Assemblage, size and abundance bias in a novel sandy shore macro-infaunal sampling technique

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Sampling sandy shore macro-invertebrate fauna is critical in enhancing our understanding of beach ecology and conservation, and is a common monitoring approach. The traditional, and almost universal, method of sampling involves sieving sand to locate infauna, but here we describe a novel Hydraulic Sampling Device (HSD), a candidate method for future macro-invertebrate sampling, which has the potential to be faster and more effective at sampling invertebrates. We compared the results obtained by these two methods. Macro-invertebrate fauna of six beaches on Phillip Island, southern Victoria, Australia were sampled in the upper and lower beach. On average, the HSD sampled a smaller size range of fauna than the sieving method, perhaps because of longer handling times and escape of larger individuals. The sieving method found more individuals and a higher species richness. The methods we describe do not produce directly comparable results. On balance, the sieving method is simpler, apparently not as prone to ‘escape bias’, and reports higher abundances and richness of beach infauna.

Introduction

Monitoring and understanding the ecology of sandy beaches will enable enhanced protection of these vulnerable, dynamic ecosystems and the fauna they support, as well as inform ecological research in general (Schlacher et al. 2015). This includes understanding not only the large, charismatic elements such as turtles, birds and fish, but also macro-invertebrate fauna (MIF); organisms retained on a 1 mm mesh (McLachlan & Brown 2006). These organisms play a vital role in the functioning of sandy beach ecosystems, being a critical component of food webs and thus carbon processing (Pavesi et al. 2007, Schlacher et al. 2008, Bessa et al. 2014). In a recent review, MIF were highlighted as one of the faunal groups critical for monitoring the ‘health’ of sandy shore ecosystems (Schlacher et al. 2014).

al. 2007, Rodil et al. 2007, Chaouti et al. 2008, Schoeman et al. 2008, Goncalves et al. 2009, Sivadas et al. 2012). These studies use various designs (Schooler et al. 2013) and adopt a variety of sampling methods including a range of corers or quadrats of varying sizes, sampling depths and different sampling effort and designs. However, one aspect generally held in common is the use of a 1 mm mesh sieve to retrieve macro-invertebrate fauna from sand (Schlacher et al. 2008).

Determining the efficiency and accuracy of chosen sampling protocols prevents a lack of power in analysis and wastage of resources (James & Fairweather 1996). The sieving method is currently the most widely used method of sampling beach invertebrates and is used as a standard sampling technique (Schlacher et al. 2008). However, as for any standard technique, new promising methods are proposed from time to time (e.g., Gray et al. 2014). Novel or emergent methods, or new applications of existing processes, are often compared with existing techniques to assess the effectiveness of new approaches and to benchmark new techniques against traditional ones (e.g., De Bondi et al. 2010). Currently, there are few studies that discuss the effectiveness of sandy shore sampling devices (but see Eleftheriou & Moore 1984), despite the fact that sandy shore ecologists have urged vigilance for promising advances in sampling methodologies (Schlacher et al. 2008). Hydraulic expansion of substrate (back-washing) to separate materials of differing specific gravities is a long-standing technique used in the water treatment industry to separate sand and biological material (Degremont Acfi 1960). Here, we examine the efficacy of a potential improvement in sampling MIF from sandy shore substrates using an apparatus employing the hydraulic expansion principal.

Traditional sieving for MIF on sandy shores is time consuming, relying on hand capture or removal of MIF from the sieved sample, with the potential to miss or lose MIF resulting in possible sample bias. Although ‘closed’ sieves exist (and are recommended; Schlacher et al. 2008), open sieves are frequently used on beaches thus there is the possibility of escape of more mobile MIF. Sieving is especially difficult where sand grain sizes are larger (Schlacher et al. 2008). Given the challenges associated with traditional means of sorting samples on sandy shores, and the ready availability of nearby seawater, we wondered whether a hydraulic solution to sampling might be possible which could improve the efficiency of sampling. We designed and constructed the hydraulic sampling device (HSD; Fig. 1), which represents a promising new sampling method for sandy shore MIF. The HSD is a prototype
designed to extract MIF from sand and if proven more effective than the sieving method, could offer a suitable alternative for sampling sandy beach MIF. The HSD is operated by dropping a sand core sample in through the top of a cylindrical chamber. Water is poured in through an attached hose creating velocity that fluidizes the sand sample ejecting MIF contained within the sand core out through a spout onto a collection plate (see Methods and Fig. 1 for details).

This study aims to determine the efficacy of our newly-designed HSD as a candidate alternative to the traditional sieving method for sampling sandy shores by comparing the methods with respect to: (1) the body size of organisms retained by each method, (2) the abundance of individuals and species richness determined by each method, and (3) species composition determined by each method.

**Methods**

**Sampling design**

The study was undertaken on six beaches on Phillip Island in southern Victoria, Australia: Anzacs Beach (38°32′16.71″S, 145°19′57.69″E), Cadigan Road (38°28′20.92″S, 145°09′37.61″E), Grossard Point (38°27′53.01″S, 145°10′29.10″E), Berrys Beach (38°31′07.69″S, 145°12′14.34″E), Surf Beach (38°30′36.24″S, 145°17′00.29″E) and Smiths Beach (38°30′19.14″S, 145°15′26.75″E). These beaches are similar in form and were selected because they are broadly representative of beaches in southern Australia. At each site a 15 metre-long stretch of beach was sampled and divided into upper and lower zones. The upper zone was above the last high-tide mark, the lower zone was below the mark. The upper limit of the sampled area was the base of the primary sand dune and the lower limit was the top of the swash zone. Sampling was conducted in late autumn and early winter 2011, within two hours either side of low-water.

Sand cores were collected with a perspex cylindrical corer, 10 cm diameter by 15 cm deep. Cores were taken at random locations at a minimum distance of 1 m from any other core. For each sampling method, 12 cores were collected from both the upper and lower zones at each site (288 in total). Systematic sampling was used to collect cores, alternating samples between methods in each zone.

**Sampling methods**

**The sieving method**

Core samples were passed through a 1 mm mesh sieve in situ. Retained invertebrates were collected, placed in vials containing 70% alcohol, labelled and taken back to the laboratory for measuring and identification to family level (Jaramillo et al. 1995, Hacking 2007, Chaouti et al. 2008, Schlacher et al. 2008, Schoeman et al. 2008, Goncalves et al. 2009, Bessa et al. 2014). Body lengths were measured using a stereo-microscope with an attached camera and inbuilt length measuring device.

**Hydraulic sampling**

The design of the HSD was adapted by a hydraulic engineer (BB) from an established process for purifying water that uses a back-washing system (Degremont Acfi 1960). This HSD was optimised by informally trialling and adjusting the prototype on the beaches of Phillip Island. In addition to its sampling efficacy, we required the device to be light-weight (to enable investigators to carry it to sampling locations), simple and efficient to use and resistant to saline conditions. In terms of simplicity, weight and potential applications on remote beaches, we preferred a device that did not require power and had limited electronic and mechanical complexity. The HSD was constructed from a Perspex cylindrical chamber 10 cm in diameter (Table 1 and Fig. 1). Core samples were dropped into the chamber through an opening in the top. Sea-water was poured into a funnel connected by a hose to the base of the cylinder. The quantity of water added depended on how readily particle separation occurred within the chamber. Sand cores with small sediment grain size generally required two buckets of water, large grain size generally required one. We ceased adding water only when MIF had stopped
accumulating on the collection plate, which we considered was a suitable standard protocol for operating the HSD. As the water was being poured, the funnel was held up at shoulder height so gravity increased the velocity of the water flow, thus fluidizing particles (the velocity of the water was roughly constant as the spout was held at a similar distance above the cylinder). The sample separated according to the specific gravity and particle size of its components. Organic material, including macrofauna, was flushed out through a spout at the top of the cylinder onto a plate. The invertebrates retained on the plate were then collected, placed in vials containing 70% alcohol, labelled and taken back to the lab for measuring and identification to family level.

Statistical analysis

The statistics package R (R Core Team 2012) and the R package lme4 (Bates et al. 2012) were used to perform linear mixed effects models to analyse the relationship between the independent fixed variables; method (two levels; sieving and HSD), and zone (two levels; upper and lower), and the response variables: body length, abundance and species richness. Beach was a random effect with six levels, and zone was also random as it was nested within beach. These random effects were not of primary interest but are included to account for correlation structures in the data that result from the experimental design. Model assumptions were checked including normality of response variables. Log-transformation was required for body length.

Consistency in species composition reported by each method across the upper and lower zones was modelled using non-metric multidimensional scaling (NMDS) using the R ver. 1.15-4 package vegan (Oksanen et al. 2008). Species presence/absence vectors were fitted onto the ordination using the function envfit (Oksanen et al. 2008). The fitted vectors are arrows shown on the NMDS, where the arrow points to sampling units where species are present (Oksanen 2013). The length of the arrow; proportional to the correlation between ordination and vector; represents the strength of the gradient ($r^2$). This is calculated using 999 random permutations (Oksanen 2013). Statistical significance was tested at the $\alpha = 0.05$ level.

Results

The sieving method found a wider size range of some species, a greater abundance of MIF and higher species richness than the HSD.

Overall, MIF found by the sieving method had average body lengths greater than those found by the HSD (Table 2 and Fig. 2).

Table 1. Specifications of the Hydraulic Sampling Device (see Fig. 1 for the arrangement of parts). The overall dry weight (excluding peripherals) was less than 2 kg.

<table>
<thead>
<tr>
<th>Reference (Fig. 1)</th>
<th>Component/region</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Base to clear Perspex viewing cylinder</td>
<td>Standard PVC flanges</td>
</tr>
<tr>
<td>2</td>
<td>Fittings to connect inlet hose to nozzle</td>
<td>20 mm diameter, with PVC washers</td>
</tr>
<tr>
<td>3</td>
<td>Filter nozzle</td>
<td>Standard 1 mm slots</td>
</tr>
<tr>
<td>4</td>
<td>Bolts and nuts to hold base assembly together</td>
<td>4 x (10 x 80 mm) galvanised bolts</td>
</tr>
<tr>
<td>5</td>
<td>Inlet water hose</td>
<td>Clear, 3 m long, 20 mm diameter, clips to suit</td>
</tr>
<tr>
<td>6</td>
<td>Bucket</td>
<td>10 litre x 3 [2 for seawater, 1 for water collection]</td>
</tr>
<tr>
<td>7</td>
<td>Strainer plate</td>
<td>1 mm diameter</td>
</tr>
<tr>
<td>8</td>
<td>Mesh cover</td>
<td>Proposed fine wire mesh or lid to prevent escape of collected invertebrates</td>
</tr>
<tr>
<td>9</td>
<td>Outlet hose</td>
<td>Clear, 1 m long, 20 mm diameter, clips to suit</td>
</tr>
<tr>
<td>10</td>
<td>Perspex clear tube</td>
<td>100 mm diameter</td>
</tr>
<tr>
<td>11</td>
<td>Outlet fittings</td>
<td>100 mm reducing to 20 mm, standard PVC fittings</td>
</tr>
<tr>
<td>12</td>
<td>Elbow</td>
<td>PVC fittings to connect outlet tube</td>
</tr>
<tr>
<td>13</td>
<td>Funnel</td>
<td>Plastic, 200 mm connecting to 20 mm diameter</td>
</tr>
</tbody>
</table>
versely the sieving method appeared not to sample the smaller invertebrates as well as the HSD in the upper zone (Fig. 2). On average, the upper zone had larger macrofauna than the lower zone (Table 2). The interaction between method and zone shows a difference in body length of infauna found by each method in the lower zone, but not much difference in body length between methods in the upper zone (Table 2).

We found a higher abundance and species richness of MIF (Table 2, Figs. 3 and 4) by the sieving method compared with the HSD method. The effect of method on abundance and richness is the same in each zone, however more MIF and

**Table 2.** Results of three linear mixed-effect models for body length (logged), abundance and species richness of invertebrates found in the upper and lower zones of beaches (‘zone’) using the HSD and sieving methods (‘method’) and the interaction between these two variables. Significant effects are in boldface. Results of random terms are presented in Appendix.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Predictor variable (reference level)</th>
<th>df</th>
<th>Coeff.</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length</td>
<td>Method (sieving)</td>
<td>349</td>
<td>0.436</td>
<td>0.121</td>
<td>3.59</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Zone (upper)</td>
<td>349</td>
<td>0.377</td>
<td>0.166</td>
<td>2.27</td>
<td>0.0237</td>
</tr>
<tr>
<td></td>
<td>Method × Zone</td>
<td>349</td>
<td>-0.507</td>
<td>0.128</td>
<td>-3.95</td>
<td>0.0001</td>
</tr>
<tr>
<td>Abundance</td>
<td>Method (sieving)</td>
<td>13</td>
<td>1.146</td>
<td>0.271</td>
<td>4.23</td>
<td>0.0010</td>
</tr>
<tr>
<td></td>
<td>Zone (upper)</td>
<td>13</td>
<td>1.617</td>
<td>0.461</td>
<td>3.51</td>
<td>0.0039</td>
</tr>
<tr>
<td></td>
<td>Method × Zone</td>
<td>13</td>
<td>-0.736</td>
<td>0.367</td>
<td>-2.01</td>
<td>0.0660</td>
</tr>
<tr>
<td>Species richness</td>
<td>Method (sieving)</td>
<td>13</td>
<td>0.528</td>
<td>0.210</td>
<td>2.51</td>
<td>0.0259</td>
</tr>
<tr>
<td></td>
<td>Zone (upper)</td>
<td>13</td>
<td>0.728</td>
<td>0.267</td>
<td>2.73</td>
<td>0.0173</td>
</tr>
<tr>
<td></td>
<td>Method × Zone</td>
<td>13</td>
<td>-0.464</td>
<td>0.284</td>
<td>-1.63</td>
<td>0.1270</td>
</tr>
</tbody>
</table>

**Fig. 2.** Median (logged) length ± 1.5 × IQR (interquartile range) of invertebrates found in the upper (dark boxes) and lower (light boxes) zones of beaches using the HSD and sieving methods. Dots indicate outliers.

**Fig. 3.** Median ± 1.5 × IQR number of invertebrates found in the upper (dark boxes) and lower (light boxes) zones of beaches using the HSD and sieving methods. Dots indicate outliers.
higher richness occurred in the upper zone than the lower zone (Table 2, Figs. 3 and 4).

The NMDS of species composition within samples shows strong trends indicating that the HSD gives an inconsistent representation of species assemblage, with HSD samples widely distributed around the NMDS plot space (Fig. 5). In comparison, the sieving samples are close together in the NMDS plot space. Difference in species composition between the HSD and sieving methods across the upper and lower zones is largely driven by the invertebrate families: Exoedicerotidae (Amphipoda) ($r^2 = 0.62$, $p = 0.004$), Cirolanidae (Isopoda) ($r^2 = 0.65$, $p = 0.001$), Hyalidae (Amphipoda) ($r^2 = 0.35$, $p = 0.027$) and Actaeciidae (Isopoda) ($r^2 = 0.54$, $p = 0.001$).

Discussion

Clear differences existed between the sampling methods tested in this study. Larger body lengths were underrepresented in macrofauna captured by the HSD in the lower zone. This may be a result of increased handling time inherent in the operating procedures of the HSD, which involves injection of water and collection of invertebrates from the collection plate (pers. obs.). Handling time is a critical aspect of sampling benthic macrofauna as longer handling time increases the chance of macrofauna escaping capture (Thorson 1957, Degraer et al. 2007).

A difference in body length between sampling methods was apparent in the lower but not upper zone. A possible explanation of this involves: (1) the escape of larger animals associated with longer handling times associated with the HSD, and (2) greater size variation of animals in the lower zone, such that in the lower zone, escapes of larger animals influence the measures of average length in the sample. Mainly larger, highly mobile crustaceans, such as talitrid amphipods, inhabit the upper zone of beaches (McLachlan et al. 1998, Rodil et al. 2006) whereas the lower zone is generally characterised by a broader range of different sized species, including smaller amphipods, isopods and polychaetes (Rodil et al. 2006).
Therefore, escape of larger amphipods is likely to influence the measure of animal size in the sample in the lower but not upper zone. Both methods capture similar sized invertebrates in the upper zone.

The sieving method found a higher abundance and species richness of MIF than the HSD method. This suggests ineffective separation of MIF from the sand sample by the HSD resulting in the capture of fewer invertebrates. The failure of the HSD to effectively separate MIF from sand samples may be a result of escape. Many sandy beach invertebrates have jumping or burrowing abilities to enable tidal migration, avoid predation and desiccation, and to prevent being washed away by wave action (Barnard & Karaman 1991, Marques et al. 2003, Serejo 2004, Bouslama et al. 2007, Rossano et al. 2009, Defeo & McLachlan 2011). Amphipods may escape capture by jumping; either off the collection plate or within the chamber. Other invertebrates such as isopods and polychaetes may escape by burrowing in the sand within the HSD chamber, against the in-flow of water. While we detected the occasional escape, MIF on the collection plate appeared largely immobile; during optimisation of the device we did not consider this would have a major effect on performance.

Whilst the sieving technique captured a reliably consistent suite of species, results of the NMDS show the HSD gives a more variable representation of species composition. The invertebrate families driving the difference between methods in species composition are all species that have significant jumping and/or burrowing abilities (Friend & Richardson 1986, Barnard & Karaman 1991, Lewis & Green 1994, Serejo 2004): Exoedicerotidae (Amphipoda), Cirolanidae (Isopoda), Hyalidae (Amphipoda) and Actaeicidae (Isopoda). The sieving method consistently overcomes these adaptations with a fast and simple mode of operation; the HSD does not. Longer handling time and a complex operating procedure provide opportunities for escape resulting in an inconsistent species composition.

There are a number of improvements to the HSD system that may increase its efficacy and utility. The analysis described here has revealed that some invertebrates may escape from the collection plate and this could be lidded to prevent animals jumping out (Table 2). More regular removal and cleaning of the inlet nozzle and the inclusion of an inlet valve would be likely to improve the separation process further. The process works most efficiently with water that is suspension-free, unlike the water used here which was taken from active surf beaches. However, the transportation of suspension free water to the study sites was deemed infeasible.

Converse to findings of several previous studies describing zonation on sandy beaches (Rodil et al. 2006, 2013), the current study found a higher abundance and species richness in the upper zone. This pattern was unrelated to the sampling method used. The discrepancy may be due to physical differences between beaches such as beach energy, sediment grain size and slope (Rodil et al. 2006, Hacking 2007, McLachlan & Dorvo 2007, Defeo & McLachlan 2011), and biological differences such as wrack deposits (Urban-Malinga et al. 2008, Rodil et al. 2013, Ruiz-Delgado et al. 2014); all important characteristics influencing abundance and species richness on sandy beaches (Rodil et al. 2006, Hacking 2007, McLachlan & Dorvo 2007, Defeo & McLachlan 2011). Seasonal zonation may also have contributed to the zonation pattern found (Bouslama et al. 2009, Ayari & Nasri-Ammar 2011); sampling was conducted in late autumn/early winter, when amphipods occupy the upper zone to avoid inundation by increased wave and storm action (Bouslama et al. 2009).

In summary, the HSD is an inferior sampling technique. It is more complicated to use and less accurate than the sieving method. Overall it also gives a smaller average body size, lower abundance and richness, and an inconsistent species assemblage compared to those given by the standard sieving method. Downfalls of the HSD lie in its inability to effectively separate MIF from sand samples, and to sample the more mobile invertebrate species. These shortcomings may be overcome with future design or protocol adjustments. While it is conceivable to enclose and reduce MIF escape from the collection plate, escape within the chamber seems more difficult to prevent without pressurising the water flow which would involve pumps and power supply. The latter adjustments would render the device less practical. The standard sieving technique...
therefore remains the preferred method for sampling MIF on sandy beaches.

Acknowledgments

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Appendix. Results of random effects included in the models. Fixed effects are reported in Table 2.

<table>
<thead>
<tr>
<th>Response variable analysed</th>
<th>Term</th>
<th>SD</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>(Intercept)</td>
<td>0.207</td>
<td></td>
</tr>
<tr>
<td>Zone (upper)</td>
<td>0.261</td>
<td>–0.711</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abundance</td>
<td>(Intercept)</td>
<td>0.590</td>
<td></td>
</tr>
<tr>
<td>Zone (upper)</td>
<td>0.910</td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.428</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richness</td>
<td>(Intercept)</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>Zone (upper)</td>
<td>0.391</td>
<td>–0.493</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.332</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>