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Early signals of *Posidonia oceanica* meadows recovery in a context of wastewater treatment improvements

Bockel Thomas [1, 2, *], Marre Guilhem [1], Delaruelle Gwenaëlle [1], Agel Noémie [1], Boissery Pierre [3], Guilhaumon François [2], Mouquet Nicolas [2, 4], Mouillot David [2], Guilbert Antonin [1], Deter Julie [1, 2]

[1] Andromède océanologie, 7 place Cassan, Carnon plage, 34130 Mauguio, France
[2] MARBEC, UMR IRD-CNRS-UM-IFREMER 9190, Université Montpellier, 34095 Montpellier Cedex, France
[3] Agence de l'Eau Rhône-Méditerranée-Corse, Délégation de Marseille, immeuble CMCI, 2 rue Henri Barbusse, CS 90464, 13207 Marseille Cedex 01, France
[4] FRB - CESAB, Institut Bouisson Bertrand, 5, rue de l'École de médecine, 34000 Montpellier, France

* Corresponding author : Thomas Bockel, email address : thomas.bockel@andromede-ocean.com

Abstract :

Natural ecological restoration is a cornerstone of modern conservation science and managers need more documented “success stories” to lead the way.

In French mediterranean sea, we monitored Posidonia oceanica lower limit using acoustic telemetry and photogrammetry and investigated the descriptors driving its variations, at a national scale and over more than a decade.

We showed significant effects of environmental descriptors (region, sea surface temperature and bottom temperature) but also of wastewater treatment plant (WWTP) effluents proxies (size of WWTP, time since conformity, and distance to the closest effluent) on the meadows lower limit progression.

This work indicates a possible positive response of P. oceanica meadows to improvements in wastewater treatment and a negative effect of high temperatures. While more data is needed, the example of French wastewater policy should inspire stakeholders and coastal managers in their efforts to limit anthropogenic pressures on vulnerable ecosystems.

Highlights

► French *Posidonia oceanica* meadow lower limits are progressing in some areas. ► Acoustic telemetry and photogrammetry allow to monitor *Posidonia oceanica* lower limits. ► Conformity of wastewater treatments has a positive effect on the lower limit. ► Recovery of Posidonia meadows is observed after ambitious environmental policies. ► Data on wastewater impact on *Posidonia oceanica* is missing.

Keywords : Mediterranean sea, Posidonia oceanica, Natural restoration, Wastewater treatment plant, Acoustic telemetry, Photogrammetry

Introduction

The European Parliament voted in July 2023 the “Nature Restoration Law” whose objectives are to restore ecosystems, habitats and species across the EU’s land and sea by 2050. Ecological restoration strategies, which aim at protecting and enhancing biodiversity (Gann et al. 2019), are traditionally subdivided into two categories: “natural” restoration (or spontaneous), where the only action is to stop the cause of the degradation, and “assisted” or “reconstructive” restoration, where other human interventions assist habitat and biodiversity recovery. The former ambiguous “active “ *vs.* “passive” terminology should be avoided (Atkinson and Bonser 2020). Evaluating restoration actions is crucial but often challenging to perform or unachieved (Wortley et al. 2013), in part due to missing guidelines to assess success or failure of those actions (Boudouresque et al. 2021), or to the lack of appropriate quantitative targets and indicators, and missing long-term fundings. Lately, several practices that first promote the implementation of natural restoration, possibly accompanied later by assisted or reconstructive restoration have emerged to achieve defined ecological targets (Jones et al. 2018; Larkin et al. 2019). However, prior to any restoration action, a detailed knowledge of the threats at the origin of the degradation is required (Boudouresque et al. 2021) in order to start reducing/removing them.

Land-based pollution is a major anthropogenic threat to coastal marine ecosystems which has been modeled and mapped at the global (Halpern et al. 2008), Mediterranean (Micheli et al. 2013) and French scale (Holon et al. 2015b). In 1991, the European directive for the treatment of residual urban waters (91/271/CEE) fixed objectives of water quality to prevent damages on receiving ecosystems, and required member states to provide action plans to comply with those objectives. Since then, French wastewater collection networks were improved reducing direct untreated outputs in the environment (French Water Agency personal communication), and wastewater treatment plants (WWTP) progressively modernized their treatment systems to include a biological stage after the preliminary physical treatments (www.assainissement.developpement-durable.gouv.fr). While physical treatment was often limited to processes like filtration and sedimentation, biological treatment allowed the biodegradation of organic matter with the help of microorganisms (https://environment.ec.europa.eu/topics/water/urban-wastewater_en) (Dhote et al. 2012).

Since 2000, another Directive, the European Water Framework Directive (2000/60/EC) requires member states to monitor the water quality within their territory, based on homogenous water bodies regarding ecological and chemical quality. Very sensitive to any change in their environment, *Posidonia oceanica* meadows are used as a proxy to monitor coastal water quality for this Directive but also for the Marine Strategy Framework Directive (2008/56/EC) that aims to protect the marine ecosystem and biodiversity upon which our health and marine-related economic and social activities depend.

Posidonia oceanica L. Delille is an endemic seagrass species of critical ecological importance in the Mediterranean sea (Boudouresque et al. 2006, 2012) *P. oceanica* covers 1 225 707 ha across the Mediterranean Sea, 42 % in western basin and 58 % in the eastern basin, with more abundant available mapping data in the north-western and central part (Telesca et al. 2015). *P. oceanica* meadows provide many important ecosystem services (Campagne et al. 2015) among which carbon sequestration (Pergent-Martini et al. 2021). *P. oceanica* grows between the surface and an average depth of 40 m, depending on light availability. It does not tolerate too strong hydrodynamics (waves physical damages and/or matte erosion (Boudouresque et al. 2006; Ruju et al. 2018)), and extreme salinity values (desalination (Boudouresque et al. 2006) as well as hypersaline waters (Capó et al. 2020; Blanco-Murillo et al. 2023)). *P. oceanica* is also sensible to extreme water temperatures with living *P. oceanica* observed at temperatures ranging from 9 to 29 °C (Boudouresque et al. 2006) and signs of warming and heat waves impacts on plant morphology and growth (growth limited above 27 °C) (Guerrero-Meseguer et al. 2017; Stipcich et al. 2022). While the species shows a promising resilience (Bennett et al. 2022; Stipcich et al. 2023), global warming is therefore a major threat to *P. oceanica* meadows through increases in water temperature (Litsi-Mizan et al. 2023), but also sea level rise, exotic species introduction and seagrass communities replacement (Pergent et al. 2014; Stramska and Aniskiewicz 2019). Anthropogenic pressures can also impact *P. oceanica* meadows (Boudouresque et al. 2009; Marbà et al. 2014) either directly through habitat degradation, such as sea bottom trawling (Pasqualini et al. 2000) or anchoring (Deter et al. 2017), or indirectly through water quality degradation due to coastal development (Holon et al. 2015a) or wastewater effluents (Boudouresque et al. 2006). Horizontal growth of Posidonia meadows is very slow (approx. 1 cm/year) (Marbà and Duarte 1998), making its natural recolonization on damaged areas very long (Cunha et al. 2004). Due to its high sensitivity to changes in environmental conditions, relatively stable at this depth (as opposed to the upper limit, characterized by more fluctuating environmental conditions), the lower limit of the meadow, i.e. the deepest extension limit, deserves specific attention (Boudouresque et al. 2000), in particular for long term monitoring.

In link with the Water Framework Directive needs, important efforts are made to monitor the health status of *Posidonia oceanica* meadows in the French Mediterranean sea, including the TEMPO network composed of 73 lower limits monitored every three years since 2011 (www.medtrix.fr, “TEMPO” project). Innovative and operational methods were developed to accurately localize and map the lower limit of the meadow such as acoustic telemetry (Descamp et al. 2011) and photogrammetry (Marre et al. 2019, 2020). These methods allow the accurate mapping of *P. oceanica* lower limits with a precision of up to 1 cm using acoustic telemetry (Descamp et al. 2011), and 0.5 cm using photogrammetry (Marre et al. 2020).

Posidonia oceanica meadows on the French Mediterranean coast experienced a decline in the past decades, accompanied by a retreat of the lower limit, mainly due to important anthropogenic pressures such as coastal development, pollution and anchoring (Boudouresque et al. 2009; Telesca et al. 2015; Holon et al. 2015b, a). However, although very slow, the natural recovery of meadows after pressure removal can be observed (Agostini et al. 2002): signs of recovery were reported in recent studies (de los Santos et al. 2019), consistent with field observations along the French coastline (Andromède océanologie 2021). For instance, recovery following improved wastewater treatments has been reported at the upper limit of the meadow (Boudouresque et al. 2000) and very close to the wastewater effluent (Boudouresque et al. 2021). This work is the first, to our knowledge, to directly test the link between wastewater treatment proxies and a change in surface at the lower limit of *Posidonia oceanica* meadows. We expect that wastewater treatment improvements participated, over more than a decade, in creating adequate environmental conditions for the meadows to start recovering. Yet, many environmental (e.g. sea surface temperature) covariates may also influence the recovery of *P. oceanica* meadows. In this work, we analyzed the influence of environmental and anthropogenic pressures on the variation of the surface of the meadow lower limit, using linear mixed models, at a national scale and over more than a decade of data. We aimed at first identifying the anthropogenic and environmental parameters driving *P. oceanica* surface change at its lower limit, and then investigating the possible effect of improved wastewater effluents quality for the surrounding meadows. The results of this study highlight the importance of threat removal as a natural restoration action, and help reveal the local (environmental and anthropogenic) context under which restoration investment returns can be expected.

Material and methods

Surface covered by the Posidonia oceanica meadow at the lower limit

Annual changes in the surfaces covered by *Posidonia oceanica* meadows at the lower limit were recorded within the TEMPO monitoring network (www.medtrix.fr, “TEMPO” project), in the French Mediterranean sea (1800 km of coastline). The monitoring sites of the TEMPO network were initially defined to be homogeneously localized along the coastline, representative of the surrounding Water Framework Directive waterbody, and to be balanced between pristine and anthropized areas. The average depth of the lower limit monitoring sites is equal to 27 meters. Surveys occurred every three years on each French marine subregion ((Provence-Alpes-Côte-d’Azur called PACA), Corse and Occitanie) since 2011. The dataset is composed of a total of 121 observations (site x year) (not every site was monitored at every survey due to a constant evolution of the network) for 50 distinct sites (50 sites only had interpretable photogrammetric results out of the 73 of the network), 11 years (2012-2022) and three regions (PACA (77 observations/29 sites), Corse (35 observations/18 sites), Occitanie (9 observations/3 sites)) (Figure 1). This heterogenous number of sites reflects the heterogeneous areas covered by *P. oceanica* in each region (26 225 ha in PACA, 52 672 ha in Corse and 133 ha in Occitanie according to the most accurate and up to date biocenosis map (www.medtrix.fr, “DONIA expert” project)). The regions are subdivided into waterbodies (37 in total), containing between 1 and 3 sites each. The position of the meadow lower limit was evaluated using telemetry (2011-2018) and photogrammetry (2016-2022) . For telemetric surveys, the boundary of the meadow was pointed by a scuba diver using AQUA-METRE D100, with an average of one point every 40 cm, adjusted locally to the complexity of the limit (Descamp et al. 2011). For photogrammetric surveys, photographic acquisitions were conducted on each site by a scuba diver at an average distance of 2 meters from the sea bottom, using a 16 Mega Pixel Nikon D4 in a waterproof Seacam housing, mounted with a Nikon RS 20 - 35 mm lens (set to 20 mm). To achieve a sufficient balance between depth of field, sharpness, and exposure, we used the following camera settings: shutter speed = 1/250 s, aperture = F12, sensibility = 1200 ISO on average (Marre et al. 2019). The photographs were processed with Agisoft Metashape Professional Version v1.8.4 (Agisoft 2022). This commercial software has been extensively used by the scientific community (Burns et al. 2015b, a; Marre et al. 2019), and allows to get through the whole photogrammetric workflow: automatic identification of key points on all photos, bundle adjustment, point cloud densification, mesh building and texturing / orthomosaic production. Position of the lower limit (for telemetry) and orthomosaics of the meadow cover (for photogrammetry) were then superimposed between each survey for each site and the variations in meadow cover were manually digitized and quantified using GIS tools (QGIS 3.16) (Figure 2). The final retained indicator was the net variation rate (progression - regression) between each survey, in percentage of the total *P. oceanica* surveyed area, divided by the number of years between each survey. This indicator will be referred in this work as “annual rate of surface change”. Surveys were realized each year at the same period (May – June) to avoid differences in leaf growth stage.

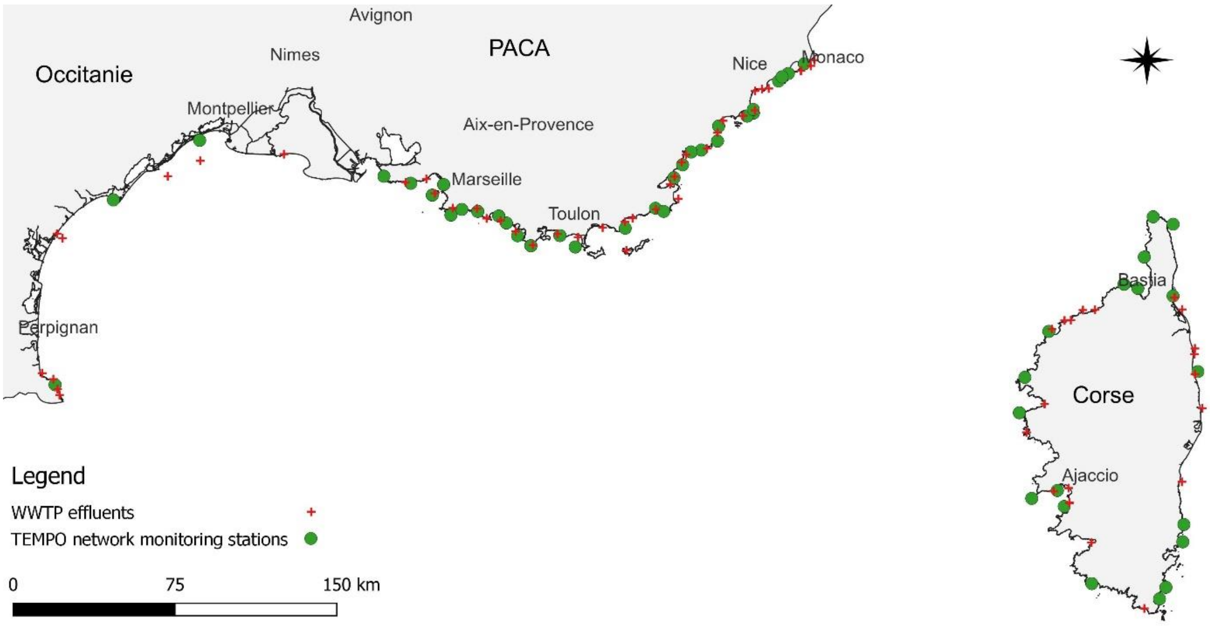

Figure 1 Localization of TEMPO lower limit monitoring sites, wastewater treatment plants (WWTP) effluents, and the three regions (Occitanie, PACA, Corse) in the French Mediterranean sea

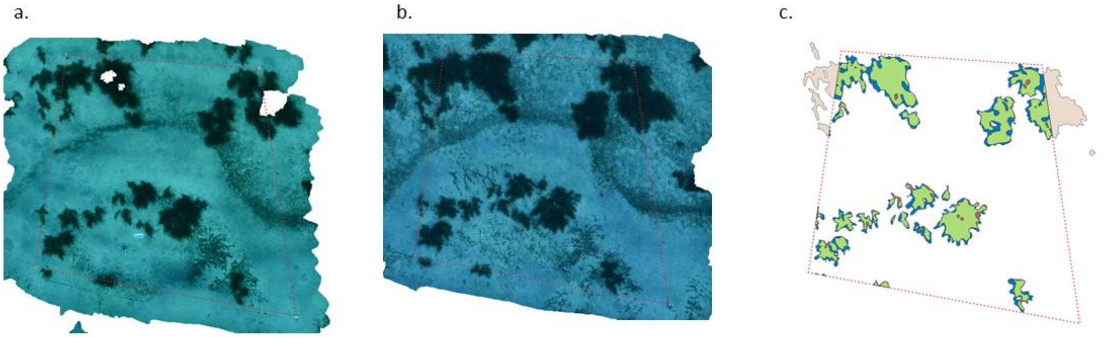

Figure 2 Illustration for the "Cap Sicié Ouest" monitoring site in region PACA of photogrammetric orthomosaics and study site delimitation for a. 2018 and b. 2021. c. Digitalization of concordant areas (in green), positively discordant areas (in blue) and negatively discordant areas (in red) between 2018 and 2021, respectively interpreted in terms of stability, progression and regressions to calculate the “annual rate of surface change”.

Local, anthropogenic and environmental descriptors

Local (Table 1), anthropogenic (Table 2) and environmental (Table 3) descriptors were used in this study to explain observed surface change at the meadows lower limit.

Table 1 Local descriptors for each survey site (AERMC = Agence de l'eau Rhône Méditerranée Corse, the French water agency)

Descriptors	Unit/modality
depth	m
distance to shore	m
region	Occitanie, PACA, Corse
waterbody	AERMC waterbody
site	TEMPO site
year	2011-2022

The descriptors of the anthropogenic pressure due to WWTP effluent were extracted from the French government collective sanitation website (www.assainissement.developpement-durable.gouv.fr). For each survey site, all descriptors in Table 2 were calculated for the WWTP with the closest effluent. The variable "type of treatment of WWTP" is a decreasing score ranging from 4 to 0, reflecting the complexity of the following treatments: settling (4), biological simple/biological with nitrification (3), biological with nitrification and denitrification (2), biological with nitrification, denitrification and dephosphatation (1), and physico-chemical (0). Descriptors of treatment efficiency were calculated as the ratio of the pollution entering over the pollution leaving the WWTP after treatment. Descriptors of cumulative output were calculated as the sum of the pollution leaving the WWTP after treatment over the years. Descriptors of treatment efficiency and cumulative output were calculated over the last 20 years (from 1999) before each survey (when data was available).

Table 2 Descriptors of wastewater treatment plants (WWTP) effluents (calculated for the closest effluent for each survey site)

Descriptors	Unit	Temporal range
size of wastewater treatment plant (WWTP)	population equivalent (pe)	Date of survey
total population (permanent + seasonal)	number	Date of survey
type of treatment of WWTP	-	Date of survey
distance to WWTP effluent	km	Date of survey
time since WWTP creation	years	Date of survey
time since WWTP conformity	years	Date of survey
mean Suspended Matter (SM) purification efficiency	%	1999-2020
mean Chemical Oxygen Demand (COD) purification efficiency	%	1999-2020
mean Nitrogen (N) purification efficiency	%	1999-2020
mean Phosphorus (P) purification efficiency	%	1999-2020
SM cumulative output	Kg	1999-2020
COD cumulative output	Kg	1999-2020
N cumulative output	Kg	1999-2020
P cumulative output	Kg	1999-2020

Environmental descriptors included sea surface temperature, chlorophyll a, turbidity, bottom temperature, salinity and seawater velocity (Table 3). Mean and standard deviation of each environmental descriptor were calculated over the last 20 years before each survey (when data was available (see Table 3 for temporal range of data)). The dataset was subsampled to 1 day per week because of computational limits during data acquisition. Diffuse attenuation coefficient of light at 490 nm (Kd490) was used as a proxy for turbidity, as already shown appropriate in the literature (Shi and Wang 2010). All environmental descriptors, except bottom temperature, were estimated for the sea surface.

Table 3 Environmental descriptors for each survey site

Descriptors	Unit	Spatial resolution	Temporal range	Temporal resolution	Source
sea surface temperature (SST)	Celsius degree	0.01 degree	2002-2021	daily	Nasa
chlorophyll a (CHLA)	$mg.m^{-3}$	0.042 degree	2002-2021	daily	Nasa
turbidity (Kd490)	m^{-1}	0.042 degree	2002-2021	daily	Copernicus
bottom temperature	Celsius degree	0.083 degree	2000-2020	daily	Copernicus
salinity	psu	0.083 degree	2000-2020	daily	Copernicus
seawater velocity	$m.s^{-1}$	0.083 degree	2000-2020	daily	Copernicus

Statistical analyses

All descriptors were centered around mean and scaled by standard deviation. The correlation between each pair of descriptors was checked to remove too strongly correlated covariates (> 0.7), by keeping in this case the variable with the strongest correlation to the response variable (Supplementary file, Figure S1). As most sites were surveyed several times across the period, and some waterbodies (AERMC waterbodies) contained more than one site, we built a linear mixed model (Bunnefeld and Phillimore 2012; Brown 2021) to evaluate the effects of each descriptor on the response variable (annual rate of surface change of the meadow at the lower limit). The full model integrated the waterbody and site (nested within waterbody) as random effects (random intercept), to consider the ecological and environmental conditions specific to each waterbody and site of survey. The full model integrated all other predictors as fixed effects, ordered by their participation to the full model R^2. Model complexity was then reduced using backward elimination of fixed effect terms (Supplementary file, Table S1). Spatial autocorrelation in the model residuals was tested using the moran's index (Gittleman and Kot 1990). We then checked for homogeneity of variance and normality of the model residuals (Supplementary file, Figure S2). We finally plotted the marginal effect of each descriptor significantly influencing the response variable.

We also calculated mean SST and bottom temperature for each year and study site and estimated average yearly increase of both parameters on the study area using regression (Supplementary file, Figure S3).

Statistical analyses were performed using the statistical software R version 4.1.2 and R Studio version 2022.07.2 (R Core Team 2022), and the R packages lmerTest 3.1.3, DHARMa 0.4.6 and effects 4.2.2.

Results

The mean annual rate of surface change of the meadow at the lower limit is highly heterogeneous among regions and years with Occitanie and PACA showing the highest mean values (M = mean, SD = standard deviation, N = number of samples) in the recent years : Occitanie (2022: M = 6.0, SD = 0, N = 1; 2021: M = 8.5, SD = 1.34, N = 2; 2018: M = 9.3, SD = 14, N = 2) and PACA (2021: M = 5.0, SD = 3.2, N = 9; 2017: M = 7.0, SD = 10, N = 2) (Figure S4).

The descriptors Suspended Matters (SM) cumulative output, Chemical Oxygen Demand (COD) cumulative output, Nitrogen (N) cumulative output, Phosphorus (P) cumulative output, chlorophyll a (CHLA) mean and standard deviation, salinity mean and standard deviation, sea surface temperature (SST) standard deviation, bottom temperature standard deviation and mean turbidity were removed from the model because they were too strongly correlated. The descriptors distance to shore, depth, time since WWTP creation, type of treatment of WWTP, standard deviation of turbidity, mean Suspended Matter (SM) purification efficiency, mean Nitrogen (N) purification efficiency, mean Phosphorus (P) purification efficiency and total population were removed from the model during backward elimination.

The final model explaining the annual rate of surface change contained random intercepts for the descriptors waterbody and site (nested within waterbody), and the following descriptors as fixed effects: region, size of WWTP, mean seawater velocity, distance to WWTP effluent, time since WWTP conformity, mean bottom temperature, mean COD purification efficiency, and mean SST. The proportion of variance explained by the model including fixed and random effects was 66 %. Fixed effects alone explained 38 % of the variance. The variance of the model intercept among waterbodies was equal to 4.8, and 1.1 among sites.

The most influent descriptors were the time since conformity of the WWTP ($\beta = 1.7$, $t(85) = 3.9$, $p = 0.0002$), the distance to the WWTP effluent ($\beta = -1.9$, $t(53) = -3.3$, $p = 0.002$), the size of the WWTP ($\beta = -1.5$, $t(40) = -3.3$, $p = 0.002$), and the region (significant difference between Corse and PACA ($\beta = 7.4$, $t(48) = 3.4$, $p = 0.001$)) (Figure 3, Figure 4 and Table S2).

The residuals of the final model did not exhibit spatial autocorrelation (Moran's I = 0.009, p = 0.64).

We estimated an average yearly increase over the study area of 0.025 °C for the SST ($R = 0.2$, $p = 7.2\ 10^{-10}$) and 0.035 °C for the bottom temperature ($R = 0.24$, $p = 3.5\ 10^{-13}$) (Figure S3).

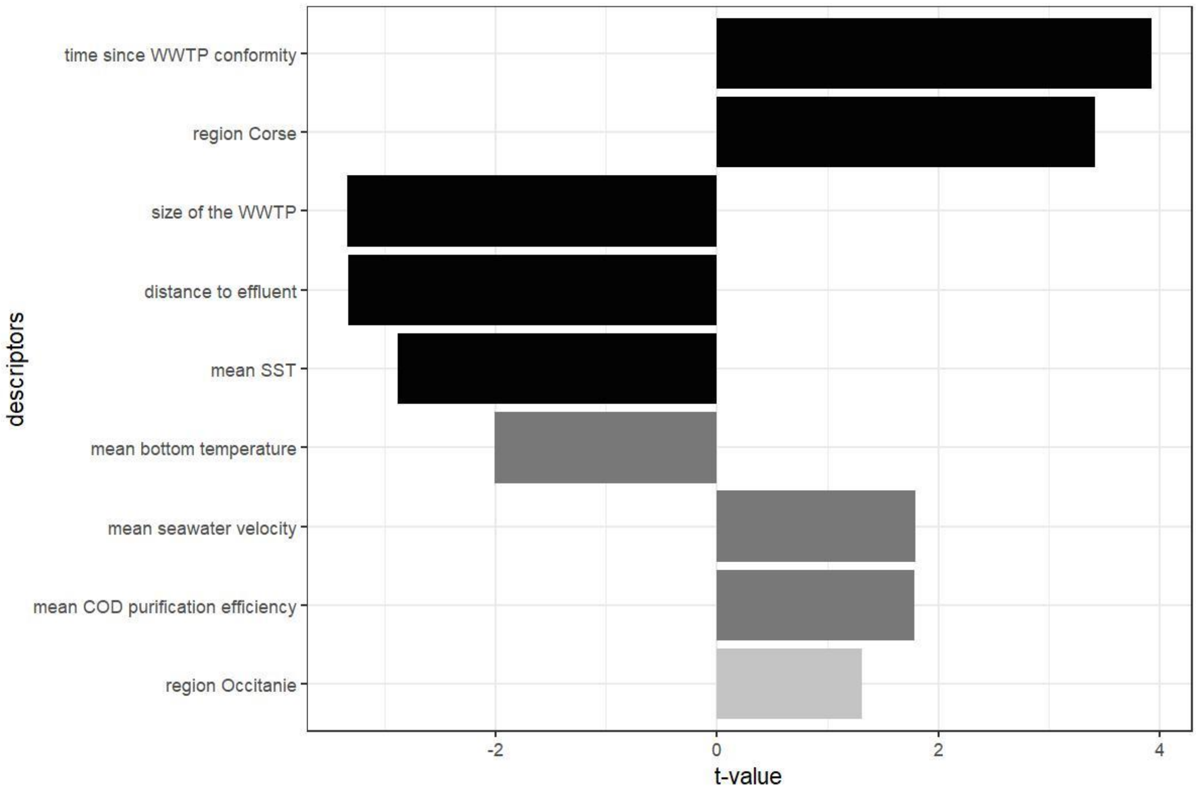

Figure 3 T-value and significance of the estimate for each descriptor used in the model. Black bars represent significant descriptors ($p < 0.05$), dark grey bars represent marginally significant descriptors ($0.05 < p < 0.1$) and light grey bars represent non-significant descriptors ($p > 0.1$).

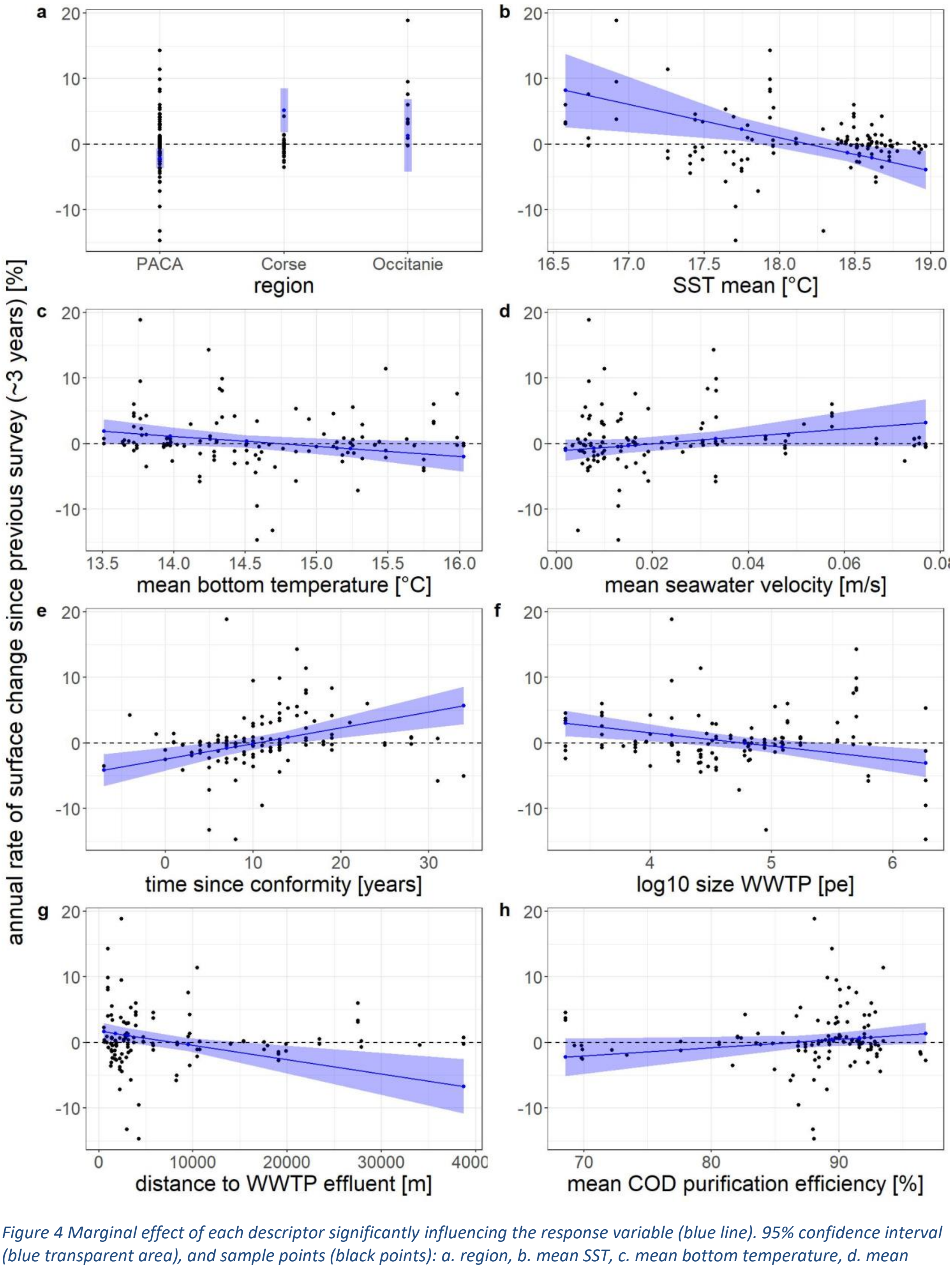

Figure 4 Marginal effect of each descriptor significantly influencing the response variable (blue line). 95% confidence interval (blue transparent area), and sample points (black points): a. region, b. mean SST, c. mean bottom temperature, d. mean seawater velocity, e. time since conformity, f. log10 of the size of the Wastewater Treatment Plant (WWTP), g. distance to the WWTP effluent, h. mean COD purification efficiency. The black horizontal dashed line represents a null annual rate of surface change.

Discussion

Threats reduction is the first step of ecological restoration and is considered a prerequisite to any further assisted or reconstructive restoration actions. Long term benefits of natural restoration are however too rarely evaluated and documented in the literature. Besides anthropogenic pressures, better understanding adequate environmental conditions and their effect will benefit future protection and restoration actions. According to our hypotheses, we showed significant effects of WWTP effluents proxies (size of WWTP, time since conformity, and distance to the closest effluent) but also of environmental descriptors (region, sea surface temperature and bottom temperature) on the meadows lower limit progression temporal trend.

Signs of progressions

Our work shows numerous recent progressions (above 5 %) in surface of the *Posidonia oceanica* meadow at the lower depth limit along the French Mediterranean coastline. Those patterns have been particularly observed in the region Occitanie (2022: 6 %, 2021: 8.5 %, 2018: 9.3 %) and PACA (2021: 5 %; 2017: 7 %) and are coherent with field observations (Andromède océanologie 2021). Progressions were also observed in the literature (de los Santos et al. 2019), often linked with management plans leading to nutrient input reductions and water quality improvements (e.g. Danish fjords 1990-2010, catalonia coast 2000-2010).

Role of the environment

An important part of the variation of the lower depth limit position was explained by random effects, especially waterbodies. Those waterbodies, defined in the framework of the European Water Framework Directive (2000/60/EC), are considered homogeneous regarding ecological functioning and anthropogenic pressures. This indicates differences between locations in the response of the meadow to its environment, possibly driven by local and historical conditions (McDonald et al. 2023) that are not already captured by the other descriptors.

The region had a significant effect on the predicted annual rate of surface change of the meadow lower limit, with higher average values predicted for Corse ($p = 0.001$) and Occitanie ($p = 0.2$) than for PACA. These results suggest that the conservation policy should be adapted to the regional context and its specificities (e.g. island geography for Corse, influence of the Rhône river for Occitanie). The progressive dynamics observed in Occitanie, corresponding to previously highly damaged meadows (Deter et al. 2022), could for example require a closed monitoring of the progression rates, and an eventual assistance with restoration actions if needed (Jones et al. 2018; Larkin et al. 2019) such as protection against anchoring or planting fragments (wreck fragments (www.medtrix.fr, “REPIC” project) or fragments sampled from the meadow (Pergent-Martini et al. 2022)) or germinated seeds (Bacci and La Porta 2022).

The mean SST and mean bottom temperature followed an increasing trend on the study area and period (+ 0.025 °C / year for SST and + 0.035 °C / year for bottom temperature). The mean SST and mean bottom temperature had a significant negative influence on the predicted annual rate of surface change of the meadow lower limit ($p = 0.006$ and $p = 0.05$). Negative values of surface change were predicted for mean values of SST above 18 °C and for mean values of bottom temperature above 14.5°C. These average temperatures are far from the species observed thermal range (Boudouresque et al. 2006). *P. oceanica* partial resilience to thermal stress is documented in the literature (Bennett et al. 2022; Stipcich et al. 2023), depending on the local environmental and physical conditions such as depth (Marín-Guirao et al. 2016). As a consequence of climate change, sea temperature and sea level (also affecting the meadows through light availability) are expected to keep rising (Pergent et al. 2014; IPCC 2023). In this context, Posidonia meadows lower depth limits require particular monitoring attention.

The mean seawater velocity had a marginally significant positive effect on the predicted annual rate of surface change of the meadow lower limit (p = 0.08). Negative values of surface change were predicted for values of seawater velocity below 0.02 m/s in average. The scientific literature agrees on the negative effect of strong hydrodynamics on Posidonia meadows (Boudouresque et al. 2006), but there is a lack of study regarding very weak hydrodynamics conditions. This result is nevertheless not surprising as a certain amount of current may help (Vacchi et al. 2012), at least through the control of suspended matter concentration in the water column and hence light availability. The maximum calculated mean seawater velocity in this study (approx. 0.1 $m.s^{-1}$) is moreover low and most probably far from the species tolerance limit.

Role of the Wastewater Treatment Plants (WWTP)

This work shows that while environmental conditions play a major role in the ecological status of the Posidonia meadow (Houngnandan et al. 2020), anthropogenic pressures, in this case WWTP effluents, also strongly influence the dynamic of progression of this highly sensitive habitat mainly through conformity, size, and distance.

The time since conformity of the WWTP to the European directive 91/271/CEE had a significant positive effect on the predicted annual rate of surface change of the meadow lower limit (p = 0.0002). Positive values of surface change were predicted on average 10 years after compliance. This result seems to indicate the efficiency of the regulation in preserving the environment receiving the treated wastewaters. The 10 years lag in the observed response moreover corresponds to the history of change of wastewater treatments in the French Mediterranean, with an important number of modernization projects around 2010 (20 years after the Directive) and signs of progression of the meadow observed in this study mostly from 2020 onwards (10 years after the modernization).

The size of the WWTP had a significant negative influence on the predicted annual rate of surface change of the meadow lower limit (p = 0.002). Larger WWTPs have to treat more important quantities of wastewater and discharge at the same outlet larger quantities of treated freshwater within the marine environment while *P. oceanica* is very sensitive to changes in salinity. While French regulation fixes high standards of elimination of organic pollution (75 % of DCO) and suspended matters (90 %) for large WWTPs (French decree 22/06/2007), some of this pollution remains in the treated water and is released in the environment, with higher quantities around bigger WWTPs. On the basis of these results, favoring several small outlets rather than a large one is therefore a question that arises for future developments. Although the influence of the size of the WWTP is expected, documented reports of this impact are limited in the literature, more focused on the benefits of new WWTP installation where previously not existing (Pergent-Martini et al. 2002; Boudouresque et al. 2021).

The distance to the WWTP effluent had a significant negative influence on the predicted annual rate of surface change of the meadow lower limit (p = 0.002). This counter-intuitive result in the first place is probably due to large areas of dead matte available for the recovery of the meadow on the most impacted sites in the past (near the effluents), by effluent pipes construction and/or poor effluent quality (Boudouresque et al. 2021). A recovery is indeed much more probable on a previously degraded meadow. The proximity of the WWTP effluent was moreover measured without considering the spatial distribution of the effluent plume. Hydrodynamic modeling the effluent plumes (Bedri et al. 2014) could provide interesting inputs to further investigate the effect of distance from the effluent.

The mean COD purification efficiency had a marginally significant positive effect on the predicted annual rate of surface change of the meadow lower limit (p = 0.08). COD is a direct proxy of organic matter concentration. This result again seems to indicate a positive response of the meadow to more efficient wastewater treatments and organic matter reduction, leading to better water quality. This result is however contrasted by mandatory surveys of the receiving sites, mostly indicating no organic matter accumulation in the sediments under open sea conditions (French Water Agency personal communication). Monitoring the organic matter in treated wastewater should be continued, so as research efforts towards better organic matter reduction processes such as the use of nanomaterials and nanofiltration (Zahmatkesh et al. 2023) and electrocoagulation (Mousazadeh et al. 2021).

An example of effective natural restoration

This study highlights signs of starting natural restoration of the Posidonia meadow after the limitation of wastewater output pressure. The results of this study in fact indicate that the influence of the improvement of wastewater treatments and the progressive achievement of conformity to the European directive 91/271/CEE, not only induce positive effects at the meadows upper limits as previously suggested (Boudouresque et al. 2000) but might also benefit the meadows at their lower limit and thus certainly participate to an improvement of the whole meadow ecosystem.

However, despite its 20 years of data, this work suffers from a lack of past data to quantify historical regressions for sites close to effluents, and possibly anterior or linked to effluents construction. Despite WWTP operators being forced by the regulation to conduct periodical surveys of the different ecological compartments of the receiving sites (Andral et al. 2011), the data coming from those surveys are rarely shared with the scientific community. Better transparency, data storage, metadata documentation, and availability of those datasets is crucial for a better understanding of the complex impacts of WWTP effluents on their receiving environment, eventually leading to a virtuous circle towards better protection of the marine ecosystems.

Conclusion

This work shows that while increasing sea temperature negatively influences *Posidonia oceanica* meadows lower limits, improvements of wastewater treatments can have a positive effect.

This case study should inspire stakeholders for new regulations and coastal managers for better enforcement, in their efforts to limit anthropogenic pressures on vulnerable ecosystems. French WWTPs are now in most cases equipped with conform secondary treatment (approx. 98% in 2022 (https://www.assainissement.developpement-durable.gouv.fr/pages/data/carteIntSteu.php)) able to efficiently reduce the organic matter present in the wastewater. This is far from being true when looking at the Mediterranean scale and even more at the global scale. “UN Water” estimates an average of 70 % of wastewater treated for high-income countries, 33 % for middle-income countries and 8 % for low income countries (European Investment Bank. and Environment and Natural Resources Department. 2022).

This work urges the necessity of treating wastewaters before their release in the marine environment, and strongly advises the implementation of adapted secondary treatments. This study shows an average time lag of 20 years between european directive and WWTPs equipment conformity, and 10 more years before any positive observed response of the meadows. No time is therefore to be lost before giving back high-quality wastewaters to the water cycle that first provided clean freshwater to our faucets. This is particularly true in a climate change context inducing warming coastal waters with possible negative effects on *Posidonia oceanica* meadows.

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Contributors

TB realized the statistical analysis and wrote the scientific paper. GM built the photogrammetric models and helped in designing the statistical analysis. GD helped the GIS analysis and the results interpretation. NA performed the GIS analysis. PB, NM and DM helped designing the research question and interpreting the results. FG helped designing the statistical analysis. AG supervised most of the fieldwork and provided precious advice. JD supervised the work, helped designing the research question and interpreting the results. All authors helped improving the manuscript.

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Supplementary material for

Early signals of *Posidonia oceanica* meadows recovery in a context of wastewater treatment improvements

Author names and affiliations : Thomas Bockel[a,b]., Guilhem Marre[a], Gwenaëlle Delaruelle[a], Noémie Agel[a], Pierre Boissery[c], François Guilhaumon[b], Nicolas Mouquet[b,d] , David Mouillot[b], Antonin Guilbert[a], Julie Deter[a,b]

[a] Andromède océanologie, 7 place Cassan, Carnon plage, 34130 Mauguio, France

[b] MARBEC, UMR IRD-CNRS-UM-IFREMER 9190, Université Montpellier, 34095 Montpellier Cedex, France

[c] Agence de l'Eau Rhône-Méditerranée-Corse, Délégation de Marseille, immeuble CMCI, 2 rue Henri Barbusse, CS 90464, 13207 Marseille Cedex 01, France

[d] FRB - CESAB, Institut Bouisson Bertrand. 5, rue de l'École de médecine, 34000 Montpellier, France

*Corresponding author (thomas.bockel@andromede-ocean.com)

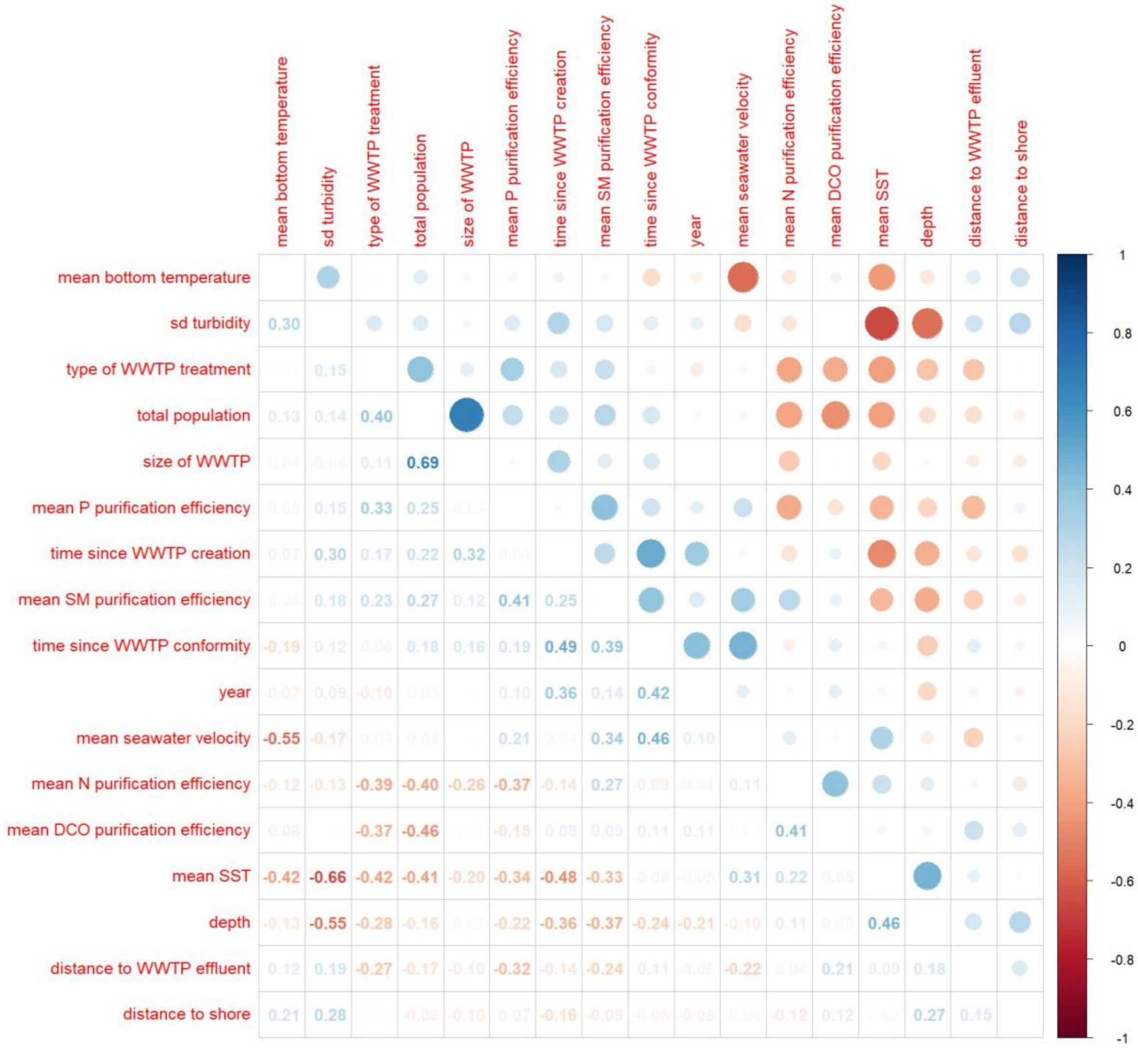

Figure S1 Correlation plot for descriptors used in the model

Table S1 Anova like elimination table for fixed effects obtained after performing backward elimination using the step function of the lmerTest R package

	Eliminated	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
mean SM purification efficiency	1	0,71	0,71	1	120,93	0,11	0,74
total population	2	2,92	2,92	1	81,45	0,46	0,50
mean N purification efficiency	3	3,82	3,82	1	108,04	0,60	0,44
distance to shore	4	5,46	5,46	1	118,62	0,84	0,36
sd turbidity	5	3,29	3,29	1	42,39	0,50	0,48
depth	6	5,14	5,14	1	69,06	0,79	0,38
time since WWTP creation	7	5,67	5,67	1	66,55	0,87	0,36
mean P purification efficiency	8	16,62	16,62	1	103,23	2,48	0,12
type of WWTP treatment	9	13,14	13,14	1	74,46	1,89	0,17
region	0	132,85	66,42	2	47,66	9,37	0,00
time since WWTP conformity	0	109,44	109,44	1	84,88	15,43	0,00
mean seawater velocity	0	22,73	22,73	1	39,81	3,21	0,08
size of WWTP	0	79,02	79,02	1	39,68	11,14	0,00
mean SST	0	58,95	58,95	1	46,99	8,31	0,01
mean bottom temperature	0	28,61	28,61	1	47,57	4,03	0,05
mean DCO purification efficiency	0	22,50	22,50	1	58,90	3,17	0,08
distance to WWTP effluent	0	78,48	78,48	1	53,30	11,07	0,00

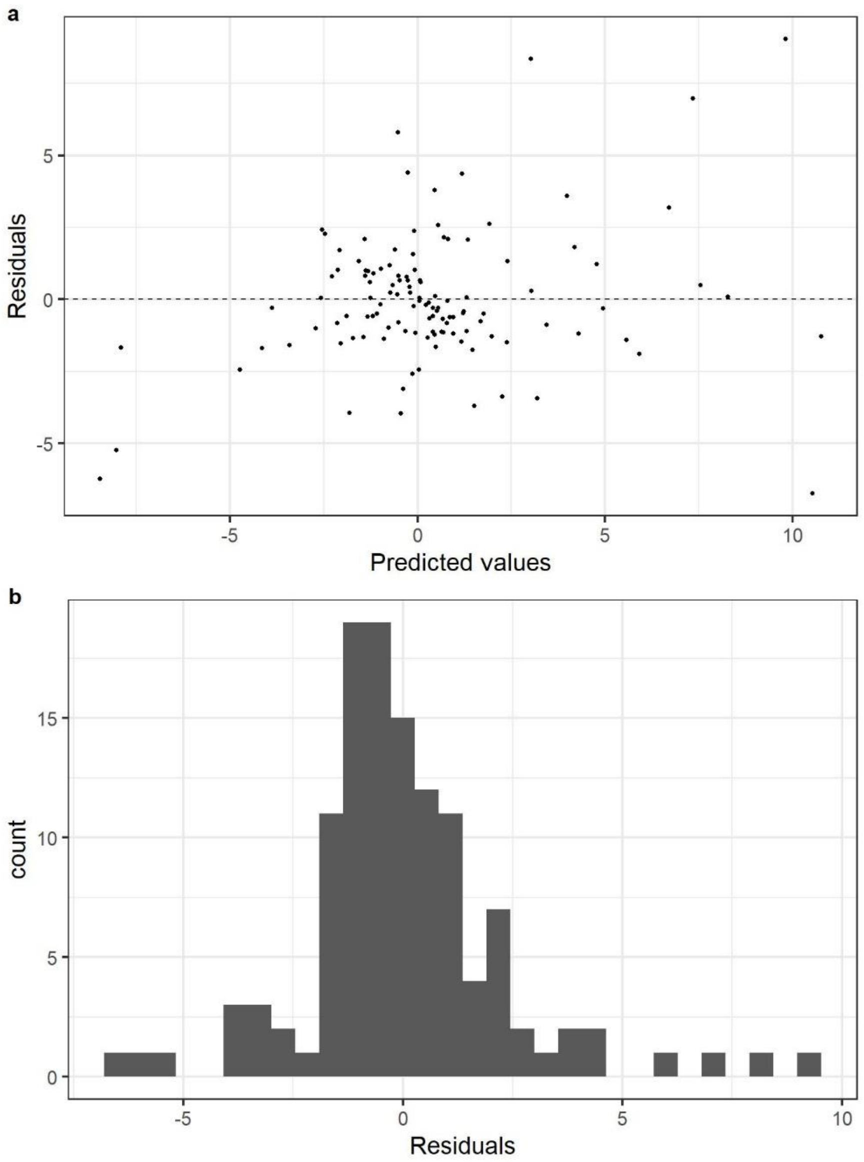

Figure S2 Validation plots for the final linear mixed model: a. residuals vs predicted values; b. histogram of residuals

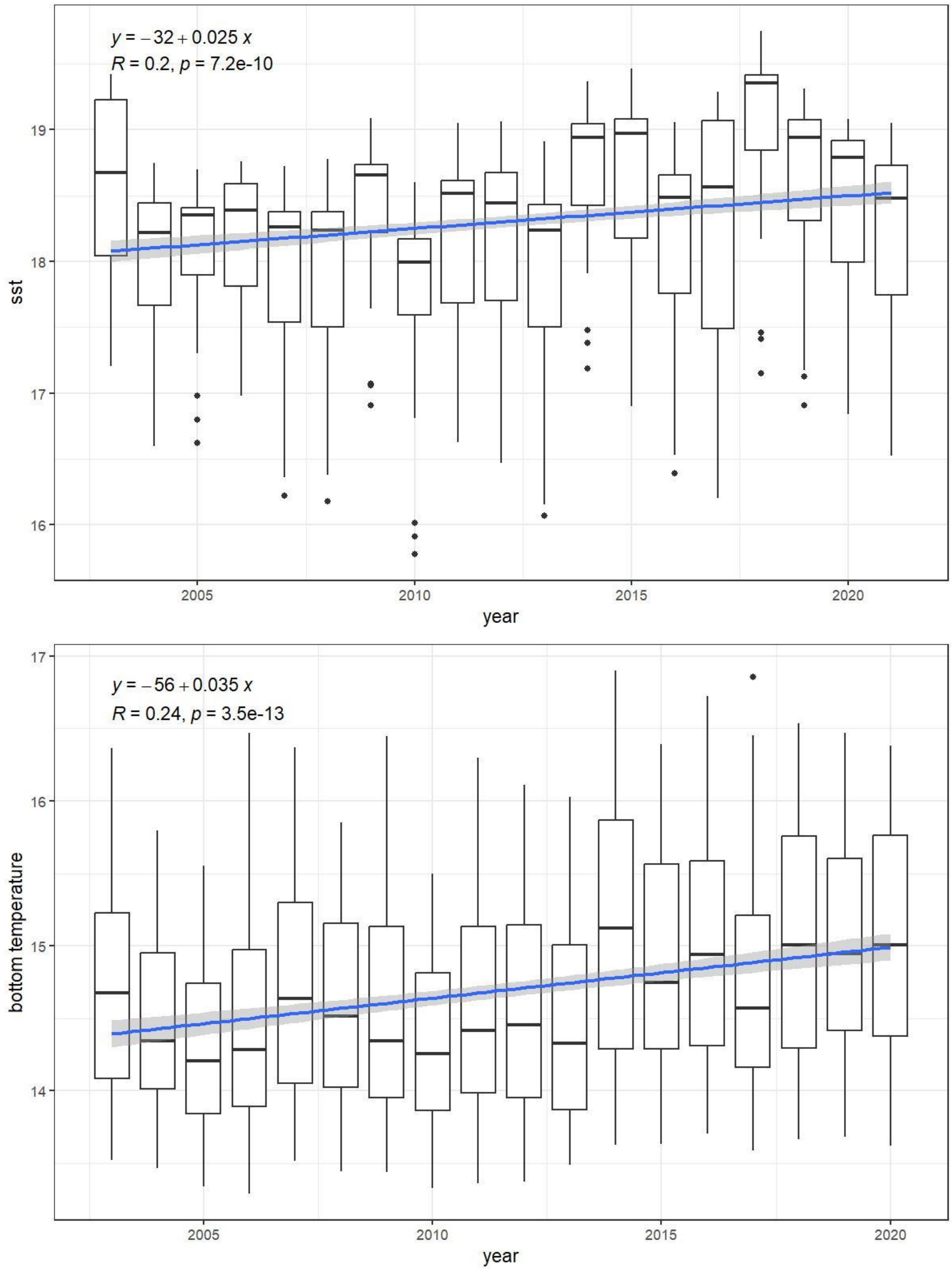

Figure S3 Distribution of annual average SST (top) and bottom temperature (bottom) over the study sites. The blue line represents the fitted regression (equation, R and p value at the top left corner of each plot)

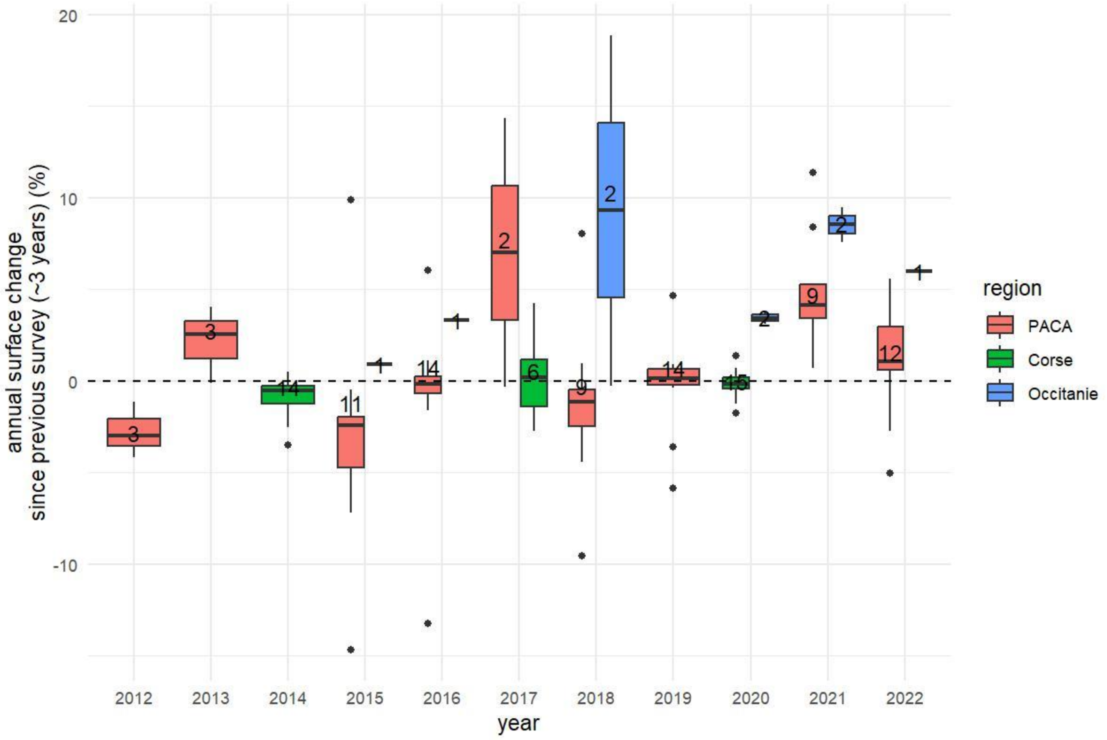

Figure S4 Annual surface change at the lower limit of meadow since previous survey (~3 years) [%], for each region, and for each year. The black horizontal dashed line represents a null net surface change.

Table S2 Fixed effects coefficients for the final linear mixed effects model

	Estimate (β)	**std. Error**	**df**	**t-value**	**p**
(Intercept)	-2.203	0.752	49.216	-2.931	0.005
region Corse	7.384	2.16	47.544	3.418	0.001
region Occitanie	3.505	2.675	41.32	1.31	0.197
time since WWTP conformity	1.72	0.438	84.88	3.929	0.0002
mean seawater velocity	1.193	0.666	39.815	1.791	0.081
size of the WWTP	-1.467	0.439	39.677	-3.338	0.002
mean SST	-3.08	1.068	46.988	-2.883	0.006
mean bottom temperature	-1.075	0.535	47.57	-2.009	0.05
mean COD purification efficiency	0.819	0.46	58.9	1.781	0.08
distance to effluent	-1.86	0.559	53.299	-3.327	0.002