



FEATURE ARTICLE

Biotic dispersal in eelgrass *Zostera marina*

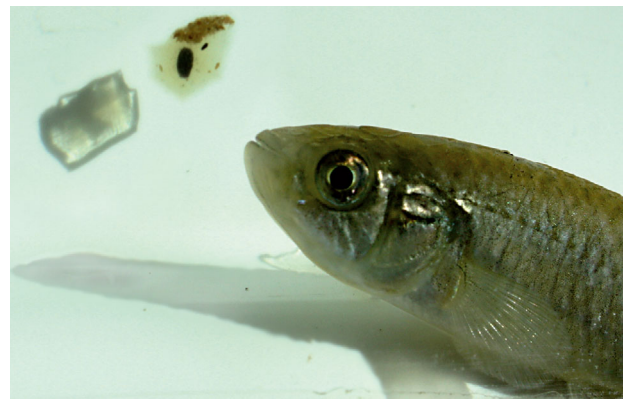
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ABSTRACT: Dispersal is a critical process in the life history of nearly all plant species and can be facilitated by both abiotic and biotic mechanisms. Despite an abundance of vertebrate fauna utilizing seagrass meadows as a feeding area and thus capable of consuming and excreting seeds, little work has been conducted on biotic seed dispersal mechanisms. The objectives of this study were to (1) determine whether seeds of the seagrass *Zostera marina* could pass through the digestive systems of resident and transient vertebrates of a seagrass bed and remain viable and (2) determine seed retention times in the guts of each species to estimate dispersal distances of *Z. marina* seeds by vertebrate dispersers. Excretion and germination rates of consumed seeds for 3 fish species (*Fundulus heteroclitus*, *Sphoeroides maculatus*, *Lagodon rhomboides*), 1 turtle species (*Malaclemys terrapin*) and 1 waterfowl species (*Aythya affinis*) showed *Z. marina* seeds could survive passage through species' digestive systems and successfully germinate. Excretion rates were generally highest for *F. heteroclitus*, *S. maculatus*, and *M. terrapin*, lowest for *A. affinis*, and moderate for *L. rhomboides*. Analyses suggest seeds were significantly affected by species' digestive tracts. Maximum dispersal distances are estimated to be 200, 60, 1500, and 19 500 m for *F. heteroclitus*, *L. rhomboides*, *M. terrapin*, and *A. affinis*, respectively. Data here provide strong evidence that biotic dispersal can occur in *Z. marina*, and biotically transported seeds can be dispersed to isolated areas unlikely to receive seeds via abiotic mechanisms. Biotic dispersal may rival or exceed abiotic mechanisms. Future seagrass dispersal models should incorporate biotic dispersal as a seed transport mechanism.

KEY WORDS: Seeds · Dispersal · Vertebrates · *Zostera marina*

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Fundulus heteroclitus were fed *Zostera marina* seeds in a squid gelatin matrix.

Image: Scott Marion

INTRODUCTION

Dispersal is widely recognized as a critical process in the life history of nearly all plant species (Levin et al. 2003, Nathan et al. 2008). Its benefits have profound impacts on plant populations by establishing new populations away from the parent plant, thereby increasing chances of survival for the dispersing propagule (Howe & Smallwood 1982). Dispersal mechanisms can be abiotic or biotic. Each plant species may rely on one specific dispersal mechanism based on life-history characteristics (e.g. wind- or current-dispersed seeds), although, it is more likely that seeds of many species are dispersed by multiple mechanisms, both abiotic and biotic (e.g. wind and animal dispersers; Howe & Smallwood 1982, Herrera 2002), prior to entering the seed bank (Chambers & MacMahon 1994, Wilkinson 1997, 1999, Higgins et al. 2003). Recent evidence suggests long-distance

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dispersal events may be more common than previously considered, regardless of the life-history traits of plants (Clark et al. 1998, Nathan et al. 2008).

In terrestrial systems, biotic dispersal of propagules occurs through ingestion and excretion of viable seeds or via seeds externally attached to the body of the dispersing agent (Chambers & MacMahon 1994, Debussche & Isenmann 1994, Figuerola et al. 2002, Figuerola & Green 2004). Biotic dispersal distances can range from meters to kilometers, depending on the dispersal agent (e.g. ants and deer, respectively; Myers et al. 2004). Despite knowledge of terrestrial biotic dispersal processes, little is known about them in marine angiosperms, the seagrasses, and whether seeds can actually survive passage through the gut of different species.

Seagrasses are found in most shallow coastal waters around the world (Green & Short 2003) and can reproduce both asexually (rhizome elongation) and sexually (seeds). Currently, mechanisms of dispersal in seagrasses have been described as primarily abiotic, via winds and currents acting on floating propagules (Churchill et al. 1985, Orth et al. 2006, Kendrick et al. 2012). Few studies have highlighted biotic dispersal mechanisms, e.g. waterfowl (Agami & Waisel 1986, 1988, Charalambidou et al. 2003). A number of vertebrate species, including sireniens, turtles, waterfowl, and fishes (Cottam et al. 1944, Adams 1976, Kendrick et al. 2012), are known to consume seagrass through either direct feeding on shoots which may contain seeds or indirect feeding on the associated epiphytes and epifauna (Thayer et al. 1984). Several feeding studies of fauna that inhabit seagrass beds have found either seed fragments or entire seeds in their guts (Adams 1976, Figuerola et al. 2003, Sumoski & Tulipani pers. obs.), providing evidence that seeds are ingested in the foraging process. Observations indicate that much of the ingested material, including seeds if present, may be excreted with minimal damage (Thayer et al. 1984).

This study investigates biotic dispersal mechanisms in the seagrass *Zostera marina* (eelgrass). *Z. marina* is widely distributed in the North Atlantic and Pacific Oceans and in the Mediterranean Sea (Green & Short 2003). It is abundant in the Chesapeake Bay region, USA, where it produces seeds in flowering shoots from late May to early June. Currently, reported dispersal mechanisms for *Z. marina* are abiotic and include floating seeds, floating reproductive shoots with mature seeds, and seeds moving across the sediment from currents (Churchill et al. 1985, Orth et al. 1994, Harwell & Orth 2002). However, *Z.*

marina supports dense populations of animals, including fishes and waterfowl, acting as both habitat and a food source for these occupants (Adams 1976, Thayer et al. 1984, Moore & Short 2006, authors' pers. obs.).

While some studies have examined the consumption of seeds of several different seagrass species by fauna (Agami & Waisel 1986, 1988, Charalambidou et al. 2003), none have examined biotic dispersal of *Zostera marina* nor estimated the dispersal distances of resident and non-resident species that may consume seeds. Our objectives in this study were to (1) determine whether *Z. marina* seeds can pass through the guts of different resident and transient vertebrates and remain viable and (2) determine seed retention times in the guts of each species to estimate dispersal distances of *Z. marina* seeds by vertebrate dispersers.

MATERIALS AND METHODS

Zostera marina seeds for the feeding experiments were obtained from mature flowering shoots present in *Z. marina* meadows in late May to early June, 2009 to 2011, in South Bay on the seaside of the Delmarva Peninsula, Virginia, USA (37° 16' 20" N, 75° 48' 51" W). Seeds were separated from reproductive shoots and stored using methods described by Marion & Orth (2010). Prior to feeding trials, seeds were assessed for viability by gently squeezing them and checking individual fall velocities (Marion & Orth 2010).

Feeding trials

Five possible biotic dispersal species representing a range of vertebrate types found in *Zostera marina* beds within Chesapeake Bay were chosen for feeding trials based on literature reports of the presence of *Z. marina* seeds in guts, abundance in seagrass beds, foraging strategy that would allow for the ingestion of seeds, or personal observations (Table 1). Three species were assumed to have a short dispersal potential of <1 km: *Fundulus heteroclitus* (mummichog), *Sphoeroides maculatus* (northern puffer), and *Lagodon rhomboides* (pinfish); and 2 species showed the potential to disperse seeds at distances >1 km: *Malaclemys terrapin* (diamondback terrapin) and *Aythya affinis* (lesser scaup) (Adams 1976, Orth & Heck 1980, D. Tulipani unpubl. data). These species can ingest seeds either by feeding directly on *Z. marina* (*L. rhomboides* TL: >80 mm, *A. affinis*), or by

Table 1. Number of individuals for each species used in the feeding trials and total number of seeds consumed for each trial year

Taxon	Trial year	No. of individuals	No. of seeds consumed
<i>Fundulus heteroclitus</i>	2009	14	207
	2010	17	176
<i>Malaclemys terrapin</i>	2009	12	32
	2010	5	36
<i>Sphoeroides maculatus</i>	2009	1	3
	2010	2	28
	2011	9	84
<i>Lagodon rhomboides</i>	2010	14	66
	2011	10	106
<i>Aythya affinis</i>	2011	4	969

indirectly consuming seeds by feeding on epiphytes and epifauna (*L. rhomboides* < 80 mm, *F. heteroclitus*, *S. maculatus*, *M. terrapin*). Feeding trials were conducted from July to November in 2009, 2010, and 2011, based on the availability of species each year (Table 1).

Fishes and terrapins were collected from nearby *Zostera marina* meadows by trawling, seining, or using a minnow trap. Fishes were selected for length to ensure seed consumption, particularly *Lagodon rhomboides* which had been found to have *Z. marina* seeds present in their guts (Table 2 in Adams 1976). *L. rhomboides* undergoes an ontogenetic shift in feeding strategy from omnivory to herbivory between 80 and 120 mm (Stoner & Livingston 1984). We used specimens that would be considered herbivorous (Table 2). Specimens were transported to the laboratory in aerated holding tanks. Individuals were kept in separate aerated aquaria. Test specimens were offered seeds placed in a feeding matrix (*Fundulus heteroclitus*: squid gelatin; *L. rhomboides*, *Sphoeroides maculatus*: shrimp; *Malaclemys terrapin*:

blue crab claw). Prior to each experiment, test specimens were placed in individual aquaria and starved for 12 to 48 h. At the initiation of each feeding trial, viable *Z. marina* seeds were placed in feeding matrices and subsequently fed to the test specimens. *M. terrapin*, *S. maculatus*, and *L. rhomboides* were fed 3 to 5 seeds for each feeding trial, while *F. heteroclitus* were fed seeds until satiated. Total number of seeds used for each species each year is given in Table 1. Non-consumed seeds were enumerated by either counting and removing fallen seeds at the bottom of the tank or by siphoning the bottom of the tank for seeds. In the 2010 and 2011 feeding trials, a mesh screen separated fish specimens from the bottom of each tank to prevent re-consumption of seeds. This behavior was noted during the 2009 *F. heteroclitus* feeding trials. Fishes were left undisturbed for 24 to 48 h after feeding, at which time all excreted seeds, including damaged seeds or seed coats, were removed from tank bottoms, in order to determine excretion rates. Terrapin cages were cleaned daily, and water was sieved to extract excreted seeds, both whole, as well as damaged. Intact seeds were stored in 25 ml vials containing each specimen's appropriate aquarium water. Lengths of feeding trials varied for all species, but generally were conducted until all seeds were excreted, or in a few individual cases, an additional 24 h beyond the species observed seed retention time (S. Sumoski pers. obs.).

Specific retention time of seeds in the guts of fishes was estimated by feeding either a single seed or glass bead mimic (~1 mm²) to ensure no digestion occurred. We noted no difference in excretion times between seeds and beads when incorporated into the test. Fishes were then monitored hourly for seed or bead excretion. Retention time of seeds in the guts of *Malaclemys terrapin* was estimated by monitoring excretion of seeds at frequent intervals during feeding trials.

Aythya affinis were held at the United States Geological Survey—Patuxent Wildlife Research Center, Maryland, USA. Individuals were fed seeds in a food slurry using Lafeber's Emerald Exotic Carnivore Diet (Lafeber Company) to ensure their stomachs were full during the feeding trial (R. Therrien pers. comm.). Specimens were then placed in individual 50 gallon (ca. 190 l) tubs with ambient seawater filled halfway, a mesh screen separating them from the bottom, and an

Table 2. Number of specimens, mean fork length (mm), and mean weight (g) of specimens used in the experiments for each species during the experimental period. nd = no data

Taxon	Years	No. of specimens	Mean fork length ± SD (mm)	Mean weight ± SD (g)
<i>Fundulus heteroclitus</i>	2009–2010	31	84.23 ± 8.66	nd
<i>Sphoeroides maculatus</i>	2010–2011	4	143.75 ± 26.57	nd
<i>Lagodon rhomboides</i>	2010–2011	14	123.36 ± 13.57	nd
<i>Malaclemys terrapin</i>	2009–2010	14	nd	263.78 ± 75.30
<i>Aythya affinis</i>	2011	4	nd	681.25 ± 15.48

opaque cover to minimize stress to the animal. Individuals were allowed to swim undisturbed for 6 to 7 h (observed seed retention time: 2 to 5 h) after which the individuals were removed and the tubs were drained, with all water siphoned into a 1.0 mm sieve to retain excreted seeds—both damaged and intact. Retention time of seeds in the guts of *A. affinis* was determined by mixing a dye marker in the carnivore diet and monitoring for excretion of the marker.

Control experiments included 3 to 5 seeds being placed in each feeding matrix and salt water during each feeding trial for at least 2 h before being removed and placed in a vial for storage. A second control ('seawater control') consisted of seeds held in plain seawater. Controls for *Aythya affinis* consisted of seeds placed in the food slurry and seeds placed in the food slurry and dye combination.

Germination tests

Excreted seeds were stored in 25 ml vials with seawater until November of each trial year, which immediately preceded the natural germination time of *Zostera marina* in Virginia (Moore et al. 1993). Seeds were planted in 1.0 mm sieved sediment collected from the York River, Virginia, USA. Seed planting depth was approximately 5 to 10 mm. The location of each seed was recorded by specimen to ensure proper identification of each emergent seedling. Sediment containers with seeds were placed in an outdoor tank with standing water and covered with a shade cloth. Temperature and salinity were monitored and kept at ambient conditions matching those of the neighboring York River. Ice was removed as necessary. Seedling emergence was monitored weekly until March, at which time the sediment was sieved through a 1.0 mm sieve and all seedlings and ungerminated seeds were removed and counted. Seedlings were identified based on the presence of a cotyledon or green shoot. Seeds which had not germinated were stripped of their seed coat and soaked in a 1.0% Tetrazolium staining solution for 24 h to determine the presence of living tissue, an indication of seed viability (Conacher et al. 1994).

Dispersal distances of each species were estimated using reported movement rates based on literature values and observed seed retention times in the guts of individual species. These estimates were then compared to distances reported for abiotic dispersers.

Analysis

Germination rates were compared among the 5 species by conducting survival analysis using the Lifetest procedure in SAS (independent variable: specimens used, dependent variable: seed excretion rate; SAS Institute). This test estimates the survival distributions and equality of the given variables, while making no assumptions about the given distributions (Dixon & Newman 1991).

Relationships between specimen length (*Fundulus heteroclitus*, *Sphoeroides maculatus*, *Lagodon rhomboides*) or weight (*Malaclemys terrapin*, *Aythya affinis*) (Table 2) and seed evacuation rates were investigated using logistic regressions in R statistical software. The binary response variables allowed consumed seeds to be placed in either an 'excreted' or 'non-excreted' category.

In order to determine the combined effects of seed loss during gut passage and subsequent mortality prior to germination, excretion and germination rates were multiplied for each species. This proportion analysis was compared to germination rates of each control for the respective species to assess the overall germination among controls or biotic dispersers. A survivorship analysis was applied using the Cox proportional hazards model (Newman & Dixon 1996, Proc PHREG) to each species germination rate through time while in the sediment and at the end of the experiment. This test typically assesses the probability of death throughout a given interval of time. It is used here to compare the germination of seeds that have passed through the gut of a specimen versus control seeds, thus resulting in hazard ratios where a value of 1 = a specimen-consumed seed and control seed are equally likely to germinate by the next point in time, <1 = a control seed is more likely to germinate than a consumed seed by the next point in time, and >1 = a specimen-consumed seed is more likely to germinate than a control seed by the next point in time.

RESULTS

Excretion and germination rates of viable *Zostera marina* seeds varied by species and trial year and were generally highest for *Fundulus heteroclitus*, *Sphoeroides maculatus*, and *Malaclemys terrapin*, lowest for *Aythya affinis*, and moderate for *Lagodon rhomboides*. Excretion rates for *F. heteroclitus* were higher in 2010 (99%) than in 2009 (76%), while they decreased for *S. maculatus* between 2010 and 2011. *M. terrapin* had relatively high excretion rates, yet

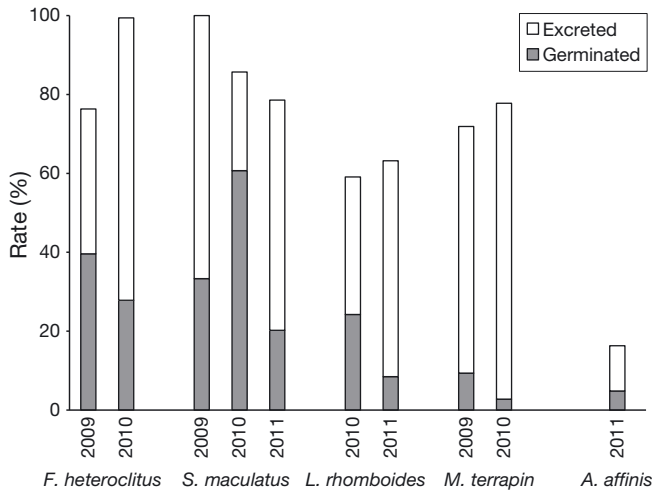


Fig. 1. Excretion and germination rates of *Zostera marina* seeds consumed by *Fundulus heteroclitus*, *Spherooides maculatus*, *Lagodon rhomboides*, *Malaclemys terrapin*, and *Aythya affinis*. Feeding trials were conducted in 2009, 2010, and 2011

low germination success. Overall, germination rates were highest among fish species and lowest in *A. affinis* (Fig. 1).

Observed seed retention times for passage of single seeds or beads were 15 to 20, 7 to 10+, 7 to 10+, and 2 to 5 h in *Fundulus heteroclitus*, *Spherooides maculatus*, *Lagodon rhomboides*, and *Aythya affinis*, respectively. Retention times were estimated to be between 24 and 144 h for *Malaclemys terrapin*.

Germination success of *Zostera marina* seeds was assessed across all 5 species and including all trial years for each species. Post-gut passage success of seeds varied significantly by species ($\chi^2 = 20.926$, $df = 4$, $p = 0.0003$). *Malaclemys terrapin* had the lowest success rate among the 5 species; *Fundulus heteroclitus* and *Spherooides maculatus* had among the highest germination success (Table 3).

Germination rates of consumed seeds compared to control seeds indicated the feeding matrix had an effect on seed survival in *Malaclemys terrapin* (in 2010) and *Lagodon rhomboides* and *Spherooides maculatus* (both in 2011) (Fig. 2). Controls suggest gut passage has little effect on survival in *S. maculatus* (in 2010) and *Fundulus heteroclitus* (in 2010), yet increases the chance of seed mortality when passing through guts of *Aythya affinis* (Fig. 2).

In 5 instances hazard ratios had significant values, indicating the seed survivorship is likely being affected by the gut of the animal: *Malaclemys terrapin* (in 2010), *Lagodon rhomboides* (in 2010, 2011), and *Spherooides maculatus* (in 2011) when comparing the feeding trial against the seawater control, and

Table 3. Total proportion of seeds germinated for all species and individuals used throughout the trial years

Taxon	Trial years	No. of individuals	Proportion germinated
<i>Fundulus heteroclitus</i>	2009–2010	31	0.39
<i>Malaclemys terrapin</i>	2009–2010	17	0.14
<i>Spherooides maculatus</i>	2009–2011	12	0.37
<i>Lagodon rhomboides</i>	2010–2011	24	0.32
<i>Aythya affinis</i>	2011	4	0.30

Aythya affinis when comparing the feeding trial against the control with food and dye (Table 4). Hazard ratios indicated better germination via the consumed seed when feeding trials were compared to both controls in *S. maculatus* (in 2010). Values were closest to being equal for all feeding trials against all controls and all years in *Fundulus heteroclitus* (Table 4).

Specimen size had little effect on seed survival during gut passage: *Fundulus heteroclitus* (in 2010) ($Z = 0.143_{0.101,175}$, $p = 0.886$), *Lagodon rhomboides* ($Z = 1.549_{0.025,61}$, $p = 0.121$) and *Spherooides maculatus* ($Z = 1.858_{0.016,56}$, $p = 0.063$). Fish length did have a significant effect on seed emergence post-consumption in *F. heteroclitus* trials in 2009 when seeds were possibly re-ingested ($Z = 3.889_{0.03,201}$, $p = 0.0001$; Table 4). Seed passage time was not correlated with the weight of *Malaclemys terrapin* ($Z = 0.249_{0.005,60}$, $p = 0.803$), while weights of *Aythya affinis* did have a significant effect on seeds ($Z = 5.064_{0.008,969}$, $p = 4.11 \times 10^{-7}$).

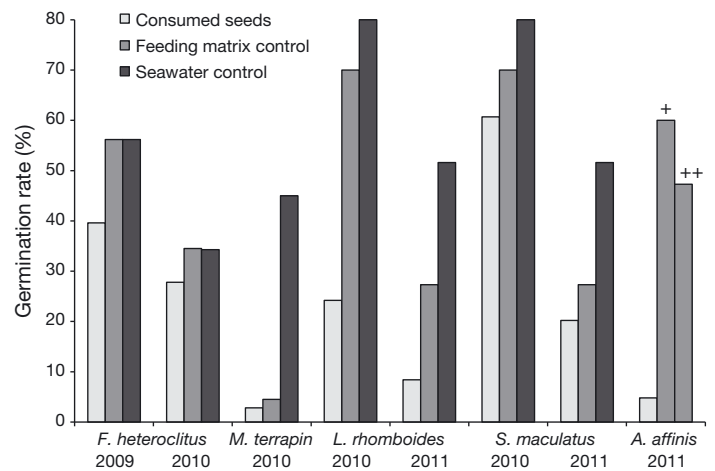


Fig. 2. Germination rate (%) of *Zostera marina* seeds in controls compared to experimental treatments for seeds consumed by *Fundulus heteroclitus*, *Malaclemys terrapin*, *Lagodon rhomboides*, *Spherooides maculatus*, and *Aythya affinis*. For *A. affinis*: +: control seeds with feeding matrix and dye marker; ++: control seeds with feeding matrix

Table 4. Cox proportional hazard model survivorship analysis for each species and year of feeding trial compared to controls with and without feeding matrices. Hazard models are used here to determine germination of consumed versus control seeds. PE: parameter estimate; SE: standard error; *H*: hazard ratio; +: control seeds with feeding matrix and dye marker; ++: control seeds with feeding matrix; **p* ≤ 0.05

Taxon	Year	Consumed seeds versus feeding matrix control					Consumed seeds versus seawater control				
		PE	SE	χ^2	p	<i>H</i>	PE	SE	χ^2	p	<i>H</i>
<i>Fundulus heteroclitus</i>	2009	-0.017	0.351	0.002	0.961	0.983	-0.196	0.351	0.311	0.577	0.822
	2010	-0.161	0.346	0.218	0.64	0.876	-0.132	0.321	0.169	0.681	0.851
<i>Malaclemys terrapin</i>	2010	-0.519	1.414	0.135	0.713	0.595	-2.788	1.054	6.99	0.008*	0.062
<i>Lagodon rhomboides</i>	2010	-0.519	0.367	2.0015	0.157	0.595	-0.788	0.337	5.452	0.019*	0.455
	2011	-0.587	0.476	1.523	0.217	0.556	-1.286	0.453	8.042	0.004*	0.276
<i>Sphoeroides maculatus</i>	2010	0.378	0.378	1.001	0.317	1.46	0.119	0.349	0.116	0.734	1.126
	2011	-0.137	0.387	0.125	0.723	0.872	-0.8173	0.359	5.167	0.023*	0.442
<i>Aythya affinis</i>	2011	-0.868+	0.305+	8.11+	0.004+*	0.42+	-0.427++	0.364++	1.378++	0.24++	0.652++

Table 5. Seed retention times estimated from single seeds or beads, recorded daily movement and maximum distances traveled for each species, are combined to estimate seed dispersal distances for each species. nd = no data

Species	Observed gut retention time (h)	Daily movement (m)	Maximum distance traveled (m)	Dispersal estimate (m)	Sources
<i>Fundulus heteroclitus</i>	15–20	200	600–3600	200	Skinner et al. (2005)
<i>Sphoeroides maculatus</i>	7–10+	nd	nd	nd	Present study
<i>Lagodon rhomboides</i>	7–10+	nd	60	0–60	Potthoff & Allen (2003)
<i>Malaclemys terrapin</i>	24–144	1430	8000	1500	Ernst & Lovich (2009), D. Tulipani (pers. comm.)
<i>Aythya affinis</i>	2–5	2000–37500	93500 d ⁻¹	2000–19500	Herring & Collazo (2005), Afton (2009)

In the 2010 feeding trials, 204 seeds did not germinate, but 30 still had intact and rigid seed coats. None of these 30 seeds stained with tetrazolium, suggesting these seeds were also non-viable. In the 2011 germination trials, 131 seeds did not germinate, but 35 had intact and rigid seed coats. Only 3 stained positively with tetrazolium, suggesting these seeds were viable.

Maximum dispersal distances were calculated as the product of literature reported values of species

movements and estimates of observed seed retention times in guts (Table 5) for *Fundulus heteroclitus*, *Lagodon rhomboides*, *Malaclemys terrapin*, and *Aythya affinis* and were determined to be 200, 60, 1500, and 19500 m, respectively (Fig. 3). No movement data were available for *Sphoeroides maculatus*, but a dispersal distance may be expected in the range of *F. heteroclitus* and *L. rhomboides* (Able & Fahay 2010).

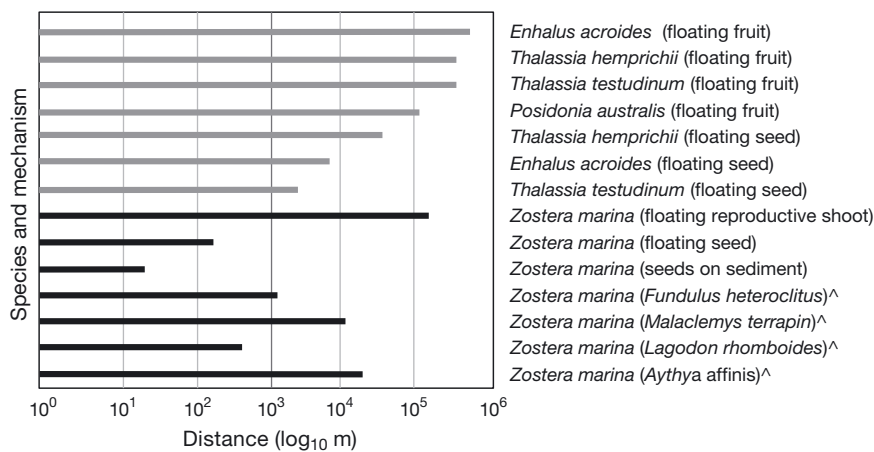


Fig. 3. Abiotic and biotic dispersal mechanisms and distances of seagrass species (modified from Kendrick et al. 2012). Black bars represent dispersal of *Zostera marina* seeds; gray bars represent seed dispersal in seagrasses other than *Z. marina*. [^]: biotic dispersal

DISCUSSION

Three years of feeding experiments indicated that *Zostera marina* seeds could survive passage through the guts of species in very different functional groups (fishes, turtles, birds) and successfully germinate. This is the first study to date to demonstrate survival of *Z. marina* seeds after ingestion by an animal, and provides evidence that biotic dispersal of *Z. marina* seeds is possible and needs to be considered in the dispersal dynamics of this species.

Seed excretion and germination

Excretion and germination rates varied among the 5 species both within and between years and may be a function of both foraging ecology and gut morphology of the individual species. Excretion rates of *Lagodon rhomboides* were lowest of the fish species used, and survivorship analyses yielded hazard ratios suggesting loss of seeds due to gut morphology in both 2010 and 2011. Other species showing significant effects from digestive tracts on seeds included *Malaclemys terrapin* (in 2010), *Spherooides maculatus* and *Aythya affinis* (both in 2011), suggesting mechanical or chemical weakening of the seed coat while in the gut of these species (Baskin & Baskin 1998). Similar effects were apparent when comparing germination rates of experimental treatments to controls. Results of each test suggest either gut morphology or feeding behavior is damaging some of the seeds in the species used in these experiments. *L. rhomboides* are primarily herbivorous at the size range used here, with dentition to crush seeds prior to entering the digestive tract and an extended intestine for further breakdown of plant material (Stoner & Livingston 1984, Luczkovich et al. 1995). *A. affinis* also feeds directly on seagrass and contains a gizzard comprised of grit to grind and break down plant cell walls (Cottam et al. 1944). *M. terrapin* selectively chooses epifauna, such as barnacles (D. Tulipani pers. comm.), which are broken down in the buccal cavity before entering the digestive tract. Secondarily consumed seeds are subject to this process (Bels et al. 1998). *S. maculatus* have beak-like jaws used to feed on and crush shelled invertebrates (Targett 1979). In addition, it is possible that our use of feeding matrices influenced overall seed mortality; it is likely that fewer seeds are destroyed when offered in combination with other food. However, despite our observations that all species used here have the capacity to damage seeds during the feeding and

digestive processes and that the added feeding matrices may have influenced overall mortality, our data show that a number of seeds can successfully pass through their guts and germinate.

Lengths and weights of species generally had little effect on seed survival. Specimen length had a significant effect on seed excretion rate for *Fundulus heteroclitus* in the 2009 experiments, but not in 2010, when re-consumption of seeds within the 24 to 48 h feeding was eliminated, suggesting that fish length may not be a factor here. Weights of *Aythya affinis* also had a significant effect on excretion rates. It is more likely that the small sample size contributed to this value.

Seed-dispersal distances

A critical component of dispersal is the distance a seed moves from its parent to a location suitable for germination, growth, and survival (Harper 1977, Nathan & Muller-Landau 2000). In biotic dispersal that distance is a function of how long a seed remains in the gut and how far the individual moves during that time period (Figuerola et al. 2010). Estimated dispersal distances assume linear movement, which could overestimate a dispersal calculation if a species changes directions during movements (Table 5). Our estimates compare favorably with several mechanisms of abiotic dispersal for *Zostera marina* and other seagrass species (Fig. 3) (Kendrick et al. 2012), with the possible exception of *Aythya affinis* and *Malaclemys terrapin* where dispersal may exceed abiotic distances. We think dispersal distances of waterfowl may exceed what we have noted here. The seed retention times we observed for *A. affinis* were relatively short compared to published literature values which exceed 24 h in some species (Charalambidou et al. 2003, Figuerola et al. 2010), suggesting that waterfowl that ingest *Z. marina* seeds may disperse seeds much further than the reported distances for *A. affinis*.

While the majority of biotic consumers will likely transport seeds shorter distances than abiotic mechanisms (Fig. 3), we suggest there may be distinct advantages for seeds dispersed by biotic mechanisms. Biotic dispersers are typically either permanent or transient residents of seagrass beds and are free to move within or between beds (Orth & Heck 1980). In dense seagrass areas, much of the habitat range of a biotic disperser may also be suitable for seagrass growth. Species foraging either directly or indirectly on the grass will likely reside within suit-

able growth areas to remain close to food sources, thus increasing their effectiveness as a dispersal agent. In contrast, abiotic dispersal is limited in movement by wind and currents (Kendrick et al. 2012), and, once exported from the seagrass bed, seeds could arrive in areas not suitable for seagrass growth, e.g. intertidal areas or regions of low salinity. Occasionally, biotic dispersers may aid in seagrass survival when scarification due to acidification or chemical weathering in the gut prompts early germination (Baskin & Baskin 1998, Herrera 2002). In many cases successful biotic dispersal adds to the genetic diversity of existing seagrass beds or may establish new seagrass populations in areas distant from established beds (Howe & Smallwood 1982, Olivieri et al. 1995, Herrera 2002).

Biotic dispersal of seagrass seeds may be more common than previously considered (Kendrick et al. 2012). Most seagrass beds support dense assemblages of small and large consumers (Thayer et al. 1984, Valentine & Heck 1999), a number of which are seasonally transient (Adams 1976, Orth & Heck 1980) and are potential consumers of seeds. A number of field studies have reported terrestrial and macrophyte seeds in waterfowl feces and digestive tracts that are either directly consumed or indirectly when foraging for benthic invertebrates (Guppy 1906, Baldwin & Lovvorn 1994, Ntiamoa Baidu et al. 1998, Mueller 1999, Green et al. 2002, Figuerola et al. 2010). In addition, tropical seagrass beds support omnivorous fishes and megafauna, such as manatees, dugongs, and green turtles, capable of applying intense grazing pressure and possibly consuming large quantities of seeds in the process (Thayer et al. 1984, Preen 1995, Valentine & Heck 1999, McDermid et al. 2007, Kendrick et al. 2012). These megafauna forage within ranges of 0 to 15 km² and 0 to 50 km² for manatees and dugongs, respectively, or travel 0.6 to 3.3 km h⁻¹ for green turtles (Godley et al. 2002, Deutsch et al. 2003, Sheppard et al. 2006). Seeds surviving gut passage of these vertebrates are capable of being dispersed distances greatly exceeding those of abiotic mechanisms (Fig. 3).

CONCLUSIONS

Our first-order experiments conducted here in a laboratory setting conclusively prove that *Zostera marina* seeds can be ingested, excreted whole, and germinate successfully, by a variety of vertebrate species. Given that several studies have found seagrass seeds in guts of fishes and birds, we suggest

that successful dispersal via biotic mechanisms may be more common than previously considered (Kendrick et al. 2012). As it is highly likely that many seeds are ingested indirectly during foraging activities of a particular species, subsequent studies should begin to focus on seed ingestion rates under field conditions when species have a diversity of food choices. The density of grass consumers also influences the likelihood and frequency of indirect seed dispersal. Seed ingestion rates may be high in tropical areas where grazing is more intense, particularly by large herbivores, such as dugongs and turtles. More importantly, the ability of seeds to successfully pass through the gut of a waterfowl species offers support for a mechanism of long-distance dispersal, as this group of vertebrates is numerically important in many areas of the world (Clausen et al. 2002, Green et al. 2002). While seeds will suffer mortality during the ingestion and digestive processes, some proportion of seeds can be expected to survive, germinate, and grow to adult plants, leading to the colonization of new habitats (Nathan et al. 2008). Finally, our data suggest that future seagrass dispersal models should incorporate biotic dispersal mechanisms.

Acknowledgements. This work was funded by a Virginia Institute of Marine Science (VIMS) student assistantship and the 2011 VIMS student research grant. The authors thank personnel from the USGS Patuxent Wildlife Research Center, the VIMS SAV laboratory, S.E.S.'s thesis committee, fellow VIMS students, especially Diane Tulipani, and faculty for their assistance in collecting specimens and analyzing data. We appreciated the excellent comments from 3 anonymous reviewers. This is Contribution No. 3253 from the VIMS.

LITERATURE CITED

- Able KW, Fahay MP (2010) *Sphoeroides maculatus* (Bloch and Schneider) northern puffer. In: Ecology of estuarine fishes: temperate waters of the western North Atlantic. The Johns Hopkins University Press, Baltimore, MD, p 494–500
- Adams SM (1976) Feeding ecology of eelgrass fish communities. *Trans Am Fish Soc* 105:514–519
- Afton AD (2009) Chronology and rates of migratory movements, migration corridors, and habitats used throughout the annual cycle by female lesser scaup radio-marked on pool 19 of the Mississippi River. Annual Progress Report, 30 November, 2009. USGS Louisiana Cooperative Fish and Wildlife Unit, Baton Rouge, LA
- Agami M, Waisel Y (1986) The role of mallard ducks (*Anas platyrhynchos*) in distribution and germination of seeds of the submerged hydrophyte *Najas marina* L. *Oecologia* 68:473–475
- Agami M, Waisel Y (1988) The role of fish in distribution and germination of seeds of the submerged macrophytes

- Najas marina* L. and *Ruppia maritima* L. *Oecologia* 76: 83–88
- Baldwin J, Lovvorn J (1994) Expansion of seagrass habitat by the exotic *Zostera japonica*, and its use by dabbling ducks and brant in Boundary Bay, British Columbia. *Mar Ecol Prog Ser* 103:119–127
- Baskin CC, Baskin JM (1998) Seeds. Ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, CA
- Bels VL, Davenport J, Renous S (1998) Food ingestion in the estuarine turtle *Malaclemys terrapin*: comparison with the marine leatherback turtle *Dermochelys coriacea*. *J Mar Biol Assoc UK* 78:953–972
- Chambers JC, MacMahon JA (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annu Rev Ecol Syst* 25:263–292
- Charalambidou I, Santamaria L, Langevoord O (2003) Effect of ingestion by five avian dispersers on the retention time, retrieval, and germination of *Ruppia maritima* seeds. *Funct Ecol* 17:747–753
- Churchill AC, Nieves G, Brenowitz AH (1985) Flotation and dispersal of eelgrass seeds by gas bubbles. *Estuar Coasts* 8:352–354
- Clark JS, Fastie C, Hurtt G, Jackson ST and others (1998) Reid's paradox of rapid plant migration. *Bioscience* 48: 13–24
- Clausen P, Nolet BA, Fox AD, Klaassen M (2002) Long-distance endozoochorous dispersal of submerged macrophyte seeds by migratory waterbirds in northern Europe—a critical review of possibilities and limitations. *Acta Oecol* 23:191–203
- Conacher CA, Poiner IR, Butler J, Pun S, Tree DJ (1994) Germination, storage, and viability testing of seeds of *Zostera capricorni* Aschers from a tropical bay in Australia. *Aquat Bot* 49:47–58
- Cottam C, Lynch JJ, Nelson AL (1944) Food habits and management of American sea brant. *J Wildl Manag* 8:36–56
- Debussche M, Isenmann P (1994) Bird-dispersed seed rain and seedling establishment in patchy Mediterranean vegetation. *Oikos* 69:414–426
- Deutsch CJ, Reid JP, Bonde RK, Easton DE, Kochman HI, O'Shea TJ (2003) Seasonal movements, migratory behavior, and site fidelity of West Indian manatees along the Atlantic coast of the United States. *Wildl Monogr* 151:1–77
- Dixon PM, Newman MC (1991) Analyzing toxicity data using statistical models for time-to-death: an introduction. In: Newman MC, McIntosh AW (eds) *Metal ecotoxicology: concepts and applications*. Lewis Publishers, Chelsea, MI, p 207–242
- Ernst CH, Lovich JE (2009) *Turtles of the United States and Canada*. Johns Hopkins University Press, Baltimore, MD
- Figuerola J, Green AJ (2004) Effects of seed ingestion and herbivory by waterfowl on seedling establishment: a field experiment with widgeongrass *Ruppia maritima* in Doñana, south-west Spain. *Plant Ecol* 173:33–38
- Figuerola J, Green AJ, Santamaria L (2002) Comparative dispersal effectiveness of widgeongrass seeds by waterfowl wintering in south-west Spain: quantitative and qualitative aspects. *J Ecol* 90:989–1001
- Figuerola J, Green AJ, Santamaria L (2003) Passive internal transport of aquatic organisms by waterfowl in Donana, south-west Spain. *Glob Ecol Biogeogr* 12:427–436
- Figuerola J, Charalambidou I, Santamaria L, Green AJ (2010) Internal dispersal of seeds by waterfowl: effect of seed size on gut passage time and germination patterns. *Naturwissenschaften* 97:555–565
- Godley BJ, Richardson S, Broderick AC, Coyne MS, Glen F, Hays GC (2002) Long-term satellite telemetry of the movements and habitat utilization by green turtles in the Mediterranean. *Ecography* 25:352–362
- Green EP, Short FT (2003) *World atlas of seagrasses*. University of California Press, Berkeley, CA
- Green AJ, Figuerola J, Sánchez MI (2002) Implications of waterbird ecology for the dispersal of aquatic organisms. *Acta Oecol* 23:177–189
- Guppy HB (1906) *Observations of a naturalist in the Pacific between 1891 and 1899*. Vol 2, Plant dispersal. Macmillan, London
- Harper JL (1977) *Population biology of plants*. Academic Press, New York, NY
- Harwell MC, Orth RJ (2002) Long-distance dispersal potential in a marine macrophyte. *Ecology* 83:3319–3330
- Herrera CM (2002) Seed dispersal by vertebrates. In: Herrera CM, Pellmyr O (eds) *Plant animal interactions: an evolutionary approach*. Blackwell Publishing, Malden, MA, p 185–210
- Herring G, Collazo JA (2005) Habitat use, movements and home range of wintering lesser scaup in Florida. *Waterbirds* 28:71–78
- Higgins SI, Nathan R, Cain M (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* 84:1945–1956
- Howe HF, Smallwood J (1982) Ecology of seed dispersal. *Annu Rev Ecol Syst* 13:201–228
- Kendrick GA, Waycott M, Carruthers TJB, Cambridge ML and others (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. *Bioscience* 62:56–65
- Levin SA, Muller-Landau HC, Nathan R, Chave J (2003) The ecology and evolution of seed dispersal: a theoretical perspective. *Annu Rev Ecol Syst* 34:575–604
- Luczkovich JJ, Norton SR, Gilmore RG (1995) The influence of oral anatomy on prey selection during the ontogeny of two percoid fishes, *Lagodon rhomboides* and *Centropomus undecimalis*. *Environ Biol Fishes* 44:79–95
- Marion SM, Orth RJ (2010) Innovative techniques for large-scale seagrass restoration using *Zostera marina* (eelgrass) seeds. *Restor Ecol* 18:514–526
- McDermid KJ, Stuercke B, Balazs GH (2007) Nutritional composition of marine plants in the diet of the green sea turtle (*Chelonia mydas*) in the Hawaiian Islands. *Bull Mar Sci* 81:55–71
- Moore KA, Short FT (2006) *Zostera*: biology, ecology, and management. In: Larkum AWD, Orth RJ, Duarte CM (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, p 361–386
- Moore KA, Orth RJ, Nowak JF (1993) Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. *Aquat Bot* 45:79–91
- Mueller H (1999) Common snipe *Gallinago gallinago*. In: Grill F (ed) *The birds of North America*, No. 417. The Birds of North America Inc., Philadelphia, PA
- Myers JA, Vellend M, Gardescu S, Marks PL (2004) Seed dispersal by white-tailed deer: implications for long-distance dispersal, invasion, and migration of plants in eastern North America. *Oecologia* 139:35–44
- Nathan R, Muller-Landau HC (2000) Spatial patterns of seed

- dispersal, their determinants and consequences for recruitment. *Trends Ecol Evol* 15:278–285
- Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsao A (2008) Mechanisms of long-distance seed dispersal. *Trends Ecol Evol* 23:638–647
- Newman MC, Dixon PM (1996) Ecologically meaningful estimates of lethal effect in individuals. In: Newman MC, Jagoe CH (eds) *Ecotoxicology a hierarchical treatment*. CRC Press, Boca Raton, FL, p 225–254
- Ntiamo-Baidu Y, Piersma T, Wiersma P, Poot M, Battley P, Gordon C (1998) Water depth selection, daily feeding routines and diets of waterbirds in coastal lagoons in Ghana. *Ibis* 140:89–103
- Olivieri I, Michalakis Y, Gouyon PH (1995) Metapopulation genetics and the evolution of dispersal. *Am Nat* 146: 202–228
- Orth RJ, Heck KL Jr (1980) Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay—fishes. *Estuaries* 3:278–288
- Orth RJ, Luckenbach M, Moore KA (1994) Seed dispersal in a marine macrophyte: implications for colonization and restoration. *Ecology* 75:1927–1939
- Orth RJ, Harwell MC, Inglis J (2006) Ecology of seagrass seeds and seagrass dispersal processes. In: Larkum AWD, Orth R, Duarte CM (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, p 111–133
- Potthoff MT, Allen DM (2003) Site fidelity, home range, and tidal migrations of juvenile pinfish, *Lagodon rhomboides*, in salt marsh creeks. *Environ Biol Fishes* 67: 231–240
- Preen A (1995) Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. *Mar Ecol Prog Ser* 124:201–213
- Sheppard JK, Preen AR, Marsh H, Lawler IR, Whiting SD, Jones RE (2006) Movement heterogeneity of dugongs, *Dugong dugon* (Müller) over large spatial scales. *J Exp Mar Biol Ecol* 334:64–83
- Skinner MA, Courtenay SC, Parker WR, Curry RA (2005) Site fidelity of mummichogs (*Fundulus heteroclitus*) in an Atlantic Canadian estuary. *Water Qual Res J Canada* 40: 288–298
- Stoner AW, Livingston RJ (1984) Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows. *Copeia* 1984:174–187
- Targett TE (1979) A contribution to the biology of the puffers *Sphoeroides testudineus* and *Sphoeroides spengleri* from Biscayne Bay, Florida. *Fish Bull* 77:292–295
- Thayer GW, Bjorndal KA, Ogden JC, Williams SL, Ziemann JC (1984) Role of larger herbivores in seagrass communities. *Estuaries* 7:351–376
- Valentine JF, Heck KL Jr (1999) Seagrass herbivory: evidence for the continued grazing of marine grasses. *Mar Ecol Prog Ser* 176:291–302
- Wilkinson DM (1997) Plant colonization: Are wind dispersed seeds really dispersed by birds at larger spatial and temporal scales? *J Biogeogr* 24:61–65
- Wilkinson DM (1999) Birds and seed dispersal; a response to comments by F. M. Chambers. *J Biogeogr* 26:429–430

*Editorial responsibility: Kenneth Heck Jr.,
Dauphin Island, Alabama, USA*

*Submitted: June 11, 2012; Accepted: October 26, 2012
Proofs received from author(s): November 30, 2012*