SCI. MAR., 66 (1): 53-58 SCIENTIA MARINA 2002

A quantitative technique for sampling motile macroinvertebrates in beds of the seagrass *Posidonia oceanica* (L.) Delile*

J.A. BORG[1, 2], M.J. ATTRILL[1], A.A. ROWDEN[1,3], P.J. SCHEMBRI[2] and M.B. JONES[1]

[1] Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, United Kingdom.
E-mail: jborg@plymouth.ac.uk
[2] Department of Biology, University of Malta, Msida, Malta.
3 National Institute of Water and Atmospheric Research, P.O. Box 14-901 Wellington, New Zeland.

SUMMARY: Techniques for sampling motile macroinvertebrates associated with *Posidonia oceanica* seagrass meadows have mainly involved the use of hand-nets and suction samplers or collection by hand. These techniques give unreliable quantitative estimates or have practical difficulties. A large cylindrical saw-rimmed corer was designed and used successfully to obtain quantitative samples of macroinvertebrates from both foliage and root-rhizome matrix of a *Posidonia oceanica* meadow in Malta (central Mediterranean). Choice of the appropriate sample unit size was assessed by comparing the relative accuracy, precision and efficiency of three different core diameters: 25 cm, 35 cm and 45 cm. The results suggest that for comparison of macrofaunal species richness and abundance between different meadows/sites the 25 cm diameter corer is recommended. For surveys aimed at estimating total diversity within a particular site, the 35 cm diameter corer is more appropriate.

Key words: sampling, seagrass, macroinvertebrates, *Posidonia oceanica,* corer, Mediterranean Sea, Malta.

INTRODUCTION

Scientific research on seagrass dates back to the late 19th century, but over the last three decades interest in the ecology of macroinvertebrates associated with seagrass meadows has increased (Phillips and Meñez, 1988). This has resulted in the development and use of an array of remote and *in-situ* sampling devices and techniques, including dredges, trawls, push-nets, hand-nets, drop-nets, drop-traps, corers, suction-samplers and box-samplers (see Heck and Wilson, 1990). The area or volume sampled varies greatly between these techniques, which hinders comparison of the results obtained from different studies (Virnstein, 1987; Attrill *et al.*, 2000). Frequently, the choice of sampler and sampling procedure lacks critical appraisal, despite the importance of these aspects in the design of ecological studies (Andrew and Mapstone, 1987). The need to assess the appropriateness of sampling methodology and design is even more evident in the Mediterranean, where the bulk of seagrass ecological research has focused on meadows of the endemic species *Posidonia oceanica* (L.) Delile (e.g. Boudouresque *et al.*, 1984, 1989). *Posidonia oceanica* is a large seagrass, with leaf lengths sometimes exceeding one metre (Drew and Jupp, 1976), which can occur in very high shoot densities (e.g. 1,200

*Received November 20, 2000. Accepted June 15, 2001.

shoots m^{-2} at a depth of 5 m, Mazzella *et al*., 1992). Additionally, it forms a dense matrix of tough, lignified roots and rhizomes admixed with sediment (called 'matte' by French workers) that can be several metres thick (Romero *et al*., 1994). This robust morphology makes sampling very difficult (Ott, 1990) and is probably responsible for the lack of effort towards improving techniques for sampling the associated macroinvertebrate assemblages from both leaf and root-rhizome strata. Most studies on the ecology of macroinvertebrates associated with *P. oceanica* meadows have employed hand-held nets and/or suction samplers, or removal of the plant and transfer to collection bags by hand. However, these techniques have severe limitations and are open to criticism.

The hand-held net technique (e.g. Ledoyer, 1962; Mazzella *et al*., 1989), is semi-quantitative and collects mainly the macroinvertebrates inhabiting the foliar stratum (Gambi *et al*., 1992). Suction samplers have also been commonly employed, sometimes in combination with the hand-held net technique (e.g. Gambi *et al.*, 1995). Although suction sampling has been shown to give reproducible, quantitative results (e.g. Brook, 1978), the device is usually bulky and difficult to transport and operate underwater, especially at depths exceeding 20 m. Its efficiency in capturing species inhabiting the deeper layers of the *P. oceanica* root-rhizome matrix is also questionable, and the fauna collected usually suffers extensive damage from the pronounced turbulence generated in the airlift and collection bag, making later identification difficult or even impossible. Manual removal of portions of the root-rhizome matrix, together with attached living shoots, has been adopted by some workers (e.g. Garcia-Raso, 1990) but the more mobile component of the macrofauna may be lost during sampling. Sampling techniques that utilise 'enclosure devices', such as corers, provide the most reliable and reproducible quantitative data on motile macrofauna associated with submerged aquatic vegetation, due to their high catch efficiency (Rozas and Minello, 1997) and have been used successfully in quantitative studies of seagrass-associated macroinvertebrates (e.g. Stoner, 1980; Lewis and Stoner, 1981). However, with rare exceptions (e.g. Vaccarella, 1981), few Mediterranean studies have utilised corers to sample macrofauna associated with *P. oceanica*, with the result that the potential of these techniques for studying Mediterranean seagrass ecosystems has remained largely ignored.

This paper presents the results of a study using a corer specifically designed for the quantitative sampling of the total macroinvertebrate community associated with Maltese *P. oceanica* meadows, which are known to have some of the highest values of shoot density in the Mediterranean (Borg and Schembri, 1995). The aim of this study was to establish an appropriate sample unit size and to determine the number of replicates required to achieve two predetermined levels of precision, given the importance of these aspects in ecological experimental design (Lewis and Stoner, 1981; Andrew and Mapstone, 1987).

MATERIALS AND METHODS

Establishment of the sample size and number of replicates collected is often a trade-off between precision and effort/cost expenditure. The sampler used must have an adequate construction and design, and be practical to deploy in the field. To meet these demands, a cylindrical corer was designed which had a simple, yet sturdy, construction and was light and easy to use in the field. Three different corer diameters were selected and the corresponding sample unit sizes were assessed for relative: (a) accuracy, (b) precision, and (c) efficiency of sampling, as defined by Andrew and Mapstone (1987). Choice of the three sampler diameters was based on setting: (i) a lower sample unit size (0.05 m^2), which was at least an order of magnitude larger than the upper size limit (40 mm) of the macroinvertebrates studied (Andrew and Mapstone, 1987); and (ii) an upper sample unit size (0.16 m^2) which corresponded to the dimension limit that the operator could handle reasonably well. Furthermore, consideration was given to the practicality of use of the sampler in *P. oceanica* meadows, especially as regards penetration of the dense root-rhizome matrix. Therefore, three cylindrical corers, differing only in diameter (25, 35 and 45 cm) and height (40, 50 and 63 cm respectively), were constructed using galvanised sheet metal (0.55 mm thickness) (Fig. 1). A band-saw blade (25 x 1mm) was welded along one of the cylinder's open ends, whilst a reinforcing metal bar (20 x 5mm) was welded at the other end. Two strong handles were riveted to the cylinders, one on each side. A 0.5 mm mesh collecting bag was attached to the top of each sampler and held in place by a length of twine inserted in the bag's seam.

FIG. 1. – The 25 cm diameter sampler with collecting bag attached (scale = 30 cm).

Fieldwork was carried out during September 1998 in a pre-designated area measuring 50 x 100 m in Mellieha Bay (35°58.73'N, 14°21.85'E), Malta, central Mediterranean. This area supports a continuous *P. oceanica* meadow at a depth of 11-12 m. Mean shoot density was estimated at 646 ± 108 m^{-2} by counting the number of shoots enclosed by five replicate 35 x 35 cm quadrats. Sampling was carried out by SCUBA diving, during which six replicates were taken randomly using each of the three different corers. Adjacent samples were at least 10 m apart and collections were always made in the afternoon. During each collection, the sampler was placed over the *P. oceanica* and the serrated edge was lowered quickly onto the bed. The sampler was held by the handles and driven slowly into the root-rhizome stratum by a turn-cutting action until a vertical penetration of 10 cm was achieved. A 'Bushman garden knife' was then used to make vertical incisions in the matte surrounding the sampler to produce a small 4-5 cm wide gap between the matte and the outer wall of the corer into which the diver's hand could be inserted. Incisions in the matte angled under the corer were also made to separate the matte core from the rest of the root-rhizome matrix. Complete detachment of the sample was achieved by insertion of the diver's hand down the sides of the corer and then under the lower edge of the device. Once this was achieved, the sampler was removed from the bed, quickly inverted and the sample was pushed into the collecting net. In this way, the core of excised root-rhizome matrix serves as a 'plunger', pushing down the whole sample into the net. The net was then removed from the corer and closed tight with the draw-string before sealing with a knot.

In the laboratory, each sample was washed in seawater, and the shoots and root-rhizome were separated and examined carefully to remove the motile macroinvertebrates. The remaining sediment and washings were then passed through a 0.5 mm sieve and the retained material was sorted in trays under a x5 magnifying lens. Macroinvertebrates were fixed in 10% formaldehyde in seawater and later transferred to 70% ethanol prior to identification to species level. Sessile macroinvertebrates (sponges, cnidarians and bryozoans) and fishes were also col-

lected but were not included in the present analyses.

Throughout the different sampling stages, the time taken to achieve each of the following was recorded: (i) collection, (ii) washing and sieving; (iii) sorting. Total time per replicate was taken as the sum of these components. The highest mean estimates obtained for standardised total abundance and for the number of species recorded were taken as the greatest estimates of accuracy (Andrew and Mapstone, 1987). Such an approach to the estimation of accuracy is based upon the assumption that one cannot count what is not present and, therefore, overestimation is very unlikely (Caughley, 1977). Differences between mean values of total abundance and species richness (untransformed data, checked for homogeneity of variances) obtained for the three different core sizes were analysed using one-way ANOVA (at $\alpha = 0.05$). Precision was calculated from the ratio SE/x, where SE is the Standard Error and x is the Sample Mean (Pihl and Rosenberg, 1982; Morin, 1985). Precision increases as the value approaches 0 (for example, 0.1 is more precise than 0.2). The number of replicates n required to achieve two levels of precision, 0.1 and 0.2, was calculated for each of the three core sizes using $n = [s/(px)]^2$, where s is Standard Deviation and p is the pre-established precision (Andrew and Mapstone, 1987). Efficiency was estimated by multiplying 'n' by the mean total time taken to process samples collected by the respective corers (James and Fairweather, 1996). A one-way ANOSIM permutation analysis (Clarke and Green, 1988) was carried out using the species-abundance matrix to test for differences in community composition within and between groups of replicate samples collected by each of the three corer sizes. SIMPER analysis (Clarke, 1993) was also carried out to identify the species contributing to the observed similarity within, and dissimilarity between, groups of replicate samples.

RESULTS

Field sampling with the 25 cm and 35 cm corers was carried out without difficulty; intact samples of *P. oceanica* root-rhizome matrix with attached shoots were obtained easily. However, considerable difficulty was experienced using the 45 cm corer, since separation of an intact core was not achieved without difficulty and, on most occasions, portions of the sample dropped out of the corer and were lost. This loss of sample is reflected in the reduced mean total time taken for collection and processing of samples collected using the 45 cm diameter compared with the 35 cm diameter corer (Table 1).

TABLE 1. – Mean time taken for collection, washing and sorting of samples collected using the three different samplers (diameter 25, 35 and 45 cm). n = 6 for each sampler, and values stated are ±1SE.

Sampler	Mean time (minutes)			
	Collection	Washing	Sorting	Total
25 cm	10.2 ± 1.1	48.8 ± 3.2	213.2 ± 12.1	272.2 ± 10.0
35cm	12.2 ± 0.9	65.5 ± 8.7	330.2 ± 27.9	407.8 ± 25.1
45cm	19.3 ± 1.7	81.0 ± 8.9	240.0 ± 56.1	340.3 ± 52.5

A total of 1018 individual motile macroinvertebrates (2 nemerteans, 330 polychaetes, 439 crustaceans, 233 molluscs and 14 echinoderms) belonging to 154 species was collected from the 18 samples. ANOVA indicated that significant differences in macroinvertebrate abundance were recorded between the 25 cm and the 35 cm diameter corers ($F = 10.9$, $p < 0.01$) and between the 25 cm and 45 cm diameter corers ($F = 14.16$, $p < 0.01$), but not between the 35 cm and 45 cm diameters corers. Significant differences were also detected in the mean number of species recorded from groups of replicate samples between the 25 cm and 35 cm diameter corers cm ($F = 7.16$, $p < 0.05$), and between the 25 cm and 45 cm diameter corers cm ($F = 7.65$, $p < 0.05$), but not between the 35 cm and 45 cm diameter corers.

Comparison of the relative accuracy showed that the estimate based on abundance was highest for the 25 cm diameter corer, whilst that based on number of species was highest for the 35 cm diameter corer (Figs. 2a and 2b). The 25 cm diameter sampler gave the best estimates of precision (Figs. 2c and 2d) and efficiency (Figs. 2e and 2f) for both abundance and number of species. Table 2 shows the total number of replicates required for each core size to attain the two levels of precision (0.1 and 0.2).

ANOSIM results indicated a significant difference ($R = 0.221$, $p < 0.06$) in the composition of the macroinvertebrate assemblages between groups of replicates collected by the 25 cm and the 45 cm diameter corers, but not between either the 25 cm and 35 cm or the 35cm and 45 cm diameter corers. SIMPER analysis revealed that the species contributing most to the difference between the 25 cm and 45 cm diameter core replicates were the amphipod *Lysianassa longicornis* (1.76%), the polychaete *Stylarioides eruca* (1.64%), the thalassinidean shrimp *Gourretia minor* (1.53%) and the polychaete *Notomastus* cf *latericeus* (1.52%).

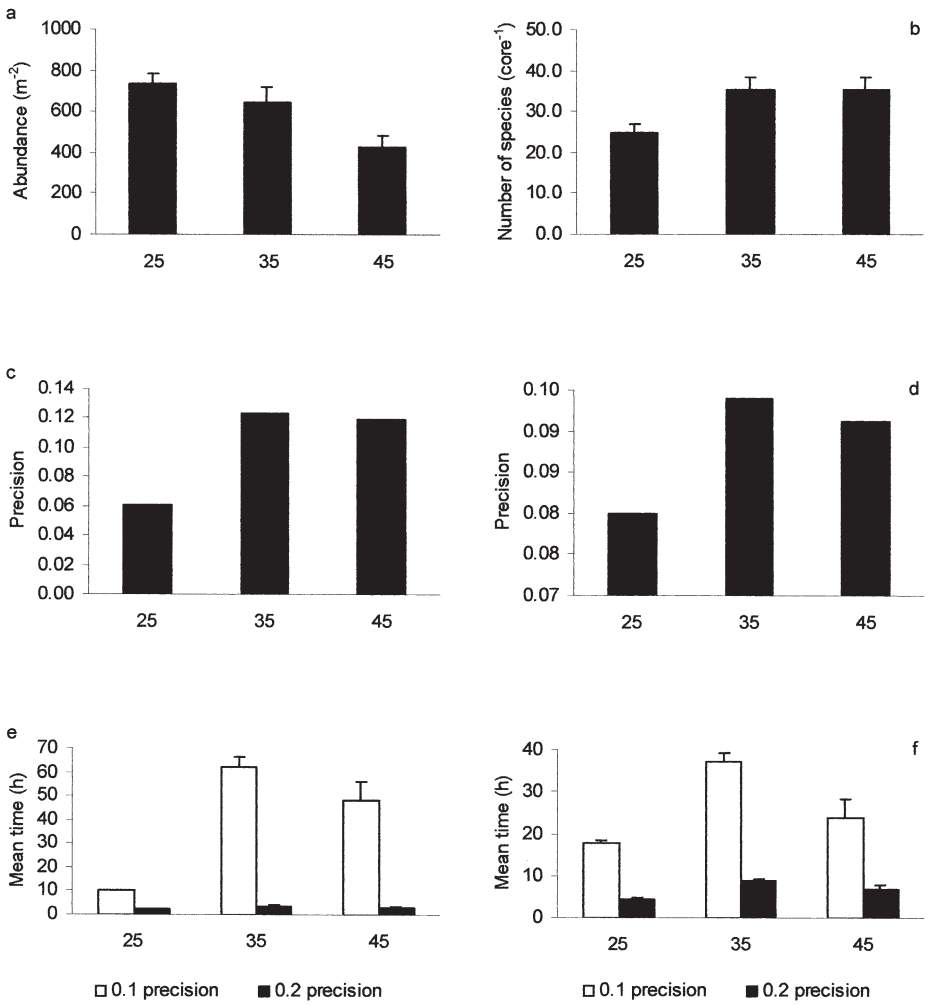

FIG. 2. – Estimates of accuracy (a, b), precision (c, d) and efficiency (e, f) for the three core sizes (25, 35 and 45 cm diameter). a, c and e: estimates based on abundance. b, d and f: estimates based on number of species. Error bars are ±1SE.

TABLE 2. – Total number of replicates required of each core size estimated for two pre-established levels of precision. Estimates are for total mean abundance and number of species.

Core size (cm)	Precision level	Nº replicates (abundance)	Nº replicates (species)
25	0.1	2.2	3.9
35	0.1	9.2	5.3
45	0.1	8.5	5.0
25	0.2	0.6	1.0
35	0.2	2.3	1.3
45	0.2	2.1	1.2

DISCUSSION

This study demonstrated that a cylindrical enclosure sampler, not much different in design from a basic corer, is a useful and practical device for quantitative sampling of the macroinvertebrates associated with beds of the seagrass *P. oceanica*. Of the three samplers tested, the smallest (25 cm diameter) gave the highest precision and efficiency estimates for both abundance and number of species. In their comparative study of the relative efficiency of three core sizes (5.5, 7.6 and 10.5 cm diameters) in sampling the macrofauna of *Thalassia testudinum* dominated seagrass meadows, Lewis and Stoner (1981) also concluded that the smaller corer collected significantly more macroinvertebrates in relation to size than either of the other two. The 25 cm diameter sampler used in the present study also gave the highest accuracy estimate for abundance, but not for number of species, suggesting that this sample unit size is suitable for comparative studies between different meadows/sites but not for surveys in which complete species richness and abundance estimates for a specific area are required. In the latter case, use of the 35 cm diameter corer may be more appropriate since it gave the highest accuracy estimate for number of species.

Serious difficulties in using the largest corer (45 cm diameter) were experienced. Besides being more cumbersome to use in the field than the smaller corers, there was often partial loss of the sample during collection. This loss is clearly reflected in the relative densities of deep burrowing macrofauna such as the polychaetes *Stylarioides eruca* and *Notomastus* cf. *latericeus*, and the thalassinidean shrimp *Gourretia minor*, which were the species contributing most to the differences between samples collected by the 25 and the 45 cm diameter samplers.

Overall, this pilot study indicates that a suitably modified cylindrical corer, with a diameter of between 25 cm and 35 cm provides robust, quantitative samples of the macroinvertebrates associated with the both the foliar and root-rhizome strata of *P. oceanica* meadows. It is suggested, therefore, that such a device be employed in studies aimed at investigating the total macroinvertebrate fauna associated with this seagrass species.

ACKNOWLEDGEMENTS

We thank Mr Mike Kendall (Plymouth Marine Laboratory, UK), Prof Michele Glemarec and Mr Jacques Grall (University of Brest, France), Ms Maria Pitas (University of Vigo, Spain), Ms Miraine Rizzo (University of Malta, Malta) and Mr Constantine Mifsud (Malta) for help with identification of the macrofauna. We also thank two anonymous referees for their useful comments and suggestions. J.A. Borg was supported by a long-term academic scholarship granted by the University of Malta.

REFERENCES

Andrew, N.L. and B.D. Mapstone. – 1987. Sampling and the description of spatial pattern in marine ecology. *Oceanogr. Mar. Biol. Ann. Rev*., 25: 39-90.

Attrill, M.J., J.A. Strong and A.A. Rowden. – 2000. Are macroinvertebrate communities influenced by seagrass structural complexity? *Ecography*, 23: 114-121.

Borg, J.A. and P.J. Schembri. – 1995. Preliminary data on bathymetric and temporal changes in the morphology of a Maltese *Posidonia oceanica* (L.) Delile meadow. *Rapp. Comm. Int. Mer Médit*., 34: 20.

Brook, I.M. – 1978. Comparative macrofaunal abundance in turtlegrass (*Thalassia testudinum*) communities in South Florida characterised by high blade density. *Bull. Mar. Sci*., 28(1): 212-217.

Boudouresque, C.F., A. Jeudy de Grissac and J. Olivier. – 1984. *International workshop on* Posidonia oceanica *beds*, GIS Posidonie Publ., Fr., 1, 454 pp.

Boudouresque, C.F., A. Meinesz, E. Fresi and V. Gravez. – 1989. *International workshop on* Posidonia oceanica *beds*, GIS Posidonie Publ., Fr., 2, 321 pp.

Caughley, G. - 1977. *Analysis of vertebrate populations*. John Wiley & Sons, New York, 234 pp.

Clarke, K.R. – 1993. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.*, 18: 117-143.

Clarke, K.R. and R.H. Green. – 1988. Statistical design and analysis for a 'biological effects' study. *Mar. Ecol. Prog. Ser.*, 92: 205-219.

Drew, E.A. and B.P. Jupp. – 1976. Some aspects of the growth of *Posidonia oceanica* in Malta. In: E.A. Drew, J.N. Lithgoe and J.D. Woods (eds.), *Underwater Research*, pp 357-369. Academic Press, London.

Gambi, M.C., M. Lorenti, G.F. Russo, M.B. Scipione and V. Zupo. – 1992. Depth and seasonal distribution of some groups of the vagile fauna of the *Posidonia oceanica* leaf stratum: Structural and trophic analyses. *P.S.Z.N.I. Mar. Ecol.*, 13(1): 17-39.

Gambi, M.C., A. Giangrande, M. Martinelli and L.A. Chessa. – 1995. Polychaetes of a *Posidonia oceanica* bed off Sardinia (Italy): Spatio-temporal distribution and feeding guild analysis. *Sci. Mar.*, 59(2): 129-141.

Garcia-Raso, J.E. – 1990. Study of a Crustacea Decapoda taxocoenosis of *Posidonia oceanica* beds from the southeast of Spain. *P.S.Z.N.I. Mar. Ecol.*, 11: 309-326.

Heck, K.L. and K.A. Wilson. – 1990. Epifauna and infauna: biomass and abundance. In: R.C. Phillips and C.P. McRoy, *Seagrass research methods*, pp 125-128. UNESCO, France.

James, R.J. and P.G. Fairweather. – 1996. Spatial variation of intertidal macrofauna on a sandy ocean beach in Australia. *Est. Coast. Shelf Sci*., 43: 81-107.

Ledoyer, M. – 1962. Etude de la faune vagile des herbiers superficiels de Zosteracées et de quelques biotopes d'algues littorales. *Rec. Trav. St. Mar. Endoume* 25(39): 173-216.

Lewis, G.F. III and A.W. Stoner. – 1981. An examination of methods for sampling macrobenthos in seagrass meadows. *Bull. Mar. Sci*., 31(1): 116-124.

Mazzella, L., M.B. Scipione and M.C. Buia. – 1989. Spatio-temporal distribution of algal and animal communities in a *Posidonia oceanica* meadow. *P.S.Z.N.I. Mar. Ecol.*, 10 (2), 107-129.

Mazzella, L., M.C. Buia, M.C. Gambi, M. Lorenti, G.F. Russo, M.B. Scipione and V. Zupo. – 1992. Plant-animal trophic relationships in the *Posidonia oceanica* ecosystem of the Mediterranean Sea: a review. In: D.M. John, S.J. Hawkins and J.H. Price (eds.), *Plant-Animal interactions in the marine benthos*. Systematics Association Special Volume No 46, pp. 165-187. Clarendon Press, Oxford, U.K.

Morin, A. – 1985. Variability of density estimates and the optimization of sampling programs for stream benthos. *Can. J. Fish. Aquat. Sci.*, 42: 1530-1534.

Ott, J.A. – 1990. Biomass In: R.C. Phillips and C.P. McRoy (eds.), *Seagrass research methods*, pp. 55-60, Unesco, France.

Phillips, R.C. and E.G. Meñez. – 1988. *Seagrasses*. Smithsonian Contributions to the Marine Sciences No. 34, 104 pp.

Pihl, L. and R. Rosenberg. – 1982. Production, abundance and biomass of mobile epibenthic marine fauna in shallow waters, Western Sweden. *J. Exp. Mar. Biol. Ecol*., 57: 273-301.

Romero, J., M. Perez, M.A. Mateo and E. Sala. – 1994. The below ground organs of the Mediterranean seagrass *Posidonia oceanica* as a biogeochemical sink. *Aquatic Botany*, 47: 13-19.

Rozas, L.P. and T.J. Minello. – 1997. Estimating densities of small fishes and decapod crustaceans in shallow estuarine habitats: a review of sampling design with focus on gear selection. *Estuaries*, 20(1): 199-213.

Stoner, A.W. – 1980. The role of seagrass biomass in the organisation of benthic macrofaunal assemblages. *Bull. Mar. Sci.*, 30(3): 537-551.

Vaccarella, R., A.M. Pastore and V. De Zio. – 1981. Metodologie di prelievo: popolamenti a polychaeti in "mattes" di *Posidonia*. *Thalassia Salentina*, 11: 1-13.

Virnstein, R.W. – 1987. Seagrass-associated invertebrate communities of the southeastern USA: a review. *Fla. Mar. Res. Publs.*, 42: 89-116.

Scient. ed.: E. Ballesteros