APPENDIX A

EVALUATION OF A MODIFIED ACTIVITY TRAP FOR INVERTEBRATE SAMPLING IN SHALLOW WETLANDS

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ABSTRACT

Standard design of invertebrate activity traps limits their use to water depths that exceed the maximum foraging depth of most shorebirds. Assessment of nektonic invertebrate availability in shallow waters is desirable because shorebird diets may include these organisms. Thus, we designed a modified activity trap to assess invertebrate abundance in water depths ≤10 cm. We tested our design by collecting paired activity trap and benthic core samples during spring 1996 in moist-soil impoundments at Back Bay National Wildlife Refuge, Virginia. Twenty-four taxa were detected by 1 or both samplers. Four relatively mobile taxa occurred with greater frequency in activity traps, whereas 3 benthic taxa occurred with greater frequency in core samples. Number of individuals per sample was correlated (r = 0.43 to 0.78, $P \leq 0.004$) between samplers for five taxa. Our results indicate that standard invertebrate sampling methods may underestimate availability of mobile taxa. Further, core samples may fail to detect large, mobile organisms that may substantially contribute to the diet of large shorebirds. We recommend that shallow-water activity traps be used as a supplement to benthic core sampling to more accurately describe shorebird food availability.

INTRODUCTION

Temperate zone habitats used by migrant shorebirds include sandy beaches, tidal mud flats, and moist-soil impoundments. Aquatic invertebrates are a primary component of their diet, including both benthic and nektonic organisms (Baker 1977, Rundle 1982, Baldassarre and Fischer 1984, Weber and Haig 1997*a*). Assessment of shorebird food availability typically focuses on benthic invertebrates through collection of core samples (Schneider and Harrington 1981, Hicklin and Smith 1984, Mercier and McNeil 1994, Weber and Haig 1996, 1997*b*, Safran et

al. 1997). To the extent that nektonic organisms make a substantial contribution to shorebird diets, methods for sampling these organisms also are desirable.

Swanson (1978*a*), Euliss et al. (1992) and Mackay and Euliss (1993) described modified core samplers designed to quantitatively sample benthic and nektonic organisms, including those found at the mud-water interface. Nonstationary sampling devices (e.g., core samplers and sweep nets) disturb the water column and substrate upon deployment, and instantly collect samples. Murkin et al. (1983) discuss 3 potential biases associated with nonstationary devices: (1) organisms capable of rapid movement may avoid moving samplers; (2) diel shifts in horizontal and vertical distribution are not accounted for by instantaneous sampling; and (3) collector's bias is introduced because no two samples can be identically collected, even by the same investigator. Stationary samplers may reduce these biases because (1) sampling over time does not preclude collection of mobile organisms; and (2) investigator bias is reduced because sample collection does not depend on deployment motion.

Activity traps are common stationary samplers that typically consist of a 2-L bottle with an inverted funnel affixed to the mouth (Ross and Murkin 1989, Murkin et al. 1983). These samplers are suspended in the water column, requiring a minimum water depth equal to the horizontal profile of the bottle (about 11 cm for a 2-L soda bottle). Water depth is an important predictor of shorebird distribution; mean water depths favored by most North American species are ≤10 cm (Weber and Haig 1996, Safran et al. 1997). Although the usefulness of activity traps in assessing waterfowl food availability is recognized (e.g., Armstrong and Nudds 1985, Cox et al. 1998), application of the technique to studies of shorebird foods is constrained by lack of an adequate sampler design.

Activity traps continually collect organisms through the period of deployment, and thus do not allow estimation of absolute invertebrate density (Murkin et al. 1983). They are, however, useful for identifying mobile taxa and making relative comparisons of invertebrate availability among habitats. Given the advantages of activity trap sampling and the potential significance of nektonic organisms to shorebirds, a modified design that facilitated use in shallow water wetlands was desirable. The goal of this study was to evaluate the efficiency of a modified shallow-water

activity trap relative to standard benthic invertebrate sampling methods.

METHODS

Sampler Design

We constructed activity traps from 225-cm^2 polyethylene tissue culture flasks (Corning #431081; use of trade names does not imply U.S. Government endorsement). These flasks are designed for sterile applications and are often discarded by research laboratories after a single use. Each trap was constructed from 2 flasks. One flask served as the body of the trap. The funnel consisted of the neck and shoulder portion sawed from a second flask (Fig. A.1). Necks of the body and funnel were joined by a 5-cm length of 3.2-cm diameter plastic tubing. The tubing fit snugly over the flask necks such that no additional securing devices were required. Flask volume was approximately 900 ml.

Field Testing

We conducted field testing of our activity trap design at Back Bay National Wildlife Refuge, Virginia, in conjunction with an ongoing study of aquatic invertebrate responses to discing of moist-soil impoundment vegetation. Sampling occurred on 16 randomly located 30-m x 75-m permanent study plots that were equally distributed among 2 freshwater impoundments (537 and 475 ha, respectively) and 2 experimental treatments (disced and undisced). Sampling locations varied in substrate composition and vegetative cover, and accordingly varied in use by migrant shorebirds.

Sampling was conducted during 3 periods (19-20 May, 31 May-2 Jun, and 13-15 Jun 1996). During each period, 1 activity trap was set at a random location in each plot. Random locations were constrained to areas with water depths of approximately 10 cm. Traps were deployed by submerging the body portion at an angle, allowing the trap to slowly fill from the surface of the water column. Air bubbles were eliminated by lightly tapping or squeezing the trap body (Swanson 1978*b*). Once completely filled, the trap was allowed to rest horizontally on the substrate (Fig. A.1). The funnel and tubing were then submerged and affixed to the body. Traps were oriented with the funnel opening opposite the prevailing wind direction to prevent

accumulation of organisms from wind-induced drift. Off-center flask necks allowed funnels to rest directly on the substrate (Fig. A.1).

After 24 hr, activity traps were retrieved and a benthic core sample (10 cm deep x 10 cm diameter) was collected from an area within 0.25 m of each trap location. Prior to removing activity traps from the water, the funnel and tubing were removed and the threaded flask cap was replaced. This method prevented invertebrates remaining in the funnel and tubing from entering the trap during retrieval. Contents of activity traps and core samples were washed over a self-cleaning 550-μm sieve (Euliss and Swanson 1989). Material retained on the sieve was placed in 250-ml plastic jars, and preserved in 95% ethanol stained with Rose Bengal. Samples were kept cool until they were sorted in the laboratory. Core and activity trap samples were hand sorted in shallow plastic pans under bright light. All organisms encountered were removed and identified using Merritt and Cummins (1996) and Thorp and Covich (1991). We classified most organisms to Class or Order, except where Family level taxonomy was of potential significance (e.g., Diptera). A category for Diptera egg masses was included because they are a known shorebird food (Baker 1977).

Under the null hypothesis of no difference in efficiency between core samples and activity traps, taxa should occur with similar frequency in both types of samples. We tested this hypothesis using McNemar's χ^2 test for paired-sample nominal data, using frequency of occurrence among trap-days as the response variable (Zar 1996). Although actual density estimation from activity traps is unreliable, we also predicted that the number of individuals occurring in activity trap samples should be correlated with density estimates from paired core sample locations (Murkin et al. 1983). Thus, we used correlation analysis to describe the relationship between number of individuals/trap for the 2 samplers. As the consequences of Type II error were deemed minimal, we accepted statistical significance at $P \leq 0.10$.

RESULTS

We collected 37 paired activity trap and core samples across the 3 sampling periods. Samples were not obtained for 11 potential locations because plots were too dry. Twenty-four taxa were collected, of which frequencies were adequate to allow χ^2 analysis of relative

occurrence rates for 15. Seven taxa were disproportionately represented ($P \leq 0.08$) between paired samples (Table A.1). Oligochaeta, Chironomidae, Ceratopogonidae, and Amphipoda occurred more frequently ($P \leq 0.052$) in core samples, whereas Corixidae, Coleoptera adults, and Coleoptera larvae occurred more frequently ($P \leq 0.08$) in activity traps. Collection rates of four common taxa (Gastropoda, Cladocera, Copepoda and Ostracoda) did not differ ($P \geq 0.13$) between samplers. Nine uncommon taxa were collected in only 1 sampler; 6 in activity traps, and 3 in core samples (Table A.1). Rank order of taxa occurrence rates did not differ between samplers (Wilcoxon signed rank test, $P = 0.7015$). Mean number of taxa/sample collected in activity traps (6.9, SE = 0.4) and core samples (6.7, SE = 0.2) were not different ($t = 0.39$, $P = 0.70$).

Despite the apparent differences in efficiency, number of individuals/trap of 5 taxa was correlated ($r = 0.43$ to 0.78, $P \leq 0.004$; Table A.1) between the 2 sampling methods. These taxa included 3 macroinvertebrates that were also disproportionately sampled (Oligochaeta, Coleoptera larvae and Chironomidae), and 2 crustaceans with similar occurrence rates (Cladocera and Copepoda; Table A.1). However, total number of invertebrates/sample was not correlated ($r = 0.14$, $P = 0.25$) between samplers.

DISCUSSION

Disproportionate occurrence rates of a given taxon may be a consequence of several factors. Although the distribution of many aquatic organisms is heterogeneous, the paired nature of our sampling design should minimize variance in density between samplers. Further, our sample size ($n = 37$) should provide adequate power to test the hypothesis of no difference in occurrence rates between samplers. Thus, we conclude that disproportionate occurrence rates did not result solely from spatial variation in invertebrate distribution between activity trap and core sample locations.

Mobility and habit (Merritt and Cummins 1996) may more adequately explain the observed differences in occurrence rates. Taxa more common in activity traps were generally more mobile (e.g., Coleoptera adults and larvae, Corixidae). Conversely, taxa occurring more

frequently in core samples were either benthic or epiphytic (Amphipoda, Oligochaeta, Chironomidae, Ceratopogonidae). That the 2 samplers would disproportionately collect taxa due to mobility differences is not unexpected. However, this analysis shows the importance of implementing several sampling methods where development of accurate species lists is desirable.

When our results were combined across taxa, number of taxa/sample did not differ between samplers. Further, the overall rank order of occurrence rates was similar for both samplers. Differences between samplers were only evident within taxa and among samples. These analyses may be the most ecologically significant, because fine scale variation in prey abundance is an important predictor of shorebird distribution (Colwell and Landrum 1993).

Low occurrence of some taxa in both samplers may similarly result from inefficiency of both samplers or low overall abundance on the study area. Taxa collected only in activity traps included 3 highly mobile organisms: brine shrimp (Anostraca), fish (Osteichthyes) and tadpoles (Amphibia). Occurrence rates for these taxa were at least an order of magnitude higher in activity traps (Table A.1) than in 720 core samples concurrently collected on the study area (0.0%, 0.1%, and 0.7%, respectively; M. Sherfy, unpublished data). Although low collection rates in this study precluded statistical analysis for these taxa, we believe their occurrence only in activity traps was indicative of higher sampler efficiency. The potential for these organisms to be consumed by shorebirds (Stenzel et al. 1976) indicates the need for adequate assessment of their presence in foraging areas.

Several families of Coleoptera adults and larvae were represented in our samples (Haliplidae, Dytiscidae, and Hydrophilidae). Statistical analysis of data for separate families was precluded by small sample sizes. These families are not generally associated with benthic habitats, but showed a weaker pattern of disproportionate occurrence than did Corixidae, a more mobile family that occurs more frequently in the water column (Merritt and Cummins 1996). Amphipoda generally forage in benthic habitats and exhibit pronounced daily movements through the water column (Thorp and Covich 1993). Although they were detected more frequently in core samples ($P = 0.052$), they occurred in activity traps more frequently than most other taxa (Table

A.1). Timing of our sample collection (generally in late afternoon) may have coincided with a daily period of preferential use of benthic habitats, leading to greater occurrence in core samples.

Corixidae and Coleoptera adults and larvae may compose a substantial portion of shorebird diets (Rundle 1982, Baldassarre and Fischer 1984, Weber and Haig 1997*b*). To the extent that core samples fail to detect these organisms, shorebird food availability and use-days may be underestimated. Although core sampling more accurately represents the substrate probing of some shorebirds, activity traps may more accurately detect some prey items. Lack of significant correlation between number of individuals/sample for Coleoptera adults and Corixidae further emphasizes the inadequacy of core samples for accurate density estimation of these taxa.

Consumption of invertebrates by predatory organisms caught in activity traps may bias density estimation of prey (Swanson 1978*b*, Murkin et al. 1983). Similar collection rates between samplers for benthic organisms, combined with consumption of these organisms by predators in activity traps, could cause apparent lack of occurrence for some prey taxa. Because we based our between-sampler comparisons on occurrence rather than density, a predator would have to remove all individuals of a taxon to produce such a false negative. Among the 4 benthic taxa for which we found disproportionately low occurrence rates in activity traps, about 85% of samples contained >1 individual/trap. Further, there was no correlation between density of these 4 taxa and predator density in activity traps ($r = 0.16$, $P = 0.32$). Although density of prey may have been artificially depressed by the presence of predators, we conclude that absence of some organisms from activity traps was not solely a function of predation. Removing air bubbles prior to deploying traps eliminates the air supply to adult beetles (Swanson 1978*b*). However, some adult beetles and all fish, tadpoles and odonates in our activity traps were alive when traps were retrieved.

The modified core samplers described by Euliss et al. (1992), Mackay and Euliss (1993) and Swanson (1978*a*) may be appropriate where accurate nekton density estimation is desirable. However, several highly mobile taxa were either not detected or detected at significantly lower rates in our core samples. Modified coring devices designed to simultaneously sample benthic and nektonic habitats may be similarly biased against some taxa. These devices are also costly and contain moving parts that may break down with continued use. Because we used discarded

flasks, our activity traps cost less than $1 each and could be constructed in a few minutes.

Significant correlation between number of individuals/sample for the 2 methods suggests that activity traps could be used to assess the relative abundance of some taxa among habitat types. Murkin et al. (1983) demonstrated a significant correlation between invertebrate abundance in activity traps and sweep nets. Their comparison demonstrated sampling variation between 2 samplers designed to sample nektonic organisms. Conversely, we tested samplers that target different habitats, and demonstrated that between-sampler variation in taxon occurrence rates is reflective of mobility and life-history characteristics. Our results corroborate those of Whiteside and Lindegaard (1980), who showed disproportionate collection of some taxa by activity traps. Similarly, Cheal et al. (1993) found low species richness in benthic core samples relative to sweep nets and tow nets.

The interface between benthic and nektonic habitats may not be clearly demarcated, especially where water is shallow and soils are highly organic. Invertebrate sampling may be highly desirable in these areas, as they may be the most productive (Nelson and Kadlec 1984) and the most favored shorebird foraging areas (Weber and Haig 1996). Methods that sample across habitat types are desirable where some organisms actively move between habitats (Cheal et al. 1991). However, pooling data from different sampling devices can introduce bias because interface organisms are double-sampled (Euliss et al. 1992). Several interface taxa (e.g., Chironomidae, Amphipoda) were among the most commonly occurring organisms in this study (Table A.1).

One drawback to activity trap sampling in shallow waters is a the potential for trap failure. Warm, sunny weather is common on our study area during late shorebird migration, making small pools of standing water susceptible to rapid evaporation. However, the low labor and monetary costs required to employ our sampler make collection of many samples feasible. Our activity traps were successfully deployed in water depths of 5 - 20 cm with no anchoring devices, and were not displaced during windy conditions.

There are both advantages and disadvantages to invertebrate sampling with activity traps. No single sampler can collect all taxa without bias. Given the potential importance of both benthic

and nektonic organisms to shorebird diets, we recommend that shallow-water activity traps be used as a supplement to benthic core sampling to more accurately describe shorebird food availability.

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Table A.1. Frequency of occurrence (%) and abundance for 25 aquatic taxa in paired activity trap and core samples (*n*=37) from freshwater moist-soil impoundments, Back Bay National Wildlife Refuge, Virginia Beach, Virginia.

	Frequency of Occurrence[a]				Abundance[b]					
					Activity Trap		Core Sample			
	Activity Trap	Core Sample	χ^2	*P*	Mean	SE	Mean	SE	*r*	*P*
Mollusca										
Bivalvia	0.0	16.2			0	0	0.3	0.1		
Gastropoda	91.9	78.4	2.286	0.130	16.1	4.5	5.9	1.2	0.12	0.49
Oligochaeta	56.8	89.2	7.562	0.006	37.3	16.4	18.1	4.7	0.52	<0.001
Arachnida	10.8	0.0			0.1	0.1	0	0		
Insecta[c]										
Ephemeroptera	2.7	0.0			<0.1	<0.1	0	0		
Odonata	5.4	2.7	<0.001	>0.999	<0.1	<0.1	<0.1	<0.1	-0.04	0.815
Hemiptera										
Corixidae	81.1	21.6	18.375	<0.001	16.6	5.7	2.3	1.8	0.03	0.83
Coleoptera										
Larvae	43.2	21.6	3.062	0.080	1.1	0.2	0.3	0.1	0.43	0.008
Adults	32.4	10.8	4.083	0.043	0.5	0.2	0.2	0.1	-0.04	0.81
Diptera										
Chironomidae	48.7	78.4	7.692	0.005	4.5	1.7	25.3	7.7	0.46	0.004
Ceratopogonidae	5.4	29.7	4.923	0.026	<0.1	<0.1	0.5	0.1	-0.13	0.45
Other Families	0.0	10.8			0	0	0.2	0.1		
Pupae	21.6	21.6	<0.001	>0.999	0.4	0.2	0.4	0.2	0.17	0.323
Adults	0.0	2.7			0	0	<0.1	<0.1		
Egg Masses	16.2	13.5	<0.001	>0.999	0.3	0.1	1.8	1.2	-0.05	0.74
Hymenoptera	2.7	0.0			<0.1	<0.1	0	0		
Crustacea										
Amphipoda	64.9	89.2	3.765	0.052	18.9	9.2	12.9	3.6	-0.05	0.75
Branchiopoda										
Cladocera	43.2	62.2	1.714	0.190	38.1	25.8	1.9	0.5	0.78	<0.001
Anostraca	2.7	0.0			<0.1	<0.1	0	0		

Table A.1, cont'd.

	Frequency of Occurrence[a]				Abundance[b]					
					Activity Trap		Core Sample			
	Activity Trap	Core Sample	χ^2	P	Mean	SE	Mean	SE	r	P
Copepoda	40.5	37.8	<0.001	>0.999	33.3	22.5	3.4	1.5	0.52	0.001
Isopoda	24.3	8.1	2.50	0.114	7.2	6.5	0.4	0.2	-0.04	0.80
Ostracoda	89.2	78.4	0.9	0.343	263.7	146.7	20.4	10.5	0.19	0.25
Osteichthyes	2.7	0.0			<0.1	<0.1	0	0		
Amphibia[d]	8.1	0.0			0.1	<0.1	0	0		

[a] (Number of samples containing taxon / 37) x 100.
[b] Number of individuals/trap.
[c] Nymphs or larvae, unless otherwise noted.
[d] Tadpoles.

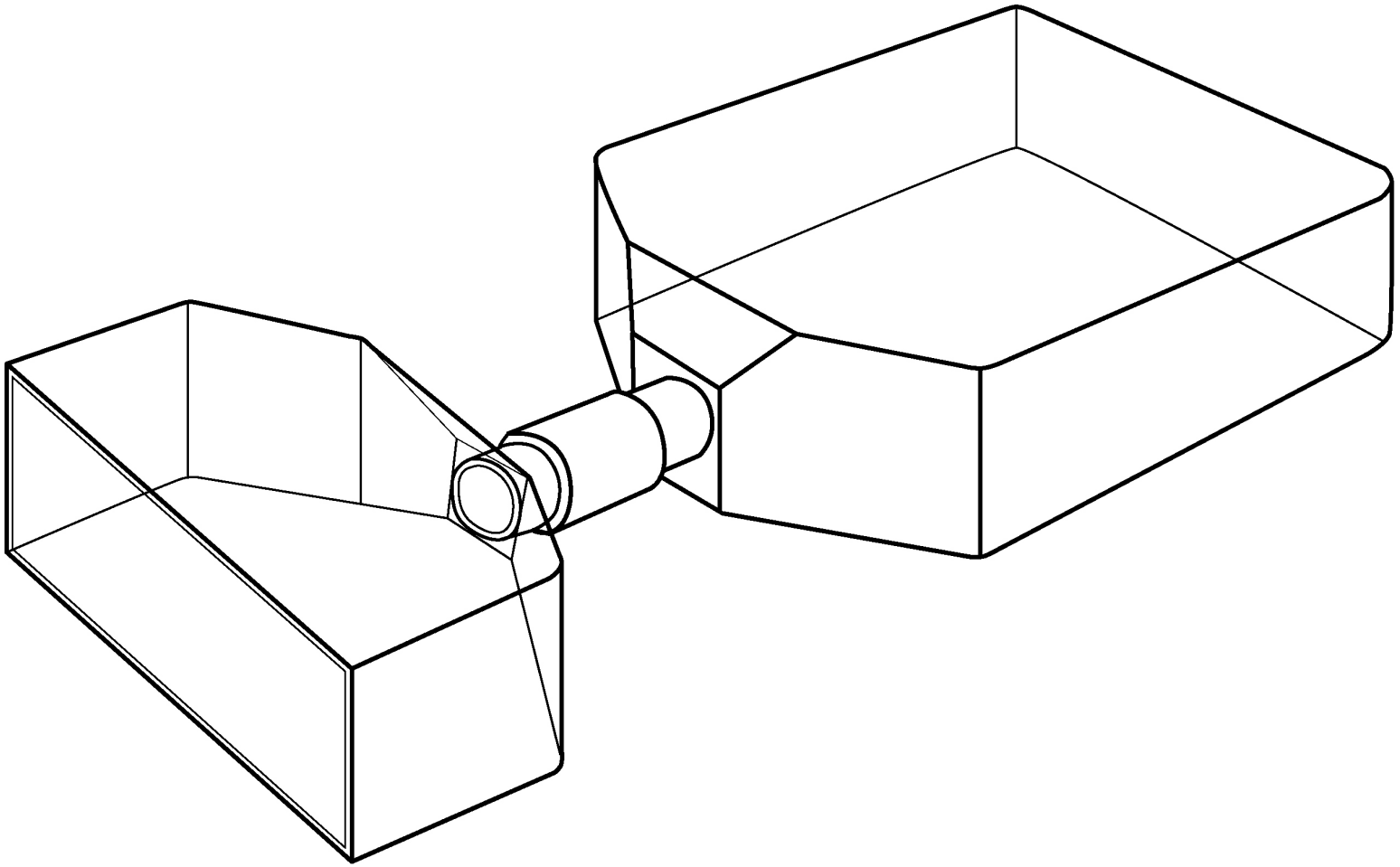

Figure A.1. Construction details for shallow-water activity traps.

APPENDIX B

ASSESSING SHOREBIRD FOOD AVAILABILITY:

CORE SAMPLING DEPTH AND CHIRONOMID VERTICAL DISTRIBUTION

(Submitted to *The Wildlife Society Bulletin, July 28, 1998)*

ABSTRACT

Benthic core samples are widely used to assess availability of invertebrate prey to shorebirds. Accepted methodology includes sampling to a depth of 10cm, although many shorebirds are morphologically incapable of acquiring prey at this depth. Given the time savings that would accrue from collecting smaller samples, we assessed the validity of sampling to a depth of 5cm. Samples (n = 72) were collected from moist-soil impoundments at 2 Atlantic coastal study sites being managed for migrant shorebirds. Each sample consisted of a 10-cm core that was split into 2 5-cm layers. Chironomids in the surface layer accounted for 62% and 68% of within-sample density and biomass, and explained 94% and 87% of the variation in among-sample chironomid density and biomass, respectively. Variance estimates among samples for density and biomass did not differ between 5-cm and 10-cm cores. We conclude that collection of benthic core samples at a depth of 5cm is a biologically and statistically valid sampling approach, and recommend that this method be employed in future studies of shorebird food availability.

INTRODUCTION

Abundance of invertebrate foods is recognized as an important determinant of habitat quality for migrant shorebirds. Chironomid larvae (Diptera:Chironomidae) are among the most widely distributed benthic macroinvertebrates, and are common in the diets of migrating and wintering shorebirds (Baker 1977, Baldassare and Fischer 1984, Batzer et al. 1993, Weber and Haig 1997). Temperate zone habitats used by shorebirds include moist-soil impoundments, which may undergo various management regimes to increase chironomid production and facilitate shorebird access to available prey. Many sampling methods have been developed for assessing wetland macroinvertebrate production (Merritt and Cummins 1996).

Evaluation of aquatic macroinvertebrate sampling methods has focused primarily on comparative efficiency of techniques (Paterson and Fernando 1971*b*, Milbrink and Wiederholm 1973, Murkin et al. 1983, Cheal et al. 1993, Francis and Kane 1995, Brinkman and Duffy 1996) and description of new sampling devices (McDaniel 1974, Swanson 1978, 1983, Miller and Bingham 1987, Euliss et al. 1992, Mackay and Euliss 1993). However, there have been few attempts to improve sampling efficiency through refinement of traditionally accepted methods. Collecting benthic core samples is a common technique for assessing shorebird forage availability, particularly where sandpipers (Tribe Calidridini) and plovers (Tribe Charadriini) are species of management concern.

The heterogeneous distribution of benthic macronivertebrates often requires substantial sampling effort to statistically demonstrate differences in abundance between habitat types (Paterson and Fernando 1971*a*, Elliot 1977). Labor requirements for sorting core samples may impose practical limits on the number of samples that can be collected in the field. Sorting labor is a function of sample volume, and may thus be reduced by collecting smaller samples. Although 10 cm is generally accepted as the appropriate depth for collection of core samples in studies of shorebird food availability (Stenzel et al. 1976, Duffy et al. 1981, Grant 1981, Schneider and Harrington 1981, Hicklin and Smith 1984, Mercier and McNeil 1994, Weber and Haig 1996), the validity of sampling to shallower depths (i.e. 5 cm) has not been assessed. Collecting 5 cm deep sample cores would substantially reduce sorting labor, but is predicated on the assumption that similar patterns of invertebrate distribution are revealed by 5-cm and 10-cm cores.

Studies of chironomid vertical distribution have been conducted in a variety of habitats, including ponds (Danks 1971, Paterson and Fernando 1971*a*), inland lakes (Paterson and Fernando 1971*a*, Shiozawa and Barnes 1977, Nalepa and Robertson 1981, Takács and Tokeshi 1994), intertidal lagoons (Mercier and McNeil 1994) and freshwater streams (Ford 1962). These studies generally indicate that the highest density and biomass of chironomid larvae occur in the upper few centimeters of substrate, although data from freshwater marshes are lacking. Spatial distribution of chironomids is affected by a variety of habitat characteristics, including grain size, organic matter content and temperature of the substrate, distribution of algae and plant litter, and

water quality (Hilsenhof and Narf 1968, Danks 1971, Wiley 1981, Campeau et al. 1993, Baker and Ball 1995, Bazzanti et al. 1997). Given that seasonal water level fluctuations may affect habitat characteristics of importance to chironomids, we questioned the assumption that previously demonstrated patterns of chironomid vertical distribution are applicable to moist-soil impoundments.

The goal of this study was to determine whether collecting core samples at a 5cm depth is a biologically and statistically valid approach to sampling benthic chironomids in Atlantic coastal moist-soil impoundments. Specifically, we compare 5-cm and 10-cm cores relative to: (1) chironomid density and biomass estimates; and, (2) among-sample variance in chironomid density and biomass.

METHODS

Study Sites

We collected substrate core samples from moist-soil impoundments at Prime Hook National Wildlife Refuge, Milton, Delaware, and Back Bay National Wildlife Refuge, Virginia Beach, Virginia. Impoundments at both refuges are typically drawn down during early spring to encourage germination of moist-soil plants and provide foraging habitat for migrant shorebirds, and are flooded in late fall to provide waterfowl access to moist-soil plant seeds. All core samples were collected from permanent study plots, with the exception of November 1996 samples from Prime Hook NWR (see below).

Prime Hook NWR is located on the coastal plain of Delaware, and is bordered by cropland, woodland, tidal marshes and Delaware Bay. Precipitation is the primary source of impoundment water, although salt water occasionally intrudes through water control structures. The impoundments are slightly brackish, with deep, highly organic soils. Vegetation communities are typically dominated by cattail (*Typha spp.*), three-square (*Scirpus olneyi*), bur marigold (*Bidens spp.*), water grasses (*Echinochloa spp.*), and common reed (*Phragmites australis*). Sampling for this study was conducted in Unit III (1012 ha) and Unit II (607 ha).

Back Bay NWR is located in southeastern Virginia, and is bordered by the Atlantic Ocean and Back Bay, a non-tidal freshwater sound. Fresh water is pumped from Back Bay into a central

storage pool, from which impoundments are flooded by gravity. Soils are typically fine to coarse sand with a distinct surface organic layer 1-10 cm deep. Dominant vegetation includes black needlerush (*Juncus roemerianus*), switchgrass (*Panicum virgatum*), spikerushes (*Eleocharis spp.*), salt meadow hay (*Spartina patens*), rushes (*Juncus spp.*) and bulrushes (*Scirpus spp*.). Sampling was conducted in Pool A (537 ha) and Pool C (475 ha).

Sampling Methods

Samples were collected during fall 1996, winter 1997 and spring 1997, in conjunction with scheduled sampling for ongoing research at each refuge. The permanent plots we selected for this study varied in plant community, soil composition, previous treatment and hydrology. Thus, our sampling was not random with respect to refuge-wide habitat availability, but was representative of habitats under management for migrant shorebirds.

November 1996 samples from Prime Hook NWR (n=20) were collected from an approximately 5 ha area (Unit III) dominated by bur marigold. Samples were collected from random locations within this area. An initial starting location was randomly selected, and subsequent samples were collected at random distance and azimuth from previously sampled locations. April 1997 samples from Prime Hook NWR (n=12) were collected from permanent sampling stations in a circular 50-m^2 study plot (Unit II). January and April 1997 samples (n=20 per month) from Back Bay NWR were collected from permanent sampling stations in 30m x 75m study plots. One plot (Pool A) was sampled during both months, and 2 plots (1 each in Pool A and Pool C) were sampled during 1 month only.

All samples were collected using 10-cm diameter PVC pipe driven to a depth of 10 cm. Following extraction of the substrate core, each sample was split into halves, representing 0-5 cm and 5-10 cm depth intervals. Samples were slowly extruded from the bottom of the core sampler, and manually separated after a 5-cm portion was visible. The remaining 5-cm half was then removed from the sampler. Sample halves were washed in the field over a 550-µm seive, placed in separate 500-ml plastic jars, preserved in 95% ethanol stained with Rose Bengal, and stored on ice in the field and in a walk-in cooler at the laboratory. At each sample location, we visually estimated percent cover of emergent vegetation and measured water temperature (± 0.5 ºC) and

water depth (± 1 cm). In the laboratory, samples were washed a second time over a 550-µm seive, placed in shallow plastic pans, and sorted by hand under bright light. All chironomid larvae were removed, placed in plastic vials with 95% ethanol, and refrigerated. We controlled observer bias by having the same individual sort both halves of each core sample. Total chironomid biomass (±0.01 mg) was determined for each core sample half after drying for 24 hours at 60 ºC.

Statistical Analysis

For each sample, we determined chironomid density in the 0-5 cm (TOP) and 5-10 cm (BOTTOM) layers, total chironomid density per sample (TOTAL = TOP + BOTTOM) and the percent contribution of surface layer chironomids to total density (PCTDENS = [TOP / TOTAL] * 100). Similarly, we determined total chironomid biomass for each sample layer (TOPMASS and BOTMASS), total biomass per sample (TOTMASS = TOPMASS + BOTMASS), mean biomass per chironomid in each layer (TOPCHI = TOPMASS / TOP, BOTCHI = BOTMASS / BOTTOM), and the percent contribution of surface layer biomass to total biomass (PCTMASS = [TOPMASS / TOTMASS] * 100). Nine core samples contained no chironomids in either layer, leading to division by zero errors for calculation of PERCENT and PCTMASS. These observations were eliminated from analyses of PERCENT and PCTMASS, but were retained for other analyses. Samples for which no chironomids occurred in 1 layer were assigned a value of 0 for the respective density and biomass variables, and were retained for density and total biomass analyses. Direct measures of biomass could not be obtained for 14 sample halves due to the occurrence of only 1 or 2 chironomids. For these samples, the mean value of the appropriate chironomid size measure from the remaining samples (TOPCHI or BOTCHI) was applied to generate an estimate of total biomass. These samples were eliminated from analysis of trends in chironomid size. Data transformations were selected based on reduced skewness and kurtosis and improved normality (Sokal and Rohlf 1995). Based on these criteria, we selected a $\log_{10}$ (x + 1) transformation for density variables and a $\log_{10}$ (x + 0.01) transformation for biomass variables.

We used Type III sums of squares from analysis of covariance (ANCOVA; PROC GLM, SAS Institute 1990) to evaluate variation in chironomid density and biomass with habitat variables (water temperature, plant cover and water depth). We used linear regression and F-tests for

homogeneity of variance to describe density and biomass relationships between 5-cm cores and 10-cm cores. For these analyses, we compared two potential sampling scenarios: (1) collection of 5-cm cores (i.e., independent variable for density = TOP), and, (2) collection of 10-cm cores (i.e., dependent variable for density = TOTAL). This approach violates linear regression and F-test independence assumptions, because the independent variable is a subset of the dependent variable. However, we employed these statistical methods not to test null hypotheses regarding relationships between variables, but in a descriptive manner intended to reveal the magnitude of presumed relationships. We judged that these comparisons most accurately reflected field sampling methods, and that their biological relevance outweighed statistical concerns. Because we sampled opportunistically, our data set was unbalanced with respect to study plots, time, and study areas. Thus, we are unable to make valid inferences regarding spatial and temporal trends in chironomid distribution. We therefore considered each 10-cm core an independent sampling unit, and restricted our analyses to within-sample variation in chironomid distribution.

RESULTS

We did not detect an influence of habitat variables on chironomid vertical distribution (PERCENT; $F = 0.72$, df = 3,59, $P = 0.546$), total chironomid density (TOTAL; $F = 1.71$, df = 3,68, $P = 0.173$), or chironomid density in the 0-5 cm layer (TOP; $F = 1.52$, df = 3,68, P = 0.218). The ANCOVA model for chironomid density in the 5-10 cm layer was significant (BOTTOM; $F = 2.95$, df = 3,68, $r^2 = 0.12$, $P = 0.0387$), but the only significant covariate was plant cover ($F = 6.41$, df = 1,68, $P = 0.0136$). The regression slope estimate for plant cover ($b = -0.00519$) indicated a negative relationship between plant cover and chironomid density in the 5-10 cm layer.

Mean chironomid density (paired t-test, $t = 5.22$, $P < 0.001$) and biomass ($t = 3.23$, $P = 0.002$) were higher in the 0-5 cm layer than in the 5-10 cm layer (Table B.1). However, mean biomass per chironomid did not differ ($t = 1.159$, $P = 0.254$) between sample layers. The mean contribution of surface layer density to total density was 61.7% (PERCENT; 95% CI = 53.1 - 70.8), whereas surface layer biomass averaged 68.5% of total biomass (PCTMASS; 95% CI = 66.5 - 70.5). Measures of chironomid density and biomass in the surface layer were good predictors of total sample density and biomass. Explained variation was 93.7% for chironomid density (Fig.

B.1), 87.4% for total chironomid biomass (Fig. B.2), and 72.4% for biomass per chironomid (Fig. B.3). Variance did not differ between 5-cm and 10-cm cores for mean chironomid density (TOP vs. TOTAL; $F = 1.04$, df = 71,71, $P = 0.859$) or biomass (TOPMASS vs. TOTMASS; $F = 1.09$, df = 71, 71, $P = 0.710$).

DISCUSSION

Chironomid Distribution

The results of this study are consistent with previous investigations that have shown high chironomid density and biomass in surface sediments (e.g., Paterson and Fernando 1971*a*, Mercier and McNeil 1994). Many chironomid subfamilies burrow into soft sediments and feed on organic detritus at the mud-water interface, although seasonal fluctuations in hydrology, salinity and temperature may affect their vertical distribution (Oliver 1971). Our samples were spatially and temporally distinct, yet showed little evident variation among locations and time periods (Figs. 1-3), and a general lack of strong evidence for habitat effects on chironomid distribution. These observations suggest that the patterns we observed may be broadly applicable to Atlantic coastal impoundments, although we are unable to statistically test this hypothesis.

Although we did not determine subfamily composition of chironomids, few epiphytic subfamilies likely occurred in our samples due to low abundance of submerged aquatic vegetation. Habitats in which algae or submerged aquatics dominate the plant community may contain greater proportions of chironomids at or above the substrate surface (Campeau et al. 1994), reducing the importance of benthic individuals as a potential shorebird food source. Among benthic chironomids, sediment penetration is a function of soil saturation, oxygen concentration, and sediment grain size distribution (Wiley 1981). Knowledge of habitat conditions for invertebrates is thus an important consideration in selecting sampling methods.

Five-cm core samples explained substantial variation in chironomid density and biomass of 10-cm core samples (Figs. 1 & 2), despite containing only slightly more than 50% of chironomid density (lower limit of 95% CI = 53%). Chironomid distribution among 5-cm core samples should thus be reflective of distribution among 10-cm core samples. Both sampling depths also provided similar variance estimates for density and biomass. Thus, power to detect differences in relative

abundance among habitats or experimental treatments using parametric statistical tests should not be reduced by reducing sample volume.

Shorebird Foraging Ecology

Bill morphology sets an upper limit on the foraging depth of shorebirds, but does not constrain long-billed species to foraging only in deeper substrates. Short-billed shorebirds, such as plovers and sandpipers, typically forage for soft-bodied invertebrates at or immediately below the substrate surface (Recher 1966, Burton 1974, Baker 1977, Hicklin and Smith 1984). Although the bill morphology of other species (e.g., dowitchers, godwits, curlews) may facilitate foraging at depths greater than 5 cm (Johnsgard 1981), long-billed species may also use visual cues and glean organisms from the substrate surface (Burton 1974, Baker 1977, Hicklin and Smith 1977). In a controlled laboratory experiment, predation rates of Sanderlings (*Calidris alba*) were highest (about 60%) on isopods at 0.5 and 1.0 cm depths, but were nearly zero at depths (3.0 cm) near the maximum bill length (range 2.4 - 2.7 cm) (Myers et al. 1980). These observations suggest that invertebrates >3 cm below the substrate surface would be similarly unavailable to other short-billed shorebirds, such as Least Sandpipers (*C. minutilla*), Western Sandpipers (*C. mauri*), and Semipalmated Sandpipers (*C. pusilla*) (range of bill lengths 1.7 - 2.8 cm [Johnsgard 1981]).

The inability of many shorebirds to extract benthic macroinvertebrates from depths >3cm may confound exclosure studies using 10-cm cores to assess resource depletion by shorebirds. Because at least 70% of the volume of a 10-cm core sample may be unavailable to short-billed species, inability of such studies to detect predation (e.g., Duffy et al. 1981, Mercier and McNeil 1994) may be due to collection of a large sample fraction that is unavailable to birds. Predation effects that occur in the surface layer may be masked by similar invertebrate densities in the lower layer between exclosed and open sample locations. Similarly, collection of 10-cm cores may overestimate invertebrate availability to short-billed species where significant resource depletion near the substrate surface has occurred.

Knowledge of vertical distribution patterns of benthic macroinvertebrates may prove useful in predicting habitat selection patterns by shorebirds. Shorebird habitat use and guild segregation vary according to habitat characteristics such as water depth (Baker 1979, Weber

and Haig 1996), horizontal prey distribution (Colwell and Landrum 1993), tidal cycle (Burger et al. 1977) and substrate composition (Harrington 1982). Among-habitat distribution of shorebird species is also a function of leg length (Baker 1979), although bill length may explain some within-species variation in habitat use (Harrington 1982). These observations suggest that abundance of prey at or near the substrate surface may be an important proximate factor determining shorebird habitat use.

Size-selective predation is a factor affecting shorebird foraging ecology (Weber and Haig 1997), suggesting that differential distribution of prey size classes may be an important predictor of habitat use. We did not detect differences in mean biomass per chironomid between the 0-5 and 5-10cm layers of our samples, suggesting that shorebirds are unlikely to preferentially forage deeper than 5 cm. Rather, organisms at or near the substrate surface are likely to be more energetically profitable due to reduced search and handling time.

RESEARCH AND MANAGEMENT IMPLICATIONS

This study provides a biological and statistical basis for collection of 5-cm deep benthic core samples. However, our sampling was restricted to 2 study sites and 1 invertebrate prey. Distribution patterns of other invertebrate prey (e.g., nereid polychaetes and amphipods; Grant 1981, Weber and Haig 1997) may differ from those of chironomids. Sampling efficiency will be maximized where techniques consider life history characteristics of both shorebirds and their prey. Chironomids are an ideal model organism for designing benthic sampling approaches, as they are among the most abundant benthic macroinvertebrates in shallow freshwater marshes, and are favored shorebird foods.

Three lines of evidence support the conclusion that 5-cm core samples are a desirable modification of accepted benthic sampling methods: (1) chironomid density and biomass in 10-cm cores were strongly predicted by density and biomass in the 0-5 cm layer; (2) chironomids were more abundant in the 0-5 cm layer, where they are most vulnerable to shorebird predation (Myers et al. 1980); and, (3) short-billed shorebirds are often numerically dominant on coastal staging and wintering areas (Clark et al. 1993, Colwell 1993, Dodd and Colwell 1996, Weber and Haig 1996), where resource limitations are most likely to occur. Further, the savings in sample

processing labor would enhance efficiency and facilitate sampling over larger spatial and temporal scales. We therefore recommend that 5cm deep cores be employed in future assessments of shorebird food availability, particularly where short-billed species are of management concern.

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Table B.1. Mean chironomid density (chironomids/sample) and biomass (mg) in upper (0-5 cm) and lower (5-10 cm) layers of benthic core samples from freshwater impoundments at Back Bay and Prime Hook National Wildlife Refuges. Means and 95% confidence intervals were back-transformed from common logarithms of raw data.

Sampling	Chironomids per Sample			Total Biomass per Sample			Mean Biomass per Chironomid		
Interval	Mean	*n*	95% CI	Mean	*n*	95% CI	Mean	*n*	95% CI
0-5 cm	4.6	72	3.1 - 6.8	0.086	72	0.052 - 0.138	0.025	47	0.018 - 0.034
5-10 cm	2.0	72	1.4 - 2.8	0.043	72	0.028 - 0.066	0.029	43	0.022 - 0.039
0-10 cm[a]	6.8	72	4.6 - 9.7	0.150	72	0.096 - 0.234	0.030	56	0.023 - 0.038

[a] Sum of upper and lower layers within samples.

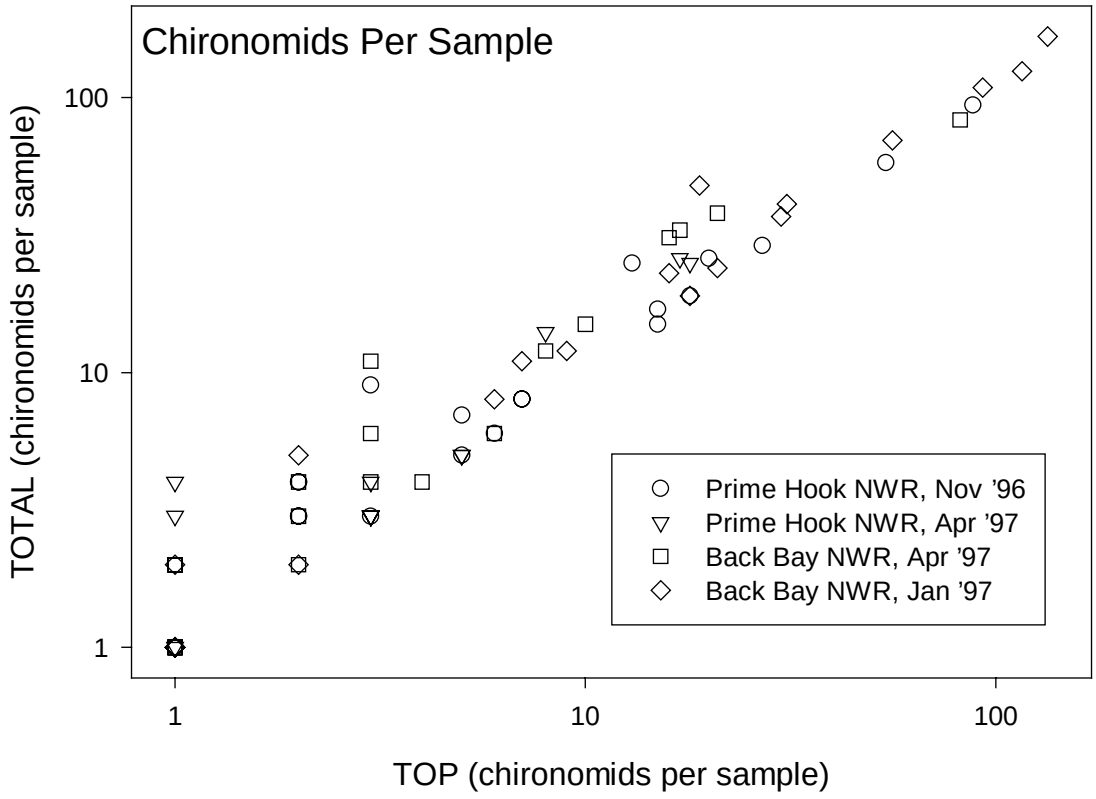

Figure B.1. Total chironomid density (chironomids per sample) in 5-cm (TOP) and 10-cm (TOTAL) benthic core samples from freshwater moist-soil impoundments at Back Bay and Prime Hook National Wildlife Refuges.

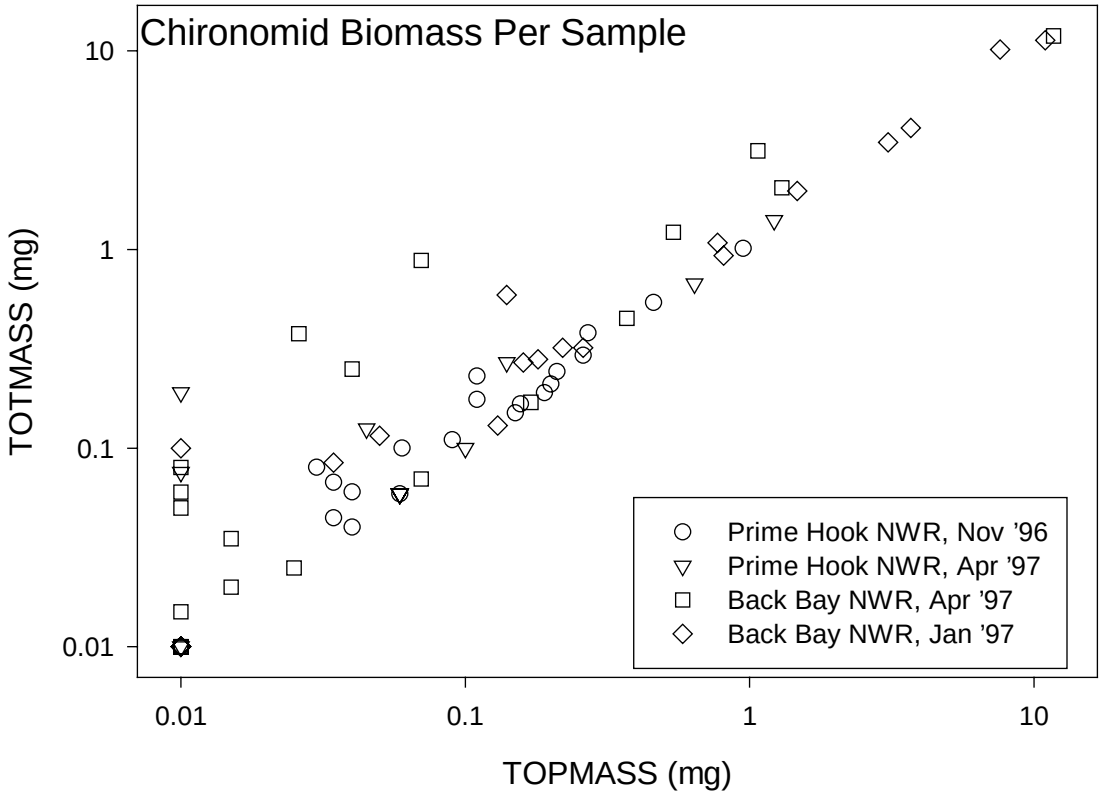

Figure B.2. Total chironomid biomass (mg) in 5-cm (TOPMASS) and 10-cm (TOTMASS) benthic core samples from freshwater moist-soil impoundments at Back Bay and Prime Hook National Wildlife Refuges.

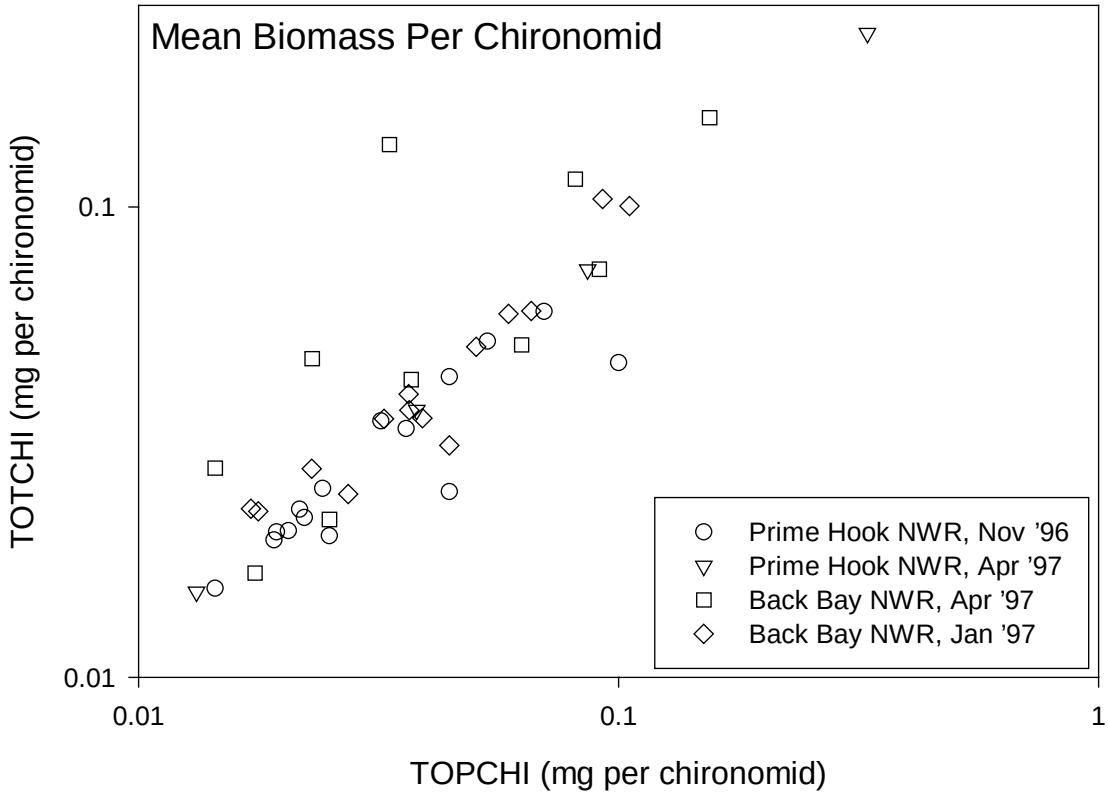

Figure B.3. Mean biomass per chironomid (mg) in 5-cm (TOPCHI) and 10-cm (TOTCHI) benthic core samples from freshwater moist-soil impoundments at Back Bay and Prime Hook National Wildlife Refuges.

APPENDIX C

ADDITIONAL REGRESSION EQUATIONS FOR PREDICTING SEED YIELD OF MOIST-SOIL PLANTS

(Submitted to *Wetlands*, July 31, 1998)

ABSTRACT

Laubhan and Fredrickson (1992) describe a regression-based method for predicting seed yield of moist-soil plants from measurements of inflorescence morphology and provide predictive equations for 11 species. Using methods similar to Laubhan and Fredrickson (1992), we developed predictive equations for seed production of 6 additional herbaceous wetland species. Five of our 6 equations had precision greater or equal to those of Laubhan and Fredrickson (1992). Thus, applying the 2 sets of available regression equations simultaneously should not produce biased seed yield estimates. We evaluated the effect of assessing seed production with both sets of equations using a data set collected at Back Bay National Wildlife Refuge, Virginia. Addition of seed production estimates for the 6 species we describe resulted in a 12% higher total seed production, suggesting that the originally published method may underestimate total seed yield for some plant communities. We encourage other investigators to develop and publish similar regression models to enhance the utility of this method across habitats and regions.

INTRODUCTION

Seasonal manipulation of moist-soil impoundment hydrology often is implemented to provide habitat conditions that favor germination of seed-producing annuals (Fredrickson and Taylor 1992). High seed production also is recognized as a goal for impoundment manipulation methods such as disking and mowing (Reid et al. 1989, de Szalay and Resh 1997, Gray 1995). Moist-soil plant seeds are important foods for wintering waterfowl (Euliss and Harris 1987, Combs and Fredrickson 1996) and may be an important proximate factor leading to greater waterfowl use of managed than unmanaged wetlands (Haukos and Smith 1993, Gordon et al. 1998). Obtaining accurate estimates of seed production is thus a desirable component of waterfowl habitat quality assessment.

Laubhan and Fredrickson (1992) describe a method (hereafter, "the LF Method") for estimating moist-soil plant seed production, using a 0.0625-m^2 sampling frame to quantify density of several species. A representative individual of each species within the frame is selected, and inflorescence dimensions are used to predict seed yield from regression equations (Laubhan and Fredrickson 1992). This technique facilitates rapid collection of seed production data, but predictive equations are available for only a limited number of species. Seed production for species not described by Laubhan and Fredrickson (1992) could be determined directly (i.e., by collecting and weighing seed), but this method is labor-intensive and requires sampling when seeds are mature. Further, bias in seed production estimates will be minimized where similar estimation methods (i.e., regression equations) are used for all species.

In conducting a study of impoundment seed production, we encountered several species for which equations for predicting seed yield were not available. The goal of this study was to generate predictive equations for these species, using methods similar to those of Laubhan and Fredrickson (1992).

METHODS

Predictive Equations

We collected specimens of 6 plant species (Table C.1) from impoundments at Back Bay National Wildlife Refuge (NWR) (Virginia Beach, Virginia) and Prime Hook NWR (Milton, Delaware) during September-October 1996. Dimensions of inflorescences were measured using digital calipers or a clear plastic ruler. Inflorescence width and height were measured at the widest and highest point, respectively. Plant specimens were air-dried in paper bags, and seed was manually stripped and separated from chaff. Although complete removal of chaff was not possible (Laubhan and Fredrickson 1992), associated error was judged to be minimal because most of the species we sampled produce relatively large seeds that were easily separated by hand. Thus, we did not assign plants to seed mass categories as Laubhan and Fredrickson (1992) did, but used the recorded seed mass for each plant as the dependent variable in predictive equations. Seed from each plant was weighed on an electronic balance (± 0.0001 g).

Predictive equations were generated using stepwise linear regression (PROC REG, SAS

Institute 1990) with a zero intercept constraint (Laubhan and Fredrickson 1992). Total seed biomass was the dependent variable, and potential independent variables included direct measures of inflorescence dimensions as well as several estimates of inflorescence volume based on approximation to geometric forms (e.g., cone or cylinder). Three species (*Spartina patens*, *Scirpus americanus*, and *S. pungens*) contained multiple inflorescences per plant. For these species, we recorded dimensions of each inflorescence and determined total inflorescence volume as the sum of volumes of all inflorescences. Similarly, total seed production for these species was calculated as the sum of seed production for all inflorescences. We used the selected regression equation to predict seed mass for each plant, and calculated Pearson's correlation coefficient between actual and predicted seed mass (Laubhan and Fredrickson 1992).

Field Assessment of Seed Production

We evaluated the effect of including our regression equations in seed production estimates, using data collected from 8 30 m x 75 m study plots in 2 impoundments at Back Bay NWR. Within each plot, we placed a 0.0625-m^2 sampling frame at 1 random location within each of 5 30 m x 15 m plot strata. Seed production was estimated for 13 species (Table C.1) for which regression equations were available. Total seed production for each sampling location (n = 160) was calculated as the sum of seed production for each species. Sampling was conducted during September and October 1996 and 1997.

We generated 3 estimates of seed production for each location: 1 (designated LF) included 7 species described in Laubhan and Fredrickson (1992), 1 (SK [i.e., Sherfy-Kirkpatrick]) included the 6 species for which we generated predictive equations, and 1 (LF+SK) included all 13 species (Table C.1). As temporal changes in seed production were not of interest for this investigation, we generated estimates of total mean seed production across all sampling locations and years. Sampling locations were considered subsamples within plots for the purposes of computing total mean seed production. We computed Pearson's product-moment correlation for seed production between LF and SK, using plots within years as independent sampling units. Seed production data were $\log_{10}$-transformed (Sokal and Rohlf 1995) prior to correlation analysis. Means are presented ± 1 SE.

RESULTS

Significant ($P < 0.0001$) regression equations were obtained for all 6 new species, with explained variation >90% for all species except square-stemmed spikerush (Table C.2). A geometric estimate of inflorescence volume was retained as a significant predictive variable in equations for common three-square and three-square bulrush, whereas the equation for white-topped sedge included a measure of horizontal cross-sectional area (Table C.2). The switchgrass and salt meadow hay equations included inflorescence width and height as the only significant predictive variables, respectively. Actual and predicted seed mass were correlated ($0.42 \leq r \leq 0.97$, $P \leq 0.0003$; Table C.2) for all species.

Mean seed production on the 8 study plots at Back Bay NWR was 408 ± 201 kg/ha for LF species, 48 ± 22 kg/ha for SK species, and 456 ± 211 kg/ha for LF+SK species. Seed production of LF and SK species was not correlated ($r = 0.15$, $n = 16$, $P = 0.578$).

DISCUSSION

Reinecke et al. (1989:236) suggest that 450 kg/ha is a "reasonable estimate of average food production" for Mississippi Alluvial Valley moist-soil impoundments, yet present a literature review that shows marked variation in seed production within and among plant species (Reinecke et al. 1989:220-221). Thus, in the absence of predictive models, gross assumptions regarding seed yield of a given species probably are not valid. Bias in estimates of seed yield will be minimized when similar estimation methods are applied among species. Our equations eliminate the need for direct seed production measurement for 6 additional species, thereby enhancing the applicability of the LF Method.

Although we lacked sufficient sample size to assess model accuracy using holdout methods (cf Laubhan and Fredrickson 1992), 5 of our regression equations had precision (r^2 = 0.91 - 0.98; Table C.2) equal to or higher than the original LF Method equations (r^2 = 0.79 - 0.96). Sample size for our square-stemmed spikerush model ($n = 70$) was higher than for any other species, yet actual seed mass had a higher coefficient of variation (106%) than any of the other 5 species (range 31% - 51%). High variance in seed production likely contributed to relatively low precision of this model, and may have resulted from variation in phenology among the sampled

plants. This model also produced the lowest correlation coefficient between actual and predicted seed mass (Table C.2), although our correlations were within the range reported by Laubhan and Fredrickson (1992).

Estimated total seed production on our study area was about 12% higher when all 13 species were considered (LF+SK estimate) than for the 7 LF species alone. Applying the assumptions of Reinecke et al. (1989:236), estimated duck use-days per hectare would be 3065 and 3476 for LF and LF+SK species, respectively. The LF Method may be considered conservative in that not all potential waterfowl foods are considered. However, the potential biological relevance of the difference in estimated duck-use days per hectare (411) indicates the value of considering additional plant species. Higher estimated seed production is a given when additional species are considered, but this exercise illustrates the need for a sampling approach that is appropriate to local plant community composition. Conclusions of seed production studies should thus be tempered according to the potential yield of unsampled species.

The LF Method was developed with species collected in New Mexico and the Upper Mississippi Valley, and accordingly focused on species with a midcontinental distribution. The species we studied are common in Atlantic Coastal moist-soil impoundments, but may vary in their relative value as waterfowl foods. Salt meadow hay is generally considered an undesirable species in coastal marshes managed for waterfowl (Hindman and Stotts 1989), although seeds of salt-tolerant plants may be consumed by American black ducks (*Anas rubripes*) (Mendall 1949) and other waterfowl (Afton et al. 1991). Conversely, marsh management practices in this region are often designed to encourage production of common three-square and three-square bulrush (Hindman and Stotts 1989). Lack of correlation between LF and SK seed production estimates suggests that the two groups of species may differ broadly in their habitat requirements.

Based on similar model precision (r^2) and correlation between actual and predicted seed yield, we conclude that reliability of our regression equations is similar to those presented by Laubhan and Fredrickson (1992). Our models also enhance the regional applicability of the LF Method by describing common coastal species that are important waterfowl foods. We encourage other investigators to develop and publish similar regression models to enhance the

utility of this method across habitats and regions.

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Table C.1. Moist soil plant species included in three seed production estimates for impoundments at Back Bay National Wildlife Refuge, Virginia. All species were included in the LF+SK estimate, whereas the LF and SK estimates contained only species from Laubhan and Fredrickson (1992) and this study, respectively.

Common Name	Scientific Name	Source
Red-rooted flatsedge	*Cyperus erythrorhizos* Muhl.	Laubhan and Fredrickson (1992)
Chufa flatsedge	*Cyperus esculentus* L.	Laubhan and Fredrickson (1992)
Rice flatsedge	*Cyperus iria* L.	Laubhan and Fredrickson (1992)
White-topped sedge	*Dichromena colorata* (L.) Hitchc.	This study
Barnyardgrass	*Echinochloa crusgalli* (L). Beauv.	Laubhan and Fredrickson (1992)
Square-stemmed spikerush	*Eleocharis quadrangulata* (Michx.) R. & S.	This study
Fall panicum	*Panicum dichotomiflorum* Michx.	Laubhan and Fredrickson (1992)
Switchgrass	*Panicum virgatum* L.	This study
Swamp smartweed	*Polygonum hydropiperoides* Michx.	Laubhan and Fredrickson (1992)
Common three-square	*Scirpus pungens* Vahl	This study
Three-square bulrush	*Scirpus americanus* Pers.	This study
Bristlegrass	*Setaria* spp.	Laubhan and Fredrickson (1992)
Salt meadow hay	*Spartina patens* (Ait.) Muhl.	This study

Table C.2. Regression equations developed for predicting seed biomass of 6 moist-soil plant species collected at Back Bay and Prime Hook National Wildlife Refuges, and Pearson correlation coefficients for actual vs. predicted seed mass. Seed biomass (g / plant) is the response variable for all equations. When used in conjunction with the 0.0625 m^2 sampling frame of Laubhan and Fredrickson (1992), multiply by [HEADS * 16] (HEADS = number of seed heads in the frame) to obtain estimates of yield in g / m^2.

	Regression				Correlation	
Species	**Equation**	**n**	**r^2**	***F*** [a]	**r**	**P**
Dichromena colorata	$0.0002619 * \pi * (WIDTH^b * 0.5)^2$	28	0.92	298.97	0.66	<0.0001
Eleocharis quadrangulata	$(-0.001934 * HEIGHT^c) + (0.0004083 * \pi * (WIDTH^c * 0.5)^2 * HEIGHT^c)$	70	0.56	43.93	0.42	0.0003
Panicum virgatum	$0.01832 * (WIDTH^b)$	30	0.91	292.87	0.54	<0.0001
Scirpus pungens	$0.4212 * \Sigma^d (\pi * (WIDTH^c * 0.5)^2 * HEIGHT^c)$	35	0.92	421.38	0.79	<0.0001
Scirpus americanus	$0.0004808 * \Sigma (\pi * (WIDTH^c * 0.5)^2 * HEIGHT^c)$	14	0.98	565.32	0.97	<0.0001
Spartina patens	$0.007956 * TOTINFHT^e$	30	0.95	550.91	0.78	<0.0001

[a] All $P < 0.0001$.
[b] WIDTH = Inflorescence width (cm).
[c] WIDTH = Infloresence width at widest point (mm), HEIGHT = Inflorescence height at longest point (mm).
[d] Denotes sum of paranthetic quantity for all inflorescences on each plant.
[e] TOTINFHT= Total height of inflorescence (cm) from point of attachment of lowest inflorescence to stem to the tip of the terminal inflorescence.

APPENDIX D

EFFECT OF GRIT AND HARD SEED SUPPLEMENTS ON TRUE METABOLIZABLE ENERGY OF WATERFOWL FOODS

INTRODUCTION

Inorganic grit may be retained in avian gizzards as an aid to mechanical breakdown of foods, and also may be an important source of calcium and magnesium during egg formation (Kopischke 1966, Kopischke and Nelson 1966). Evidence for the former role is provided by the positive relationship between grit and seed consumption in birds (Robel and Bisset 1979, Trost 1981, Gionfriddo and Best 1996). Beer and Tidyman (1942) also inferred that hard seeds may substitute for grit, based on an inverse relationship between quantities of grit and hard seeds in gizzards of gallinaceous birds. The "seed substitution hypotheses" was formalized by Peres and vanRoosmalen (1996) to explain consumption and dispersal of seeds low in nutritional value by tropical birds. This hypothesis proposes that birds consume some hard seeds as an aid to mechanical digestion of other foods, despite their potentially low nutritional value (Peres and vanRoosmalen 1996, Foster and Delay 1998). However, high abundance of hard seeds in gizzards with little grit (Beer and Tidyman 1942) provides only weak evidence for the seed substitution hypothesis, as these observations also may be a consequence of low digestion rates in the absence of grit. Despite its presumed digestive benefit, few studies have examined the nutritional consequences of grit consumption. Further, the "seed substitution hypothesis" remains untested in a controlled study.

Petrie et al. (1997) predicted that the value of grit as an aid to mechanical digestion would be evident through increased true metabolizable energy (TME) estimates for seed diets in Canada geese (*Branta canadensis*) provided with grit. Contrary to this prediction, their TME estimates for milo and smartweed (*Polygonum pensylvanicum*) were 14% and 52% higher, respectively, in grit-free birds than in birds with free access to grit. Petrie et al (1997) concluded that lower TME estimates in grit-free birds resulted from retention of a portion of the test diet in the gizzard as an aid to digestion. In contrast, Robel and Bissett (1979) found that metabolizability of shrub

lespedeza (*Cassia nictitans*) did not differ in northern bobwhites (*Colinus virginianus*) with and without access to grit. However, Robel and Bissett fed their birds the test diet for 3 days prior to excreta collection, suggesting that seed substitution from the grit-free diet may have occurred in grit-free birds. These studies suggest that gizzard grit may provide variable nutritional benefits among taxa, although data from other bird species are lacking.

Previous studies of nutritional consequences of grit consumption have used birds maintained on highly digestible pelleted diets (Petrie et al. 1997), or have exposed birds to seed diets for <3 days prior to data collection (Robel and Bissett 1979). Because grit consumption increases when diets contain high quantities of seeds (Trost 1981, Gionfriddo and Best 1996), pre-experimental exposure to a seed diet may alter the influence of grit on metabolizable energy of seed diets. The potential influence of grit and hard seed consumption on digestive efficiency also has important implications for the value of these diet items to wild birds. Consequently, this study included pretrial diets both with and without grit and hard seeds to facilitate detection of an interaction between effects of these supplements on true metabolizable energy of seed diets in waterfowl. An additional objective was to assess the implications of test diet regurgitation on measures of true metabolizable energy. Specific hypotheses tested in this study and predictions that follow from these hypotheses include the following:

H_1: Consumption of grit aids digestion of hard seeds.

A) TME should be higher in birds with access to grit than in grit-free birds.

H_2: Previous consumption of a diet high in hard seeds enhances digestion of hard seed diets.

A) TME should be higher in birds provided with milo than in milo-free birds. Rejection of this hypothesis would suggest that birds either retain seeds from the pretrial diet as an aid to digestion, or that long-term exposure to a seed diet conditions the digestive tract in a manner that enhances metabolizability.

H_3: Hard seeds are retained in the gizzard by grit-free birds as an aid to digestion.

A) TME should be lower in birds provided only with grit than in grit- and milo-free birds. Rejection of this hypothesis would suggest that birds retain force-fed seeds in the gizzard to compensate for lack of grit (Petrie et al. 1997).

B) TME should be higher for birds provided with milo than in grit-free birds not provided with milo. Rejection of this hypothesis would suggest that birds retain pretrial milo seeds as an aid to digestion.

H_4: True metabolizable energy can be measured in birds that regurgitate a portion of the test diet without a loss of accuracy, regardless of the test diet being used.

A) Frequency and extent of regurgitation should not vary among diets.

B) TME estimates should not differ when obtained from birds that regurgitate and those that do not.

METHODS

Experimental Design

Adult blue-winged teal (*Anas discors*, *n* =32) were randomly assigned to 1 of 4 pens on 4 Apr 1997, and each pen was randomly assigned 1 of 4 pretrial treatments. Randomization was constrained to equalize sex ratio (4M:4F) among pens. Treatments consisted of a 2 x 2 factorial arrangement of the presence and absence of grit and milo supplements. "Treatments" are hereafter referred to as CONTROL (no supplements), GRIT (grit supplement only), MILO (milo supplement only), and GRITMILO (both grit and milo supplements), whereas "diets" reflect the specific food (milo, millet, or smartweed) for which TME was measured during TME trials. Similarly, combinations of treatments with common supplements are referred to as "Grit birds" (GRIT + GRITMILO), "No-Grit birds" (CONTROL + MILO), "Milo birds" (MILO + GRITMILO), and "No-Milo birds" (CONTROL + GRIT).

All treatments received *ad libitum* quantities of a pelleted, nonmedicated chicken starter daily (minimum crude protein 20.6%, minimum crude fat 3.0%, maximum crude fiber 7.0%; Big Spring Mills, Elliston, VA). Food was provided in 3 ceramic bowls per pen. Birds receiving grit were provided with a 4th bowl that contained an *ad libitum* quantity of granite grit (approximately 100g). A small quantity of granite grit (approximately 25g) also was sprinkled on the surface of feed bowls in these pens daily. Birds receiving milo were fed a mixture of chicken starter and milo (2:1 v/v). Milo (Kester's Wild Game Food Nursery Inc., Omro, Wisconsin) was thoroughly mixed with chicken starter to promote consumption. CONTROL birds received chicken starter with no

supplements. Birds generally emptied 1 of the 3 bowls daily, and did not appear to avoid consuming milo. Rather, the surface layer of feed in pens receiving milo frequently contained no milo, suggesting that birds may have preferentially consumed milo seed. Wet or powdery feed was removed from bowls daily and replaced with fresh feed. A detailed description of facilities and daily husbandry is provided in Chapter 1.

Due to limited availability of isolation cages, 6 of the 8 birds were randomly selected from each treatment prior to the first TME feeding trial. The initial goal was to measure TME of each test diet in a balanced design, using these 24 birds for all test diets. The remaining 2 birds in each treatment were used to replace birds lost to mortality or escape from captivity during the study. An attempt was made to evaluate TME for all diets on the replacement birds. Data were retained from birds that died or escaped, except where cause of death appeared to potentially confound TME assays (e.g., esophogeal impaction). Thus, the final data set was unbalanced with respect to birds and test diets.

Metabolizable energy of each test diet was evaluated in a series of 3 feeding trials. Each feeding trial was conducted over a 3-day period, following the protocol outlined by Sibbald (1975). Birds were randomly assigned to 1 of 24 cages constructed of vinyl-coated chicken wire. Cage assignments were retained for subsequent trials within each series of trials, but were randomized again for subsequent series of trials. Briefly, birds were fasted and weighed on the first day of the trial, and the “fed” group was force-fed the test diet on the second day of the trial. The “fasted” group did not receive any food during the second trial day. Water was available *ad libitum* during the trials. Excreta were collected from all birds 24hr after force feeding of “fed” birds. Regurgitated feed was manually separated from excreta and collected. Excreta samples were frozen >24hr, freeze-dried >36hr, manually ground, and weighed. Gross energy was analyzed using a Parr model 1241 adiabatic bomb calorimeter. Further details on feeding trial methods and calculation of TME are provided in Chapter 1.

Trials were conducted from 7 Jul - 9 Aug (milo), 3 Sep - 5 Nov (millet), and 14 Nov - 22 Dec 1997 (smartweed) and thus, diet was confounded with time period. To control for variation among individuals in endogenous losses, each bird served as its own control for each series of

trials (Kaminski and Essig 1992; see also Chapter 1). For the first feeding trial with each diet, half of the birds were randomly selected to be force-fed, and the remaining birds were starved for measurement of endogenous losses. For the second trial, the force-fed and starved groups were switched. This approach minimized confounding with time within diets, while generating measures of excretory energy in both the fasted and fed condition for each bird. TME was calculated for each bird using estimates of fasted and fed excretory energy from these 2 trials. Trials were separated by >10 days to minimize weight loss of birds.

Regurgitation of test diets occurred frequently (see Results). Omission of birds that regurgitated (Jorde and Owen 1988, Petrie et al. 1997, 1998) would have substantially reduced sample sizes. Because TME should be independent of diet intake (Sibbald 1975), adjusting intake level for unconsumed (i.e., regurgitated) food should not affect TME estimates. Thus, regurgitated food was collected separately from excreta, air-dried >24hr, and weighed. Mass of regurgitated food was subtracted from initial food mass, and TME was calculated based on net intake. This approach assumes 1) collection and weighing of regurgitated food without error; and, 2) no physiological consequences of regurgitation that would alter metabolic efficiency. The validity of these assumptions was tested by adding a third feeding trial to each series of trials, using all birds that had regurgitated when fed the test diet. The third trial was conducted using identical methods to the first 2 trials in the series, except that all birds were force-fed the test diet.

Statistical Analysis

Data were analyzed using mixed linear models (PROC MIXED, Littell et al. 1996), with true metabolizable energy as the response variable, birds as a random factor, and test diet, pretrial grit exposure and pretrial milo exposure as fixed factors. The mixed model approach was preferable to general linear models (PROC GLM, SAS Institute 1990) for 2 reasons. First, the handling of missing observations by PROC MIXED is preferable to that of PROC GLM. The goal of this study was to measure TME for each diet in all birds, producing a balanced data set. Experimental difficulties (e.g., bird mortality, spilled samples) produced missing observations for some bird x test diet combinations. PROC MIXED retains experimental units (i.e., birds) for which some observations are missing, increasing statistical power over PROC GLM, in which any

experimental unit with missing data is deleted from analysis (Littell et al. 1996). Second, the mixed model approach more accurately accounts for the presence of both fixed (i.e. pretrial grit and milo exposure) and random (i.e. birds within pretrial diets) effects than do general linear models (Bennington and Thayne 1994, Littell et al. 1996). Hypotheses H_1-A and H_2-A were tested by examining *P*-values for the Grit and Milo main effects, respectively. The Grit x Milo term and all interactions between Diet, Grit and Milo were retained to assess variation among diets in response to treatments. Hypotheses H_3-A and H_3-B were tested using linear contrasts (Littell et al. 1996) of the appropriate treatments within diets.

Cochran's Q-test was used to test for differences in frequency of regurgitation among test diets (H_4-A). This test generated 2 x 3 contingency tables (frequency of regurgitation x test diets) that control for individual variation among birds (Sokal and Rohlf 1995). Frequency of regurgitation was measured as the total number of birds that regurgitated each test diet divided by the number of birds fed that diet. Percentage of the test diet regurgitated also was calculated for each observation (%REGURG = [Regurgitated Food Mass / Initial Food Mass] * 100). Variation among test diets in %REGURG was analyzed using a mixed linear model that was structured identically to the model used to assess variation in TME, including retention of interaction terms (see above). Arcsine-square root transformation of %REGURG data was employed prior to analysis (Sokal and Rohlf 1995). Pearson's correlation coefficient was calculated for %REGURG of the 3 test diets.

A dataset was generated that consisted of all birds that regurgitated when initially force-fed a test diet, but that did not regurgitate the same diet when force fed a second time. The dataset included a variable (DIFF) that consisted of the difference in TME measurements from these 2 observations. Because sample size was low within diets (n = 4 per diet), hypothesis H_4-B was initially tested using a Kruskal-Wallis test with the χ^2 approximation (Schlotzhauer and Littell 1987), using DIFF as the response variable and diet as the class variable. As this analysis indicated significant variation in DIFF among diets, a Wilcoxon signed-rank test was performed on DIFF for each diet.

An assumption inherent in the foregoing analyses is that birds within experimental treatments are independent. This is not strictly true, because all birds on a given experimental treatment were assigned to the same holding pen (Hurlbert 1984). A statistically preferable design would have been to keep each bird in a separate cage to ensure independence. However, limited cage space at the facility rendered this option infeasible. Random assignment of birds to pens should minimize, but not eliminate, spurious effects that could arise from the confounding of pens and treatments. All husbandry practices (e.g., feeding times, food and water availability) also were identical among pens.

RESULTS

Metabolizable Energy

Mean TME for each diet was highest in the GRIT treatment, but there was little variation among treatments (Table D.1). Coefficients of variation within treatments were substantially higher for smartweed (range 55.3 - 72.4 %) than for millet (range 3.6 - 12.0 %) or milo (range 6.1 - 12.8%). Mean TME did not differ between GRIT and CONTROL birds (H_3-A) or MILO and CONTROL birds (H_3-B) for any test diet (Table D.1), but differed significantly among diets (F = 1.32, $P < 0.001$, Table D.2). No interaction terms were significant in the analysis (Table D.2). Overall mean TMEs (± SE) (millet = 2.76 ± 0.12 kcal / g, milo = 3.51 ± 0.12 kcal / g, smartweed = 1.38 ± 0.39 kcal / g) were substantially lower than those reported by Petrie et al. (1997) (Table D.3).

Regurgitation

Mean percentage of the test diet regurgitated was significantly lower for the milo diet than for the millet and smartweed diets, but regurgitation occurred in fewer birds on the milo diet than on the millet and smartweed diets (Table D.4). Consequently, mean percentage of the test diet regurgitated did not differ among diets for birds that regurgitated (F = 0.11, df = 2,14, P = 0.8931; Table D.4). The data included 17 birds for which TME was measured for all 3 diets. Of

Table D.1. Mean true metabolizable energy (kcal / g) of 3 test diets fed to blue-winged teal provided pre-trial supplements of grit and milo.

	True Metabolizable Energy											Tests of linear contrasts					
	CONTROL			GRIT			MILO			GRITMILO		H_3-A[1]			H_3-B[2]		
Test Diet	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	*F*	*df*	*P*	*F*	*df*	*P*
Millet	6	2.72	0.13	6	2.90	0.14	6	2.68	0.11	3	2.74	0.06	0.01	1,31	0.913	0.32	1,31	0.578
Milo	8	3.49	0.07	5	3.61	0.14	6	3.58	0.10	6	3.38	0.18	0.10	1,31	0.756	0.14	1,31	0.709
Smartweed	6	1.42	0.42	6	1.60	0.36	5	1.38	0.44	4	0.97	0.31	0.01	1,31	0.913	0.30	1,31	0.588

[1] Comparison of GRIT mean to CONTROL mean.

[2] Comparison of MILO mean to CONTROL mean.

Table D.2. Results of mixed model analysis of variance for main effects of pre-trial supplements (Grit and Milo) on true metabolizable energy of 3 seed diets (Diet) in blue-winged teal.

Source	*df*	*F*	*P*
Grit	1,31	1.32	0.259
Milo	1,31	0.01	0.934
Grit * Milo	1,31	1.44	0.239
Diet	2,31	83.28	<0.001
Diet * Grit	2,31	0.35	0.709
Diet * Milo	2,31	0.22	0.801
Diet * Grit * Milo	2,31	0.21	0.814

Table D.3. Mean true metabolizable energy (kcal/g) of 3 seed diets fed to captive blue-winged teal on pre-treatment diets with and without pre-trial grit and milo supplements. Each mean represents combined data from 2 treatment groups (see footnotes). Data from a similar study with Canada geese are provided for comparison (Petrie et al. 1997).

	Blue-winged teal (This study)												Canada Geese (Petrie et al. 1997)	
	Milo[1]			No Milo[2]			Grit[3]			No Grit[4]			Grit	No Grit
Test Diet	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	Mean	Mean
Millet	9	2.85	0.10	12	2.70	0.08	9	2.70	0.08	12	2.81	0.09	3.46	3.62
Milo	11	3.48	0.11	14	3.53	0.06	12	3.50	0.10	13	3.53	0.07	3.70	4.23
Smartweed	10	1.35	0.26	11	1.40	0.29	9	1.20	0.28	12	1.51	0.26	1.60	2.43

[1] MILO and MILOGRIT birds combined.

[2] CONTROL and GRIT birds combined.

[3] GRIT and MILOGRIT birds combined.

[4] MILO and CONTROL birds combined.

Table D.4. Percent of initial food mass regurgitated by blue-winged teal during true metabolizable energy feeding trials. Birds from 4 experimental pre-trial diets were combined for analysis.

	All Birds			Regurgitators Only		
	n	Mean	SE	n	Mean	SE
Millet	21	18.8	4.1	16	24.7	4.4
Milo	25	6.6	3.4	7	23.7	9.8
Smartweed	21	18.5	4.0	15	25.8	4.3
Test Statistic		$F = 6.20$		$Q = 11.427$	$F = 0.11$	
df		2,37		2	2,14	
P		0.0047		0.003	0.8931	

these birds, 8 regurgitated millet and smartweed but did not regurgitate milo, and only 1 bird failed to regurgitate any of the 3 diets. Compared within birds, percent mass of smartweed and millet regurgitated was significantly correlated (r = 0.646, *P* = 0.002), but percent mass of milo regurgitated was not correlated with either smartweed (r = -0.22, *P* = 0.368) or millet (r = -0.08, *P* = 0.762).

Paired measures of TME were obtained for 12 birds (*n* = 4 per diet) that regurgitated when initially force-fed, but did not regurgitate when force-fed the same diet a second time. Because diet influenced the difference in TME between the 2 measures (Kruskal-Wallis test, χ^2 = 6.615, *df* = 2, *P* = 0.037), data were analyzed separately by diet. However, this analysis did not reveal differences in median difference of the 2 TME measures within diets (Wilcoxon signed-rank tests, all *P* > 0.125). Percent difference between the paired TME measures was > 20% for 4 birds; 3 of these occurred during smartweed feeding trials and the fourth occurred during the millet trial (Table D.5). Within paired observations, TME estimates were higher when the bird had regurgitated than when it had not (i.e., negative value for percent difference) for 6 observations; these included all observations from the millet trials, but only 1 each from the milo and smartweed trials (Table D.5).

DISCUSSION

Influence of Grit on Metabolizability (H_1)

There was no evidence for a direct influence of pretrial grit consumption on TME of test diets, nor did the influence of grit consumption vary among test diets (Diet * Grit interaction; Table D.2). Consquently, this study provides no evidence upon which to base a rejection of H_1. Mean TME was 26% higher in No-Grit than in Grit birds for smartweed (Table D.3). Similarly, Petrie et al. (1997) observed a 52% higher TME for smartweed in Canada geese on No-Grit than in Grit treatments (Table D.3).

Coefficient of variation ranged from 55 - 72% among treatments for smartweed, but only 6 - 12% and 3 - 12% for milo and millet, respectively. Given the low variation in TME for 2 of 3 diets, it is unlikely that variation in consumption of dietary supplements within treatments

Table D.5. Paired measures of true metabolizable energy (TME; kcal / g) of 3 test diets fed to blue-winged teal on 4 pre-trial treatments, calculated from paired observations in which each bird did and did not regurgitate.

			TME						
Bird #	Diet	Treatment[1]	Regurgitation[2]	No Regurgitation[2]	Absolute Difference[3]	Percent Difference[4]	Initial Food Mass[5]	Regurgitated Food Mass[6]	Percent Regurgitation[7]
812	Milo	GRITMILO	3.56	3.47	-0.09	-2.6	3.83	1.16	30.2
741	Milo	MILO	3.47	3.54	0.07	1.9	3.91	1.78	45.4
756	Milo	GRITMILO	3.48	3.61	0.13	3.6	3.45	0.60	17.4
888	Milo	MILO	3.63	3.73	0.10	2.8	3.92	2.68	68.3
	Milo Mean		3.54	3.59	0.05	1.43	3.78	1.55	40.33
740	Millet	GRIT	2.48	2.01	-0.48	-23.8	5.25	0.96	18.2
745	Millet	CONTROL	2.65	2.45	-0.20	-8.3	4.39	0.20	4.6
747	Millet	GRITMILO	2.82	2.81	-0.01	-0.3	4.00	0.54	13.6
899	Millet	CONTROL	3.20	2.70	-0.50	-18.5	4.49	1.43	31.9
	Millet Mean		2.79	2.49	-0.30	-12.73	4.53	0.78	17.07
734	Smartweed	MILO	0.91	1.97	1.05	53.5	3.58	0.64	17.9
735	Smartweed	CONTROL	1.05	1.89	0.85	44.8	4.48	1.16	26.0
818	Smartweed	MILO	2.15	2.13	-0.02	-0.8	4.68	1.27	27.1
899	Smartweed	CONTROL	0.58	1.68	1.10	65.5	4.06	1.67	41.1
	Smartweed Mean		1.17	1.92	0.75	40.76	4.20	1.19	28.03
	Grand Mean		2.50	2.67	0.17	9.82	4.17	1.17	28.48

[1] Pre-trial dietary supplements: CONTROL = none, GRIT = granite grit, MILO = milo, GRITMILO = granite grit + milo.

[2] Independent observations from separate feeding trials with force-fed birds.

[3] Absolute difference = No Regurgitation - Regurgitation.

[4] Percent difference = [(No Regurgitation - Regurgitation) / No Regurgitation] * 100.

[5] Initial Food Mass = Mass of test diet force fed at the beginning of feeding trials.

[6] Regurgitated Food Mass = Mass of test diet regurgitated during excreta collection period.

[7] Percent Regurgitation = (Regurgitated Food Mass / Initial Food Mass) * 100.

contributed to lack of significance of treatment effects. Daily observations of feeding bowls suggested that Milo birds did not select against milo, and grit was frequently observed in the excreta of Grit birds. However, consumption of grit and milo was not directly measured.

Robel and Bissett (1979) also found that grit did not influence apparent metabolizability of shrub lespedeza seeds by northern bobwhites. Their metabolizability estimates for shrub lespedeza (51-55%) were similar to or higher than estimates obtained in CONTROL birds in this study for millet (65%) and smartweed (34%). Grit-induced enhancement of digestive efficiency would be expected to occur for low metabolizability foods such as these, yet grit did not provide an apparent digestive benefit to either bobwhites or teal.

The Seed Substitution Hypothesis (H_2)

Because the seed substitution hypothesis predicts that birds retain hard seeds in the gizzard, birds provided with a hard seed diet should retain these seeds and use them as an aid to digestion. However, the Milo and Milo x Diet effects in this study were nonsignificant, suggesting that such substitution did not occur in the test birds. The high metabolizability of milo by CONTROL birds (92%) suggests that teal realized a greater nutritional benefit from digesting this diet than retaining it in the gizzard as an aid to digestion. The foregoing evidence suggests that the seed substitution hypothesis does not apply to blue-winged teal, at least for relatively low-fiber seeds.

Retention of Test Diets by Grit-Free Birds (H_3)

Petrie et al. (1997) observed significantly higher metabolizability of milo and smartweed in grit-free Canada geese than in birds provided with grit (Table D.3). They concluded that TME of these foods in grit-free birds was elevated because seeds were retained in the gizzard beyond the excreta collection period, resulting in an artificially depressed estimate of excretory energy. Data from the present study suggest that similar processes in blue-winged teal did not occur. Similar TME estimates for GRIT and CONTROL birds for all foods (Table D.1) could occur only if excretory energy were similar for these groups, which in turn implies that gizzard retention of test diets did not differ between treatments. Further, lack of difference in TME estimates for MILO and CONTROL groups suggests that milo seed provided in pretrial diets was not retained as an aid to

digestion. These observations contradict the conclusions of Petrie et al. (1997), in that no evidence is provided for gizzard retention of either pretrial or force-fed seeds in blue-winged teal. However, Canada geese are primarily grazers and would thus be expected to be less efficient digesters of seed diets than are blue-winged teal. Greater nutritional benefits of mechanical digestion may therefore accrue to geese than to teal. Further, the relatively rapid food passage rates characteristic of small-bodied species such as teal (Robbins 1983) may negate any potential nutritional benefits of seed retention in the gizzard.

Effect of Regurgitation on Accuracy of TME Assays (H_4)

Low sample size for the paired-observations dataset precluded parametric statistical analysis, preventing testing for significant interaction between test diet and percent error in TME measurements on regurgitating birds (Table D.5). The range of error for regurgitating birds for the milo test diet was within the range of calorimetry error (<5%), suggesting that TME was measured essentially without error for this diet. However, mean percent difference between the 2 measures suggested that, in regurgitating birds, TME was substantially overestimated for millet and underestimated for smartweed (Table D.5). Viewed in light of the mean percent of initial test diet regurgitated (Table D.5), these observations do not suggest that variability in collection of regurgitated feed contributed to TME measurement error. Conversely, TME measurements in regurgitating birds were most accurate for the diet in which birds tended to regurgitate the highest percentage of the intial food mass (milo). All 4 birds for which paired observations were obtained on the milo diet were provided pretrial milo supplements (i.e., Milo birds; Table D.5), suggesting that pretrial exposure to this diet does not reduce the extent of regurgitation when it occurs. However, a significantly lower percentage of birds regurgitated the milo diet (28%) than regurgitated either then millet or smartweed diets (76% and 71%, respectively). Further, the data do not support the conclusion that high regurgitation rates contribute to low precision of TME estimates. Percent of the test diet regurgitated was nearly identical for millet and smartweed (Table D.4), yet variation in TME estimates among individuals was substantially higher for smartweed (mean coefficient of variation among treatments = 66.0%) than for millet (mean CV = 9.3%).

These results suggest that, at least for some diets, TME can be measured in birds that regurgitate without a loss of accuracy. However, the high error in TME measurements for millet and smartweed should lead future investigators to question whether this assumption holds for all diets. Where measures of TME can be obtained on birds that do not regurgitate, a potentially substantial source of variation will be eliminated.

CONCLUSIONS

The results of this study suggest that measures of true metabolizable energy of seed diets in blue-winged teal are not influenced by pretrial provision of grit or hard seed diets. Because it is difficult to ensure that captive birds are grit-free (Robel and Bissett 1979), providing grit to captive birds is advantageous to maintaining controlled conditions for feeding trials (Petrie et al. 1997). Data from Milo birds showed no influence of this treatment on TME, suggesting that pretrial "familiarity" with a diet does not markedly improve metabolic efficiency. It should also be noted that the test diets in this study were fed intact and dry, which may not accurately represent the condition in which wild birds obtain food. Blue-winged teal obtain seed diets from the surface of flooded habitats. Short-term flooding leads to seed deterioration (Fredrickson and Reid 1988, Nelms and Twedt 1996), suggesting that concomitant changes in metabolizability may occur. Flooding may reduce hardness of high-fiber foods to the point that grit is unneccessary as an aid to mechanical digestion. Clearly, feeding trial methods that accurately represent the foraging mode of wild birds are needed.

Despite using similar pretrial treatments and identical test diets, the influence of grit on TME differed markedly between this study and that of Petrie et al. (1997). This suggests that proximate causes of grit consumption may vary within taxonomic families. Although only 2 data points are currently available, these data suggest that a gradient in body size, and thus in passage rate, may influence the nutritional benefits of grit consumption. Gionfriddo and Best (1996) showed that grit consumption is common among many avian families, including nectarivores (e.g., hummingbirds). This observation suggests that similar variation among taxa in the nutritional benefits of grit consumption occurs. That there appears to be no universal hypothesis explaining grit consumption by birds argues for further research into the nutritional benefits of grit.

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