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► **To cite this version:**

Simone Mirto, Silvia Bianchelli, Cristina Gambi, Maja Krzelj, Antonio Pusceddu, et al.. Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects. *Marine Environmental Research*, 2009, 69 (1), pp.38. 10.1016/j.marenvres.2009.07.005 . hal-00547650

HAL Id: hal-00547650

<https://hal.science/hal-00547650>

Submitted on 17 Dec 2010

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Accepted Manuscript

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PII: S0141-1136(09)00104-4
DOI: [10.1016/j.marenvres.2009.07.005](https://doi.org/10.1016/j.marenvres.2009.07.005)
Reference: MERE 3361

To appear in: *Marine Environmental Research*

Received Date: 2 February 2009
Revised Date: 24 July 2009
Accepted Date: 28 July 2009

Please cite this article as: Mirto, S., Bianchelli, S., Gambi, C., Krzelj, M., Pusceddu, A., Scopa, M., Holmer, M., Danovaro, R., Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects, *Marine Environmental Research* (2009), doi: [10.1016/j.marenvres.2009.07.005](https://doi.org/10.1016/j.marenvres.2009.07.005)

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4 1 **Fish-farm impact on metazoan meiofauna in the Mediterranean Sea:**
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7 2 **analysis of regional vs. habitat effects**
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37 15 *Marine Environmental Research*
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27 **ABSTRACT:**

28 The worldwide exponential growth of off-shore mariculture is raising severe concerns about the
29 impacts of this industry on marine habitats and their biodiversity. We investigated the metazoan
30 meiofaunal response to fish farm impact in four regions of the Mediterranean Sea. Meiofaunal
31 assemblages were investigated in two habitats (seagrass meadows of *Posidonia oceanica* and
32 non-vegetated soft bottoms) comparing sites receiving faeces and uneaten food pellets from fish
33 farms to control sites. We report here that, consistently across different regions, the meiofaunal
34 abundance typically responded positively to fish-farm effluents. Biodeposition caused also
35 significant changes in assemblage structure and the reduction in the richness of higher meiofaunal
36 taxa, but the multivariate analysis of variance revealed that the effects were region- and habitat-
37 specific. In non-vegetated systems, three of the four regions investigated displayed significant
38 effects of the fish farms on richness of meiofaunal taxa. In vegetated habitats, meiofauna did not
39 respond to biodeposition (except in one region), suggesting that seagrass meadows can mask the
40 effects of fish-farm effluents on benthic biodiversity. We conclude that different indicators of
41 fish-farm impact are needed in vegetated and non-vegetated benthic systems.

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44 **Key-words:** fish-farm impact, meiofauna, soft sediments, *Posidonia oceanica*, Mediterranean
45 Sea.

1. Introduction

Eutrophication is recognized as one of the most important emerging problems of the coastal oceans and, during the past four decades, has increased exponentially in intensity, geographical area and environmental consequences (Cloern, 2001). Eutrophication is typically related to the increase of nutrient and organic matter loads, which cause a progressive decrease of dissolved oxygen concentrations (Dell'Anno et al., 2008). The accumulation of large amounts of organic matter in coastal sediments, associated with eutrophication processes, also induce significant changes in the composition of sediment organic matter, which can have putative effects on the benthos (see Pusceddu et al., 2009 for a review).

Aquaculture is a fast-growing industry which, through the release of organic and inorganic N and P contributes to the progressive eutrophication of coastal areas (see Holmer et al., 2008 and citations therein). Aquaculture activities are now relevant at local and regional scales, and recent estimates indicate that, in Mediterranean coastal areas, the release of nutrients from fish farming contributes for up to 7 and 10% of N and P total discharge, respectively (Pitta et al., 1999).

Aquaculture installations can produce relevant shifts of the whole natural environment (Boyra et al., 2004; Machias et al., 2004), threatening the environmental quality of coastal zones (Gowen and Bradbury, 1987) and generating conflicts between aquaculture and the conservation of marine habitats, including the protection of large benthic primary producers, such as the seagrass *Posidonia oceanica*.

The organic enrichment of the sediments immediately beneath the sea cages is a direct result of the sedimentation of particulate waste products from the fish farm (Hargrave et al., 1997; Karakassis et al., 1998; Holmer et al., 2008) and a decreasing concentration with the increasing distance from the point source is typically observed (Hargrave et al., 1993; Mazzola

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3 71 et al., 1999; Pusceddu et al., 2007; Holmer et al., 2007). The continuous flow of faeces and
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5 72 food pellets from fish cages alters the quantity and the biochemical composition of sediment
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8 73 organic matter, but with potentially different effects in different regions and/or habitats.
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10 74 In fact, previous studies, carried out in non-vegetated and seagrass habitats of four
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12 75 Mediterranean regions (Cyprus, Greece, Italy and Spain), have shown that the response of the
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15 76 benthic biochemistry to aquaculture biodeposition is idiosyncratic, and significant increase in
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17 77 the organic load can be detected only in those regions characterized by biopolymeric C contents
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20 78 typically, 2 mg C g⁻¹ (Pusceddu et al., 2007).
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22 79 To identify the changes induced by aquaculture activities on benthic ecosystems, we
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24 80 investigated meiofaunal assemblages in different regions of the Mediterranean Sea along a
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27 81 longitudinal gradient of trophic conditions (in terms of primary productivity, Pusceddu et al.,
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29 82 2007). Metazoan meiofauna, for their ecological importance in the benthic ecosystems and the
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32 83 lack of larval dispersion, are considered a sensitive tool for investigating structural and functional
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34 84 changes of natural and anthropogenically-impacted ecosystems (see Vincx and Heip, 1987 for a
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36 85 review).
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39 86 Here we tested, using a hierarchical sampling strategy under uni- and multi-variate
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41 87 contexts, the hypothesis that the presence of fish farms influences significantly the meiofaunal
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43 88 assemblages in terms of abundance, community structure and diversity (i.e. richness and
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46 89 evenness of taxa) and that different patterns can be expected in the four regions according to their
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48 90 different trophic backgrounds.
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93 2. Materials and Methods

94 2.1 Study site and sampling strategy

95 To cover different environmental conditions characterizing Mediterranean coastal zones along an
96 east-to-west longitudinal transect (ca. 3,500 km wide), four regions were selected: Akrotiri Bay
97 (Cyprus; 34° 39' N, 34° 04' E; July, 2002), Sounion Bay (southern Greece; 37°39' N, 24°01' E;
98 July, 2003), Pachino Bay (Italy; 36°43' N, 15°05'E; September, 2002), and Gulf of Alicante
99 (Spain; 38° 24' N, 0° 24' W; September, 2003) (Figure 1). All of these regions, located at similar
100 latitudes and depths were selected on the basis of the presence of the fish farms, which have been
101 previously characterized in terms of their main environmental features (Table 1). In each of the
102 sampling regions, the effects of the fish farm on the meiofauna were investigated in two different
103 habitats: meadows of the seagrass *Posidonia oceanica*, and soft non- vegetated bottoms. In each
104 region, a preliminary survey was carried out to ascertain the presence of both the seagrass and the
105 soft substrates, and to characterize the environmental settings of the areas in terms of mean depth
106 and temperature, bottom currents, sediment type and porosity, and chlorophyll-a and inorganic
107 nutrient concentrations in the water column (Karakassis et al., 2005). In each habitat, the impact
108 was quantified by contrasting the fish-farm sites with control sites characterized by relatively
109 pristine conditions and by environmental features comparable to those found beneath the cages
110 (Table 1). Controls were located upstream of the main currents, and at least 1,000 m from the fish
111 farms. It has been demonstrated that this distance avoids any potential effect of fish farms
112 (Holmer et al., 2008) and allows maintaining the spatial variability in background (control)
113 conditions as low as possible.

114 At each site, three replicates were collected randomly from the central area of each fish-
115 farm site (i.e. beneath the cages) and in each control site, by means of manual corers (diameter
116 3.7 cm, 10.7 cm² surface area, down to a depth of 10 cm) operated by SCUBA divers.

117 2.2 Sediment biochemistry

118 Sediment protein, carbohydrate and lipid contents were determined spectrophotometrically and
119 concentrations were converted into carbon equivalents using 0.40 and 0.49 and 0.75 g C g⁻¹
120 conversion factors, respectively, and their sum reported as biopolymeric C (Pusceddu et al.,
121 2009). Data relative to the quantity and biochemical composition (i.e. protein, carbohydrate and
122 lipid contents) of organic matter in the sediments under scrutiny have been detailed elsewhere
123 (Pusceddu et al., 2007). In this study, we focused our attention on the biopolymeric C
124 concentrations and on the values of the protein to carbohydrate ratio as descriptors of the
125 quantity and quality of sediment organic matter, respectively (Pusceddu et al., 2009).

127 2.3 Meiofaunal analyses

128 Each sediment sample was fixed with 4% buffered formaldehyde (in filtered seawater solution)
129 and was sieved through 1000 µm sieve (to retain macrobenthos and macroalgae) and 32 µm sieve
130 (to retain smaller meiofauna) in laboratory. The sample fraction retained by a 32 µm mesh net
131 was added to Ludox HS 40 (density arranged to 1.18 g cm⁻³), for density centrifugation extraction
132 (10 min, 800 × g, for 3 times) from the sediment (Heip et al., 1985). All metazoan animals were
133 counted and classified per taxon under a stereomicroscope using Delfuss cuvettes, after staining
134 with Rose Bengal (0.5 g L⁻¹). Community evenness was calculated as the Pielou's index using the
135 different meiofaunal taxa as entries, by means of the PRIMER software (Clarke and Gorley,
136 2003).

138 2.4 Statistical analyses

139 Differences between control and impact sediments were assessed for all of the investigated
140 variables using a three-way analysis of variance (ANOVA). The analysis treated the factor region

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3 141 (R, 4 levels) as random, habitat (H, 2 levels) as fixed and crossed with R, and impact (I, 2 levels)
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6 142 as fixed and orthogonal to R and H. When significant differences ($p < 0.05$) were observed, a post-
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8 143 hoc Student-Newman-Kuels' test (SNK) was also performed. ANOVA and SNK tests were
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10 144 carried out using the GMAV software (University of Sidney).

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13 145 Distance-based permutational multivariate analyses of variance (PERMANOVA,
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15 146 Anderson, 2001) were used to quantify in the two benthic habitats the effects of the fish farms on
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17 147 i) the organic matter quantity and quality and ii) the meiofaunal assemblages. The analysis treated
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19 148 the factor region (R, 4 levels) as random, habitat (H, 2 levels) as fixed and crossed with R, and
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22 149 impact (I, 2 levels) as fixed and orthogonal to R and H. For organic matter, the data set included
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24 150 48 observations on concentrations of biopolymeric C and the values of the protein to
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27 151 carbohydrate ratio. For meiofauna, the data set included 48 observations and the abundance of all
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29 152 the taxa. Since the interaction term $R \times H \times I$ was seen to be significant either for organic matter
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31 153 and meiofauna, pairwise comparisons, which also used 499 random permutations to obtain P-
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33 154 values, were also carried out to ascertain differences between control and impact sediments in all
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36 155 regions, separately. The P-values were calculated using 499 Monte Carlo draws from the
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39 156 asymptotic permutation distribution (Anderson and Robinson, 2003). PERMANOVA tests were
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41 157 carried out (either in the uni- and multivariate contexts) also removing the effects of the main
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43 158 environmental variables (i.e., water depth, current speed, sediment type, porosity and water
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46 159 content, biopolymeric C and protein to carbohydrate ratio) as covariates.

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48 160 In order to assess whether and how much environmental variables explained changes in
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50 161 meiofaunal abundance, richness and evenness of taxa, a non-parametric multivariate multiple
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52 162 regression analysis that was based on Euclidean distances was carried out using the routine
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55 163 DISTLM forward (Mc Ardle and Anderson, 2001). The forward selection of the predictor
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58 164 variables was carried out with tests by permutation. P values were obtained using 4,999
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3 165 permutations of raw data for the marginal tests (tests of individual variables), while for all of the
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5 166 conditional tests, the routine uses 4,999 permutations of residuals under a reduced model.
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8 167 9 10 168 **3. Results**

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13 169 Three-way univariate ANOVA revealed that biopolymeric C concentrations and values of the
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15 170 protein to carbohydrate ratio displayed a significant Region (R) \times Habitat (H) \times Impact (I)
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17 171 effect (Table 2), with differences between impacted and control sites varying between the two
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19 172 habitats and the four geographical areas. In particular, the SNK tests revealed that biopolymeric
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21 173 C concentrations in farm-impacted sediments increased consistently in vegetated sediments in
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23 174 Cyprus, in both habitats in Greece and in non-vegetated sediments in Italy (Figure 2A),
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25 175 whereas values of the protein to carbohydrate ratio increased in impact sites in non-vegetated
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27 176 sediments in Cyprus, in both habitats in Italy and in non-vegetated sediments in Spain and
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29 177 decreased in impact sediments of both habitats in Greece (Figure 2B).
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34 178 A general increase of meiofaunal abundance was observed in impacted sediments
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36 179 (Figure 3A) and the ANOVA revealed significant R \times H \times I variations in total meiofaunal
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38 180 abundance (Table 3). Moreover, the SNK tests indicated that total meiofaunal abundance
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40 181 increased significantly in impact sites in vegetated sediments in Cyprus and non-vegetated
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42 182 sediments in Italy, whereas no significant differences between impact and control sediments
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44 183 were observed in all other regions and habitats (Figure 3A).
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48 184 Abundance of all of the meiofaunal taxa in the sediments are reported in Table 4.
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51 185 ANOVA revealed significant R \times H \times I effects on the abundance of all of the meiofaunal taxa
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53 186 with differences between impact and control sites differing in extent and direction in different
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55 187 regions and habitats and for the different taxa (Table 5). Significant interaction of the R \times H \times I
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57 188 factors, indicating idiosyncratic responses to fish-farm impact in different regions and habitats,
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3 189 was observed for polychaetes, gastrotrichs, bivalves, cumaceans and tanaidaceans. The SNK
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6 190 tests (data not shown) revealed increasing nematode abundance beneath the cages in vegetated
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8 191 sediments in Cyprus and in both habitats in Greece. Copepods increased in both fish-impacted
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10 192 habitats in Cyprus. The abundance of ostracods varied between impacted and control sites only
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13 193 in Spain (in both habitats), whereas oligochaetes, kinorhynchs and amphipods did not display
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15 194 any significant difference between impact and control sediments in any of the investigated
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18 195 region and habitat. Turbellarians' abundance exhibited significant differences only between
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20 196 regions.

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22 197 The PERMANOVA test applied on the meiofaunal community composition showed a
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25 198 significant $R \times H \times I$ effect, indicating that the differences between control and fish-farm
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27 199 impacted sites varied between the two habitats and the four regions (Table 6, Figure 3B-C).
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29 200 The analysis carried out separately for all of the four regions and the pairwise comparison tests
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32 201 revealed that differences between impact and control sites in the meiofaunal community
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34 202 composition were significant only in non-vegetated sediments either in Cyprus and Greece.

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36 203 At all sites nematodes and copepods were the most dominant taxa, followed by
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39 204 polychaetes, ostracods, turbellarians, oligochaetes, gastrotrichs and all other taxa.

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41 205 The richness of meiofaunal taxa decreased significantly in impacted sites in all non-vegetated
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44 206 sediments, whereas no significant differences between impact and control sites were observed
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46 207 in seagrass sediments (Table 7 and Figure 4A). On the other hand, significant differences in
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49 208 community evenness were observed between regions and between impact and control sites only
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51 209 in non-vegetated sediments in Cyprus (Table 7, Figure 4B).
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4. Discussion

4.1 Impacts of fish-farm effluents on meiofaunal abundance

Ecosystem alterations induced by different typologies of disturbance, including human impacts, generate variable shifts in benthic species, assemblages and community composition. Fish farming, by modifying the whole attributes of the benthic environment beneath the cages, produces relevant modifications in the abundance, biomass, species composition and evenness of meio- and macrofauna (e.g. Mirto et al., 2002; Kalantzi and Karakassis, 2006).

The responses of metazoan meiofauna to various ecosystem alterations are clearly detected at the highest taxonomic level, with a resolution similar to that provided by the analysis of lower taxonomic levels (Kennedy and Jacoby, 1999; Warwick 1988), also under the influence of fish farms (Sutherland et al. 2007).

In this study, we analysed the potential effects of fish farm effluents on the abundance and community composition of meiofauna, by comparing, for the first time, two different habitats in four different regions with different background trophic conditions. The general outcome from the available literature is that fish-farm effluents typically alter meiofaunal abundance, diversity, biomass, and species composition (Duplisea and Hargrave, 1996; Kennedy and Jacoby, 1999; Mirto et al., 2000; -2002; La Rosa et al., 2001; Sutherland et al., 2007).

However, changes associated with the presence of fish-farm effluents are often not consistent, as meiofaunal abundance may either increase or decrease beneath the fish cages, depending on the site or the farm characteristics.

In the present study, the idiosyncratic meiofaunal responses to fish farm impact was confirmed. Although total meiofaunal abundance was generally higher in the fish-farm than in the control sediments, such changes were not always statistically significant. This result is apparently in contrast to previous investigations reporting a decrease of meiofaunal abundance in

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3 234 non-vegetated systems subjected to bio-deposition from fish cages (Mirto et al., 2002; Sutherland
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6 235 et al., 2007). The general positive response of meiofaunal abundance to fish-farm biodeposition
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8 236 could be related to the relatively limited organic enrichment in the sediments beneath the cages,
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10 237 as reported in sediments subjected to mussel farm bio-deposition, where the organic inputs to the
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13 238 bottom were similarly low (Danovaro et al., 2004).

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15 239 The results of the PERMANOVA, carried out in the univariate context, revealed that
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17 240 differences in total meiofaunal abundance between impact and control sites were due to the
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20 241 effects of the environmental covariates (Table 8). The effect of organic matter enrichment in fish-
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22 242 farm sediments was significant only in oligotrophic regions (i.e., Cyprus and Greece). The
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25 243 multiple regression analysis, indeed, revealed that, among the different environmental variables,
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27 244 only the current speed and the biopolymeric C concentrations explained significant proportions
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29 245 (15% and 9%, respectively) of the total meiofaunal abundance (Table 9). This result supports the
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32 246 expectation that changes in meiofaunal abundance in fish-farm sediments are linked to the
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34 247 organic matter inputs released by the fish farm, as well as to the local hydrodynamism.

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36 248 The results of this study suggest that the impact of aquaculture effluents on the
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39 249 meiofaunal abundance is both region- and habitat-specific and also highlight that the direction
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41 250 and intensity of the response of total meiofaunal abundance to aquaculture effluents depend on
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44 251 the environmental conditions.

45 46 252 47 48 253 *4.2 Impacts of fish-farm effluents on meiofaunal communities*

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51 254 The results presented here provide evidence that, in all investigated regions, consistent
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53 255 significant effects of fish-farming effluents on meiofaunal biodiversity can only be detected in
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55 256 non-vegetated sediments. In all soft bottoms beneath fish farms the richness of higher taxa
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58 257 decreased significantly when compared to control sites (from 13-16 in all control sediments to a
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3 258 minimum of 9 taxa in non-vegetated impact sediments in Spain), whereas no significant
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6 259 differences were observed in terms of taxa evenness. The taxa that disappeared beneath the cages
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8 260 changed amongst regions, but always included the rare taxa (i.e., taxa representing <1% of the
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10 261 total meiofaunal abundance; Figure 3C) of the control sites. Meiofaunal taxa disappeared in fish-
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13 262 farm sediments included kinorhynchs (only in non-vegetated sediments in Spain), cumaceans (in
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15 263 37.5% of the total cases, i.e., in non-vegetated sediments in Cyprus, Italy and Spain), isopods
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17 264 (37.5%, in non vegetated sediments in Cyprus and Greece and in vegetated sediments in Spain),
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19 265 thanaidaceans (37.5% in non-vegetated sediments in Cyprus and both habitats in Greece),
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21 266 amphipods (50%, in non-vegetated sediments in Greece, Italy and Spain and in vegetated
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23
24 267 sediments in Italy), and tardigrades (25% of the total cases: in non-vegetated sediments in Cyprus
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26
27 268 and Spain). These results do not allow identifying specific taxa as indicators of biodeposition
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29 269 impact.

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31
32 270 The analysis of meiofaunal assemblage attributes (meiofaunal abundance, community
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34 271 composition, richness of taxa and community evenness) within seagrass sediments revealed that
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36 272 all of these variables responded idiosyncratically to the presence of fish farming activities. The
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39 273 lack of a clear and consistent meiofaunal response to the fish-farm biodeposition in seagrass
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41 274 sediments could have several possible explanations. *Posidonia oceanica* is a seagrass endemic of
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43 275 the Mediterranean Sea, which covering ca 50.000 km², plays a key ecological role for a wide
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46 276 range of organisms and assemblages, preserving their biodiversity and protecting the integrity of
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48 277 the coastal areas (Hemminga and Duarte, 2000). The effects of fish-farm biodeposition on
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51 278 seagrass meadows can be difficult to detect due to the fact that the presence of the seagrass masks
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53 279 the changes in organic matter concentrations and composition (Pusceddu et al., 2007). However,
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55 280 the presence of a large number of filter feeders and detritus feeders associated with the seagrass
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58 281 bed can also act as a buffer for the organic enrichment, by consuming a large fraction of the
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3 282 biodeposits (Balata et al., 2008). Moreover, the presence of a large assemblage of prokaryotes,
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6 283 protozoa and meiofauna within the seagrass sediments makes it difficult to detect the presence of
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8 284 significant changes in these components (Danovaro 1996, Bongiorni et al., 2005). *Posidonia*
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10 285 *oceanica* is known to be highly sensitive to anthropogenic impacts, and it has been demonstrated
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13 286 that, in the long term (i.e. years to decades), the increased sedimentation of waste particles and
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15 287 the accumulation of organic matter lead to the deterioration of the seagrass system (Pergent-
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18 288 Martini et al., 2006). In the regions investigated, the seagrass systems displayed a clear impact
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20 289 and several indicators of deterioration of the *Posidonia* meadows were reported (Holmer et al.,
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22 290 2008). However, the effects of biodeposition on meiofaunal variables were not evident, indicating
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25 291 that in seagrass systems the search for meiofaunal indicators of impact is unnecessary as it
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27 292 provides, if any, a slow or late response to the fish-farm impact.
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29 293 30 31 32 294 *4.3 Conclusions and perspectives*

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34 295 In this study we showed that fish-farm biodeposition in the Mediterranean Sea can
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36 296 provoke changes in meiofaunal abundance, community structure and the biodiversity at high
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39 297 taxonomic levels. However, the hierarchical sampling conducted here demonstrated that the
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41 298 meiofaunal responses varied among regions and habitats. In all non-vegetated sediments the
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44 299 richness of meiofaunal taxa decreased in response to fish-farm biodeposition, and therefore
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46 300 independently from the background environmental conditions. Conversely, in seagrass
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49 301 sediments, the idiosyncratic response did not allow any clear indicator of impacts from fish-
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51 302 farm bio-deposition to be identified. The results of this study highlight the importance of using
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53 303 different indicators of fish-farm impact in vegetated and non-vegetated systems.
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305 Acknowledgements

306 The authors thank the EU project MedVeg (n° EU Quality of Life & Management of Living
307 Resources Programme, Contract No. Q5RS-2001-02456.).

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401 **Figure Captions**

402 **Figure 1.** Sites of the four fish-farms investigated in Cyprus, Italy, Greece and Spain in the
403 Mediterranean Sea.

404 **Figure 2.** Biopolymeric C concentrations (A) and values of the protein to carbohydrate ratio (B)
405 in the investigated sediments. According to the SNK tests (at $p < 0.05$), + = increase; - =
406 decrease; ns = not significant. Data are extracted from Pusceddu et al. (2007).

407 **Figure 3.** Total meiofaunal abundance (A), meiofaunal community composition (B) and the
408 relative abundance of rare taxa (C) in Cyprus, Greece, Italy and Spain in non-vegetated
409 and vegetated habitats, in control (C) and impact (I) sediments.

410 **Figure 4.** Taxa richness (A) and community evenness (B) in Cyprus, Greece, Italy and Spain in
411 non-vegetated and vegetated habitats, in control (C) and impact (I) sediments. The
412 statistical significance of the differences among bars (SNK test: + = increase $p < 0.05$, - =
413 decrease $p < 0.05$, ns = not significant) are also reported.

1 **Table 1** - Environmental characteristics reported in all sampling sites (distance of the sampling sites from the fish farm, water depth,
 2 current speed, sediment type, porosity, water content and grain size).

3

Region	Habitat	Site	Distance from fish farm (m)	Water Depth (m)	Current speed (cm sec ⁻¹)	Sediment type Silt/Clay (%)	Sediment Porosity (%)	Sediment Water Content (%)	Grain Size (Mean Diameter, mm)
Cyprus	non- vegetated	Impact	0-20	30	20-40	80.7	1.2	42.9	0.01
	Control	4000 westward	30	20-40	75.9	1.2	44.9	0.02	
	Impact	300 northward	20	20-40	68.4	1.2	46.4	0.02	
	Vegetated Control	1000 eastward	20	20-40	2.5	1.1	36.5	0.10	
Italy	non- vegetated	Impact	0	22	20	5.7	1.2	41.6	0.47
	Control	3200 north-east	22	20	3.9	0.8	13.5	0.84	
	Impact	5	22	20	4.6	1.2	46.9	0.45	
	Vegetated Control	1000 northward	22	20	4.8	1.1	33.7	0.90	
Greece	non- vegetated	Impact	0	16	6.3	6.7	1.1	29.5	0.38
	Control	800 westward	16	6.3	2.2	1.6	18	1.89	
	Impact	5	16	6.3	20.2	1.0	27.3	0.33	
	Vegetated Control	1000 southward	15	6.3	13.1	1.1	34.4	0.76	
Spain	non- vegetated	Impact	0	29	4.7	7.7	1.1	31.7	0.32
	Control	1000 westward	29	4.7	15.6	1.2	42.3	0.43	
	Impact	10	29	4.7	16.2	1.1	32.5	0.40	
	Vegetated Control	1000 southward	29	4.7	15.3	1.1	32.4	0,9	

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5 **Table 2** - Results of ANOVA testing for differences between impact and control sites in quantity
 6 and quality of sediment organic matter in different habitats and regions. R = region; H = habitat; I
 7 = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; F = F value; P =
 8 probability level: *** = $P < 0.001$; ns = not significant.

	Source	SS	DF	MS	F	P
Biopolymeric C	R	38.74	3	12.91	28.63	***
	H	3.54	1	3.54	0.73	ns
	I	4.40	1	4.39	0.53	ns
	R × H	14.53	3	4.84	10.74	***
	R × I	25.01	3	8.34	18.48	***
	H × I	3.99	1	3.99	1.00	ns
	R × H × I	11.99	3	3.99	8.86	***
	Residuals	14.43	32	0.45		
	Total	116.64	47			
Protein to carbohydrate ratio	R	12.83	3	4.28	285.87	***
	H	1.723	1	1.72	2.65	ns
	I	0.05	1	0.05	0.01	ns
	R × H	1.95	3	0.65	43.42	***
	R × I	10.66	3	3.55	237.45	***
	H × I	1.23	1	1.23	5.12	ns
	R × H × I	0.72	3	0.24	16.04	***
	Residuals	0.48	32	0.02		
	Total	29.64	47			

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12 **Table 3** - Results of ANOVA testing for differences between impact and control sites in total
 13 meiofaunal abundance in different habitats and regions. R = region; H = habitat; I = impact; SS =
 14 sum of squares; DF = degrees of freedom; MS = means square; F = F value; P = probability level:
 15 *** = $P < 0.001$; ns = not significant.

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Source	DF	SS	MS	F	P
R	3	27255106	9085035	11.7511	**
H	1	909986	909986	0.2431	ns
I	1	13917504	13917504	4.4563	ns
R × H	3	11228668	3742889	4.8413	**
R × I	3	9369245	3123082	4.0396	*
H × I	1	347142.6	347142.6	0.1521	ns
R × H × I	3	6848726	2282909	2.9528	*
Residuals	32	24739857	773120.5		
Total	47	94616234			

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20 **Table 4** - Meiofaunal taxa abundance (ind 10 cm⁻²) in fish-farm impacted and control sites across
 21 the four regions and the two habitats. Reported are average value \pm standard deviation.

Site	Location	Site	Nematoda	Copepoda	Polychaeta	Ostracoda	Turbellaria	Oligochaeta	Gastrotricha	Bivalvia
Cyprus	Non-vegetated	Control	1903 \pm 499	192 \pm 43	64 \pm 14	3 \pm 0	9 \pm 8	9 \pm 6	2 \pm 0	1 \pm 0
		Impact	1751 \pm 365	438 \pm 56	69 \pm 5	0 \pm 0	4 \pm 2	53 \pm 20	20 \pm 17	1 \pm 0
	Vegetated	Control	898 \pm 157	186 \pm 26	52 \pm 0	3 \pm 2	1 \pm 1	8 \pm 6	2 \pm 2	2 \pm 0
		Impact	1919 \pm 145	312 \pm 65	90 \pm 2	5 \pm 2	2 \pm 2	7 \pm 7	9 \pm 2	1 \pm 0
Greece	Non-vegetated	Control	813 \pm 248	595 \pm 253	37 \pm 13	27 \pm 17	16 \pm 3	20 \pm 6	61 \pm 4	1 \pm 1
		Impact	3406 \pm 1798	757 \pm 147	549 \pm 217	45 \pm 47	23 \pm 6	155 \pm 73	344 \pm 26	1 \pm 1
	Vegetated	Control	583 \pm 262	582 \pm 258	87 \pm 53	15 \pm 5	7 \pm 2	13 \pm 12	43 \pm 8	86 \pm 40
		Impact	1218 \pm 201	784 \pm 809	87 \pm 103	19 \pm 27	18 \pm 6	35 \pm 16	224 \pm 80	0 \pm 0
Italy	Non-vegetated	Control	367 \pm 69	409 \pm 48	52 \pm 18	14 \pm 3	1 \pm 0	2 \pm 0	5 \pm 1	0 \pm 0
		Impact	291 \pm 7	394 \pm 88	65 \pm 13	16 \pm 0	5 \pm 0	6 \pm 4	13 \pm 4	1 \pm 1
	Vegetated	Control	723 \pm 95	284 \pm 145	69 \pm 42	16 \pm 10	3 \pm 1	5 \pm 2	12 \pm 8	0 \pm 0
		Impact	938 \pm 239	255 \pm 39	77 \pm 36	21 \pm 9	2 \pm 0	6 \pm 1	28 \pm 11	0 \pm 0
Spain	Non-vegetated	Control	1467 \pm 521	539 \pm 63	144 \pm 32	6 \pm 6	3 \pm 4	24 \pm 3	150 \pm 131	0 \pm 0
		Impact	2342 \pm 737	305 \pm 107	222 \pm 108	1 \pm 2	1 \pm 1	72 \pm 52	10 \pm 12	0 \pm 0
	Vegetated	Control	2084 \pm 639	488 \pm 186	129 \pm 65	7 \pm 2	2 \pm 3	252 \pm 102	11 \pm 10	0 \pm 0
		Impact	3381 \pm 406	432 \pm 420	125 \pm 60	37 \pm 12	4 \pm 5	33 \pm 27	41 \pm 47	0 \pm 0
			Kynorhyncha	Tardigrada	Cumacea	Amphipoda	Isopoda	Tanaidacea	Acarina	Nemertina
Cyprus	Non-vegetated	Control	11 \pm 3	0 \pm 0	0 \pm 0	0 \pm 1	0 \pm 0	1 \pm 0	0 \pm 0	20 \pm 7
		Impact	9 \pm 9	0 \pm 0	0 \pm 0	0 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0	5 \pm 3
	Vegetated	Control	1 \pm 0	0 \pm 0	1 \pm 0	1 \pm 1	1 \pm 0	0 \pm 0	0 \pm 0	4 \pm 4
		Impact	3 \pm 0	2 \pm 2	0 \pm 0	1 \pm 1	1 \pm 0	2 \pm 0	1 \pm 1	7 \pm 7
Greece	Non-vegetated	Control	19 \pm 10	9 \pm 9	0 \pm 0	2 \pm 1	0 \pm 0	1 \pm 1	2 \pm 2	46 \pm 34
		Impact	22 \pm 7	2 \pm 2	0 \pm 0	0 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0	292 \pm 160
	Vegetated	Control	15 \pm 5	12 \pm 9	44 \pm 17	0 \pm 1	2 \pm 0	5 \pm 3	0 \pm 0	0 \pm 0
		Impact	14 \pm 5	20 \pm 15	2 \pm 1	0 \pm 1	1 \pm 0	0 \pm 0	4 \pm 3	62 \pm 46
Italy	Non-vegetated	Control	2 \pm 2	36 \pm 4	0 \pm 0	1 \pm 1	1 \pm 0	7 \pm 3	7 \pm 0	1 \pm 1
		Impact	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 0
	Vegetated	Control	7 \pm 1	27 \pm 12	1 \pm 1	0 \pm 1	1 \pm 0	3 \pm 2	3 \pm 2	5 \pm 4
		Impact	0 \pm 0	14 \pm 8	0 \pm 0	0 \pm 1	0 \pm 0	0 \pm 0	1 \pm 0	5 \pm 1
Spain	Non-vegetated	Control	9 \pm 7	26 \pm 19	1 \pm 1	2 \pm 1	1 \pm 0	1 \pm 0	5 \pm 3	4 \pm 2
		Impact	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 1	0 \pm 0	2 \pm	0 \pm 0	77 \pm 56
	Vegetated	Control	8 \pm 0	44 \pm 26	0 \pm 0	2 \pm 1	1 \pm 0	2 \pm 0	1 \pm 1	4 \pm 2
		Impact	8 \pm 0	66 \pm 57	0 \pm 0	1 \pm 1	0 \pm 0	2 \pm 0	3 \pm 3	30 \pm 23

24 **Table 5** - Results of ANOVA testing for differences between impact and control sites in
 25 abundance of the meiofaunal taxa in different habitats and regions. R = region; H = habitat; I =
 26 impact; SS = sum of squares; DF = degrees of freedom; MS = means square; F = F value; P =
 27 probability level: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; ns = not significant.
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	Source	SS	DF	MS	F	P		Source	SS	DF	MS	F
Nematodes	R	13.15	3	4.38	38.98	***	Turbellarians	R	18.88	3	6.29	12.84
	H	0.06	1	0.06	0.04	ns		H	2.28	1	2.28	3.64
	I	2.66	1	2.66	4.47	ns		I	0.41	1	0.41	1.94
	R × H	4.34	3	1.45	12.86	***		R × H	1.86	3	0.62	1.24
	R × I	1.78	3	0.59	5.29	**		R × I	0.65	3	0.22	0.44
	H × I	0.10	1	0.10	0.36	ns		H × I	0.03	1	0.03	0.03
	R × H × I	0.82	3	0.27	2.42	ns		R × H × I	1.96	3	0.65	1.34
	Residuals	3.60	32	0.11				Residuals	15.66	32	0.49	
Total	26.50	47				Total	41.73	47				
Copepods	R	1116932.28	3	372310.76	6.23	**	Oligochaetes	R	59266.22	3	19755.41	1.84
	H	17542.98	1	17542.98	1.01	ns		H	56.14	1	56.14	0.00
	I	30403.35	1	30403.35	0.38	ns		I	223.39	1	223.39	0.00
	R × H	52331.00	3	17443.67	0.29	ns		R × H	40553.05	3	13517.68	1.24
	R × I	237589.60	3	79196.53	1.33	ns		R × I	41567.71	3	13855.90	1.24
	H × I	1332.47	1	1332.47	0.11	ns		H × I	34558.65	1	34558.65	3.44
	R × H × I	35031.09	3	11677.03	0.20	ns		R × H × I	29968.09	3	9989.36	0.94
	Residuals	1912625.14	32	59769.54				Residuals	349658.16	32	10926.82	
Total	3403787.91	47				Total	555851.41	47				
Polychaetes	R	170.08	3	56.69	8.01	***	Gastrotrichs	R	49.57	3	16.52	19.54
	H	39.79	1	39.79	1.39	ns		H	0.66	1	0.66	0.54
	I	96.90	1	96.90	2.63	ns		I	5.48	1	5.48	1.84
	R × H	85.71	3	28.57	4.04	*		R × H	3.67	3	1.22	1.44
	R × I	110.65	3	36.88	5.21	**		R × I	9.05	3	3.02	3.54
	H × I	66.82	1	66.82	1.12	ns		H × I	1.80	1	1.80	0.44
	R × H × I	179.26	3	59.75	8.44	***		R × H × I	12.30	3	4.10	4.84
	Residuals	226.52	32	7.08				Residuals	27.00	32	0.84	
Total	975.73	47				Total	109.54	47				
Ostracods	R	24.69	3	8.23	16.96	***	Nemertins	R	33.06	3	11.02	23.54
	H	1.53	1	1.53	0.60	ns		H	5.29	1	5.29	0.84
	I	0.01	1	0.01	0.04	ns		I	14.88	1	14.88	2.04
	R × H	7.58	3	2.53	5.21	**		R × H	19.28	3	6.43	13.74
	R × I	0.40	3	0.13	0.28	ns		R × I	22.38	3	7.46	15.94
	H × I	1.75	1	1.75	1.39	ns		H × I	1.25	1	1.25	0.94
	R × H × I	3.77	3	1.26	2.59	ns		R × H × I	4.16	3	1.39	2.94
	Residuals	15.53	32	0.49				Residuals	15.00	32	0.47	
Total	55.26	47				Total	115.29	47				

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	Source	SS	DF	MS	F	P		Source	SS	DF	MS	F	P
Acarines	R	39.94	3	13.31	6.11	***	Isopods	R	1.12	3	0.37	1.77	ns
	H	0.76	1	0.76	0.16	ns		H	2.99	1	2.99	3.12	ns
	I	11.47	1	11.47	0.68	ns		I	3.89	1	3.89	3.91	ns
	R × H	14.28	3	4.76	2.18	ns		R × H	2.87	3	0.96	4.57	**
	R × I	50.90	3	16.97	7.78	**		R × I	2.99	3	1.00	4.75	**
	H × I	50.51	1	50.51	11.65	*		H × I	0.04	1	0.04	0.65	ns
	R × H × I	13.01	3	4.34	1.99	ns		R × H × I	0.17	3	0.06	0.26	ns
	Residuals	69.78	32	2.18				Residuals	6.70	32	0.21		
Total	250.64	47				Total	20.76	47					
Amphipods	R	4.06	3	1.35	2.78	ns	Kinorynchs	R	1566.82	3	522.27	20.47	***
	H	0.04	1	0.04	0.03	ns		H	40.43	1	40.43	0.42	ns
	I	4.25	1	4.25	8.04	ns		I	39.30	1	39.30	1.42	ns
	R × H	4.27	3	1.42	2.92	*		R × H	285.49	3	95.16	3.73	*
	R × I	1.59	3	0.53	1.09	ns		R × I	83.07	3	27.69	1.09	ns
	H × I	3.03	1	3.03	3.46	ns		H × I	2.49	1	2.49	0.08	ns
	R × H × I	2.62	3	0.87	1.79	ns		R × H × I	97.20	3	32.40	1.27	ns
	Residuals	15.58	32	0.49				Residuals	816.26	32	25.51		
Total	35.44	47				Total	2931.04	47					
Bivalves	R	15.59	3	5.20	79.49	***	Tanaidaceans	R	1.94	3	0.65	5.74	**
	H	2.58	1	2.58	1.16	ns		H	0.10	1	0.10	0.25	ns
	I	2.37	1	2.37	0.72	ns		I	2.28	1	2.28	1.57	ns
	R × H	6.67	3	2.22	34.01	***		R × H	1.27	3	0.42	3.76	*
	R × I	9.89	3	3.30	50.41	***		R × I	4.35	3	1.45	12.85	***
	H × I	3.50	1	3.50	1.22	ns		H × I	0.05	1	0.05	0.07	ns
	R × H × I	8.61	3	2.87	43.89	***		R × H × I	2.02	3	0.67	5.96	***
	Residuals	2.09	32	0.07				Residuals	3.61	32	0.11		
Total	51.30	47				Total	15.64	47					
Cumaceans	R	9.05	3	3.02	63.63	***	Tardigrades	R	41.73	3	13.91	27.05	***
	H	5.28	1	5.28	1.25	ns		H	18.12	1	18.12	11.49	*
	I	2.94	1	2.94	2.79	ns		I	9.57	1	9.57	2.81	ns
	R × H	12.65	3	4.22	88.95	***		R × H	4.73	3	1.58	3.07	*
	R × I	3.17	3	1.06	22.28	***		R × I	10.23	3	3.41	6.63	**
	H × I	0.91	1	0.91	0.54	ns		H × I	12.22	1	12.22	12.82	*
	R × H × I	5.05	3	1.68	35.48	***		R × H × I	2.86	3	0.95	1.85	ns
	Residuals	1.52	32	0.05				Residuals	16.46	32	0.51		
Total	40.57	47				Total	115.91	47					

31 **Table 6** - Results of PERMANOVA testing for differences in the whole set of meiofaunal taxa.
 32 The analysis was done on the distance matrix calculated using Bray-Curtis similarities on
 33 untransformed data. Each term was tested using 4,999 random permutations of the appropriate
 34 units (***) = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant). DF = degrees of
 35 freedom, MS = mean square, F = F-value; P (MC) = Monte Carlo probability level. Reported are
 36 also the results of the pairwise comparisons testing differences between impact and control sites
 37 in the two habitats separately for the four regions (* $P < 0.02$). R = region; H = habitat; I = impact
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PERMANOVA outputs

	DF	MS	F	P(MC)
R	3	5835.49	19.49	***
H	1	1111.62	0.76	ns
I	1	3614.80	3.56	ns
R × H	3	1455.63	4.86	**
R × I	3	1016.26	3.39	**
H × I	1	418.29	0.51	ns
R × H × I	3	818.36	2.73	**
Residuals	32	299.37		
Total	47			

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Pairwise comparisons: Impact vs. Control sites

Habitat	Region	P
Cyprus	Non-vegetated	**
	Vegetated	ns
Greece	Non-vegetated	**
	Vegetated	ns
Italy	Non-vegetated	ns
	Vegetated	ns
Spain	Non-vegetated	ns
	Vegetated	ns

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45 **Table 7** - Results of ANOVA testing for differences between impact and control sites in richness
 46 and evenness of meiofaunal taxa in different habitats and regions. R = region; H = habitat; I =
 47 impact; SS = sum of squares; DF = degrees of freedom; MS = means square; F = F value; P =
 48 probability level: *** = $P < 0.001$; ns = not significant.
 49

	Source	SS	DF	MS	F	P
Number of taxa	R	47.5	3	15.8	126.67	***
	H	24.1	1	24.1	9.32	ns
	I	48.0	1	48.0	32.00	*
	R × H	7.8	3	2.6	20.67	***
	R × I	4.5	3	1.5	12.00	***
	H × I	30.1	1	30.1	15.70	*
	R × H × I	5.8	3	1.9	15.33	***
	Residuals	4	32	0.1		
	Total	171.7	47			
Community Evenness	R	0.327	3	0.109	27.31	***
	H	0.018	1	0.018	8.59	ns
	I	0.003	1	0.003	0.53	ns
	R × H	0.006	3	0.002	0.53	ns
	R × I	0.015	3	0.005	1.28	ns
	H × I	0.027	1	0.028	4.48	ns
	R × H × I	0.018	3	0.006	1.54	ns
	Residuals	0.128	32	0.004		
	Total	0.543	47			

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52 **Table 8** - Results of PERMANOVA testing for differences between impact and control sites in
 53 total meiofaunal abundance in different habitats and regions, after the removal of the covariate
 54 effects. R = region; H = habitat; I = impact; SS = sum of squares; DF = degrees of freedom; MS
 55 = means square; F = F value; P = probability level: *** = $P < 0.001$; ns = not significant.
 56 Covariates included: biopolymeric C contents, protein to carbohydrate values, water depth,
 57 sediment porosity and water content and current.

Source	DF	SS	MS	F	P
Covariables	6	7.395	1.232	7.45	**
R	3	0.406	0.135	1.28	ns
H	1	0.082	0.081	0.09	ns
I	1	1.128	1.127	-0.78	ns
R × H	3	489.232	163.077	-38.78	ns
R × I	3	-12.148	-4.049	2.22	ns
H × I	1	1.851	1.851	-0.35	ns
R × H × I	3	0.609	0.203	0.61	ns
Residual	26	8.521	0.327		
Total	47	13.398			

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61 **Table 9** – Results of the multiple regression analysis of meiofaunal abundance (square root
 62 transformed), taxa richness (untransformed) and community evenness over the environmental
 63 variables. Prop = proportional; Cu, = cumulative.

64

	Variable	pseudo-F	P	Explained variance (%)	
				Prop	Cum
Meiofaunal abundance	Current	8.17	0.012	15.08	15.08
	Biopolymeric C	5.13	0.032	8.7	23.77
	Protein to carbohydrate ratio	2.64	ns	4.31	28.09
	Water depth	0.13	ns	0.21	28.30
	Sediment porosity	0.07	ns	0.11	28.41
	Sediment water content	0.01	ns	0.01	28.42
Taxa richness	Water depth	11.19	0.004	19.56	19.56
	Current	9.07	0.014	13.49	33.05
	Protein to carbohydrate ratio	12.81	0.002	15.09	48.14
	Sediment porosity	0.58	ns	0.69	48.84
	Biopolymeric C	0.30	ns	0.36	49.20
	Sediment water content	0.87	ns	1.05	50.25
Community evenness	Current	19.71	0.002	29.99	29.99
	Water depth	14.85	0.002	17.37	47.37
	Biopolymeric C	1.15	ns	1.34	48.71
	Sediment porosity	0.90	ns	1.06	49.76
	Protein to carbohydrate ratio	0.85	ns	0.99	50.76
	Sediment water content	0.07	ns	0.09	50.84

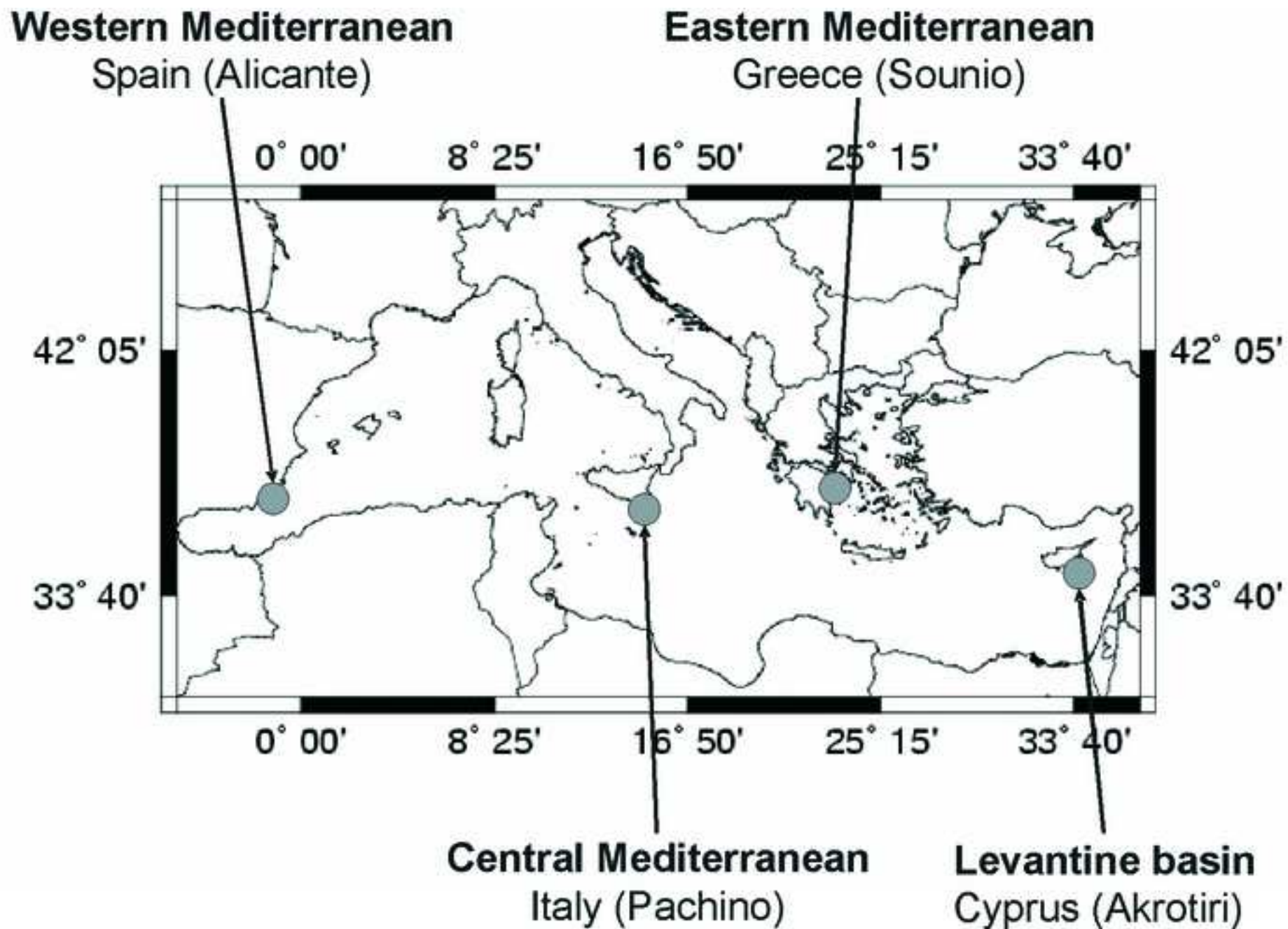
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Figure 1



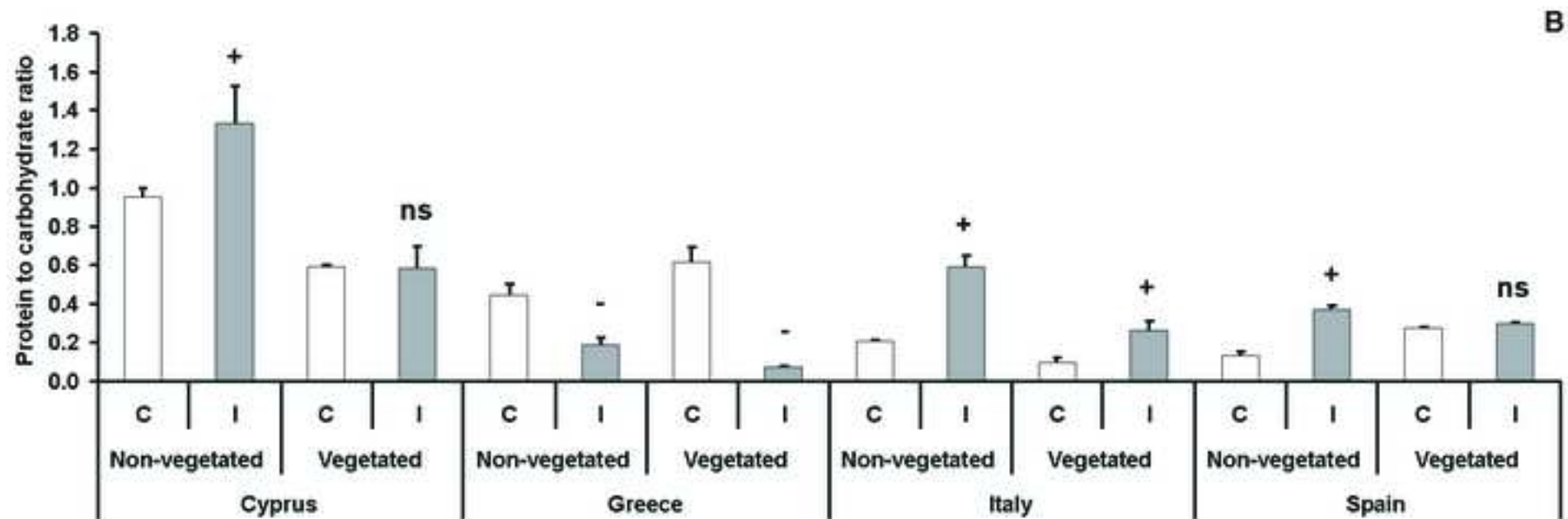
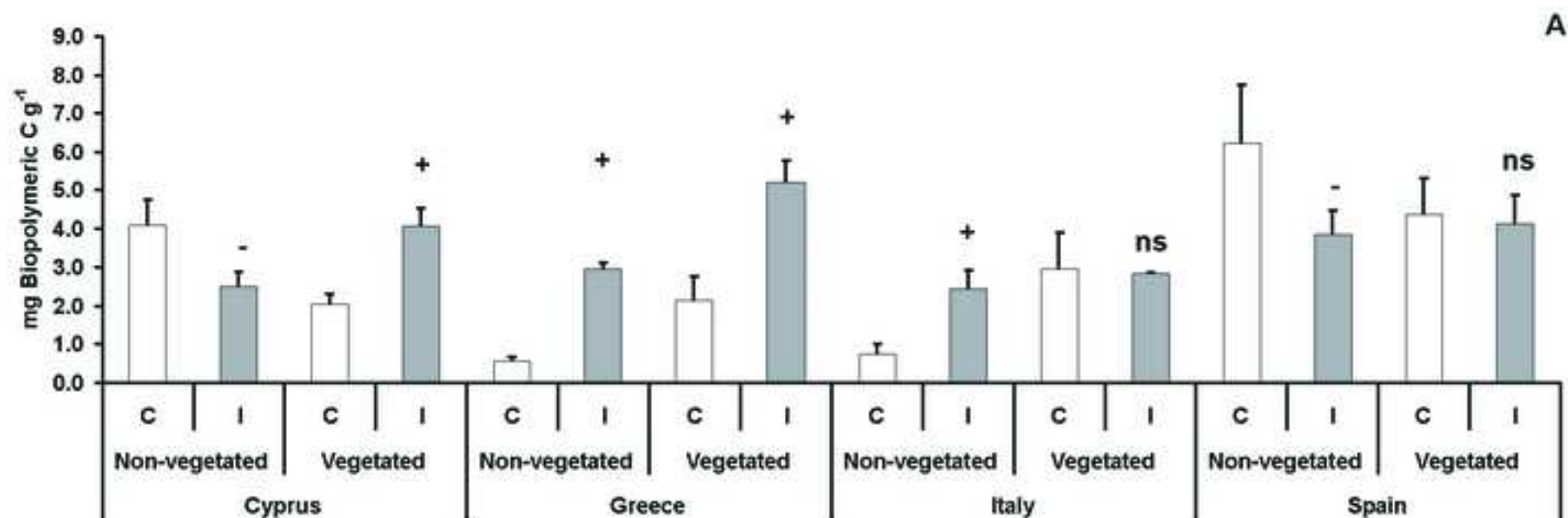


Figure 3

