Public Summary: The goal of the project was to protect guard Long Island Sound from the introduction of non-native organisms that may be imported via fishing bait worms and the seaweed packing material known as wormweed (Ascophyllum nodosum). The project examined bait for non-native invertebrate animals, macroalgae (also known as seaweeds), and harmful, toxin-producing microalgae. Bait was purchased from retail bait shops at locations ranging from northeastern Long Island Sound along the Connecticut shoreline to the southwestern part of the Sound in Long Island. Using a combination of visual and microscopic inspection, and sophisticated molecular biological techniques to detect the presence of microalgal cells, the study questioned whether (i) non-native organisms were being imported via bait worms, and if so whether; (ii) non-native organisms vary according to purchase location, or; (iii) time of year.

Overall, 14 species of macroalgae, two species of harmful microalgae (Alexandrium fundyense, and Pseudo-nitzschia multiseries), and 23 different categories of invertebrate animals were discovered among the wormweed. Only one of the microalgal species was not native to Long Island Sound. Overall, location (eastern vs. western, northern vs. southern Long Island Sound) did not affect the number of algal or invertebrate species. Temperature did affect algal diversity and abundance, however, both in post-collection incubation (5° < 15° = 25°) and seasonally (summer produced highest numbers). Invertebrates were most abundant in summer as well.

The Gulf of Maine now harbors a diverse suite of non-native organisms. These may be exported to other areas of the U.S. via national bait wholesalers and cause ecological harm to the receiving ecosystem. In addition to potential ecological impacts associated with the import of non-native organisms, economic harm is also possible. For example, commercial shellfishing beds may be closed when harmful microalgae bloom in coastal waters. With ca. 470 retail bait shops in NY and CT, the chances of introduction of harmful non-natives is not trivial. For example, in our 18 month study of four locations, we discovered the harmful non-native microalga Pseudo-nitzschia multiseries in 58% of our samples.

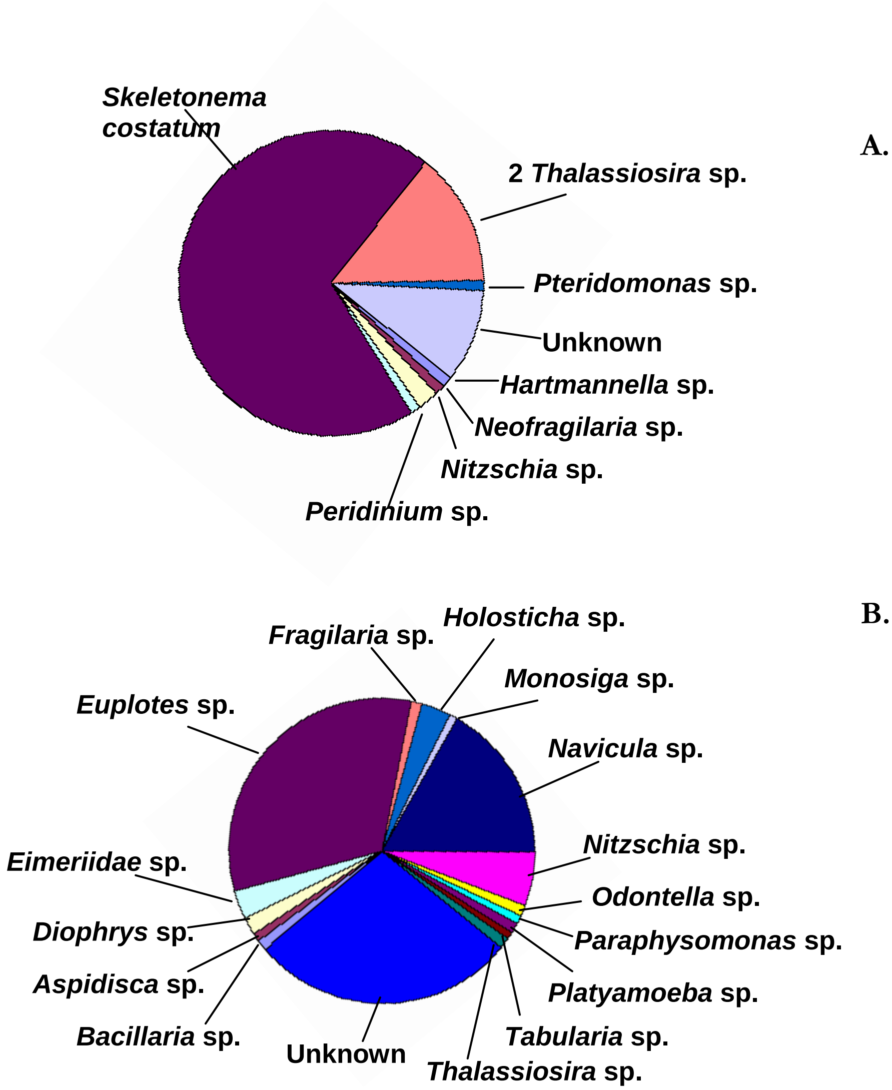


Figure. 2: Microalgal species found through DNA sequencing in DBT 5 (A) and FW15 (B).

There were no significant differences ($p > 0.05$) in total number of algal species (seaweed and microalgae combined) between sites in New York vs. those in Connecticut, between northern sites (all excluding DBT) vs. southern sites (DBT), or between eastern sites (KT, CB, and RE) vs. western locations (all remaining sites). The 10-day incubation increased the number of epiphytes found; significantly more species were found after the incubation period for both years ($p < 0.001$). One-way ANOVA for both the HAB and macroalgae species showed no effect of incubation temperature on the number of HAB species found ($p > 0.05$). However, temperature had a strong effect on the number of macroalgal species detected ($p < 0.001$); the 5°C incubation produced fewer species (avg = 0.5 species) than the 15°C (avg = 1.3 species) and 25°C (avg = 1.5 species) incubations. A two-way ANOVA revealed no interaction between sampling date and incubation temperature on the number of total algal species found (macro- and micro-algae combined). . A one-way ANOVA test revealed that no incubation temperature was more likely to produce HAB-forming species ($p > 0.05$).

Invertebrate Animals: For each sampling date, contents of all six boxes obtained from each tackle shop were combined into one container and evenly divided up between macro-algae analysis, the micro-algae analysis, and the invertebrate analysis. The seaweed was examined for invertebrates and any dislodged invertebrates were collected from the tray and preserved in a 70% ethanol solution until identification. Several weeks following preservation, all invertebrates were identified to lowest practical taxonomic category and enumerated using a 40x dissecting microscope and relevant taxonomic keys (Gosner, 1979, Weiss, 1995) Species diversity was represented using the Shannon-Weiner index.

Nine categories of invertebrates were identified in the boxes of baitworms: isopods, amphipods, bivalves, annelids, gastropods, arachnids (mites), ostracods, copepods and insect larvae (Table 1). In general, overall species composition of invertebrates obtained from the baitworm boxes did not vary between the tackle shops. A total of 23 separate invertebrate taxa were found in the samples and all samples were typically dominated by three species: the gastropod *Littorina saxatilis*, the amphipod *Hyale nilssoni* and isopod *Jaera marina*.

The total number of individuals found in the baitworm boxes did display considerable variability between sampling locations, although there was no consistent pattern among sampling periods. In most instances, however, the highest numbers of invertebrates were recorded between the months of June and August when invertebrate abundance is generally known to be highest in coastal New England waters (Fig. 1). Species diversity estimates typically varied from 1.0 to 2.5 and there were no consistent patterns between sampling locations and sampling date (Fig. 2). Decreases in species diversity usually correlated with samples containing large numbers of *Jaera marina* during July and August (Fig. 2).

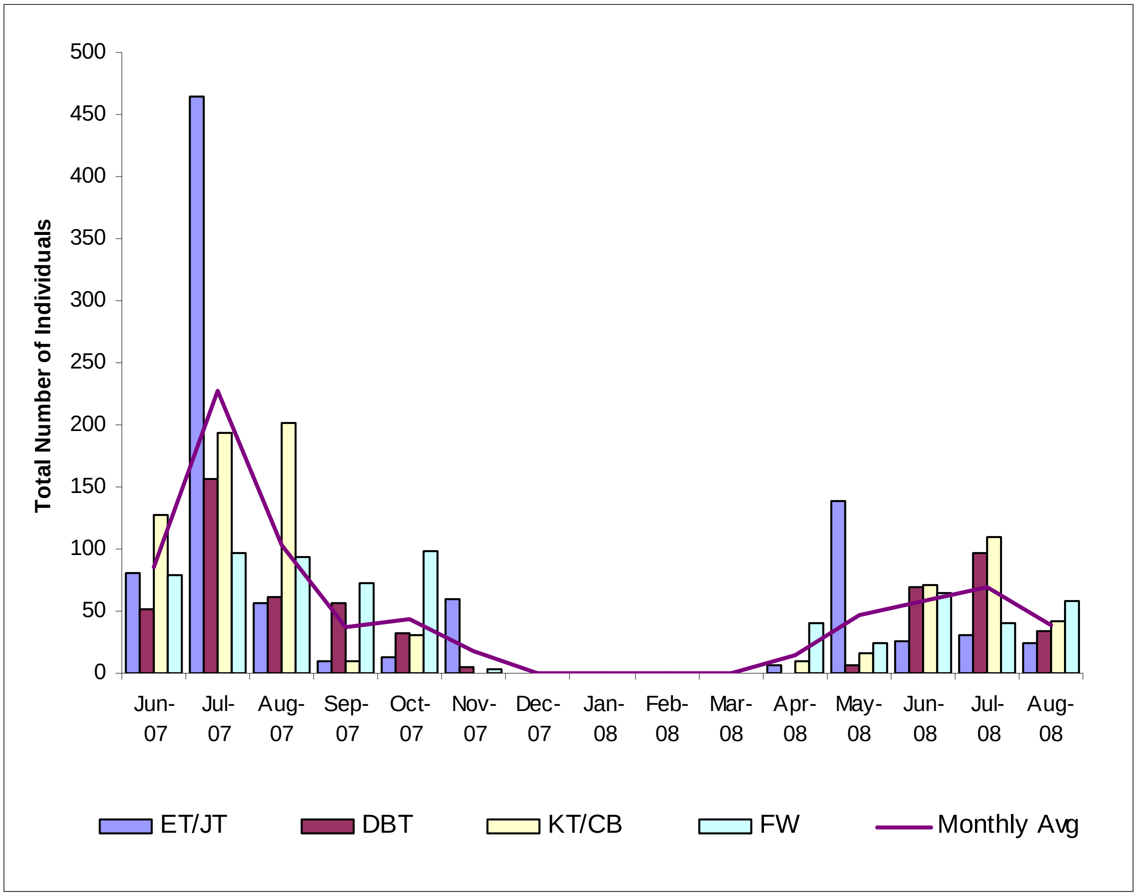
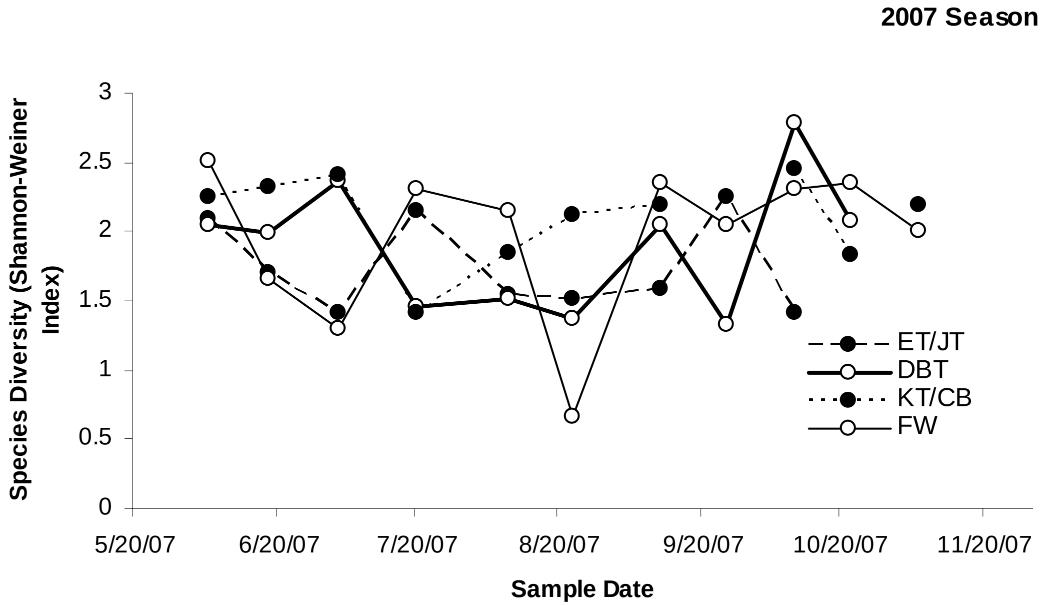


Figure 1: The total number of individuals present every month in all samples from each tackle shop. No samples were obtained between December 2007 and March 2008. The summer months appear to have the greatest number of individuals per sample than the fall months, despite the high variability between tackle shops.

Table 1: Species present in the samples collected from the four tackle shops.

		Sample Site			
	Species	**ET**	**DBT**	**FW**	**KT**
Amphipods	*Caprella penatis*	✓	✓		✓
	Eulimnogammarus obtusatas	✓	✓	✓	✓
	Gammarid Amphipod (unk)		✓	✓	
	Hyale nilssoni	✓	✓	✓	✓
	Jassa falcata				✓
Gastropods	*Hydrobia* spp	✓	✓		✓
	Littorina littorea			✓	
	Littorina obtusata	✓	✓	✓	✓
	Littorina saxatilis	✓	✓	✓	✓
Bivalves	*Gemma gemma*				✓
	Mercenaria mercenaria		✓	✓	
	Mya arenaria	✓	✓	✓	
	Mytilus edulis	✓	✓	✓	✓
Annelids	*Enchytraeus albidus*	✓	✓	✓	✓
	Oligochaete	✓	✓	✓	✓
	Spirorbis spirillum	✓			
Arachnids	Trombidiid mite	✓	✓	✓	✓
	Halacarus sp	✓	✓	✓	✓
Isopod	*Jaera marina*	✓	✓	✓	✓
Copepod	*Trigiopus*	✓	✓	✓	✓
Ostracod	*Unknown ostracod*	✓	✓		✓
Insects	Chironomid larvae	✓	✓	✓	✓
	Dipertan larvae				✓



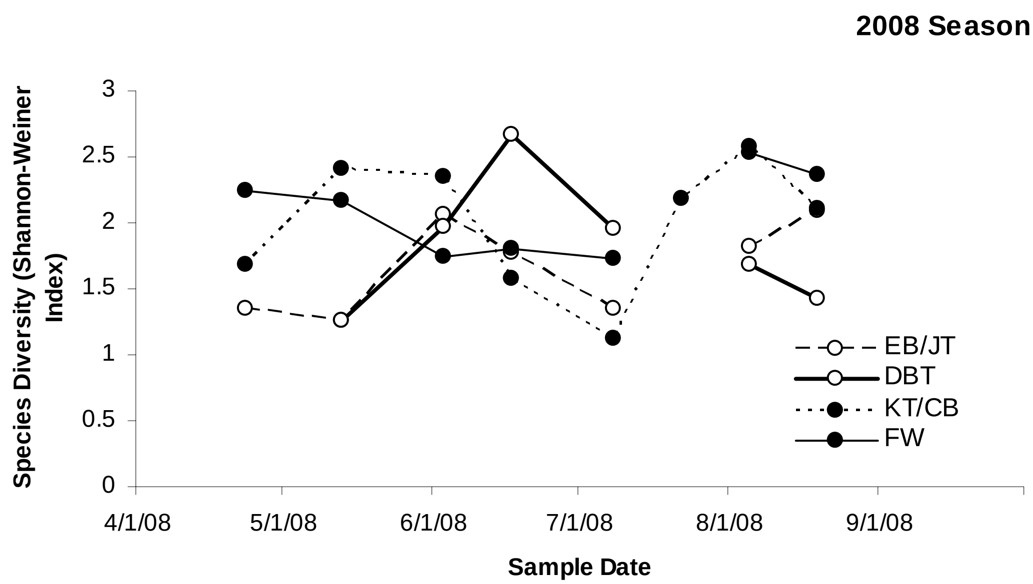


Figure 2: Species diversity calculated from the samples taken from the four tackle shops. Breaks in the data are the result of too few species in the sample to run the analysis or the inability to obtain a sample on that date. The decreases in the species diversity correlate with large numbers of *Jaera marina*, *Hyale nilssoni* and/and *Littorina saxatilits*. Species diversity increased in most of the 2007 samples during the fall where the abundance of the dominant species decreased substantially or they were absent from the samples.

10. Conclusions: Previous studies have shown that bait worm packaging can be a vector of non-native and potentially invasive species (Cohen *et al.* 2001, Carlton *et al.* 2001). Our study further demonstrated the extent of this threat to the Long Island Sound. Although the macroalgal species found post-incubation are all indigenous to LIS, four genera, *Chaetomorpha, Cladophora, Ulva*, and *Pilayella*, cause blooms in temperate waters (Valiela *et al.* 1997, Mathieson and Dawes, 2002). These species can shade habitat-forming benthic algae and submerged aquatic vegetation, decreasing their photosynthesis and growth (Wallentinus and Nyberg 2007). Even though the macroalgal species found within the bait worm packaging are native to LIS, this does not imply an absence of risk. Populations of the same species may be genetically distinct from each other. Thus, this vector could still be introducing non-native macroalgal ecotypes which could have a more opportunistic life history than the native ecotype, thereby having an effect on the ecosystem (Lüning, 1990, Mathieson et al. 2003).

Two harmful microalgae were found within the bait-worm packaging: *Alexandrium fundyense* and *Pseudo-nitzschia multiseries*. *A. fundyense* has formed toxic blooms in the Gulf of Maine every year (Anderson et al. 2005 and references therein). In recent years, its bloom extended southward, with severe toxic outbreaks occurring along the south shore of Long Island Sound in 2008 (http://www.longislandsoundstudy.net/newsmail/june_08_onlinev.htm). This species was detected in the bait-worm packaging throughout the study period, with higher occurrence in July and August 2008 corresponding with a PSP outbreak on August 1, 2008 that eventually closed Maine's shellfish beds (Fitzpatrick 2008). Although it is unclear how *A. fundyense* was introduced into LIS, our results indicate that bait worm products are a potential vector. It is interesting to note that the most severe bloom in LIS has occurred in Northport Harbor where fishing has been very active.

The diatom *Pseudo-nitzschia multiseries* has not yet been recorded in large numbers in LIS, but the presence of this HAB is been detected occasionally (S. Lin, unpublished observation). In our study, *P. multiseries* was detected via microscopic examination in one sample and through molecular analysis for many samples. Clearly, bait worm products could be a vector for its introduction into LIS.

There were no significant differences in the number of algal species carried by *A. nososum* bait packing between sampling sites throughout the study, indicating a similar risk of purchasing NIS-contaminated bait worms at the four sites around LIS. In addition, incubation of the samples for the 10 d period at 5, 15, and 25°C demonstrated that organisms contained with *Ascophyllum nodosum* were capable of growing under a variety of conditions. This incubation period tested the viability of the hitchhiking micro- and macroalgal species. We conclude that many are likely able grow and perhaps establish viable populations. The first stage of a successful species invasion is for the organism to arrive, survive, and establish itself within a body of water, while the second stage is to spread and affect the native species (Allendorf and Lundquist 2003). The observation that *P. multiseries* was present in the packaging and survived the 10-day incubation at LIS's range of temperatures indicate that once introduced, it can survive in LIS and the surrounding waters. If it ever forms blooms, it would exert great ecological and economic impacts on LIS because it is a toxigenic organism.

The temperature incubations demonstrated growth of macroalgal epiphytes and endophytes associated with the *Ascophyllum nodosum* over a range of temperatures that reflect the seasonal range in LIS (Pedersen et al. 2007), though the likelihood of culturing macroalgal species at 5°C was less than at the other temperatures. This suggests that the greatest threat exists during the warmer months. In fact, many of the species found in this study are eurythermal north Atlantic taxa with warm temperature affinities (Lüning 1990). This is important because the main fishing season is during the summer and fall, during which water temperatures would be favorable for these organisms. For the HAB species, survival and growth occurred under all incubation temperatures. This could indicate that these microalgae could be introduced throughout the year. In fact, there was no significant interaction between season of sampling and incubation temperature with respect to species richness, implying that there is similar risk of introducing these species throughout the fishing season.

Our findings may have implications for other areas because Maine exports bait worms and *Ascophyllum nodosum* throughout the U.S.A. However, we recognize that the transportation process may affect the viability of the NIS and HAB organisms and should be examined. The current geographic distributions of both *Alexandrium fundyense* and *Pseudo-nitzschia multiseries* should also be taken into account when analyzing these data. *Ascophyllum fundyense* is only found in the northern hemisphere within a North American clade (John *et al.* 2003). This species is found along the northeast coast of North America. However, as the populations move towards the south, blooms become more infrequent and less toxic (Colin and Dam 2002). As a consequence, it is considered to be a cold-water species and is not expected to thrive at warmer water temperatures. In our study, *Alexandrium fundyense* was found within the 25°C incubation samples, indicating it is capable of growing at higher temperatures. Future work would need to assess the toxicity potential of *A. fundyense* at this warmer temperature. *P. multiseries,* on the other hand, is a cosmopolitan species found throughout the world's oceans at a large range of temperatures (Hasle 2002). It is present in both hemispheres and extends from the northern to the southern latitudes. As such, it is capable of surviving in a large range of environments. This is reflected in this study by *P. multiseries* presence at each incubation temperature throughout the study period.

Examination of the Lugol's-preserved samples revealed a high diversity of organisms within the *Ascophyllum nodosum* packaging material. A complex microalgal community is, therefore, transported by this vector. Many of these microalgal and protozoan species would not have been found without sequencing the samples because of their low abundance and inconspicuous habit. Although differences between these DNA samples could not be directly compared because they were from different incubation temperatures (5° and 15°C) and each underwent a different DNA-extraction method, the diverse flora found in both samples indicate the potential of introducing a complex microbial assemblage that could survive in a new environment. At present, we cannot ascertain whether any of those species may become harmful in other systems.

While bait worm boxes containing *Ascophyllum nodosum* transfer of a variety of benthic invertebrates between regions in the United States (Miller, 1969, Crawford 2001), to date, we found no NIS transferred between the Gulf of Maine and LIS. However, *J. marina*, a dominant isopod in all samples, is considered a cryptogenic species (Maclellan 2005). This species distributional range has been described as the north side of Cape Cod to Newfoundland with some southward extension, but the specific southern-most location has yet to be confirmed (Pollock 1998).

<u>Implications</u>. There are approximately 470 bait shops currently in Connecticut and New York States. The likelihood of a non-native species to be introduced into a habitat increases with the number of release events (Allendorf and Lundquist 2003), and recreational fishing is a very common activity within LIS. Educating both retailers and fishermen about the dangers of bait worm packaging and steps they can take to reduce the risk of invasive species introductions could have an immediate benefit to LIS (Padilla and Williams 2004). Weigle et al. (2004) surveyed bait businesses and found that 60% of retailers who import non-local bait worms receive them packaged with seaweed. They also noticed non-target (non-worm) species

included within the packaging. Yet, nearly half of those surveyed did not know of the concept of invasive species and the environmental damage they can cause. Recently, the Connecticut Sea Grant Program has begun a sticker and education programs in which bait shops receive warning stickers to place on their boxes of bait and display posters in their stores (N. Balcom, pers. comm.).

Although this study specifically targeted species which could potentially be harmful to LIS, it is important to note that bait worms are shipped from Maine to coastal locations throughout the United States and Europe. Given this, the species which were found within and on the *Ascophylllum nodosum* could potentially be dispersed to other habitats. Survival of the HAB species at different temperature in this study underlines the potential threat to different areas. Both seaweed invasions and HAB-forming phytoplankton can dramatically affect ecosystem structure and function, thus posing major challenges for coastal management of these marine habitats (Valentine *et al.* 2007). Prevention programs similar to what Connecticut Sea Grant is conducting would help reduce the probability that potential invaders could be introduced and established within coastal waters. In addition to the algae included with the samples, it is possible that the worms themselves are vectors of non-native organisms. If these were found to be carriers of harmful species, then individual states would need to assess the risk of importing these worms into their marine coastal systems. Recommendations or suggestions could be made to develop a system of certification and best practice guidelines to include guarantees that wholesalers and retailers market "invasives-free" bait-worm products and take active steps to reduce the risk of invasive species introductions (USGS 2003). In addition, other potential vectors need to be considered in taking preventative measures against invasive species. Among others, shellfish aquaculture has been found to be one of the major vectors to spread invasive organisms (Carlton 1999). This vector is very similar to bait-worm packaging in that both transport specific marine organisms across continents that may have non-native and invasive species included in the shipment.
It is noteworthy that invasive species have been introduced into Maine's coastal waters, and these have the potential to be further spread to other areas through *Ascophyllum nodosum* packaging. Among documented invaders are: the green crab (*Carcinus maenus* Linnaeus), the Asian shore crab *(Hemigrapsus sanguineus* De Haan), several tunicates (*Didemnum* Savigny sp., *Botrylloides violaceus* Oka, *Styela clava* Herdman), a green algae (*Codium fragile* (van Goor) P.C. Silva), an oyster parasite (*Haplosporidium nelsoni*), a salmon virus (*Orthomyxovirus*), and a bryozoan (*Membranipora membranacea* Linnaeus) (Thayer and Stahlnecker 2006), and even some Asiatic *Porphyra* Agardh species (Neefus *et al.* 2008). Although these species were not found throughout this study, there is the potential for these organisms to be moved throughout the country in bait-worm packaging, and additional research will be needed to address this threat. Baitworms boxes containing *Ascophyllum* is well recognized to be an important potential vector for the transfer of a variety of benthic invertebrates between regions in the United States (Miller, 1969, Crawford 2001). To date, however, no non-native species have been found being transferred in the bait boxes between the Gulf of Maine and Long Island Sound.

Summary. The results of the tests of the hypotheses are here:

1. Hypothesis 1a H_0: Worm baits sold along Connecticut and New York shores of LIS do not contain NIS seaweeds or HAB microalgae – **REJECTED; although no NIS macroalgal species were discovered in the initial inspection of the worm bait packages or after 10 d growth at 5°, 15°, or 25°C, molecular methods (and one microscope ID) detected two HAB microalgae species (*Alexandrium fundyense* and *Pseudo-nitzschia multiseries*)**

2. Hypothesis 1b H_0: Worm baits sold in LIS contain similar taxonomic suites of NIS seaweeds and potential HAB microalgae - **PROVISIONALLY NOT REJECTED; no pattern in the detection of macroalgal or microalgal species was observed as a function of bait purchase location**
3. Hypothesis 1c H_0: Worm bait vectors show no seasonality in associated NIS seaweed flora or HAB microalgae – **REJECTED; macroalgae and HAB microalgae were more frequently detected during warmer months (May-Oct)**
4. Hypothesis 2a H_0: Worm baits sold along Connecticut and New York shores of LIS do not contain non-native invertebrate animals – **NOT REJECTED; all invertebrates recorded during the study are current residents of LIS**
5. Hypothesis 2b H_0: Worm baits contain similar taxonomic suites of non-native invertebrate animals *(not relevant since no NIS invertebrates were recorded in bait purchases)*
6. Hypothesis 2c H_0: Worm bait vectors show no seasonality in associated non-native invertebrate animals. *(not relevant since no NIS invertebrates were recorded in bait purchases)*

11. Presentations/Publications/Outreach

Presentations:

Haska, Christina L., Charles Yarish, and Senjie Lin. Assessing the role of bait worm packaging as a potential vector of invasive species to Long Island Sound. Annual Conference of the Phycological Society of America. July, 2008.

Haska, Christina L. Assessing Bait Worm Packaging as a Potential Vector of Invasive Species Introductions into Long Island Sound. Feng Graduate Student Colloquium, Avery Point, CT. May, 2008.

Haska, C., Yarish, C., and Lin, S. 2008. Assessing bait worm packaging as a potential vector of invasive species to Long Island Sound. Presentation at the Long Island Sound Research Conference, New London, CT, October, 2008.

Publications: Manuscripts are currently in preparation.

Outreach: Two workshops have been conducted to begin dissemination of the project results. On November 5, 2008, the results of the study were presented in an open forum (i.e., including interested public) to officials from the Connecticut DEP in Old Lyme. On November 18, a similar presentation was made to the Marine Resources Advisory Council in Setauket, NY. The latter meeting included both officials from the Department of Environmental Conservation and commercial fishermen. An additional presentation will be made to the annual meeting of the New York Lobstermen's Association at the Cornell Cooperative Extension Offices, Riverhead on January 24, 2009 in Riverhead, NY.

Additionally, we have been assisting Nancy Balcom of Connecticut Sea Grant with dissemination of the inserts for the bait-worm boxes that alert fishermen to the potential invasive threat and simple means for minimizing the threat (disposal in land-based garbage containers). This inserts are part of a program funded separately by NOAA called the "Don't Dump Bait Campaign."

12. Other Information: Four undergraduate students participated in the project as Research Assistants, helping with sample acquisition, initial processing, and culturing tasks. The students included Yusuff Abdu and Al Rakiposki (University of Connecticut), and Francisco Cerqueira and Andrew Payne (Purchase College).

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