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Research Article

Vector management reduces marine organisms transferred with live saltwater bait

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Abstract

The global transfer of live bait creates a potent vector (mechanism) for invasion of marine species, including associated biota ("hitchhikers") not intended for shipment. Unlike other vectors of non-native species transfer in coastal marine systems (e.g., ship ballast water), vector management strategies to reduce transport of associated biota with live bait are lacking. In this study, we experimentally tested whether simple, inexpensive treatment methods could reduce hitchhiker abundance and richness with live bait shipments, using the Maine live baitworm trade as a model. The Maine bait industry ships locally-harvested polychaete worms and packing algae to coastal regions of the United States, Europe, and Asia, and may unintentionally transfer associated hitchhikers, including known invaders. We exposed packing algae to three osmotic shock treatments (tap water, hypersaline water, and tap + hypersaline water), and measured abundance and richness of all live and dead macroinvertebrate taxa, as well as the condition of baitworms, after shipment to two locations (Maryland and California). Compared to controls, experimental treatments lowered average abundances by up to 99% and up to 93% for richness, and appeared to have no negative effects on bait or algae quality. The simplest treatment, tap water, was statistically as effective as more complicated treatments in reducing associated biota. We suggest that simple osmotic shock treatments on live packing algae prior to shipping could reduce the prevalence of hitchhikers associated with live trade vectors both nationally and internationally with little impact on the respective industries or their stakeholders.

Key words: baitworms, invasion, live trade, hitchhikers, osmotic shock, wormweed

Introduction

The anthropogenic movement of marine species continues to rise as a result of enhanced human transport vectors throughout the world's oceans (Ruiz et al. 2000a; Seebens et al. 2013). While much attention has focused on major vectors like commercial shipping (e.g., ballast water, hull fouling) that transport diverse communities of marine biota around the globe (Carlton and Geller 1993), "seemingly insignificant vectors" (Carlton 2001) like live trade can also pose considerable threats to natural systems and local economies, especially when they result in impactful introductions like the European green crab (*Carcinus maenas*) and the IndoPacific lionfish (*Pterois volitans*) (Grosholz and Ruiz 1996; Carlton and Cohen 2003; Padilla and Williams 2004; Lovell et al. 2007; Arias-González et al. 2011). Although the overall volume and flux of entrained organisms is typically smaller, live trade vectors can still be potent mechanisms of transport for viable organisms, especially when they operate year-round, are less environmentally harsh to entrained biota, and introduce reproductively active adults, including brooding females (Carlton 2001; Williams et al. 2013).

Live trade industries are widespread across terrestrial and aquatic systems around the world. For example, the live plant trade is a $500 billion/yr industry and a well-known vector for insects and diseases in terrestrial systems (Liebhold et al. 2012), and aquarium and live food/bait trades are well-described vectors of species introductions to lakes, rivers, and coasts (Carlton 1992; Rixon et al. 2005; Clapp et al. 2012). In marine systems, live trade includes several industries, like bait, seafood, and aquarium pets—all of which have been associated with species introductions (Cohen et al. 2001; Crawford 2001; Harley et al. 2013). The live saltwater bait trade, in particular, is an economically valuable industry in some areas of North America, like Maine, with the potential to transfer new species, genotypes, and symbionts worldwide (Stephenson and Wilson 2006; Cohen 2012; Haska et al. 2012). In contrast to other marine introduction vectors (e.g., ship ballast water, aquaculture) where strategies exist to reduce species transfers and minimize invasions (Gollasch et al. 2003; IMO 2004), management of the live bait trade is mostly lacking. Yet the invasion risk associated with this vector can be quite high given limited regulation on the sale and transfer of live organisms both to and from distant, evolutionarily distinct regions around the globe (Williams et al. 2013).

Here, we use the American saltwater bait industry as a model for examining the composition of hitchhiking biota entrained in live bait vectors and to establish viable strategies for reducing species transfers associated with live bait. In the USA, Maine is the largest supplier of saltwater baitworms for recreational fishing (Stephenson and Wilson 2006), and saltwater bait harvesting is one of the ten most valuable fisheries in the state (Maine Department of Marine Resources 2016). Polychaete worms (mostly the common bloodworm, *Glycera dibranchiata*) are harvested by hand from coastal mudflats and sold to bait dealers in the region (Cohen et al. 2001; Crawford 2001). Harvesters also collect marine algae, targeting the free-living form of the brown alga *Ascophyllum nodosum* ecad *scorpioides* (or "worm-weed"), for use as packing material to keep worms moist and protected during transport (Crawford 2001; Stephenson and Wilson 2006). Dealers ship large quantities of polychaetes and wormweed via overnight mail services to locations around the USA and also abroad to Europe and Asia (Creaser et al. 1983; Gambi et al. 1994; Olive 1994; Cohen et al. 2001; Costa et al. 2006; Fowler et al. 2016). After bait is sold to anglers, the packing algae and baitworms may be discarded into coastal marine environments (Lau 1995), where hitchhiking species can be inadvertently introduced (Cohen et al. 2001; Crawford 2001; Haska et al. 2012; Cohen 2012). Over 150 distinct taxa have been detected in wormweed from bait shipments to date, including several known invaders (see tables in Cohen 2012; Haska et al. 2012; and Fowler et al. 2016) like the packing algae *A. nodosum* itself (Miller et al. 2004), the marine snail *Littorina saxatilis* (Carlton and Cohen 1998), and the European green crab *Carcinus maenas* (Cohen et al. 1995), all of which are non-native in west coast habitats like San Francisco Bay.

Despite recognition and concern about the potential for invasion from the live saltwater bait trade, strategies to reduce the transfer of species associated with the vector have not advanced. One proposed solution is to replace packing algae with alternative packing materials, like newsprint, charcoal, or sawdust (Crawford 2001; Carlton 2001). Though a promising strategy, such substitutions of alternative materials for live algae could be economically detrimental to the livelihoods of numerous seaweed harvesters throughout the region, given the history as a cottage industry. To address this issue, we investigated a vector management strategy that could reduce hitchhiking abundance and richness without adversely affecting the traditional operation of the industry—by testing simple, affordable treatment applications for the packing algae prior to shipping. Given the broad salinity tolerance of wormweed (range = 0 to 40 psu; Chock and Mathieson 1979), we hypothesized that temporary exposure to fresh and/or hypersaline water could induce stressful osmotic shock in hitchhikers without negatively affecting the packing algae. Osmotic shock methods have been widely used and/or recommended for reducing invasion risk in other marine introduction vectors, particularly ballast water (Ruiz and Reid 2007; Santagata et al. 2008).

In our study, we partnered with a commercial saltwater bait dealer to ship baitworms and treated or untreated algae to two US States, Maryland and California, where saltwater baitworm shipments are common and fishing is popular, given the large estuaries located in both States (Chesapeake Bay and San Francisco Bay, respectively). For each shipment, we explored whether the abundance and richness of entrained organisms could be reduced by simple, inexpensive osmotic shock treatments (i.e., tap water, hypersaline (hyper) water, and tap water followed by hypersaline (tap+hyper) water). We hypothesized that all three osmotic shock treatments would significantly reduce the abundance and richness of hitchhiking organisms in the packing algae compared to untreated controls; however, we predicted that the tap+hyper treatment would be the most effective strategy, given the greater osmotic shock induced by this treatment. We also predicted baitworm condition would be similar between treatments and controls.

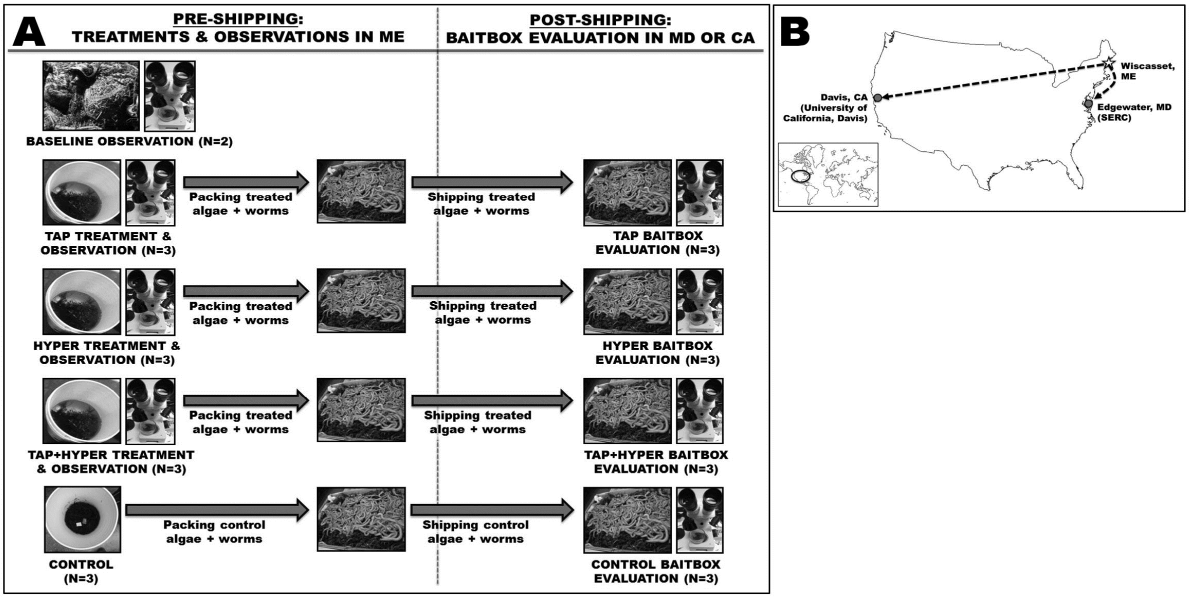

Figure 1. (A) Study experimental design and (B) map of shipping and destination regions. (A) The first row of images depicts the baseline richness and abundance observations at the Maine (ME) source in which purchased wormweed was immediately assessed for associated biota; this occurred just for the June 2012 experiment. The next three rows represent the experimental treatments (tap, hypersaline, tap plus hypersaline) on wormweed, with the first column of images depicting abundance and richness observations of dislodged or killed organisms prior to shipping; these pre-shipping assessments were just taken for the June 2012 experiment. Treated wormweed was packed with baitworms (per commercial standards) and shipped via overnight mail either to Maryland (MD) or California (CA). On the day of arrival, baitboxes were evaluated for richness and abundance of hitchhiking organisms, and worm condition was assessed. The last row of images represents untreated controls that were packed with worms and shipped to either MD or CA where they were evaluated for hitchhiking richness and abundance. Note: the hypersaline treatment was not performed during the second experiment (July 2012). (B) The star represents the baitworm distributor's ME location, and the grey circles represent our two recipient locations in MD and CA, which are common areas receiving baitworm shipments due to popular fishing spots in the large estuaries of Chesapeake Bay and San Francisco Bay.

Altogether, our study demonstrates the effectiveness of osmotic shock methods as a management tool for lowering the abundance and richness of living biota that hitchhike with live bait, without imposing undue hardship on traditional seaweed harvesting and packaging practices. Such strategies could be employed in multiple live trade industries around the world. Importantly, our experiments deepen the understanding of live trade vectors and the types of organisms (including known invaders) that could be transferred if operations continue as per the current status quo.

Methods

Two separate experiments were conducted in summer 2012 near the contemporary (and historic) epicenter of the Maine bait industry (Boothbay and Wiscasset) to determine the effect of exposure to fresh and/or hypersaline water on biota associated with the euryhaline wormweed. The first experiment was conducted in June 2012 with shipments to the Smithsonian Environmental Research Center (SERC), Edgewater, Maryland (MD) (latitude: 38.53ºN, longitude: 76.32ºW), and this experiment was replicated in July 2012 with shipments to the University of California (CA), Davis (latitude: 38.32ºN, longitude: 121.44ºW). Figure 1 visually demonstrates our methodology, which is described in detail below.

Wormweed treatments and pre-shipping richness and abundance observations

Freshly harvested packing algae (wormweed) was purchased from a Wiscasset, Maine (ME) baitworm distributor (latitude: 44.00ºN, longitude: 69.39ºW). The wormweed was then driven 32-km to the Darling Marine Center (Walpole, ME; latitude: 43.56ºN, longitude: 69.34ºW) and held in a 4.4 ºC walk-in refrigerator. All experimental treatments were carried out within five days of wormweed purchase. Our three treatments included: a tap water soak ("tap" (T)) at 0 psu; a hypersalinity water soak ("hyper" (H)) at 60 psu; and a tap water soak at 0 psu, followed by a hypersalinity water soak at 60 psu ("tap+hyper" (TH)). For each treatment, 750-g of wormweed was soaked in separate 20-L buckets for 12-hr at 4.4 ºC (note: tap+hyper was soaked for 24-hr;

12-hr per treatment). There were three replicates per treatment, comprised of algae taken from two large storage bags purchased from the Wiscasset distributor. Hypersaline water was prepared by mixing Instant Ocean aquarium salts (United Pet Group, Blacksburg, Virginia) with de-ionized water from the Darling Marine Center. Salinity was measured using a YSI 8525 meter (Yellow Springs Instruments, Yellow Springs, Ohio). Tap water was untreated potable well water from the Darling Marine Center (Tim Miller, pers. comm.). Experimental controls consisted of the same amount of wormweed in 20-L buckets without water at 4.4 ºC, akin to wormweed storage by distributors.

After the allotted treatment time, each replicate (except untreated controls) was lightly shaken by hand for 5 seconds in the treatment bucket, thus treated algae included an osmotic shock and mechanical shaking (the latter done to help dislodge any heavier, shelled organisms like snails). Treated algae were then removed from their respective treatment buckets and placed into new buckets, where they were soaked for another 2-hr in filtered seawater (30 psu) to restore algae to background salinity levels. Meanwhile, the water from each treatment bucket—containing live and dead organisms that had fallen out of the algae during the treatment process (soak and shaking)—was sieved using a 63 micron mesh. All the dislodged biota were then observed under a dissecting stereomicroscope at $6.5^{\times}$ magnification, where they were counted, preliminarily identified, and assessed as “live” or “dead” based on response to touch or visible deterioration (when live/dead categorization was uncertain, specimens were placed into a separate bowl at full salinity for 1-hr to look for signs of mobility). Specimens were then stored in glass vials with 95% ethanol for later identification. While these pre-shipping observations did not contribute to the statistical analysis of treatment effect, they provided an understanding of the abundance, richness and viability of organisms that were removed from the wormweed at the source during the treatment process.

Finally, to gain a general understanding of the abundance and richness of associated organisms found in unmanipulated wormweed, we also performed a baseline (abbreviated as B-ME) observation of wormweed purchased from the Maine distributor at the source. Two replicate volumes (750-g each) of wormweed were taken directly from the two storage bags, placed into a plastic bin, and manually searched for associated biota; i.e., every frond was systematically inspected by eye for attached organisms, which were moved to glass bowls and examined under the microscope, counted, and then preserved per the above methodology. The bin itself was also rinsed with seawater and sieved for any dislodged organisms, which were also counted and identified.

Post-shipment baitbox assessments

All treated and untreated wormweed were transported (40-min) to our Wiscasset distributor, who packed the algae with bloodworms into baitboxes using standard commercial protocols. Baitboxes were shipped overnight to SERC in Maryland (June 2012) or UC Davis in California (July 2012). Three shipments (randomized by treatments and controls) occurred each day for a total of four days. A HOBO temperature logger (Onset Industries, Bourne, Massachusetts) was also included in one replicate baitbox per treatment/control for the June 2012 experiment. Following delivery by 10 am the next morning, baitboxes were placed in refrigeration (4.4 ºC) until examination that same day (<8-hr after delivery). Though we were unable to mimic all the physical conditions associated with the standard operation of the baitworm industry, our partnering with a commercial bait dealer closely emulated the typical processing and handling of the weed and worms for shipping, including overnight shipments.

Post-shipment processing of each baitbox included the following steps. First, bloodworms were removed from each box and visually examined to assess condition on a 3 point scale: 1 = poor condition (high mortality, poor coloration, severed worms, low mobility); 2 = good condition (low mortality, fairly normal coloration, mostly intact worms, moderate mobility); 3 = excellent condition (no mortality, normal coloration, intact worms, high mobility). Second, bloodworms were rinsed in artificial seawater to dislodge any attached organisms; this “worm-water” was sieved, and associated organisms were moved to glass bowls. Third, the treated or untreated algae were removed from each baitbox, rinsed, and every algal frond was systematically examined by eye for attached organisms, which were placed into glass bowls. Fourth, the baitbox was rinsed using seawater and sieved for any dislodged organisms. Finally, all glass bowls were examined under a stereomicroscope for living and dead biota, which were counted and preserved for later identification (as per methodology above).

Statistical analyses

Taxonomic richness and abundance of associated marine invertebrates in treated and untreated algae were evaluated post-shipment. In the June 2012 experiment, the tap, hyper, and tap+hyper treatments were all assessed, while in the July 2012 experiment, just the tap and tap+hyper treatments were performed due to time limitations; however, as the hyper treatment was the least successful of the three analyses, its non-inclusion did not affect our overall conclusions (see Results). Using JMP 9.0.2 (SAS Institute, Inc.), four one-way ANOVAs were performed to examine the effect of treatment on response variables (richness and square-root trans-formed abundance) for live organisms; pairwise comparisons were also examined using post-hoc Tukey's tests. In addition, the effect of treatment on community structure was explored using PRIMER 6 (Primer-E Ltd.) for the two experiments and also the Maine source data. These analyses included a Bray-Curtis similarity resemblance matrix non-metric multi-dimensional scaling (nMDS) plot and a Similarity Percentage (SIMPER) analysis. Live and dead abundance and richness were both analyzed to determine which taxa were removed by the treatments at the source, which taxa exhibited the highest survival post-shipment, and which taxa arrived dead and which arrived alive in post-shipment baitboxes.

Results

Baitbox temperatures and worm condition

Mean temperatures for baitboxes [C-MD = 14.8 (±0.3) ºC; T-MD = 13.3 (±0.4) ºC; H-MD = 14.0 (±0.4) ºC; and TH-MD = 15.6 (±0.3) ºC] were not significantly different among treatments during transit (F=1.33, df=3, p=0.26) and were within the typical temperature tolerance ranges for temperate intertidal species in Maine during May, where mean air temperatures ranged from 5–18ºC (http://www.currentresults.com) and mean sea surface temperatures ranged from 12–16 ºC (http://www.nsof.class.noaa.gov). Based upon visual assessment, all worms were in excellent condition upon arrival, and there were no differences in worm condition between controls and treatments.

Abundance and richness of hitchhikers in post-shipment baitboxes

We found significant differences among treatments for live abundance and richness in our first experiment (shipment to MD) (abundance: F = 8.94, df = 3, p = 0.006; richness: F = 11.15, df = 3, p = 0.003), and post-hoc Tukey's tests revealed significantly (p<0.05) lower abundance and richness for tap and tap+hyper treatments compared to the control. In our second experiment (shipment to CA), taxa richness was significant (F = 11.18, df = 2, p = 0.01), but abundance was not (F = 2.43, df = 2, p = 0.169); post-hoc tests revealed significantly (p<0.05) lower richness in the tap+hyper treatment versus the control (Figure 2). The hyper treatment did not have a significant effect on abundance or species richness in the first experiment, and as described above, this treatment was not included in the second experiment.

In post-shipment baitboxes, comparisons of average abundance and richness of hitchhiking individuals in treated versus untreated algae revealed a large reduction in both measures for treated boxes compared to control boxes (Table S1), especially for the tap+hyper treatment which induced the greatest osmotic shock. In the two experiments, we observed 99% and 94% reductions of live abundance in treated algae compared to controls, respectively, and 97% and 93% reductions of total (live+dead) richness. These losses (live only, or live and dead combined) were also apparent across individual taxonomic groups, including the most common taxa (amphipods, isopods, mites, and snails; supplementary material Table S1).

Community assemblages

In nMDS plots, the Maine baseline assessment (B-ME) and post-shipment controls for the first experiment (C-MD) were closely aggregated, suggesting that shipment did not adversely affect species assemblages during transit. However, post-shipment controls in the second experiment (C-CA) shifted to a different position in the state space (Figure 3), probably due to seasonal differences in assemblages from spring to summer (see discussion). Compared to live and dead assemblages in treated packing algae from MD baitboxes, those from ME source treatment buckets were tightly clustered, indicating that the treatments were effective at eliminating fairly similar levels of diversity at the source. The most disparately spaced treatments from their respective controls were the post-shipment MD-TH and CA-TH treatments (Figure 3), which as described above, were the most effective treatments at reducing or eliminating live hitchhiking diversity.

In SIMPER analyses (Tables S2 and S3), two species consistently showed the highest contributions to live and dead assemblages in pre-shipment observations in ME and also in treated and control post-shipment baitboxes: the amphipod *Hyale nilssoni* and the isopod *Jaera albifrons*. However, both species had higher average abundances in pre-shipment observations than in any of the post-shipment treated baitboxes indicating the elimination of many individuals prior to shipping; for example, live *H. nilssoni* were 57% more abundant in T-ME versus T-MD and were absent altogether in the TH-MD, T-CA and TH-CA treatments. In addition, both species' live average abundances were lower in treated algae

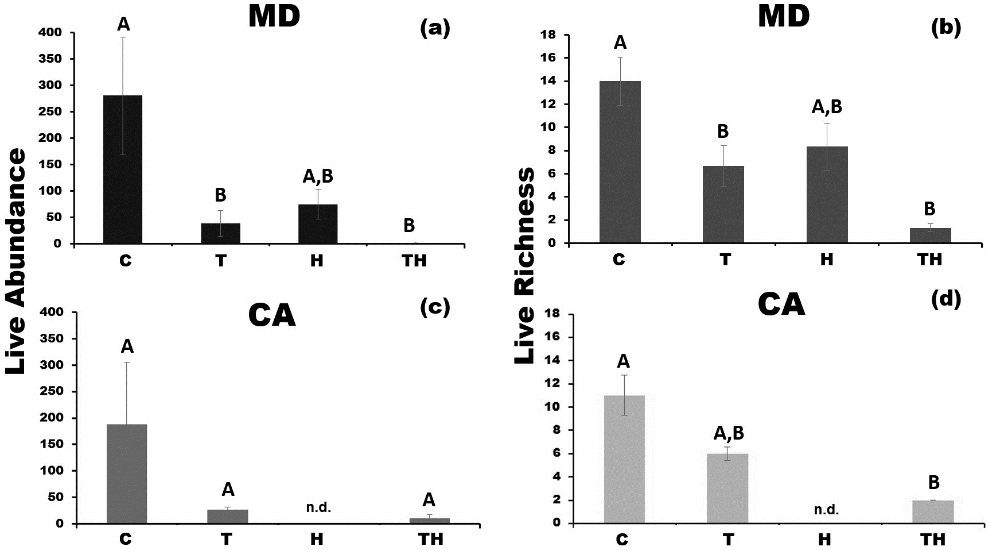

Figure 2. Average (±SE) live and dead square-root transformed abundance (a,b) and richness (c,d) of associated organisms per treatment. Letters represent pairwise significant differences ($p<0.05$) based on post-hoc Tukey's tests for treatments and recipient locations: Maryland (MD), and California (CA).

versus untreated controls in post-shipment baitboxes, and in several cases, were absent or found dead in treated algae. Other live taxa in post-shipment baitboxes included amphipods, mites, the barnacle *Balanus crenatus*, the isopod *Philoscia vittata*, and unidentified nematodes; however, their abundances were all low in baitboxes of treated wormweed, with the exception of halacarid mites, though these were still substantially lower in post-shipment baitboxes than pre-shipment observations. Three periwinkle snails (*Littorina littorea*, *L. obtusata*, and *L. saxatilis*) were also detected in post-shipment baitboxes. *Littorina littorea* (common periwinkle) and *L. obtusata* (smooth periwinkle) were each found in just one T-CA baitbox, while *L. saxatilis*, one of the more abundant live organisms in pre-shipment (ME) observations and in post-shipment control boxes, was found in very low abundances in the H-MD treatment, absent altogether from the T-MD and TH-MD treatments, and only found dead in one T-CA and one TH-CA baitbox.

Discussion

Reducing live species transfers via osmotic shock

Our study corroborates prior investigations showing Maine's live bait vector to be a potent mechanism of propagule transfer, especially when unimpeded (Haska et al. 2011; Cohen 2012; Fowler et al. 2016). We detected high abundance and richness of live hitchhikers in untreated baitboxes after shipment to two locations (Maryland and California), and in one control box, we identified as many as 500 live individuals from 20 different taxonomic groups. However, our investigation also demonstrated that the prevalence of live hitchhikers can be significantly reduced by simple osmotic shock treatments prior to shipping—thus revealing a promising vector management strategy to lessen the propagule pressure associated with live trade vectors, while still maintaining the traditional operation of those industries. In testing three osmotic shock treatments (tap water, hypersaline water, and tap + hypersaline water), we predicted that our tap+hyper treatment would be most effective at reducing live abundance and richness in packing algae since the osmotic shock induced by this treatment is the most severe. As anticipated, we found both abundance and richness of live hitchhiking organisms were reduced by up to 99% in the tap+hyper treatment. Yet, even our simplest treatment (tap water) eliminated more than 85% of live abundance in post-shipment baitboxes, suggesting that this approach can provide a straightforward, rapid, and economical procedure to substantially lessen abundance and richness of live hitchhikers entrained in the vector. Importantly, these methods did not negatively affect the condition of baitworms in any shipment, and we noted the worms remained in good condition for multiple days after their delivery dates to both shipment locations.

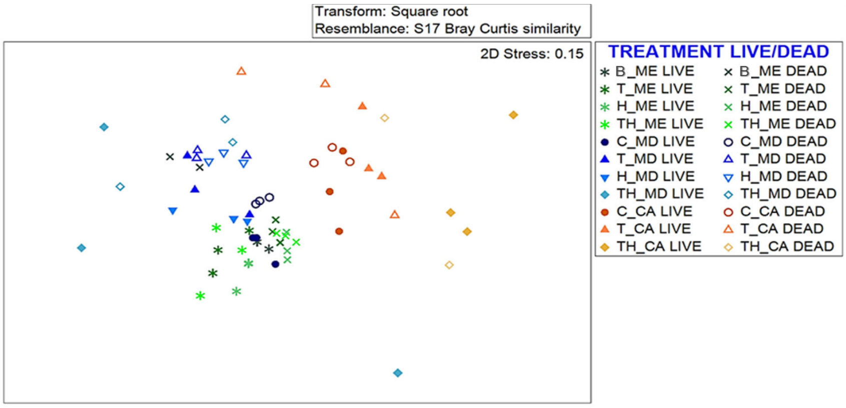

Figure 3. nMDS plot using square root transformed abundance data and Bray-Curtis similarity for live and dead biota. These analyses spatially explore live and dead diversity for pre-shipment source (ME=Maine) and post-shipment baitboxes (MD=Maryland, CA=California). Symbols closer together have more similar species assemblages than those further away.

In fact, some of the worms were utilized by local community members for nearby fishing activities (i.e., SERC hosted free baitworm giveaways to help educate the public about proper packing algae disposal; e.g., see http://www.serc.si.edu/labs/marine_invasions/feature_story/May_2012.aspx).

Our results are also consistent with other marine vectors, including ballast water, demonstrating osmotic shock treatments to successfully and significantly reduce hitchhiking biota. For example, the transfer of live organisms in ship's ballast tanks is a global biosecurity concern—negatively impacting commercial species, ecosystems, human health, and human infrastructure (Hallegraeff and Bolch 1992; Ruiz et al. 2000b; Hayes and Sliwa 2003; DiBaccio et al. 2012). Ballast water exchange has therefore been advocated or mandated as a management strategy in numerous countries worldwide due to its effectiveness at reducing live transfers of marine and freshwater organisms between ports (Wonham et al. 2005; Costello et al. 2007; Gray et al. 2007; Albert et al. 2013). This strategy works by replacing coastal water from a source port with fully marine water at sea. Because salinities in coastal ports (typically protected bays, estuaries, and lakes) and the open ocean are often considerably different, osmotic shock can be induced in organisms located in the ballast tanks (Ruiz and Reid 2007). Such osmotic shock strategies contribute to lowering propagule pressure in recipient locations and consequentially can reduce the invasion risk to recipient communities (Ruiz and Reid 2007; Santagata et al. 2008; DiBacco et al. 2012).

Species assemblages pre- and post- shipment

The most commonly detected taxa in live algal packing materials in ours and other live bait studies (Haska et al. 2011; Cohen 2012; Fowler et al. 2016) are crustaceans and gastropods, particularly isopods, amphipods, and snails. Crustaceans and gastropods are also some of the most frequently introduced coastal marine species worldwide across numerous anthropogenic vectors (Ruiz et al. 2000a; Pysek et al. 2008). In our study, a repeatedly observed species at the source and in post-shipment control boxes was a marine gastropod, the rough periwinkle snail (*L. saxatilis*), which is the most geographically widespread of all Littorinidae snails, including native, introduced, and cryptogenic populations in North America, Europe, and Africa (Carlton and Cohen 1998; Panova et al. 2011). This snail's reproductive strategy of brooding live crawl-away young is believed to have contributed to its widespread distribution and invasion success (Johannesson 1988; Chang et al. 2011). Indeed, we found live *L. saxatilis* in all pre-shipment observations and all post-shipment control baitboxes. However, our treatments eliminated all living individuals of this snail from post-shipment treated baitboxes, pointing to the effectiveness of our treatments in removing living individuals of a widespread species and known invader from this active vector.

Two other commonly observed species, the amphipod *H. nilssoni* and isopod *J. albifrons*, are also brooders, and we observed live brooding individuals of both species in post-shipment baitboxes of untreated algae. This is of concern, not only considering the high abundances of these species, but also because the transfer of reproducing individuals to new regions enhances the likelihood for successful establishment in novel regions. At this point, it is unclear whether these two common hitchhikers have successfully established outside their native ranges (North Atlantic). Neither are listed as "exotic" on two major databases for aquatic non-natives: the National Exotic Marine and Estuarine Species Information System (NEMESIS) (http://invasions.si.edu/nemesis/browseDB/searchTaxa.jsp), nor the USGS nonindigenous aquatic species (NAS) list (http://nas.er.usgs.gov/queries/SpeciesList.aspx?group=Crustaceans&genus=&species=&comname=&Sortby=1). Yet given their abundances in post-shipment baitboxes in our study and in prior investigations (e.g., Fowler et al. 2016), the likelihood of successful establishment elsewhere could be high. Thus close monitoring of these, and many other commonly associated marine invertebrate species in coastal regions importing live polychaete baitworms, is an important endeavor.

While we observed strong reductions in richness and abundance in treated baitboxes, the composition of species assemblages arriving to each were somewhat disparate. Given that all shipments were made overnight and temperatures within baitboxes were consistent, assemblage differences as a result of transit seem implausible. Instead, we attribute such differences to the timing of the two experiments (June for Maryland versus July for California) that reflect natural shifts in species assemblages in wormweed habitats throughout the year (Fowler et al., unpublished data) and are also supported by baseline observations of wormweed in the Maine source (Figure 3). Such seasonal shifts in species pools across the months intimate that different assemblages are entrained by the vector throughout the year, thereby increasing annual hitchhiker diversity and potential risk of successful transfer and invasion (Miller and Ruiz 2009). Because the Maine bait vector is a year-round operation, it differentially samples the environment throughout the year, with the greatest available diversity expected in warmer months.

Conclusions

Our investigation, along with prior studies (Costa et al. 2006; Haska et al. 2011; Cohen 2012; Fowler et al. 2016), have shown the live saltwater bait vector to be a potent conveyor of vast quantities of individuals across numerous marine taxa nationally and internationally. Importantly, this vector remains active, unregulated, and operational year-round, providing continuous transfer of associated biota through a broad-scale distribution network in North America and worldwide (Crawford 2001). Despite strong evidence demonstrating potential impacts on recipient coastal communities (e.g., Cohen et al. 2001; Weigle et al. 2005), management strategies are largely lacking to prevent the unintentional transfer of hitchhiking organisms. It would therefore seem prudent to take direct steps to lessen the propagule pressure inherent in this vector, and thereby its invasion risk. Artificial packing materials (e.g., seawater soaked newsprint, paper towels, charcoal, or sawdust) could be a possible alternative to live algae (Crawford 2001), and some of these materials have been shown to nearly eliminate live hitchhiking diversity; for example, Fowler et al. (2016) found a 99% reduction of hitchhiking abundance and richness in baitboxes from a Maine dealer who packed worms in saltwater-soaked, shredded newsprint rather than wormweed. However, such alternative packing materials may be less appealing to many baitworm dealers since they do not preserve the traditional operation of the industry and may increase costs (AEF, pers. comm.). Our study demonstrates that simple, inexpensive osmotic shock treatments of algal packing materials could also reduce the unintentional transfer of hitchhikers by up to 99% (thereby abating the likelihood of new invasions and associated impacts), while maintaining the traditional use of packing algae (wormweed) in the industry. Further, these simple osmotic shock strategies could be employed in other live trade vectors that utilize packing algae in shipments of living biota, or ship algae as a product (e.g., the aquarium trade). In sum, given the strong reductions of live hitchhiking abundance and richness of treated algae in our experiments, we offer this approach as a viable strategy for consideration by the Maine bait-worm industry, the fishing community, and recipient states and regions worldwide, given the considerable benefit it could provide to at-risk ecosystems globally.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Abundance and richness of hitchhiking taxa across treatments in recipient regions, Maryland and California.

Table S2. SIMPER analysis of associated live and dead biota in packing algae post-shipment bait boxes.

Table S3. SIMPER analysis of live and dead biota in wormweed from baseline observations at the Maine source and for sieved water from treatment buckets in Maine before shipment.

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