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How precise are estimates of intertidal macroinfaunal density and spatial dispersion when converted to larger areas?

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Abstract. The precision of density and spatial dispersion (Morisita's index) of an economically important intertidal bivalve (*Anomalocardia flexuosa*) and mangrove macroinfauna was evaluated after converting the density data to larger areas. For both faunal groups, converted densities always overestimated "true" density (a direct count in the larger area), especially when the sampled area and that to which the data were converted were very different. In comparison with random and contagious dispersions, conversions were more precise with regular dispersion, especially when densities were low. *A. flexuosa* densities were more precise when converted from the arithmetic mean, whereas those from the harmonic mean underestimated true density. In contrast, mangrove macroinfauna density conversions from the harmonic mean were more precise, whereas those from the arithmetic mean and median overestimated true density. There was no difference in the contagious dispersion of either *A. flexuosa* or mangrove macroinfauna, regardless of whether or not the density was converted. Converted densities, commonly used in studies of the benthic macrofauna, may incorrectly estimate true densities but appear not to change estimates of spatial dispersion, at least when using Morisita's index. Given the above, and the ecological and functional importance of the macroinfauna, their densities in intertidal soft sediments are probably better expressed in relation to their original sampling units and conversions to larger areas should be avoided.

Key words: zoobenthos, abundance, spatial arrangement, estimating density, mangrove

Resumo. Como são precisas as estimativas de densidade e dispersão espacial da macroinfauna de entremarés quando convertidas para áreas maiores?. A precisão da densidade e dispersão espacial (índice de Morisita) de um bivalve de importância econômica (*Anomalocardia flexuosa*) e da macroinfauna de manguezal foi avaliada após expressar os valores em relação a uma área maior. Para ambos os grupos faunais, densidades convertidas sempre superestimaram densidades verdadeiras, especialmente quando a área amostrada e a área à qual os dados foram convertidos eram muito diferentes. Na dispersão regular, as conversões foram mais precisas, especialmente em densidades baixas. Densidades de *A. flexuosa* foram mais precisas quando convertidas da média aritmética, enquanto que aquelas da média harmônica subestimaram a densidade verdadeira. Em contraste, conversões da densidade da macroinfauna do manguezal com a média harmônica foram mais precisas, enquanto que aquelas da média aritmética e mediana superestimaram a densidade verdadeira. Não houve diferenças na dispersão contagiosa de *A. flexuosa* e da macroinfauna do manguezal, independentemente da densidade ser convertida ou não. Densidades convertidas, frequentemente utilizadas em estudos da macrofauna bentônica, podem fornecer estimativas incorretas das densidades verdadeiras, mas aparentemente não afetam estimativas da dispersão espacial, pelo menos com o índice de Morisita. Dado o acima exposto e a importância ecológica e funcional da macroinfauna, suas densidades em sedimentos não-consolidados de entremarés são provavelmente melhores expressas em relação às suas unidades de amostragem originais e conversões para áreas maiores devem ser evitadas.

Palavras chave: zoobentos, abundância, arranjo espacial, estimando densidade, manguezal

Introduction

Management and conservation strategies for estuarine areas have often relied on estimates of the density and spatial dispersion of the benthic macrofauna in different habitats (De Grave & Casey 2000, Katsanevakis 2007). Due to time constraints in the field, limited financial resources, attempts to limit damage to the habitat and the need for comparisons with other studies, these estimates are often made using sampling units (cylindrical tubes, quadrats, dredges, and so on) that sample a conveniently small area or volume (Cabral & Murta 2004, Ferraro & Cole 2004, Beukema & Dekker 2012). Macroinfaunal studies use sampling units with different areas or volumes and/or shapes, depending on the target species size and mobility, type of habitat as well as the scale of the study (Andrew & Mapstone 1987, Southwood & Henderson 2000). In this context, for comparative purposes, expressing benthic faunal density in relation to a standard area that is larger (usually 1 m^2) than what was sampled, is a common practice in the literature (Southwood & Henderson 2000, Dittmann 2001, Degraer *et al*. 2003, Neves & Bemvenuti 2006, Beseres & Feller 2007). In such cases, the density of individuals in the sediment collected with the sampling unit is converted to a new value expressed in terms of a larger standard area.

Conversions assume densities and patterns of spatial dispersion in the larger area or volume are similar to those in the sampled portion of the habitat, which is often not the case due to the high spatial heterogeneity in macrofaunal abundance (New 1998, Cabral & Murta 2004). Thus, the use of such conversions can sometimes result in incorrect estimates of density (and also diversity) (Colwell *et al*. 2012) that can compromise the reliability and applicability of management and conservation strategies based on such estimates. The size and spatial dispersion of organisms (and the appropriate statistical model describing these properties) should be evaluated before converting estimates of density (Andrew & Mapstone 1987, De Grave & Casey 2000). Although sampling error is well known and acknowledged (New 1998, Southwood & Henderson 2000, Cabral & Murta 2004) conversions may increase the risk of obtaining a density estimate that is very different from the "true" population density. We can say with a certain degree of confidence that the latter value may lie within an interval, such as the standard error, around the mean (Andrew & Mapstone 1987, Zar 1999). However, conversions may increase the size of the interval (by increasing variability) and thus reducing the precision of the estimate.

Intertidal areas are dynamic environments (Short 1999), where soft sediments present a diverse benthic community (Little 2000) that contributes significantly to animal biomass and nutrient recycling (Snelgrove & Butman 1994). In the present study, we examine the effects on the precision of estimates of density and spatial dispersion expressed in terms of a larger standard area, using count data of an economically important and relatively large intertidal bivalve (*Anomalocardia flexuosa*) and the smaller and more numerous mangrove macroinfauna. Specifically, we investigate whether (1) converted estimates of density and spatial dispersion are reliable in the natural habitat, (2) density and the type of spatial dispersion affect the precision of the converted estimate, and (3) the number of replicates and the type of central tendency statistic influence the precision of converted estimates of density and spatial dispersion.

Intertidal regions and mangrove environments are currently threatened by increasing anthropogenic disturbance (Lee 1999). In low intertidal areas, for example, natural beds of bivalves are frequently exploited by coastal communities (Nishida *et al*. 2006, Silva-Cavalcanti & Costa 2009). In mangroves, anthropogenic disturbances result in pollution (Allen *et al*. 2001), forest clearing and conversion of mangroves for aquaculture (Ellison & Farnsworth 1996). Such disturbances may greatly influence the abundance and spatial arrangement of the benthic fauna in coastal habitats (de Boer & Prins 2002) and attempts to describe such changes, as well as apply management practices based upon them, may contain flaws as a result of using density conversions.

Materials and Methods

2.1 Study site and sampling dates in the natural habitat

The study was carried out at two different sites, both located in the northeastern coast of the State of Pará, northern Brazil, although with different faunal groups. At each site, the study area was marked out and mapped, as follows: Area 1 – sampling of a 200 m² area within a natural bed (total area 9250 m²) of the venerid bivalve *Anomalocardia flexuosa* at Emboraí bay, Felipa Island, municipality of Augusto Corrêa (46° 27' 38.6"W and 01° 00' 55.7"S) was carried out over 4 days during spring tides in both September 2009 and March 2010, corresponding to the dry and wet seasons, respectively. Area 2 – sampling of macroinfauna in a 200 m² area adjacent to the mangrove tidal channel Furo do Meio situated in the central portion of the

Ajuruteua Peninsula, municipality of Bragança (46º 38' 59.00"W and 00° 52' 26.00"S) was carried out in June 2010, May 2011 and October 2011, the former two corresponding to the wet season and the latter to the dry season. The mangrove macroinfauna is generally dominated by a few deposit-feeding capitellid polychaetes, followed by lower abundances of a larger number of other polychaetes, two abundant species of bivalves (a mussel and a small clam) and a more diverse assemblage of crustaceans, dominated by brachyuran decapods (Beasley *et al*. 2010). Although individual taxa have different abundances and spatial dispersions, these are usually highly correlated (Englund & Cooper 2003) and we used the total number of individuals of the mangrove macroinfauna, assuming this represents the sum of the interactions among the individual taxa. Sampling of both *A. flexuosa* and mangrove macroinfauna was carried out in both seasons in order to take into account annual variation in abundance and dispersion. A description of this coastal area and general characteristics typical of the study areas can be found in Souza-Filho *et al.* (2009) and Saint-Paul & Schneider (2010).

2.2. Sampling method

2.2.1 Precision of converted estimates of density and spatial dispersion in natural habitats

Three sampling units were used to estimate density: a 0.007854 m² PVC tube with a diameter of 10 cm and a height of 20 cm (Rebelo 1986), a 0.25 m² quadrat and a 1 m² quadrat. On each sampling occasion, random sediment cores of bivalves (n= 30) and macroinfauna (n=20) were obtained in each study area, whereby the 1 m^2 quadrat was placed in a random position within which the PVC tube was inserted in a random position to a depth of 20 cm in the sediment. The sediment retained by the tube was sieved through a 0.3 mm mesh, as meshes smaller than 0.5 mm are recommended for benthic macroinfaunal studies (Schlacher & Wooldridge 1996). The 0.25 m² quadrat was also randomly placed within the 1 m² quadrat and dug to a depth of 20 cm and sieved. Finally, the sediment remaining in the 1 m² quadrat was removed to a depth of 20 cm and sieved.

The total number of individuals was counted for each sampling unit either in the field (bivalves) or in the laboratory (macroinfauna). In the case of the 1 m^2 quadrat, the total number of individuals was the sum of the number of individuals in sediment from the tube, the 0.25 m^2 quadrat and sediment remaining in the 1 m^2 quadrat after the other sampling units were removed. In the case of the 0.25 m^2 quadrat, the total number of individuals was the sum of the number of individuals in sediment from the tube and in sediment remaining in the 0.25 m^2 quadrat after the tube was removed. Henceforth, these totals will be referred to as the direct counts in the 1 m^2 and 0.25 m^2 areas. Mangrove macroinfauna, coarse sediment and debris that remained after sieving each sample to remove fine sediments was packed in plastic bags and fixed in a solution of magnesium chloride (5% $MgCl_2$) to anesthetize the animals. In the laboratory, sediment, debris and associated animals were fixed in 5% formalin for up to 24 hours, washed in water, counted and preserved in 70% ethanol.

Densities in the sampling unit were expressed in relation to a larger area (converted) as follows: 1) tube to 0.25 m² quadrat, 2) tube to 1 m² quadrat, and 3) 0.25 m² quadrat to 1 m² quadrat. The density of faunal groups in the larger area (0.25 or 1 m²), N_e, was obtained by converting the density in the sampling unit (tube or 0.25 m²) to the larger area using the following formula:

$$N_e = \left(\frac{n_1}{A_1}\right) \times A_2$$

where, n_1 is the number of individuals counted in the sampling unit (tube or 0.25 m^2 quadrat) and A_1 is the area of that sampling unit, A_2 is the larger area to which n_1 is being expressed (0.25 m^2 or 1 m^2).

The direct count of *A. flexuosa* and macroinfauna in the larger area was compared with the converted count using the t-test for paired observations adjusted for heterogeneity of variances with Welch's correction, if necessary. The standardized Morisita index of dispersion (I_{STM}) (Elliott 1983, Andrew & Mapstone 1987, Hurlbert 1990) was calculated using the *vegan* package (Oksanen *et al*. 2014) to determine the type of spatial dispersion in samples of direct and converted counts for all three types of conversions. I_{STM} values are independent of sample size and range between -1 and +1 where $I_{STM} < 0$ represents a regular dispersion, $I_{STM} = 0$ represents a random dispersion, and $I_{STM} > 0$ represents a contagious dispersion.

2.2.2 Precision of converted estimates with experimental variation in density and spatial dispersion

In July 2012, an experiment was carried out to verify the effect of variation in density (low, medium, high) and type of spatial dispersion (regular, random, and contagious) on the precision of conversions. Empty shells of *A. flexuosa* (and a small number of assorted venerid bivalves) and biodegradable objects (cereal) that simulated the size and form of *A. flexuosa* and macroinfauna, respectively, were manipulated in the laboratory to represent regular, random and contagious dispersion at low (n=60), medium (n=140) and high (n=220) densities of *A. flexuosa* and low (n=625), medium (n=1050) and high (n= 1350) densities of objects representing macroinfauna in a 1 m^2 quadrat. Though somewhat artificial, this experiment allowed control over numbers and dispersion in a way that would have been impossible in the field. The densities chosen reflect the range of variation observed in the natural habitat. For each combination of spatial dispersion (regular, random and contagious) and density (low, medium and high) a random sample (n=5) was taken with the tube, totaling 45 replicas for each faunal group in which shells and objects were counted in the tube. The number of shells or objects was converted from the tube to 1 m^2 and compared with the total number (direct count) placed in the 1 m^2 quadrat, using paired t-tests with Welch correction for heterogeneous variances.

2.2.3 Effect of sample size and central tendency statistics on precision of converted estimates

In April 2012, the effects of sample size and type of central tendency statistic on the precision of conversions, from the tube to 1 m² quadrat, of counts of *A. flexuosa* and macroinfauna in their natural habitats were investigated. The PVC tube was inserted in three, six and nine random positions within the 1 m² quadrat. Sediment was removed and sieved and the number of individuals determined in the same manner as described above for the tube and 1 m^2 quadrat (Section 2.2.1). Four different estimates of central tendency, the arithmetic mean, median, harmonic mean and geometric mean, were calculated for counts in the tube for each of the three sample sizes (n=3, 6 or 9), and each converted to a larger 1 m^2 area. The direct count in the 1 m^2 quadrat was compared with the converted count and differences among all counts were evaluated using one-way ANOVA. If a significant difference was found, Dunnett's test with a critical value of q'=2.50 with 4 (Counts) and 70 (Error) degrees of freedom (Zar 1999) was used to compare the direct count with each of the converted counts made with the four different estimates of central tendency. All data were analyzed with the software GNU-R (R-Project 2014), with the exception of Dunnett's test.

Results

3.1 Precision of converted estimates of density and spatial dispersion in natural habitats

Anomalocardia flexuosa. A total of 12125 individuals were counted during sampling. Spatial dispersion in direct and converted counts was always contagious and Morisita's standardized index was generally higher in converted counts (Table I). All three conversions yielded counts of *A. flexuosa* that were always greater than direct counts (Fig. 1a). Variances were heterogeneous ($F_{59,59}$=0.17, p=0.001) for the comparison of the conversion from the tube with the direct count in the 0.25 m² quadrat and the mean difference of 88 individuals between counts was significant (t_{59}=8.82, p<0.001). In the comparison of the conversion from the tube with the 1 m² quadrat, variances ($F_{59,59}$=0.11, p<0.001) were heterogeneous and the mean difference of 380 individuals was significant (t_{59}=8.73, p<0.001). With the conversion from the 0.25 m² quadrat to the 1 m² quadrat, variances were homogeneous ($F_{59,59}$=0.68, p=0.14) although the mean difference of only 26 individuals between direct and converted counts was significant (t_{59}=3.14, p<0.001) (Fig. 1a).

Mangrove macroinfauna. A total of 105138 individuals were counted during sampling. Spatial dispersion in direct and converted counts was always contagious and the standardized Morisita index was usually higher in converted counts (Table I). All three converted counts of mangrove macroinfauna were greater than direct counts (Fig. 1b). Variances were heterogeneous ($F_{59,59}$=0.014, p<0.001) for the comparison of the conversion from the tube with the direct count in the 0.25 m² quadrat. In this case, the mean difference of 1050 individuals between counts was significant (t_{59}=8.39, p<0.001). In the comparison of the conversion from the tube with the 1 m² quadrat, the mean difference was 3950 individuals, whereas in the conversion from the 0.25 m² quadrat with the 1 m² quadrat, this difference was much smaller (550 individuals). Both variances ($F_{59,59}$=0.076, p<0.001 and $F_{59,59}$=0.157, p<0.001) and mean counts (t_{59}=35.15, p<0.001 and t_{59}=7.19, p<0.001) were significantly different in the latter comparisons of conversions with direct counts, respectively (Fig. 1b).

3.2 Precision of converted estimates with experimental variation in density and spatial dispersion

For both *A. flexuosa* and objects representing mangrove macroinfauna, regular dispersion appeared to be more suitable for converted counts, which were more similar with direct counts in contrast with counts from random and contagious dispersions (Table II, Fig. 2ab). Moreover, for both faunal groups, direct and converted counts were more similar at low densities for all three types of dispersion in contrast with counts from medium and high densities and differences became greater for random and contagious dispersions (Table II, Fig. 2ab). Significant differences between direct and converted counts of *A. flexuosa* were found at all densities with all types of dispersion (Table II). However, differences between direct and converted counts of objects representing mangrove macroinfauna were not significant for densities with regular dispersion (Table II). For both faunal groups, spatial dispersion of converted counts differed from the experimentally controlled dispersion after converting the density to 1 m^2. Experimental regular dispersion became random at all densities. Random dispersions became contagious at low and high densities but remained random at medium density. Finally, contagious dispersion remained contagious at low and high densities but became random at medium density.

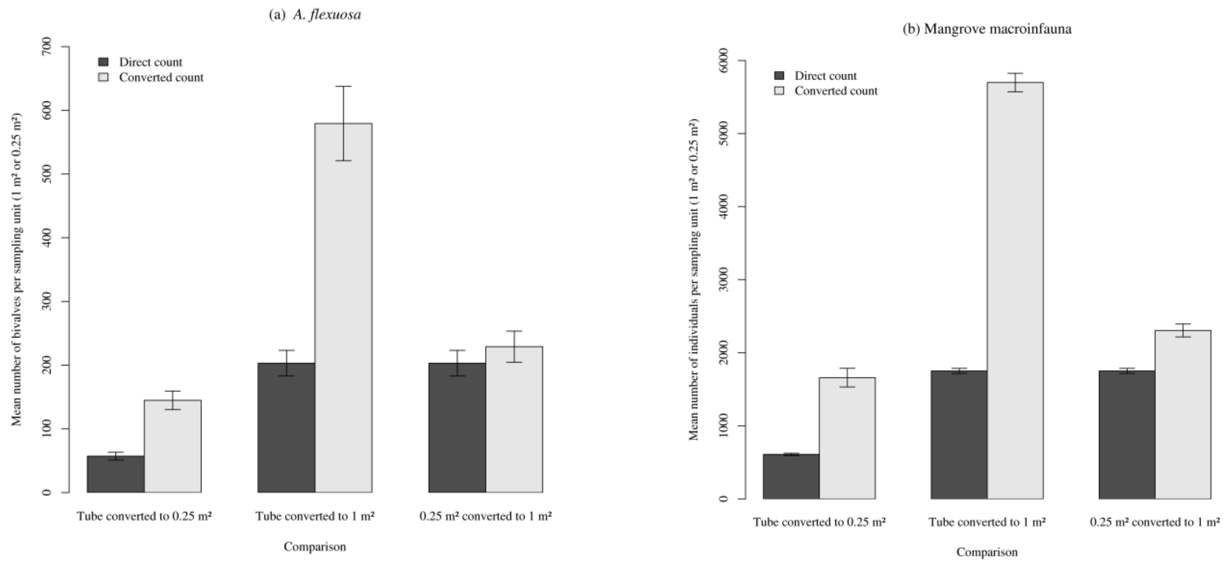

Figure 1. Mean values ± s.e. (n=60) of direct and converted (from a 0.007854 m^2 tube and 0.25 m^2 quadrat) counts of (a) *Anomalocardia flexuosa* and (b) mangrove macroinfauna in 0.25 m^2 and 1 m^2 quadrats.

3.3 *Effects of sample size and central tendency statistics on the precision of converted estimates*

Anomalocardia flexuosa. A total of 21550 individuals were counted in this experiment. With 3 random positions of the tube, there were significant differences among counts using different measures of central tendency ($F_{4,70}$=11.1, p<0.001). The converted estimate most similar to the direct count was that made with the arithmetic mean. There were no significant differences between direct and converted counts estimated by the arithmetic mean (q'=0.44 n.s.), sample median (q'=1.31, n.s.) and geometric mean (q'=1.48, n.s.) (Fig. 3a). The least similar converted estimate was that made with the harmonic mean (Fig. 3a), which was significantly lower than the direct count (q'=2.99, p<0.05).

With 6 random positions of the tube, there were significant differences among counts ($F_{4,70}$=18.1, p<0.001). The converted estimate most similar to the direct count was that made with the median and the least similar converted estimate was that made with the harmonic mean (Fig. 3b). However, there were no significant differences between direct and estimated counts using the arithmetic mean (q'=2.18 n.s.), median (q'=1.30, n.s.), harmonic mean (q'=2.41, n.s.) and geometric mean (q'=0.17, n.s.) (Fig. 3b).

Table I. The standardized Morisita index of dispersion (I_{STM}) for 6 samples of abundance data of direct and converted counts of *Anomalocardia flexuosa* (n=60) and mangrove macroinfauna (n=60) using three types of conversions (tube to 0.25 m^2, tube to 1 m^2 and 0.25 m^2 to 1 m^2) in natural habitats. Spatial dispersion was contagious in all cases.

	0.25 m² quadrat		1 m² quadrat			
Faunal group	Direct count	Converted from tube	Direct count	Converted from tube	Direct count	Converted from 0.25 m² quadrat
A. flexuosa	0.5055	0.5050	0.5048	0.5050	0.5048	0.5056
Mangrove macroinfauna	0.5003	0.5029	0.5002	0.5002	0.5002	0.5007

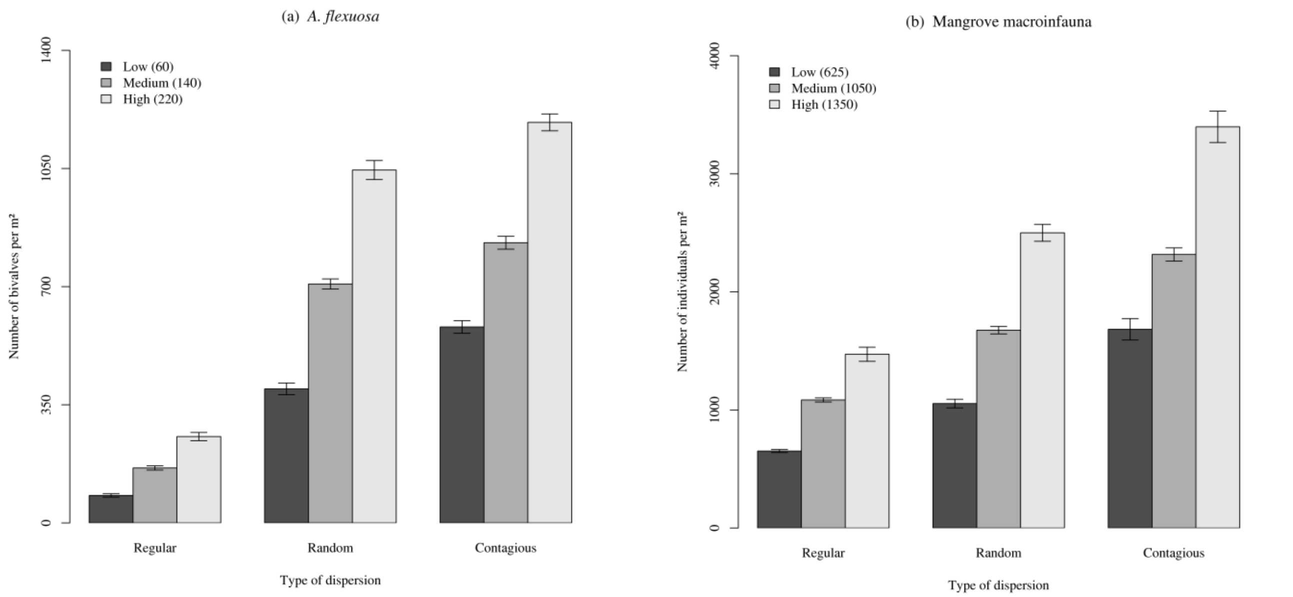

Figure 2. Mean (± s.e.) converted counts of (a) *Anomalocardia flexuosa* and (b) objects representing mangrove macroinfauna in 1 m^2 quadrats at low, medium and high densities and with regular, random and contagious dispersion. The direct counts (in 1 m^2) for each faunal group is indicated in the respective figure legends.

With 9 random positions, there were significant differences among counts ($F_{4,70}$=27.8, p<0.001). The converted estimate most similar to the direct count was that made with the arithmetic mean. No significant differences between direct and converted counts were found for the arithmetic mean and the geometric mean (q'=0.69, n.s. and q'=2.44, n.s., respectively) (Fig. 3c). The least similar converted estimate was that made with the harmonic mean. Significant differences were found between direct and converted counts made with the median and the harmonic mean (q'=2.51, p<0.05 and q'=4.68, p<0.05, respectively) (Fig. 3c). In general, converted estimates made from the harmonic mean always greatly underestimated the direct count, whereas those from the arithmetic mean produced a much closer estimate. The geometric mean and median gave intermediate estimates.

Mangrove macroinfauna. A total of 66888 individuals were counted in this experiment. With 3 random positions of the tube, there were significant differences among counts using different measures of central tendency ($F_{4,70}$=30.5, p<0.001). The converted estimate most similar to the direct count was that made with the harmonic mean, and the least similar converted estimate was that made with the geometric mean (Fig. 3d). There was no significant difference between the direct count and that converted from the harmonic mean (q'=1.69, n.s.). However, significant differences were found between direct and converted counts for the arithmetic mean (q'=4.57, p<0.05.), sample median (q'=5.34, p<0.05) and geometric mean (q'=2.69, p<0.05).

With 6 random positions of the tube, there were significant differences among counts ($F_{4,70}$=16.7, p<0.001). The converted estimate most similar to the direct count was that made with the harmonic mean, and the least similar converted estimate was that made with the arithmetic mean (Fig. 3e). There was no significant difference between the direct count and that converted from the harmonic mean (q'=1.66, n.s.). However, counts were significantly greater for conversions made from the arithmetic mean (q'=4.23, p<0.05), median (q'=3.49, p<0.05) and geometric mean (q'=2.95, p<0.05) (Fig.3e).

With 9 random positions of the tube, there were significant differences among counts ($F_{4,70}$=200.3, p<0.001) and none of the converted estimates were similar to the direct count (Fig. 3f). Differences between direct and converted counts were significant for all measures of central tendency (arithmetic mean: q'=12.6, p<0.05, median: q'=14.8, p<0.05, harmonic mean: q'=6.82, p<0.05 and geometric mean: q'=9.91, p<0.05) (Fig. 4f). In general, estimates converted from the arithmetic and geometric means always greatly overestimated the direct count, whereas those made from the harmonic mean produced a much closer estimate. The sample median gave an intermediate estimate. Spatial dispersion in direct counts of both *A. flexuosa* and mangrove macroinfauna remained contagious after conversion (Table III).

Table II. Summaries of paired t-tests of comparisons of converted counts (from the tube sampling unit to 1 m^2) of *Anomalocardia flexuosa* and objects representing mangrove macroinfauna with direct counts in 1 m^2 using samples (n=5) with different densities (low, medium and high, see text for details) and spatial dispersions (regular, random and contagious). The mean difference between counts *d* is rounded to a whole number. Values of the standardized Morisita index of dispersion (I_{STM}), significance and type of dispersion are given for converted counts.

		A. flexuosa			Mangrove macroinfauna		
		Dispersion			Dispersion		
Density		Regular	Random	Contagious	Regular	Random	Contagious
Low	t	4.21	19.53	27.82	2.45	11.88	11.69
	p	0.013	<0.001	<0.001	0.07	<0.001	<0.001
	d	21	337	521	32	434	1063
	I_{STM}	0.1658 n.s. Random	0.5002 p<0.05 Contagious	0.5000 p<0.05 Contagious	0.0821 n.s. Random	0.5003 p<0.05 Contagious	0.5013 p<0.05 Contagious
Medium	t	3.68	38.24	36.22	2.01	19.81	22.69
	p	0.021	0.001	<0.001	0.11	<0.001	<0.001
	d	23	568	690	35	626	1267
	I_{STM}	0.0541 n.s. Random	0.1560 n.s. Random	0.3324 n.s. Random	0.1049 n.s. Random	0.5000 n.s. Random	0.5002 n.s. Random
High	t	2.96	29.29	38.74	2.03	10.54	15.44
	p	0.041	0.001	0.001	0.11	<0.001	<0.001
	d	36	825	967	122	1211	2048
	I_{STM}	0.5000 n.s. Random	0.5001 p<0.05 Contagious	0.4544 p<0.05 Contagious	0.5006 n.s. Random	0.5003 p<0.05 Contagious	0.5007 p<0.05 Contagious

Discussion

Our results clearly showed that conversions generally overestimate bivalve and mangrove macroinfauna abundances, generally leading to overestimates of their densities. As sampling of the macroinfauna is carried out using a smaller unit and converted to a larger area, the method assumes that the density of individuals in any part of the larger area is similar to that of the sampling unit. Converted density estimates, however, are not usually reliable simply because this assumption is unlikely to be met (Colwell *et al*. 2012). High spatial heterogeneity in abundance is virtually the rule in benthic macroinfaunal populations (Andrew & Mapstone 1987, Morrisey *et al*. 1992, Netto & Lana 1994), which naturally form dense aggregations (Mann 2000, Cabral & Murta 2004) in places with conditions optimal for survival, growth and reproduction (Elliott 1983).

The spatial distribution and abundance of intertidal benthic macrofaunal assemblages have been associated with physical and biological factors, such as level of exposure (Quan *et al.* 2009), hydrodynamics (Alongi & Christoffersen 1992, Rodil *et al.* 2008) substrate heterogeneity (Netto & Lana 1994, Ourives *et al.* 2011), reproductive behavior (Rainer & Wadley 1991), food availability (Boehs *et al.* 2004) and interspecific competition (Alongi 1987, Dittmann 2001, Boehs *et al*. 2004). In the specific case of mangroves, predation by epifauna (Alongi 1989) forest type (Alongi 1987, Alongi & Christoffersen 1992), substrate properties (Guerreiro *et al.* 1996) and chemical defense by mangroves (Alongi 1989) are among the main factors that regulate density and spatial dispersion of macroinfauna (Lee 1999). Such populations usually have a contagious (aggregated or clumped) dispersion and abundance distributions described by the negative binomial model (Southwood & Henderson 2000). With contagious dispersion, individuals may be densely concentrated in some

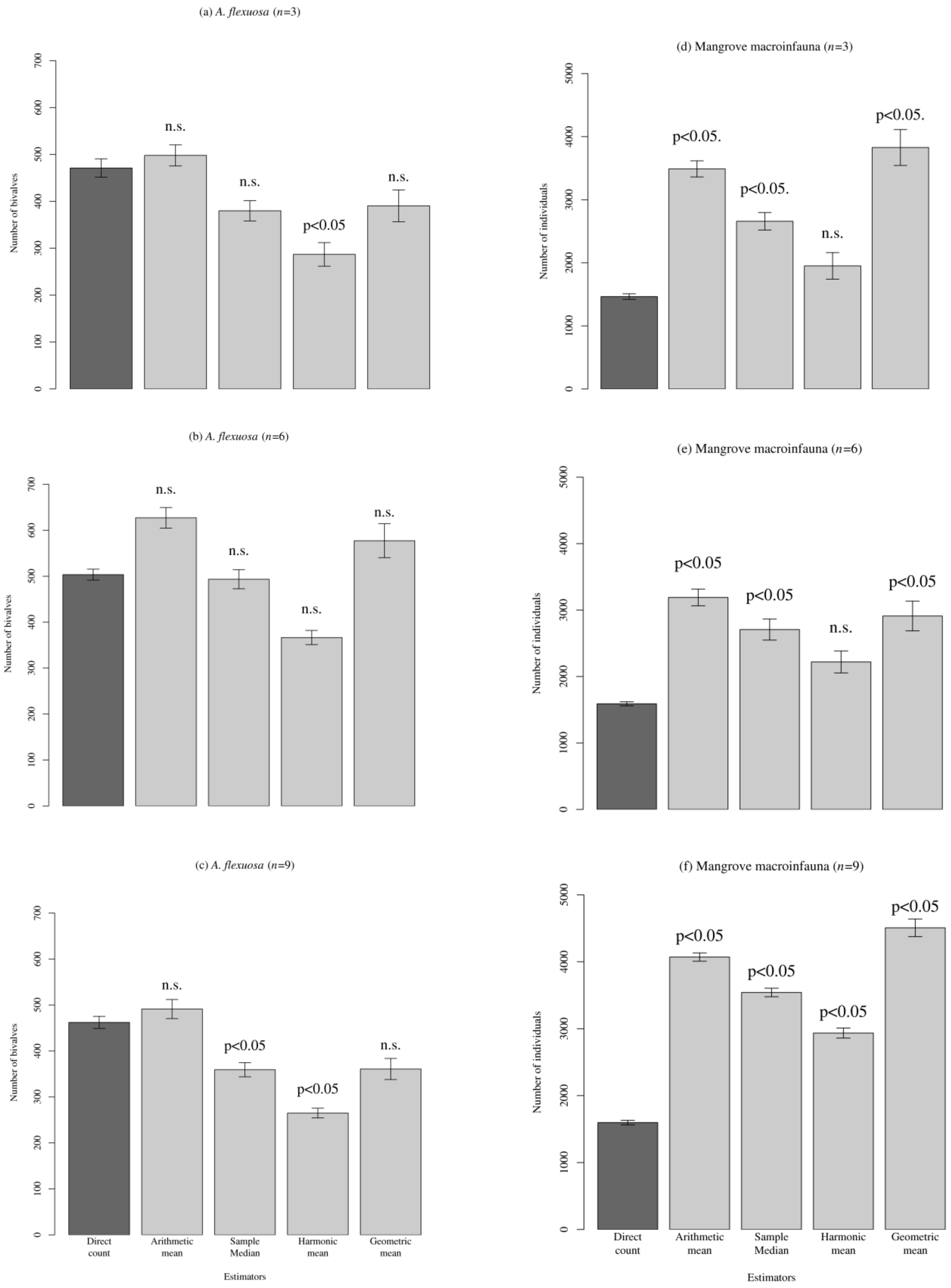

Figure 3. Comparison of mean (± s.e.) direct (dark gray) and converted (light gray) counts, using the arithmetic mean, sample median, geometric mean and harmonic mean, of (a,b,c) *Anomalocardia flexuosa* and (d,e,f) mangrove macroinfauna in samples of *n*=3, 6 and 9 replicas, respectively, in 1 m^2 quadrats. The significance (p) of Dunnet's test for the comparison of the direct count with each converted count, is given above the bar.

areas, very sparse in others and in a large part of the habitat no individuals occur (Southwood & Henderson 2000). If the sampling unit is placed by chance in an area with either many or a few individuals, the converted number will be either much higher or much lower, respectively than the true number of individuals in the larger area. Our results suggest that for contagious dispersion, random sampling only increases variability among replicate counts (Mann 2000, Cabral & Murta 2004), increasing the error associated with conversions. Suggestions have been made towards an optimal sampling protocol for benthic macroinfauna, taking into account costs (time and resources), accuracy and precision of density estimates (Mann 2000, Cabral & Murta 2004, Ferraro & Cole 2004). However, as there is large spatio-temporal variability in benthic macrofaunal abundance (Andrew & Mapstone 1987, Morrisey *et al.* 1992, Netto & Lana 1994), an optimal sampling design for one study may not be useful in another (Ferraro & Cole 2004).

Table III. The standardized Morisita dispersion index (I_{STM}) calculated from direct counts of *Anomalocardia flexuosa* and mangrove macroinfauna in 1 m^2 quadrats and converted counts, converting from the arithmetic mean, sample median, harmonic mean and geometric mean of samples of different sizes (n=3, 6 and 9) obtained with a tube in natural habitats. Individuals of both faunal groups were contagiously dispersed in all samples.

Faunal group	n	Direct count	Arithmetic mean	Sample median	Harmonic mean	Geometric mean
	3	0.5007	0.5009	0.5036	0.5036	0.5014
A. flexuosa	6	0.5001	0.5005	0.5019	0.5007	0.5008
	9	0.5003	0.5008	0.5018	0.5005	0.5007
	3	0.5004	0.5006	0.5027	0.5058	0.5013
Mangrove macroinfauna	6	0.5001	0.5007	0.5029	0.5027	0.5017
	9	0.5002	0.5001	0.5004	0.5003	0.5001

There is a need for appropriate model-based methods to convert count data from a contagious dispersion. For example, the size of a population of the endangered bivalve *Pinna nobilis* was estimated with higher precision using a density surface model with GIS based on line transect survey data, than with the traditional distance sampling method since the model related abundance to spatial covariates of interest, such as bathymetry (Katsanevakis 2007). The implications of this apply not only to estimates of variation in density but also to estimates of spatial dispersion, which may be important indicators of environmental impacts (Rosenberg 1974, Chintiroglou *et al.* 2000) or the effects of harvesting on benthic macrofauna (Nishida *et al.* 2006).

Conversions may have different effects on different faunal groups since our results show that the effect of conversion is more severe with mangrove macroinfauna than with the bivalve *A. flexuosa*. This may have more to do with the particular spatial dispersion and biological characteristics of the fauna or taxon with different consequences for assemblages dominated by certain taxa and those that are more equitable and diverse (Watling *et al.* 1978, Benedetti-Cecchi *et al.* 1996). We did not examine the responses of individual taxa in relation to conversions and further studies are needed to evaluate the precision of conversions with different taxa and in different types of macroinfaunal assemblages.

Although we did not control for effects of the shape of the sampling unit (circular) in relation to the larger area (square), many intertidal benthic studies convert macroinfaunal densities in sediment cores with a variety of shapes (circular, rectangular or square) to densities expressed in terms of a standard 1 by 1 m square (De Grave & Casey 2000). The latter study found no difference between densities of macroinfauna sampled using circular and square corers with similar areas (0.028 m^2) but there was a difference between the circle/square data and that obtained with a rectangular corer of similar area (De Grave & Casey 2000).

Estimating mean macrofaunal density per sampling unit and inferring the estimate to be representative of the study area is quite different to converting densities to a larger area and then estimating mean density. The latter involves an extra step that may result in incorrect final estimates because the conversion usually causes observations used to estimate mean and variability to be greater than what they would be if direct counts had been made in the larger area. Even when raw numbers of individuals per sampling unit are used to estimate a mean and variability, if these estimates are then converted, there will still be error as converted densities always overestimate direct counts. Conversions are regularly used in studies of the density of the intertidal benthic fauna (Dittmann 2001, Degraer *et al.* 2003, Dolorosa & Schoppe 2005, Neves & Bemvenuti 2006, Beseres & Feller 2007, Mendonça *et al.* 2008). The latter studies clearly describe how samples were taken using a sampling unit with stated dimensions and how densities were then expressed in relation to a larger area, usually 1 m^2 for comparative purposes. With such information, published densities may be converted back to the area or volume of the original sampling unit. However, other studies are less clear on how, or even whether or not, the density data were converted (Fernandes & Soares-Gomes 2006, Santos *et al*. 2010, Rosa-Filho *et al*. 2011).

Our results are consistent with other studies (Dittmann 2001, Degraer *et al*. 2003, Dolorosa & Schoppe 2005, Neves & Bemvenuti 2006, Beseres & Feller 2007, Mendonça *et al.* 2008) that show that the precision of conversions is best with regular dispersion because the number of individuals tends to be very similar (low variability) among sampling units in any randomly selected part of the habitat and thus conversion to a larger area will result in a estimate that is close to the “true” number of individuals in the larger area. Regular dispersion patterns may result from competitive interactions, especially in homogeneous environments (Rickefs & Miller 2000) and have been associated with populations of invertebrates (Gotelli & Ellison 2012) where mortality may reduce the degree of aggregation of individuals in a population, causing a tendency towards regular dispersion (Southwood & Henderson 2000). Anthropogenic exploitation of populations of intertidal bivalves from mangrove estuaries may cause the typically aggregated dispersion to change to regular or random (Nishida & Leonel 1995, Santos *et al*. 2010). However, since macroinfaunal populations are usually contagiously dispersed (Andrew & Mapstone 1987, Cabral & Murta 2004), regular dispersions are of little practical benefit in improving the precision of conversions of their densities.

Conversions appear to be more precise at low densities, especially with regular dispersion for both *A. flexuosa* and mangrove macroinfauna. Spatial dispersion is associated with density and benthic invertebrate populations with higher densities tend to be more contagiously dispersed (Rosenberg 1974), whereas those with low densities tend toward regular dispersion (Southwood & Henderson 2000). As greater variability occurs in samples with higher mean densities (Zar 1999, Southwood & Henderson 2000), conversions are expected to be much less precise with random and aggregated dispersions.

Our findings suggest that the arithmetic mean is the best estimator for converting a sample of count data of *A. flexuosa*, whereas, the most appropriate estimator for conversions of mangrove macroinfauna counts is the harmonic mean. The arithmetic mean has been used in conversions of density data of the benthic fauna (Morrisey *et al*. 1992, Zar 1999, Dittmann 2001, Beseres & Feller 2007, Santos *et al.* 2010, Smith & Crabtree 2010), but few other estimators have been used (Beukema 1976, Levinton & Lopez 1977, MacRae 1984, Seber 1986). Conversions from a single count of individuals is by far the most frequently used method in the literature (Degraer *et al.* 2003, Dolorosa & Schoppe 2005, Neves & Bemvenuti 2006, Mendonça *et al*. 2008).

Sample size may also be important for obtaining a reliable estimate of density (Peckarsky 1984, Zar 1999). With both the smaller (n=3, less representative) and larger sample sizes (n=9, greater variability), conversions of densities of *A. flexuosa* are less precise than the intermediate sample size (n=6). With mangrove macroinfauna, as sample size increased, conversions became less precise, which was probably related to strong aggregation with higher densities and greater variability with larger sample size (Rosenberg 1974). The size of the sampling unit also affects the precision of density estimates where smaller units tend to produce more variability, especially with contagious dispersion as they either contain zero or a few individuals or very many individuals, if the sampling unit is located in a clump (Andrew & Mapstone 1987). More information on benthic macrofaunal patch distribution and size is needed to determine the optimal size of the sampling unit (Ferraro & Cole 2004) and to compare the efficiency of different sampling designs (Cabral & Murta 2004).

Our study concludes that densities of mangrove macroinfauna from sampling units expressed in relation to larger areas are not precise and should be avoided, especially when there is contagious dispersion. Although of limited use in studies of the macroinfauna of intertidal soft sediments where animals are contagiously dispersed and it is not practical to take large replicate sediment cores, conversions appear more accurate with regular dispersion, and when the area to which the conversion is made is not much greater than the sampled area. With species richness estimates, conversions appear reliable only for sample sizes (abundances and/or sampled area) from 1.8 to 3 times the reference sample (Melo *et al.* 2003, Colwell *et al.* 2012). Many surveys of intertidal benthic macroinfaunal density use conversions to areas or volumes that far exceed these limits (Degraer *et al.* 2003, Dolorosa & Schoppe 2005, Smith & Crabtree 2010). Macroinfaunal organisms are very small relative to the standard area of 1 m^2, commonly used for comparisons among different studies, and expressing benthic density in terms of such a large area may not be useful due to the low precision of converted estimates (i.e. a large difference between the converted count and the direct count in the larger area). A suitable sampling method appropriate for the size of the organisms under investigation is important for obtaining reliable estimates of density and spatial dispersion of the benthic fauna (Peckarsky 1984). An area of $0.01m^2$, a little larger than our tube area, was optimal for sampling benthic macrofauna in sediment (Ferraro & Cole 2004). Although an even larger area of 0.1 m^2 might be useful for comparisons among different benthic macrofaunal studies (Ferraro & Cole 2004), careful consideration should be given to the size of the sampling unit taking into account the type of fauna, spatial aggregation and habitat (Andrew & Mapstone 1987). Even the shape of a sampling device may influence estimates of density (De Grave & Casey 2000). Thus, the adoption of a standard area or volume smaller than 1 m^2 in studies of intertidal soft sediment macroinfaunal abundance may eliminate the need for density conversions and allow more reliable comparisons among similar studies in different locations.

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