



Posidonia oceanica habitat loss and changes in litter-associated biodiversity organization: A stable isotope-based preliminary study



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ABSTRACT

1. *Posidonia oceanica* (L.) Delile meadows are experiencing severe decline, with significant effects on *P. oceanica* meadow biodiversity, food webs and associated ecological processes. Despite the importance of this habitat, very little information with which to comprehend and predict the effects of habitat loss on the trophic traits of meadow-dwelling species is available.
2. The study addressed the effect of changes in *P. oceanica* cover on the trophic niche of macroinvertebrate litter-associated species and on their consumption of various basal resources along the upper limit of a *P. oceanica* meadow in the central Tyrrhenian Sea.
3. Census data and C and N stable isotope analysis were used to characterize changes in the trophic niche of species and the contribution of basal resources to food webs along a meadow coverage gradient.
4. Falling *P. oceanica* cover was associated with lower species richness and animal density. In the low-coverage area of the meadow, the organic matter content of basal resources decreased, and the trophic generalism of species increased while their trophic niche overlap declined. In addition, consumption of living *P. oceanica* plants and epiphytes decreased, and consumption of sediment-derived organic matter increased.
5. The results suggest that the community associated with *P. oceanica* litter is particularly sensitive to meadow degradation, which affects trophic structuring and nutrient pathways within the food web.

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1. Introduction

Posidonia oceanica (L.) Delile meadows are recognized as the most productive marine ecosystems in the Mediterranean region (Pergent et al., 1994; Duarte and Chiscano, 1999), providing many services of socio-economic importance and playing a fundamental role in maintaining and organising marine biodiversity (Hemminga and Duarte, 2000). Seagrass meadows are currently experiencing a worldwide decline (Orth et al., 2009). In Mediterranean coastal

areas, natural and anthropogenic disturbance has resulted in severe habitat loss and degradation (Marbà et al., 1996; Green and Short, 2003; Montefalcone et al., 2010; Gatti et al., 2012), producing significant modifications to both abiotic and biotic characteristics, with significant effects on *P. oceanica* meadows and their associated biodiversity (Mazzella et al., 1992; Bowden et al., 2001; Boström et al., 2006; Borg et al., 2010).

Studies of the effects of *Posidonia oceanica* meadow degradation have reported discordant results, depending on the animal groups of interest, type of meadow, area of meadow addressed (i.e. core or margin) and spatial scale of the study (Bowden et al., 2001; Barberà-Cebrián et al., 2002; Tanner, 2005; Borg et al., 2010). Despite the

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importance of leaf litter habitats in *P. oceanica* ecosystems (Mancinelli and Rossi, 2002; Covazzi-Harriague et al., 2006; Como et al., 2008), most studies focus on the fauna associated with living plants, providing little information about the food web organization of the benthic community associated with seagrass leaf litter (Gallmetzer et al., 2005; Lepoint et al., 2006). Moreover, there is very little information with which to comprehend and provide meaningful projections of the effects of *P. oceanica* habitat loss on the trophic traits of meadow-dwelling species, nutrient pathways within the food web and associated ecological processes (Boström et al., 2006; Stachowicz et al., 2007).

The trophic dimension of the ecological niche is crucial to the stable coexistence of species in natural communities (Rossi, 1985), and studying changes in trophic niches and trophic interactions can provide useful information regarding community dynamics (Layman et al., 2007a; Calizza et al., 2012, 2013). Understanding the effects of *Posidonia oceanica* habitat loss on (1) the trophic organization of communities, including community wide metrics and the trophic niche overlap of species, and (2) the configuration of nutrient flow pathways within the food web can provide important information for ecosystem management and conservation (Stachowicz et al., 2007).

Recently, stable isotope analysis has been successfully used in marine ecology to study the diet of fauna associated with *Posidonia oceanica* and to evaluate the relative contributions of various basal resources to food web carbon and nitrogen input (Moncreiff and Sullivan, 2001; Vizzini et al., 2002; Sturaro et al., 2010). Isotopic data from specimens of various species have provided interesting quantitative information on the trophic niche at population and community level (Bearhop et al., 2004; Layman et al., 2007b; Semmens et al., 2009; Jackson et al., 2011).

This study addressed the effect of *Posidonia oceanica* cover reduction on the macrobenthic community associated with *P. oceanica* leaf litter. To this purpose, three areas differing in vegetation cover were chosen along the upper limit of a *P. oceanica* meadow off the central Tyrrhenian coast of Italy. Carbon and Nitrogen stable isotope analysis was integrated with census data to reconstruct the trophic interactions between macroinvertebrates and basal resources. The null hypothesis to be tested was that changes in *P. oceanica* cover, with associated changes in both resource availability and disturbance (Gacia et al., 1999; Harris et al., 2004; Heck and Orth, 2006), had no effect on the species richness and animal density, or on the niche width and niche overlap (trophic redundancy), of macroinvertebrate species and the range of basal resources used by them. Moreover, since substrate structural complexity (sensu Como et al., 2008) has been acknowledged as a fundamental factor promoting the high biodiversity of seagrass systems (Como et al., 2008; Hansen et al., 2011), additional sampling was carried out using structurally simpler leaf litter in order to test the specific importance of *P. oceanica* leaf litter to its associated biodiversity.

2. Materials and methods

2.1. Study area

The study area was located in the central Tyrrhenian sea, along the coastline of the Lazio region of Italy, near the “Salt marshes Natural Reserve” of Tarquinia (Vt) which occupies an area of 1700 km² (42°12'21"N 11°42'42"E). The *Posidonia oceanica* meadow extends discontinuously for about 40 km. We selected three study locations, and two study sites per study location, along an extended stretch of the southern portion of the meadow, at a fixed depth of 6 m. In this stretch the meadow's upper limit is 3 m deep and shoot density is 300–350 shoots/m². The upper limit of the meadow is

characterized by continuous and reticulated patches, with seagrass coverage ranging from 75–100% to 0–25% (Valentini et al., 2013) and shoot density from 300 to 350 shoots/m², regardless of *P. oceanica* coverage. Within our study area no matte patches were present, seagrass leaf detritus was present within vegetated patches, and bare unvegetated patches of sediment were interposed between patches of *P. oceanica*. The three study locations were chosen on the basis of *P. oceanica* coverage, assessed by *in situ* measurement by two scuba divers. Three circular patches 60 m in diameter were chosen on the basis of this preliminary visual survey and a coverage gradient across sampling locations was identified, comprising a high coverage patch (location “D”), with 92.5 ± 2.5% coverage; an intermediate coverage patch (location “I”), with 70 ± 5% coverage; and a low coverage patch (location “F”), with 50 ± 5% coverage. The three different sampling locations were 1850 m apart, and were characterized by identical abiotic conditions. Location I was positioned roughly half-way between D and F.

2.2. Sample collection

Macroinvertebrates were sampled using litter bags with a mesh size of 1.0 cm, sufficient to prevent material loss and to allow the entry of large macroinvertebrates. Each litter bag contained 20 g of *Posidonia oceanica* leaf litter, previously harvested near each study site and oven dried at 60 °C for 72 h before immersion. Litter bags were positioned and anchored to the meadow bed within *P. oceanica* patches in 12 replicates for each sampling location (6 litter bags per sampling site). Taking account of the spatial scale considered and possible spatial variability in macroinvertebrate distribution, to preserve data independence, the litter bags were placed 10 m apart. As the study sought to test the effects of *P. oceanica* cover reduction on the leaf detritus-associated macroinvertebrate community, sampling with litter bags was preferred to direct sampling of natural detritus. This enables better control over leaf litter features, avoiding the possible confounding effects of differences in detritus composition, status, quantity and retention time arising from differences in *P. oceanica* cover within the meadow (Gallmetzer et al., 2005).

To test the specific importance of *Posidonia oceanica* leaf litter structural complexity (sensu Como et al., 2008; see also Hansen et al., 2011) to macroinvertebrate richness, abundance and species assemblage, we replicated the sampling using allochthonous, structurally simpler *Phragmites australis* leaf litter collected in the nearby “Salt marshes Natural Reserve” of Tarquinia. Probably originating in the nearby natural reserve, where *P. australis* is abundant, *P. australis* leaves were often observed within the *P. oceanica* meadow upper limit. Before being placed in litter bags, both *P. australis* and *P. oceanica* litter was conditioned in sea water in the laboratory (7 days). All litter bags were left on the seabed for 10 days to allow animal colonization and were then carefully retrieved by SCUBA divers and placed in plastic bags for transport to the laboratory. Macroinvertebrates from each litter bag were sorted and counted separately and specimens were identified to the lowest possible taxonomic level.

Samples of bottom sediment were collected in 6 replicates from each sampling location (3 replicates per sampling site) in sterilized plastic 125 ml jars. Coarse (C, >1 mm), fine (F, between 1 mm and 0.056 mm) and ultra fine (Uf, <0.056 mm) sediment fractions were separated by sieving. *Posidonia oceanica* leaves and leaf litter and bottom sediment were harvested in each sampling location (3 replicates per two sampling sites per location). *P. oceanica* leaves were separated depending on the presence/absence of epiphyte colonization, and leaf detritus was separated into “green” (i.e. non-decomposed) and “brown” (evidently decomposed). Epiphytic

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