

Notes

Subsampling Reduces Sorting Effort for Waterfowl Foods in Salt-Marsh Core Samples

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Abstract

Waterfowl researchers often use soil core samples to estimate food availability in foraging habitats, and these estimates are needed for bioenergetic models of carrying capacity. However, core sampling is frequently a time- and resource-intensive process, and some researchers have suggested that subsampling may be a valuable way to reduce processing time. We evaluated whether 10% and 25% by mass subsampling are appropriate techniques for reducing core-sorting effort while maintaining precision for samples taken in six separate habitat types along the Delaware bayshore. We found no significant difference between biomass found in 100% sorted cores and estimated biomass obtained by 10% and 25% subsampling. We found that 10% subsampling offered the greatest time savings, reducing mean sorting times by 77% (from 13.7 hours to 3.3 hours) from 100% sorted cores. We recommend that researchers consider subsampling to reduce core-sorting effort and cost, particularly when processing large numbers of cores.

Keywords: Bioenergetics; carrying capacity; core sample; food availability; invertebrate; moist-soil seed; waterfowl

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Introduction

Waterfowl are one of the most intensively managed bird groups in North America, and regional Joint Ventures (interagency working groups, <http://www.fws.gov/birdhabitat/Jointventures/index.shtml>) are tasked with their conservation. A primary goal of Joint Ventures is to reach and maintain population objectives set by the North American Waterfowl Management Plan (U.S. Fish and Wildlife Service 2012). Joint Ventures managing wintering birds are primarily concerned with providing foraging habitat because food is assumed to be a limiting factor on wintering grounds. To determine how much habitat is required to meet population objectives, Joint Venture scientists often use bioenergetics modeling, which combines landscape energy availability and waterfowl energy demand to estimate regional waterfowl carrying capacities. Thus, estimating how much food is provided by a given habitat type is a critical component in determining available energy for waterfowl populations and is necessary to inform management decisions (Central Valley Joint Venture 2009).

Core sampling is a common technique used to quantify seed and invertebrate availability in wetlands (Swanson 1983; Anderson and Smith 2000; Stafford et al. 2006). Typically, substrate samples are collected in the field using a cylindrical coring device and transported to a laboratory for preservation and processing at a later date. Cores are then meticulously sorted under dissecting microscopes to remove food items. Food items are dried and weighed, and biomass is extrapolated to the landscape level to estimate food availability in a given region (Anderson and Smith 1999; Cramer et al. 2012). Sorting core samples is frequently a tedious, resource-intensive process, especially for cores collected in sites with high densities of small seed or invertebrate species. Thus, it is advantageous to develop methodologies that reduce the time and effort involved in soil core sampling. Subsampling may reduce both time and money required to process soil cores.

If there is no difference in food estimates achieved by extrapolating from subsampling versus sorting the entire core, then subsampling may be a useful tool for reducing time and effort associated with core sampling (Kross et al. 2008; Hagy et al. 2011; Stafford et al. 2011).



Hagy et al. (2011) and Stafford et al. (2011) have assessed the time savings associated with subsampling in wetlands in the Mississippi Flyway, which are characterized by high densities of seeds. However, because invertebrate foods are more prevalent than seeds in many saltmarsh systems (Cramer et al. 2012), it is important to ensure that subsampling accurately represents both seed and invertebrate biomass. Our goal was therefore to determine if subsampling cores is an appropriate substitute for sorting complete cores collected in habitats associated with an Atlantic coastal saltmarsh system and compare time savings associated with subsampling different percentages of the larger core sample.

Study Site

We collected core samples across a ~60 km span of the Delaware bayshore between Kent and Sussex counties (39°17'N, 75°27'W to 38°48'N, 75°12'W) during October, January, and April of 2011–2012 and 2012–2013. This region is of critical conservation importance to migrating shorebirds and wintering waterfowl, such as the American Black Duck (*Anas rubripes*). Our system was characterized by a range of habitat types that varied in salinity and management regime. We collected samples in several impounded marshes in addition to unmanaged saltmarshes, which we separated into five habitat categories: subtidal, mudflat, low marsh, high marsh, and quasi-tidal pools. Subtidal habitats included areas below the mean low tide line and were irregularly exposed. Mudflat habitats included regularly flooded and exposed expanses largely devoid of vegetation. Low marsh habitat consisted of regularly flooded areas between mean high and low tide, dominated by *Spartina alterniflora*. High marsh consisted of irregularly flooded areas above mean high tide line, dominated by *Spartina patens*. Quasi-tidal pools were present within high marsh habitats and were relatively constant areas of standing water subject to some tidal exchange (Cramer et al. 2012).

Methods

We collected cores using a custom-built PVC corer measuring 5.1 cm in diameter by 12.7 cm in depth. Upon collection, we transported samples to the laboratory and refrigerated them for ≤ 3 days before washing and fixing them with a 10% formalin solution and Rose Bengal dye. We stored cores in plastic cups for sorting at a later date. Prior to processing, we washed samples to remove formalin and then passed the material through a size 10 (2 mm opening) sieve and a size 60 (0.251 mm opening) sieve. We categorized material remaining in the size 10 sieve as “large”, and material remaining in the size 60 sieve as “small”. Using forceps, we sorted cores to separate seeds and invertebrates from nonfood material under 6 \times magnification dissecting microscopes. We did not dry cores prior to subsampling and sorting to avoid desiccating invertebrate foods. We sorted portions of cores in petri dishes, and kept the remainder of each core in an

airtight cup to prevent desiccation while sorting. The first 10 samples sorted by each technician were checked by experienced technicians to ensure that all food items were removed and identified.

We randomly selected 12 cores (two per habitat type) for a 25% by mass subsample and 12 additional cores (two per habitat type) for a 10% by mass subsample using methods similar to Hagy et al. (2011) and Stafford et al. (2011). Following their design, we first sorted through 100% of “large” material, removed seeds and invertebrates, separated them at the lowest possible taxonomic level to which they could be identified (typically genus-level for seeds and order-level for invertebrates), and dried and weighed them to 0.0001 g. Second, we subsampled “small” material, which is the most time-consuming to process. For the two subsampling methodologies, we weighed the “small” material to 0.1 g and removed and sorted 25% or 10% by mass. We separated, dried, and weighed seeds and invertebrates to 0.0001 g and multiplied biomass by 4 or 10 (respectively) to estimate total mass. To assess whether there were differences in subsample estimates versus the complete core, we then sorted the remaining 75% or 90% of the “small” material in our subsampled cores to determine the actual mass of each item in the complete core sample. We added subsampled or fully-sorted “small” biomass to “large” material biomass, resulting in a total estimated and actual biomass value for each sample (see *Supplemental Material*, Table S1, DOI: <http://dx.doi.org/012014-JFWM-002.S1>).

We combined cores from all habitats to test whether subsampling was effective regardless of habitat type, and because sample sizes were too small to test for differences in individual habitat types. We conducted paired Student's t-tests using R (R Core Development Team, Version 2.15.3, 2013) to determine if there was a significant difference between biomass as estimated by our subsampling methods and actual biomass in each core. We also quantified the amount of time spent sorting 10%, 25%, and 100% to assess the time savings associated with subsampling. We recorded sorting time for each approach for the cores used in the 10% and 25% subsampling tests, as well as for additional cores collected from our field sites during a concurrent study, to increase sample size and provide more accurate estimates of time savings.

Results

On average, “small” material made up 49.57% (\pm SE 7.02%) of food biomass in the 25% subsampling test, and 71.29% (\pm 6.01%) of food biomass in the 10% subsampling test, with the remainder composing “large” material. Thus we concluded that our subsampling technique operated on a large enough proportion of each sample such that our total biomass estimates were not dominated by “large” unsorted material. For our 25% subsampling test, invertebrates composed 22.62% (0.0114 ± 0.0043 g) of total core biomass on average, with seeds composing the remainder (0.0390 ± 0.0165 g, Figure 1). For our 10% subsampling test, invertebrates

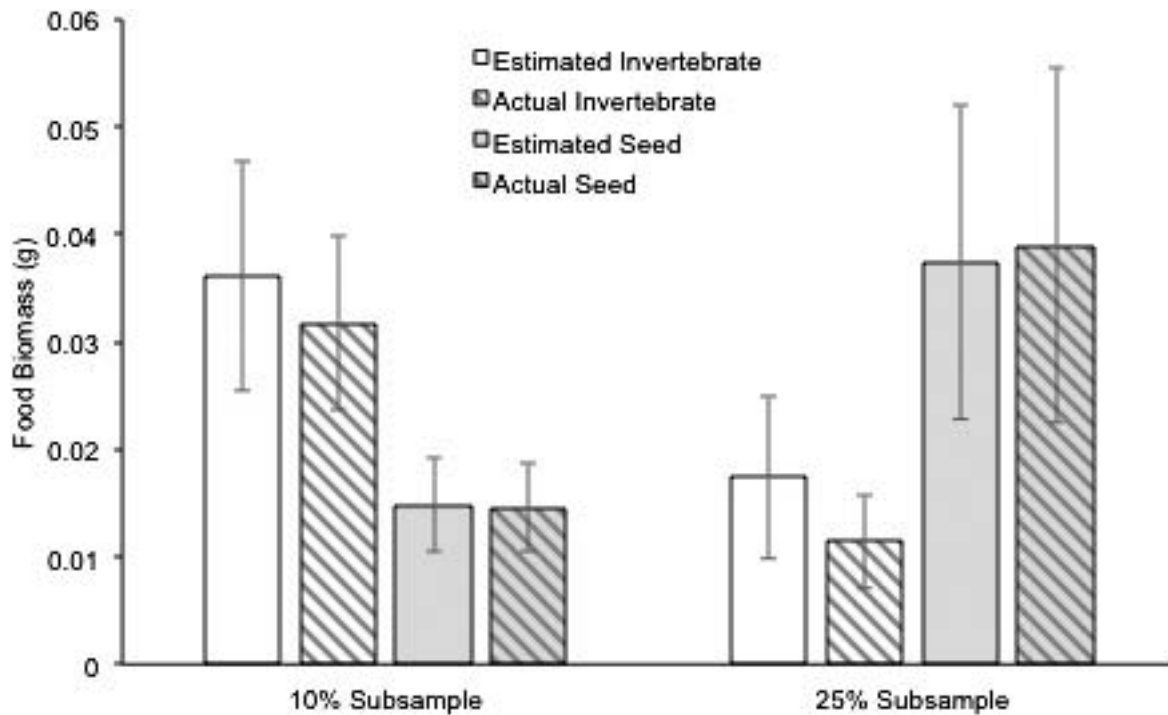


Figure 1. Average estimated versus actual food biomass for seeds and invertebrates in 10% subsampling trial ($n = 12$) and 25% subsampling trial ($n = 12$) from soil cores collected in October, January, and April 2011–2012 and 2012–2013 across a ~60 km span of the Delaware Bayshore.

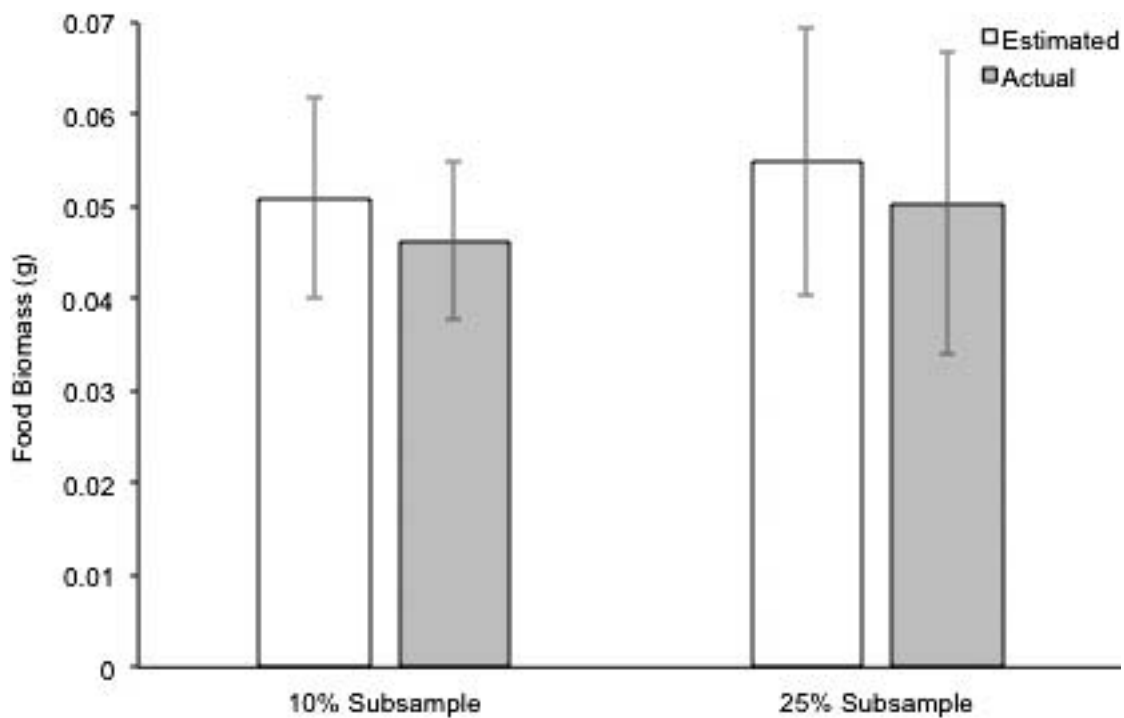


Figure 2. Average estimated total food biomass versus actual total food biomass for 10% subsampling trial ($n = 12$) and 25% subsampling trial ($n = 12$) from soil cores collected in October, January, and April 2011–2012 and 2012–2013 across a ~60 km span of the Delaware Bayshore.

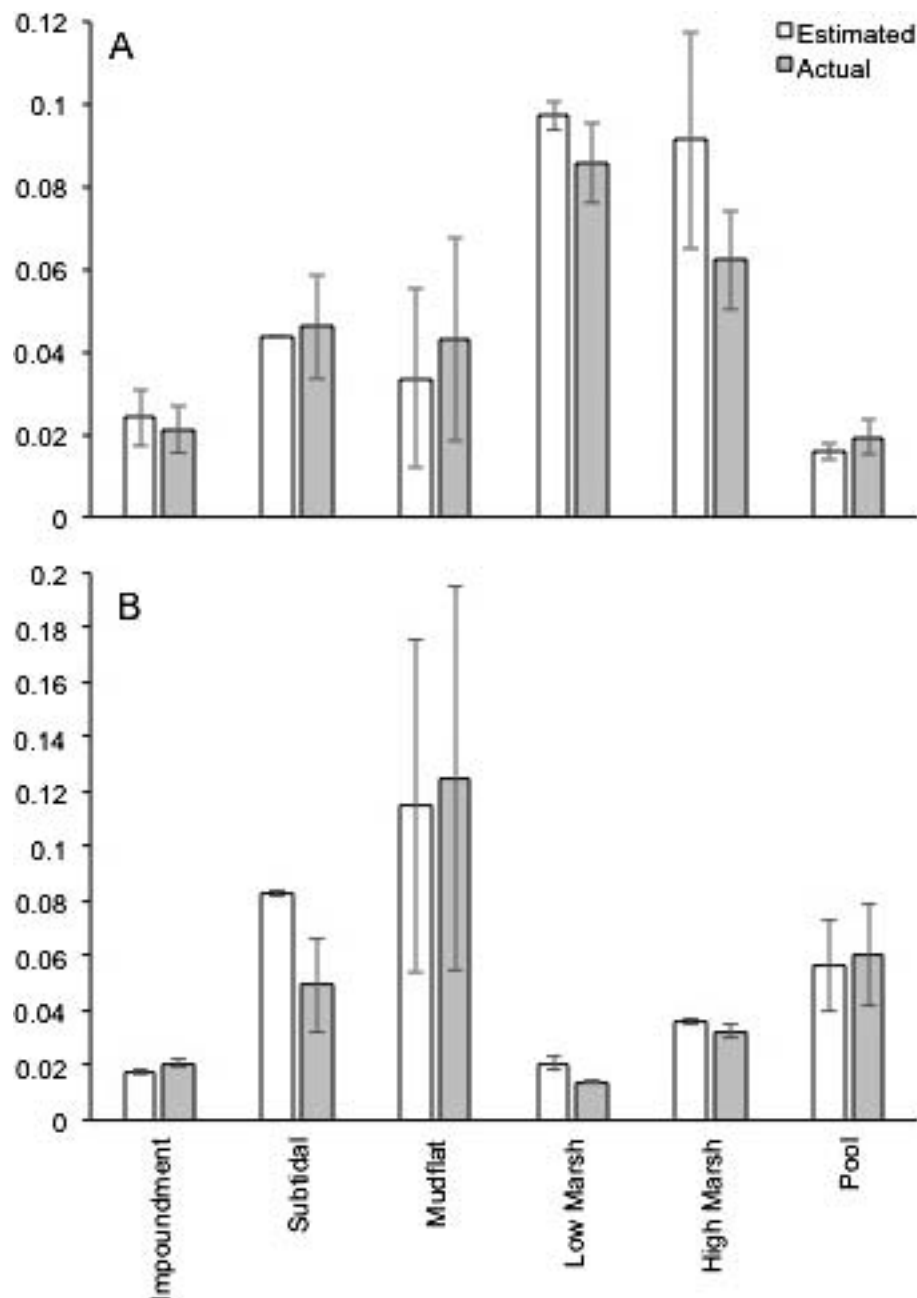


Figure 3. Mean and standard error of estimated food biomass versus actual food biomass by habitat type ($n = 2$ per habitat) for 10% subsampling trial (A) and 25% subsampling trial (B) from soil cores collected in October, January, and April 2011–2012 and 2012–2013 across a ~60 km span of the Delaware Bayshore.

composed 68.68% (0.0318 ± 0.0081 g; seed biomass 0.0145 ± 0.0041 g, Figure 1). Although we noted a large difference in proportion of seeds to invertebrates between our 10% and 25% subsampling tests, these biomass estimates came from two different sets of cores, and so these differences reflect inherent variation between the two sets of cores. Subsampling accurately predicted both seed and invertebrate biomass independently (Figure 1).

Estimated biomass for the 25% subsampling test (0.0549 ± 0.0146 g) was not significantly different from the actual biomass of the fully sorted core (0.0504 ± 0.0164 g; $t_{11} =$

0.80 , $P = 0.44$). Estimated biomass for the 10% subsampling test (0.0510 ± 0.0109 g) was not significantly different from the actual biomass of the fully sorted core (0.0463 ± 0.0086 g; $t_{11} = 0.91$, $P = 0.38$, Figure 2). For the 25% test, estimated biomass was 0.0045 g higher than actual biomass on average. For the 10% test, estimated biomass was 0.0047 g higher on average. While sample size was too small to statistically examine subsampling techniques per individual habitat type, we anecdotally found little difference between subsamples and complete cores in any of the various habitat types (Figure 3).

Table 1. Processing times and statistics for soil cores from five habitat types for 100% sorted cores (Full Core), 25% subsampled cores, and 10% subsampled cores, collected in October, January, and April 2011–2012 and 2012–2013 across a ~60 km span of the Delaware Bayshore. Sorting time data was collected for the 24 cores used in the 25% and 10% subsampling tests, as well as for additional cores from ongoing studies. Time = Mean sorting time in hours, SE = standard error in hours, n = number of cores, ΔT = difference in sorting time between 100% sorted and subsampled cores, and % Time = percentage of time saved on average compared to 100% sorted cores.

Habitat type	Time	SE	ΔT	% time	n
Full core					
Impoundment	10.2	1.3			78
Subtidal	21.7	5.3			14
Mudflat	16.3	6.1			14
Low Marsh	11.6	3.2			15
High Marsh	14.2	3.4			15
Pool	8.3	1.8			14
Average	13.7	3.5			
25% subsample					
Impoundment	7.3	1.1	−2.9	28%	283
Subtidal	9.5	1.7	−12.2	56%	43
Mudflat	5.5	1.0	−10.8	66%	41
Low Marsh	8.0	1.2	−3.6	31%	43
High Marsh	9.4	1.6	−4.8	34%	43
Pool	7.7	1.6	−0.6	7%	41
Average	7.9	1.4	−5.8	37%	
10% subsample					
Impoundment	2.6	1.1	−7.6	74%	9
Subtidal	7.0	1.7	−14.7	68%	7
Mudflat	2.0	1.0	−14.3	88%	6
Low Marsh	3.2	1.2	−8.4	72%	6
High Marsh	4.2	1.6	−10.0	70%	6
Pool	1.0	1.6	−7.3	88%	3
Average	3.3	1.4	−10.4	77%	

We found that subsampling cores reduced processing times significantly (Table 1). Using 25% subsampling reduced mean sorting time by 37% ($\Delta T = -5.8$ hours) across all habitat types; using 10% subsampling reduced mean sorting time by 77% ($\Delta T = -10.4$ hours). In some cases, subsampling led to dramatic time savings: 10% subsampling of mudflat habitat decreased sorting time by 14.3 hours (88% reduction) per core.

Discussion

To our knowledge, all evaluations of subsampling prior to this study have been conducted on cores collected in freshwater wetlands dominated by seeds (Hagy et al. 2011; Stafford et al. 2011). Because Atlantic saltmarshes have a high invertebrate to seed biomass ratio compared to mid-continental freshwater wetlands (Plattner et al. 2010; Cramer et al. 2011), it is important to test the efficacy of subsampling on cores collected from the Atlantic region. We conclude that because there is no statistically significant difference between actual core biomass and biomass estimated via subsampling, both

10% and 25% subsampling techniques may be appropriate for reducing core sorting time in cores collected from invertebrate dominated saltmarsh systems. Nevertheless, the low sample sizes presented in this study limit the power of our analysis; we encourage future researchers to confirm our results using larger sample sizes. Our results are consistent with Stafford et al. (2011), who found a strong correlation between seeds found in completely sorted versus subsampled cores. Quantifying waterfowl food abundance along the Atlantic coast has become a research priority in recent years (Sherfy 1999; DiBona 2007), and our results may help reduce the time and expense of future studies.

We noted that the proportion of invertebrates to seeds differed between the 10% and 25% subsampling tests. We can think of no *a priori* reasons why 10% versus 25% subsampling may be biased towards either seeds or invertebrates. Thus, we conclude that these differences are due to inherent variation in food biomass between the two sets of cores; the subsampled (estimated) and fully sorted (actual) biomass values are similar for both seeds and invertebrates

(Figure 1), and so subsampling apparently captures this natural variation.

Hagy et al. (2011) found that subsampling 25% by mass reduced sorting times by 59% (from 219.1 min to 89.1 min; H. M. Hagy, Illinois Natural History Survey, personal communication), consistent with our time savings for subtidal and mudflat habitats (Table 1). Subsampling was an effective means of reducing sorting times for all other habitat types as well. Subsampling 10% by mass was most effective, reducing sorting times from 13.7 hours to 3.3 hours on average, representing a 77% reduction. Bioenergetics studies often involve processing a large number of core samples; for example, Cramer (2009) collected 1,020 cores to assess food resource availability in southern New Jersey. Each sample required approximately 13.7 hours of processing time to completely sort, but only 3.3 hours to sort using 10% subsampling (average across all habitat types). At a hypothetical technician salary of \$12.00/hour, 10% subsampling potentially saves more than \$120,000 in technician costs. Subsampling is thus a valuable tool researchers should consider if core sample processing effort proves to be temporally and financially prohibitive.

Supplemental Material

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Table S1. Actual and estimated invertebrate and seed biomass data in grams for 25% and 10% subsampling trials for core samples collected in Atlantic coastal marsh habitats.

Found at DOI: 10.3996/http://dx.doi.org/012014-JFWM-002.S1 (18 KB XLSX)

Reference S1. Central Valley Joint Venture. 2009. Central Valley Joint Venture Monitoring and Evaluation Plan.

Found at DOI: 10.3996/http://dx.doi.org/012014-JFWM-002.S2; also available at http://www.centralvalleyjointventure.org/assets/pdf/CVJV_Wintering_Waterfowl_Monitoring_Evaluation_Plan.pdf (370 KB PDF)

Reference S2. U.S. Fish and Wildlife Service. 2012. North American Waterfowl Management Plan.

Found at DOI: 10.3996/http://dx.doi.org/012014-JFWM-002.S3; also available at <http://nawmprevision.org/sites/default/files/NAWMP-Plan-EN-may23.pdf> (2670 KB PDF)

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