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SHORT COMMUNICATION

Some recommendations for an accurate estimation of *Lanice conchilega* density based on tube counts

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Abstract The tube building polychaete *Lanice conchilega* is a common and ecologically important species in intertidal and shallow subtidal sands. It builds a characteristic tube with ragged fringes and can retract rapidly into its tube to depths of more than 20 cm. Therefore, it is very difficult to sample *L. conchilega* individuals, especially with a Van Veen grab. Consequently, many studies have used tube counts as estimates of real densities. This study reports on some aspects to be considered when using tube counts as a density estimate of *L. conchilega*, based on intertidal and subtidal samples. Due to its accuracy and independence of sampling depth, the tube method is considered the prime method to estimate the density of *L. conchilega*. However, caution is needed when analyzing samples with fragile young individuals and samples from areas where temporary physical disturbance is likely to occur.

Keywords *Lanice conchilega* · Tube counts · Belgian Continental Shelf

Introduction

The tube building polychaete *Lanice conchilega* (Pallas 1766) is a dominant species of European intertidal and shallow subtidal sands (Ropert 1996; Van Hoey et al. 2004). The species is of high ecological importance since (1) its dense populations affect sediment properties (Jones and Jago 1993) and oxygen transport (Forster and Graf 1995), (2) it alters the composition of benthic communities (Zühlke 2001), and (3) it is an important food item for birds and fish (Petersen and Exo 1999). The worms build very characteristic tubes, made of cemented sand grains and shell breccia (Ziegelmeier 1952). The top of the tube usually projects a few centimeters above the sediment and carries a ragged fringe. *Lanice conchilega* itself can grow up to a length of 30 cm, while its tube can reach a length of 65 cm (Ziegelmeier 1952). When disturbed, the worm can rapidly retract into its tube to depths of more than 20 cm (Ziegelmeier 1952; Dales 1955). Consequently, it is very difficult to sample *L. conchilega* individuals with corers for intertidal sampling (Heuers et al. 1998; Petersen and Exo 1999; Ropert and Dauvin 2000; Strasser and Pieloth 2001; Zühlke 2001; Callaway 2003) and certainly with a Hamon grab (Ropert and Dauvin 2000) or a Van Veen grab in the case of subtidal populations (Buhr and Winter 1977; this study). Generally, a Van Veen grab (50–70 kg, not loaded with extra weight), frequently used in macrobenthic studies, penetrates 10–15 cm into the sediment, depending on the type of substrate (Beukema 1974). When using this sampling device, an underestimation of the real density by specimen counts could thus be expected.

Observations in the field and in fixed samples, however, indicated that unoccupied tubes are different from tubes occupied by *L. conchilega*, which can be discerned by the existence of a certain rigidity of the tube, caused by cement mucus produced by *L. conchilega*. Observations also suggested that the

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tube top with the fringe is destroyed shortly after the worm had died or abandoned the tube, although remains of tubes can still be found in the sediment until several months later (Zühlke 2001). Therefore, many studies use the number of tubes with a well developed ragged fringe and certain rigidity, as a direct reflection of the abundance of *L. conchilega* (Ropert and Dauvin 2000; Strasser and Pieloth 2001; Zühlke 2001; Callaway 2003).

This paper reports on some aspects to be considered when using tube counts as an estimate of *Lanice conchilega* density.

Methods

Three subtidal sampling stations (O1, WK2 and WK1) with high local abundance of *L. conchilega* were selected for this study, in the coastal zone of the Belgian Continental Shelf (BCS). All three stations were sampled monthly (September–April) or biweekly (May–August) during the period March 2002–September 2003 (23 sampling dates). The macrobenthic samples were taken with a Van Veen grab (0.1026 m^2) on board of the research vessel 'Zeeleeuw'. The samples were fixed in 8% formaldehyde-seawater and sieved over a 1 mm and 0.5 mm sieve. The samples were treated with care in order to avoid destruction of the *L. conchilega* tubes.

The volume of the sediment was recorded to estimate the sampling depth of the Van Veen grab for each sample. The grain size distribution of a sub sample from the Van Veen was determined with a LS Coulter particle size analyzer: median grain size of the fraction 2–850 μm and mud content (volume percentage with particles < 64 μm) were used as granulometric variables.

A field study at the Flemish beach nature reserve "Baai van Heist" (Belgium) was set up to test the relationship between tube counts and number of *L. conchilega* individuals as a function of excavated depth. Cores (0.0031 m^2) were inserted into the sand down to different depths (5, 10, 15, 20 and 30 cm). Three replicate samples were collected per depth and sieved over a 1 mm sieve.

In this study the *L. conchilega* individuals (further referred to as specimen counts) as well as the tubes with a well developed ragged fringe (further referred to as tube counts) were counted in the samples. These two methods can be used to estimate the real density of this polychaete at a certain place and time. Valid tube counts were based on the following criteria, irrespective of tube length: (1) tubes have a well developed ragged fringe at the end, (2) they have a good rigidity, and (3) they are not filled with sand.

The non-parametric Mann–Whitney *U* test and median or Kruskal–Wallis test was used to test for significant differences between tube and specimen counts at different depths, and Spearman rank was used to trace correlations between variables.

Results

Field study

By digging out sampling cores down to different depths, a sampling-depth dependent relationship between tube and specimen counts was found (Fig. 1). At a depth of 5 cm, on average 82% less specimens than valid tubes were found (Mann–Whitney *U* test: $P = 0.049$). At a depth of 10 cm (9% less specimens than valid tubes), no significant differences between the two counting methods was found (Mann–Whitney *U* test: $P = 0.83$). From 15 cm depth onwards, equal values for the two counting methods were found [Mann–Whitney *U* test: $P = 0.66$ (15–20 cm) and $P = 1$ (30 cm)], indicating a good estimate of the real density of *L. conchilega*. Counts of tubes with fringes were not dependent of depth (Median test: $P = 0.8925$), whereas specimen counts were (Median test: $P = 0.0469$).

Abiotic variables

The median grain size of most of the samples varied between 150 and 250 μm, whereas the mud content varied mostly between 0 and 20% (muddy, fine sandy sediment) (Fig. 2). There was no correlation between sampled volume and median grain size (Spearman rank: $P = 0.9$), whereas a positive, though low correlation was found between sampled volume and mud content (Spearman rank: $P = 0.036$).

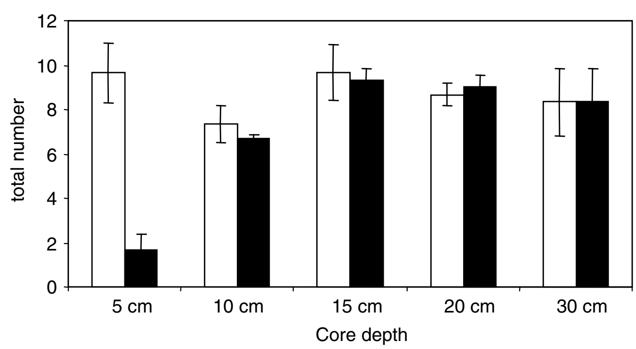

Fig. 1 Tube counts (*white columns*) and specimen counts (*black columns*) at different sampling depths, with indication of the standard error taken in the field

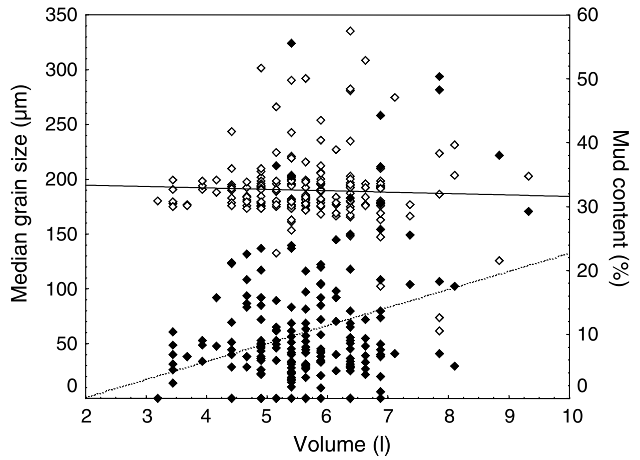

Fig. 2 Median grain size (µm) (*open rhombus*, *black trend line*) and mud content (%) (*black rhombus*, *dotted trend line*) against sample volume (liter), with indication of a trend line

The sampled volume ranged between 3.19 and 9.33 l, and the variance between the different sampling stations was low (average volume of 5.97 ± 1.1 l (SD) for station O1, 5.51 ± 0.93 l for station WK2, 5.4 ± 0.92 l for station WK1). The maximum volume of a Van Veen grab was about 15.05 l at the maximal sampling depth of 18.5 cm. The volume range obtained for the three stations is in accordance with a sampling depth of the Van Veen grab varying between 5.5 (3.03 l) and 12 cm (9.23 l).

Valid tube counts versus specimen counts

More tubes than specimens were counted at most sampling occasions for the three separated stations (Fig. 3). However, no correlation was found between the percentage differences between tube and specimen counts and the sampled volume (Spearman rank: $P = 0.24$) (Fig. 4a). On a few sampling occasions there were more specimens than fringed tube counts. These sampling occasions were characterized by the absence of tubes with well developed fringes or by high densities of small tubes (spring–summer). A significant difference was found between the percentage differences between tube and specimen counts and the season of sampling (Kruskal–Wallis test: $P = 0.0121$). Most occasions where tube counts were lower than specimen counts were in spring and summer (Fig. 4b).

Discussion

The intertidal field study at the beach of Heist showed that counting fringed tubes is a good method for estimating *Lanice conchilega* density. This is mainly

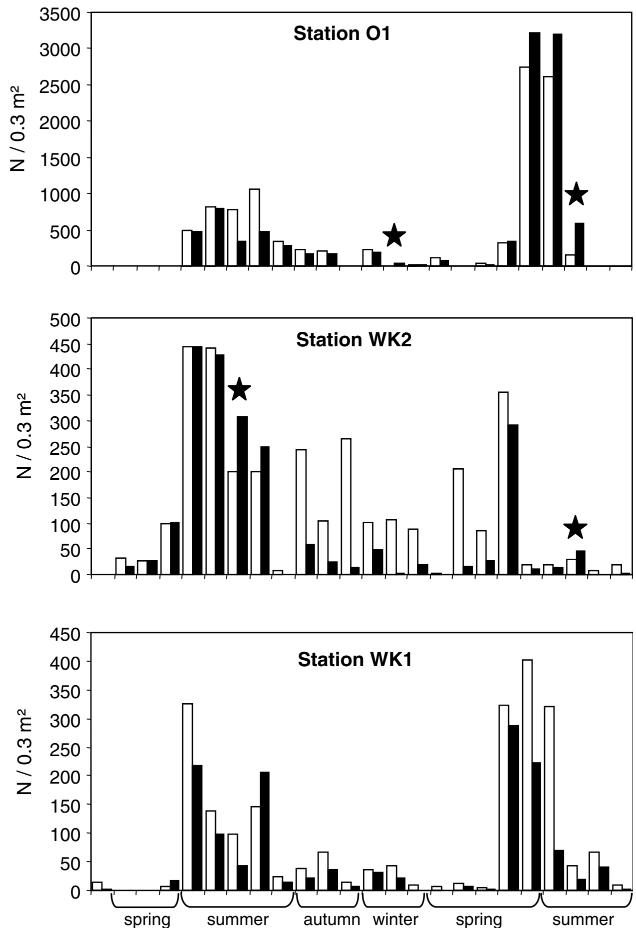

Fig. 3 Differences between tube counts (*white column*) and specimen counts (*black columns*), expressed in ind/0.3 m^2. The star indicates samples with absence of tubes with well developed fringes

due to the sediment-depth independency of the method, which is not the case for specimen counts. However, not every sampling method reaches adequate depths of at least 10 cm. In the case of sampling with a Van Veen grab, a strong underestimation of the real density is given by counts of larger worms, such as *L. conchilega*, due to the low sampling efficiency (Beukema 1974). Sampling efficiency is only high for species that live in the upper five centimeters of the bottom (Ursin 1956; Lie and Pamatmat 1965; Beukema 1974). Additionally, the average sediment content of a Van Veen depends on the coarseness of the sediment: the grab penetrates relatively deep in both very fine (sand with a high proportion of mud) and very coarse sand, whereas relatively small volumes were obtained from fine sands that had low proportions or no mud at all (Beukema 1974). In our study the sedimentology of the three stations was characterized by fine sands with a varying mud

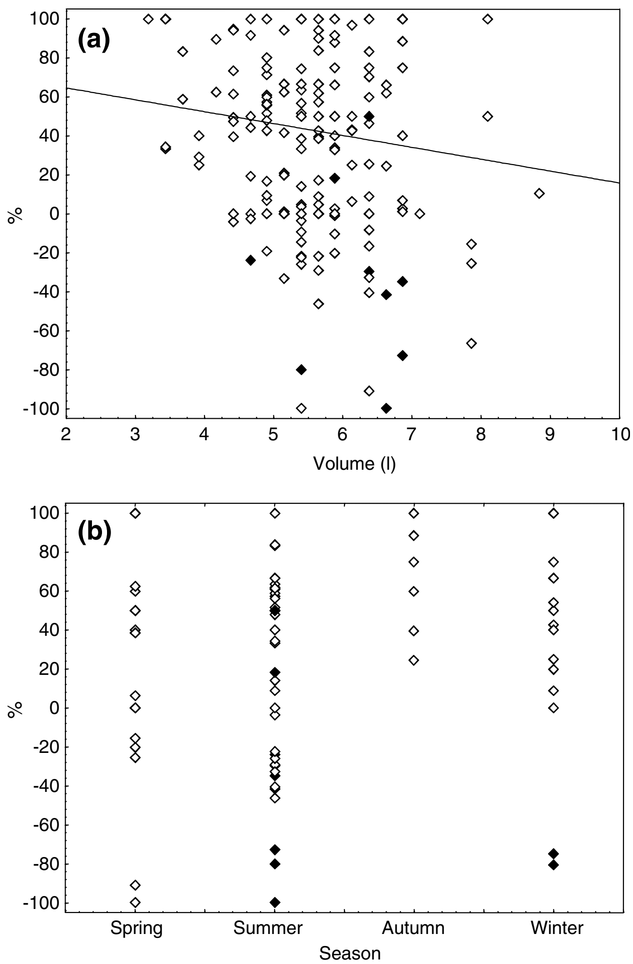

Fig. 4 **a** Scatter plot of sample volume against the percentage differences between tube and specimen counts (N (tube) – N (specimen)/N (max) × 100). *Black rhombus* indicates samples lacking tubes with well developed fringes. **b** Scatter plot of the season against the percentage differences between tube and specimen counts. *Black rhombus* indicates samples lacking tubes with well developed fringes

content (0–20% or more), and the sampled volume increased with increasing mud content. The average sampled volume at the three stations corresponded with a Van Veen grab penetration depth of 5–12 cm. Although a Van Veen grab, under ideal sampling conditions, makes a horizontal rather than a semicircular cut on a sandy substrate (Lie and Pamatmat 1965), personal observations showed that a semi-circular cut is not unusual at our sampling sites. Due to this characteristic shape of the Van Veen grab, *L. conchilega* tubes at the outside of the grab are sampled less efficiently, even when the Van Veen reaches its maximal sampling depth (18.5 cm). Therefore, it is advisable to use the tube tops with fringes, irrespective of tube length, as an estimate of *L. conchilega* density, as was already done in previous studies (Ropert and Dauvin 2000; Strasser and Pieloth 2001; Zühlke 2001; Callaway 2003).

Strasser and Pieloth (2001) also found a strong linear correlation (Spearman rank $r = 0.97$, $P < 0.001$) between the number of tube top counts and living worms, although the tube counts slightly overestimated the number of animals present (trend line above the 1:1 ratio). This supports the idea that the shape of the tubes (U- or W-type) is independent of density (Strasser and Pieloth 2001). Therefore, the authors proposed a correction factor of 0.73 to estimate real densities. However, in the case of high *L. conchilega* densities, this correction is too strong, whereas for low densities patches the correction factor is too weak. That is why a correction factor should be replaced by a regression equation depending on the *L. conchilega* abundance. Furthermore, since both methods in our field study rendered exactly the same density estimates at a sampling depth of 30 cm (Mann–Whitney *U* test: $P = 1.000$), it can be expected that this number reflects the real density of *L. conchilega*. This conclusion confirms the one to one relationship between the number of fringed tubes and the real density of *L. conchilega*, at least in high density patches such as the ones at the beach of Heist. The one to one relationship in high density patches also supports the idea that the shape of *L. conchilega* tubes is indeed density dependent (no U- or W-shaped tubes).

Higher numbers of tubes compared to specimens were found on most occasions, as expected based on the sampling efficiency of a Van Veen grab. However, sometimes lower numbers of tubes than specimens were recorded. Two hypotheses might explain these deviations from the general pattern. Firstly, in some cases this may be mainly due to the absence of tubes with well developed fringes. The destruction of the tube fringe could be caused by previous disturbances, like beam-trawl fishery (sampling areas are characterized by a high beam-trawl frequency; personal observations) or even storm conditions (not the case on the discussed sampling dates). *Lanice conchilega* normally rebuilds its tube very quickly (within 48 h), but the form of the fringes of the tubes can differ according to the water condition (Nicolaidou 2003). In still water the fringe of the new tube was thinner and extended to all directions, while under a wave regime the individual branches were thicker and positioned perpendicularly to the direction of the waves. Secondly, tube tops of young adults (tube diameter 1.0–1.5 mm) are more fragile and less developed (personal observations) and therefore more sensitive to destruction during sieving or sorting. This factor may explain the lower number of tubes during periods with high abundances of young individuals (i.e., spring–summer).

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