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LAYING A GAS PIPELINE THROUGH A *POSIDONIA OCEANICA* MEADOW: AN EXAMPLE OF ITS EFFECTS ON PLANT RECOVERY AND EPIFAUNAL DIVERSITY

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SEAGRASS
LONG TERM RESEARCH
EPIFAUNA
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ECOSYSTEM FUNCTIONING
RECOVERY
DREDGING

ABSTRACT. – We report on a case of natural *Posidonia oceanica* recovery after partial meadow destruction due to the laying of a submarine gas pipeline covered with rubble. Ten years later, the new patches were mapped combining the use of an underwater photogrammetry technique and GIS; at the same time, the ecosystem function of the new settled plants was assessed by analyzing major elements of the *Posidonia* motile invertebrate fauna (amphipods, isopods and mollusks). Recruitment of detached plants from nearby meadows was only successful on rubble compared with on adjacent sandy zones. In a subarea of 736.83 m², 184 new *Posidonia* spots were established. A high complexity of patch structure (*i.e.*, shoot/unit area) and community richness (*i.e.*, number of species, abundance and diversity) was detected in comparison with other historic shallow stands with the same geographical exposure. The richness in the epifaunal population and the assemblage composition of the three main epifaunal taxocenes also point to a good recolonization capacity. These results call for additional investigations to assess the functioning of the *P. oceanica* ecosystem through the associated epifauna; however, the success of reforestation on rubble along a channel with intensive shipping activity can suggest a solution to manage human requirements and landscape integrity at low cost and with natural donors.

INTRODUCTION

Posidonia oceanica (Linnaeus) Delile meadows represent one of the most productive coastal systems in the Mediterranean basin, but for almost 30 years the seagrass has been included in the Habitat Directive (1992/43/EEC) list and its habitat has been under legal protection, as it is continuously facing significant threats, driven by increasing human activity as well as global warming (Pergent *et al.* 2012). The fragmentation of the *P. oceanica* meadows is considered one of the main issues relative to their decline, also affecting the connectivity and diversity of associated communities and therefore the main trophic fluxes and ecosystem functioning (Mazzella *et al.* 1992, Boudouresque *et al.* 2006, Personnic *et al.* 2014). For these reasons, in order to mitigate *P. oceanica* loss caused by coastal works and infrastructure settlements and to renovate its ecosystem goods and services, several restoration operations have been undertaken (Cunha *et al.* 2012). As this seagrass is a slow-growing species and natural recovery by damaged plants may require decades, several transplanting techniques have been employed involving the introduction of shoots or meadow blocks (Bacci *et al.* 2014). Varying rates of success have been achieved but always at high financial cost when applied at large scales, because of the high work time requirements (both in diving activities and in the lab). Moreover, the large number of shoots that have to be removed from a

donor meadow, especially in a marine protected area, is a major concern. It has already been reported (Balestri & Lardicci 2008, Balestri *et al.* 2011) that the use of plant fragments detached after storms seems to have major advantages over traditional restoration techniques.

Here we report preliminary results on the long-term success of ramets naturally arriving on a trench dredged through a *P. oceanica* meadow to lay a gas pipeline between the Phlegrean island of Ischia (Gulf of Naples, Italy) and the mainland. Moreover, the ability of new patches to support an epifaunal community comparable with that typically associated with this seagrass system has been assessed for the first time. Results could be useful as a basis for integrating other ecosystem-based approaches for the purpose of assessing the ecological functioning of this key ecosystem.

MATERIALS AND METHODS

The study sites: The submarine gas pipeline was deployed in 2009 between the island of Ischia (Gulf of Naples, Italy) and the mainland (Fig. 1). It runs on the sea bottom up to the entrance of the harbor of Ischia, where a *Posidonia oceanica* meadow had developed (GAS) (Fig. 1). Along its shallowest stands (from 7.5 to 5 m depth), settled on a mat 1.5 m high, the *P. oceanica* system was removed, and in a channel about 300 m long and 6 m wide the pipeline was laid and covered by rubble.

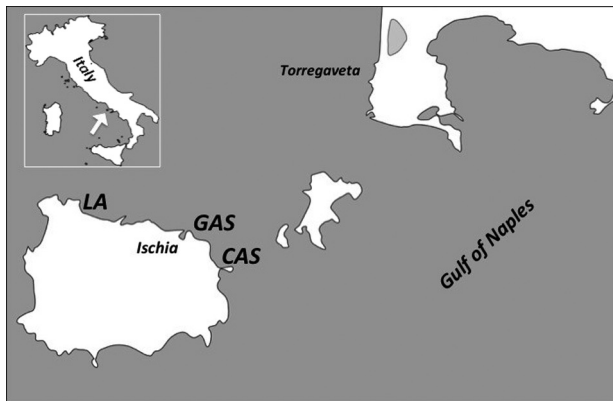


Fig. 1. – Map of the study area in the Gulf of Naples (Italy) with the locations of the monitored *Posidonia* recovery in front of the harbor of Ischia (GAS) and of the other two compared meadows at Lacco Ameno (LA), and Castello Aragonese (CAS).

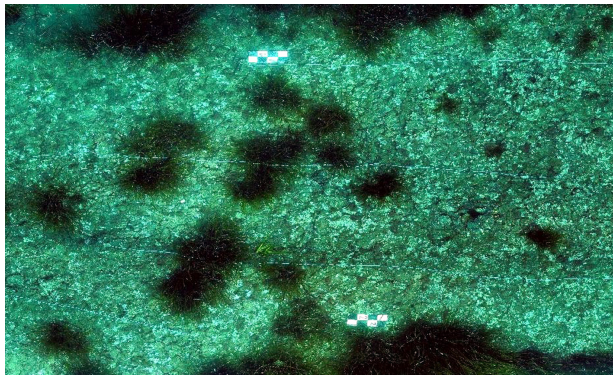


Fig. 2. – Scuba diving operations to map the new *Posidonia* patches and an example of the resulting ortho-photosaia.

Mapping of *Posidonia oceanica* recovery: After ten years (2009-2019), the results of the natural recovery of the *Posidonia oceanica* by resettlement plants coming from nearby stands were quantified and georeferenced in its deepest sector. The new patches were mapped by the Digital Photogrammetry technique and their cover was determined by GIS according to Cotugno *et al.* (2019). Generally, 5 ropes per time were displayed on the bottom, 2-3 meters apart from each other. Four white/black targets were placed on the corners of the working sub-area and their positions were georeferenced using a GPS (model Garmin Etrex 30), kept on the sea surface, directly above each underwater target. A small camera (Garmin Virb Ultra) able to record video in 4 k at 25 fps was used by a diver moving along the ropes at a constant distance from the bottom in order to obtain at least 70 % of overlapped pictures (Fig. 2). In the lab, 300 to 1,800 frames were extracted from each film sequence and then processed with the Agisoft Photoscan software that also provided us with the automatic calibration to correct focal length and lens aberration. By using structure from motion algorithms (SfM), 3D models of the bottoms were produced and the Orthophoto mosaics were georeferenced using the open source software QGIS. By means of the geometrical QGIS tools, we obtained the surface area and the total cover of the newly settled *Posidonia oceanica* patches.

Patch structure and epifaunal community: Patch structure (number of shoots per unit area) and epifaunal community were sampled in June 2019 within the same two randomly selected plots (0.16 m² each). Epifauna was collected first using a diver-operated suction sampler (for a description of the method used, see Garrard *et al.* 2014). The material obtained was stored in ethanol and then sorted into coarse taxonomic groups. Overall, samples were numerically dominated by three groups (mollusks, amphipods and isopods), which together made up 76 and 75 % of the total epifaunal abundance from the two replicates, respectively. Following this, the three groups were identified at the LPT (lowest possible taxon) on an expert basis.

The composition and structure of the three taxocenes was then compared with those from two historically established beds located on the northern coast of Ischia, *i.e.*, off Lacco Ameno (denoted as LA) and Castello Aragonese (CAS), respectively (Fig. 1), which were sampled using the same method and in the same season as the GAS one, although in a different year and at a shallower depth (3 m depth) (Garrard 2013). Notwithstanding the difference in depth, the communities from the three sampled stations can be considered as belonging to a same coenotic unit (shallow stand; Mazzella *et al.* 1992).

Summary variables of the three selected taxocenes (N, number of individuals; S, number of species; H'log_e, Shannon-Wiener diversity index) are presented using bar graphs. A statistically reliable data analysis was not possible owing to the small sample size (two replicates). However, standard deviations are shown in the graph as an indication of sample variability.

Multivariate analyses of the structure of assemblages were conducted following PRIMER v6 (Primer-E Ltd., Plymouth, UK) procedures on square root-transformed abundance data. SIMPER (Similarity Percentage test) was used to determine the species that contributed the most to similarity within each assemblage as an indication of their typification ability. nMDS (non-metric multidimensional scaling) plots, overlaid with circles resulting from a previous CLUSTER analysis tested for significance with SIMPROF (Similarity Profile routine), were used for a graphical representation of similarities between samples.

RESULTS

Mapping and patch complexity

The deepest section of the channel (7.5-6.3 m depth) was measured and mapped using the Digital Photogrammetry technique. It measured 128 m in length with a surface area of 736.83 m²: 184 new patches, of different size, had been settled, covering a surface area of 67.76 m² (9.2 %) (Fig. 3). The structural complexity of the patches was found to be high, with a shoot density higher than those recorded in LA and CAS meadows (Table I). Since 2009, no new ramet had settled on bare sand but they only colonized the rubble (Fig. 3).

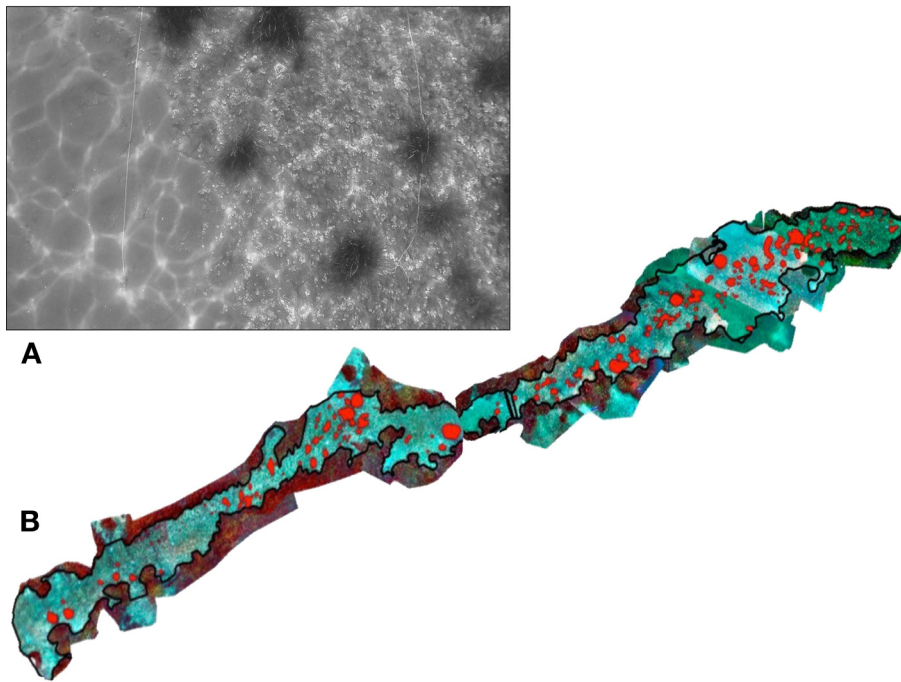


Fig. 3. – Colonization of drifted ramets on rubbles only (A) and map of the new *Posidonia* patches (red spots) along the monitored track (B).

Table I. – Values of the two replicates of *Posidonia oceanica* density at the three different sites.

	GAS		LA		CAS	
	a	b	a	b	a	b
No. shoots in 0.16 m ²	143	134	49	55	62	56

Epifaunal community

On the whole, GAS featured the highest abundance of all three taxocenes with respect to the other sites (Fig. 4A). The same was the case for the number of species (Fig. 4B) while the Shannon diversity did not show a clear difference (Fig. 4C).

In the ordination nMDS plots of the amphipod taxocene, samples from GAS were clearly separated (47 % similarity) from the other two sites, which in turn showed a significant separation at the 53 % level of similarity (Fig. 5a). Species which most characterize the assemblages are shown in Table II. Major contributors to the typification of GAS were a suite of species dominated numerically by *Apolochus neapolitanus* (Della Valle, 1893) and *Orchomene humilis* (Costa, 1853). *A. neapolitanus* was also the major contributor to similarity at CAS, followed by *Elasmopus pocillimanus* (Spence Bate, 1862). At LA, *Lembos websteri* (Spence Bate, 1857) ranked first in both abundance and contribution to similarity within the taxocene.

Also in the case of mollusks, GAS samples grouped separately in a significant manner (14 % similarity) from the other two sites (Fig. 5B). GAS was characterized by the high contribution of *Vitreolina philippi* (de Rayneval & Ponzi, 1854) whereas *Rissoa italiensis* Verduin, 1985

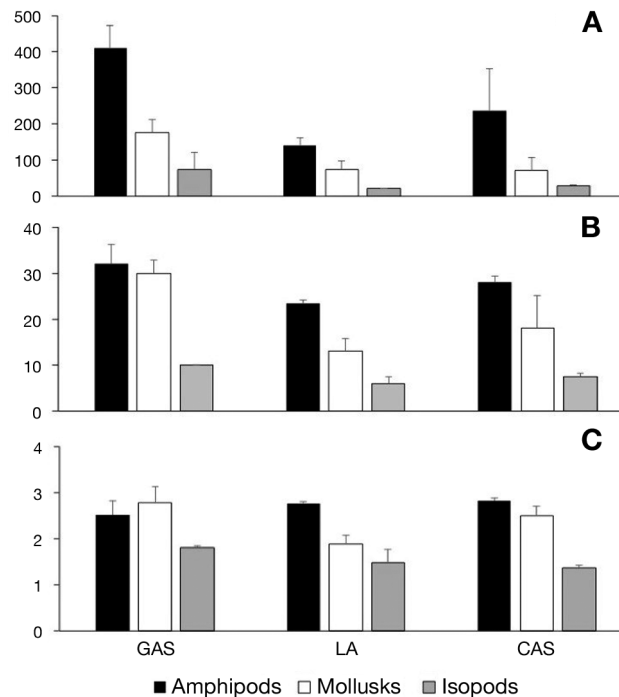


Fig. 4. – Abundance (A), number of species (B), and Shannon Diversity (C) of the three taxocenes at the three sites.

dominated at LA and a group of species including *Steromphala umbilicaris* (Linnaeus, 1758), *Rissoa auriscalpium* (Linnaeus, 1758) and *Alvania lineata* (Risso, 1826) were the main contributors to similarity at CAS.

No significant grouping was found for isopods (Fig. 5C). Juveniles of the species *Cymodoce hanseni* Dumay, 1972 accounted for the highest contribution

Table II. – Species which most contribute to the typification of the sites (based on SIMPER, cut-off 50 %). a: average abundance (mean of two replicates), b: percent contribution to total similarity (species nomenclature based on International Commission on Zoological Nomenclature).

Species	GAS		LA		CAS	
	a	b	a	b	a	b
Amphipods						
<i>Apolochus neapolitanus</i>	135.5	16.66	8.5	7.35	27	10.33
<i>Orchomene humilis</i>	56	10.47	0.5		11.5	6.08
<i>Liljeborgia dellavallei</i>	5		9.5	7.35	27.5	7.30
<i>Ericthonius</i> spp.	11.5	5.29	5		15.5	7.84
<i>Aora</i> spp.	13.5		11.5	8.22	6.5	
<i>Megamphopus cornutus</i>	11.5		11.5	7.80	8.5	
<i>Apherusa</i> cfr. <i>chierghinii</i>	18	6.38	7.5		4.5	
<i>Monocorophium sextonae</i>	0		0		29.5	7.02
<i>Lembos websteri</i>	0		26	13.00	2.5	
<i>Gammaropsis palmata</i>	17.5	6.38	2		8	
<i>Quadrimaera</i> cfr. <i>inaequipes</i>	2		6		19	7.84
<i>Iphimedia minuta</i>	7.5		7	6.88	6.5	
<i>Elasmopus pocillimanus</i>	1		1.5		18.5	8.59
<i>Peltocoxa marioni</i>	12.5	5.53	2.5		3.5	
Mollusks						
<i>Vitreolina philippi</i>	43.5	13.94	0		0	
<i>Rissoa italiensis</i>	0		27	31.10	3	
<i>Alvania lineata</i>	0		11		12	11.75
<i>Rissoa auriscalpium</i>	10	6.36	0.5		8	12.87
<i>Steromphala umbilicaris</i>	0		4.5		9	13.90
<i>Jujubinus striatus</i>	0		7.5	14.66	5	
<i>Tricolia pullus</i>	8	7.53	1		2	
<i>Alvania cimex</i>	1		0		9	10.51
<i>Parvicardium exiguum</i>	0		9	15.84	0	
<i>Nassarius incrassatus</i>	8.5	6.36	0		0	
<i>Chauvetia brunnea</i>	8	6.36	0		0	
<i>Calliostoma laugieri</i>	4		0		3.5	9.10
<i>Rissoa guerinii</i>	6.5	6.36	0.5		0	
<i>Limaria tuberculata</i>	4.5	5.69	0.5		0.5	
Isopods						
<i>Cymodoce hansenii</i>	27.5	26.37	9.5	53.95	3	16.97
<i>Joeropsis brevicornis</i>	18.5	21.53	3		16.5	36.66
<i>Gnathia</i> juv. indet.	5.5	15.23	0.5		1	

to similarity within the taxocene at GAS and LA, while the asellote *Joeropsis brevicornis* Koehler, 1885 ranked first in abundance and contribution to similarity at CAS (Table II).

DISCUSSION

The natural recovery of *Posidonia oceanica* after the deployment of the gas pipeline ten years ago represents a relevant result, due to the paucity of long term data on this topic, apart those recorded at Capo Feto (Sicily) (Badala-

menti *et al.* 2006), and to the peculiar environmental conditions of the site, located at the entrance of the harbor of Ischia and along a channel with a very intense vessel traffic. Results confirm the success of the natural recruitment of this seagrass by propagules drifted from nearby stands (Balestri & Lardicci 2008) and often observed in these years along the trench. The role played by the stones to capture and trap the buoyant ramets testifies to the importance of the type of substratum in determining the success of new plant settlement (Di Carlo *et al.* 2005, Badalamenti *et al.* 2011). In particular, the higher rate of plant recovery observed at Capo Feto than at Ischia may be due

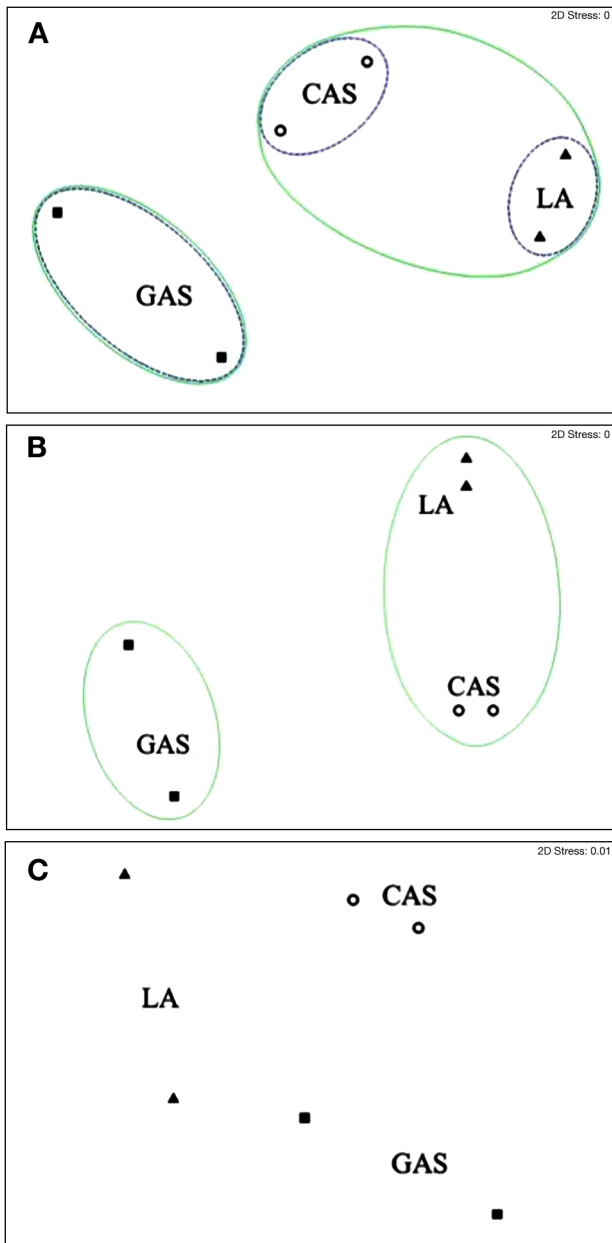


Fig. 5 — Multidimensional scaling (nMDS) plots on Bray-Curtis similarity matrix of assemblage data (A: Amphipods; B: Mollusks; C: Isopods). Symbols represent plots sampled at the three sites (squares: GAS; triangles: LA; circles: CAS). Ovals include points, which show a significant similarity in cluster analysis.

to the deeper location of that meadow and to the different geological origins of the rubble. In Sicily, the trench was covered by calcareous stones while at Ischia volcanic rubble was used, the latter probably more economic as characteristic of the Phlegrean area. Volcanic rocks, more consistent, probably needed more time to be suitable for colonization by pioneer species before being able to facilitate the settlement of the seagrass.

The method used to map the *P. oceanica* recolonization has been previously applied in different benthic systems. In the Mediterranean Sea, photogrammetry has

been used for studies on coralligenous ecosystem and few experiments have been done to map seaweeds and to follow structural and morphological characteristics of the *P. oceanica* meadow (Rende *et al.* 2015, Abadie *et al.* 2018). The results we obtained, although on a limited area, indicate that underwater photogrammetry can be a useful technique applied to conservation purposes and therefore can be coupled with classical monitoring procedures.

While aware of the exploratory nature of our study of epifauna, a number of observations can be made. First, attributes such as the number of individuals and of species from the new formation show higher values compared with those from the “old” beds while levels of diversity are within the same range. Higher shoot density at GAS, as a descriptor of habitat complexity (Attrill *et al.* 2000), may be one determinant of this relative richness, possibly coupled with the effect of patch size and the inputs from the nearby meadow edges (Tanner 2006, Bostrom *et al.* 2006). A major effort was allocated to the taxonomic resolution of the assemblage analysis. This is warranted by the importance of detecting those species, which may be associated with a particular condition of the beds and may point to the restoration of a healthy/typical population in new *P. oceanica* formations. The structure of amphipod and mollusk assemblages at GAS differs from those from the other two sites, which in turn show some degree of similarity with each other. Overall, dominant species at all three sites are known to be commonly associated with *P. oceanica* meadows although with specific peculiarities. Among amphipods, *A. neapolitanus* is a small-sized species which may be favored by the high complexity of the new patch while the greater weight of *L. websteri* at LA and of species such as *E. pocillimanus* and *Monocorophium sextonae* (Crawford, 1937) at CAS may be explained by a substantial presence of detritus and the relative proximity of rocky reefs, respectively. This is consistent with the notion that local factors may influence epifaunal composition (*e.g.*, Borg *et al.* 2010). On the whole, carnivores and scavengers (*A. neapolitanus*, *O. humilis*) seem to prevail in the new formation while tube-building detritivores and deposit-suspension feeders (*L. websteri*, *M. sextonae*, *Erichthonius* spp.) dominate the other sites possibly benefitting from the presence of an established *matte*, which is lacking at GAS. Species dominating the mollusk assemblages at the three sites also mostly belong to the typical stock of *P. oceanica* (*e.g.*, Russo *et al.* 1984, Albano & Sabelli 2012). However, the new patch is characterized by the small eulimid *V. philippi*, which is a parasite on sea urchins and ophiuroids (Oliverio *et al.* 1994). Its presence should be indicative of a remarkable echinoderm frequentation. Isopods do not show a clear distinction between assemblages, seemingly due to the consistent occurrence of *C. hanseni*, which constitutes a substantial component of the isopod taxocene in northern Ischia meadows (Gambi *et al.* 1992, Garrard 2013), and

of *J. brevicornis* which features a broader habitat range and whose presence may be favored by the relative nearness of rocky reefs similar to the above mentioned amphipod species.

In conclusion, while differences occur seemingly related to the fragmentation status, the age and the location of the studied beds, we may say that the new formation is able to host a rich and diversified epifauna with compositional traits of a typical *P. oceanica* community. The next step, which is in progress, is the analysis of the nearby meadow, which may act as a source of epifaunal forms.

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