Diversity and Distributions, (Diversity Distrib.) (2016) 22, 30–42

BIODIVERSITY RESEARCH

Opening Pandora's bait box: a potent vector for biological invasions of live marine species

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ABSTRACT

Aim For over 80 years, the Maine baitworm trade has shipped live polychaete worms and packing algae 'wormweed' to distributors world-wide, while also consistently transferring a wide diversity and abundance of hitchhiking organisms of all life stages to numerous recipient communities. Here, we investigate this potent, yet underestimated, invasion vector using an important recipient region (the Mid-Atlantic) to examine the stepwise species transfer and survival along four stages of the vector.

Location Maine and Mid-Atlantic region (New Jersey, Delaware, Maryland, Virginia and North Carolina), USA.

Methods We quantified taxonomic identities and abundances of organisms associated with packing algae at four stages along the vector pathway during summer 2011: (1) Maine source habitats; (2) bait boxes from Maine distributors; (3) bait boxes from distributors in five Mid-Atlantic States; and (4) bait bags from retailers in five Mid-Atlantic States. We also examined functional diversity based on significant physical and life history characteristics and assessed genetic diversity for two common hitchhiking snail species.

Results We identified 17,798 live macro-organisms across 58 taxa, including marine macro-invertebrates, macroalgae, vascular plants and semi-terrestrial or aquatic invertebrates, present in bait boxes and bags. In all measures of diversity and abundance, we observed decreases of live marine macro-invertebrates across sequential stages of the vector from source to recipient regions. Significant differences in community composition were also observed between stages and were driven by isopods (taxonomic diversity) and isopods, amphipods and some gastropods (functional diversity).

Main conclusions The lack of management in the face of the sheer magnitude and diversity of organisms that are transported via the live marine bait trade underscores how this is an underappreciated vector that could be a considerable source of successful invasions globally.

Keywords
biological invasions, bloodworm, introduced, marine, Mid-Atlantic (USA), packing algae, wormweed.

INTRODUCTION

The accidental movement of organisms by human aided transport mechanisms (vectors) has become a major contributor to the homogenization of marine biotas globally (Ruiz & Carlton, 2003; Lockwood *et al.*, 2005). In coastal ecosystems, vectors such as ships' hull fouling and ballast water are recognized as important conveyors of organisms across natural dispersal barriers, contributing strongly to invasion dynamics (Carlton & Geller, 1993; Ruiz *et al.*, 2000). However, a diverse

DOI: 10.1111/ddi.12376
http://wileyonlinelibrary.com/journal/ddi

Diversity and Distributions
A Journal of Conservation Biogeography

range of other vectors can also be potent mechanisms of biological invasion and subsequent ecological impact (Carlton 1992; Weigle *et al.*, 2005). One such vector is the live bait trade, which delivers bait and associated organisms via overnight shipping to commercial distributors, bait shops and individual patrons world-wide. Bait vectors differ from other types of live trade, because bait and associated organisms are often used and sometimes released in natural habitats where they have opportunities to successfully establish and spread (Lau, 1995).

Due to their large size, marine polychaetes are used as live bait by recreational fishermen for a wide variety of fish species (Brown, 1993). In the USA, Maine is the largest supplier of marine bait for recreational fishing (Cohen *et al.*, 2001). The Maine industry harvests 'wild-caught' polychaetes [primarily bloodworms *Glycera dibranchiata* Ehlers 1868 and sandworms *Nereis (Alitta) virens* Sars 1835] from intertidal mudflats throughout coastal Maine. Worms are then packed in shallow newspaper-lined cardboard boxes, which are filled with 'wormweed' [i.e. a free-living growth form of the brown algae *Ascophyllum nodosum* (Linnaeus) Le Jolis 1863 ecad *scorpioides*] and frozen ice packs and shipped overnight to distributors world-wide (Creaser *et al.*, 1983; Crawford, 2001).

While the freshwater live bait trade has been explored as a mechanism for introducing both vertebrates and invertebrates (e.g. Keller *et al.*, 2007; Drake *et al.*, 2015), the Maine marine baitworm trade differs significantly as a transport mechanism because it transfers a wide diversity and abundance of hitchhiking organisms of all life stages (including gravid females) that are naturally associated with wormweed, resulting in the relocation of relatively intact communities. Previous studies have identified > 50 taxa, comprised of algae, invertebrates, fungi and protists (including harmful microalgae) in bait boxes (Cohen *et al.*, 2001; Haska *et al.*, 2011). In San Francisco Bay, Lau (1995) found that 40% of anglers discarded both leftover bait and wormweed into the water and introductions of multiple species have been associated with this vector there (see Discussion). Other than the study by Lau (1995), the end-user behaviour of marine fishermen using wormweed is an unknown variable but is presently being investigated by other researchers (M. Paolissio, pers. comm.). While past research has demonstrated the vector's operation and some understanding of entrained species richness (e.g. Cohen *et al.*, 2001; Haska *et al.*, 2011; Cohen, 2012), the potential scope of biotic transfer may be vastly underestimated, especially in terms of live species richness and abundance of organisms delivered to recipient regions.

More broadly, the Maine bait trade has well-known source and recipient regions, resulting in consistent, predictable and repeated transfers of biota over time. These characteristics have important implications for invasion dynamics and also provide a highly tractable system to examine the stepwise species transfer and survival process. In our study, we evaluate the scope and operation of this vector by measuring diversity (taxonomic, functional and genetic), abundance and community composition at four stages along the vector pathway: from Maine field sites (i.e. wormweed habitats) to Maine distributors to Mid-Atlantic distributors and finally to Mid-Atlantic retailers. Our investigation provides a model for understanding similar vector pathways that distribute biota world-wide.

METHODS

To assess the richness and abundance of marine invertebrates transported through the live Maine marine baitworm trade, we quantified the taxonomic identities and number of individuals at four discrete stages along the vector pathway from Maine to the Mid-Atlantic, USA (Fig. 1), including: (1) natural wormweed source habitats in Maine [e.g. saltmarsh and shoreline habitats where free-living *Ascophyllum* grows and is collected (Maine field – MEf)]; (2) bait boxes packed with wormweed purchased directly from Maine distributors (MEd) and shipped to the Smithsonian Environmental Research Center (SERC, Edgewater, Maryland); (3) bait boxes purchased directly from Mid-Atlantic distributors (MAd) in five Mid-Atlantic states: New Jersey, Delaware, Maryland, Virginia and North Carolina; and (4) bait bags purchased from two local bait retailers (MAr) in each of the five states. In addition to measures of taxonomic diversity, we also assigned all taxa to functional categories and calculated functional diversity across the four stages of the vector. Finally, we assessed the genetic diversity of two common hitchhiking snail species and measured haplotype diversity across the first three stages of the vector.

Sample collection

For each of the four stages, we collected samples during summer 2011 to coincide with the season of expected maximum abundance and species richness for field sites at temperate latitudes and the greatest number of hitchhikers in Maine baitworm shipments (Haska *et al.*, 2011). Details for collections by stage are outlined below.

Maine Field (MEf)

We selected five sites (Fig. 1) that are actively used for wormweed collections (P. Thayer, Maine Department of Marine Resources, pers. comm.). At low tides in July 2011, we collected wormweed from six randomly positioned 0.5 m^2 quadrats along a 30-m intertidal transect line running parallel to the water's edge at the elevation where the free-living *Ascophyllum* occurred at highest densities. All wormweed was collected from each quadrat. When empty quadrats were encountered, haphazard re-positioning of the quadrat to the nearest location with wormweed present augmented the collection. Wormweed from the six quadrats was combined and randomly divided into three replicate quarts (0.95 L) per site. Within 12 h of collection, all wormweed

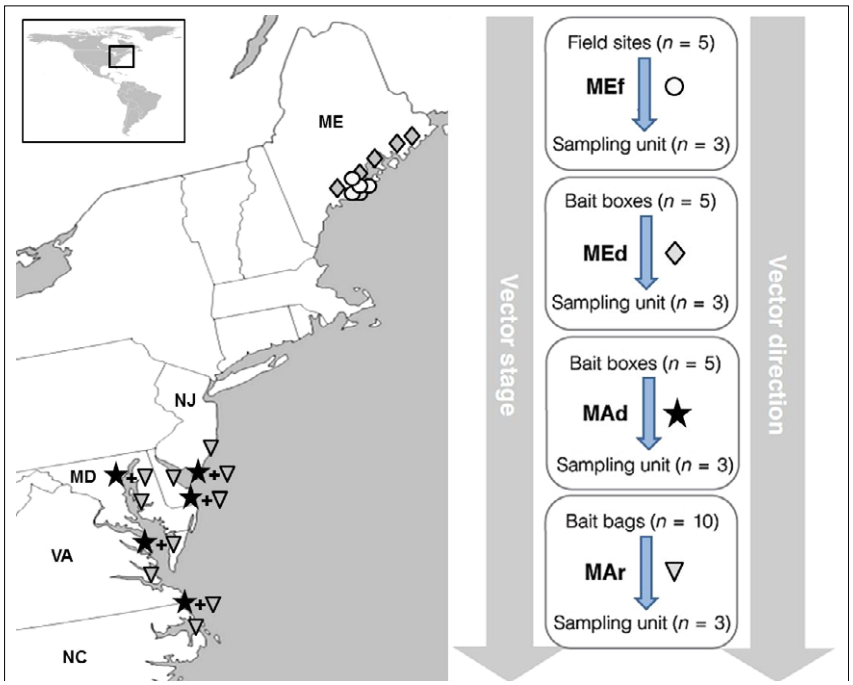

Figure 1 Map and schematic of sampling regime for the four stages of the baitworm vector from the source (Maine field) to recipient regions (Mid-Atlantic) during summer 2011. MEf, Maine field sites; MEd, Maine distributors; MAd, Mid-Atlantic distributors; MAr, Mid-Atlantic retailers. In some cases, bait bags were bought from the same MAd and MAr (i.e. these Mid-Atlantic distributors are also Mid-Atlantic retailers), and these MAd/MAr are represented by a star symbol plus an inverted triangle.

was closely examined for associated biota (see 'Sample Analysis' below).

Bait boxes from Maine Distributors (MEd)

We identified five Maine baitworm distributors from the Boothbay region northwards (Fig. 1). Each distributor shipped a bait box of 250 worms with wormweed to SERC every week for 3 weeks. The distributors were aware they were sending bait boxes to SERC, but did not know the premise of this study.

Bait boxes from Mid-Atlantic Distributors (MAd)

We identified one distributor in each of five Mid-Atlantic States (NJ, DE, MD, VA and NC) from whom we purchased a bait box of 250 worms every week for 3 weeks (Fig. 1). All bait boxes were purchased from the distributor, placed in an insulated cooler and driven to SERC for analysis.

Bait bags from Mid-Atlantic Retailers (MAr)

We obtained five bait bags (a plastic bag containing a small amount of wormweed and 10–12 worms) from 10 Mid-Atlantic retailers (Fig. 1) every week for 3 weeks (total = 150 bait bags). Collectively, the volume of wormweed in five bait bags equalled one quart. Five of these retailers were the same MAds from whom we purchased bait boxes, and five were additional bait shops, one from each Mid-Atlantic state. We categorized all of the bait bag sources as Mid-Atlantic retailers (MAr = 10). Both MAds and MArs were chosen based on their advertised sale of live bloodworms, proximity to major recreational fishing areas and geographic location in the Mid-Atlantic region. Bait bags were purchased, placed in a cooler and driven to SERC for analysis.

Sample analysis

Taxonomic richness and abundance

Replicate samples of wormweed from each vector stage were refrigerated (5 °C) to keep wormweed, associated organisms and bloodworms (when applicable) cool while awaiting examination (≤ 12 h). For bait boxes and bags, bloodworms were separated from wormweed, and wormweed was separated into three 1-quart containers to create three replicate volumes for each box. Replicates of wormweed and bloodworms were rinsed separately in artificial saltwater (30 ppt) to dislodge any associated organisms. Rinse water was sieved (63 μm) to retrieve dislodged biota. Wormweed from each replicate was then visually inspected for all biota, which were removed, placed into glass specimen dishes and examined under a stereomicroscope (40×). Organisms were assigned to coarse taxonomic groupings (e.g. family or genus) based on clear morphological signatures, assessed (and separated) as 'live' or 'dead' based on movement in response to stimuli (light and touch) and preserved in 70% ethanol for further examination and identification to lowest possible taxonomic level (species whenever possible). Counts were recorded to provide measures of richness and abundance per taxa.

Functional diversity

Functional groups were classified using five dimensions which describe significant physical and life history characteristics in marine benthic communities (See Table S1 in Supporting

Information; adapted from Wahl, 2009): body size (small, medium, large or very large); growth form (encrusting, massive, bushy or filamentous); trophic type (autotroph, suspension feeder, deposit feeder, predator or grazer); modularity (solitary or colonial); and motility (attached, crawling, swimming, drifting or burrowing). These traits could theoretically generate 800 functional groups.

Genetic diversity

Two frequent hitchhikers in the baitworm vector, *L. saxatilis* (rough periwinkle) and *L. littorea* (common periwinkle), were chosen for genetic diversity analyses because they were common across the vector and thereby had sufficient numbers for population genetics analyses. Furthermore, they both occur as non-native populations in California (Carlton & Cohen, 1998; Chang *et al.*, 2011), and *L. saxatilis* is thought to have been introduced via the Maine bait vector (Carlton & Cohen, 1998). Snails were collected from MEf, MEd and MAd. We also included supplementary population data from nearby Maine field sites which was previously sampled and sequenced for *L. littorea* (Blakeslee *et al.*, 2008) and *L. saxatilis* (A.M. Blakeslee, unpublished).

Mitochondrial DNA was extracted from the snail's foot using a standard CTAB method (France *et al.*, 1996). For *L. littorea*, a 624-bp fragment of the cytochrome b gene was amplified using primers and protocols from Blakeslee *et al.* (2008). For *L. saxatilis*, we amplified a 757-bp fragment of the cytochrome oxidase I gene using the following forward and reverse primers (A.M. Blakeslee, unpublished): LSCOIBLA-F: TTCTCCCTGGGTTTGGTATG; LSCOIBLA-R: AAATGGGCTTTTGTTCATCG. PCR protocols followed those used previously for *L. littorea* (Blakeslee *et al.*, 2008). Sequencing was completed at the Smithsonian Institution's Laboratory of Analytical Biology (Suitland, Maryland, USA) in both forward and reverse directions. Sequences were assembled and inspected by eye for ambiguities using LaserGene DNASTAR, Inc., Madison, WI, USA (10.1.1).

Statistical analyses

Data from one Maine distributor were an outlier and excluded from the analyses below; this distributor used unprinted newspaper (rather than wormweed) for packaging, which was largely devoid of live hitchhikers (one live snail and one live cricket across all bait boxes). The remaining data were analysed using univariate and multivariate methods. While we documented occurrences of both marine and terrestrial macro-organisms, our analyses focused on marine macro-invertebrates only, which comprised the majority of individuals and were best resolved taxonomically. One-way ANOVAs tested differences across vector stages for several dependent variables: abundance, taxonomic richness and functional diversity. Homogeneity of variances and normality was tested using the Levene Median test and Shapiro–Wilk test, respectively. Kruskal–Wallis ANOVA on ranks using Dunn's test was used for all pairwise comparisons when data were not normally distributed, and significance was assigned for $P < 0.05$.

To investigate community composition at all four vector stages, abundance was square-root-transformed to decrease the importance of very abundant species. Transformed values were used to create a resemblance matrix using S17 Bray–Curtis similarity index (Bray & Curtis, 1957). These data were compared using the different vector stages of sampling as factors, and non-metric multidimensional scaling plots (MDS) were generated to visualize differences among vector stages. One-way analysis of similarity (ANOSIM) and multiple pairwise comparisons were used to determine whether significant differences in taxa assemblages existed between pairs of stages. SIMPER analysis was conducted on the square-root-transformed data to determine which taxa were driving the differences observed. ANOSIM, MDS and SIMPER analyses were performed using PRIMER 6 (Clarke & Gorley, 2006).

To minimize the underestimation of local taxonomic richness, rarefaction analyses were employed to construct accumulation curves using PRIMER 6 (Clarke & Gorley, 2006) and the second-order jackknife estimator (Jack2; Smith & van Belle, 1984). Jack2 has been shown to be the best estimator for characterizing marine benthic communities (Canning-Clode *et al.*, 2008). Because a clearly asymptoting accumulation curve indicates complete capture of the total taxa richness in a population (Gotelli & Colwell, 2001), Jack2 estimator curves and taxa accumulation curves converging on the same asymptote were assumed to reflect adequate sampling (Walther & Morand, 1998).

Littorina spp. sequences were aligned using Geneious 7.1.7 (Biomatters Ltd., Auckland, New Zealand) and collapsed into haplotypes using TCS 1.21 (Clement *et al.*, 2000). For each species, we calculated fixation indices for population pairs based on pairwise differences between haplotypes (Φ_{ST}), testing significance of differentiation in Arlequin 3.11 (Excoffier *et al.*, 2005). Pairwise Φ_{ST} spatial patterns were explored using MDS analysis (PRIMER 6; Clarke & Gorley, 2006) between and among the four stages. Finally, we estimated expected haplotype richness (compared to observed haplotype richness) for each stage using EstimateS 8.20 (Colwell, 2009).

RESULTS

We identified 58 live taxa that were alive in bait boxes and bags at distributors (MEd, MAd, MAr), excluding field collections prior to distribution (MEf) (see Table S2). In addition to baitworms and wormweed, live biota included marine macro-invertebrates (37 taxa), macroalgae (8 taxa), vascular plants (2 taxa) and semi-terrestrial or aquatic invertebrates (11 taxa). Live marine invertebrates included gravid individuals for some taxa and egg cases for snails and flatworms. Eight additional marine invertebrate taxa were only found as dead individuals in bait boxes or bags, and further additional taxa were detected only in the field collections (see Table S2).

In total, we detected and characterized 17,798 live macro-organisms; 99% of these were marine invertebrates, which

constituted 96.7–99.7% of each vector stage (see Table S3). Below, we examine further measures of abundance and diversity that focus on marine invertebrates alone, due to the dominance and relatively high taxonomic resolution for this group. When considering all taxonomic groups combined, the same overall patterns are seen across vector stages, but the level of taxonomic identification is highly uneven outside the marine invertebrates (see Table S3).

Abundance

The mean number of live marine macro-invertebrates per standardized sample decreased across sequential stages of the vector (Fig. 2a). Pairwise comparisons revealed that abundance of live organisms in the initial stage in Maine (MEf) differed statistically from that at the Mid-Atlantic distributors (MAd, MAr), but other comparisons were not significant. The relatively high abundances of live organisms in Maine field (MEf) samples were driven by large numbers of isopods (*Jaera albifrons* Leach 1814, range 14–805), Littorine snails (*L. saxatilis*, range 0–336; and *L. obtusata*, range 0–102), marine mites (e.g. Halacaridae, range 0–491), flatworms (range 0–364) and free-living nematodes (range 0–716). The dominance of these particular organisms at the MEf stage was also reflected in their mean abundances, compared to those for other taxa, but was less pronounced at subsequent stages (see Table S2).

Mortality of organisms contributed partly to the overall abundance decline across stages, as indicated by the decline in percentage of total live organisms. There was an overall significant difference among stages, such that mean percentage live marine macro-invertebrates from MEf (84%) was significantly higher than all other stages; the other three stages were not significantly different from one another (58–67%) (see Table S3).

Taxonomic richness

Both total and average taxonomic richness decreased from MEf to MAr for live marine macro-invertebrates. There was a significant decline in the mean taxonomic richness of live marine macro-invertebrates between Maine vector stages (MEf, MEd) and Mid-Atlantic stages (MAd, MAr) (Fig. 2b; see Table S3). Rarefaction analyses further indicated that a much larger species pool existed at the MEf stage than was included in our samples. The difference between the observed (38 taxa) and expected (57 taxa) values at this stage represented a 50% increase in taxonomic richness (Fig. 3; see Table S3). In contrast, the expected values of taxonomic richness for the other three vector stages were only 9–19% above observed values, suggesting samples at the distributor and retail levels captured the majority of taxa.

The combination of taxonomic richness and abundance defined live marine macro-invertebrate community structure, which differed significantly across the four different stages (Fig. 4a, see Table S4). The most similar taxa compositions were between MEd and MAd, and the most dissimilar were MEf and MAr (see Table S4). A single species, the isopod

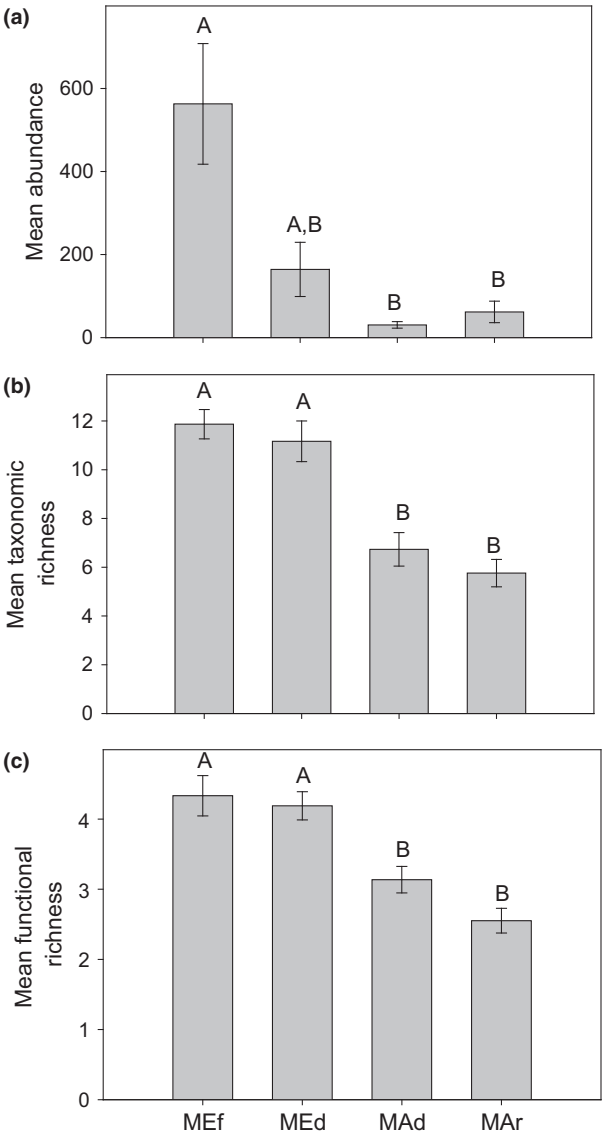

Figure 2 Mean abundance (± SE) (a), taxonomic richness (± SE) (b) and functional richness (± SE) (c) of live marine invertebrates found in replicate quarts during summer 2011 at each stage of the vector: Maine field (MEf), Maine distributor (MEd), Mid-Atlantic distributor (MAd) and Mid-Atlantic retailer (MAr). Capitalized letters above the bars indicate pairwise significant differences ($P < 0.05$).

J. albifrons, contributed the most (18.4–21.51%) to total average dissimilarity between stages (see Table S4), except for the comparison of MAd and MAr (where community composition was similar).

Functional diversity

Mean functional richness decreased from Maine stages (MEf, MEd) to Mid-Atlantic stages (MAd, MAr) for live marine macro-invertebrates (Fig. 2c, see Table S3). Similar to trends observed in taxonomic richness, rarefaction curves suggested that 25% more functional groups exist in MEf than were

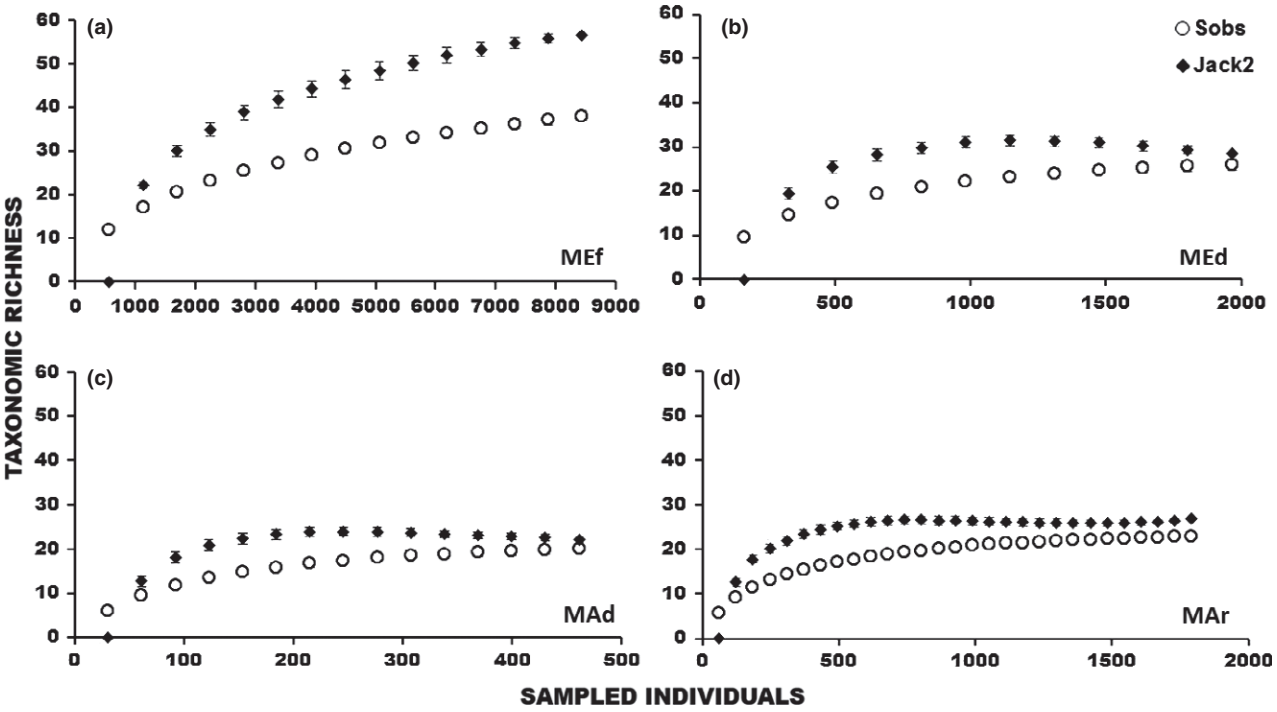

Figure 3 Rarefaction curves for live marine invertebrates across the four stages of sampling from replicate quarts of packing algae: Maine Field (MEf) (a), ME Distributor (MEd) (b), Mid-Atlantic distributor (MAd) (c), Mid-Atlantic Retailer/Bait shop (MAr) (d). Circles represent the accumulated observed richness (sobs) (± SE), and diamonds represent jackknife second-order (Jack2) estimated richness (± SE) across sampled individuals. The *y*-axis scale is standard across the four panels, but the *x*-axis is scaled differently to demonstrate the patterns and the abundance of individuals at each stage.

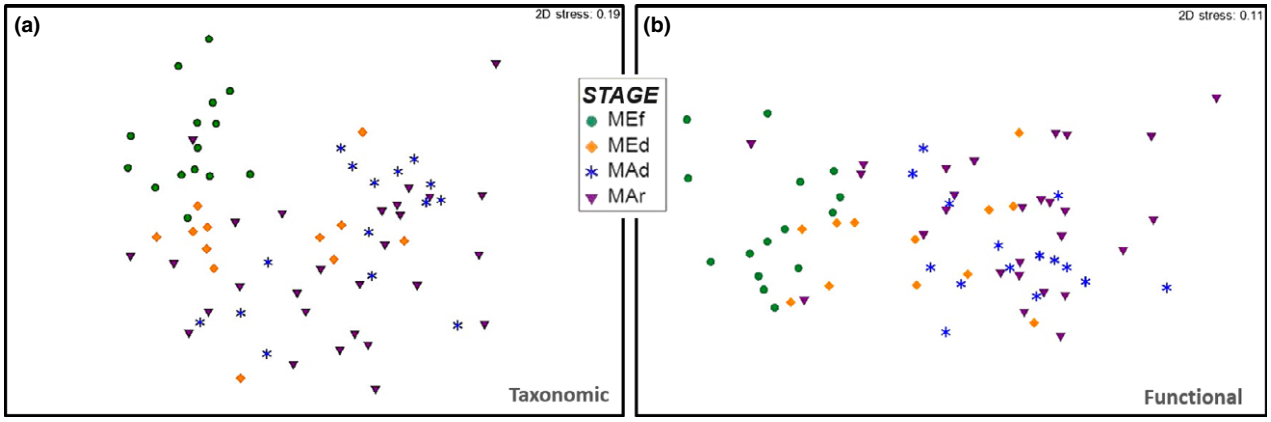

Figure 4 Multidimensional scaling plot of abundances of live marine invertebrates during summer 2011 across the four stages of sampling from replicate quarts: Maine Field (MEf), ME Distributor (MEd), Mid-Atlantic distributor (MAd), Mid-Atlantic Retailer/Bait shop (MAr) using two measures of diversity: taxonomic (a) and functional (b). (a) Taxonomic diversity: ANOSIM global $R = 0.21$; significance level of sample statistic = 0.1%; pairwise tests between all stages at the R significance level are between 0.1 and 1.9%, except for MAd and MAr (86.5%) and MEd and MAr (53.8%). (b) Functional diversity: ANOSIM global $R = 0.32$; significance level of sample statistic = 0.1%; pairwise tests between all stages at the R significance level are between 0.1 and 3.9%, except for MAd and MAr (84.3%). One outlier (MEd) with 0 abundance was excluded from this analysis.

actually observed, but increases between observed and expected functional richness also existed for MEd (12.5%) and MAd (25%) (see Table S3). Only MAr showed no increase between observed and expected values.

Functional diversity also defined live marine macro-invertebrate community structure and differed significantly across the four different stages (Fig. 4b, see Table S4). The stages with the most similar functional group compositions were MAd and MAr, and the most dissimilar were MEf and MAr (see Table S4). In all cases, MMGSC (medium body size, massive growth form, grazing trophic type, solitary modularity and crawling motility) (e.g. amphipods, isopods and

some gastropods) contributed the most to average dissimilarity (37.86–46.25%) (see Tables S4 and S1).

While not included above in comparisons of functional diversity across vector stages, the distribution of reproductive modes for the cumulative species pool is also noteworthy. Of the 41 marine macro-invertebrate taxa recorded in our study, 54% have direct development (see Table S2). Another 36% brood larvae, lay eggs or have the capacity for asexual reproduction. Only 10% of all marine macro-invertebrate taxa observed are considered free-spawning.

Genetic diversity of *Littorina* spp.

We had greater success obtaining sequence data for *L. saxatilis* than *L. littorea*, and both species were encountered more frequently in field samples than bait box stages, resulting in greater numbers of samples for MEf than for MEd or MAd. Altogether, we obtained 177 sequences for *L. saxatilis* (105 MEf, 30 MEd, 42 MAd) and 64 sequences for *L. littorea* (53 MEf, 0 MEd, 11 MAd).

In MEf, *L. saxatilis* is represented by two abundant haplotypes (red and blue in Fig. 5a), which are also the most abundant (and sometimes exclusive) haplotypes found in MEd and MAd. The red haplotype is more prevalent in southern than northern Maine populations. Such variation in haplotype frequencies was also seen at the distributor level (MEd, MAd), reflecting different geographic sources of wormweed. In comparison, *L. littorea* had much greater haplotype diversity in MEf, with a relatively small subset detected in MAd due to low sample size from distributors and relatively high haplotype diversity (Fig. 5c).

Both periwinkle species declined in observed and expected genetic diversities across all vector stages (MEf, MEd, MAd). Rarefaction curves suggest that both species are under-sampled for haplotype richness in MEf. Rarefaction curves on MEd and MAd samples suggest very few additional

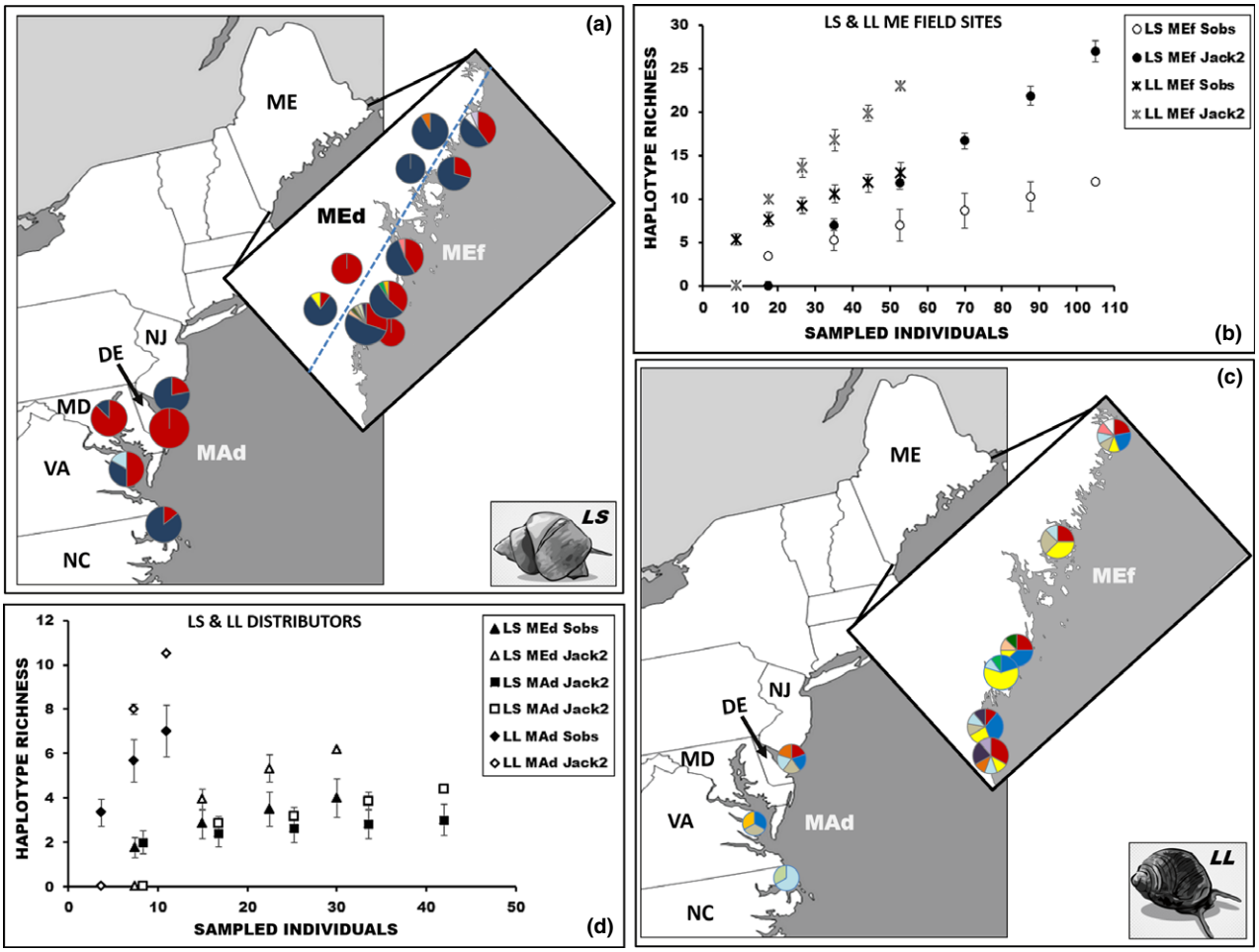

Figure 5 Haplotype frequency maps and rarefaction curves of observed and estimated haplotype richness for two of the most common species found in the live bait vector, the marine snails, *Littorina saxatilis* (*LS*,a–b) and *Littorina littorea* (*LL*, c–d), across three stages of sampling (MEf, MEd and MAd, except for *L. littorea* MEd because sequence data could not be obtained). Map a and c: haplotype frequency for each snail found from MEf, MEd and MAd. The rarefaction curves showing observed haplotype richness (Sobs) (± SE) and expected haplotype richness (Jack2) (± SE) are depicted in b at the MEf stage and in d at the MEd and MAd stage for both snail species.

haplotypes would be expected in the MEd or MAd stages for *L. saxatilis*, while the MAd stage for *L. littorea* suggests more haplotypes would be expected with further sampling. However, fewer *L. littorea* were found at this stage than for *L. saxatilis*, suggesting a possible artefact of low sampling for *L. littorea* (see Table S3, Fig. 5b, d).

DISCUSSION

The Maine live bait worm trade is a potent vector for the ongoing introduction of live marine organisms around the globe (Weigle *et al.*, 2005; Haska *et al.*, 2011; Cohen, 2012), especially when wormweed is used for packing material and contains a diverse range of associated organisms. To date, at least 114 taxa have been found alive in baitworm shipments from Maine to other geographic regions, including 58 taxa in this study and 56 additional taxa reported in previous studies (Haska *et al.*, 2011; Cohen, 2012). Moreover, the cumulative species richness associated with these shipments is still underestimated, particularly as (1) the microorganisms, epibiota and parasites have received relatively little in-depth analysis, and (2) the species pool and environmental conditions change through time.

While doubling the known number of live species transferred by the Maine baitworm vector, our study provides the first formal analysis of changes in biota associated with sequential stages of the vector and insight into the species pool at each stage, focusing on macro-invertebrates. The highest mean (per sample) values for abundance, species richness and functional richness were observed in Maine (MEf, MEd), and these were significantly lower upon arrival to the Mid-Atlantic (MAd, MAr). Rarefaction curves also indicate that the cumulative species pools were smallest in the Mid-Atlantic (MAd, MAr) and were well characterized by our sampling effort, with observed and predicted species richness reaching the same asymptote (Fig. 3). In contrast, the observed species richness was higher for Maine field samples than Mid-Atlantic distributors and retailers, and the predicted species richness was > 50% greater than the observed value for Maine field samples. Taken together, these data indicate that species richness is underestimated in Maine field samples, even for well-studied macro-invertebrates, and only a subset of species have been detected downstream at the Mid-Atlantic (MAd, MAr) stages. Similar patterns existed when considering genetic diversity for the snails *Littorina* spp. (Fig. 5).

Studies to date provide only brief snapshots in time of the biota transferred by this vector (e.g. Haska *et al.*, 2011; Cohen, 2012). While rarefaction curves indicate more taxa are available in Maine for transport than were detected in our samples, this is likely to be influenced by temporal and spatial variation (i.e. when and where wormweed is being collected) over both short and long time-scales. This variation affects the cumulative taxonomic richness entrained in wormweed at Maine distributors and, ultimately, which taxa arrive to recipient regions due to several mechanisms. First, invertebrate populations undergo temporal (seasonal and annual) fluctuation in abundance such that the probability of collecting resident species should increase over time (Soberon & Llorente, 1993). Second, fluctuations in abundance and environmental conditions should affect the number of individuals (per taxa) arriving to Maine distribution hubs as well as the probability that some taxa will survive one or more transfer events to recipient regions. Third, the actual taxonomic richness in Maine is changing through time, due to invasions and range expansions (e.g. Thayer & Stahlnecker, 2006; Neefus *et al.*, 2008), which further increase the cumulative number of taxa being entrained over longer time periods. For example, although not observed in our samples to date, several marine invaders have successfully colonized Maine's coastal waters from other locations [e.g. the Asian shore crab (*Hemigrapsus sanguineus*), several tunicates (*Didemnum* sp., *Botrylloides violaceus*, *Styela clava*) and a bryozoan (*Membranipora membranacea*)] and could be spread through the global transfer of wormweed (Yarish *et al.*, 2009).

While we have focused on taxonomic richness, the same concept applies to genetic or intraspecific diversity, whereby an increasing number of distinct genotypes should be transferred over time (e.g. *Littorina* spp.) including possible new invading haplotypes (e.g. *Carcinus maenas*; Darling *et al.*, 2008). Moreover, both taxonomic richness and genetic diversity can ultimately influence functional diversity. Most taxa successfully transported through the live bait pathway exhibited medium body size, massive growth form, grazing trophic type, solitary modularity and crawling motility (e.g. amphipods, isopods and some gastropods) as functional traits. However, these patterns could also be temporally and spatially influenced. Specifically, while we focused on the transfer of organisms during the summer, different functional traits could be common in other seasons (e.g. Frainer *et al.*, 2014). Seasonal or interannual changes in abundance and diversity have not yet been explored.

Scaling Up: scope of the maine baitworm trade

The potential for species invasions is affected not only by the number of species transferred but also the duration and magnitude of transfers. In general, the probability of an invasion event should increase with the number and abundance of species being introduced. Furthermore, duration of vector operation also serves to increase the cumulative species richness transferred and the frequency of introductions, which increase probability of establishment (Lockwood *et al.*, 2005; Colautti *et al.*, 2006; Hedge *et al.*, 2014). In this context, it is useful to note that the Maine baitworm vector has been in operation since the 1930s (Creaser *et al.*, 1983). Since its inception the exact number of worms shipped is unknown, but Creaser *et al.* (1983) report the annual number of landed bloodworms from 1946 to 1964 (16,197,316 ± 2,081,476), and the Maine Department of Marine Resources (2014) reported that an average of 563,939 ± 142,553 pounds of

worms were landed from 1964 to 2013. In our study, there were 250 bloodworms (~2 lbs) per bait box, suggesting an average of 168,807 ± 10,613 boxes have been shipped per year since 1946, with a cumulative number of over 11 million boxes from 1946 to 2013. Based on average numbers of live marine macro-invertebrates found in bait boxes on arrival to the Mid-Atlantic (Fig. 3), over 1.2 billion live macro-invertebrates may have been transferred with Maine bait-worms in the past 67 years (1946–2013). However, due to the lack of reporting requirements for the movement of this vector across state lines, the exact number of bait boxes that arrive into particular states is unknown.

While a primary market for Maine's live bait trade is the US Atlantic coast, shipments are also made to the US Gulf and Pacific coasts, Italy, France and Spain (Creaser *et al.*, 1983; Crawford, 2001). The history and magnitude of shipments to different regions is not fully known, but some estimates exist for worms and associated macro-invertebrates for California and Spain (Cohen *et al.*, 2001 and Costa *et al.*, 2006, respectively), raising concern about potential invasions. We note that the mode of live bait transport has changed through time (now dominated by overnight shipment), which likely reduces in-transit mortality and increases the international reach of this vector.

Distinctive attributes of the vector

The Maine bait vector is fundamentally different than many other well-known marine vectors in several respects (Weigle *et al.*, 2005). First, unlike ballast water or hull fouling, bait transfers are designed explicitly to maximize in-transport survival by providing favourable environmental conditions and rapid delivery. Second, bait and associated organisms are released frequently into the wild, given their utilization in natural environments (Lau, 1995). A recent study by Drake *et al.* (2015) found that freshwater live bait anglers released unused bait into the environment (rather than discarding it) due to convenience; moreover, there was a misconception that released bait could actually provide ecological benefits to natural resources. Third, a large component of the species transferred with Maine baitworms are non-target species associated with wormweed, constituting a habitat occupied by resident biota. Fourth, most of the invertebrates we observed transferred with bait are adults or juveniles, and many reproduce by direct development or brooding. As parasite prevalence is often size/age-dependent, the transfer of parasitized invertebrates is also likely enhanced in the bait vector (Chang *et al.*, 2011; Blakeslee *et al.*, 2012) compared with ballast water, where larval forms are common. In fact, several live snails found in bait boxes were parasitized (data not shown). In addition, the reproductive mode for the majority of bait-borne invertebrates (i.e. direct development) may reduce Allee effects (and increase establishment probability) as compared to those that reproduce via free-spawning long-duration planktonic larvae (Miller *et al.*, 2007). Fifth, bait shipments originate from a single source region and operate year-round, resulting in repeated inoculation to the same recipient waters over years to decades. While such repeated inoculation increases probability of establishment (Drake & Lodge, 2004; Von Holle & Simberloff, 2005) due to variation in environmental or biotic conditions, continued transfer of novel genotypes can also alter invasion dynamics (Saltonstall, 2002; Cox, 2004; Roman & Darling, 2007). Finally, such sustained inoculation to many different global regions also serves to increase the likelihood of invasion opportunity beyond that found in a single recipient region.

Impact and management of the vector

Bait boxes have the potential to become a real-life Pandora's box, in which the contents of this seemingly insignificant vector can have far-reaching negative consequences. In San Francisco Bay, a global hotspot for invasions, multiple invasions have been attributed to the Maine algal packing material (wormweed). These include the packing alga itself, *Ascophyllum nodosum* (Miller *et al.*, 2004), the periwinkle snail *Littorina saxatilis* (Carlton & Cohen, 1998), and the European green crab *Carcinus maenas* (Lau, 1995; Cohen *et al.* 1995, Cohen *et al.*, 2001). While we found only two dead *C. maenas* in bait bags, Cohen *et al.* (2001) 'occasionally' observed individuals in bait boxes shipped to California, and others have reported live individuals in wormweed at Maine baitworm dealerships (Cohen *et al.*, 2001; Crawford, 2001).

These three introduced taxa have population and community level impacts in various global regions. *Littorina saxatilis* has been shown to impact algal bloom dynamics (Lotze & Worm, 2000), compete with other snail species (Reid, 1996), prey on newly settled barnacles (Carlton & Cohen, 1998) and host several parasite species, even in non-native populations (Blakeslee *et al.*, 2012). The European green crab, *Carcinus maenas*, which is now established on all continents except Antarctica (Carlton & Cohen, 2003; Hidalgo *et al.*, 2005; Roman, 2006), has been documented to compete with and prey upon ecologically and commercially important molluscs and crustaceans and cause habitat modification through direct and indirect mechanisms (e.g. Grosholz *et al.*, 2000; Breen & Metaxas, 2008; Sungail *et al.*, 2013; Garbary *et al.*, 2014). *Carcinus maenas* has been estimated to cause over $22 million dollars of damage each year in the USA alone (Williams & Grosholz, 2008). Both attached and free-living tufts of *Ascophyllum nodosum* have been reported periodically in introduced regions [e.g. Chesapeake Bay (Orris, 1980), San Francisco Bay (Miller *et al.*, 2004) and North Carolina (Schneider & Searles, 1991)]. *Ascophyllum nodosum* has a major structuring role in marine intertidal and subtidal habitats, having both direct and indirect effects as the canopy provides shelter, shade and protection (Jenkins *et al.*, 1999).

While significant momentum and regulations exist for managing some marine vectors, such as ballast water (IMO, 2003; ICES, 2005), similar efforts have not emerged for marine bait, which remains largely unmanaged in both the

source and recipient regions (Weigle *et al.*, 2005; Yarish *et al.*, 2009; Cohen, 2012). In addition, some US states have regulations on the use, sale or transport of live bait in freshwater systems (Litvak & Mandrak, 1993, 2000; Meronek *et al.*, 1995; Kerr *et al.*, 2005; Peters & Lodge, 2009), but such efforts are lagging in marine ecosystems. This relative lack of management and policy attention in marine systems probably stems from the fact that the majority of species are relatively small, inconspicuous and understudied (and possibly undetected) in packing materials, despite documented impacts of significant bait-associated invasions. The United Nations recently called for voluntary risk assessments and appropriate measures to manage that risk for the import or transport of live bait (UN, 2014).

With the apparent lack of current regulations on the live marine bait trade, there are several possibilities that could be employed voluntarily by either the source or recipient regions to decrease the number of hitchhikers. One Maine live bait dealer packages bloodworms in unprinted newspaper dipped in salt water, which virtually eliminates all hitchhikers and keeps the worms in good condition (AEF, personal observation). Other alterative packaging exists, such as charcoal or sawdust, which are both used in Europe to transport live bait (Crawford, 2001). While a complete change in packaging materials could have negative impacts on the livelihood of wormweed harvesters, there are other possible methods to retain wormweed and decrease hitchhikers. These include requiring all distributors and retailers to dispose of wormweed and provide customers with worms only (Cohen, 2012), washing wormweed in tap water or hypersaline water to create an osmotic shock (Blakeslee, AMH; Fowler, AE; Couture, JL; Grosholz, ED; Ruiz, GM; Miller AW, unpublished data), or freezing wormweed for several days prior to shipment. While each of these methods may seem simple, managers, industry and the public will need to weigh the cost and practicality of changing packing materials in an industry that has used wormweed since at least the 1950s (Creaser *et al.*, 1983) against the risk of new invasions and unwanted impacts. Due to the complicated nature of vector management operating across different sectors and geographic boundaries, an integrated approach is needed along these axes to explore viable options that minimize such unintended species transfers. Given the scale of species transfer with Maine baitworms, combined with the other bait distribution networks, there is an urgent need to rapidly advance management strategies for coastal marine ecosystems and their associated invasion risks.

ACKNOWLEDGEMENTS

We thank the SERC Marine Invasions Laboratory, numerous interns and other personnel. We also thank Pete Thayer for field expertise and industry knowledge and Norm Woodley for identifying insect larvae, pupae and adults. We appreciate laboratory space, accommodations and support provided by the Darling Marine Center. We also thank three anonymous referees for comments that significantly improved the manuscript. This research was supported by Maryland Sea Grant awards NA10OAR4170072 and SA7528120. Algae and associated organisms were collected under ME DMR Special License Numbers ME 2011-77-00 and ME 2012-14-01.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 The five ecological traits used to determine functional groups for this study that are the most relevant to benthic communities.

Table S2 Mean cumulative abundance separated by species or taxa sampled from the four stages of the live bait pathway during summer 2011.

Table S3 Diversity measures and observed/expected values for all taxa and marine invertebrates sampled from the four stages of the live bait pathway during summer 2011.

Table S4 SIMPER analysis for live marine invertebrates collected from the four stages of the live bait pathway during summer 2011.

BIOSKETCH

The Marine Invasions Laboratory at the Smithsonian Environmental Research Center seeks to understand biological invasion patterns and processes in marine ecosystems through vector, population, and community ecology, biogeography and the synthesis of information on non-native species and transfer mechanisms.

Author contributions: AEF, AMHB, JCC, FM, GMR and AWM conceived the ideas; AEF, AMHB, JCC, MR, JTC and AMP collected the data; AEF and AMHB analysed the data; and AEF, AMHB, JCC, GMR and AWM led the writing.

Editor: Hugh MacIsaac