ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/257537516

Bait worm packaging as a potential vector of invasive species

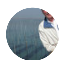
Article *in* Biological Invasions · February 2011

DOI: 10.1007/s10530-011-0091-y

CITATIONS
46

READS
270

7 authors, including:



Charles Yarish
University of Connecticut

234 PUBLICATIONS **7,518** CITATIONS

SEE PROFILE

George P. Kraemer
Purchase College, State University of New York

65 PUBLICATIONS **3,299** CITATIONS

SEE PROFILE

Noreen Blaschik
University of Connecticut

1 PUBLICATION **46** CITATIONS

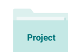
SEE PROFILE

Huan Zhang
University of Connecticut

175 PUBLICATIONS **3,482** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Symbiosis and parasitism of algae View project

Algae-bacteria relationship View project

All content following this page was uploaded by George P. Kraemer on 21 May 2014.

The user has requested enhancement of the downloaded file.

Author's personal copy

Biol Invasions (2012) 14:481–493
DOI 10.1007/s10530-011-0091-y

ORIGINAL PAPER

Bait worm packaging as a potential vector of invasive species

Christina L. Haska · Charles Yarish · George Kraemer · Noreen Blaschik · Robert Whitlatch · Huan Zhang · Senjie Lin

Received: 21 December 2010 / Accepted: 17 August 2011 / Published online: 14 September 2011
© Springer Science+Business Media B.V. 2011

Abstract Invasive species have become an increasingly greater concern for the ecological health of coastal ecosystems, yet vectors of these introductions often are unclear. This project evaluated the potential for the brown seaweed *Ascophyllum nodosum* ecad *scorpiodes* (Hauck) Reinke, packaged with bait worms (*Nereis virens*) harvested from the coast of Maine (USA), as a vector of invasive marine fauna and flora. Often, the seaweed and contents of the bait boxes are discarded into the water by recreational fishermen after using the bait worms, and any included non-native species may then be introduced. Bait boxes were purchased from several commercial vendors in Connecticut and New York over a two-year period. Subsamples of the seaweed were placed in laboratory culture and the growth of associated macro- and microalgae was monitored. Marine invertebrate species present in the samples were also identified and quantified. Results indicated 13 species of macroalgae and 23 species of invertebrates were associated with baitboxes. Among the highly diverse microbial assemblage detected, two species of potentially toxic marine microalgae, *Alexandrium fundyense* Balech and *Pseudonitzschia multiseries* (Hasle) Hasle, were found both prior to and after incubation at various temperatures, indicating these harmful algae are brought to and can survive in receiving waters. These findings highlight the need to consider alternative choices of bait box packaging materials or appropriate disposal methods of the seaweed in order to minimize the transport of species which are not native to the receiving coastal waters.

Keywords Invasive species · *Alexandrium fundyense* · *Pseudonitzschia multiseries* · *Ascophyllum nodosum* · Bait worms

Electronic supplementary material The online version of this article (doi:10.1007/s10530-011-0091-y) contains supplementary material, which is available to authorized users.

C. L. Haska (✉) · N. Blaschik · R. Whitlatch · H. Zhang · S. Lin (✉)
Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Rd, Groton, CT 06340, USA
e-mail: christina.haska@gmail.com

S. Lin
e-mail: senjie.lin@uconn.edu

Present Address:
C. L. Haska
Great Lakes Fishery Commission, 2100 Commonwealth Blvd Ste 100, Ann Arbor, MI 48105, USA

C. Yarish
Department of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT 06901, USA

G. Kraemer
Departments of Environmental Studies and Biology, Purchase College, 735 Anderson Hill Rd, Purchase, NY 10577, USA

Author's personal copy

Introduction

Introductions of non-native species are threatening the economic and ecological well-being of coastal marine ecosystems. Because of this, potential transport vectors of non-natives must be identified and evaluated (Pimental et al. 2005; Chapin et al. 2000). Recognition of these vectors will help environmental managers reduce introductions of non-native species, which is often a considerably less expensive strategy than attempting to restore an area after a non-native introduction (Ricciardi and Rasmussen 1998).

Seaweed packaging of bait worms (*Nereis virens* Sars and *Glycera dibranchiata* Ehlers) can be a vector of potentially invasive species. Packing seaweed such as the brown alga *Ascophyllum nodosum* ecad *scorpiodes* (Hauck) Reinke is used to reduce the stress to bait worms from increased temperatures and desiccation during transport. Unintentionally, this seaweed can also enable the survival of other organisms contained within or attached to the seaweed. Unlike other industries, in which introductions are often accidental, live-bait products and their packaging are destined to be released into the water, thereby raising the probability of non-native species introductions (Weigle et al. 2005). For example, Lau (1995) found that 40% of anglers discarded leftover bait worms and seaweed into the water.

The State of Maine is currently one of the world's largest exporters of marine bait worms for recreational fishing (Brown 1993; Thayer and Stahlnecker 2006). At present, this industry is valued at $7.3 million annually and harvests over 5 million kilograms of *A. nodosum* annually for packing material (Maine Department of Marine Resources; www.maine.gov/dmr/commercialfishing). Bait worms are shipped from Maine to locations throughout the continental United States and Europe (compiled by Cohen et al. 2001) for retail sale. These worms are also available for sale via the Internet (Olson 2001). Species established within the coastal areas of Maine (whether native or non-native to the region), therefore, have the potential to be introduced to a vast array of coastal regions and habitats in the USA and throughout the world.

Previous studies have indicated that bait worm packaging is a potential vector of invasive species nationwide (Silva 1979; Dawson and Foster 1982; Carlton 2001). Cohen et al. (2001) examined the contents of bait worm boxes shipped from Maine to the San Francisco Bay area of California and found 38 distinct species. *A. nodosum* was also found along the shoreline of San Francisco Bay and is thought to have been introduced through the use of bait worm boxes. It has since been successfully eradicated, although the site is still being monitored in order to assess the long-term effectiveness of the eradication program (Miller et al. 2004).

In this study, bait worms were purchased from several retail shops throughout Connecticut and New York. The associated seaweed were analyzed to determine whether or not the nereid or glycerid bait worm packaging (primarily *A. nodosum*) acts as a vector transporting macro- or microalgae or invertebrates from the Gulf of Maine, and whether or not these species could survive upon introduction to foreign waters.

Materials and methods

Acquisition of bait boxes and initial processing

Sandworm (*Nereis virens*) bait boxes were purchased from five retail shops from New York (NY) and six from Connecticut (CT) (Table 1), with an attempt to sample from two shops in CT and two in NY on each of the 19 sampling dates, with the exception of the last date in 2007 when only three shops were sampled (Online Resource 1). Information was unavailable as

Table 1 Bait retail shop locations and acronyms used throughout the study

	Acronym	Latitude/longitude
Connecticut		
Groton1	CT-A	41°20′N, 72°4′W
Groton2	CT-B	41°20′N, 72°4′W
Old Saybrook	CT-C	41°17′N, 72°21′W
Norwalk	CT-D	41°6′N, 73°24′W
Stamford	CT-E	41°5′N, 73°34′W
Greenwich	CT-F	41°30′N, 73°39′W
New York		
Glenwood Landing	NY-A	40°49′N, 73°38′W
Port Chester	NY-B	41°N, 73°39′W
New Rochelle	NY-C	40°55′N, 73°47′W
Bronx1	NY-D	40°51′N, 73°52′W
Bronx2	NY-E	40°51′N, 73°52′W

Author's personal copy

to whether or not these shops sold worms that originated from the same area of Maine; this uncertainty was dealt with by randomly selecting shops along the Long Island Sound coast to use as sampling locations. To study seasonal variations in the presence of associated fauna and flora in the bait boxes, sampling was conducted twice a month during the main fishing season (i.e., June through October) and once a month during the beginning and end of the fishing season (Online Resource 1). Bait worms were purchased in six ½ dozen boxes or 3 one-dozen boxes containing *Ascophyllum nodosum* as the packing material, depending on availability. When possible, the bait boxes were purchased on the same day; however, circumstances sometimes required them to be purchased on different days. In these cases, the bait boxes were kept at 5°C until the following day (Online Resource 1). On one occasion, the retail shop did not have bait boxes containing *N. virens*, so boxes containing bloodworms (*Glycera dibranchiata*) were substituted since they are also packaged with *A. nodosum* (Online Resource 1).

The bait worms were first removed from the packaging seaweed and wet weights of the seaweed were recorded to determine if large differences in quantity existed among bait shops and sampling dates. It is important to note, however, that this project was not intended to measure quantitative differences in diversity between samples, but rather to provide a glimpse into how diverse the species composition is within bait boxes. This was strictly a presence/absence study; therefore, sample sizes of each site on each date were similar but not exactly the same, resulting in a conserved diversity estimation.

Macroalgal sampling and incubation

Data were gathered on the common epiphytes and endophytes found associated with *Ascophyllum nodosum* along the coastline of Maine (see Online Resource 2). *A. nodosum* from the bait boxes was initially examined to determine if any of these epiphytic or endophytic macroalgae were present prior to incubation. Approximately 1/3 of the packaging material was removed and cultured to promote the growth of macroalgae present in microscopic stages (i.e., thalli or spores): at least three ca. 1 cm pieces of the basal, apical, and branch portions of the

Table 2 Dates, sites, and species of *Fucus* included in the bait-worm packaging *Ascophyllum nodosum* (see Table 1 for sample-location codes)

	CT-B	CT-D	CT-E	CT-F	NY-A	NY-B
5-Jun-07						
8-Jun-07				✔▲		
2-Jul-07					▲	
19-Jul-07		✔				
8-Aug-07		✔				✔
22-Oct-07		✔			✔	
5-Nov-07		✔				✔
2-Apr-08	✔	✔	✔		✔	
1-May-08	✔					
16-Jun-08					✔	
7-Jul-08	✔	✔			✔	

▲ *Fucus vesiculosus*

✔ *Fucus spiralis*

A. nodosum thalli were included in each culture dish. Often *Spartina* sp. and macroalgae (mainly *Fucus* sp.) were found mixed within the seaweed in the bait boxes, so they were also divided among the incubation vessels (Table 2). Two hundred milliliters of enriched von Stosch (VSE) media was placed in each 400 ml deep Petri dish (Ott 1965). These cultures were placed in three different temperatures which would mimic a variety of conditions throughout the United States (5, 15, 25°C) under a 12:12 L:D photoperiod with a photon flux rate of 40 µmol photon $m^{-2}s^{-1}$. The thalli were incubated for 10 days and reexamined for growth of epiphytic or endophytic marine macroalgae. If a positive identification could not be made at that time, the material was placed back into culture until morphological identification could be done using Villalard-Bohnsack (1995) and Sears (2002) keys.

Microalgal sampling and incubation

For microalgal analyses, approximately 1/3 of the seaweed packaging and associated *Spartina* or *Fucus* spp. from each sampling site was added together to a 1L Erlenmeyer flask containing 500 ml of 0.45 µm-filtered, autoclaved seawater and shaken to release any microalgal cells (i.e., vegetative cells or cysts) contained within or on the packing material. This

Author's personal copy

seawater was then sieved through a 50-μm filter to remove sediment, and the filtrate was distributed into 50 ml conical tubes. One tube was preserved with Lugol's solution for subsequent microscopic examination, three tubes were used for culture purposes, and 2–4 tubes were used for DNA extraction. These DNA samples will be referred to as the "initial" samples.

The day following the sampling date, the contents of the 50 ml tubes labeled for culture were added to 200 ml F/2 media (Andersen 2005) in 250 ml tissue culture flasks (BD Falcon: Franklin Lakes, NJ). The flasks then were incubated at the same temperatures as the macroalgae (5°, 15°, 25°C); however, the light intensity was increased to 80 μmol photon $m^{-2}s^{-1}$. These flasks were incubated for 10 days, at the end of which their contents were prepared for DNA extraction. The DNA samples that were extracted after the incubation period are referred to as "post" samples.

DNA extraction

Two hundred milliliters of each initial sample and 50 ml of each post sample was centrifuged at $4{,}000\times g$ for 10 min. The supernatant was removed, and the pellet was re-suspended in approximately 1 ml of residual liquid. After transferring into a 1.5-ml tube and centrifuging at 12,000 rpm for 3 min, the supernatant was removed, and the pellet was suspended in DNA lysis buffer (10 mM Tris pH 8.0, 100 mM EDTA pH 8.0, 0.5% SDS, 200 μg/ml proteinase K). DNA extractions were performed using a CTAB protocol (Zhang and Lin 2005) for samples collected in 2007. Upon completion of the extraction, the DNA was eluted with 80 μl 10 mM Tris–HCl. DNA concentration and quality were measured spectrophotometrically using a Nanodrop (Thermo Scientific; Waltham, MA). DNA quality was further examined by PCR using a universal 18S rDNA primer set (see Online Resource 3). If the PCR failed, the DNA solution was extracted again with phenol–chloroform and run through the Zymo column.

Despite the extensive efforts to obtain PCR-amplifiable DNA, some of the samples failed in PCR, particularly with the initial samples. This failure probably resulted from inhibitory compounds from sediment and other debris associated with the *Ascophyllum nodosum,* which were rich in phenolic compounds. To alleviate this problem, a Soil Microbe Kit (Zymo Research) was used to extract DNA for samples collected in 2008. The samples were centrifuged as above; however, with this method, the pellet was added to the Kit lysis buffer and homogenized at 6.5 m s^{-1} for 45 s. The protocol included with the Kit was followed, continuing through the last step of centrifugation through the IV-HRC spin filter. DNA was eluted with 100 μl of the elution buffer provided.

Polymerase chain reaction (PCR)

PCR was run on the microalgal samples to determine if particular target species were present. Primers and annealing temperatures for each reaction are listed in Online Resource 3. First, DNA quality was tested using PCR with universal primers, as mentioned previously. PCR inhibitors were often found within the samples; therefore, this amplification was critical in determining whether or not those samples could be amplified, thereby ensuring there would be no false negatives (Lin 2008). Once the DNA was deemed clean enough to amplify, PCR was run for seven individual species. Specifically, six dinoflagellates (*Alexandrium fundyense* Balech, *Karlodinium veneficum* (D. Ballantine) J. Larsen, *Pfiesteria piscicida* K. A. Steidinger & J. M. Burkholder, *Pseudopfiesteria shumwayae* (Glasgow & Burkholder) Litaker, Steidinger, Mason, Shields & Tester, *Akashiwo sangiunea* (K. Hirasaka) G. Hansen & Ø. Moestrup, *Karenia brevis* (C. C. Davis) G. Hansen & Ø. Moestrup) and one diatom (*Pseudonitzschia multiseries* (Hasle) Hasle) were targeted for analysis.

Both the universal 18S rDNA and *Alexandrium*-specific PCRs were run using Takara Hot Start Ex Taq system with 1 μl DNA. Amplification for universal 18S rDNA was done in 35 cycles of 95°C for 25 s, 56°C for 30 s, 72°C for 40 s, followed by an additional extension step of 72°C for 5 min. For *A. fundyense*, the cycle program was 35 cycles of 95°C for 20 s, 58°C for 25 s, 72°C for 30 s, followed by a final step of 72°C for 5 min. PCR for the other target species was run through a Bio-Rad iQ iCycler system (BioRad; Hercules, CA) to achieve higher through-put. This program included an initial denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 s, annealing temperature for 25 s, and 72°C for 20 s, with a final melting curve analysis run from 55 to 95°C. The annealing temperatures can be found in Online Resource 3 for each individual PCR reaction. To validate the positive

Author's personal copy

signals for the targeted species, the PCR products were cloned and sequenced (see below), and the results were aligned to previously known sequences for the species.

Microalgal microscopic analysis

The samples preserved in Lugol's were kept in the dark at 4°C until analysis. A 1 ml sample was placed on a Sedgewick Rafter slide and observed using an Olympus BX51 compound microscope. The most prevalent species present were grouped according to taxonomic class (Tomas 1997; Graham and Wilcox 2000).

Cloning and sequencing of selected samples and targeted species

After analyzing the Lugol's preserved samples, two were chosen for universal 18S rDNA cloning and sequencing to investigate general eukaryotic diversities. The purpose was to look more broadly for potential HABs and other microalgal species that might: (1) escape microscopic analysis because of low abundance or small cell size, or (2) escape molecular detection because they were not one of the target species. One sample for this analysis was taken from October of 2007 and the other was from June of 2008. These samples were chosen because they contained a wide taxonomic group of organisms as found microscopically in the Lugols'-preserved samples. The DNA was PCR-amplified using the universal 18S rDNA primers as described above. The PCR product was purified and cloned into a T-vector (Takara: Shiga, Japan). One hundred and twenty clones were randomly picked and sequenced on an ABI Prism automated sequencer at the Yale University DNA Facility (New Haven, CT, USA). In addition, to validate the positive result on the targeted species, the PCR products were also cloned and sequenced as just described. The sequences were then BLAST-searched against GenBank nr database to match previously reported sequences. E values of 0 with 98% sequence identity were used to consider a genuine match at the species level; e values higher than E-50 and sequence identity <50% were considered unknown; those in between were categorized as a hit organism at the genus or higher taxonomic level. To assess if our sampling reached the species diversity in the seaweed packaging microbial community, the curve of the cumulative number of unique taxa versus clone number was analyzed.

Invertebrate sampling

After proceeding with the protocols for the macro- and microalgal analysis, the remaining seaweed (approximately 1/3 of the starting material) was rinsed over a 300 µm mesh-sieve to separate the algae from any non-epiphytic organisms. The sample was then examined for invertebrates and any dislodged invertebrates were collected and preserved in a 70% ethanol solution until identification. Several weeks following preservation, all invertebrates were identified to the lowest practical taxonomic category and enumerated using a 40× dissecting microscope and relevant taxonomic keys (Maclellan 2005; Pollock 1998). Species diversity was calculated using the Shannon-Weiner index.

Statistical analyses

For the algal component of this study, the general objective was to determine whether or not a significant difference among sampling sites and incubation temperatures existed, since this would address questions of geography and potential survivability of the introduced species. This was done by performing *t* tests to examine site and incubation temperature differences between samples collected from New York vs. Connecticut, between retail sites on the northern shore of Long Island Sound (LIS) versus the southern shore, and between the eastern and western ends of LIS. A one-way ANOVA also examined whether the 10-day incubation revealed a larger number of species compared to the initial sample inspection. Finally, incubation temperature and season were tested for their effects on the total species number by a two-way ANOVA to determine if seasonality could be a risk factor for survival of the hitchhikers (Gotelli and Ellison 2004). SPSS and Microsoft Excel were used to calculate these analyses, and the data complied with the assumptions of the *t* tests and ANOVAs.

DNA sequence data submission

The 18S rDNA sequences obtained in this study have been deposited to GenBank under accession numbers GU385505-GU385695.

Author's personal copy

Results

Detection of epiphytic macroalgae

The *Ascophyllum nodosum* variant ecad *scorpioides* was the principal packaging material for the bait worm boxes. On some occasions, *Fucus spiralis*, *Fucus vesiculosis,* and *Spartina* sp. were mixed with the *A. nodosum* (Table 2). Overall, no significant differences existed in the mass of packaging materials among sampling dates ($P > 0.194$, one-way ANOVA) or sampling sites among dates ($P > 0.41$, one-way ANOVA). So although the sampling sizes were not specific quantities of seaweed for each sampling date, there was a high consistency with the volume of samples, resulting in a methodical division of the seaweed.

On the day of sampling, the *A. nodosum* (and other material associated with it) was examined to determine if any detectable epiphytic or endophytic algae were present. On only one sampling date were any epiphytic or entangled macroalgae found before incubation: *Cladophora ruchingeri* Kützing was epiphytic on the *A. nodosum* on July 2, 2007 from the NY-A site (refer to Table 1 for the site information).

After each 10-day incubation, the samples were re-inspected for the presence of macroalgae. Throughout this study, a total of 13 different macroalgal species were found within the cultures (Table 3; Online Resource 4). There were five different *Ulva* species found; however, these species identifications required incubations longer than 10-days. When this occurred, the *Ulva* would be placed back in culture to incubate and grow further. Upon re-examination, the *Ulva* would either have grown to a point for species identification or it did not survive in culture any longer (see Table 3: indicated on the last line). In addition to the *A. nodosum*, the *Fucus* and *Spartina* spp. were also found to have epiphytic macroalgae after the incubation was complete. *Spartina* had, on average, twice as many species of epiphytes or endophytes than *A. nodosum*.

Detection of target microalgae

DNA extracted from the microalgal samples first underwent a universal 18S rDNA PCR to determine which samples were amplifiable. From the 2007 samples, 100 of the 172 samples (58%) were successfully amplified; however, none of the 44 initial samples were successful. The 2008 samples had a higher percentage of success with 112 of the 128 samples (88%) amplifying for 18S rDNA, and of these 18 were from the 32 initial samples. Overall, 70% of the DNA samples were positively amplified for universal 18S and therefore were examined for the presence of species-specific molecular sequences.

Seven species were targeted molecularly with PCR: *Alexandrium fundyense*, *Karlodinium veneficum*, *Pfiesteria piscicida*, *Pseudopfiesteria shumwayae*, *Akashiwo sanguinea*, *Karenia brevis,* and *Pseudo-nitzschia multiseries*. Two of these species were consistently found throughout the study: *Alexandrium fundyense* and *Pseudo-nitzschia multiseries* (Table 4; Online Resource 5). Sequences obtained from two selected PCR products (NY-A-25°C from July 19, 2007 and CT-D-15°C from July 22, 2008 for *A. fundyense* and NY-B-5°C from July 19, 2007 and CT-D-25°C from August 18, 2008 for *P. multiseries*) confirmed that what were amplified were, indeed, the target species.

General microbial community

The samples preserved with Lugol's solution revealed a highly diverse community of microorganisms. Among the genera found commonly throughout the study were diatoms such as *Cocconeis, Thalassiosira, Chaetoceros, Navicula, Caloneis*, *Melosira*, *Nitzschia*, and *Cylindrotheca* (Table 4). Of the samples examined, the 5°C sample from NY-A on October 22, 2007 and the 15°C sample from the CT-D site on June 2, 2008 contained a wide taxonomic group of organisms and thus were selected for further molecular analysis. Based on the 90 clones sequenced, the NY-A sample contained a large community of diatoms, with *Skeletonema* accounting for approximately 70% of the microalgae present (Fig. 1a). The next dominant species included *Thalassiosira* and *Nitzschia*. Of the 102 clones from the CT-D sample, however, the sequences showed a mixture of both ciliates and diatoms (Fig. 1b), with the ciliate *Euplotes* being the most dominant lineage, followed by *Navicula, Nitzschia, Holosticha,* and *Diophrys*. In addition, a large proportion of the sequences had no matches in GenBank.

With >2% sequence difference as the delineating cutoff of a unique taxon, the cumulative number of

Author's personal copy

Table 3 Summary of macroalgae associated with *Ascophyllum nodosum* packaging material found post-incubation

Species	2007											2008							
	5-Jun	18-Jun	2-Jul	19-Jul	8-Aug	23-Aug	10-Sep	24-Sep	8-Oct	22-Oct	5-Nov	22-Apr	12-May	2-Jun	16-Jun	7-Jul	22-Jul	4-Aug	18-Aug
Chaetomorpha linum Kützing						✔													
Cladophora ruchingeri Kützing			✔							✔	✔		✔				✔	✔	
Ectocarpus siliculosus Lyngbye				✔	✔		✔				✔	✔		✔	✔		✔		✔
Myrionema corunnae Sauvageau	✔														✔		✔		
Percursaria percursa Bory de Saint-Vincent	✔			✔			✔		✔		✔			✔					✔
Pilayella littoralis (Linnaeus) Kjellman									✔										
Rhizoclonium tortuosum (Dillwyn) Kützing	✔	✔	✔	✔		✔	✔	✔	✔			✔	✔	✔	✔		✔	✔	✔
Ulothrix flacca (Dillwyn) Thuret	✔	✔	✔	✔	✔	✔			✔		✔	✔	✔		✔				
Ulva clathrata Le Jolis						✔	✔		✔		✔		✔						
Ulva compressa Agardh								✔	✔			✔			✔				
Ulva flexuosa (Agardh) Wynne						✔	✔		✔			✔		✔					
Ulva intestinalis (Linnaeus) Link	✔			✔	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔			✔	✔
Ulva prolifera O. F. Müller														✔					
Ulva distromatic blade	✔		✔	✔	✔	✔	✔	✔			✔		✔	✔	✔	✔	✔		✔

unique taxa found versus the number of clones sequenced was plotted. These plots showed that the number of unique taxa found in sample NY-A (24 taxa) was approaching a plateau, whereas that in CT-D was still increasing with the number of clones sequenced, even though 49 unique taxa had been retrieved (Fig. 2).

Invertebrate assemblages

Invertebrates identified in the bait boxes included isopods, amphipods, bivalves, annelids, gastropods, arachnids (mites), ostracods, copepods and insects (Table 5). The greatest numbers of individuals were observed between the months of June and August, when population abundances of these invertebrates in the wild are known to be at their highest (Fig. 3). While a total of 23 separate invertebrate taxa were found in the samples, samples were typically dominated by the gastropod *Littorina saxatilis*, the amphipod *Hyale nilssoni* and the isopod *Jaera marina*. Species diversity estimates typically varied from 1.0 to 2.5 and there were no consistent temporal patterns of species diversity among sampling locations and sampling dates (data not shown). Decreases in species diversity are explained by large numbers of

Author's personal copy

J. marina, H. nilssoni and/or *L. saxatilits* found during July and August; however, species diversity increased in most samples during the 2007 fall months when the abundance of the dominant species decreased substantially or were absent from those samples.

Effects of sampling site, date, and incubation

There were no significant differences in the number of pooled algal species (both macro- and microalgae) present (*t* test) between New York versus Connecticut sites ($P > 0.45$), between northern sites (all excluding NY-A) vs. southern sites (NY-A) ($P > 0.096$), or between eastern sites (CT-A, CT-B, and CT-C) vs. western locations (all remaining sites) ($P > 0.14$). To determine if the 10-d incubation increased the number of epiphytes found, another *t* test was run on pre-incubation versus post-incubation species numbers; significantly more species were found after the incubation period for both years combined ($P < 0.001$). Approximately 94% of all detections occurred post-incubation.

A one-way ANOVA for both the HAB and macroalgal species revealed no effect of incubation temperature on the number of HAB species found ($P > 0.11$); however, there was a strong effect of temperature on the number of macroalgal species detected ($P < 0.001$). Specifically, the 5°C incubation had statistically fewer species than the 15°C and 25°C incubations ($P < 0.001$), and there were approximately 2.8-times more species found in the higher temperatures. A two-way ANOVA determined that

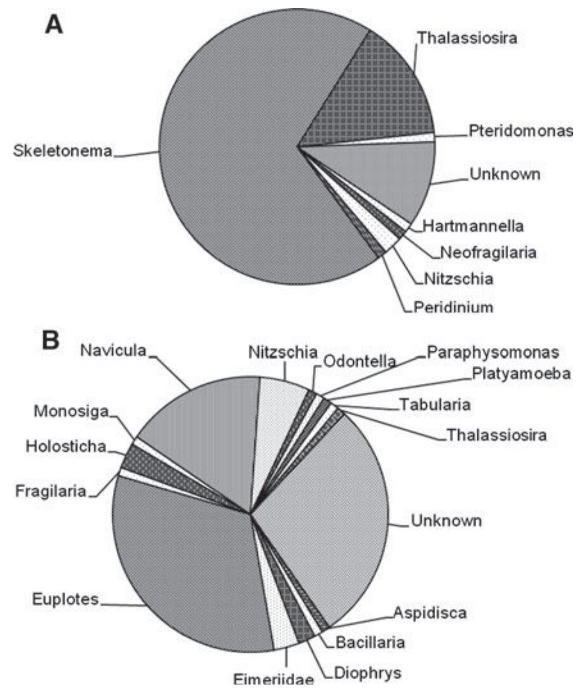


Fig. 1 Microalgal species found associated with *Ascophyllum nodosum* packaging material through DNA sequencing in NY-A-5°C (**a**) and CT-D-15°C (**b**)

Table 4 Summary of microalgal species found to be associated with the *Ascophyllum nodosum* packaging material samples

Species	2007											2008							
	5-Jun	18-Jun	2-Jul	19-Jul	8-Aug	23-Aug	10-Sep	24-Sep	8-Oct	22-Oct	5-Nov	22-Apr	12-May	2-Jun	16-Jun	7-Jul	22-Jul	4-Aug	18-Aug
Alexandrium fundyense	✔		✔	✔			✔					✔		✔	✔	✔	✔	✔	✔
Pseudonitzschia multiseries	✔	✔	✔	✔	✔	✔	✔	✔		✔		✔		✔	✔	✔	✔	✔	✔
Caloneis sp.		✔	✔	✔	✔	✔	✔	✔	✔			✔		✔	✔	✔		✔	
Chaetoceros sp.			✔					✔		✔			✔			✔			
Cocconeis sp.		✔	✔	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔		✔	✔	✔	✔
Cylindrotheca sp.			✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Melosira sp.		✔	✔	✔					✔		✔		✔		✔		✔		
Navicula sp.		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Nitzschia sp.		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔		✔	✔	✔	✔			
Thalassiosira sp.		✔	✔	✔	✔	✔	✔	✔		✔		✔	✔	✔	✔		✔	✔	✔

Samples were not preserved with Lugols' solution on June 5, 2007

Author's personal copy

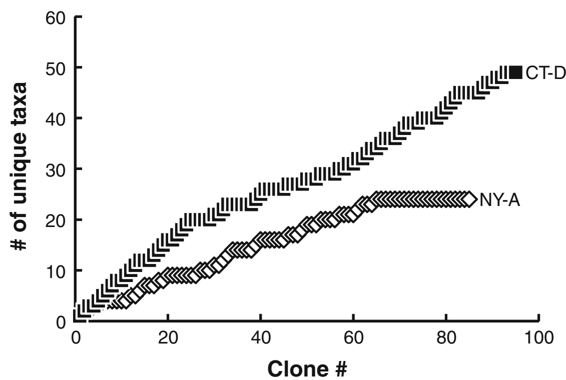


Fig. 2 Relationship between the cumulative number of unique taxa and the number of clones sequenced for NY-A and CT-D samples. Unique taxa were defined as > 2% difference in the 18S rDNA sequence

interactions between sampling date and incubation temperature had no significant effect on the number of macro- and microalgal species recorded ($P > 0.86$). Finally, a one-way ANOVA test revealed that incubation temperature did not affect the detection of either microalgal HAB-forming species ($P > 0.39$ for *A. fundyense* and $P > 0.37$ for *P. multiseries*).

Discussion

Bait worm packaging as a vector for transporting non-native algal species

Bait worm packaging has previously been shown to be a vector of transport for non-native and potentially invasive species (Cohen et al. 2001; Carlton 2001). This study further demonstrates the extent of this potential threat to bodies of water that receive imported bait worms for recreational fishing. Many marine algae have microscopic stages whereby they withstand oversummering or overwintering conditions. By exposing the samples to multiple temperatures, many taxa were captured that would have been otherwise undetectable. It is likely that most visible epiphytes were detached from the *A. nodosum* before being transported with the worms, either through accidentally being brushed off or purposely removed by the harvesters.

Of the macroalgal species found post-incubation, four genera, *Chaetomorpha, Cladophora, Ulva*, and *Pilayella*, have been known to cause blooms in temperate waters (Valiela et al. 1997; Mathieson and Dawes, 2002). When in bloom, these species may shade other benthic algae, thereby decreasing photosynthesis and growth (Wallentinus and Nyberg 2007). This may influence competitive interactions, change algal community composition, and alter habitat and food sources for native consumers. Large blooms of seaweeds can also lead to habitat degradation and hypoxia (Sfriso et al. 1987; Fletcher 1990; Yarish et al. 1991; Valiela et al. 1997; Pang et al. 2010).

The detection of two harmful microalgae within the bait worm packaging also clearly indicates the seaweed packaging is a potential vector of transport. *Alexandrium fundyense,* which has regularly formed toxic blooms in the Gulf of Maine in recent decades (Townsend et al. 2001; McGillicuddy et al. 2005), was detected throughout the study period. Its presence in more samples in July and August 2008 was coincident to an algal bloom recorded on August 1, 2008 in Maine (Fitzpatrick 2008). The second harmful microalgae species, the diatom *P. multiseries,* produces domoic acid (Bates and Trainer 2006). In our study, this species was detected microscopically in one sample and through molecular analysis in approximately 50% of all amplifiable samples.

The temperature incubations indicated which conditions might facilitate the growth and reproduction of potential invaders upon introduction. The temperatures chosen in this study mimicked those commonly found throughout the US during any given year. It is important to note that the beginning stages of a successful species invasion is for the organism to arrive, survive, and establish itself within a body of water, while the later stages spread and affect the native species (Allendorf and Lundquist 2003). This incubation period tested the ability of the hitchhiking organisms to undergo cell division (verified) and establish viable populations (yet to be determined). For macroalgae, the frequency of finding a species at 5°C was lower than those at the higher temperatures. This may indicate the macroalgae would be more apt to initiate growth during warmer months or in a warmer climate as compared to colder seasons and northern climates. This finding is supported by the fact that many of the species found throughout this study are eurythermal North Atlantic taxa with warm temperature affinities (Lüning 1990). This has significant implications because the main fishing season in many states is during the summer and fall, during

Author's personal copy

Table 5 Invertebrate species present in the samples collected from the six tackle shops from June 2007 to August 2008

Species	2007											2008							
	5-Jun	18-Jun	2-Jul	19-Jul	8-Aug	23-Aug	10-Sep	24-Sep	8-Oct	22-Oct	5-Nov	22-Apr	12-May	2-Jun	16-Jun	7-Jul	22-Jul	4-Aug	18-Aug
Caprella penantis (Am)				✔	✔			✔											
Dexiospira spirillum (An)									✔										
Echinogammarus obtusatus (Am)			✔	✔	✔			✔	✔	✔	✔			✔	✔	✔	✔	✔	
Enchytraeus albidus (An)	✔	✔	✔	✔			✔		✔		✔	✔	✔	✔	✔	✔		✔	✔
Gemma gemma (B)			✔					✔											
Halacarus sp (Ar)	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔			✔	
Hyale nilssoni (Am)	✔	✔	✔	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Hydrobia spp (G)	✔	✔	✔						✔			✔	✔	✔			✔		
Jaera marina (Is)	✔	✔	✔	✔	✔	✔	✔	✔	✔			✔	✔	✔	✔	✔	✔	✔	✔
Jassa falcata (Am)		✔																	
Littorina littorea (G)									✔	✔									
Littorina obtusata (G)		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Littorina saxatilis (G)	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Mercenaria mercenaria (B)															✔				✔
Mya arenaria (B)						✔	✔			✔									
Mytilus edulis (B)		✔	✔	✔	✔	✔	✔				✔	✔	✔						
Tigriopus sp. (C)	✔	✔	✔		✔		✔	✔	✔										
Chironomid larvae (In)			✔	✔	✔		✔	✔	✔	✔	✔		✔	✔	✔				✔
Dipteran larvae (Is)			✔																
Gammarid amphipod (Am)								✔		✔									✔
Oligochaete (An)	✔		✔		✔	✔	✔				✔								
Ostracod (C)			✔	✔				✔	✔	✔			✔			✔		✔	
Trombidiid mite (Ar)	✔	✔	✔	✔	✔	✔	✔		✔		✔	✔	✔	✔	✔	✔	✔	✔	✔

Codes are as follows: *An* annelid, *Am* amphipod, *Ar* arachnid, *B* bivalve, *c* copepod, *G* gastropod, *In* insect, *Is* isopod

Author's personal copy

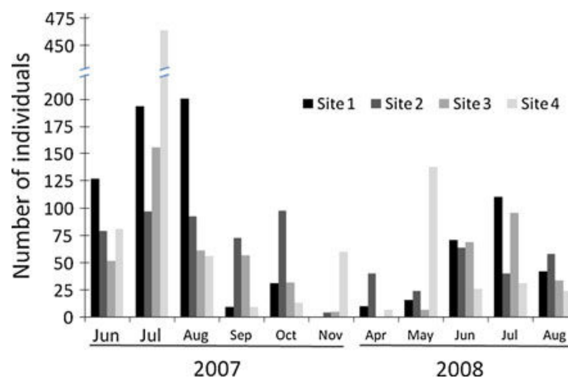


Fig. 3 The total number of invertebrate individuals present every month from each sampling site. Site 1 is CT-A, B, or C; Site 2 is CT-D, Site 3 is NY-A, and Site 4 is CT-E or F or NY-B,C,D, or E (refer to Online Resource 1 for specific sampling information). Note the break in the y-axis

which water temperatures would be favorable for these organisms. The observation that *Pseudo-nitzschia multiseries* was present in the packaging and survived the 10-day incubation at the range of temperatures also indicate that once introduced, it has the potential of developing year-round sustainable populations in surrounding waters.

Maine exports bait worms and *Ascophyllum nodosum* throughout the USA and Europe (Carlton 1979; Crawford 2001; Miller et al. 2004). This study illustrated that there were no significant interactions between season of sampling and incubation temperature with respect to the species number, implying that there is equal risk of introducing these species to different latitudes as well as different fishing seasons. The transportation method, however, for bait worms could differ between states or countries (i.e., air travel and ground shipping would have different stressors), and its effect on the survival of the organisms should be examined. Although the similarity among samples in this study does suggest there is a likelihood the species would be transported to other parts of the country, analyses of bait boxes in southern or western states may provide different species composition and/or different dominant species than what was found here. This was not a quantitative study and our goal was simply to provide a conserved diversity estimate.

The current geographic distributions of both *Alexandrium fundyense* and *Pseudo-nitzschia multiseries* should also be taken into account when interpreting these data. *A. fundyense* (a distinct species aside from the more global *A. tamarense,* although the distinction is still being debated) is only found along the northeast coast of North America. As a consequence, *A. fundyense* is considered to be a cold-water species and is not expected to thrive at warmer water temperatures. In this study, however, *A. fundyense* was found in the 25°C incubation samples, indicating it is capable of growing in warmer waters. Future work should assess the toxicity potential of *A. fundyense* at warmer temperatures. *P. multiseries,* in contrast, is a cosmopolitan species and is found at a large temperature range (Hasle 2002). Its wide distribution is reflected by its detection at each incubation temperature used in the present study period.

Bait worm packaging as a vector for transporting a diverse microbial assemblage

Microscopic examination of the samples revealed a high diversity of microorganisms within the *Ascophyllum nodosum* packaging material. The 18S rDNA sequencing also revealed a highly diverse community of eukaryotic microorganisms. Many of these microalgal and other protistan species would not have been found without sequencing because of their low abundance. The unique taxon cumulation curve appeared to allow us to retrieve the majority of the eukaryotic species diversity for the NY-A sample because the data approached a plateau with 24 taxa. The curve for the CT-D sample, however, still showed an increasing trend, indicating the species diversity in the seaweed packaging microbial community was even higher than the 49 taxa retrieved. Although differences between the two samples sequenced should not be directly compared because they were from different incubation temperatures (5° and 15°C), were sampled during different times of year (summer and fall), and each underwent a different DNA extraction method, the diverse eukaryotic protistan communities found in both samples indicate the potential of bait packaging seaweed to introduce a complex microbial assemblage to various water bodies. To fully understand the sources and implications of variation over space and time for the microbial assemblages, additional samples would need to be cloned from a variety of dates and temperatures. The NY-A and CT-D samples simply indicate there is a high diversity of microbes existing in the bait worm packaging.

Author's personal copy

Bait worm packaging as a vector for transporting non-native invertebrates

Bait boxes can be an important vector for the transfer of a variety of benthic invertebrates. The summer months have the greatest number of individuals per sample than the fall months, despite the high variability between bait shops. No consistent temporal differences were found among sampling sites.

Management implications

Many anglers prefer live baits, including sandworms, and the likelihood of a non-native species being introduced into a habitat increases with the number of release events (Allendorf and Lundquist 2003). Weigle et al. (2005) surveyed bait businesses and found that 60% of retailers who import non-local bait worms receive them packaged with seaweed. The same percentage noticed non-target species included within the packaging. Yet, nearly half of those surveyed did not understand the possible ecologic and economic impacts of invasive species and the environmental damage they can cause. Educating both retailers and fishermen about the dangers of discarding bait worm packaging into the sea and feasible steps to properly dispose of the packaging could have an immediate benefit (Padilla and Williams 2004; Balcom and Yarish 2009). In addition to the packaging seaweed, it is possible that the bait worms themselves are vectors of non-native organisms. If verified, individual states would need to assess the risk of importing these worms into their marine coastal systems. Recommendations could be made to develop a system of certification and best practice guidelines for wholesalers and retailers to market “invasive-free” bait worm products, which would consequently reduce the risk of invasive species introductions.

Acknowledgments Yunyun Zhuang kindly helped with DNA sequence data analysis. This work was aided by the following undergraduate assistants: Yusuff Abdu, Frank Cerqueira, Andrew Payne, Ryan Patrylak, and Allen Rakiposki. Jang K. Kim and Rebecca Gladych assisted with macroalgal identifications. Nancy Balcom provided insights from Connecticut SeaGrant. Dr. Gary Wikfors (Northeast Fisheries Center, NOAA) assisted with manuscript revisions. The work was funded by grants from the U.S. EPA (No: LI-97149601), University of Connecticut-CESE, National Oceanic and Atmospheric Administration, and the Connecticut SeaGrant.

References

Allendorf FW, Lundquist LL (2003) Population biology, evolution, and control of invasive species. Conserv Biol 17(1):24–30

Andersen RA (2005) Algal culturing techniques. Elsevier Academic Press, pp 578

Balcom N, Yarish C (2009) DON’T DUMP BAIT: marine bait worms as a potential vector of non-native species. Connecticut Sea Grant Publication, pp 2. http://web2.uconn.edu/seagrant/whatwedo/ais/btwrms.pdf

Bates SS, Trainer VL (2006) The ecology of harmful diatoms. In: Granéli E, Turner J (eds) Ecology of harmful algae. Ecological studies, vol 189. Springer-Verlag, Heidelberg, pp 81–93

Brown B (1993) Maine’s baitworm fisheries: resources at risk? Am Zoologist 33:568–577

Carlton JT (1979) History, biogeography, and ecology of the introduced marine and estuarine invertebrates of the Pacific coast of North America. Ph.D. thesis. University of California, Davis

Carlton JT (2001) Introduced species in the US coastal waters: environmental impacts and management priorities. Pew Oceans Commission, Arlington, Virginia, pp 36

Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. Nat 405:234–242

Cohen AN, Weinstein A, Emmett MA, Lau W, Carlton JT (2001) Investigations into the introduction of non-indigenous marine organisms via the cross-continental trade in marine baitworms. A report for the US Fish and Wildlife Service, San Francisco Bay Program, pp 29

Crawford SE (2001) Live rockweed (*Ascophyllum*) used as a shipping medium for the live transport of marine baitworms from Maine. In: Paust BC, Rice AA (eds) Marketing and shipping live aquatic products: proceedings of the second international conference and exhibition, November 1999. Seattle, Washington, pp 95–97

Dawson EY, Foster MS (1982) Seashore plants of California. University of California Press, Berkeley, CA, pp 226

Fitzpatrick A (2008) Maine Department of Marine Resources, PSP closed area No. 100-A. http://www.maine.gov/dmr/rm/public_health/closures/closedarea.htm

Fletcher RL (1990) The “green tide” problem: a review. In: Proceedings from a second workshop: marine biotechnology on eutrophication and biotransformation in coastal waters. Sorrento, Italy, 18–24 November 1990. Ecolmare, pp 100–105

Gotelli NJ, Ellison AM (2004) A primer of ecological statistics. Sinauer Associates Inc. Sunderland, Massachusetts, pp 510

Graham LE, Wilcox LW (2000) Algae. Prentice Hall, Upper Saddle River, New Jersey, USA, pp 640

Hasle GR (2002) Are most of the domoic acid-producing species of the diatom genus *Pseudo-nitzschia* cosmopolites? Harmful Algae 1(2):137–146

Lau W (1995) Importation of baitworms and shipping seaweed: vectors for introduced species? In: Sloan D, Christensen M, Kelso D (eds) Environmental issues: from a local to a global perspective. Environmental Sciences Group Major, University of California, Berkeley, CA, pp 21–38

Author's personal copy

Lin S (2008) Use of molecular markers for early warning detection of harmful algal blooms. Int J Environ Pollut 33: 381–400

Lüning K (1990) Seaweeds—their environment, biogeography, and ecophysiology. In: Yarish C, Kirkman H (eds) Edited Translation of the German language edition Meeresbotanik: Verbreitung, Okophysiologie und Nützung der marinen Makroalgen by Klaus Lüning. John Wiley and Sons Inc, New York, pp 527

Maclellan J (2005) Long Island Sound invasive species list. EPA Long Island Sound Study, pp 3

Mathieson AC, Dawes CJ (2002) *Chaetomorpha* balls foul New Hampshire, USA beaches. Algae 17:283–292

McGillicuddy DJ Jr, Anderson DM, Lynch DR, Townsend DW (2005) Mechanisms regulating large-scale seasonal fluctuations in *Alexandrium fundyense* population in the Gulf of Maine: results from a physical-biological model. Deep Sea Res 52(19–21):2698–2714

Miller AW, Chang AL, Cosentino-Manning N, Ruiz GM (2004) A new record and eradication of the northern Atlantic alga *Ascophyllum nodosum* (Phaeophyceae) from San Francisco Bay, California, USA. J Phycol 40:1028–1031

Olson AM (2001) Do live marine products serve as pathways for the introduction of nonindigenous species? In: Paust BC, Rice AA (eds) Marketing and shipping live aquatic products: proceedings of the second international conference and exhibition, November 1999, Seattle, Washington, pp 243–247

Ott FD (1965) Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. Va J Sci 16: 205–218

Padilla DK, Williams SL (2004) Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Front Ecol Environ 2(3):131–138

Pang SJ, Liu F, Shan TF, Xu N, Zhang ZH, Gao SQ, Chopin T, Sun S (2010) Tracking the algal origin of the Ulva bloom in the Yellow Sea by a combination of molecular, morphological and physiological analyses. Mar Environ Res 69(4): 207–215

Pimental D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associates with alien-invasive species in the United States. Ecol Econ 52:273–288

Pollock L (1998) A practical to the marine animals of Northeastern North America. Rutgers University Press, New Jersey, pp 394

Ricciardi A, Rasmussen JB (1998) Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can J Fish Aquat Sci 55:1759–1765

Sears JR (2002) NEAS keys to the benthic marine algae of the northeastern coast of North America from Long Island Sound to the Strait of Belle Isle. Express Printing, pp 161

Sfriso A, Marcomini A, Pavoni B (1987) Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon. Mar Environ Res Lond 22(4):297–312

Silva PC (1979) The benthic algal flora of central San Francisco Bay. In: Conomos TJ (ed) San Francisco Bay: The Urbanized Estuary, pp 287–345

Thayer PE, Stahlnecker JF (2006) Non-native invasive marine species in Maine: a report to the Maine State Legislature. Marine Resources Committee and Natural Resources Committee, pp 22

Tomas CR (1997) Identifying marine phytoplankton. Academic Press, New York, pp 858

Townsend DW, Pettigrew NR, Thomas AC (2001) Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp., in the Gulf of Maine. Cont Shelf Res 21(4):347–369

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42(5):1105–1118

Villalard-Bohnsack M (1995) Illustrated key to the seaweeds of New England. The Rhode Island Natural History Survey, pp 145

Wallentinus I, Nyberg CD (2007) Introduced marine organisms as habitat modifiers. Mar Pollut Bull 55:323–332

Weigle SM, Smith LD, Carlton JT, Pederson J (2005) Assessing the risk of introducing exotic species via the live marine species trade. Conserv Biol 19(1):213–223

Yarish C, Kilar JA, Merrill JE (1991) The management of eutrophication through aquaculture and natural beds of marine algae. In: Hinga KR, Stanley DW, Cline DJ, Lucid DT, Katz MJ (eds) The national estuarine eutrophication project: workshop proceedings. Rockville, MD. National Oceanic and Atmospheric Administration and the University of Rhode Island Graduate School of Oceanography, pp 40–41

Zhang H, Lin S (2005) Development of a cob-18S rRNA gene real-time PCR assay for quantifying *Pfiesteria shumwayae* in the natural environment. Appl Env Microbiol 71(11): 7053–7063

View publication stats