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Improving Management Support Tools for Reintroducing Bivalve Species (Eastern Oyster [*Crassostrea virginica* Gmelin]) in Urban Estuaries

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ABSTRACT

Successful reintroduction of "ecologically extinct" bivalve species into anthropogenically impaired urban estuaries is problematic when employing existing management tools used in estuaries where bivalves are present (GIS-based restoration models, expanding existing shellfish beds, placement of shell substrate, physical oceanographic parameters). A significant management challenge is appropriate site selection. We are proposing the inclusion of a biological parameter (evaluation of tissue histopathology) in an inexpensive and rapid site selection model to inform management decision making and identify sites with the greatest potential for reintroduction success. Use of biological biomarkers is not a new concept, but it is important that they be included in a multitiered management approach to bivalve reintroduction. This Case Study tested adult Eastern Oysters (*Crassostrea virginica* Gmelin) from locations that supported comparable short-term survival rates by evaluating growth and tissue health and/or disease. Biomarkers indicated oyster tissues at one site were normal, the female: male sex ratio was 50:50, and female oysters were in spawning condition. Conversely, oyster tissues at the second site exhibited multiple abnormalities, samples were 100% male, and the incidence of disease was high. Using the biomarker tool, we evaluated 4 additional sites where oysters exhibited short-term (1 year) survival. At 2 locations, we observed chronic health impacts that would preclude reintroduction, including samples from one site where a wild population was surviving. We also analyzed tissue and shell heavy metal contents. Soft tissue metal concentrations in Meadowlands samples were at the high range of scientific literature values, averaging 1.1% of total body weight, whereas tissue metal concentrations at the Keyport site were within acceptable ranges. Although initial survival and growth rates at both locations were comparable, site-specific urban stressors reduced oyster fitness at 1 of the 2 locations. We are proposing an Estuarine Reintroduction Site Selection Model, which includes a biological in situ parameter, to increase the probability of successfully managing a sustainable oyster reintroduction before commencing expensive large-scale restoration activities. *Integr Environ Assess Manag* 2014;10: 555–565. © 2014 SETAC

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INTRODUCTION

Urban estuaries represent some of the most degraded habitats known (Jackson et al. 2001; Lotze et al. 2006), where intense coastal development followed oyster fishery exploitation and collapse (Kirby 2004). Loss of natural estuarine habitat and associated marine species occurred as multiple competing uses related to industry and transportation caused anthropogenic alterations in harbors worldwide. Today, re-establishment of marine species back into urban harbors is a global issue (Gummer et al. 1994; Craig 2002; Liang et al. 2006; Shelmerdine and Leslie 2009; White et al. 2009; Snelgrove et al. 2012; Thurstan et al. 2013). It is possible that 40 years after passage of the Clean Water Act, it may now be feasible to consider reintroduction of "ecologically extinct" species in some US estuaries where they were historically present.

Eastern oyster (*Crassostrea virginica* Gmelin) reintroduction in Atlantic estuaries could contribute desirable ecosystem services (Coen et al. 2007; Grabowski et al. 2012). However, oyster reintroduction may be problematic in urban estuaries due to major alterations of natural hydrologic patterns, as well as the presence of historic contaminants (Simenstad et al. 2005; Mann and Powell 2007; Beck et al. 2009; Brumbaugh and Coen 2009). The critical management question is whether existing conditions at a potential reintroduction site can support long-term survival and reproductive success.

Typically, oyster restoration is undertaken to support an existing fishery resource, and management tools include enhancement of existing reefs through the addition of oysters and/or placement of hard substrate for larval settlement (Kennedy et al. 2011). It is also common to use geographic information system (GIS) models based on physiochemical

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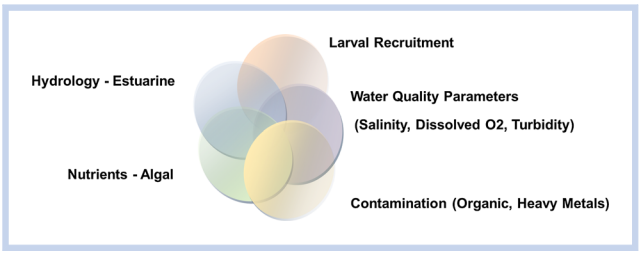

Figure 1. Venn diagram illustrating the interaction of multiple environmental parameters that determine oyster survival in urban estuaries.

characteristics suitable for oyster survival and settlement. Restoration models correlate environmental parameters with the presence and/or absence of oysters, with high values corresponding to areas supporting native populations (Barnes et al. 2007; Pollack et al. 2012). However, in anthropogenically altered urban estuaries, the absence of adult oyster populations, insufficient larval density, atypical hydrology, and/or the presence of historic or emerging contaminants may overshadow physiochemical factors required for reintroduction success (Figure 1).

Oyster restoration projects are expensive. The US Army Corps of Engineers (USACE) estimates that multiyear restorations, installed on a variety or river and tributary types, can range in cost from $200 000 to $2 000 000 per acre; the National Oceanic and Atmospheric Administration (NOAA) places the cost at $1.5 million per mile of constructed oyster reef (Table 1). From a management point of view the question is "Where should reintroduction occur in order to achieve long-term sustainability and maximize reintroduction efforts that may be quite costly"? When an estuary lacks a self-sustaining oyster population and reintroduction success is uncertain, relying solely on physiochemical management approaches used to maintain an existing fishery is problematic.

Habitat selection is a critical issue in successful animal reintroductions (Mihoub et al. 2009), which often fail because "apparently similar" habit is actually unsuitable; identifying suitable sites can be challenging, often due to environmental changes since local extinction of the target species (Hodder and Bullock 1997). Additionally, the pre-extinction food web components may be missing or replaced with species that do not meet nutritional requirements of the reintroduced species. Under conditions commonly found in urban estuaries, sites available for reintroduction are typically limited (Simenstad et al. 2005). In short, reintroduction of an "extinct" urban species requires augmenting existing management methods with cost-effective approaches that help elucidate a site's potential for reintroduction success.

Hudson-Raritan Estuary case study

After 2 centuries of anthropogenic activities the once extensive Hudson-Raritan Estuary (HRE) oyster fishery, an important component of the shallow water ecosystem, is "ecologically extinct" (McCay 1998; MacKenzie 1992; CRP 2009). The draft USACE Comprehensive Restoration Plan (CRP) for the estuary calls for reestablishment of 200 acres of Eastern Oyster reef by 2020 (CRP 2009). HRE environmental conditions are similar to those in many degraded urban estuaries (Jeffries 1962; Kennish 1992; Davis and Kidd 2012). Heavy metal and organic contaminants (including PCBs, PAHs, and dioxins) are still present in the estuary's sediments (Bonnevie et al. 1994; Cooper and Brown 1995; Adams and Benyi 2003 and references therein; CARP 2007), as well as in its shellfish (Kimbrough et al. 2008).

Although small isolated HRE native oyster populations have been reported (T Medley, PhD Thesis, City University of New York, New York, USA, personal communication), researchers testing hatchery-reared seed oysters have observed initial growth and survival followed by population crashes (J Levinton, Stony Brook University, Stony Brook, NY, USA, personal communication). Using the System-Wide Eutrophication Model (Miller and Wands 2009) and Jamaica Bay Eutrophication Models (NYCDEP 2011 and references therein), which are based on 4 physiochemical parameters (salinity, dissolved O_2, total suspended solids, bathymetry), the CRP (2009) identified over 50 000 acres of potential HRE oyster restoration sites (Figure 2). Starke et al. (2011) also developed a GIS model using habitat characteristics to create a Restoration Suitability Index to identify potential HRE oyster restoration locations.

Since placement of shell substrate in 1999, attempts to reintroduce oysters to the HRE have been unsuccessful for a variety of reasons including sediment burying oysters placed in unsuitable locations, shell piles washing away due to high energy events, and lack of larval recruitment. The HRE Oyster Restoration Research Project placed shell substrate at 5 NY test locations and experienced similar reintroduction challenges (low recruitment, oyster survival rates unacceptably low, and shell substrate lost due to high energy events) at 4 of the 5 test sites (Grizzle et al. 2013). The process of identifying HRE reintroduction locations clearly needs additional refinement as evidenced by the poor success to date.

Rather than rely solely on physiochemical parameters, we developed an Estuarine Reintroduction Site Selection Model

Table 1. Oyster restoration is expensive

Location	Restoration Size	Cost	Cost/Acre	Reference
St. Lucie River, FL	2 acres	$4 million	$2 000 000	http://www.oysterrestoration.com/
Lower Neuse River	10 acres	$11.078 million	$1 107 800	http://www.usace.army.mil/Portals/2/docs/civilworks/CWRB/neuse/neuse.pdf
Gulf of Mexico	100 miles	$150 million		http://www.habitat.noaa.gov/pdf/tnc_oyster_economics_factsheet.pdf
Chesapeake, MD	7300–14 600 acres	$.87–2.85 billion	$195 205	http://www.nab.usace.army.mil/Portals/63/docs/Environmental/Oysters/MasterPlan_ExecutiveSummary.pdf
Chesapeake, VA	10 100–20 400 acres	$.97–3.63 billion	$177 941	http://www.nab.usace.army.mil/Portals/63/docs/Environmental/Oysters/MasterPlan_ExecutiveSummary.pdf

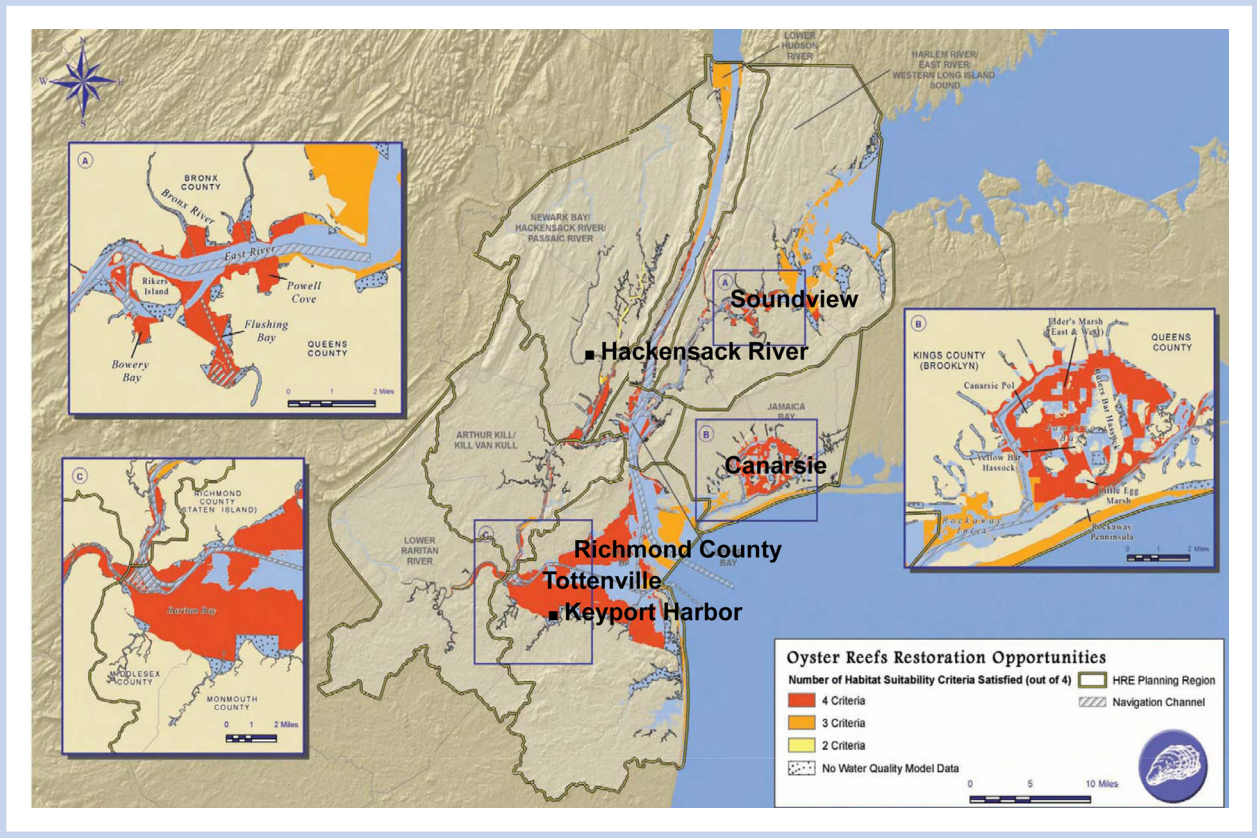

Figure 2. Map showing potential oyster restoration locations identified in the draft Hudson-Raritan Estuary (HRE) Comprehensive Restoration Plan. Locations of the Hackensack River (HR) and Keyport Harbor (KH) oyster reintroduction research sites are noted. Locations of Soundview, Canarsie, Tottenville, and Richmond County Yacht Club collection sites also noted. Map reproduced from the draft HRE Comprehensive Restoration Plan (2009).

(ERSSM) to improve the process of selecting urban oyster restoration sites (Figure 4), which includes an evaluation of in situ biological health (Wintermyer and Cooper 2003, 2007; Cooper and Wintermyer 2009). Use of biological biomarkers to evaluate shellfish health is not a new concept (NOAA Mussel Watch Program, as reported in Kimbrough et al. 2008; Edge et al. 2014), but it is important that they be included in a multitiered management approach to bivalve reintroduction. The proposed model is integrated with a Management Decision Tree (Table 2), which is composed of a series of questions to be sequentially answered before investing in an expensive large-scale urban oyster restoration. Multiple CRP reintroduction locations, identified based on physiochemical properties (Decision Tree Step 3), were tested to determine oyster short-term overwinter survival rates. Two sites (Figure 2) where survivorship was relatively high (60%–70%) were selected as Case Studies to test the inclusion of the tissue histopathology biomarker (Decision Tree Step 4) in this 2-tiered management reintroduction model.

We hypothesized that: 1) both Case Study test sites would support longer-term oyster survival, and 2) year-old adults at both locations would be capable of spawning. To test these hypotheses, we used histological tissue evaluation as the biomarker to assess health and reproductive fitness. To test use of the biomarker in assessing and ranking overall health, we also sampled 3 locations where volunteer "gardeners" were growing oysters at their docks and 1 location containing a small wild population. These sites were ranked with respect to their reintroduction suitability. To determine if known historic site-specific contaminants (Hackensack District metal contamination) would be observed in reintroduced oysters, soft tissues, and shells from both Case Study sites were analyzed and heavy metal concentrations determined.

MATERIALS AND METHODS

Study locations

The 2 Case Study sites (Keyport Harbor [KH] in Raritan Bay and the estuarine portion of the lower Hackensack River [HR] in the Meadowlands District) exhibited physiochemical parameters (J. Watson, Assistant Commissioner, New Jersey Department of Environmental Protection, personal communication; MERI 2011) within the ranges typical of a northeastern US estuary and within the ranges tolerated by *C. virginica* (salinity 5–10 and 4–26 ppt; dissolved O_2 5–10 and 6–12 mg/L; temperature 6° C–25° C and 3° C–27° C; total suspended solids 5–29 mg/L and 11–48 mg/L; HR and KH, respectively). The Meadowlands District sediments are known to retain historic heavy metal contamination (Crawford et al. 1994; Weis et al. 2004).

Juvenile seed oysters approximately 30 mm in length were purchased from Aeros Cultured Oyster (Southold, NY) and placed in a Raritan Bay aquaculture facility (NY/NJ Baykeeper, Atlantic Highlands, NJ) for acclimation. After the juveniles reached a length of approximately 40 mm, randomly selected subsamples were installed subtidally in the KH and HR locations (40° 27′ 01″ N; 74° 11′ 07″ W ; 40° 46′ 86″ N; 74° 05′ 12″ W, respectively) in September–October, 2009. Cages were constructed of 1.25 cm net mesh and oyster density was 500/m^2, selected based on naturally occurring densities (Mann

Table 2. Reintroduction management decision tree

Action steps	
1. Has a collaborative decision supported by public representatives, regulators, and NGOs been made that it is important to reintroduce a species for an economic, aesthetic, or important ecological services reason and/or reasons. Yes - Proceed to Step 2	No - stop
2. Can the introduction have a reasonable potential for success based on the state of the estuary considering multi-uses, site-specific chemical contamination, or biologic considerations? Yes - Proceed to Step 3	No - stop
3. Have potential reintroduction sites within the estuary been evaluated and potential reintroduction sites selected based on historic and physiochemical characteristics? Yes - Proceed to Step 4	
4. Has the reintroduction target species been placed at potential sites to assess in situ biological health, growth, gonadal development, disease, and histological abnormalities? Healthy - Proceed to Step 5	Unhealthy - stop
5. Have potential reintroduction sites been ranked based on the biomarker health evaluation? If ranked - Proceed to Step 6	
6. Deployment of larger scale introduction to sites highly ranked based on the overall health evaluation followed by long-term assessment and management to evaluate the health of the introduced species. Are adaptive management actions required to sustain the reintroduced population over time?	

NGO = nongovernmental organization.

et al. 2009). The cages were supported by Rebar or PVC frames and a random subset of oysters was placed in a separate cage attached to the top of each support structure, approximately 1 foot below the water surface at mean low tide; oyster samples were retrieved from these subsets. To assess oyster health, random samples were collected before spawning ($N = 10$ per site $\times$ 2 sites = 20) in June, 2010; postspawning samples ($N = 10$ per site $\times$ 2 sites = 20) were collected in late August and early October, 2010.

To test the ability of the histopathology biomarker to rank multiple locations we randomly selected 1-year-old oysters from 3 HRE New York volunteer oyster gardening sites (Figure 2): Canarsie, N = 14 (Brooklyn); Tottenville (N = 14), and Richmond County Yacht Club (N = 10) (Staten Island) and from an isolated native population in Soundview, N = 11 (Bronx River). Oyster soft tissues from the 4 locations were processed and Lesion Severity Scores were determined as described below.

Sample processing

Oysters were weighed and measured (shell length from umbo to hinge, height, and width at highest point of outer shell) before shucking. Wet weight of body tissue and water within the shell and the shell weight were obtained after shucking. Soft tissues were preserved in 10% buffered formalin, subsequently transferred to 70% ethanol solution, and embedded in paraffin for sectioning (Histocenter II manufactured by Allied Fischer Scientific). The paraffin block was cut (Reichert Histostat Rotary Microtome) into 6 μm sections and placed on a slide, stained with hematoxylin and eosin, and observed under a light microscope (Zeiss, West Germany) to examine mantle, gill, muscle, digestive tract, heart, kidney, and reproductive tissues.

Lesion Severity scores were used to determine the degree of tissue damage (Ray 1954; Ford and Tripp 1996). Tissue lesions, hyperplasia (an abnormal increase in the number of normal cells), infiltration of macrophages, parasites, altered epithelial cell type and/or tissue structures were scored using a 3-point rating system where: 1 = normal appearance of oyster cell/tissue structure, 2 = presence of minor cell and/or tissue abnormalities, and 3 = oyster's cell and/or tissue appearance highly abnormal. Slides were prepared for each sample and histopathology scores for each tissue type were calculated based on the Lesion Severity ratings.

Shell and soft tissue metal analysis

Samples evaluated biologically for histopathology were analyzed to determine metal concentrations. To obtain a representative subset, tissues and shells from 3 oysters were combined into a single composited sample; 3 composite samples were made, each containing 3 different oysters ($N = 3$ per location $\times$ 2 locations = 6 composited soft tissue samples and 6 composited shell samples). Three replicates taken from each composited sample were analyzed for 24 metals commonly investigated in our laboratory, which was more extensive than typical studies reporting values for individual metals (Table 5).

The shell and body tissues were separately digested using a MarsX microwave sample digester (CEM, Matthews, NC). Between 90–260 mg of air-dried sample was reacted with 0.5 mL of nitric acid (EMD Omni Trace, ultra high purity) and microwaved (300 W, 75% power, 5 min) until no further digestion occurred. This digestion was followed by addition of 0.25 mL of nitric acid and samples were microwaved (300 W, 100% power, up to 10 min). This digestion was followed by addition of 0.75 mL of nitric acid; the samples were sonicated 1 h and microwaved (300 W, 100% power, 20 min), cooled to room temperature, and diluted to 30 mL (Milli-Q ultra pure deionized water of 18.2 MΩ; Millipore, Massachusetts, USA). The samples were centrifuged, a few milliliters of supernatant were removed and recentrifuged and diluted 5-fold with 5% nitric acid. Samples were analyzed with an inductively coupled plasma mass spectrometer (X5, Thermo Electron) using a

multi-element scan. We note that this method required significant modification from previous microwave tissue digests in our laboratory (Buckley et al. 2003; Xie et al. 2007). The stepwise addition of nitric acid allowed for complete digestion while keeping the overall sample volume low.

Metal concentrations were determined using standard calibration curves. Commercial metal standards (NIST traceable) were purchased form High Purity Standards. Every 10 to 12 samples a quality control sample (containing NIST traceable Calibrant A and Calibrant B) was run; blanks and digestion spikes were also run. The method detection limits were determined by multiplying the lowest standard that was within 20% of the least-squares line for the calibration curve by the dilution factor for the sample weight.

Statistical analysis

All summary statistics and analysis of variance (ANOVA) were conducted using SAS GLM (SAS Software, Version 9.2). One-way ANOVA (SITE Factor: Hackensack River vs Keyport Harbor) were conducted to test metal concentrations and histopathology scoring differences in oysters grown at the 2 sites. Post-hoc means were tested using Tukey's HSD method. A significant effect was at a $p \leq 0.05$.

RESULTS

Although both test sites supported short-term oyster survival, our results suggest that adults placed at the Hackensack River test location were not healthy and that successful spawning could be unlikely.

Physical characteristics

Before spawning, HR oysters were significantly smaller (length 3.9 cm; height 0.8 cm; width 2.9 cm) than the KH oysters (Table 3). Postspawning HR oysters exhibited shell length, width, and height that were equivalent to the KH shell dimensions. However, postspawning HR shell weight and thickness (7.8 g and 0.9 mm, respectively) were significantly below those of KH samples, resulting in HR total oyster weight approximately 8 g below KH total oyster weight (Table 3).

Histopathology biomarker evaluation

A histopathology evaluation of 40 adult oysters 4–7 cm in length revealed numerous abnormalities in HR oysters. Lesion Severity scores were significantly higher in HR gill, mantel, abductor muscle, digestive gland, kidney, gonad, and connective tissues (Table 4) versus KH scores. The shell gland, responsible for formation of the shell at its outer edges, appeared normal in the KH samples but was dysmorphic in HR oysters (Table 4 and Figure 3A). The HR shell gland had a vacuolated and edematous appearance, and the epithelium was undergoing dysplasia and metaplasia in a majority of the oysters sampled; in a number of HR samples the shell gland middle lobe was twice as long as the adjacent lobe, which is normally the longest (Figure 3A). Tissue disruption was observed in the HR gill and mantle tissues (Table 4 and Figures 3B and 3C). The pathogen species *Perkinsus* (Dermo) was observed in HR epithelium and connective tissues. KH oysters exhibited only minor abnormalities, although some slight localized edema was observed.

Similar to the shell gland, HR mantle tissue was edematous and hyperplasia was observed along the epithelium at the shell edge and on the internal cavity edge of the branchial chamber (Figures 3B and 3C). HR oyster gills were smaller in size and lacked epithelial cells along the proximal length of the chitinous rods of the gill filament. Ciliated cells along the gill filaments were present but appeared to be fewer in number, and HR gills

Table 3. Physical characteristics of 1-year-old HR and KH Eastern oysters[a]

	Prespawn		Postspawn	
	June 2010	June 2010	August 2010	October 2010
Parameter	KH	HR	KH	HR
Length (cm)	4.7 ± 0.27 $F_1 = 5.74$, $p = 0.028$	3.9 ± 0.16	6.1 ± 0.38	6.2 ± 0.20
Height (cm)	1.3 ± 0.7 $F_1 = 25.10$, $p < 0.0001$	0.8 ± 0.05	1.8 ± 0.10	1.6 ± 0.07
Width (cm)	3.6 ± 0.19 $F_1 = 7.78$, $p = 0.012$	2.9 ± 0.14	4.5 ± 0.19	4.5 ± 0.21
Wet body wt (g)	2.8 ± 0.51	1.3 ± 0.09	6.1 ± 0.75	6.5 ± 0.59
	$F_1 = 8.97$, $p = 0.008$			
Shell thickness (mm)	2.0 ± 0.20	2.0 ± 0.10	3.4 ± 0.30 $F_1 = 21.14$, $p = 0.0002$	2.5 ± 0.12
Shell wt (g)	7.9 ± 1.20	2.5 ± 0.24	23.0 ± 2.28 $F_1 = 8.36$, $p = 0.097$	15.2 ± 1.40
Total wt (g)	11.9 ± 1.90 $F_1 = 16.39$, $p = 0.001$	4.2 ± 0.30	32.0 ± 3.36 $F_1 = 4.07$, $p = 0.05$	23.9 ± 2.15

HR = Hackensack River; KH = Keyport Harbor.
[a]$N = 10$ oysters from each site for each sampling date = 40.

Table 4. KH vs HR oyster lesion severity scores and prespawning histological evaluation summary

Tissue	Gonad	Gill	Labial palps	Digestive gland	Mantle	Abductor muscle	Kidney	Connective tissue	Shell gland
KH	1.25 + 0.105 *N* = 14	1.58 + 0.178 *N* = 13	1.33 + 0.333 *N* = 3	1.28 + 0.109 *N* = 18	1.53 + 0.118 *N* = 18	1.00 + 0.000 *N* = 7	1.50+ 0.136 *N* = 17	1.11 + 0.072 *N* = 19	
(*N* = 10)	80% normal sperm/egg development 50% female 50% male	90% normal appearance		75% normal appearance			100% normal appearance	20% possible *Dermo* 25% perivascular inflammation	90% normal appearance
HR	2.73 + 0.099 *N* = 15	2.56 + 0.127 *N* = 18	2.00 + 0.408 *N* = 4	2.20 + 0.156 *N* = 20	2.75 + 0.100 *N* = 20	1.88 + 0.125 *N* = 8	2.30 + 0.179 *N* = 20	1.85 + 0.167 *N* = 20	
(*N* = 10)	100% male 10% gonadal neoplasm 20% thick spermatogonia layer	100% showed small principle filaments		50% showed minor increased goblet cells			100% normal appearance	50% hyperplastic (increased number) goblet cells and *Dermo*	70% abnormal structure and vacuolization
F_1	81.04	21.25	1.43	22.64	63.79	42.47	11.97	16.20	
p value	<0.001	<0.001	NS	<0.001	<0.001	<0.001	0.0014	<0.001	

HR = Hackensack River; KH = Keyport Harbor; NS = No statistically significant difference.

had focal areas of hyperplasia (Figure 3C). HR oysters had an increase in the number of goblet cells in the epithelial linings of the mantle cavity (Figure 3C) and the digestive gland (Figure 3E). HR oysters exhibited hyperpigmentation (Figure 3D) beneath the epithelium along the mantle edge and in the labial palps; no pigmentation was observed in KH animals. Focal areas of macrophage infiltration were observed in the HR connective tissue and the number of brown cells (Figure 3D) was elevated. The HR digestive tubules in the digestive gland were severely dilated (Figure 3E).

HR reproductive abnormalities included a thick layer of spermatogonia, which is not normal when the oyster is in an advanced spawning state (Galtsoff 1964). HR oysters were all male (Figure 3F), rather than the more typical 50:50 ratio of females to males (Morton 1991) seen in KH oysters. One HR oyster exhibited a gonadal neoplasm (Figure 3F), which has been reported to occur in higher frequency after exposure to organic contaminates (Yevich and Barszcz 1977), although a cause and effect relationship has not been established.

Application of the biological model parameter to other locations

We applied the tissue histopathology evaluation biomarker to test the health of 1-year-old oysters at 4 locations identified in the CRP as potential restoration sites: a wild population from Soundview Park in the Bronx and "oyster gardened" animals raised by volunteers in Canarsie, Brooklyn, Wards Point, and Richmond County Yacht Club, Staten Island. Oyster soft tissues were evaluated and ranked in terms of predicted success of reintroduction at each location (Table 6). The tissue biomarkers were able to easily differentiate overall health at the 4 locations, and based on this qualitative evaluation, the order of most to least suitable reintroduction locations was: Canarsie > Tottenville >> Richmond County Yacht Club > Soundview.

Metal analyses

HR soft tissue metal concentrations exceeded KH concentrations for all metals analyzed except Ti (Table 5). HR oyster total body metal concentrations exceeded 1.1% of body weight; total KH soft tissue metal concentration was 0.4%. Conversely, KH shell metal concentrations were higher than those observed in the HR samples, except in the case of Mn. Given the abnormal histopathology observed in the Meadowlands samples it is possible that known historic contamination in this location may have affected test animals.

DISCUSSION

Oyster health described by the histopathology biomarker differed significantly between the 2 Case Study sites; therefore, we rejected the hypotheses that these sites could produce equally successful reintroduction outcomes, supported by longer-term survival and reproductive success. Although oysters survived and grew at the HR site, tissue abnormalities, 100% male sex ratio, visible presence of pathogens indicate this site is a poor choice for reintroduction at this time. In addition, the lack of gonadal maturation and altered sex ratio would preclude a sustainable reproducing population.

HR metal loads indicate that oysters were accumulating metals in their tissues and that their ability to maintain cellular and tissue homeostasis was somehow impaired. The decrease in shell thickness also indicated that normal formation of the shell matrix was disrupted. This is likely due in part to the lesions

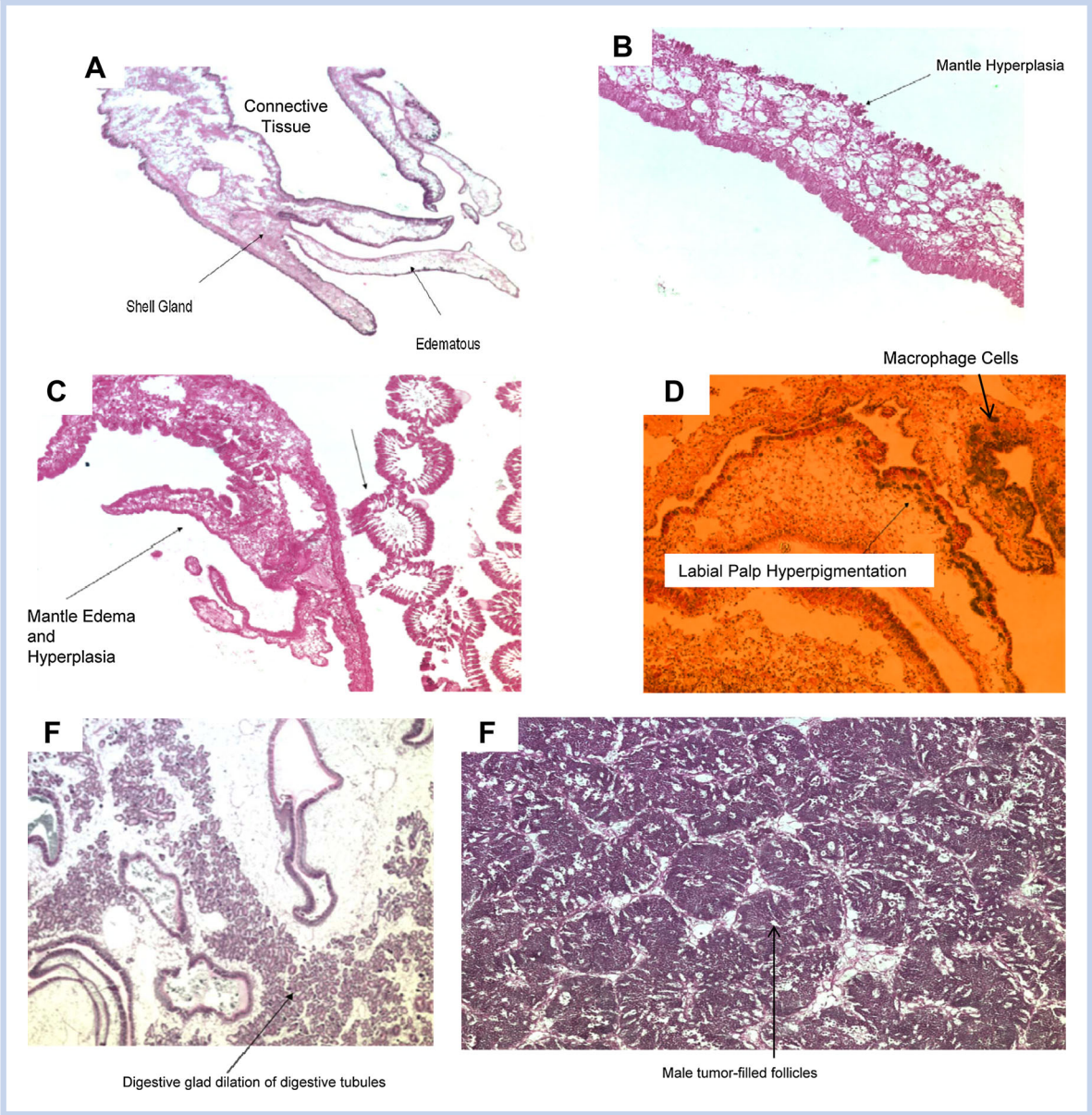

Figure 3. Examples of abnormal tissues observed in Hackensack River oyster samples. (**A**) HR shell gland showing elongated middle lobe and edematous tissue. (**B**) HR mantle showing abnormal cell structure (dysplasia) and hyperplasia. (**C**) HR sample showing mantle edema and hyperplasia in gill and mantle tissue. (**D**) HR sample showing labial palp hyperpigmentation. (**E**) HR digestive gland showing dilation of digestive tubules. (**F**) HR male gonadal follicles containing tumor cells filling the follicles.

observed in the shell gland and along the mantle, which are essential for proper shell deposition and formation. Although specific causes could not be identified, exposure to the system's legacy metal and/or organic contaminants, such as PCBs and 2,3,4,8-substituted dioxins, are likely contributors to the abnormalities observed.

Estuarine Reintroduction Site Selection Model

Since the CRP decision had been made to reintroduce oysters to the HRE (Decision Tree Step 1), there were reports of small isolated surviving populations (Decision Tree Step 2), and 50 000 acres of potential reintroduction areas have been identified based on physiochemical parameters (Decision Tree Step 3), we proceeded to assess in situ biological health (Decision Tree Step 4). Overwinter survivorship rates were determined by placing oysters in locations where the ranges of physiochemical parameters support oyster survival. This initial test placement can be done easily and cheaply in collaboration with local nongovernmental organizations (NGOs) using "citizen scientist" volunteers.

Where overwinter survivorship is high, histopathology analyses of 1-year-old adults can determine overall short-term health and reproductive fitness. Based on the results of this relatively rapid and inexpensive biological screening ($10 000–$15 000 over a 3 year period), expanded restoration activities can be undertaken where survival rates are high and health appears normal; conversely, reintroduction activities can be redirected away from locations where survivorship is low and/or animals exhibit abnormal histopathology. Should local participants wish to pursue restoration activities at sites where

Table 5. One-year-old oyster soft tissue and shell metal concentrations

	Shell		Soft tissue		Literature values		Reference
Metal (PPM)	Hackensack	Keyport	Hackensack	Keyport	Shell	Soft tissue	
Al	43 ± 4.7	194 ± 47.2	233 ± 31	140 ± 8		6–101	Sadig and Alam 1989
As	<0.16[a]	0.6 ± 0.06[b]	2.4 ± 0.30	1.5 ± 0.01		5–21 0.5 3–43	Volety 2008 Elston et al. 2005 NOAA 1987
Ba	5.7 ± 0.75	7.0 ± 0.5	3.2 ± 0.27	2.5 ± 0.10		<0.7	Sadig and Alam 1989
Cd	<0.16	<0.16	4.6 ± 0.37[o]	1.9 ± 0.02[p]		3–16 0.7–1.4 2.2 1.8–16	Frazier 1975 Volety 2008 Guzman-Garcia et al. 2009 Hayes et al. 1998
Cr	0.3 ± 0.04	0.5 ± 0.09	3.3 ± 0.42	2.2 ± 0.25		3–6 6 1 0.1–5	Volety 2008 Guzman-Garcia et al. 2009 Elston et al. 2005 NOAA 1987
Co	0.6 ± 0.01	0.8 ± 0.07	0.9 ± 0.08[q]	0.3 ± 0.00[r]			
Cu	1.5 ± 0.10[c]	2.3 ± 0.10[d]	400 ± 31[s]	60 ± 0.3[t]	160	50–225 500 98–376 56–212 31 15–1603	Frazier 1975 Frazier 1976 Volety 2008 Hayes et al. 1998 Elston et al. 2005 NOAA 1987
Ga	0.3 ± 0.00	0.3 ± 0.03	0.2 ± 0.02	0.2 ± 0.01			
Fe	121 ± 7[e]	402 ± 56.2[f]	485 ± 58[u]	139 ± 3[v]	19	200–500 600 319–628	Frazier 1975 Frazier 1976 Volety 2008
Pb	1.0 ± 0.04	1.2 ± 0.16	11.4 ± 1.27[w]	1.8 ± 0.02[x]		0.7–1.3 6 1.4–15	Volety 2008 Guzman-Garcia et al. 2009 Hayes et al. 1998
Li	1.0 ± 0.02[g]	1.8 ± 0.13[h]	0.6 ± 0.06[y]	0.2 ± 0.01[z]			
Mg	1879 ± 7	2346 ± 110	2843 ± 239[aa]	485 ± 13[bb]			
Mn	246 ± 0.5	208 ± 18.1	33.1 ± 3.13[cc]	7.1 ± 0.10[dd]	330–520 505	5–30 114 19–25	Frazier 1975 Frazier 1976 Volety 2008
Ni	4.6 ± 0.13	5.0 ± 0.3	3.2 ± 0.28[ee]	1.8 ± 0.03[ff]		0.6–1.4 0.9 0.6–13	Volety 2008 Elston et al. 2005 NOAA 1987
K	<16.0	<16.0	2269 ± 213[gg]	1234 ± 9[hh]			
Rb	<0.16	0.3 ± 0.08	1.2 ± 0.14	0.6 ± 0.02			
Se	0.1 ± 0.14	0.5 ± 0.03	3.7 ± 0.48	1.7 ± 0.23		1–1.8 0.4 0.9–5.7	Volety 2008 Elston et al. 2005 NOAA 1987
Ag	<0.16	<0.16	14.8 ± 2.0[ii]	2.8 ± 0.04[jj]		1.2–1.6 0.5 0.3–7	Volety 2008 Elston et al. 2005 NOAA 1987
Sr	960 ± 1.8[i]	1386 ± 76[j]	13.5 ± 1.13	12.2 ± 0.58		9–53	Sadig and Alam 1989
Ti	1.5 ± 0.08	43 ± 1.0	8.2 ± 0.54[kk]	17.6 ± 0.80[ll]		0.9–3	Sadig and Alam 1989
U	<0.03[k]	0.1 ± 0.01[l]	0.5 ± 0.05[mm]	0.2 ± 0.00[nn]		2–2.5	Akyil and Yusof 2007
V	<0.26[m]	0.8 ± 0.14[n]	1.1 ± 0.17[oo]	0.4 ± 0.01[pp]	0.5–1.4	0.5–1.4	Blotcky et al. 1979
Zn	<16.0	<16.0	4572 ± 348[qq]	1886 ± 51[rr]	2500	1800–5000 1495–5669 1806–2902 478 300–13 000	Frazier 1975, 1976 Volety 2008 Hayes et al. 1998 Elston et al. 2005 NOAA 1987

Significant differences ($p < 0.05$) between Hackensack River and Keyport Harbor oyster soft tissue and shell metal concentrations signified by different letters. $N = 12$ (6 composited soft tissue; 6 composited shell) × 3 replicates/sample.

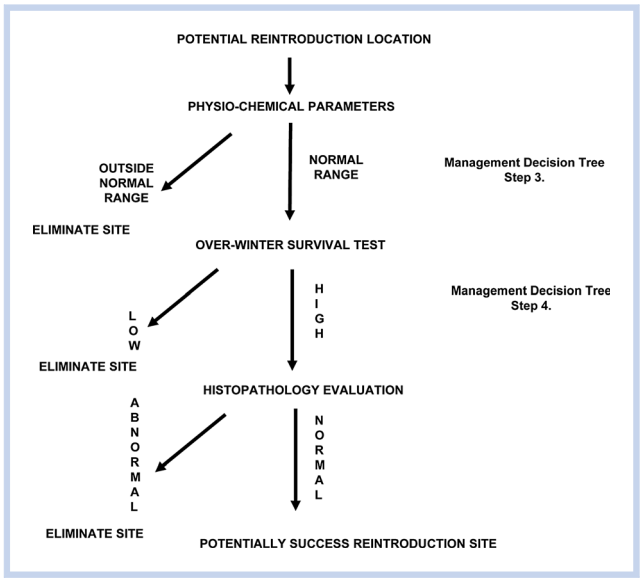

Figure 4. Schematic representation of the proposed Estuarine Reintroduction Site Selection Model (ERSSM) that includes an in situ biological parameter as a tool to aide in identifying sites with high potential for long-term sustainability.

the biological evaluation indicates poor health additional chemical analyses of known site-specific contaminants (such as our heavy metal screening) can be undertaken to identify environmental stressor(s) that could inhibit reintroduction success.

The Estuarine Reintroduction Site Selection Model (ERSSM) indicated that Keyport Harbor, NJ is a promising location for future HRE oyster reintroduction activities, but the lower Hackensack River should be excluded from bivalve reintroduction at this time. The ERSSM also indicated that although a wild population is surviving at an urban location, the overall animal health and fitness does not appear to be high. These results are important because although Soundview is currently supporting a small wild population, the biological evaluation suggests that this site would be a poor choice to invest long-term reintroduction efforts. The ERSSM proposed for selection of suitable oyster sites can be modified for any bivalve that is being considered for reintroduction in an urbanized harbor worldwide.

Conclusions

In highly urbanized estuaries natural hydrology has been greatly altered due to anthropogenic activities and oyster populations are rarely present. Oyster restoration typically occurs in estuaries where existing populations are present and often threatened; this study is unique because it took place in a heavily urbanized estuary where oysters are essentially extinct and successfully managing reintroduction of the species is the long-term objective. Commonly employed oyster restoration management approaches lacking a biological parameter are limited in determining potentially successful reintroduction locations. To augment existing management tools we propose a Management Decision Tree coupled with an ERSSM before costly investment in reintroduction initiatives based solely on physical oceanographic or historical parameters. This management tool combines currently accepted physiochemical site-selection parameters with standard oyster biological health criteria to evaluate proposed reintroduction locations, potentially reducing costs associated with unsustainable urban reintroduction efforts. The Case Study demonstrated that inclusion of a simple biological parameter to evaluate in situ oyster health can enhance management decision-making in differentiating between locations where reintroduction might or might not be successful. The Estuarine Reintroduction Site Selection Model's inclusion of a biological parameter can contribute to identification of sites where conditions may support oyster fitness, while rapidly eliminating sites where longer-term fitness would be compromised. Addition of the biomarker evaluation could potentially save hundreds of thousands of dollars at risk if large urban reintroduction initiatives are conducted in unsustainable locations.

Dedication—We would like to dedicate this article to Dr. Sammy Ray, who was Dr. Cooper's mentor and a pioneer in oyster research.

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Table 6. Tissue biomarker evaluation of oysters at 4 New York HRE locations

NYC Sites	Sample *N*	F:M ratio	Overall oyster condition
Soundview (public park)	11	7:3	Smallest oysters examined with thinnest shells; female gonads and eggs were delayed in development
Canarsie (canoe club)	15	8:7	Well developed ready to spawn; excellent condition with virtually no lesions present; no morphological damage or parasites
Tottenville (small marina)	15	7:8	Gonads well developed ready to spawn; increase in goblet cells in epithelial tissues; focal areas of macrophage infiltration and increase in brown cells in connective tissue due to some type of irritant
Richmond County Yacht Club	10	4:6	50% had underdeveloped eggs or poorly differentiated sperm with extensive macrophage infiltration in connective tissues, large number of brown cells in connective tissue and perivascular infiltration; evidence of chronic damage; mud blisters on shells; generally poor condition with disrupted gonadal development

HRE = Hudson-Raritan Estuary; NYC = New York City.

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