The effects of the restoration of Foundry Cove on the dominant, resident oligochaete, Limnodrilus hoffmeisteri

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Abstract

The following study is the first evaluation of the effects of an extensive clean-up on Foundry Cove's ecosystem. Utilizing the benthos as an indicator of ecosystem health, and thus restoration success, the study examined three factors pertaining to the current population of *Limnodrilus hoffmeisteri*: (1) population densities; (2) body burdens of cadmium; and (3) current level of metal resistance. All measurements from Foundry Cove were compared to populations in the control cove, South Cove. The results demonstrate that current cadmium concentrations in the surficial sediment of Foundry Cove are two to three orders of magnitude lower than 1983 levels. Yet, concentrations remain one order of magnitude above the control site. The current Foundry Cove population of *L. hoffmeisteri* has decreased population densities and a body burden of cadmium two orders of magnitude lower than worms collected from the control site. It remains significantly more metal-resistant than control site worms. Worms from Foundry Cove site 2 demonstrate significantly decreased metal resistance compared to metal-resistance in worms collected prior to the clean-up. Conversely, worms collected from Foundry Cove site 1 are significantly more metal-resistant compared to metal-resistance in worms collected prior to the clean-up. Clearly, the restoration efforts in Foundry Cove have triggered a dynamic period. Current cadmium concentrations are believed to be below the threshold level at which metal-resistance evolves in *L. hoffmeisteri* and the degrees of metal resistance are changing. There are three principal explanations for the observed decrease in resistance levels following the removal of cadmium: (1) mixing of previously metal-resistant and non metal-resistant populations; (2) the restoration of immigration of non-metal-resistant worms from surrounding populations; and (3) the existence of costs associated with metal-resistance resulting in competitive inferiority and a shift in gene frequency. These potential explanations cannot be narrowed until the population structure of the cove is established and a measure of gene flow is estimated. A continued decrease of resistance levels will eliminate the first explanation as the only explanatory factor. It remains unclear whether metal-resistance is a useful criterion for evaluating ecosystem restoration until further research is conducted.

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Introduction

The recent, multi-million dollar clean-up of Foundry Cove presents unique research opportunities in restoration ecology and evolutionary biology. This tidal freshwater marsh on the Hudson River had the distinction of being the most cadmium-contaminated site ever reported (Knutson et al., 1987). The Cove has consequently served as a productive research site for investigations on the effects of metal pollution on aquatic ecosystems. One of the more remarkable effects of this metal contamination was the rapid evolution of a metal-resistant oligochaete, Limnodrilus hoffmeisteri (Klerks and Levinton, 1989a). The evolution and maintenance of a population of metal-resistant worms served as a conspicuous indicator of ecosystem degradation. Our study is the first evaluation of the effects of the clean-up on the Cove's ecosystem. Utilizing the benthos as an indicator of ecosystem health, and thus restoration success, the study examines the current population of L. hoffmeisteri. In doing so, this investigation addresses the question of whether L. hoffmeisteri can be used as an indicator of ecosystem restoration, just as it is used as an indicator of ecosystem stress.

Ecosystem restoration is frequently defined as the recreation of both the structure and function of a now degraded ecosystem (Cairns, 1991). Yet, restoration success is often evaluated with respect to a single criterion pertaining to ecosystem structure (Cairns, 1991). Furthermore, the criterion is frequently a chemical parameter which as an indirect measure is frequently proven to be an insufficient gauge of ecosystem function (Lenat et al., 1980). Implicit in this approach to restoration evaluation is the assumption that once ecosystem structure is restored the restoration of ecosystem function will follow (Westman, 1991).

The long-term monitoring plan (Advanced GeoServices Corp., 1995) for Foundry Cove employs this limited method of evaluating ecosystem restoration. The plan focuses on a single criterion to evaluate restoration success, i.e., cadmium concentrations. It stipulates that once a year for five years, cadmium concentrations will be measured in water, sediment, and various biota of Foundry Cove. The evaluation of a population is limited to one species, the muskrat, Ondatra zibethicus. It is generally assumed that if cadmium concentrations are reduced, ecosystem function will be restored.

The current study broadens the scope of the evaluation to include population parameters of benthic macrofauna. The effects of the clean-up are assessed by evaluating changes in the population of the metal-resistant and dominant oligochaete, L. hoffmeisteri. Three factors pertaining to the current population of L. hoffmeisteri were measured: (1) population density; (2) body burdens of cadmium; and (3) the current level of metal-resistance. The concentration of total cadmium in surficial sediments at each sampling site was also measured. As restoration success is often evaluated by comparing various parameters with nearby non-degraded (or less degraded) sites, all results are compared to the nearby control site, South Cove.

Limnodrilus hoffmeisteri is presumed to be a particularly appropriate gauge for restoration success for a few reasons. First, the use of benthic invertebrates in the evaluation of water quality and the "health" of aquatic ecosystems is well established (Reish, 1979; Lenat et al. 1980; Morse, 1980). Macrobenthic organisms are desirable in evaluating aquatic ecosystems because they reside on, or within, sediments, are exposed directly to contaminated particles and pore waters and consequently serve as good indicators of long-term, as well as short-term, conditions in the system. Secondly, oligochaetes, Tubifex in particular, are known to be tolerant of contaminated conditions and have been recognized as "biological indicators" of ecosystem stress for over a hundred years (Reish, 1979). Thus, oligochaetes are viewed as a tool for monitoring aquatic ecosystems. Frequently, severely contaminated sites demonstrate a higher density of oligochaetes and at times, a decrease in species diversity (Klerks and Levinton, 1989b; Lenat et al. 1980). A decrease in the population density of oligochaetes and a return to historic levels of species diversity would be the anticipated indicators of ecosystem restoration in these locations.

In the case of the cadmium-laden Foundry Cove, two changes in the population of resident oligochaetes (L. hoffmeisteri) were noted. First, the worm evolved a significantly higher degree of metal-resistance compared to worms collected from the control site (Klerks and Levinton, 1989a). Additionally, L. hoffmeisteri appeared to be more densely populated in Foundry Cove than the control cove (Klerks, 1987). Although changes in population densities of oligochaetes have been used extensively in evaluation of aquatic ecosystems (Lenat et al. 1980), changes in metal resistance have not. This study is one of the first to investigate if metal-resistance is a useful criterion to evaluate the success of ecosystem restoration efforts.

In addition to providing an opportunity to study restoration ecology, the sudden removal of the strong selective agent in the evolution of metal-resistance (i.e., cadmium), provides an opportunity to investigate potential costs, or metabolic trade-offs, associated with metal-resistance. An investigation into the relative competitive abilities of resistant and non-resistant worms may provide indirect evidence of the existence or absence of costs associated with metal-resistance.

A common assumption in evolutionary biology is that there are costs to adaptation (Futuyma and Moreno, 1988; Huey and Hertz, 1984). Costs associated with adaptation are believed to be a principal force in the evolution of specialization. Although widely applied, the notion that a "jack of all trades is a master of none" (Levins, 1968; MacArthur, 1972) has rarely been demonstrated (Huey and Hertz, 1984; Mitter and Futuyma, 1983).

Most attempts to identify and measure genetic-based trade-offs in fitness have entailed rearing organisms in a variety of environments in the lab for the purposes of demonstrating a negative correlation in fitness between environments. These efforts have largely been unsuccessful due to two common experimental artifacts: (1) populations adapting to laboratory conditions, and (2) positive genetic covariances from alternative loci, masking negative correlations (Fry, 1990; 1993; Rausher, 1988; Bell and Koufopanou, 1986).

To avoid these statistical complications, Fry (1993) suggests performing selection experiments whereby a trait is artificially selected for in the lab. Once the selected trait has reached "equilibrium," the organism is cultured and bred in an environment that lacks the selective agent for multiple generations to determine whether the trait is lost over time. These trends are compared to a control. The recent clean-up of Foundry Cove may mimic this experimental design, thereby providing a large-scale, in situ experiment.

Metal-contaminated sites have been utilized repeatedly for evolutionary studies because they impose high selective pressures and often result in relatively rapid evolution. Most attempts to measure costs associated with metal resistance have utilized resistant plants (Wilson, 1988; Hickey and McNeilly, 1975). Although there are numerous examples of metal resistance in marine and aquatic invertebrates, research investigating the costs of metal resistance are lacking in aquatic organisms (Klerks and Weis, 1987). Weis and Weis (1989) provide one of the few examples of costs to metal tolerance in an aquatic animal. Their results show costs of embryonic tolerance to methyl-mercury resulting in slower growth and weakness in adult mummichogs.

Methods

Study Site/Sampling locations

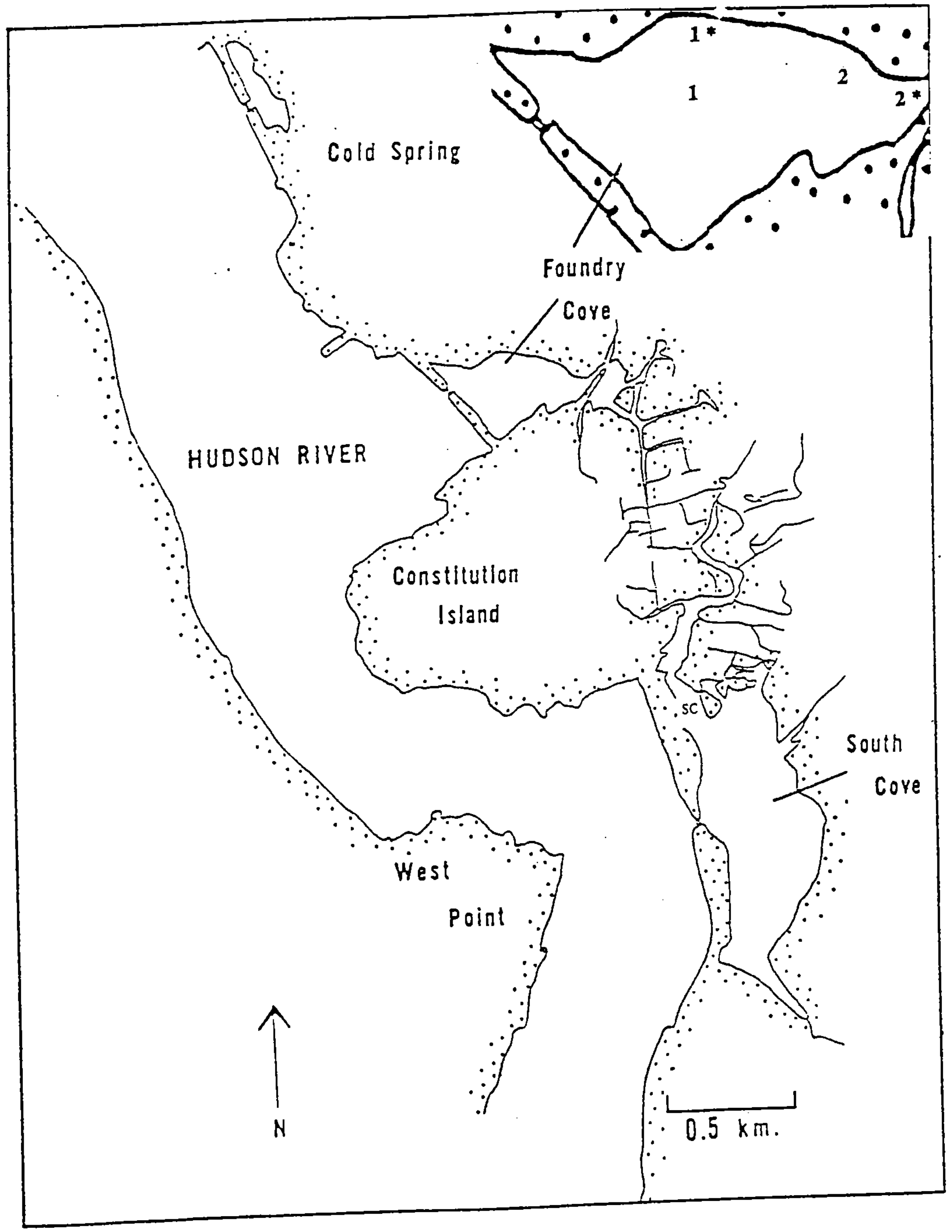
Foundry Cove is a tidal freshwater marsh on the eastern banks of the Hudson River located approximately 100 km from the Verrazano Narrows (Fig. 1). From 1952-1979, Foundry Cove was the discharge site for nickel, cadmium, and cobalt enriched waste waters from a nearby battery factory. It is estimated that 179 metric tons of cadmium were discharged into Foundry Cove in total (Resources Engineering, 1983).

In 1972, the upper 30 cm of sediment in the cove was dredged, removing approximately 10% of the cadmium from the sediment (Resource Engineering, 1983). Despite this effort, cadmium levels as high as 50,000 μg Cd per g of dry sediment were found in 1975 (Klerks and Levinton, 1993). The cove was declared a "Superfund" site in

1983. An extensive, $91 million clean-up attempt was completed in the winter of 1994. Restoration efforts involved draining, dredging, and treating contaminated sediments in the eastern cove as well as replanting acres of marshes in the surrounding area.

Sediment and worms were collected from two stations in Foundry Cove and one location in the control site, South Cove (Fig. 1). An attempt was made to sample the same sites in Foundry Cove as in previous investigations (Knutson et al., 1987; Klerks and Levinton, 1989; Wallace, 1996). The exact locations of the current sites, however, differ for two reasons. First, the configuration of the cove has changed as a result of the marsh restoration. Secondly, suitable habitat for the worms was found only off shore as the result of dredging (Fig 1). Accordingly, previous study sites are labeled Foundry Cove 1* and 2* and present study sites are labeled Foundry Cove 1 and 2.

FIGURE 1. LOCATION OF THE STUDY SITES



Population Densities

L. hoffmeisteri were collected by a corer of the top 5 cm of sediment (35 cm^2 area) in July, 1996. Following separation from sediment with a 500 μm sieve, worms were preserved in rose bengal tinted formalin. Densities were determined from number of individuals per core. Comparisons of densities between sites were determined on square root transformed data by one-way ANOVA (Sokal and Rohlf, 1981).

Cadmium Measurements

Sediment and worms were collected with an Ekman sediment grab in Foundry Cove and South Cove to an approximate depth of 15 cm. Cadmium concentrations in sediment were measured by a Perkin-Elmer inductively coupled plasma emission spectroscopy (optima 3000) following extraction by microwave digestion in sealed Teflon bombs (Kingston and Jassie, 1988) with concentrated nitric and hydrofluoric acids. To measure body burdens, approximately 0.3 grams/wet weight of worms were digested with concentrated nitric and hydrofluoric acids. Cadmium concentrations in tissues were measured by a Perkin-Elmer atomic adsorption spectroscopy (3300). Data are reported on a dry mass basis using a factor of 7.8 to relate wet and dry masses.

Laboratory Cultures

To facilitate comparison of experimental results to those of earlier investigations, the culturing methods of Klerks and Levinton (1989) were followed. Worms were sorted with a 500μm sieve and cultured in polystyrene dishes, with a one cm layer of sediment and nine cm of continually aerated Hudson River water. To remove all other macroscopic living matter, sediment collected from the same site as the animals was sieved to <500 μm. Worms were fed with fish food flakes once per week. Cultures were kept at 24° C under a 12 h light/12 h dark cycle.

Cadmium resistance measurements

Metal resistance in L. hoffmeisteri from the current Foundry Cove and South Cove populations was measured via toxicity bioassays. The methods of Klerks and Levinton (1989) and Wallace (1996) were followed to enable comparison of assays. Mature L. hoffmeisteri of approximately 1 cm were exposed to a solution containing 8.9 μM Cd (added as $CdCl_2$) in soft reconstituted fresh water (0.864 g $NaHCO_3$, 0.54 g $CaSO_4$, 1.1 g $MgSO_4$, and 0.036 g KCl in 18L of distilled water) (Klerks, 1987). Cadmium concentrations of 8.9 μM were chosen because survival times were long enough to distinguish between resistance levels and short enough to avoid mortality from other factors (e.g., depletion of oxygen) (Klerks and Levinton, 1989a). Animals were acclimated for 24 hours to the soft reconstituted water in Petri dishes where worms evacuated gut contents. At the onset of the experiment, ninety-six animals from each population were transferred individually to small well dishes containing cadmium-spiked water. Worms were placed randomly in wells and lids were placed on the dishes to prevent evaporation. Survival was recorded every hour until half of the worms had died. Death was defined as the hour when a worm did not respond to disturbance by a pipette (Wallace, 1996).

Two bioassays were performed to increase confidence in the results. Comparisons in cadmium resistance were made by pair-wise comparisons of survivorship curves using Gehan's Generalized Wilcoxon Test (Lee, 1980). This test compares distributions and is appropriate for use on partial data sets.

Measures of Molecular Variation

Population structure and gene flow will be determined eventually through the use of genetic markers. DNA was extracted from worms preserved in 70% ethanol by a CTAB/proteinase K and phenol/chloroform extraction (Palumbi et al., 1991). The cytochrome oxidase I (COI) gene was amplified using PCR primers 1490 and 2198 (Folmer et al., 1994). Amplifications were conducted on a Perkin-Elmer 2400 thermal cycler for 30 cycles utilizing a 94° C/30 seconds, 50° C/30 seconds, and 72° C/120 seconds amplification profile. The resultant PCR product was gel purified, selectively precipitated, and re-amplified at an annealing temperature of 54° C to yield a clean product. PCR products were sequenced by nonidet P-40 double strand sequencing (Palumbi et al., 1991).

Results

Population Densities

The current population densities of L. hoffmeisteri in Foundry Cove (site 1 and 2) are significantly lower than the current population density of South Cove ($p<0.05$). Current population densities of L. hoffmeisteri are approximately a third to two times lower than densities of macrobenthos prior to the clean-up in South Cove and Foundry Cove (Table 1).

Table 1: Mean Abundance of Macrobenthos Pre-Clean-Up and L. hoffmeisteri Post-Clean-Up per Square Meter

Sample Location	Mean Abundance Macrobenthos pre-clean-up (n=15) (individuals)[1]	Mean Abundance L. hoffmeisteri post-clean-up (n=15) (individuals)
South Cove	9,100	5,813 (st. dev. 9.23)
Foundry Cove 1	10,000	3,995 (st. dev. 71.3)
Foundry Cove 2	13,200	3,238 (st. dev. 6.11)

[1] Klerks (1987)

Cadmium Concentrations

The concentration of total cadmium in the sediment in Foundry Cove sites 1 and 2 is currently two and three orders of magnitude lower, respectively, than pre-cleanup levels. Furthermore, there is no longer a steep gradient of cadmium concentrations between Foundry Cove sites 1 and 2. Although no restoration efforts were conducted in South Cove, cadmium concentrations in South Cove sediment are currently an order of magnitude lower than 1983 levels. Current concentrations in Foundry Cove remain an order of magnitude higher than concentrations in South Cove (Table 2).

Table 2: Cadmium Concentrations in the Surficial Sediments of South Cove and Foundry Cove (total Cd)

Sample Location	Cadmium Concentration pre-clean-up [1]	Cadmium Concentration post-clean-up (differing values are replicates)
South Cove	15 ppm	5 ppm 6 ppm
Foundry Cove 1	500 ppm	86 ppm 69 ppm
Foundry Cove 2	10,000 ppm	56 ppm 56 ppm 67 ppm 62 ppm

[1] Knutson et al. (1987 data collected in 1983)

The concentration of total cadmium in tissues of L. hoffmeisteri from Foundry Cove (site 2) is two orders of magnitude lower than pre-cleanup levels in 1983. Current body burdens in Foundry Cove worms are one order of magnitude higher than those of South Cove worms (Table 3).

Table 3: Body Burden of total Cadmium in L. hoffmeisteri

Sample Location	Cadmium Concentration pre-clean-up[1]	Cadmium Concentration post-clean-up (differing values are replicates)
South Cove	22 ppm	2.3 ppm 1.5 ppm
Foundry Cove 2	1,100 ppm	13.6 ppm 52 ppm

[1] Klerks and Bartholomew (1991)

Degree of Metal-Resistance

Bioassay 1

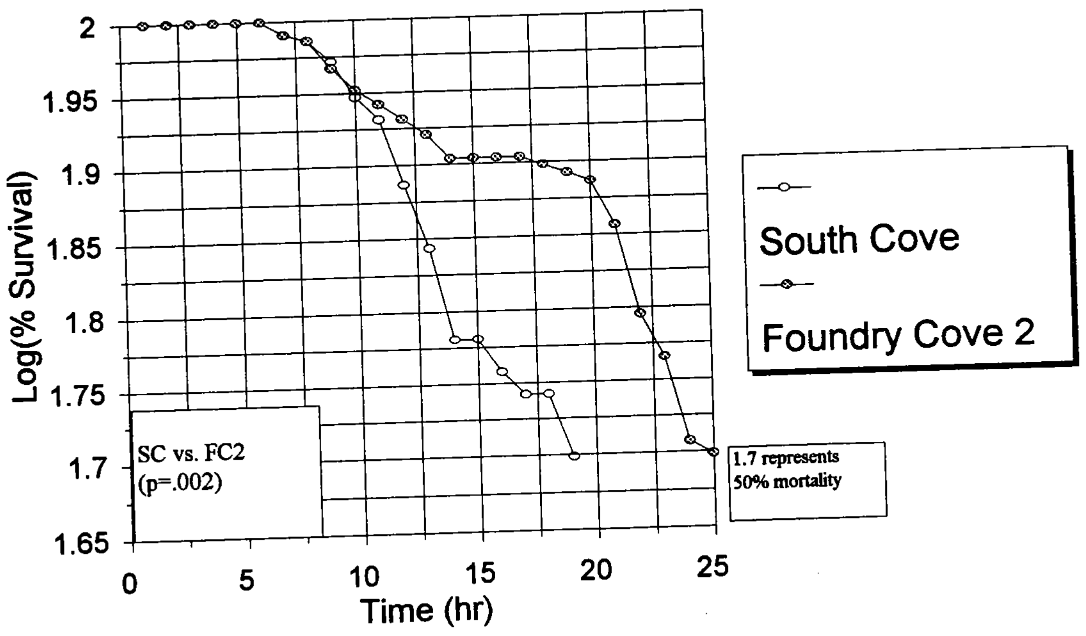
The time to 50% mortality in samples from South Cove (control) and Foundry Cove 2 was hour 19 and hour 25, respectively. There is a four hour difference between the 50% mortality time of Foundry Cove 2 and the *average* 50% mortality time of the South Cove distributions (Table 4). The comparison (Gehan's Generalized Wilcoxon Test) of survival distributions between Foundry Cove 2 and South Cove demonstrates a significant difference (p=0.002) (Fig. 2).

Table 4: Degree of Resistance

Population (Assay)	Time at 50% mortality (hours)	Difference between time at 50% mortality and the avg. South Cove time at 50% mortality (i.e., 21hr/st. dev.1.73) (hours)
South Cove pre-clean-up	19	—
South Cove post-clean-up (assay 1)	22	—
South Cove post-clean-up (assay 2)	22	—
Foundry Cove 2 pre-clean-up	30	9
Foundry Cove 2 post-clean-up (assay 1)	25	4
Foundry Cove 2 post-clean-up (assay 2)	26	5
Foundry Cove 1 post-clean-up (assay 2)	24	3

Fig 2: Toxicity Bioassay Post Clean-up

South Cove vs. Foundry Cove (assay 1)



Bioassay 2

The time to 50% mortality in samples from South Cove (control), Foundry Cove 1, and Foundry Cove 2 was hour 22, 24, and 26, respectively. A difference in 50% mortality times between the mean of the control worms and the worms of Foundry Cove sites 1 and 2 was three and five hours, respectively (Table 4). The survival distributions from each Foundry Cove site were found to be significantly different from the survival distribution of South Cove worms in this assay (p=.02 for site 1 and p=.002 for site 2) (Fig. 3).

Pre-Clean-up Bioassay vs. Bioassays 1 and 2

The 50% mortality time of Foundry Cove 2 worms in a bioassay conducted prior to the clean-up was 30 hours (Wallace, 1996). This represents a nine hour difference when compared to the average 50% mortality time of the control cove worms (Table 4). There was a significant difference between the survival distributions of Foundry Cove 2 worms and South Cove worms prior to the clean-up ($p<<<0.001$) (Fig. 4).

In pairwise comparisons, there was no significant difference between the survival distributions of South Cove worms from three different bioassays (Table 5). Similarly, there was no significant difference between the survival distributions of Foundry Cove worms (sites 1 and 2) collected after the clean-up (Table 6). Pairwise comparisons, however, demonstrated a significant difference between the survival distributions of Foundry Cove worms prior to the clean-up and after the clean-up, in sites 1 and 2 (Table 6).

Population Structure

Thus far, a suitable genetic marker demonstrating adequate genetic variation has been identified. Comparisons of a 140 base pair portion of the COI gene in one Foundry Cove worm and one South Cove worm show a single base change in a first codon position (bp538). The change is silent and maintains the production of leucine.

Fig 3: Toxicity Bioassay Post Clean-up

South Cove vs. Foundry Cove 1 & 2

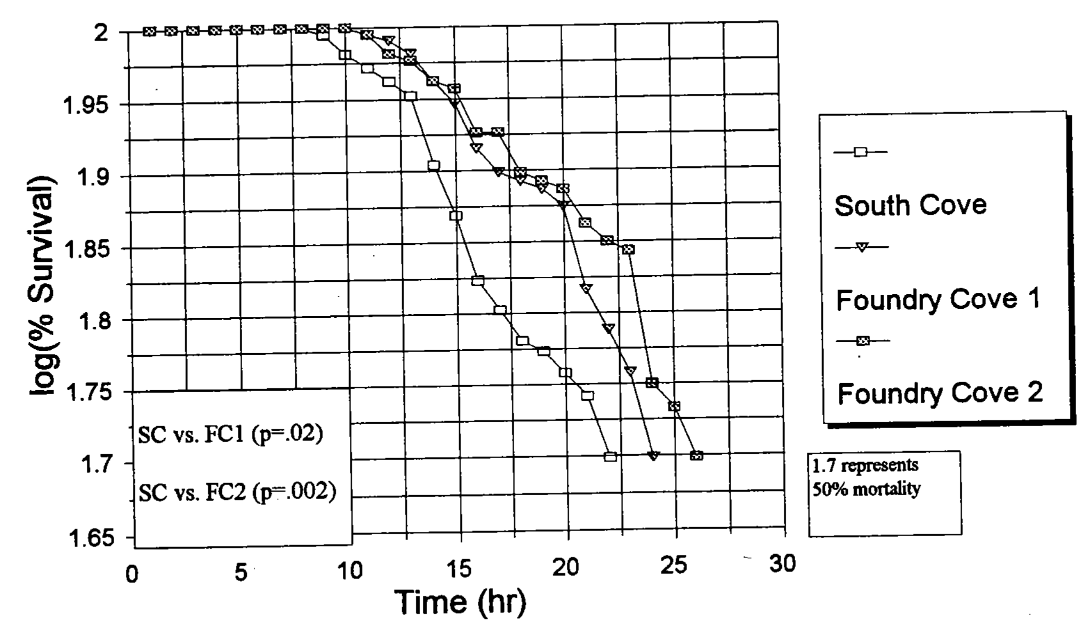


Fig 4: Survival in Three Bioassays

Post vs. Pre-cleanup Foundry Cove

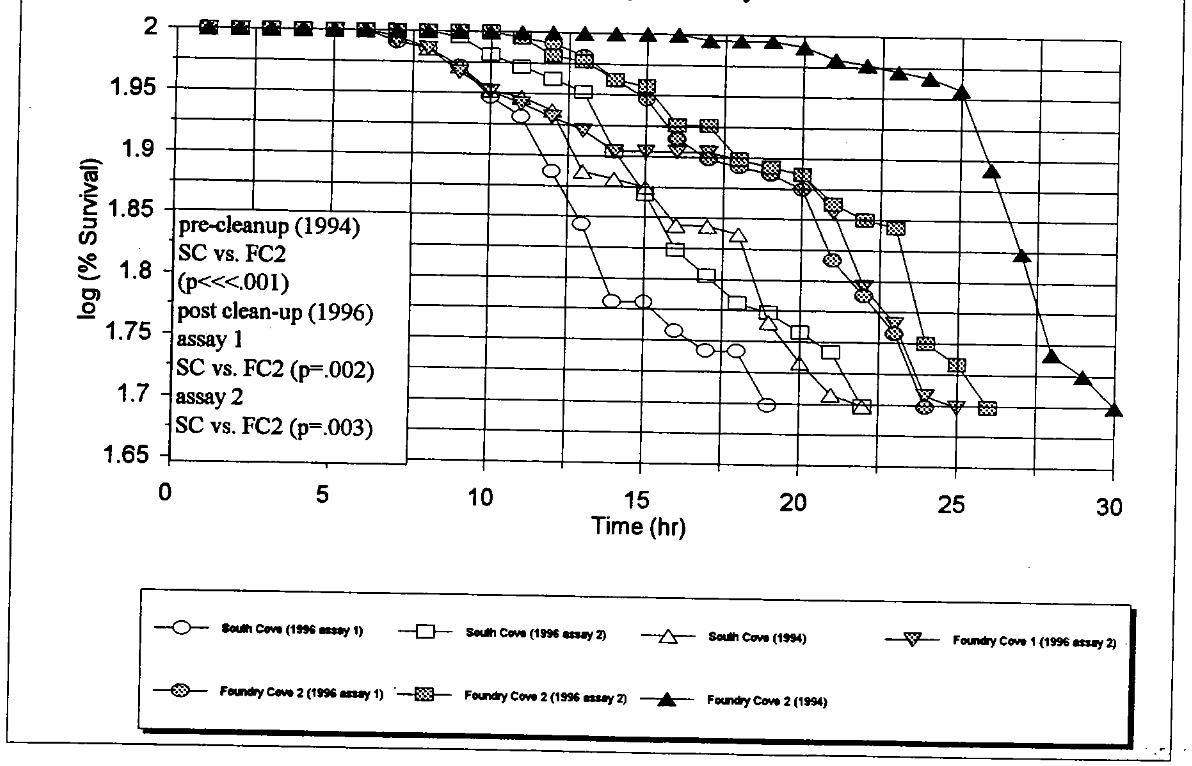


Table 5: Comparisons of South Cove Survival Distributions

Population (assay)	South Cove pre-clean-up	South Cove post-clean-up (assay 1)	South Cove post-clean-up (assay 2)
South Cove pre-clean-up	—	n.s.	n.s.
South Cove post-clean-up (assay 1)		—	n.s.
South Cove post-clean-up (assay 2)			—

Table 6: Comparisons of Foundry Cove Survival Distributions

Population (assay)	Foundry Cv 2 pre-clean-up	Foundry Cv 2 post-clean-up (assay 1)	Foundry Cv 2 post-clean-up (assay 2)	Foundry Cv 1 post-clean-up (assay 2)
Foundry Cv 2 pre-clean-up	—	p<0.001	p<0.001	p<0.001
Foundry Cv 2 post-clean-up (assay 1)		—	n.s.	n.s.
Foundry Cv 2 post-clean-up (assay 2)			—	n.s.
Foundry Cv 1 post-clean-up (assay 2)				—

Discussion

Has the population density of L. hoffmeisteri been restored?

Utilizing population density as a measure of restoration success is difficult for a number of reasons. First, as is often the case, no historic data prior to contamination exist. Additionally, no measures of population density of L. hoffmeisteri prior to the clean-up were recorded. Klerks (1987) provides measures of macrobenthos density, of which L. hoffmeisteri constitute the majority, however, no specific measures of L. hoffmeisteri were published. Secondly, there was not a strong statistical difference between the population densities of L. hoffmeisteri in Foundry Cove and the control site (Klerks, 1987). Establishing significant differences in density is difficult due to high spatial variance. Klerks and Levinton note that L. hoffmeisteri appear to be more common in the most contaminated regions of Foundry Cove as compared to South Cove (Klerks and Levinton, 1989b).

The current population densities of L. hoffmeisteri in Foundry Cove are significantly lower than densities of macrobenthos in Foundry Cove prior to the clean-up. They are also, however, lower in South Cove. The possible causes of these declines are numerous. In Foundry Cove, physical removal of worms and/or suitable habitat as the result of dredging may explain the declines. It is possible that a reduction in contamination in both Foundry Cove and South Cove is the principal cause. Recent measurements of faunal diversity show that diversity is currently higher in Foundry Cove than prior to the clean-up (Sokol et al., 1996). Natural population fluctuations can not be ruled out as an explanation.

Is the cove clean? What is the current body burden of Cd in L. hoffmeisteri?

Cadmium concentrations in Foundry Cove sediment are a great deal lower now, following the clean-up, than in 1983 (Table 2). In especially polluted sites such as Foundry Cove 2, there was a three order of magnitude decrease in cadmium concentrations. South Cove, which did not undergo restoration, demonstrated a ten-fold reduction in cadmium. Sedimentation processes over the past 13 years are the likely explanation of the changes in South Cove. Foundry Cove and South Cove remain polluted ecosystems, as they contain considerably higher concentrations of cadmium than pristine background levels which are typically in the mid part per billion range (Greenwood and Earnshaw, 1984).

Cadmium concentrations in the tissues of L. hoffmeisteri have also declined tremendously over the past ten years (two orders of magnitude in Foundry Cove and one order of magnitude in South Cove) (Table 3). It is not clear why one Foundry Cove replicate contains five times the cadmium as the other. The possibility of contamination is believed to be unlikely because cadmium was the only metal that displayed a significant spike.

Are L. hoffmeisteri still metal-resistant ?

Metal resistance, as measured by this and previous studies (Klerks, 1987; Wallace, 1996), represents a relative measure. As defined here, "metal-resistant" means significantly more metal-resistant than South Cove worms. There have been no measures of cadmium-resistance in L. hoffmeisteri collected from pristine environments. When comparing the results of the current study to those of previous studies (Wallace, 1996), it is important to note that sampling locations vary somewhat. However, changes caused by factors such as mixing as a result of dredging are much greater than these slight discrepancies in sampling locations.

The survival distributions from the three toxicity bioassays show three distinct clusters of distributions (Fig. 4). South Cove samples had the shortest average time to 50% mortality, indicating the lowest level of cadmium resistance. Foundry Cove worms that were collected approximately two years (20 months) following the clean-up demonstrated an intermediate time to 50% mortality. Lastly, Foundry Cove worms collected immediately prior to the clean-up (1994) demonstrated the longest time to 50% mortality (30 hr). There were no significant differences in pairwise comparisons within each of the clusters (Tables 5 and 6) lending confidence to the repeatability and comparability of these assays.

There was a significant difference between the survival distributions of the current Foundry Cove worms and their associated South Cove worms (Fig. 3 and 4). Thus, L. hoffmeisteri remains "metal resistant" in Foundry Cove following the clean-up. Its degree of metal resistance, however, has changed. There was a significant difference between the survival distributions of all current Foundry Cove worms and those Foundry Cove worms collected immediately prior to the clean-up ($p<0.001$) (Fig. 5). Because the 1994 toxicity bioassay was conducted on worms from site 2 in Foundry Cove, the results demonstrated that there has been a decrease in the resistance level of Foundry Cove 2 worms. Klerks (1987) reports that worms from Foundry Cove 1 (sediment [Cd] $\approx$ 500 ppm) were not significantly different from South Cove worms. Thus there was an apparent increase in resistance to cadmium in worms collected from Foundry Cove 1 (Fig. 4).

There are three principal explanations for the observed decrease in resistance levels following the removal of cadmium: (1) physical mixing of previously metal-resistant and non metal-resistant populations; (2) the restoration of immigration of non-metal-resistant worms from surrounding populations; and (3) the existence of costs associated with metal-resistance resulting in competitive inferiority and a shift in gene frequency. Any combination of these three explanations may be operating.

Given current information, the most likely explanation is that previously metal-resistant and non-resistant populations of L. hoffmeisteri in Foundry Cove were mixed during the restoration process which entailed extensive dredging. The apparent increase in metal-resistance of worms from Foundry Cove 1 and decrease in metal-resistance of worms from Foundry Cove 2 provides evidence for the homogenization of formerly distinct populations.

It is alternatively possible, however, that reduction of cadmium contamination has resulted in the restoration of successful immigration. The apparent change in metal-resistance may be a result of large numbers of non-resistant immigrants shifting the gene frequency towards non-resistance. Lastly, it is possible that the recent decrease in metal-resistance is evidence that metal-resistance imparts costs to L. hoffmeisteri that resulted in competitive inferiority in non-contaminated sites and thus caused a shift in gene frequencies. In order to understand the basis of shifts in gene frequencies within Foundry Cove, one must establish the population structure in the region and estimate gene flow into the cove. Preliminary analyses of the genetic variation of the COI gene in L. hoffmeisteri from these sites identifies the COI as an appropriate marker of genetic variation.

It will be possible to determine if physical mixing of populations during dredging was the principal cause of the reduction in metal-resistance through continued monitoring of the metal-resistance. Continued relaxation of metal-resistance over time will provide evidence for either the restoration of gene flow or a decrease in the competitive abilities of resistant worms. Until a further decline in resistance is observed, it is too soon to determine if metal-resistance is a useful criterion for evaluating ecosystem restoration. The available evidence is compelling, however.

Is there evidence to costs associated with metal resistance?

The observed decrease in metal-resistance of Foundry Cove 2 individuals sustains notions of potential costs associated with metal resistance. Cadmium has effectively been removed for a period of 20 months, translating to approximately 4-6 generations as estimated by Klerks (1987). If costs associated with metal-resistance are the basis for the decrease in resistance, the rate of the observed change indicates that the costs are quite high. Again, it will not be possible to determine if the present or continued shifts in metal-resistance are principally the result of costs until population structure is established, through additional ongoing research. Artificial selection experiments in the lab will also provide additional information on the question of costs associated with metal resistance in L. hoffmeisteri.

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