CHANGES IN THE STRUCTURE OF A *POSIDONIA OCEANICA* MEADOW AND IN THE DIVERSITY OF ASSOCIATED DECAPOD, MOLLUSC AND ECHINODERM ASSEMBLAGES, RESULTING FROM INPUTS OF WASTE FROM A MARINE FISH FARM (MALTA, CENTRAL MEDITERRANEAN)

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ABSTRACT

Nutrient inputs resulting from fish farming activities located in the vicinity of seagrass meadows can potentially alter the structure and affect the functioning of these ecosystems, however, few studies have addressed this problem. The impact of waste generated by an offshore fish farm in Malta (Central Mediterranean) on the structure of a meadow of the seagrass *Posidonia oceanica* and on the associated decapod, echinoderm and mollusc assemblages was studied by collecting samples from stations located at distances of 10 m, 30 m, 50 m, 90 m, 170 m and 330 m away from the farm. Meadow morphology and leaf epiphyte load changed with distance from the fish cages, as did the species richness and abundance of macroinvertebrates associated with the seagrass. However, while shoot morphometric measures increased significantly in value over the whole length of the transect (330 m), macroinvertebrate species richness and abundance peaked at an intermediate distance (40–160 m) from the cages. These results suggest that while waste generated from fish farms can severely alter the structure of a seagrass meadow over a large area, nutrient enrichment could increase productivity in certain parts of the same meadow, leading to a localized increase in species richness and abundance of associated macroinvertebrates. These changes result in different 'ecological zones' round the source of nutrient input.

Nutrient enrichment resulting from aquaculture operations appears to be the most common and widespread cause of change in marine coastal ecosystem structure where this activity is present (Gowen and Bradbury, 1987). Nutrient enrichment arises from the inevitable release of metabolic waste products and uneaten food into the aquatic environment (Barg, 1992; Gowen and Bradbury, 1987), most of the insoluble component of which settles below and in the vicinity of the cages (Hevia et al., 1996), where it can cause large changes in the structure of the benthic assemblages originally present (Johannessen et al., 1994; Findlay and Watling, 1995). Seagrass habitats are especially vulnerable to such impacts since they occur in shallow coastal waters, inlets, embayments and lagoons, all of which are much sought-after by fish farm operators.

Where offshore fish farms are located directly above seagrass meadows, deposition of particulate organic matter smothers the seagrass such that completely degraded meadows with only dead rhizomes have been reported under fish cages and in their immediate surroundings (Delgado et al., 1997; Mendez et al., 1997). High inputs of fish-farm waste also lead to increased turbidity in the water column (Delgado et al., 1997; Mendez et al., 1997) which, together with the shading effect of the fish cages, diminishes the amount of light reaching the seagrass and may reduce photosynthesis (Dennisson, 1987). In turn, this may cause death of the belowground organs of the plant due to a reduced flux of oxygen from the leaves (Hemminga, 1998). Additionally, in nutrient rich waters, phytoplankton blooms may occur and the epiphytic load on the seagrass leaves increases, both reducing further the amount of light reaching the plant's photosynthetic tissues,

leading to reduced productivity of the macrophyte (Neckles et al., 1993; Tomasko and Lapointe, 1991). Furthermore, a high epiphytic cover may cause the leaves to become more prone to detachment due to the increased drag (Boudouresque et al., 1984), as well as reduce the uptake of nutrients by the leaves (Sand-Jensen, 1977). Siting of fish farms in low energy environments enhances these adverse effects since waste generated by the farm tends to remain concentrated in the vicinity of the cages (Gowen and Bradbury, 1987).

A number of studies have shown that waste generated by fish farming has an adverse impact on seagrass meadows, sometimes causing extirpation of the plants (Delgado et al., 1997; Mendez et al., 1997). Studies on the impact of fish farming on benthic ecosystems other than seagrasses (e.g., Johannessen et al., 1994) have concluded that although the diversity of macroinvertebrates below the farm usually decreases compared with the situation before installation of the fish cages, the species richness and biomass of macroinvertebrates may increase a small distance away from the farm site due to the enhanced food supply. In many coastal areas worldwide, seagrass meadows are under threat due to an increase in aquaculture activities. In the Mediterranean Sea, where fish farming has been shown to have a considerable impact on the benthos (Karakassis et al., 1999; 2000), many inshore fish farms are inevitably located above or in the vicinity of meadows of the endemic seagrass *Posidonia oceanica* (L.) Delile, since this species occupies extensive areas of the seabed at depths ranging from 1 m to 40 m (Drew and Jupp, 1976). Despite the importance of seagrass meadows as highly productive ecosystems (Zieman and Wetzel, 1980) and the increased threat from the rapidly growing aquaculture industry (Barg, 1992), studies on the impact of fish farms on the faunal assemblages associated with seagrass meadows are generally lacking.

The aim of this study was to investigate changes in the morphology of a *P. oceanica* meadow and in the composition of the associated decapod, echinoderm and mollusc assemblages, resulting from inputs of waste from a marine fish farm in Malta (Central Mediterranean).

MATERIAL AND METHODS

STUDY AREA.—The study area was located in St Paul's Bay on the northwestern coast of the island of Malta (Fig. 1), where a fish farm has been growing gilthead seabream, *Sparus aurata* (L.), as the main cultured species since 1991. The farm is sited just off the northern headland of the bay and is well sheltered from the northwest (Mistral) wind, which is the predominant wind in the Maltese Islands (Chetcuti et al., 1992). The farm consists of eight cages linked together to form a single unit approximately 50 m long by 15 m wide and is located in waters 12–16 m deep. Annual fish production by the farm is around 15 t yr^{-1}. A continuous *P. oceanica* meadow was originally present below the fish farm, but plants lying directly below the cages were decimated soon after installation of the cages and initiation of fish production. However, plants some 10 m away from the perimeter of the fish cages have retained their vitality, although shoot density was reduced and the leaves had a heavy epiphytic growth, as could easily be seen by direct observation during a preliminary survey.

Sampling stations were located at the same depth (12 m) but at increasing distances from the cages along a line transect as follows: 10 m (station A), 30 m (station B), 50 m (station C), 90 m (station D), 170 m (station E) and 330 m (station F) (Fig. 1). Collection of samples along a reference transect at the same depth on the other side of the bay was not possible since the seabed in that part was predominantly rocky and the *P. oceanica* meadows there were patchy. To study the seagrass-associated macroinvertebrates, *P. oceanica* shoots and the associated root-rhizome matrix were
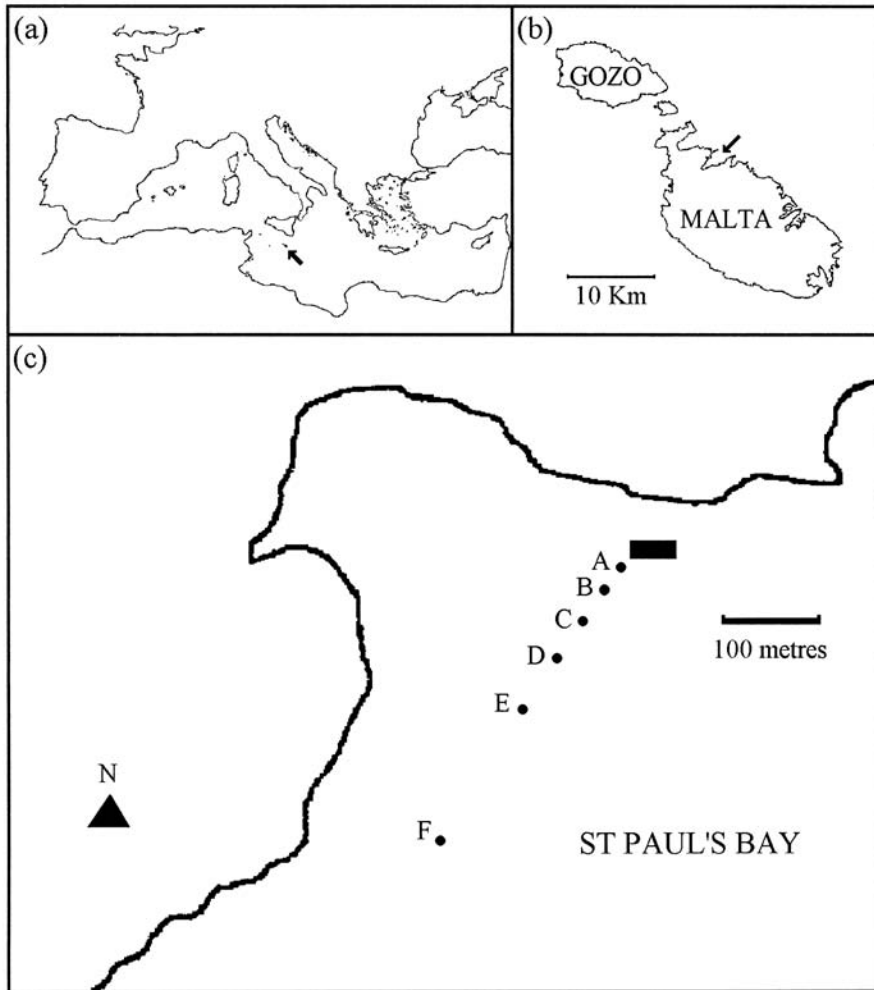
Figure 1. Map showing (a) the location of the Maltese Archipelago at the centre of the Mediterranean (arrow), (b) the location of the study area (arrow) and (c) the location of the fish cages (rectangle) and sampling stations. The Maltese islands are centered on latitude 35° 55.5′ N and longitude 14° 23.5′E.

collected using a cylindrical corer (internal diameter 35 cm, length 50 cm) to which a 0.5 mm mesh net was attached (Borg et al., 2002). Three replicate cores were collected by SCUBA divers from each station. To study meadow parameters, divers collected 12 orthotropic shoots at random from each station. Shoot density was estimated in situ at each station by counting the number of shoots present in a 35 cm × 35 cm quadrat; five replicates were counted per station. A sample of sediment for estimation of organic content and for granulometry was collected from each station using a small PVC corer (internal diameter 10 cm, length 10 cm). All fieldwork was carried out in August 1998.

In the laboratory, *P. oceanica* morphometric parameters were estimated by first separating the leaves from the rhizomes and classifying them according to Giraud's (1979) scheme as 'adult', 'intermediate' or 'juvenile', and then counting the number of leaves in each class. Leaf width and

leaf length (to the nearest mm) were recorded for each leaf class and estimates of the leaf area index were made for each station. The epiphytic load was determined by scraping off epiphytes from the leaves using a blunt blade and weighing after drying in an oven at 70°C for 48 h. The scraped leaves were also dried at 70°C for 48 h and then weighed to obtain an estimate of mean shoot dry weight at each station. The sediment samples were analyzed for grain size distribution and organic content (Walkley and Black titration method) according to the procedures in Buchanan (1984). Plant material present in the seagrass core samples was separated from sediment and washed to remove all macroinvertebrates present amongst the leaves and the root-rhizome matrix. The washing and sediment were then passed through a 0.5 mm sieve and the retained decapods, echinoderms and molluscs (which are locally the best known taxonomically) were sorted, identified to species, and counted.

One way analysis of variance (ANOVA) was used to test for significant differences (0.05 level of significance) in the meadow parameters between stations, except for number of leaves per shoot which was examined using the Kruskal-Wallis test (0.01 level of significance). The macrofaunal abundance data were analysed by non-metric multidimensional scaling (NMDS) and hierarchical agglomerative cluster analysis after double square-root transformation and calculation of the Bray-Curtis similarity measure, using the PRIMER (Plymouth Routines In Marine Ecological Research) suite of computer programs (Clarke and Warwick, 1994). Analysis of similarities (ANOSIM) was used to test for differences in faunal composition between stations (Clarke and Green, 1988). The similarities percentages procedure (SIMPER) (Clarke, 1993) was used to identify the species that contributed most to 'between station' dissimilarities and those that contributed to 'within station' similarities.

RESULTS

Values of mean total number of leaves per shoot were not significantly different between stations, but the number of adult leaves at the 10m station was significantly lower than for the other stations (Kruskal-Wallis, $P < 0.01$) (Fig. 2A). Values of mean leaf length (adult + intermediate leaves) and adult leaf length were significantly different (ANOVA, $P < 0.05$) with an overall increase in value with distance from the cages (Fig. 2B). Mean shoot density and dry weight values were significantly different (ANOVA, $P < 0.05$) with an overall increase in value with distance from the cages (Fig. 3A,B). Leaf area index values were significantly different (ANOVA, $P < 0.05$) and overall increased with increasing distance from the cages (Fig. 4A). Values of leaf epiphyte weight were significantly different (ANOVA, $P < 0.05$), with an overall decrease with increasing distance from the cages (Fig. 4B).

The sediment at all six stations was predominantly sandy, however, the gravel fraction was relatively low in the two stations closest to the fish-cages and highest at the 330 m station (Table 1). Values of percent organic carbon in the sediment showed an overall decrease with distance from the cages, except at the 170 m station where the value recorded was anomalously high (Table 1).

A total of 1151 individuals comprising 26 decapod, 8 echinoderm and 71 mollusc species, were identified. Mean abundance and mean species richness reached a peak value 90 m from the cages (Fig. 5A,B). Both cluster analysis and NMDS indicated three groups: one consisting of the 330 m station, another which included the 10 m and 30 m stations and a third with the three intermediate (50 m, 90 m and 170 m) stations (Fig. 6A,B). The ANOSIM test showed an increasing difference in faunal composition (as a trend of increasing R statistic) with increasing distance from the cages (R ranged between 0.33 and 0.89; R = 1 if all replicates at a given site are more similar to each other than to any replicate from any other site; R = 0 if there are no differences between the sites).
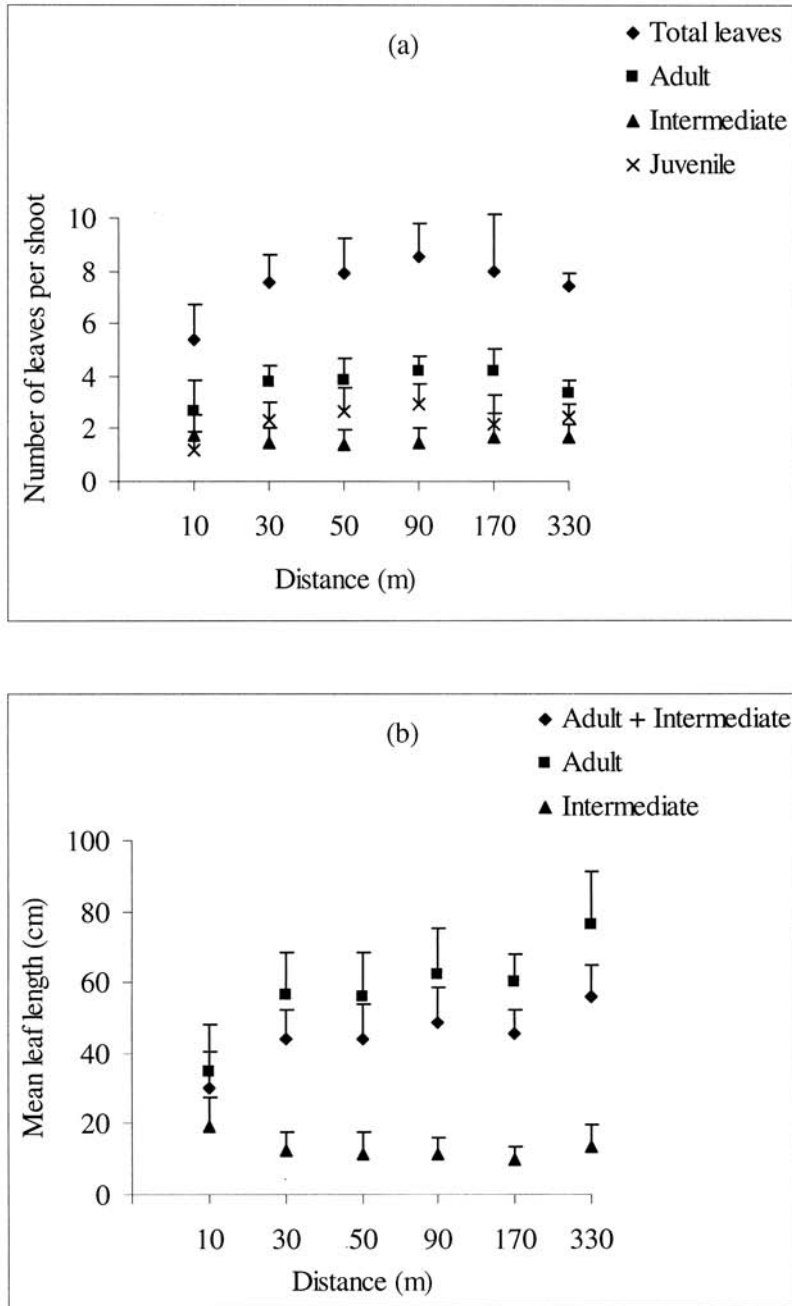
Figure 2. Changes in (a) mean number of total, adult, intermediate and juvenile leaves and (b) mean leaf length of adult + intermediate, adult and intermediate leaves with distance from the fish cages.
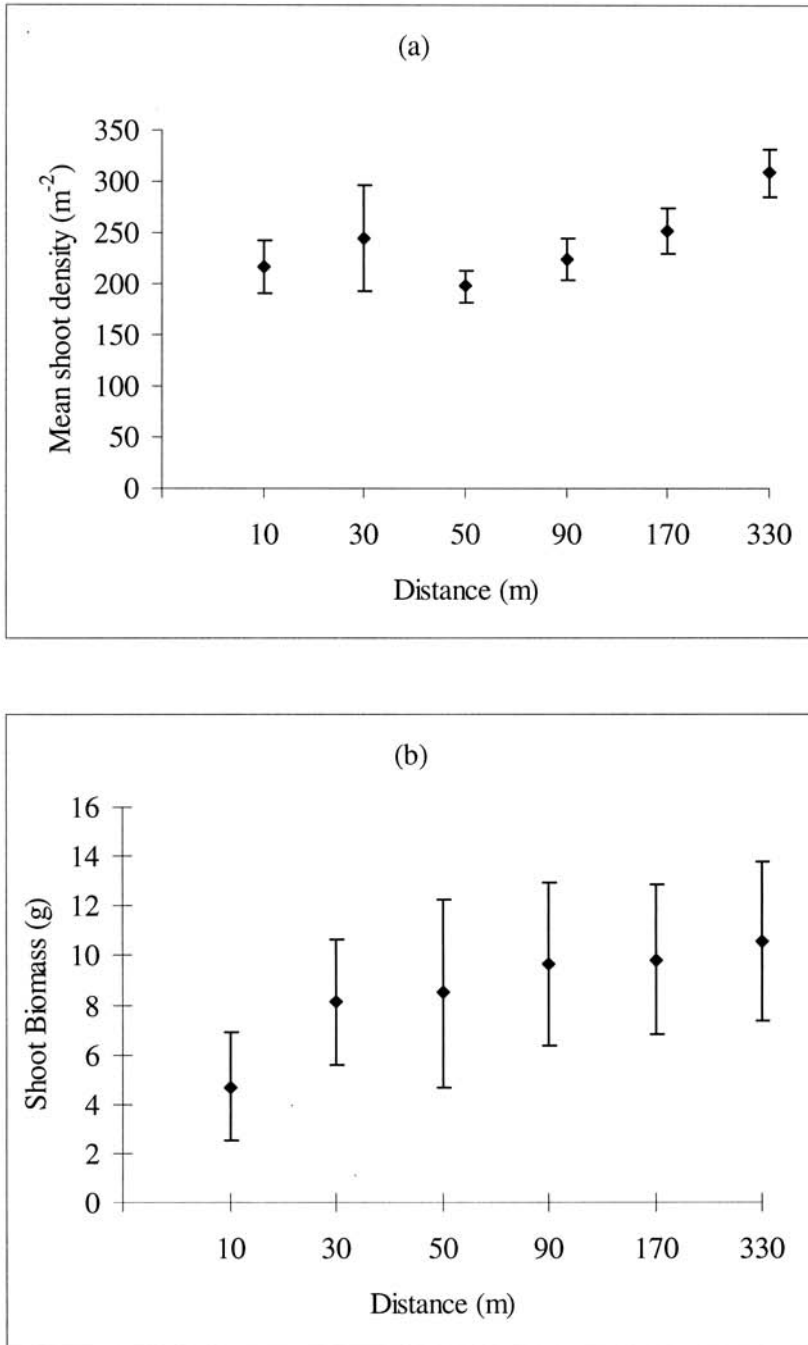
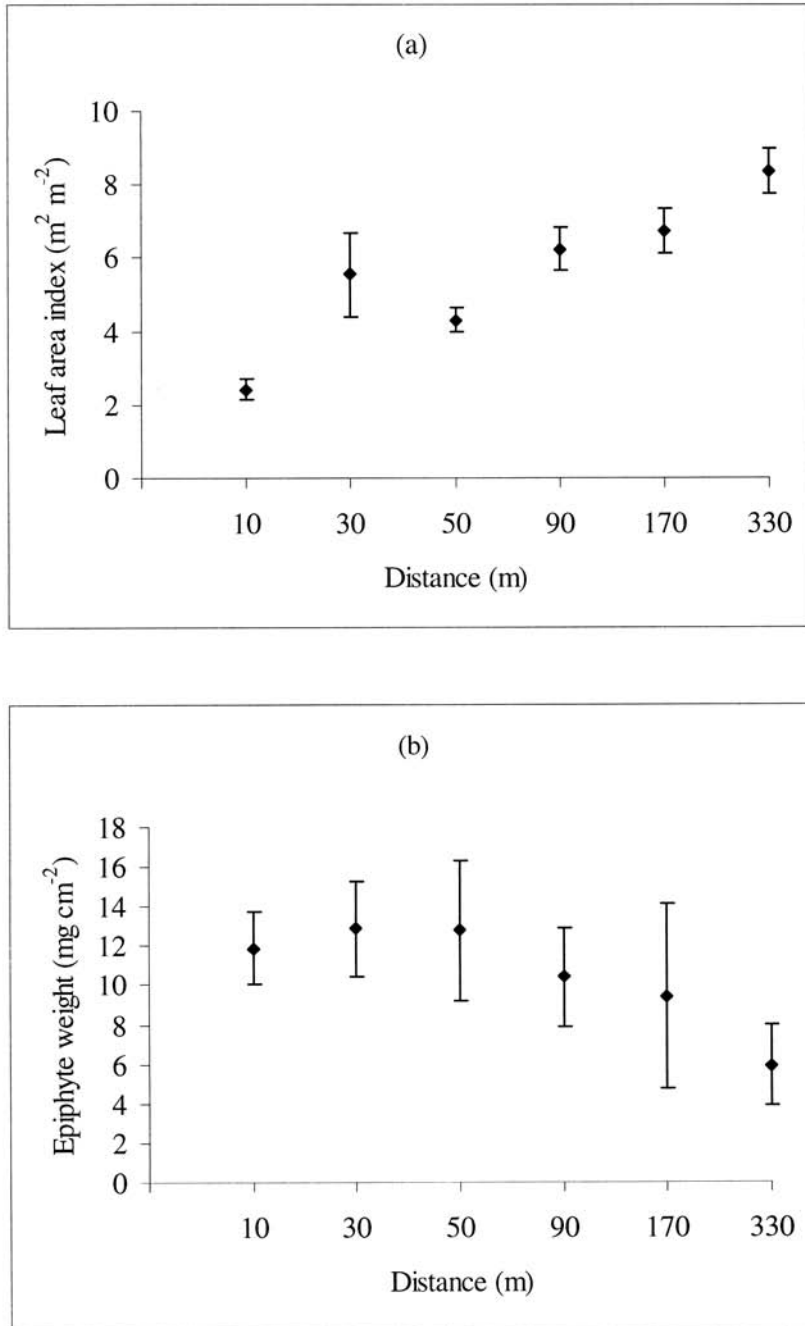Figure 3. Changes in (a) mean shoot density and (b) mean shoot biomass with distance from the fish cages.

Figure 4. Changes in (a) mean leaf area index and (b) mean leaf epiphyte load with distance from the fish cages.

Table 1. Relative abundance of gravel, sand and mud (silt + clay) and the percent organic carbon for each of the six stations.

Station code	Distance from cages (m)	Gravel (%)	Sand (%)	Silt & clay (%)	Organic carbon (%)
A	10	0.97	82.21	16.82	2.3
B	30	0.8	85.58	13.62	1.6
C	50	2.19	83.2	14.62	1.58
D	90	2.04	84.08	13.88	1.54
E	170	1.52	82.18	16.3	2.56
F	330	3.61	88.21	8.18	0.57

The SIMPER analysis showed an overall increase in 'within station' similarity with distance from the fish-cages. The three highest 'between station' dissimilarity values were: between the 50 m and 330 m stations (70%), between the 10 m and 330 m stations (69.7%), and between the 30 m and 330 m stations (68.1%). No single species had a large contributory influence to these dissimilarities (maximum = 6.3%). The species that contributed most were the bivalve *Loripes lacteus*, the chiton *Acanthochitona fascicularis*, the gastropod *Alvania cimex* and the shrimp *Hippolyte inermis*.

Discussion

P. oceanica meadow structure was considerably altered in the vicinity of the cages as shown by the overall increase in shoot density, leaf area index and shoot biomass, and the decrease in leaf epiphytic load with increasing distance from the fish farm. Although the observed values of shoot biomass and number of leaves per shoot were higher than those recorded from the same depths in other Maltese coastal areas, the shoot density and leaf area index values recorded from the present study were much lower (see Borg and Schembri, 1995). An earlier study (Cassar, 1994) had shown that sedimentation rates and nutrient levels are high close to the same farm. The presence of suspended matter in the water column causes a reduction in the amount of light reaching the seagrass, while elevated concentrations of dissolved nutrients promote a high epiphytic cover on the leaves. The overall result is a reduction in growth rate of the seagrass and gross changes in shoot morphology. Although this finding is similar to that documented by other workers studying the impact of fish farming on *P. oceanica* (e.g., Delgado et al., 1997; Mendez et al., 1997), the observed changes in meadow structure were evident over a considerably large distance (> 200 m). We partly attribute this to the low energy environment at our site and the shallow waters in which the cages are located; both factors reduce the potential for dispersal of farm waste (Gowen and Bradbury, 1987).

The multivariate analyses indicated that the composition of the invertebrate assemblages studied varied with distance from the cages and that three zones are present. Of these, zone II, which includes the intermediate stations (C, D and E), has a higher species richness and abundance than either zones I (stations A and B) and III (station F). The peaks in species richness and abundance in zone II are not a result of corresponding maxima in seagrass shoot density and biomass, since these two parameters increased steadily over the whole distance considered. Rather, it appears that nutrient enrichment at the intermediate stations is occurring in quantities that enhance productivity in this part of the meadow and thus zone II is able to support a more abundant and diverse fauna than
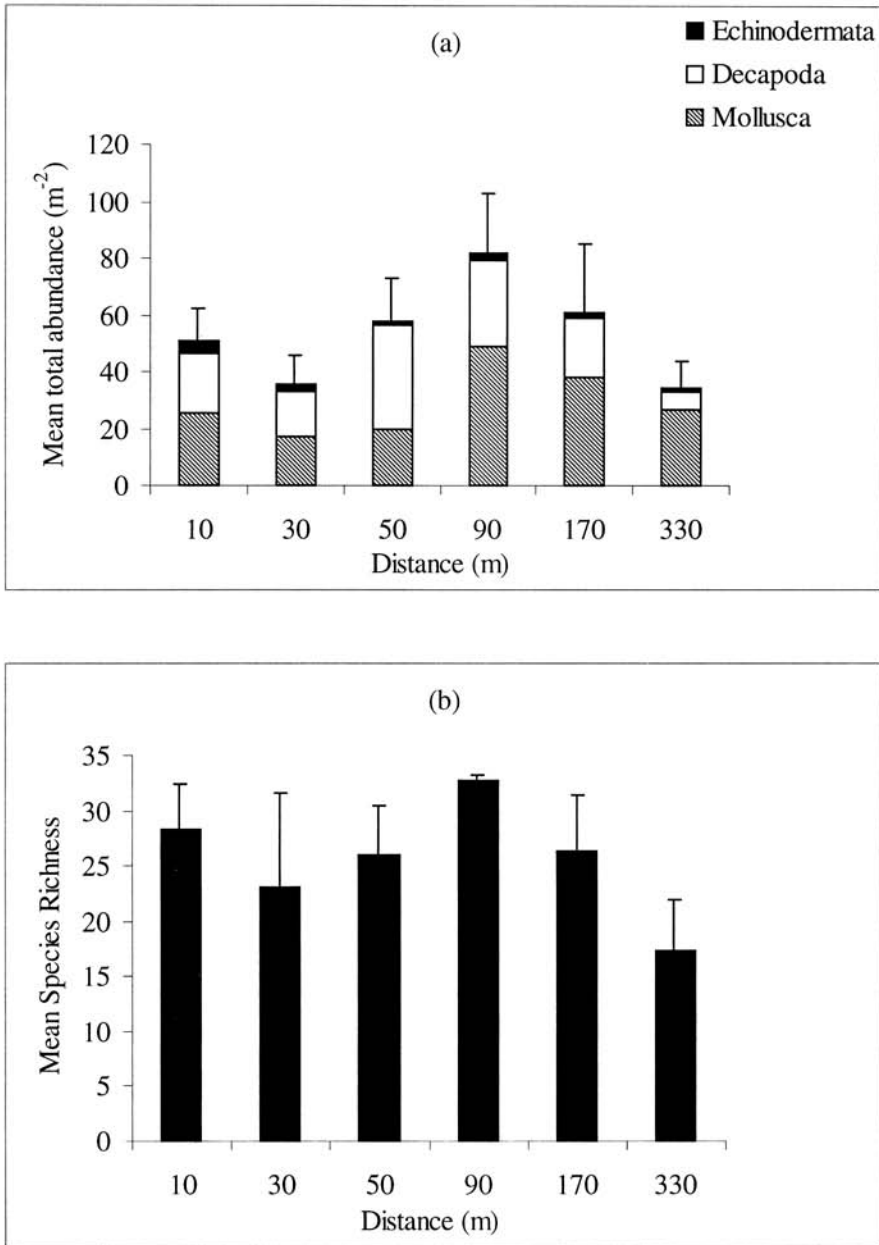
Figure 5. Variation of (a) mean macrofaunal abundance and (b) mean number of species with distance from the fish cages. Bars represent standard deviation.
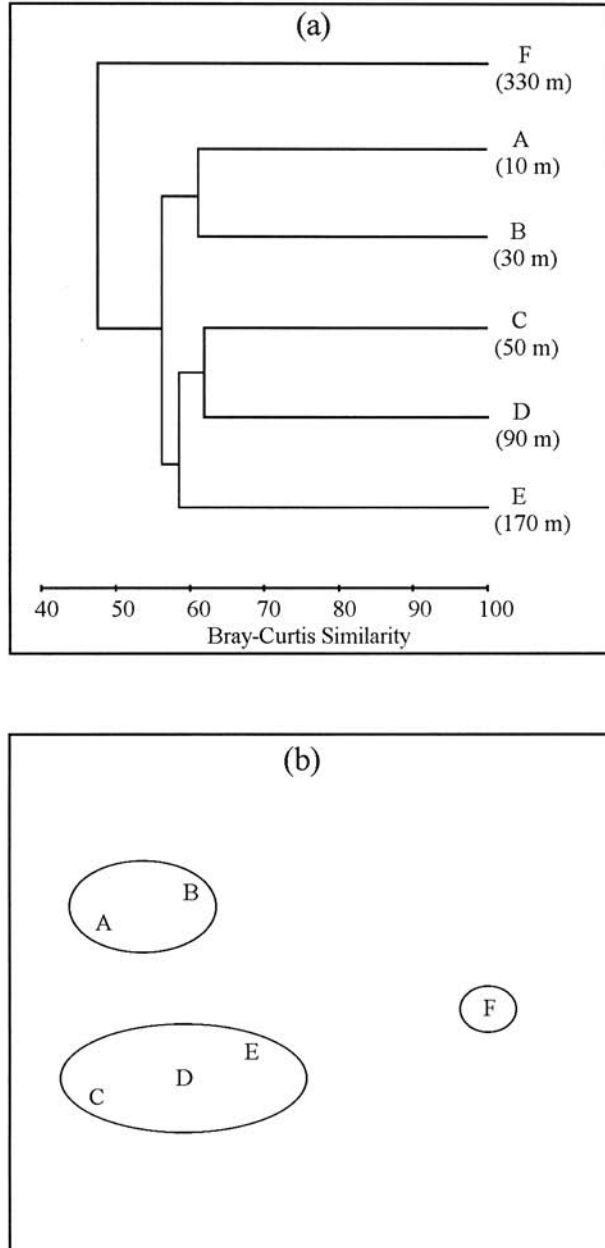
Figure 6. (a) dendrogram for the six sampling stations and (b) NMDS plot (level of stress = 0.3).

either of the adjoining two zones. Such a zonation of the macrobenthos near fish farms has been noted by other authors, with three to four zones usually being described (see Brown et al., 1987; Ye et al., 1991; Barg, 1992).

The macroinvertebrates recorded during the present study were the same as those recorded from *P. oceanica* meadows in other parts of the Mediterranean (see Mazzella et

al., 1989). However, comparison of the assemblages from the different zones shows differences in dominance of trophic groups. Zone I is dominated by grazers and deposit feeders (decapods, chitons and gastropods), which exploit the abundant epiphytes and deposited organic matter present close to the cages. In zone II, the species composition does not change much from that of zone I but the species abundance increases. On the other hand, zone III is characterized by a decrease in the abundance of grazers and deposit feeders and an increase in the abundance of other trophic groups, mostly carnivores and suspension-feeding bivalves.

Overall, our findings show that waste generated by offshore fish farms located close to seagrass meadows can cause changes in both meadow architecture and in the diversity of seagrass-associated macroinvertebrate assemblages. While an increase in macroinvertebrate diversity was recorded from one part of the meadow (zone II), loss of seagrass and a decrease in macroinvertebrate diversity was recorded from other parts of the same meadow located closer to the cages (zone I). Although, potentially, nutrient enrichment from fish farms can enhance faunal diversity in oligotrophic waters such as those of the central Mediterranean, in practice such beneficial effects are difficult to attain since they require judicious adjustment of aquaculture operations to achieve 'acceptable' or 'favorable' levels of nutrient input. This is no easy task since it requires very careful planning and consideration of numerous factors such as the size of the farm, the fish stocking densities and physical environmental factors such as the bathymetric and hydrodynamic characteristics of the chosen site (Wu, 1995). Moreover, the physiological response of the seagrass may differ depending on species and ecotype, such that equal levels of nutrient enrichment do not necessarily have the same impact on different seagrass ecosystems. Where siting of fish farms close to seagrasses cannot be avoided, studies such as the present highlight the need to apply appropriate models (Gowen and Bradbury, 1987) prior to initiation of the farm operations in order to predict impact and hence avoid nutrient overloading. Subsequent close monitoring of the state of the seagrass ecosystem is then essential to ensure early detection of adverse impact, when present, on these important habitats.

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