ER390 Final Report

# Intertidal Seagrass Restoration in the Natural Marine Park of Arcachon Bay



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Restoration of Natural Systems Diploma

University of Victoria

## Acknowledgements

I am very grateful to the Natural Marine Park of Arcachon Bay for welcoming me and allowing me to work within such an amazing team!

I would like to express my special thanks to Thomas Fauvel and Nancy Shackelford for supervising me throughout the project duration.

I also want to thank Romuald, Stéphan, and Mica for transporting us to field sites when a boat was needed.

Finally, I would like to thank Laurie, Lisa, Mathilde, François, Justine, Mégane & Ilana for accepting to assist me with the field work (also known as mud bath!). I really could not have completed this work without your help.

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## <u>Abstract</u>

Seagrass meadows are key coastal ecosystems sustaining a diversity of ecosystem services for people and nature. Arcachon Bay, a 174 km<sup>2</sup> semi-enclosed protected lagoon on the southwestern Atlantic coast of France, is home to large meadows of Zostera marina and Zostera noltii. Yet, both seagrass species have undergone a sharp regression in the 2000s under various anthropic and environmental pressures. The Natural Marine Park of Arcachon Bay, the public entity responsible for Arcachon Bay conservation, experiments with active restoration of Z. noltii meadows through seed- and transplant-based restoration. The ER390 project aimed to contribute to Z. noltii restoration by assessing transplantation performances across the Bay to investigate habitat suitability, and to a lesser extent by facilitating participatory seed collection. A protocol to monitor transplants was created and implemented over the summer 2023. Following monitoring findings, most successful tidal flats for transplantation are identified and a set of environmental factors influencing Z. noltii survival and growth is examined. Subsequent recommendations are formulated regarding future monitoring and transplantation. A large quantity of Z. noltii seeds has been collected through participatory seed collection which also contributed to improve public awareness of seagrass meadows, and to harness a sense of stewardship within the local community. Finally, avenues for public participation and information to amplify local seagrass protection and restoration are explored.

## Introduction

Seagrass meadows are highly productive and biodiverse coastal ecosystems providing a wide range of ecosystems services across the globe (Duarte, 2002; Orth et al.,2006; Waycott et al., 2009). As bioengineers, seagrasses create complex habitats which serve as nurseries, shelters, and food for a diversity of marine and avifauna species (Auby, 1991). Besides, seagrass beds improve water quality by trapping and retaining sediments from the water column. Seagrasses also stabilize the seabed, control erosion along coastlines and mitigate storm surges. As they sequester atmospheric and suspended organic carbon, these ecosystems act as natural carbon sinks. Yet, seagrasses are threatened by anthropic activities (e.g. coastal engineering, chemical and nutrient pollution), and are particularly vulnerable to climate change, particularly warmer water temperature, extreme weather events, and rising sea levels (Duarte, 2002; Repolho et al., 2017; Waycott *et al.*, 2009). This context calls for proactive seagrass conservation and restoration.

#### Study area

Arcachon Bay is a 174 km<sup>2</sup> semi-enclosed protected lagoon located in the Gulf of Biscay on the southwestern coast of France (44°40 N, 1°10 W) (*figure 1*). The Bay connects to the Atlantic ocean through narrow passes. Arcachon Bay encompasses a diversity of marine, coastal sand ecosystems, mudflats, saltmarsh, riverine and forest ecosystems. Most importantly, Arcachon Bay is home to the largest dwarf eelgrass (*Zostera noltii*) beds in Europe spanning over 4 564 hectares on intertidal sand and mudflats (Plus et al., 2010; PNMBA, 2017). Alongside dwarf eelgrass meadows are 104 hectares of eelgrass (*Zostera marina*) fringes in subtidal areas of channels. Both *Zostera* species are legally protected from harm and are part of conservation schemes (Hilly & Bajjouk, 2010).



*Figure 1.* Map of the Natural Marine Park of Arcachon Bay (the yellow line correspond to the Park boundaries while the green line represents the boundaries of the EU Natura 2000 protected area) (Parc naturel marin, n.d.a).

Seagrass meadows perform crucial ecological functions in Arcachon Bay. *Z. noltii* is responsible for 20% of the primary production in the Bay (Auby, 1991). As such, *Zostera* meadows host a diverse community of amphipods, polychaete, gastropods, fish and birds, including numerous endangered species (Auby, 1991; Bernard, 2013). Arcachon Bay has the largest population of at-risk *Hippocampus guttulatus* and *H. hippocampus* in France (Hily, 2006). Besides, *Zostera* meadows settle sediments, mitigate coastal erosion and enhance water quality in the Bay (Ganthy, 2011; PNMBA, 2017).

*Z. marina* and *Z. noltii* historically used to grow abundantly in Arcachon Bay. However, in the early 2000s, both seagrasses underwent a sharp decline initiated by the synergistic effects of unprecedented marine heatwaves and water pollution in the Bay (Auby et al., 2011; PNMBA, n.d.b). These factors have been connected to a surge of *Z. marina* mortality. The loss of *Z. marina* in subtidal areas of the Bay triggered positive feedbacks by shifting hydrodynamic conditions for *Z. noltii* in the intertidal zone. The resulting increase in shear bottom stress caused a drastic regression of *Z. noltii* (Cognat, 2019). Overall, 84 percent and 45 percent of *Z. marina* and *Z. noltii* respective ranges have been lost in Arcachon Bay (PNMBA, 2022a) (*figure 2*). Since 2014, the Bay is a marine protected area (MPA) under the management of Parc Naturel Marin du Bassin d'Arcachon (Natural Marine Park of Arcachon Bay), a public organism attached to the Office Français de la Biodiversité (French Biodiversity Agency), the national entity responsible for environmental conservation. The 435-square-kilometre MPA entirely covers Arcachon Bay and surrounding open-coast areas (PNMBA, n.d.a).





Figure 2. Drastic regression in the range of Z. noltii in Arcachon bay over 1989-2012 period (Cognat, 2019, 51).

and *Z. noltii* meadows to ecological functions and ranges equivalent to early-2000s conditions by 2032 (PNMBA, 2017). Several initiatives targeted pollution reduction and improved water

quality to a certain degree (PNMBA, 2022a). But active restoration seems a necessity to enhance recolonization by *Z. marina* and *Z. noltii* in the Bay. Shifting environmental conditions associated with the loss of seagrass engineer species appear unfavorable to natural plant recolonization and seed dispersal in some areas (Cognat, 2019). Furthermore, local seedbanks may not be sufficient for natural regeneration in areas where meadows have been lost (Meyer, 2023; PNMBA, 2022a).

The Park is now experimenting with active restoration of *Z. noltii*, which has greater stress tolerance and is easier to handle than *Z. marina* related to the stricter protection status of the latter. As part of the European Seagrass Consortium, a practitioners' network to share best practices for seagrass conservation and restoration, the park has implemented *Z. noltii* seed-based restoration following methods developed by Dutch researchers in the Wadden Sea (Govers et al., 2022; PNMBA, 2022a). Since 2021, *Z. noltii* reproductive shoots are collected in healthy meadows with volunteers during summer and seeding takes place over the following spring (PNMBA, 2022a; 2022c). As a result, new *Z. noltii* patches have established on a tidal flat in Gujan-Mestras (PNMBA, 2023b). New efforts now target transplant-based restoration to diversify efforts. Transplantations complement seed-based restoration in light of fluctuating seed production and very poor seed harvest in summer 2022 (PNMBA, 2023). Beginning in March 2023, *Z. noltii* sods have been transplanted in different sites across the Bay based on a protocol implemented by Dutch researchers simultaneously in the Wadden Sea.

#### Project goal and objectives

The ER390 project sought to contribute to the restoration of *Z. noltii* meadows in Arcachon Bay. The focus was on assessing the transplantation performances and examining habitat suitability for *Z. noltii* restoration. To a lesser extent, the project aimed at facilitating seed-based restoration specifically participatory *Z. noltii* reproductive shoot collection.

The project goals included:

#### 1) Continue ongoing *Z. noltii* transplantation in Arcachon bay.

Objective: conduct transplantation in different areas of the Bay,

<u>Objective</u>: increase the number of transplantation sites to at least 40 different transplant sites (stations) across the Bay.

#### 2) Develop and implement a post-transplantation monitoring framework.

Objective: measure the survival and growth of Z. noltii transplants,

Objective: assess the environmental factors influencing transplantation performances,

Objective: evaluate the donor bed recovery,

Objective: formulate directions for future transplantation and monitoring.

## 3) Facilitate public participation in *Z. noltii* seed-based restoration.

<u>Objective</u>: help volunteers collect reproductive shoots while limiting damage to the donor meadow,

<u>Objective</u>: inform the public on Z. noltii meadows and local restoration efforts.

## <u>Goal 1</u>

Creating a large set of transplant sites will allow to assess habitat suitability across different intertidal flats and environmental conditions in Arcachon Bay.

## <u>Goal 2</u>

Monitoring results will provide preliminary findings on the success of *Z. noltii* transplantation and the recovery of donor beds, along with environmental factors at play in transplant survival and growth in Arcachon Bay. These will improve knowledge on habitat suitability for *Z. noltii*.

### <u>Goal 3</u>

Assisting volunteers in *Z. noltii* shoot collection will help spread best practices for collection and contribute to improve public knowledge on eelgrass beds and the importance of seagrass conservation.

## **Methods**

## Transplantation efforts

*Z. noltii* transplantation was conducted between late March and mid-June 2023. Transplant locations were selected using satellite imagery and corresponded to places where *Z. noltii* used to grow in the past and intact beds remained nearby. Most sites were accessed by foot with mud shoes to prevent damage to seagrass beds and avoid sinking in the mud. Boat was used to access flats that could not be reached on foot.

The transplantation protocol replicated experiments conducted in the Wadden Sea over the same period. *Z. noltii* sods were harvested from nearby healthy beds with ten-centimetre-diameter-PVC cores. To limit detrimental impacts to the eelgrass beds, sods were dug at the center of donor meadows in the densest sections. The edges of the holes from sods were softened and left for natural sediment infilling and regeneration. In the Plage du Canal flat

(Gujan-Mestras), holes were flagged to monitor donor bed recovery. *Z. noltii* sods were immediately transplanted in test sites close to donor beds where *Z. noltii* used to grow (PNMBA, 2023a). The transplant sods were inserted in manually-dug holes. Each station consisted of nine sods arranged in three triangles set one meter apart from each other, with sods located every other 30 centimetre (*figure 3*). For monitoring purposes, transplantation sites (hereafter named as stations) were marked with flags, and their position was recorded with a GPS device.



*Figure 3.* Transplantation protocol. Zostera noltii transplant core in donor meadow (above left), transplant transport in PCV tubes (above right), transplant station organization (bottom left) and on site (bottom right) (S. Bouillaguet, L. Roudault, C. Laurent).

Fifty-three transplant stations (476 sods) were created northeast and southeast of Arcachon Bay on 14 tidal flats in Gujan-Mestras, le Teich, Audenge and Lanton.

#### Transplant monitoring protocol

A monitoring protocol was designed to assess the performances of *Z. noltii* transplants, namely survival and growth, to investigate different environmental factors at play in transplant performances, and to examine donor bed recovery.

A literature review was conducted on *Z. noltii* transplantation projects and environmental conditions influencing performances. The protocol included a description of (1) transplant survival, (2) a measurement of horizontal growth (e.g. rhizomatic expansion via asexual reproduction), and (3) a qualitative description of transplant fitness (e.g. stable or regressing following cover estimate).

Photographs allow to examine changes in vegetation in a given location over short periods of time (Auby *et al.*, 2015; Hall, 2001; Lejolivet *et al.*, 2019). As such, photo-point monitoring was used to measure transplant growth. A picture of each sod was taken during monitoring to measure transplant growth accurately (*figure 4*). A slice of PCV core used for transplantation was placed on sods to delineate the baseline transplant area on the photographs and deduce growth. Two photographs were taken for transplants covered with algae, one with algae and one after removing them. For each station, the transplant sods were numbered and distinguished following compass direction. Hand-drawn schemes specified sod number and location on monitoring sheets. A preliminary estimation of horizontal growth was visually conducted on site, followed by a detailed measurement via an analysis of photographs on *ImageJ*, an image treatment software used in other seagrass monitoring protocols (Auby *et al.*, 2015; Lejolivet *et al.*, 2019). On *ImageJ*, new plant shoots outside the PVC slice were delineated with the polygon tool to obtain the total expansion area (*figure 5*). Net growth was obtained from substituting this result with the baseline transplant area. For comparison purposes, values were normalized into net monthly growth, and averaged for each station.



*Figure 4.* Photograph of a Z. noltii transplant (station 37 on the Grand Verdura flat) with the PVC slice used as baseline for transplant size (C. Laurent).



*Figure 5.* Picture shown on figure 5 treated on ImageJ to measure net expansion area (transplant growth). (ImageJ)

Beside plant-based metrics, data was collected on environmental factors found to influence survival and growth. Environmental parameters monitored in the field included:

• Presence of algae, and if so, coverage estimate and species identification,

- Presence of bioturbation (e.g. soil physical and chemical alterations by living organisms) in the form of galleries and species presence, and if so, specific species,
- Microtopography (flat, depression, hill),
- Sediment type (sand, clay, soft mud, sandy-clay, muddy sand estimated through touch),
- Moisture (percentage of residual water cover at low tide),
- Presence of trash, particularly disaffected oyster farms commonly found within the Bay where oyster farming is widespread, which may modify hydrodynamic conditions (Cognat, 2019).

Data on other environmental parameters was extracted from existing model sets based on the location of each station, including:

- Hydrodynamic conditions (e.g. mean bottom current speed and 95<sup>th</sup> percentile for greatest values),
- Salinity,
- Water temperature,
- Emersion time,
- Contaminants.

For contaminants, fine-scale water quality data was not available. But the Leyre river which empties into the south east of the Bay is the prominent freshwater influence and a well-known source of contaminants (e.g. fertilizers, pesticides and industrial effluents) (Auby, 1991, 14). Therefore, the distance between each station and the Leyre mouth was used as a quantitative proxy for water quality with the hypothesis that the greater the distance to the Leyre mouth, the higher water quality.

Field monitoring was conducted from mid-June to the end of August. All transplant stations were monitored at least once, while 18 stations located on the tidal flats with best performances for survival and growth were monitored a second time after a month.

With regards to donor bed recovery, hole resorption in the marked beds on Salines flat was assessed. *Z. noltii* cover where holes were dug was compared with nearby meadow sections.

Using field monitoring and data collected during the ER390 project, statistical analyses were performed by the park staff on the software *R* to assess the influence of environmental parameters first on transplant survival, then on monthly growth. The contribution of multiple variables on the transplant survival and growth was analyzed with multivariate models. A binomial generalized linear model was selected for transplant survival, while a linear model was applied to assess transplant growth. Variables were selected based on the Akaike Information Criterion (AIC) to select the model with the greatest explanatory value.

## Participatory seed collection



*Figure 6.* Map of Arcacgon Bay including "I'Île aux Oiseaux" in the center of the Bay (Google maps).

Four participatory sessions were held over summer 2023 to gather reproductive shoots of *Z*. *noltii* in healthy meadows of the Bay as part of seed-based restoration. Harvesting took place at low tide on a sandy flat on *Île aux Oiseaux* ("island of birds") at the center of the Bay (*figure 6*).



Figure 7. Volunteers collecting Zostera noltii shoots on l'Île aux Oiseaux in August 2023 (M. Lebeault).

Volunteers were introduced to *Z. noltii* biology and seagrass conservation challenges in Arcachon. Based on live specimens and pictures of *Z. noltii*, volunteers were assisted to successfully identify plant sections to collect, and to avoid pulling up undesired plant material such as rhizomes or other seagrass species (*figure 7*). Reproductive shoots were collected in plastic bags. Following harvesting, reproductive shoots were stored into large tanks filled with circulating sea water for seed maturation. Then, mature seeds will fall at the bottom of the tank and will be collected via sieve filtration.

## Results

#### Transplant survival & growth

Overall, 405 out of 476 transplant sods had survived after the first monitoring, equaling nearly 85% of the sods. All 9 sods had survived in 36 stations (around 68% of the sites). As for growth, net expansion was measured for 283 sods with various growth values as part of the first monitoring, representing 59% of all transplants and 70% of the sods that had survived. On



*Figure 8. Transplant growth by location (median, 1st and 3rd quartile, minimum and maximum). The red dots represent the average monthly growth.* 

the two flats monitored twice, 156 and 64 sods had survived at the first and second monitoring respectively, while 128 and 15 sods had grown at the first and second monitoring respectively. Thus, around 60% of the sods which survived at the first monitoring had died by the second monitoring. 82% of transplants had undergone expansion at the first monitoring, but only 10% did at the second monitoring.

Transplant growth by station was illustrated graphically to show the sites with the best performances (*figure 8*). Eleven stations have an average growth superior to, or close to, 50%. Eight of these sites are located on Plage du Canal and Salines flats, located in Gujan-Mestras south of Arcachon Bay (*figure 9*). Two of these stations with highest growth are located in Lanton, and one is located on the Grand Verdura flat. Eight stations have a growth comprised between 25 and 50% and are mostly located on Plage du Canal and Salines flats. Twelve stations have an expansion between 10% and 25%, and 22 stations inferior to 10%.



*Figure 9.* Transplant with large rhizomatic growth (202,865cm<sup>2</sup> expansion area) on the Grand Verdura flat (station 37) (C. Laurent).

PARC NATUREL MARIN DU BASSIN D'ARCACHON Survie et croissance des transplants de Zostère naine

#### DOCUMENT DE TRAVAIL



**Figure 10.** Average monthly growth of transplant stations (dots) in Arcachon Bay (dark green dots are stations with an average monthly growth of 50%, light green correspond to 25-50%, yellow to 10-25%, orange equal or inferior to 10%, and red to absence of growth).

#### Environmental parameters

Sediment data collected in the field indicated that most stations had muddy sediment (29 stations), to a lesser extent sandy-mud (19 stations) and muddy-sand sediment (5 stations).

Algae presence was recorded on nearly half of the stations (25 sites) and on 148 sods with various coverage. Species included *Ulva lactuca, Ulva compressa, Vaucheria species, Cladophora species,* and *Gracilaria* species. Algal blooms occurred over the summer and thick algae mats were found to cover *Z. noltii* beds and some stations on multiple flats, for instance in le Teich and Certes (*figure 11*). A site in Salines (station 23) was completely covered with algae at the first monitoring in June, yet all but one of the sods had survived and expanded. By August, half of the transplants had died and the rest had regressed.



Figure 11. Zostera noltii bed (left) and transplant (right) covered by algae mats (Cladophora species) (C. Laurent).

Bioturbation was present on 29 out of 53 stations. Bioturbation phenomena observed on site included galleries in the sediment within *Z. noltii* sods, uprooting in some cases, and the presence of polychaete worms (tube worms species) directly inside the sods. Polychaete worms hid in the sediments at low-tide which hindered rigorous species identification. Suspected species solely based on observations of mucus-made tubes covered with shells and *Z. noltii* include *Diopatra biscayensis*, *Diopatra neapolitana* (*figures 12 and 13*), or *Marphysa sanguinea*, common species in the Bay. Worms were unexpected as they were not notice on site during transplantation.



**Figure 12.** Diopatra biscayensis (A, B, C) and Diopatra neapolitana (D, E, F, G) classified depending on decoration level, from high decoration (A, D) to medium decoration (B, E) to low decoration (C, F) (from Wethey et al., 2016).



*Figure 13.* Tube worm species found on flats and transplants in Arcachon Bay with tube decoration resembling those of D. biscayensis or D. neapolitana (C. Laurent).

#### Donor bed recovery

Hole resorption was confirmed in the monitored donor bed on Plage du Canal. *Z. noltii* leaf cover at the hole locations was found to be equivalent to intact nearby meadow.

#### Statistical analysis

Statistical analyses were conducted by the parc staff from data obtained within the ER390 project. Data exploration using ACP showed that the distance to the Leyre estuary, temperature and salinity highly correlate. Therefore, models including those parameters together were excluded from the analysis.

The best-fit model according to AIC value for transplant survival showed significant positive effect of mean bottom current speed, and significant negative effect of distance to Leyre river, immersion time, algae, bioturbation and 95% percentile of bottom speed. Survival was higher for transplants in sediment with a higher sand-to-mud ratio (*table 1*).

Parameter	Estimate	Standard	Z value	P value
		error		
Distance to Leyre river	-8.5 x10 <sup>-4</sup>	1.5x10 <sup>-4</sup>	-5.7	<0.05
Immersion	-9.2 x10 <sup>-1</sup>	1.3 x10 <sup>-1</sup>	-6.8	<0.05
Bottom current speed	3.7 x10 <sup>1</sup>	7.4	-5	<0.05
Algae	-1.4	4.1 x10 <sup>-1</sup>	-3.4	<0.05
Bioturbation	-2.2	4.1 x10 <sup>-1</sup>	-5.3	<0.05
95% perc. bottom speed	-6.9	2.4	-2.9	<0.05
Sediment (Sand)	3.1 x10 <sup>1</sup>	7.7 x10 <sup>2</sup>	4 x10 <sup>-2</sup>	0.97
Sediment (Sandy-Mud)	1.4 x10 <sup>1</sup>	2.2	6.6	<0.05
Sediment (Mud)	1.6 x10 <sup>1</sup>	2.2	7.1	<0.05
Sediment (Soft mud)	1.4 x10 <sup>1</sup>	1.9	7.2	<0.05

**Table 1.** Results of the best-fit statistical model transplant survival.

The best-fit model for monthly growth of surviving transplants according to AIC value shows significant negative effect of the occurrence of bioturbation, and positive effect of muddy sediments. Overall results are summarised in *table 2*.

	Transplant survival	Transplant growth
Positive effect	Bottom current speed	N/A
Negative effect	Distance to Leyre river	Bioturbation
	Immersion	
	Algae	
	Bioturbation	
	95% percentile bottom speed	

**Table 2.** Result summary of the influence of environmental parameters on Z. noltii transplant performances.

#### Participatory seed collection

Over 160 people volunteered over the four participatory session to collect Z. noltii reproductive shoots. Around 25 000 seeds were collected according to preliminary estimates. The sessions contributed to raise awareness of attendants on seagrass meadow conservation and to reach out to other beach users interested in the event. Media coverage also helped spreading information on seagrass conservation.

## **Discussion & Recommendations**

#### Transplant performances

At the end of the first monitoring phase, transplant performances appear satisfactory relative to previous *Z. noltii* transplantation projects (Martins et al., 2005; Suykerbuyk et al., 2016). But high mortality found on the two flats monitored twice indicates a lower survival and growth performance at the end of the summer. Besides, the monitoring results shall only be considered as preliminary. Indeed, monitoring only lasted during the first growing season, and thus do not pertain to long-term performances. Measuring survival and growth after at least a year would provide more long-term insights. Therefore, repeating transplant monitoring at least at the start and end of the next growing season (and ideally, over multiple years) will be key to assess the long-term success of the transplantations.

#### Site selection

Results largely varied across sites even at the flat-level, pointing to the complex interplay of factors influencing *Z. noltii* survival and development. But some flats had very high performances and seem better suited to restoration in the Bay, specifically Plage du Canal and Salines flats in Gujan-Mestras. Increasing transplantations on these sites will allow to continue investigating habitat suitability for restoration. Conducting more transplants on the Grand Verdura flat is also recommended as the only transplant station on the flat had very high growth.

However, as stated above, at the second monitoring in August, transplants on the most successful sites in Gujan-Mestras had regressed relatively to the first monitoring a month earlier. Causes of this sudden decline are unknown, and many doubts persist as to whether the plants actually died. Indeed, transplants may have been temporarily recovered with sediments, or defoliated earlier than nearby meadows by following a specific cycle due to the specific size and density of the transplants. Other hypotheses are that heatwave events or algal bloom may have affected negatively the transplants. Overall, further monitoring is required in 2024 to carefully re-assess these sites in Gujan-Mestras.

### Environmental factors

#### Hydrodynamics

Hydrodynamic exposure is an important factor at play in *Z. noltii* survival. In Arcachon Bay, stronger hydrodynamic conditions contributed to the regression of *Z. noltii* beds (Auby et al., 2011; Cognat, 2019). Results from transplant monitoring indicate that bottom current speed correlates positively with transplant survival, but the 95% percentile bottom speed negatively affects survival. The existing literature may help explain these contrasted findings. Increases in

bottom current speed may benefit seagrasses as it facilitates water renewal and nutrient availability with the mixing of water column (Peralta et al., 2006). However, extreme hydrodynamic pressures may harm *Z. noltii* by damaging leaves, uprooting plants, and ultimately fragmenting beds (Cognat, 2019). Continuing transplant monitoring will help to better understand the effects of hydrodynamic conditions on transplants. Monitoring is particularly important after the winter, which is associated to greater hydrodynamic pressure related to seasonal storm surges (Paul et Amos, 2011).

#### Sediments

Results indicate that transplant sites with higher sand-to-mud ratios positively correlate with survival while transplant growth is higher in muddy sediments. These preliminary findings appear in accordance with existing literature suggesting that *Z. noltii* transplants best survive in sandy sediments, yet best grow in muddy sediments in the long-term (Valle et al., 2015). But the sediment dataset for Arcachon Bay were obsolete, therefore sediment data was collected from touch tests in the field. In future monitoring and transplantation, sediment cores should be sampled to provide more accurate data, and to better assess the influence of sediments on *Z. noltii* transplants.

#### Algae

Macroalgae presence and cover is an important factor which may have adverse effects on *Z. noltii* development (Brun et al., 2003; Philippart, 1995; Suykerbuyk et al., 2018). Results suggest that algae presence negatively influences transplant survival, which aligns with some of the literature. While seagrasses tend to facilitate algae by creating a substrate and shelter for them to grow on, overgrowing of opportunistic macroalgae over *Z. noltii* has been found to smother plants by decreasing light availability, reducing photosynthesis, ultimately hindering transplant survival and growth (Brun et al., 2003; Philippart, 1995; Suykerbuyk et al., 2018). Although seagrasses tolerate temporary shading by storing nutrient reserves, smaller species, such as *Z. noltii*, have limited storage capacity, therefore, they are more vulnerable to shading (Brun et al., 2003). Furthermore, macroalgal blooms are hardly predictable as they depend upon various conditions (Gubelit & Berezina, 2010; Schramm, 1999), and they are set to occur more frequently in relation to climate change (Gao et al., 2017; Pörtner et al., 2019). In summer 2023, extensive algal blooms were likely related to exceptionally warm water temperatures (Copernicus, 2023). Continued macroalgae monitoring on transplant stations will contribute to better understand their effects on *Z. noltii* and examine mitigation options.

#### Bioturbation

Monitoring results indicate that bioturbation significantly reduces transplant survival and growth in Arcachon Bay. Cases of bioturbation by worms have been found within the literature on *Z. noltii*, including bioturbation by *Arenicola marina* in the Wadden Sea (Philippart,

1994; Suykerbuyk et al., 2012) and *Hediste diversicolor* in England (Hughes et al., 2000). Worms may damage plant roots by moving sediments, and they may also feed on seeds or leaves (Bernard, 2013; Hughes et al., 2000). There was no evidence of bioturbation in the literature regarding the suspected worm species in Arcachon Bay. However, *Z. noltii* leaves were found in the stomach of *D. neapolitana* specimens (Berke, 2022). Proper species identification based on a full specimen will allow further research progress. Besides, following methods used in other projects, future transplantations may include experiments to exclude worms from transplants, for example, with an impermeable shell layer underneath sediments (Hughes et al., 2000; Sukerbuyk et al., 2012). These experiments will help to investigate the impact of bioturbation on seagrass transplants and assess mitigation options.

#### Emersion

Results indicate that emersion correlates positively with transplant survival. This first finding seems counterintuitive regarding previous evidence in the literature. Indeed, when emerged, seagrasses are directly exposed to fluctuating air temperatures, hence are at risk of desiccation particularly during warm weather and under high irradiance (Auby, 1991). Very high air temperatures have been shown to cause *Z. noltii* mortality and decrease photosynthetic efficiency (Massa *et al.*, 2009; Repolho et al., 2017). Emersion may also create self-shading of leaves which also reduces productivity (Suykerbuyk et al., 2018). Continued monitoring of emersion will contribute to re-examine its effects on transplants.

#### Water quality, temperature and salinity

The distance between transplant stations and the Leyre estuary (e.g. proxy for water quality) is negatively correlated with transplant survival. But the latter is highly correlated to salinity and water temperature. Therefore, interpretation based on these results is difficult. The three parameters may influence transplants in various ways. Lower water quality (e.g. excess nutrients levels and biocides) is correlated with reduced *Z. noltii* survival and growth (Auby et al., 2011; Martins et al., 2005; Suykerbuyk et al., 2016). *Z. noltii* has a minimum salinity tolerance, and very high salinity concentrations can decrease growth (Martins et al., 2005). High water temperature also hinders transplant survival, development, and reduces rhizomatic healing following transplantation (Massa *et al.*, 2009; Repolho et al., 2017). Continued transplant monitoring will provide more data to further assess the effects of water quality, temperature and salinity on transplants. Instead of model sets, direct water sampling near transplant stations will provide more accurate data on these variables. Besides, future transplantations may be conducted in early spring or fall to assess the potential effects of seasonality (including temperatures) on transplant performances.

Recommendations	Rationale	Implementation			
Transplantation					
Increase transplantation efforts on Plage du Canal, Salines and Grand Verdura flats.	Focus transplantation efforts on flats with the highest transplant survival and growth performances, and further assess habitat suitability.	Upcoming transplantations.			
Include experiments to shield Z. noltii transplants from bioturbating tube worms (shells layer or mesh placed into the sediment of transplant stations).	Further examine the impact of bioturbation by worms on transplants and assess potential mitigation options.	Upcoming transplantations.			
Conduct transplantations over different seasons.	Assess the importance of seasonality and temperatures on transplant performances.	Early spring and fall.			
Monitoring					
Continue to monitor survival and growth of transplants conducted in 2023, and environmental factors, particularly on Plage du Canal and Salines flats.	Assess long-term survival and growth of transplants and the effect of various environmental factors.	At least at the start and end of the next growing season (in spring and early fall 2024).			
Include sediment cores in the transplant monitoring protocol.	Collect more accurate data across stations.	Once per station.			
Include water samples close to transplant stations in the monitoring protocol.	Collect more accurate data on water quality, temperature and salinity.	At least at the start and end of the next growing season (in spring and early fall 2024).			

**Table 3.** Synthesis of recommendations for transplant-based restoration of Zostera noltii in Arcachon Bay.

#### Seed-based restoration

Participatory seed collection attracted a large attendance and some media coverage. Large public interest in seagrass ecosystems and ecological restoration points to the importance of participatory methods to protect seagrasses in Arcachon Bay. New participatory programs could be developed to engage even more people in conservation, for example, by involving citizens in monitoring pollution, or in examining *Z. noltii* sea-wrack on beaches. However, many restoration activities are out of reach of public participation such as transplantation which implies substantial physical efforts. Public outreach with tailored educational material (e.g. videos, stories, etc.) may also contribute to increase public engagement.

#### Limitations

There are various limitations to the project results. Data collection, image treatment, environmental parameter selection, and interpretation all are associated with some biases. Placing the PVC slice back on transplants to delineate sods entailed some subjectivity. Then, some photograph quality was poorer due to field conditions, making growth measurements more difficult. Finally, selected data for each environmental factor may not have been the most representative. For example, the distance to the Leyre was not necessarily an accurate proxy for water quality.

## **Conclusion**

The ER390 project aimed at contributing to the restoration of dwarf eelgrass (*Zostera noltii*) meadows in Arcachon Bay. It consisted in transplanting *Z. noltii* in various sites across the Bay to assess habitat suitability for restoration, in monitoring transplants and donor bed recovery, and to a lesser extent in facilitating participatory *Z. noltii* seed-based restoration. Fifty-three transplant stations were created in the Bay. The monitoring protocol included data collection on transplant survival and growth and on various environmental parameters of importance and photograph analysis. Monitoring results provide preliminary insights into transplant performances and habitat suitability. At the first monitoring, transplants neared 85% survival rate and 59% growth. Sites with best transplant performances were located in Gujan-Mestras and may be prioritized for future transplantation. Several factors appeared to influence transplant survival and growth in various ways. Continued transplant monitoring (including slight protocol changes) over the upcoming year and new transplantations will refine these first findings on *Z. noltii* seed collection and demonstrated a large interest in seagrass protection and restoration, highlighting the importance of participatory restoration methods.

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