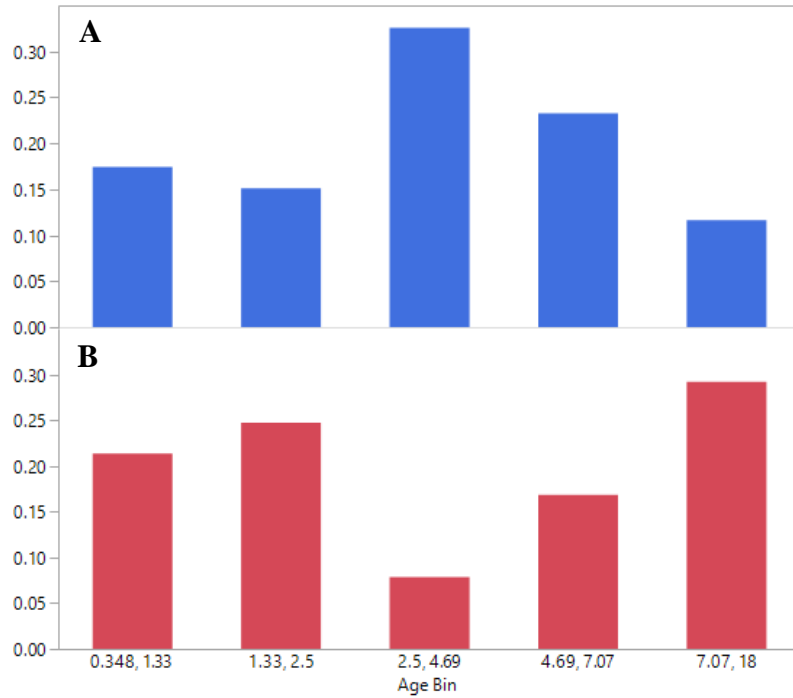
248 Site specific average number of leaves produced per year was either at or above the  
249 universal average for *P. oceanica* of 7.7 leaves year<sup>-1</sup> (Table 2; Duarte, 1991). The Orgegia  
250 sewage outfall site shoots indicated a lower annual leaf production than for all other sites with  
251 7.7 leaves yr<sup>-1</sup> (Table 2). All remaining sites were within a more equivalent range, between 8 and  
252 8.4 leaves yr<sup>-1</sup>, when sites were combined with exclusion of the Orgegia site. Shoot ages ranged  
253 from 0.4 to 22.1 years and the histograms for all sites combined and independently (Figure 2)  
254 produced fits in the lognormal family (lognormal, Weibull, and gamma distributions), which are  
255 expected fits for shoot age histograms (Hemminga and Duarte, 2000). Basic statistics for age  
256 structure of the sites are similar throughout, with the exception of Orgegia sewage outfall site  
257 having higher average, median, and quartile shoot ages. Examination of potential effects of  
258 stressor presence on shoot age structure showed evidence of effects from sewage impact

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259 presence ( $\chi^2= 23.2$ ,  $df= 4$ ,  $p< 0.001$ ), but no evidence of an effect from trawling impact presence  
 260 ( $\chi^2= 7.7$ ,  $df= 4$ ,  $p= 0.10$ ). Visual examination of the histograms (Figure 3) indicates sewage  
 261 impacted sites had far less proportional representation in the 2.5-4.69 year age bin and far greater  
 262 proportional representation in the 7.07-18 year age bin, when compared with unimpacted 17 m  
 263 depth shoots.



264  
 265 Figure 2. Age structure histograms of combined shoots for all collection sites combined, and for  
 266 individual sewage and trawling impacted and unimpacted collection sites (AR= Arenales, SJ=  
 267 San Juan, RL= Rincon de Leon, OR= Orgegia, LV= La Vilajoyosa, SP= Santa Pola).  
 268 Distributions for fitted lines are color coded- light green is log-normal, red is Weibull, and blue  
 269 is gamma. Outlier box plots above the distribution show medians, quartiles within the diamond  
 270 area, and region of highest density within the red brackets.



271

272 Figure 3. Proportions of evenly binned shoot ages for 17 m depth collection sites: unimpacted  
 273 sewage control (SC) sites (A) and sewage impacted (SI) sites (B).

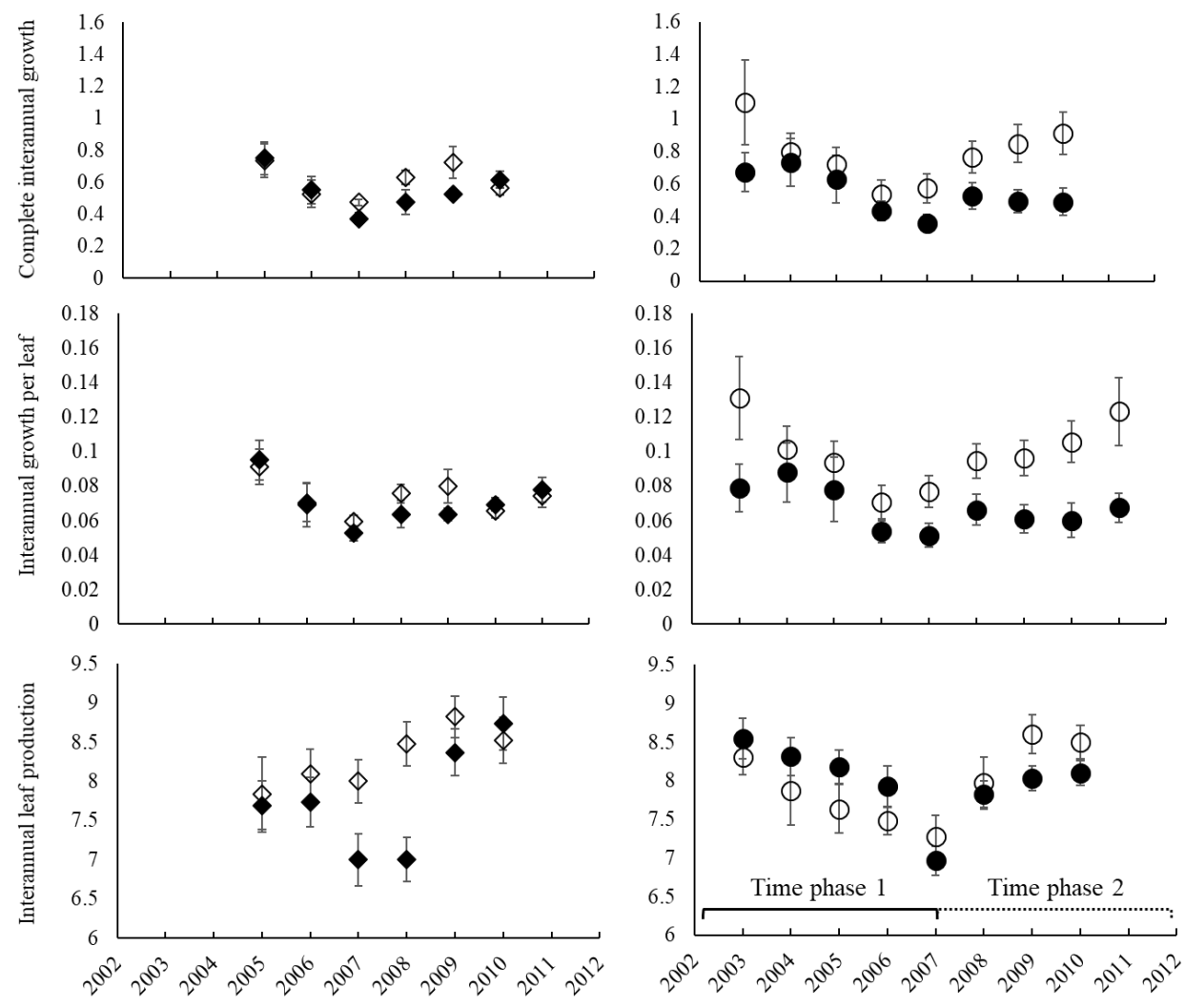
274 3.3 Interannual orthotropic growth and leaf production

275 Model selection for all responses examined resulted in a best fit linear model structure  
 276 with random slope, random intercept, and compound symmetry covariate structure. Examination  
 277 of log<sub>10</sub> transformed growth measurements between shoots from sewage outfall plume impacted  
 278 sites and 17 m reference sites shoots found no evidence of influence from an impact or time  
 279 effect for either the complete interannual growth or the interannual growth per leaf when full  
 280 models were compared with reduced models (Figure 4; Table 3). However, there was evidence  
 281 for an effect of time with annual leaf number production, with an estimated increase of 0.21  
 282 leaves per unit of time (Table 3 and 4). Comparisons between trawling impacted sites and 20 m  
 283 reference sites indicated an interaction effect of impact and time for all production metrics  
 284 (Figure 4; Tables 3 and S1). The linear models estimated that the effect of the trawling impact

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285 corresponded with 11% and 5% lower growth with every time increment (for complete  
286 interannual growth and interannual growth per leaf, respectively). Model fits also determined an  
287 interaction effect predicted annual leaf growth for 20 m shoots, with trawling effects contributing  
288 to an estimated 0.23 fewer annual leaves for each increment of time, compared with unimpacted  
289 shoots (Figure 4; Tables 3 and S1). Results for all mixed model comparisons examining the  
290 effects of anthropogenic impact on production can be found in Table S1.



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292 Figure 4. Interannual growth and leaf production of orthotropic rhizomes for 17m depth (left)  
293 sewage impacted (SI; black diamonds) and unimpacted (SC; open diamonds), 20m depth (right)

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294 trawling impacted (TI; black circles) and unimpacted (TI; open circles). Time periods used in  
295 piecewise growth analysis are shown for 20m depth shoots.

296 Models to examine the effect of depth on complete interannual growth using only  
297 reference site shoots had a significant interaction effect of depth and time such that shoots from  
298 20 m depth had 12% lower growth with each increment of time than shoots from 17 m depth  
299 (Tables 3 and S4). Interannual growth per leaf models also indicated an interaction effect of  
300 depth and time, but inclusion of zero in the 95% CI negates further interpretation of this result.  
301 Time, but not depth, predicted annual leaf production, with an estimated increase of 0.23 leaves  
302 with each time increment (Table 3, Table S4).

### 303 3.4 Mid-2000s stress event effects on interannual growth and leaf production

304 Because linear models for the 17 m depth shoots did not indicate an interaction effect of  
305 impact with time, there was no further interest in examining the possibility for combined effects  
306 of the mid-2000s stress event and sewage impact presence and therefore piecewise split slope  
307 models were not constructed for the 17 m depth shoot data. However, the linear models for 20 m  
308 depth shoots did indicate an interaction effect and the constructed piecewise split slope growth  
309 model fits were more parsimonious than the continuous slope models for interannual growth (L.  
310 ratio= 114.5,  $p < 0.0001$ ; L. ratio= 90.6,  $p < 0.0001$ , for complete annual growth and growth per  
311 leaf, respectively). This relationship was also found when examining full and reduced models of  
312 annual leaf production, with a significant interaction term that was followed by a piecewise  
313 model that provided a significantly better fit when compared with the continuous slope  
314 interaction model (L. ratio= 32.8,  $p < 0.001$ ; Figure 4; Table 3 and 4).

#### 315 3.4.1 Model analysis for separated time phases of 20 m depth shoots

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316 Mixed model comparisons for time phase 1 (stress event period) provided evidence of an  
317 impact effect for complete interannual growth and interannual growth per leaf, however the 95%  
318 CI for these metrics included zero and therefore further interpretation of this predictor was not  
319 warranted (Tables S2 and S3). Time was found to be an adequate predictor of both growth  
320 metrics during time phase 1, such that complete interannual growth had an estimated 23%  
321 decline with each increment of time and growth per leaf had an estimated 18% decline with each  
322 increment of time. Annual leaf production in time phase 1 was also adequately predicted by time,  
323 with an estimated decline of 0.35 leaves per unit of time (Table S2). Model comparisons for time  
324 phase 2 (recovery period) provided evidence of an interaction effect for complete interannual  
325 growth, such that the effect of trawling impact corresponded with an estimated 10.4% lower  
326 growth rate per unit of time when compared with unimpacted shoots (Tables S2 and S3). Model  
327 comparisons for interannual growth per leaf in time phase 2 indicated trawling reduced growth  
328 per leaf by 41% overall, but that shoot growth per leaf of all shoots increased by an estimated 8%  
329 per unit time (Tables S2 and S3). Annual leaf production measurements had a significant result  
330 for the effect of time only, with leaf number increasing 0.4 leaves per unit time (Tables S2 and  
331 S3).

### 332 3.4.2 Model analysis for depth-related effects on growth

333 Piecewise split slope models were a more parsimonious fit for complete interannual  
334 growth and interannual growth per leaf than the continuous slope models (Table S4). During  
335 time phase 1, comparisons showed significant effects of time (Table S4) with declines of 12.5%  
336 (5.7 to 19.7% CI) and 0.8% (0.2 to 1.4% CI) with each unit of time for complete interannual  
337 growth and interannual growth per leaf, respectively. Comparisons of complete interannual  
338 growth during time phase 2 gave evidence of a significant interaction effect of depth and time,



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339 such that 20 m depth corresponded with an estimate of 19.1% (7.9 to 31.8% CI) less growth per  
340 unit of time when compared with 17 m depth shoots (Table S4). Interannual growth per leaf  
341 showed no evidence of an effect of either depth or time during time phase 2 (Table S4).

342 4. Discussion

343 By comparing demographics and models of growth and leaf production between  
344 meadows exposed to a significant anthropogenic disturbance and undisturbed meadows of the  
345 same depth, we were able to detect the influence of the anthropogenic disturbance on some  
346 aspect of *P. oceanica* life history. Interestingly, the disturbance signatures for sewage and  
347 trawling were specific to stressor identity such that the two disturbance types presented in  
348 different aspects of shoot life history. Anthropogenic disturbance impacts on *P. oceanica* life  
349 history metrics manifested as distinct age structures in sewage impacted shoots and as lower  
350 rhizome vertical growth rates for trawling impacted shoots. This finding serves to highlight the  
351 general need to investigate multiple life history response metrics for a greater chance at detecting  
352 evidence of negative effects from stressors. Additionally, analysis of growth and leaf production  
353 at separate periods of time effectively highlighted the negative impact of trawling on *P. oceanica*  
354 recovery to a large-scale stressor, thus indicating lower resilience of trawling impacted shoots.  
355 Current conservation goals for *P. oceanica* throughout the Mediterranean are to identify, assess,  
356 and mitigate threats for the prevention of further meadow losses (Life, 2014). Management  
357 strategies for seagrasses in other world regions have shown that pinpointing “hotspot”  
358 vulnerability areas using indicators of declining production can enhance the efficiency of  
359 conservation efforts for this valuable habitat (Berry et al., 2003; UNEP, 2014). The examination  
360 of demographic and production metrics for *P. oceanica* orthotropic shoots in this study provided

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361 support for use of these metrics as indicators of negative effects of localized anthropogenic  
362 disturbances on new shoot production and stress event recovery.

363 Evidence of sewage outfall stress on *P. oceanica* shoot age structure was likely driven in  
364 part by the substantially higher 7 to 18-year age group representation for the outfall impacted  
365 shoots. In fact, this oldest age category represents the majority of outfall impacted shoots, which  
366 suggests that overall new shoot recruitment may be low for these impacted sites. This can derive  
367 from the high dependence of *P. oceanica* on vegetative reproduction (Arnaud-Haond et al.,  
368 2014), wherein declines in recruitment rates occur as disturbances directly affect vegetative  
369 expansion via lowered production (González-Correa et al., 2005; Marbà, 2009). High nutrient  
370 loadings often create low light conditions due to enhanced turbidity, which can negatively affect  
371 new clonal production (Díaz-Almela et al., 2008). A dearth in new shoot production during a  
372 period of poor environmental condition, such as the early 2000s stress event, could help explain  
373 the distinctly lower shoot representation between the ages of 2.5 to 4.69 in sewage outfall shoots.  
374 Evidence of these distinct age structures merits further investigation into the potential for use of  
375 demographics when investigating impacts of anthropogenic stressors on new shoot production  
376 and potential timeframes of higher stress periods.

377 The effect of trawling disturbance on *P. oceanica* shoot life history was detected in the  
378 lower rhizome vertical growth over time in trawl impacted shoots when compared with  
379 unimpacted 20 m depth shoots. With respect to determinations of estimated trawling influence  
380 for the two modeled growth rate metrics, we found interannual growth per leaf to be consistently  
381 more conservative than complete interannual growth. The benefit for dividing an annual growth  
382 rate by the number of leaves produced that year would be to reduce within-shoot variability,  
383 thereby reducing any potential bias arising from disparate shoot age structures of the populations

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384 being compared. Seagrass shoot vertical growth is typically more rapid within the first year,  
385 before stabilizing with age (Duarte et al., 1994). Therefore collections that bias towards  
386 disproportionately younger shoots for a site could have some influence on a model of growth  
387 and, in such a scenario, the more conservative interannual growth per leaf would be the more  
388 appropriate growth metric.

In addition to age dependence, vertical shoot growth of seagrasses is typically an attribute  
of sediment dynamics (Marba and Duarte, 1997; Marbà, 2009; Guidetti, 2001), because shoot  
vertical growth must match sedimentation in order to keep the photosynthetic leaves above the  
sediment line. However, declines in *P. oceanica* orthotropic shoot vertical growth rates from  
water depths of 16 to 22 m have also been attributed to organic nutrient loading linked to  
aquaculture disturbances (Delgado, 1999; Marbà et al., 2006). The lack of similar vertical growth  
declines in sewage impacted shoots was therefore somewhat unexpected, and the definitive  
explanation as to why trawling impacted shoots did exhibit lower vertical growth rates than  
reference shoots can only be speculated. Although growth rate comparisons between the 17 m  
and 20 m depth reference shoots did not consistently support depth related differences (see  
section 3.4.2), depth-related ecophysiologicals have been reported as affecting carbon balance  
comparisons between shallow and deep depth *P. oceanica* shoots exposed to an environmental  
stressor (Sandoval-Gil et al., 2014). Trawling impacts may have a larger effect on *P. oceanica*  
rhizome vertical growth rates due to the deeper depth, and therefore lower light availability, at  
which trawling is conducted in this region of the Mediterranean coast. An examination of  
recovery dynamics of a 20 m depth *P. oceanica* meadow following the deployment of anti-  
trawling measures determined that light availability was still substantially lower at impacted sites  
eight years after trawling cessation, likely related to sediment resuspension, and that low growth

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407 rates indicated meadow recovery to pre-impact conditions could take a century or more  
408 (González-Correa et al., 2005). While *P. oceanica* is likely a low-light adapted seagrass species  
409 (Olesen et al., 2002; Dattolo et al., 2014), disturbances at deeper depths have a greater negative  
410 effect on seagrass production due to the more diminished light environment.

411           Interestingly, all shoots regardless of location, depth, or anthropogenic impact presence  
412 exhibited reduced production rates during the time phase 1 stress event period, providing support  
413 that this unidentified stressor made up of singular or multiple occurrences was large-scale and  
414 possibly climactic. The Mediterranean region has been designated a “hot spot” of climate change  
415 intensification (Diffenbaugh et al., 2007), increasing the frequency of marine heat wave events  
416 (Oliver et al., 2018) that have resulted in mass mortalities of *P. oceanica* and sessile benthic  
417 invertebrates (Marbà and Duarte, 2010; Crisci et al., 2011; Rivetti et al., 2014). Also, a climate  
418 change-related increase in stratification events can severely reduce benthic community  
419 production due to water column stability and exhaustion of surface layer nutrients (Coma et al.,  
420 2009). There is no confirmation on record for a marine heat wave or mass mortality event  
421 occurring near the Alicante province during the mid-2000s, but this specific coastal region has  
422 experienced a significant warming trend (Olcina Cantos et al., 2019). High water temperatures  
423 negatively impact growth of *P. oceanica* by reducing carbohydrate storage necessary for shoot  
424 survival during periods of low production (Alcoverro et al., 2001; Lee et al., 2007; Koch et al.,  
425 2013; Marín-Guirao et al., 2016). Largescale climate events can also exacerbate more frequent  
426 natural occurrences, such as mild winter seasons where a lack of winter storms leads to a  
427 reduction in *P. oceanica* community net primary production due to a buildup of *P. oceanica* leaf  
428 litter that blocks light to benthic algae and increases respiration (Champenois and Borges, 2012;  
429 Champenois and Borges, 2021). High temperatures during an exceptionally warm summer were

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430 also found to exacerbate anthropogenic disturbance stress, further reducing carbohydrate storage  
431 of impacted *P. oceanica* (Helber et al. 2021). Therefore, although the identity of the large-scale  
432 stressor that caused synchronous shoot growth declines can only be speculated on, our findings  
433 reiterate how interactions of climate change, anthropogenic alterations, and naturally occurring  
434 conditions can negatively influence production of *P. oceanica*.

## 435 5. Conclusion

436 Our opportunistic stress event investigation produced evidence to support the conclusion  
437 that reconstructed growth metrics were able to detect lower recovery of trawling impacted  
438 shoots, implicating lower resilience of these meadows to large-scale stressors. This finding is  
439 especially important for *P. oceanica* monitoring efforts that rely primarily on areal coverage  
440 trends and may miss a stress signal that only presents in shoot-based life history metrics, such as  
441 changes in growth (Marbà and Duarte, 1997; Guidetti, 2001) and reproduction (Balestri, 2004).  
442 Mixed model repeated measures procedures also overcame common statistical challenges of  
443 working with reconstructed shoot data when using more traditional ANOVA designs; mainly,  
444 violations of normality and inter- dependence of longitudinal within-shoot measurements  
445 (Lovison et al., 2011). The life history metrics and techniques used in this study would be  
446 applied most optimally in spatial analysis efforts which combine human activity location data  
447 with *P. oceanica* coverage and environmental variables to form meadow risk assessments (see  
448 Holon et al., 2018 and Houngnandan et al., 2020). Confirmation of reduced production over time  
449 in shoots from anthropogenically impacted areas would allow for more targeted protections and  
450 policy-based actions to reduce anthropogenic stressor impacts to these valuable seagrass  
451 ecosystems.

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4 Table 1. Outfall water quality from wastewater treatment plants serving the Alicante province.

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6 Volume describes the amount of wastewater treated; Discharged BOD refers to the biological  
7 oxygen demand of discharged wastewater; % BOD removed describes wastewater treatment  
8 effectiveness for BOD removal; Discharged COD refers to chemical oxygen demand of  
9 discharged wastewater; % COD removed describes wastewater treatment effectiveness for COD  
10 removal; Discharged N describes the amount of nitrogen released in wastewater; Discharged P  
11 describes the amount of phosphorous released in wastewater. Source: All data from  
12 <http://discomap.eea.europa.eu>. Stations: Monte Orgegia and Rincon de Leon.  
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Site	Volume (m <sup>3</sup> day <sup>-1</sup> )	Discharged BOD (t y <sup>-1</sup> )	% BOD removed	Discharged COD (t y <sup>-1</sup> )	% COD removed	Discharged N (t y <sup>-1</sup> )	Discharged P (t y <sup>-1</sup> )
Rincon de Leon	7,888,640	5,522	81	29,976	94.3	30,276	2,011
Orgegia	19,267,660	34,681	95.8	125,239	91.3	76,666	8,843

Table 2. Demographic statistics of collected orthotropic shoots. Avg. leaves yr<sup>-1</sup> is the average number of leaves produced per year with 95% confidence interval; Avg. shoot age is the average age in years of orthotropic shoots; Median shoot age is the median age in years of orthotropic shoots; Percentiles are the 0.25 and 0.75 percentiles, respectively; Maximum age is the maximum age in years of orthotropic shoots; and CV is the coefficient of variation.

Site name	Site description	Avg. leaves yr <sup>-1</sup> (95% CI)	Avg. shoot age (y)	Median shoot age (y)	Percentiles	Maximum age (y)	CV
Arenales 17m	Sewage control	8.5 (7.7 – 9.3)	3.5	3.4	1.5 and 5.5	8.1	63.7
San Juan 17m	Sewage control	8.4 (8 – 8.8)	4.4	4.1	2.4 and 6.5	11.8	57.4
	Combined	8.4 (8 – 8.8)	4.0	3.8	1.9 and 5.9		
Rincon de Leon	Sewage impact	8.6 (8.2 – 9)	2.7	3.4	1.0 and 3.4	12.8	95.8
Orgegia	Sewage impact	7.3 (6.9 – 7.7)	8.3	7.2	5.2 and 12.1	18.0	59.0
	Combined	7.8 (7.4 – 8.2)	5.0	3.0	1.4 and 7.2		
Arenales 20m	Trawling control	8.5 (8.1 – 8.9)	4.1	3.5	1.4 and 6.3	9.2	66.8
San Juan 20m	Trawling control	7.7 (7.3 – 8.1)	5.8	4.8	3.8 and 7.4	22.1	62.2
	Combined	8.1 (7.7 – 8.5)	5.1	4.4	2.5 and 7.1		
Santa Pola	Trawling impact	7.9 (7.7 – 8.1)	5.5	4.0	2.1 and 7.1	16.0	64.3
La Vilajoyosa	Trawling impact	8.0 (7.8 – 8.2)	5.4	5.9	1.9 and 7.9	14.9	69.1
	Combined	8.0 (7.8 – 8.2)	5.5	4.8	2.0 and 7.8		
All sites		8.1 (7.9 – 8.3)	4.9	4.0	1.9 and 7.0		75.4

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Table 3. Model results for selected parsimonious linear mixed effects models of  $\log_{10}$  transformed interannual growth and leaf production fit with restricted maximum likelihood. Dependent variable is the response data that was tested; Fixed effects are the resulting fixed factor(s) of the final model selected; Estimate is for the coefficient; p-value corresponds to model comparisons; RE variance is the random effect variance; RS variance is the random slope variance; Within-shoot correlation coefficient corresponds to relative dependence of measurements within the same orthotropic shoot.

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Dependent variable	Fixed Effects	Estimate (95% CI)	p-value	RE variance (% of total)	RS variance (% of total)	Within-shoot correlation coefficient ( $\rho$ )
<b>Sewage Effect</b>						
Complete interannual growth	Intercept Only	-0.28 (-0.35 – 0.21)	<0.0001	11.3%	0.04%	0.35
Interannual growth per leaf	Intercept Only	0.03 (0.027 – 0.032)	<0.0001	4.4%	0.06%	0.26
Annual leaf production	Time	0.21 (0.065 – 0.35)	0.0043	0.015	0.67%	0.27
	Intercept	7.0 (6.34 – 7.61)	<0.0001			
<b>Trawling Effect</b>						
Complete interannual growth	Impact *Time	-0.05 (-0.08 - -0.02)	0.0026	16%	0.4%	0.42
	Intercept	-0.25 -0.39 - -0.11)	0.03			
Interannual growth per leaf	Impact*Time	-0.03 (-0.06 - -0.002)	0.036	18%	0.6%	0.29
	Intercept	-1.14 (-1.27 - -1.01)	<0.0001			
Annual leaf production	Impact*Time	-0.23 (-0.39 - -0.07)	0.0051	0.2%	0.1%	0.24
	Intercept	7.24 (6.79 – 7.68)	<0.0001			
<b>Depth Effect</b>						
Complete interannual growth	Depth*Time	0.05 (0.02 – 0.08)	0.003	5.5%	0.4%	0.46
	Intercept	-0.23 (-0.34 - -0.13)	<0.0001			
Interannual growth per leaf	Depth*Time	0.004 (-0.0001 – 0.007)	0.056	7.3%	1.6%	0.27
	Intercept	0.03 (0.02 – 0.04)	<0.0001			
Annual leaf production	Time	0.23 (0.15 – 0.3)	<0.0001	3.6%	<0.001%	0.39
	Intercept	6.87 (6.32 – 7.42)	<0.0001			



Table S1. Linear mixed effects parsimonious fixed factor model selection for interannual growth and leaf production. Comparisons were conducted between a full model and reduced models lacking the effect in question. Nested test numbers refer to numbered models with fixed factors as designated. Selected model factors are in bold.

Responses	Fixed Model Factors	AIC	Maximum Likelihood Ratio	Nested Test p-value
<b>Sewage Impact</b>				
Complete interannual growth	1 Impact*Time	-90.76	1 vs 2= 0.095	1 vs 2= 0.76
	2 Impact	-92.66	2 vs 3=0.75	2 v 3= 0.39
	3 Time	-93.92	3 vs 4=0.005	3 vs 4= 0.94
	<b>4 Intercept only</b>	-95.91		
Interannual growth per leaf	1 Impact*Time	-1491.25	1 vs 2=0.003	1 vs 2= 0.96
	2 Impact	-1493.25	2 vs 3=0.36	2 vs 3= 0.55
	3 Time	-1494.89	3 vs 4=0.32	3 vs 4= 0.57
	<b>4 Intercept only</b>	-1496.57		
Annual leaf production	1 Impact*Time	640.7	1 vs 2=0.65	1 vs 2= 0.42
	2 Impact	639.3	2 vs 3=3.0	2 vs 3= 0.086
	<b>3 Time</b>	640.3	3 vs 4=5.7	3 vs 4= 0.017
	4 Intercept only	643.9		
<b>Trawling Impact</b>				
Complete interannual growth	<b>1 Impact*Time</b>	-275.16	1 vs 2=6.9	1 vs 2=0.009
	2 Impact	-270.25	2 vs 3=1.2	2 vs 3=0.27
	3 Time	-271.03	3 vs 4=0.13	3 vs 4=0.72
	4 Intercept only	-272.9		
Interannual growth per leaf	<b>1 Impact*Time</b>	-410.01	1 vs 2=4.7	1 vs 2=0.03
	2 Impact	-407.32	2 vs 3=2.3	2 vs 3=0.13
	3 Time	-407.00	3 vs 4=0.04	3 vs 4=0.85
	4 Intercept only	-408.95		
Annual leaf production	<b>1 Impact*Time</b>	1257.91	1 vs 2=6.2	1 vs 2=0.013
	2 Impact	1262.09	2 vs 3=2.1	2 vs 3=0.15
	3 Time	1262.18	3 vs 4=0.54	3 vs 4=0.46
	4 Intercept only	1260.72		

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Table S2. Model results for selected parsimonious linear mixed effect model comparisons for trawling impacted sites.  
# indicates log<sub>10</sub> transformation of dependent variables.  
\*2010 for complete interannual growth and annual leaf production, 2011 for interannual growth per leaf

Dependent Variable	Parsimonious Model Fixed Effects	Estimate	95% CI (lower-upper)	Random effect variance (% of total)	Random slope variance (% of total)	Within-shoot correlation coefficient (ρ)
<b>Time 1 (2003-2007)</b>						
Complete interannual growth <sup>#</sup>	Impact	-0.17	-0.24 — 0.07	37.5%	0.8%	0.71
	Time	-0.09	-0.11 — -0.06			
	Intercept	0.13	-0.03 — 0.29			
Interannual growth <sup>#</sup> per leaf	Impact	-0.15	-0.34 — 0.04	59.0%	1.5%	0.57
	Time	-0.07	-0.09 — -0.04			
	Intercept	-0.84	-0.98 — -0.7			
Annual leaf production	Time	-0.35	-0.49 — -0.21	19.8%	0.6%	0.31
	Intercept	9.0	8.27 — 9.66			
<b>Time 2 (2007-2010/2011*)</b>						
Complete interannual growth <sup>#</sup>	Impact *Time	-0.043	-0.08 — -0.006	1%	0.6%	0.50
	Intercept	-0.63	-0.76 — -0.49			
Interannual growth <sup>#</sup> per leaf	Impact	-0.15	-0.31 — -0.012	28.7%	1.7%	0.29
	Time	0.033	0.008 — 0.06			
	Intercept	-1.14	-1.27 — -1.01			
Annual leaf production	Time	0.4	0.19 — 0.6	36.6%	1.5%	0.36
	Intercept	5.39	4.26 — 6.51			

Table S3. Linear mixed effects parsimonious fixed factor model selection for 20 m depth interannual growth and leaf production separated by Time Phases. Comparisons were conducted between a full model and reduced models lacking the effect in question. Nested test numbers refer to numbered models with fixed factors as designated. Selected model factors are in bold. \*2010 for complete interannual growth and annual leaf production, 2011 for interannual growth per leaf

Responses	Fixed Model Factors	AIC	Maximum Likelihood Ratio	Nested Test p-value
<b>Time Phase 1 (2003-2007)</b>				
Complete interannual growth	1 Impact*Time	-224.5	1 vs 2= 2.13	1 vs 2= 0.15
	<b>2 Impact</b>	-224.4	2 vs 3=8.16	2 vs 3= 0.004
	<b>3 Time</b>	-218.2	3 vs 4=9.72	3 vs 4= 0.002
	4 Intercept only	-210.5		
Interannual growth per leaf	1 Impact*Time	-227.5	1 vs 2=0.93	1 vs 2= 0.34
	<b>2 Impact</b>	-228.5	2 vs 3=9.5	2 vs 3= 0.002
	<b>3 Time</b>	-221.0	3 vs 4=8.02	3 vs 4= 0.005
	4 Intercept only	-215.0		
Annual leaf production	1 Impact*Time	444.7	1 vs 2< 0.001	1 vs 2= 0.98
	2 Impact	442.7	2 vs 3=2.4	2 vs 3= 0.12
	<b>3 Time</b>	443.1	3 vs 4=8.1	3 vs 4= 0.004
	4 Intercept only	449.2		
<b>Time Phase 2 (2007-2010/2011*)</b>				
Complete interannual growth	<b>1 Impact*Time</b>	-225.3	1 vs 2=4.63	1 vs 2=0.031
	2 Impact	-222.7	2 vs 3=2.30	2 vs 3=0.13
	3 Time	-222.4	3 vs 4=6.41	3 vs 4=0.011
	4 Intercept only	-218.0		
Interannual growth per leaf	1 Impact*Time	-327.4	1 vs 2=1.92	1 vs 2=0.17
	<b>2 Impact</b>	-327.5	2 vs 3=6.8	2 vs 3=0.009
	<b>3 Time</b>	-322.7	3 vs 4=4.51	3 vs 4=0.034
	4 Intercept only	-320.2		
Annual leaf production	1 Impact*Time	776.7	1 vs 2=0.6	1 vs 2=0.44
	2 Impact	775.3	2 vs 3=0.3	2 vs 3=0.30
	<b>3 Time</b>	774.4	3 vs 4=6.9	3 vs 4=0.009
	4 Intercept only	779.3		

Table S4. Linear mixed effects model comparison statistics for determinations of depth effects (control site shoots only) for interannual growth and leaf production across all RTs and split into time phases 1 and 2.

<b>17 m and 20 m depth comparisons across all RTs</b>	<b>Fixed Model Factors</b>	<b>AIC</b>	<b>Maximum Likelihood Ratio</b>	<b>Nested Test p-value</b>
Complete interannual growth	<b>1 Depth*Time</b>	-209	1 vs 2= 6.45	1 vs 2= 0.01
	2 Depth	-204.5	2 vs 3= 0.003	2 vs 3= 0.95
	3 Time	-206.5	3 vs 4= 2.13	3 vs 4= 0.15
	4 Intercept	-206.4		
	<b>5 Piecewise</b>	-213.3	1 vs 5= 14.4	1 vs 5= 0.01
Interannual growth per leaf	<b>1 Depth*Time</b>	-2096	1 vs 2= 4.04	1 vs 2= 0.045
	2 Depth	-2094	2 vs 3= 0.57	2 vs 3= 0.45
	3 Time	-2095	3 vs 4= 1.56	3 vs 4= 0.21
	4 Intercept	-2096		
	<b>5 Piecewise</b>	-2100	1 vs 5= 14	1 vs 5= 0.016
Annual leaf production	1 Depth*Time	879.5	1 vs 2= 2.53	1 vs 2= 0.11
	2 Depth	880.1	2 vs 3= 1.88	2 vs 3= 0.17
	<b>3 Time</b>	880	3 vs 4= 7.64	3 vs 4= 0.006
	4 Intercept	885.6		
<b>Time Phase 1</b>				
Complete interannual growth	1. Depth*Time	-83.2	1 vs 2= 0.05	1 vs 2= 0.83
	2. Depth	-85.2	2 vs 3= 0.35	2 vs 3= 0.55
	<b>3. Time</b>	-86.8	3 vs 4= 8.13	3 vs 4= 0.004
	4. Intercept	-80.7		
Interannual growth per leaf	1. Depth*Time	-645.4	1 vs 2= 4.93	1 vs 2= 0.08
	2. Depth	-644.4	2 vs 3= 3.78	2 vs 3= 0.052
	<b>3. Time</b>	-644.4	3 vs 4= 4.65	3 vs 4= 0.031
	4. Intercept	-641.7		
<b>Time Phase 2</b>				
Complete interannual growth	<b>1 Depth*Time</b>	-179	1 vs 2= 7.72	1 vs 2= 0.006
	2 Depth	-173	—	—
Interannual growth per leaf	1 Depth*Time	-1706	1 vs 2= 3.52	1 vs 2= 0.061
	2 Depth	-1704	2 vs 3= 1.37	2 vs 3= 0.24
	3. Time	-1705	3 vs 4= 1.73	3 vs 4= 0.19
	4. Intercept	-1705		