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Assessment of Secondary Production and Efficiency of Different Mesh Sizes to Study Benthic Communities Associated to a *Zostera marina* Meadow

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Authors' contributions

This work was carried out in collaboration between all authors. Author E. Solana Arellano, designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript; author V. Diaz-Castañeda gave the biological discussion of the Benthic community, help with the redaction of the manuscript and the identification of organisms; author Olga Flores-Uzeta took samples and helped with the sample processing; author H. Echavarria- Heras helped with the statistical analysis and the reviewing of the manuscript; author J. C. Rubio-Polania Helped processing samples and organism identification. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The problem addressed in this paper, was to reduce the number of sieves in order to propose appropriate methodologies for the estimations of abundance, secondary production, community composition and structure of benthic fauna associated to a *Zostera marina* meadow. Therefore we are aiming to calculate in an efficient way, secondary production of benthic communities in coastal areas associated to a *Zostera marina* meadow and examine the retention efficiency of different mesh sizes.

Study Design: Bimonthly five randomly chosen cores of 15 cm of diameter and 18 cm length (area=176.7 cm²), were collected.

Place and Duration of Study: The samples were taken during one year period from June 2008 to June 2009 in a *Zostera marina* meadow at Punta Banda estuary, a shallow coastal

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lagoon located near Ensenada, Baja California, Mexico (31º43-46' N and 116°37-40'W).
Methodology: Samples were immediately fixed with 7% buffered formalin, later, samples were washed through a nested series of sieves (5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.70, 0.50 mm) stacked in descending order of size. In the laboratory fauna collected from each sieve was sorted and taxa classified using a stereoscopic microscope, organisms were counted (abundance, density) and preserved in 70% ethanol. Wet weight was measured using a Sartorius precision balance with a resolution of 0.001 g, for each group and each sieve. A general method to obtain Ash-free-dry weight (AFDW) of each core, which is the value of the dry weight minus the weight of the ash.
Results: A total of 14760 organisms were collected and classified in 11 phyla; we found densities ranging from 17457 to 33600 ind. m^{-2}. Benthic fauna was separated using eight different sieves (5.6 mm to 0.5 mm). The observed mean annual secondary production was 14.8 g (AFDW) m^2 y^{-1}. The projected mean using equivalences was 12.2 g (AFDW) m^2 y^{-1}. A student t-test showed no significant differences between this two means. We suggest using the weight equivalences proposed by Crisp to find reliable AFDW estimates.
Conclusion: Results indicate that using only two sieves of 1.0 and 0.5 mm, allows an appropriate estimation of abundance of individuals in small size classes. Crisp equivalences procedure to assess secondary production reduces the amount of time and effort needed to obtain AFDW assessments without causing bias in the interpretation of community composition and structure.

Keywords: Estuary; benthos; secondary production; mesh sizes.

1. INTRODUCTION

Seagrass ecosystems play an important role in marine environments since they provide several ecological services, and act as ecological engineers [1,2]. These organisms can provide important ecosystem functions for associated resident species, including refuge from predation or amelioration of physically demanding conditions, thus enhancing diversity and abundance [3,4,5]. Meadow-forming seagrasses are important foundation species because they add a three dimensional structure with branching rhizomes and roots in an otherwise two-dimensional habitat, providing substrate for attachment of invertebrates, and protection against predation. Seagrasses are successful primary producers, ensuring an abundant supply of organic matter for zoobenthos. The physical structure provided by them creates a microhabitat that protects organisms from waves and currents and stabilizes sediments [6]. Argued that due to coastal development and climate change one third of seagrass meadows has disappeared since the first records in 1879 affecting resident benthic communities. These benthic communities play a critical role in the functioning of estuaries, microphytobenthos and benthic consumers are essential components of coastal ecosystems, influencing sediment biogeochemistry via uptake and release of nutrients and sediment erosion via their production of exopolymers [7]. Moreover, benthic invertebrates respond to cumulative factors of natural and anthropogenic origin, and are considered useful for detecting environmental alterations. Some benthic species are considered ecosystem engineers [8,9,10,11] which can change the availability of resources to other species by physical alteration of the environment [12]. Nevertheless, information on the macrofaunal communities and associated environmental parameters of estuaries and in particular in seagrass beds in Baja California is limited.

Many authors have stressed out the importance on the relationship between seagrass meadows and the heterogeneous faunal assemblage that inhabits them [13,14] and recognized diverse ecological characteristics, for example for *Zostera marina*, [15] found that the abundance of epifauna and infauna is positively correlated with two aspects of plant morphology: the root-rhizome mat, and the plant canopy. Similarly, [16] detected that *Zostera marina* vegetation reduces significantly the predation effects of flounders on seagrass infauna. They also found that the faunal changes in the *Z. marina* community indicated an increase in food availability, which could be associated with positive effects of coastal eutrophication.

For Punta Banda estuary and San Quintín Bay, Mexico, many studies have been made about *Zostera marina* meadows [17,18,19] (among others) but, due to the difficulties to work with benthic communities to assess body size, biomass, abundance and secondary production [20,21,22] (among others),f ew have been made with the macrobenthic fauna associated to *Zostera marina* ecosystems for our study site [23,18]. Many attempts have been made to simplify this procedure using different methods like sieving [24,25,26,27,22] using allometry [24,28,20] or both [24,25,20,29]. In particular, for ash-free dry weight assessments (AFDW), many authors have used Brey empirical formula [30] but no calibration of the formula and/or statistical analysis has been made. At the same time, equivalences of wet to dry weight and dry to ash-free-dry weight are used to estimate secondary production [31,32]. With this idea in mind, in this study we 1) describe the benthic fauna associated to a *Zostera marina* meadow at Punta Banda estuary through time, 2) prove that for our study site, data does not conform to Brey´s formula and direct equivalences are better estimates of secondary production, and 3) test the allometric relationship between biomass and sieve size which in turn will help us to assess secondary production in a more direct way, 4) describe marine benthic assemblages associated to a *Zostera marina* meadow through time.

2. MATERIALS AND METHODS

Data were collected from June 2008 to June 2009 in a *Z. marina* meadow at Punta Banda estuary, a shallow coastal lagoon located near Ensenada, Baja California, Mexico (31°43-46' N and 116°37-40'W). This estuary has a total area of 16 km^2, with 4.6 km^2 of navigable waters and 11.8 km^2 of salt marshes, mudflats, and seagrass beds [33,34,35]. Under normal conditions, evaporation exceeds precipitation, but this pattern is reversed during extreme winter storms [36,37]. As a result, the site is permanently hyperaline and tidal activity mainly controls water renewal for inter-tidal water plants [38].

Five cores of 15 cm of diameter and 18 cm high (area=176.7 cm^2) were collected bimonthly inside the meadow. Samples were immediately fixed with 7% buffered formalin. Most sieving studies use only one or two mesh sizes to describe the biomass and abundance of benthic communities but we believe that such procedures can under-sampled the community, for small and can bias the interpretation of community composition and structure. Therefore, we consider that a bigger degree of accuracy is needed. Therefore samples were washed through a nested series of sieves (5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.7, 0.5 mm) stacked in descending order of size. In the laboratory fauna collected on each sieve was sorted and taxa classified using a stereoscopic microscope, organisms were counted (abundance, density) and preserved in 70% ethanol. Wet weight was measured using a Sartorius precision balance with a resolution of 0.001 g, for each group and each sieve. A convenient method to obtain Ash-free-dry weight (AFDW) of each core [20], which is the value of the dry weight minus the weight of ashes, was obtained. Samples were heated in a muffle furnace at 470ºC for 6 hours.

We performed a repeated measures ANOVA in order to seek for differences between means of density and weight with respect to time and sieve sizes. One way ANOVA was performed to detect differences in taxa densities through time. The variables density and weight were transformed using natural logarithms in order to assess normality and homogeneity of variances.

Using the Ordinary Least Square method (OLS) for a complete year, samples were fitted using the [29] modification of [30] formula for macrobenthic secondary production, that is, we fitted the formula:

$$\log P = \alpha + \beta \log B + \delta \log A \tag{1}$$

Where, *P* is secondary production ($gm^{-2}t^{-1}$), *B,* stands for biomass (gm^{-2}), *A* is density ($individual\ s\ m^{-2}$) and $\alpha, \beta,$ and δ are the parameters to be fitted. Then, we used these parameters to obtain the mean annual production and compare the results with the observed values.

For the relationship between biomass and sieve sizes, let *B* stand for biomass in a given sieve and *S* for sieve mesh size. Then, the relationship between *B* and *S* is given by

$$B = \alpha S^{\beta} \quad (2)$$

Where α and β are the allometric parameters to be fitted. To obtain α and β of equation (2), generally log-transformation of the equation is used. However, [39] argued that equation (1) is intrinsically non-linear and disagreed with the logarithmic transformation approach to obtain a linearized form by pointing out that equivalent models fitted in arithmetic and logarithmic domains do not have equivalent least-square solutions. The transformed data will meet the requirements for parametric statistics (homogeneity of variances and normality) but transforming would generate a new distribution for the observed measurements. Moreover, the viability of back-transformed data is not always possible [40]. This back-transformation depends on the variability in the original response variable at each level for the transformed independent variable. [39] claimed that the small recorded deviations that do not fall on the line tend to be overweighed. Therefore, following their suggestions, we fitted equation (2) in its non-linear form, instead of the traditional approach of linearizing the equation through a logarithmic transformation.

3. RESULTS AND DISCUSSION

A total of 14760 organisms were counted and classified in11phyla and 10 classes and 8 orders Table 1.

Fig. 1 shows the abundance of organisms collected during the sampling period for each mesh size. The efficiency of several meshsizes in retaining benthic organisms was tested. We observe that taxa richness varied on the different mesh sizes used and that capture of organisms was higher in the smaller mesh sizes (0.5 and 0.7 mm) especially for nematodes, micro mollusks, annelids and peracarid crustaceans. These mesh sizes also increased the number of juveniles as well as meiofauna, mainly Foraminifera and Nematoda Fig. 1. The use of smaller mesh sizes than 1.4 mm proved to be more effective in collecting other taxa that were not collected with bigger meshes. The study shows that retention of marine invertebrates in this ecosystem may vary, being higher in smaller mesh sizes versus larger ones (1.4 to 5.6 mm), a higher number of individuals were collected with 0.5 and 0.7 mm. Nematoda are also best collected, in these mesh sizes particularly larger ones like Enoplidae. These enoplids live in seagrass beds using detritus derived from the vegetation; probably they are large omnivorous species exploiting the abundant organic matter. In general, it is clear those polychaetes, mollusks and crustaceans (particularly peracarids) dominated all year round (except the two smaller mesh sizes where Nematoda dominated). In the 1.0 mm mesh size Nematoda are not well collected. In all mesh sizes and sampling dates, the best represented group was Annelida Polychaeta. They constitute a dominant functional component of macrobenthic communities and reveal a wide range of adaptability to different marine and coastal habitats. The ability of these animals to survive in a wide range of environmental parameters allows them to occupy a variety of niches [41]. The fact that they dominate also in the small mesh sizes indicate that some species are reproducing almost all year round and small juveniles were collected in the 0.5 mm mesh.

Table 1. Abundance of benthic organisms collected at a *Z. marina* meadow in Estero de Punta Banda.

TAXA	Abundance	Abundance/class
Nematoda	3940	3940
Cnidaria	32	
Anthozoa		32
Mollusca	842	
Bivalvia		41
Gastropoda		801
Nemertea	209	
Anopla		209
Annelida	7994	
Polychaeta		7989
Oligochaeta		5
Arthropoda	1547	
Crustacea		
Amphipoda		381
Cumacea		89
Isopoda		150
Decapoda		25
Copepoda		9
Ostracoda		890
Stomatopoda		1
Insecta		1
Diptera		1
Echinodermata	1	
Asteroidea		1
Phoronida	34	34
Platyhelminthes	25	
Turbellaria		25
Protozoa	68	
Foraminifera		68
Chordata	3	
Osteichthyes		3

To analyze density of benthic invertebrates, MANOVA (Multivariate Analysis of Variance) among date of sampling and sieve size were applied. Results showed significant differences among sampling dates ($p<0.05$) and among sieve sizes ($p<0.05$). Interaction between factors was also proved ($p<0.05$); we found that the highest mean density was reached in April 2009 (spring), in sieve size of 0.5mm, with a mean of 284 organisms/m^2 and in which polychaetes were dominant.

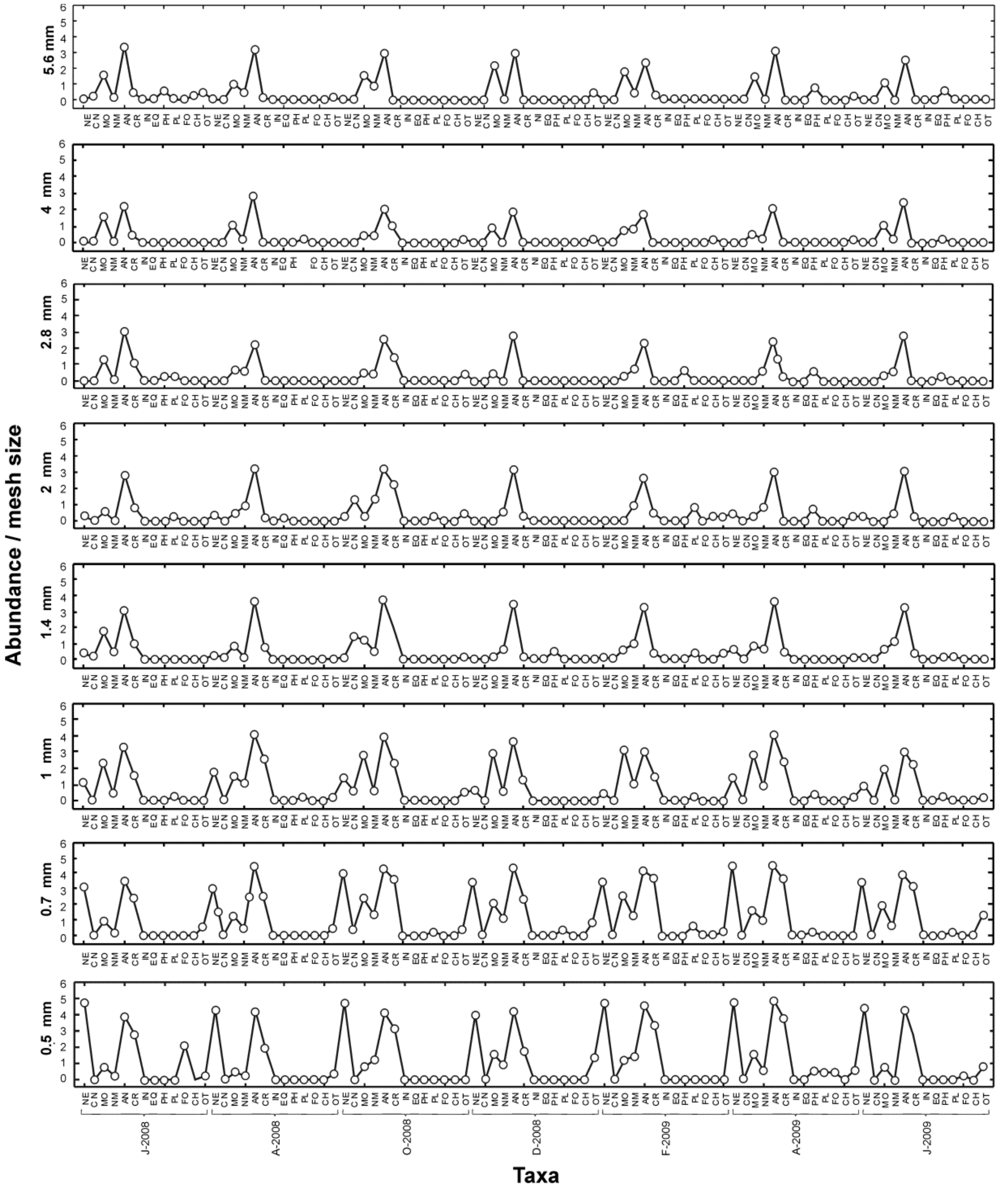

Fig. 1. Abundance of organisms through the sampling dates and mesh sizes. This Fig allows us to see the structure of benthic community through time, sieve size and taxa.

Fig. 2 shows mean density of organisms per square meter for each sampling date. A post hoc Tukey´s test established the following groups: a first group conformed by six sieve sizes (5.6, 4.0, 2.8, 2.0, 1.4, and 1.0 mm), another group with two (4.0 and 2.8 mm) and a third one with (0.71 and 0.50 mm); all these groups showed no significant differences (p>0.05).

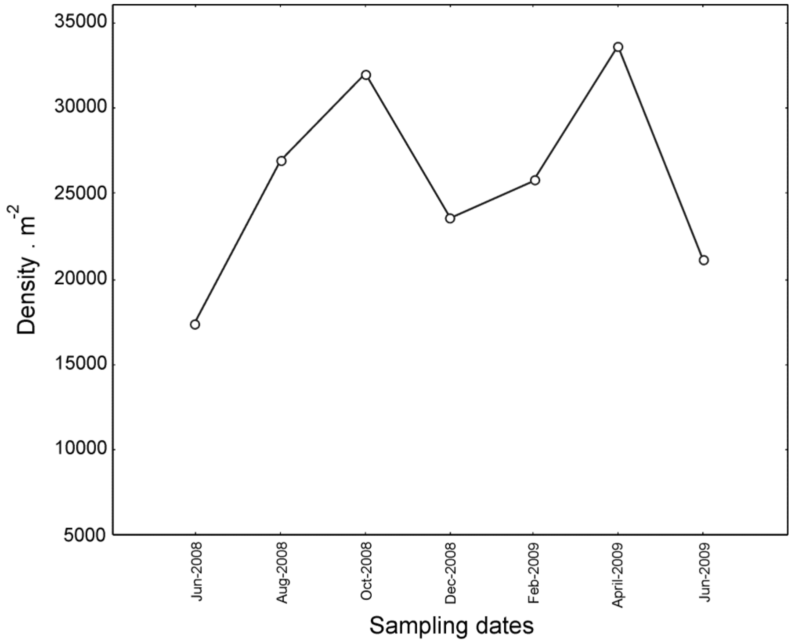

Fig. 2. Mean density per square meter of organisms for each sampling date

From the previous results we can conclude that for the density analysis of fauna associated to *Zostera marina*, we can eliminate sieve sizes 5.6, 2.8, 2.0, 1.4 and 0.71 mm and reduce the sampling processing in a significant way. Similarly, with respect to mean weight per sieve size, we performed a repeated measurement two way ANOVA using sample dates and sieve size as factors. We found significant differences in dates and sieve-size (p<0.05).February 2009 (winter) presented the main difference and had the biggest mean weigh for sieve size 5.6 (mean of 3.2g of wet weight). February 2009 was different from December 2009 and August 2008, all the rest of the dates showed no significant difference (p>0.05). From all dates, except June 2008, the only sieve size that showed significant differences was 5.6 mm; this sieve showed differences in weight with respect to the rest of the sieves and had the biggest weight in each date, and therefore we acknowledge that this sieve size is very important for weight analysis, particularly when studying megafauna. For June 2009 (early summer), we observed significant differences among all sieve sizes (p<0.05). Combining these two analyses, we suggest eliminating sieves sizes 2.8, 2.0, 1.4 and 0.71 mm from studies of benthic fauna associated to *Zostera marina* meadows in Baja California.

Since we found significant differences for time and sieve, we fitted equation (2) separately for each sample date. All fits presented high determination coefficients ranging from 0.94 to 0.99 (see Table 2). Fig. 3 shows predicted and observed values for the fits at each sample date. We found that fitted parameters from June-2008, October 2008, December 2008 and June 2009 data presented significant differences (p>0.05), while parameters fitted for August2008 and April 2009 data showed no significant differences between them. Meanwhile, the parameters for the fit of February 2009 data —which was the sample date with bigger mean sieve weight (for sieve size 5.6 mm) —turned out to be different from the rest of the fits.

Table 2. Parameters fitted for equation (2) sample date and determination coefficients

Sample date	**Parameters**	R^2
June 2008	$\alpha = 0.00002 \pm 0.00003$ $\beta = 6.1 \pm 1.02$	0.98
August 2008	$\alpha = 0.000013 \pm 0.00002$ $\beta = 5.9 \pm 0.73$	0.99
October 2008	$\alpha = 0.000002 \pm 0.000001$ $\beta = 7.5 \pm 1.97$	0.97
December 2008	$\alpha = 0.000001 \pm 0.000001$ $\beta = 8.18 \pm 1.12$	0.99
February 2009	$\alpha = 0.00001 \pm 0.0002$ $\beta = 8.71 \pm 0.99$	0.99
April 2009	$\alpha = 0.00009 \pm 0.0002$ $\beta = 4.5 \pm 1.1$	0.94
June 2009	$\alpha = 0.000004 \pm 0.000001$ $\beta = 7.1 \pm 1.11$	0.99

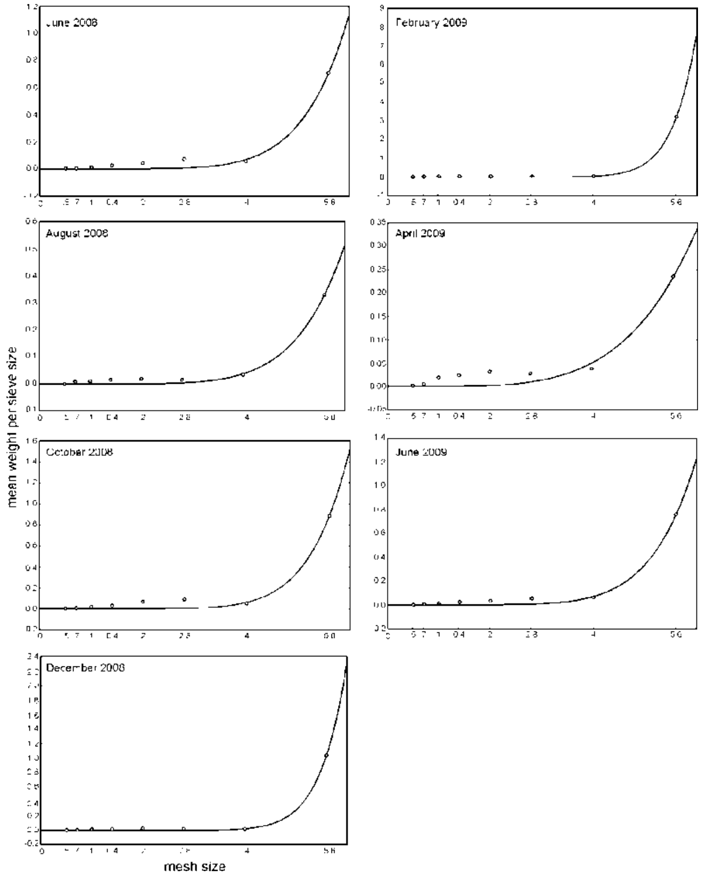

Fig. 3. Predicted (continuous line) and observed values (o) of equation (2) for the fits at each sample date. The graph shows the good correspondence between fitted and observed values

Finally, parameters from the fits of data from August2008 and April2009 showed to be statistically the same. Cluster analysis showed exactly the same result. Moreover using the parameters fitted for each date we projected the mean weight per sieve values and compared them graphically with the observed values (Fig. 4). The results indicate that it is possible to accurately predict mean weight per sieve of macrobentic fauna associated to *Zostera marina* patches.

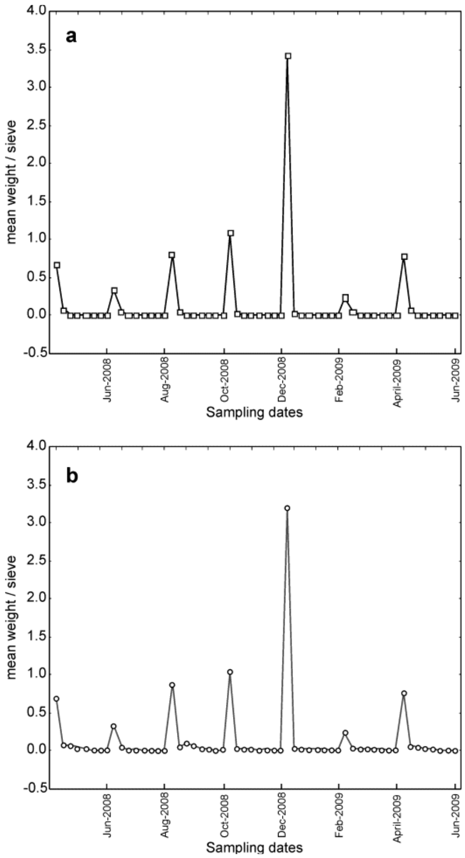

Fig. 4. Projected mean weight per sieve values using parameters fitted for equation (2) for each sample date (a) and observed mean weight per sieve values (b). Besides the good correspondence of projected and observed values this Figure shows descriptively the statically differences found

For our study site, observed and projected values using equation (1) mean weigh per square meter are given in Fig. 5.

Observed AFDW were obtained and compared with the projection using equivalences according to [42]. The observed mean annual secondary production was 14.8 $g(\mathrm{AFDW})m^{-2}y^{-1}$. The projected mean using equivalences was 12.2 $g(\mathrm{AFDW})m^{-2}y^{-1}$. Student t-test showed no significant differences between this two means (*t=-0.56, d.f=12, p>0.05).* A linear equation between projected AFDW and observed AFDW values produced a determination coefficient of 0.93with cero intercept and slope of 0.90 ± 0.10 implying a good correspondence between these two evaluations.

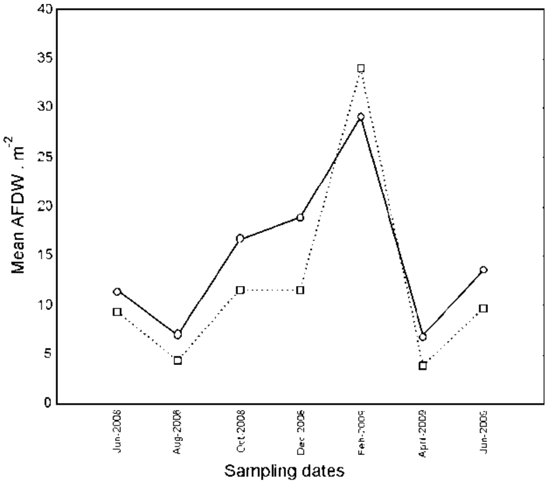

Fig. 5. Projected values using equation (1) (dashed lines) and observed (continuous lines) of mean weigh per square meter of macrobentic fauna associated to *Zostera marina* patches at each sample date

Fig. 6 shows observed and predicted values with the projection using equivalences according to [42] for mean AFDW per m^2. Finally the fit of equation (1) showed a determination coefficient of *0.96* and the following values for the fitted parameters; $\alpha = 2.37 \pm 1, \beta = 0.89 \pm 0.09, \delta = -0.53 \pm 0.23$ which are remarkably different than parameters for the [30] formula (-0.4; 0.737, and 0.27 respectively). Mean annual production using these parameters and equation (1) was found to be $11.75g(\text{AFDW})m^{-2}y^{-1}$ which is very close to observed and projected values through equivalences.

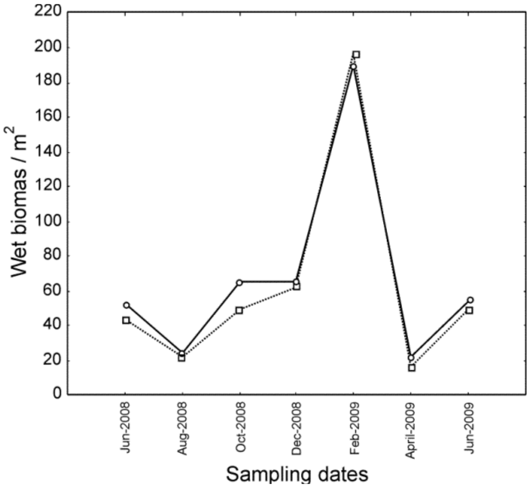

Fig. 6. Observed (continuous line) and predicted (dashed lines) using [36] equivalences values of mean AFDW m^2 at each sample date. We can see the degree of correspondence between observed and predicted values

The dominant benthic organisms collected were polychaetes, crustaceans (amphipods and isopods) and mollusks (gastropods); these organisms support nekton and bird species.

Polychaetes seem to dominate almost during all the year. [27] argued about the few studies dealing with the effect of mesh sizes in retaining benthic organisms and allowing a correct estimation of the composition and abundance of invertebrates associated to macrophytes. Organisms must find in their environment satisfaction of a number of different requirements: food, shelter and access to mates. Their needs may change over time, but food and shelter can be considered as the main factors driving habitat choice [43].

4. CONCLUSION

Seagrasses are crucial as shelters for a large number of invertebrates (they offer protection from predation) and a critical evaluation of the effect of mesh sizes is needed to discuss abundance and community structure. Although [22] reported that densities, biotic indices and biological traits are not affected by sieve mesh sizes they only compared two mesh sizes (1.0 mm and 0.5 mm), they found that a mesh size of 1 mm is sufficient in describing macroinvetebrate communities. These results are contradictory since we observed that bigger mesh sizes (5.6, 4.0, 2.8, 2.0, and 1.4 mm) under-sampled the community, especially small taxa and juveniles and can bias the interpretation of community composition and structure. Thus we might expect that in [22] study, the smaller mesh size (0.5mm) was more accurate since [26] found that, in terms of retention efficiency, the use of fine sieve mesh sizes (<0.5mm) gives a more accurate estimates of density, weights and community composition. In the present study, we found that for the density analysis of fauna associated to *Zostera marina* beds in Baja California west coast, we can obviate sieve sizes: 5.6, 2.8, 2.0, 1.4 and 0.70mm and reduce de sampling processing in a significant amount of time.

Faunal composition and densities per square meter were comparable in our study site with those reported in the literature. We found densities ranging from 17457 to 33600 in d. m^{-2}, other authors have found densities varying between 9000 to 100000 ind. m^{-2} in macrophyte beds [44,45]. This together with the results of the fitting of equations (1) and (2) can drastically reduce and simplify the calculation of the secondary production. That is, we propose to reduce the number of sieve sizes to only two sieves of 0.5 mm and a smaller (0.30 or 0.35 mm) which have proved to have good retention efficiency and allow estimating adequately abundance of individuals in small size classes. For other studies, since biomass depends on geographical location, we advise for calibration purposes, to perform a fitting of equation (2) with few replicates for the different sampling dates for the first year and use the fitted parameters to assess wet biomass in future samplings. Use the weight equivalences proposed by [42] to find reliable AFDW estimates. This procedure will reduce the amount of time and effort needed to obtain AFDW assessments without causing bias in the interpretation of benthic community composition and structure. Nevertheless, we believe that more and longer studies are needed to enhance the above proposed procedures.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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