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# Running the gauntlet: pollution, evolution and reclamation of an estuarine bay

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## Introduction

We wish to provide the student with an overview of an integrative approach to studying biotic responses to pollution in natural populations. We will use a specific polluted site to illustrate this approach, which involves the study of population responses, both in terms of response to a toxic substance and of trophic transfer through the food web.

We wish to demonstrate the following points:

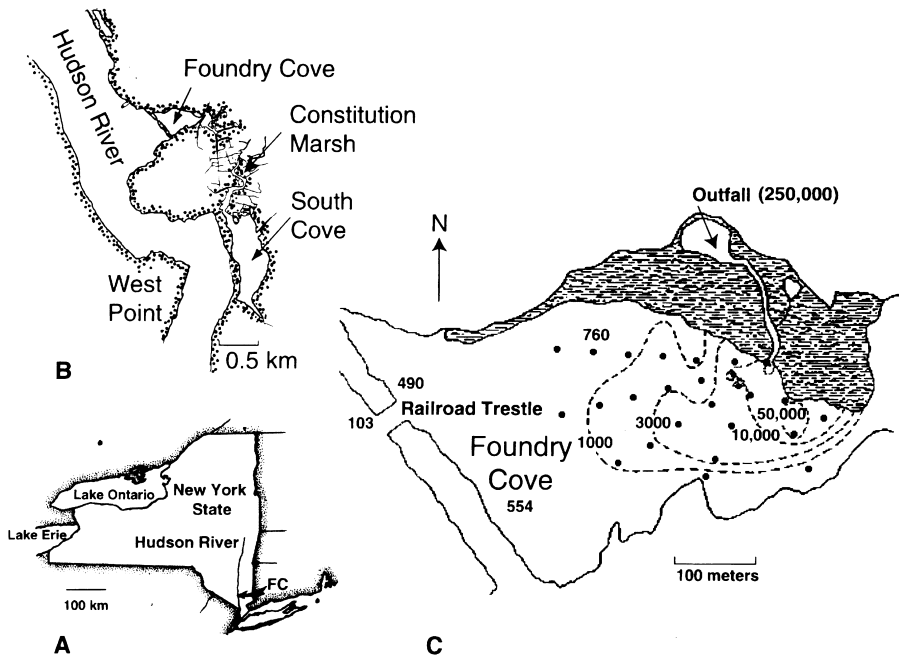
1. Pollution need not have effects that can easily be discerned as wholesale reorganizations of the entire biota. More subtle rearrangements may be at play, even though the toxic substance has strong physiological effects.
2. Toxic substances often reduce viability, but genetic variability in the degree of resistance to a toxic substance may produce consequences beyond a simple reduction of viability. The evolution of response to toxic substances and evolutionary changes in life history attributes might result in increased population fitness during exposure to the toxic substance, owing to natural selection.
3. The evolution of resistance may have a cost and reduce the productivity of the resistant population but this need not always be the case.
4. Resistant organisms may have elevated levels of a toxic substance obtained from the environment. This may have the unfortunate effect of trophic transfer of the substance upwards through the food web.
5. The advent of Darwinian selection for resistance and the loss of resistance following removal of a pollutant may be very informative of the overall physiological effects of the pollutant on the population and the cost of evolution of resistance to the toxic substance.
6. The sum total of these responses argues for a complete evolutionary and population-level understanding of ecological toxicology. More naive approaches that attempt to use model stock cultures of laboratory-reared organisms for

exposure tests may be misleading as to the ultimate effects of population-level responses to toxins and the degree of trophic transfer.

## Background of Foundry Cove

Foundry Cove (Figure 1) is a tidal, freshwater bay-marsh system on the east side of the Hudson River estuary, about 80 km north of New York City. It was named after a foundry that was established during the American Civil War, where guns, including the famous Parrot Gun that reputedly won the war for the North, were manufactured. Presumably metals were released into the cove at the time but we have no present-day evidence for this. The modern era of pollution at Foundry Cove had its origins also in military activities.

The Cold War scare of the late 1940s and early 1950s inspired the Nike missile defence system, which required large nickel-cadmium batteries as a power system. From 1953 to 1979, a series of companies manufactured nickel-cadmium batteries. During this period significant quantities of concentrated cadmium and nickel nitrate solutions, accompanied for a time by cobalt (which was used as a stabilizer; Kneip and Hazen, 1979), entered Foundry Cove and the adjacent Hudson River. The wastewater, comprising cadmium and nickel hydroxides, had a pH of 12–14 (Resource Engineering, 1983). While considerable wastes were discharged into the Hudson River, 29%, or 53 metric tonnes, were discharged directly into Foundry Cove, resulting in extremely high cadmium and nickel concentrations in the sediment, probably the highest levels of such pollution in the world. A law suit filed to force compliance with the US Clean Water Act led to the removal of about 10% of



**Figure 1.** Location of Foundry Cove, New York State, USA. (A) Location of Foundry Cove in New York State; (B) Location of Foundry and South Coves in Hudson River; (C) Map of Foundry Cove, with variation of cadmium in the surface sediments in 1974, from data collected by R. E. Hazen and T. J. Kneip, redrawn after Knutson *et al.*, 1987.

the metals in 1972–1973 (see Knutson *et al.*, 1987) but the cove remained badly polluted until a clean-up operation under the direction of the Superfund Law (United States Environmental Protection Agency Record of Decision, 30 September 1986) was completed in 1995. Until this clean-up the outfall area harboured sediments with cadmium and concentrations of  $10^3$ – $10^4$  ppm were commonplace (Knutson *et al.*, 1987). The lowest concentrations registered were around 500 ppm! Cd:Ni:Co ratios in the sediments were generally 18:20:1 (Knutson *et al.*, 1987).

## The biota do not notice the pollution

A survey in the early 1980s of the soft-bottom benthic invertebrate communities of the polluted Foundry Cove and the nearby, relatively clean, South Cove revealed a startling result (Klerks and Levinton, 1989a). With the exception of the extremely polluted sediments of the outfall area (see Klerks and Levinton, 1992), it was difficult to distinguish between Foundry Cove and South Cove, either in terms of overall benthic species diversity or animal density. Oligochaetes were slightly more abundant in Foundry Cove at the expense of other species such as aquatic larvae of chironomid flies but the effect was not strong. This led to a paradox, how could such seemingly toxic sediment support biota no less rich in species or in abundance than a similar but unpolluted cove?

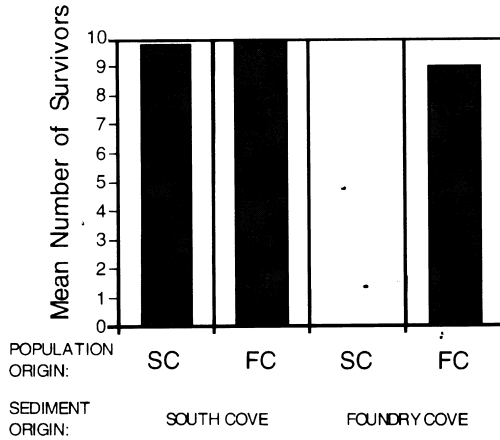
## Evolution in response to the pollution event

### *The presence of resistance*

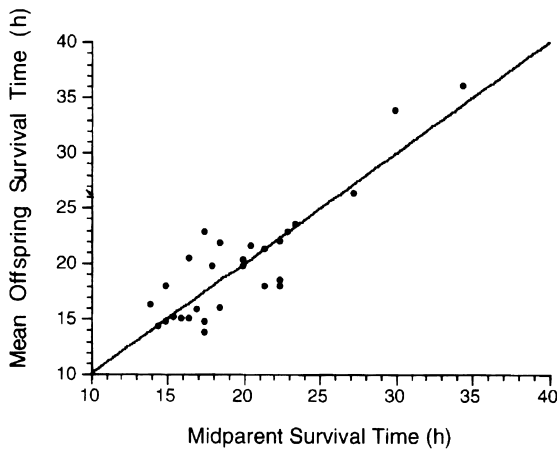
We investigated the dominant oligochaete, *Limnodrilus hoffmeisteri*, to determine its sensitivity to dissolved cadmium and nickel. *L. hoffmeisteri* occurs commonly in muddy sediments, is a simultaneous hermaphrodite, and is rarely longer than a few mm. Following copulation, embryos develop in cocoons, which can be found free in the sediment. Freshly-caught worms from Foundry Cove sediments with high cadmium concentrations survived well when exposed to Cd-rich sediments or when placed in a solution with high dissolved cadmium (Figure 2). Nearly all worms collected from the nearby South Cove, however, died when exposed to Foundry Cove sediments (Klerks and Levinton, 1989a). This difference in resistance could be attributed to a genetic inadequacy to combat exposure to metals, which the tolerant species might have gained through progressive acclimation. When we raised two more generations in clean sediments, the differences in survival to different metals were retained in the grandchildren of the field collected populations (Klerks and Levinton, 1989b). This suggested that the resistance might be the result of rapid selection, and that most of the resistance trait was genetically based.

A study of resemblance among relatives produced the surprising result that the among-allelic contribution (narrow-sense heritability) to resistance was approximately unity (Figure 3), meaning that all of the variation in resistance among individuals in a population could be explained by genetic variation. A selection experiment yielded a heritability estimate of 0.59, which is still considerable (Klerks and Levinton, 1989b).

It was possible to estimate the time that it had taken for the evolution to occur in Foundry Cove, based on estimates of mortality of worms introduced from a non-polluted site in Foundry Cove to a metal-rich site. With a conservative estimate of the heritability (0.6) and assuming that 95% of the population under selection survived to contribute to the next generation, we can calculate that the evolution we observed would have taken about 40 generations. If 40% contributed to the next generation, then the evolutionary adaptation could have occurred in a startling four generations (Klerks and Levinton, 1989b). The latter estimate is more consistent



**Figure 2.** Experiment demonstrating differential mortality of Foundry Cove (FC) worms, *Limnodrilus hoffmeisteri*, and South Cove (SC) worms, when exposed to Foundry Cove (cadmium polluted) and South Cove (clean) sediments. After Klerks and Levinton 1989a.



**Figure 3.** Correlation of survival to exposure to cadmium between parents and offspring for *Limnodrilus hoffmeisteri*\* collected in South Cove. Slope (Model I regression)=0.99. After Klerks and Levinton 1989b.

with our laboratory selection experiment which, in three generations, produced about two-thirds of the resistance we observed in Foundry Cove populations.

### How many genes?

The surprising degree of genetic determination of the resistance trait and the large range of variation in resistance explain the rapid response to selection, as we know that mortality is high when naive groups of individuals are exposed to high levels of

cadmium. Thus the essentials of natural selection — genetic variation and strong differences in fitness — are present in abundance.

It is also of interest to understand the genetic architecture of resistance, which can be understood with a further exploration of resemblance among relatives. The number of segregating genetic elements can be estimated by means of a method originally developed by Wright (Castle, 1921; Wright, 1968) and later generalized by Lande (1981). Basically, it involves crossing two parental populations resistant and non-resistant, to produce an F1 generation that is likely to express an intermediate value of the trait in question, which here is survival time to cadmium exposure. Then random pairs are established in the F1 to produce an F2 generation. The variation in resistance is likely to expand into the F2 and the degree of expansion can be used to calculate two different estimates of the number of segregating elements (Lande, 1981).

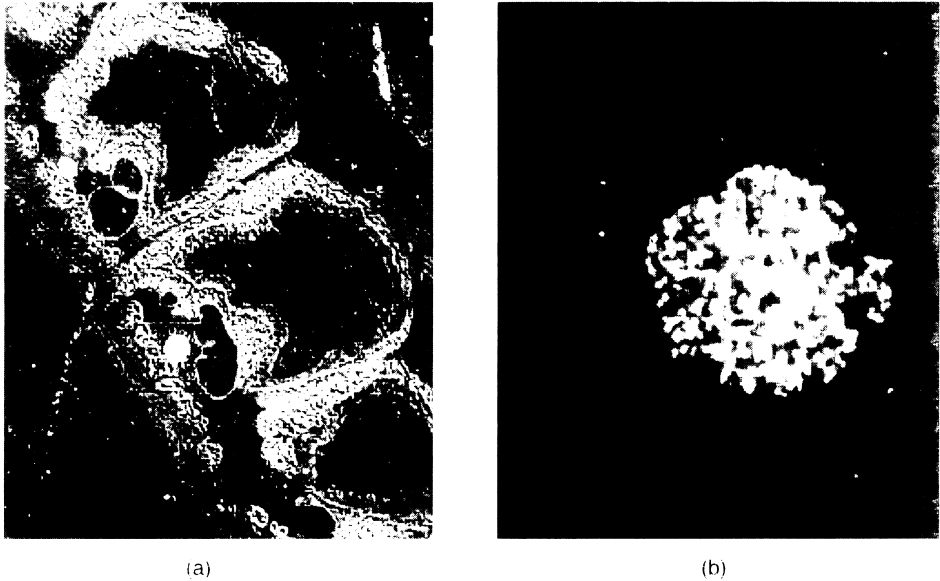
Cadmium resistance in the F2 generation turned out to be bimodal, the modes corresponding to those of the parental generation. The two estimates of the minimum number of genetic factors that contribute to the difference in resistance between the South and Foundry Cove populations of *L. hoffmeisteri* were similar: 0.344 and 0.360 (Martinez and Levinton, 1996). These values point to a major segregating factor that is likely to be responsible for the observed differences. It is possible that variations at one locus (or perhaps a series of closely inked loci) explains the rapid evolution of resistance.

This estimate is crude and a more accurate assessment would require backcrosses of the F2 to the parental stocks. Nevertheless, the recovery of both modes in the F2 suggests a fairly simple genetic mechanism involving very few loci. Unfortunately, our work on identifying the molecular mechanism is incomplete, although our HPLC study on metal-binding proteins suggests that in resistant worms cadmium is binding to a protein of low molecular weight, perhaps metallothionein (Klerks and Bartholomew, 1991). The induced nature of the protein would also support this identification, but further work is needed to confirm it.

Metallothionein provides the simplest genetic mechanism to explain resistance, which might be based upon gene duplication. Yeast cell cultures (Thiele *et al.*, 1986) and *Drosophila* embryos (Maroni *et al.*, 1987) both are more resistant to copper toxicity when they bear multiple copies of the *mt* gene. Multiple copies of the *mt* gene could also result in higher basal levels of metallothionein, which could be important of surviving the initial exposure to Cd. Multiple copies probably allow for more rapid transcription and eventual production of more of the protein.

## Physiological mechanisms of tolerance

Resistance to a toxic substance could involve two different strategies: either the avoidance of uptake or the uptake and sequestering of the substance in a non-toxic form. *L. hoffmeisteri* in Foundry Cove clearly carried high body burdens of cadmium, so it belongs to the latter category. Subsequent studies of uptake confirmed this deduction (Klerks, 1987; Klerks and Bartholomew, 1991). A crude understanding of the somatic partitioning of cadmium can be obtained by centrifugation, which allows the fractionation of cadmium in association with various cell constituents (Klerks and Bartholomew, 1991; Wallace and Lopez, 1997). A surprisingly large amount of Cd is associated with the cytosol, probably bound in a cadmium-binding protein, such as metallothionein (Wallace and Lopez, 1997). The cadmium in the cytosol fraction increases with cadmium exposure. This must involve a considerable synthesis of metal-binding protein, which is physiologically costly. A considerable amount of cadmium is also associated with metal-rich granules (MRGs, Figure 4), which appear as a pellet in the centrifuge fractions. These granules, about a micron in size, are concentrated in the chloragoc tissue surrounding the gut (Klerks and Bartholomew, 1991). These MRGs are probably composed of cadmium sulphide. Presumably these granules are eventually broken down and cadmium is excreted.



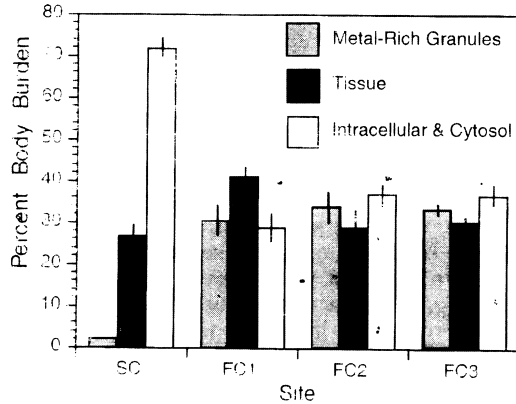
**Figure 4.** Backscattered electron images of *Limnodrilus hoffmeisteri* from Foundry Cove, exposed to cadmium-rich sediments. (a) Oblique section of *L. hoffmeisteri*, showing the distribution of Cd and S-rich granules. The vertical dimension of the photograph is approximately 500  $\mu\text{m}$ . (b) Closeup of a granular aggregate, approximately 10  $\mu\text{m}$  across. Photographs by Paul Klerks and Paul Bartholomew

## The relationship to trophic transfer

Cadmium is thus stored by *L. hoffmeisteri* in at least two discontinuous phases: dissolved and complexed with a metal-binding protein and in metal-rich granules (Klerks and Bartholomew, 1991; Wallace and Lopez, 1997). It would stand to reason that MRGs would be less easily assimilated by predators than material in the dissolved phase located in the cytosol (Reinfelder and Fisher, 1991). Thus, the partitioning of cadmium may strongly influence potential trophic transfer to predators of *L. hoffmeisteri*.

Wallace and Lopez (1997) tested the partitioning hypothesis by feeding cellular material that had been fractionated into a metal-rich granule, cell-debris fraction and a cytosol fraction, of which the latter contained dissolved Cd bound to metal-binding proteins. The respective materials were marked with gamma particle emitter  $^{109}\text{Cd}$ , which allowed one to follow the uptake by the grass shrimp *Palaemonetes pugio* *in vivo* in a gamma counter. The material was embedded into gelatin before feeding to the shrimp, which occurs abundantly in Foundry Cove and preys efficiently upon *L. hoffmeisteri* in the laboratory. Retention of Cd by the shrimp, overall, is very high for period of at least 75 h. The  $^{109}\text{Cd}$  absorption efficiency of shrimp that were fed the MRG-cell debris fraction was only 48.6%, but absorption efficiency was 84.8% when shrimps were fed the cytosol fraction. It is likely that MRGs are absorbed still less efficiently when fed without the other cell debris. Worms from the relatively non-polluted South Cove have Cd wholly associated with metal-binding proteins, whereas Foundry Cove worms have a large part of the Cd stored as MRGs (Figure 5). This results in a far more efficient trophic transfer of Cd from South Cove worms to shrimp (Wallace *et al.*, 1998).

These results demonstrated that trophic transfer of Cd from worms to shrimp depends heavily upon the dominant form in which the Cd is encountered. Although

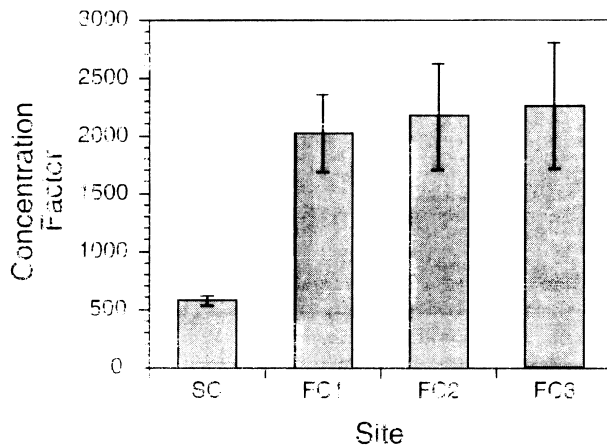


**Figure 5.** Subcellular distribution of cadmium in *Limnodrilus hoffmeisteri* collected from South Cove and three metal-polluted Foundry Cove sites. Note the large proportion of Cd in the cytosol fraction of South Cove worms, relative to Foundry Cove worms. After Wallace *et al.* (1998).

Foundry Cove worms have more Cd bound in MRGs than South Cove worms, the total Cd transferred is far greater from Foundry Cove worms, as Foundry Cove worms carry much higher body burdens of Cd. The greater uptake of Foundry Cove worms is shown clearly by the total magnification factor of uptake of cadmium, relative to availability (Figure 6). South Cove worms have a concentration factor (from cadmium-spiked water) of ca. 500, whereas Foundry Cove worms have a concentration factor of ca. 2000 (Wallace *et al.*, 1998).

## Recovery and its possible evolutionary meaning

The evolution of resistance to cadmium toxicity in *L. hoffmeisteri* was quite rapid and this was related to the strong natural selection that combined with the genetic variability present for resistance. These conclusions depend upon the assumption that our assessment of the non-resistant population of the early 1980s (Kierks and Levinton, 1989b) is an appropriate measure of the genetic variability in resistance



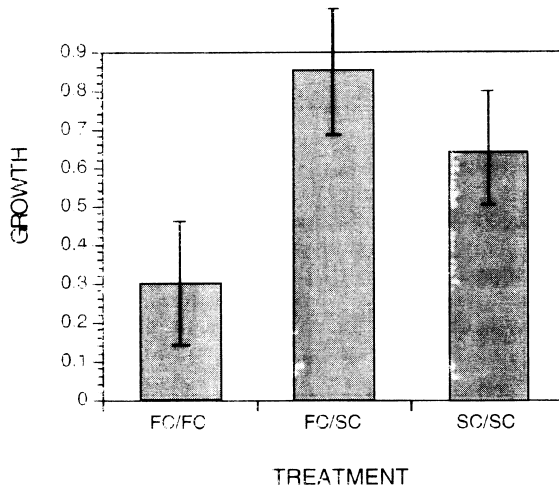
**Figure 6.** Concentration factors for *Limnodrilus hoffmeisteri* that were collected from South Cove and three cadmium-polluted Foundry Cove sites and exposed for 7 days to  $^{109}\text{Cd}$ -spiked Hudson River water. After Wallace *et al.* (1998).

that existed before the pollution of Foundry Cove. Even if the development took more than just a few generations, our study shows the time course to be a maximum of 20 years.

With such strong selection in both the laboratory and the field, combined with the geographic gradient of resistant populations over about 500 m, one might reasonably conclude that there is a cost to the evolution of the resistance trait. It is clear why non-resistant genotypes were not likely to invade the cadmium-rich zone over such a short distance: mortality would just be too high, except for the rare resistant genotypes. With modest dispersal, one might expect resistant genotypes progressively to invade the area of non-resistance. Invasion might proceed by floating individuals carried by tidal currents, although we have not made a systematic survey of such movements. Even without swimming, simple crawling would be a strong force for homogenization of the two populations. Nilsson *et al.* (1998) have examined the potential for reproduction and population spread in another oligochaete and have found that rapid extension of a population's range could occur in weeks through asexual reproduction and lateral burrowing. Sexual reproduction would slow down the process but the generation time (60 days or less) would permit production of young that could spread quite rapidly, certainly over a 200 m distance.

Thus, it seems reasonable that the cline in resistance, before the clean-up in 1994, had been maintained partially because there was a cost to natural selection and that resistant genotypes were competitively inferior to non-resistant genotypes. This mechanism has been suggested in the evolution of resistance of grasses to metals (McNeilly and Bradshaw 1968).

To test this hypothesis, Montero and Levinton (1995) examined the growth and reproductive characteristics of the Foundry Cove resistant population in clean and cadmium-polluted sediments, when compared to non-resistant individuals from South Cove. The results were surprising. *L. hoffmeisteri* from the metal-polluted site grew more slowly in metal-polluted sediments than when in clean sediments, or relative to clean-sediment-derived South Cove individuals, grown in clean sediments. However, *L. hoffmeisteri* from metal-polluted sediments grew in clean sediments as well as their South Cove conspecifics derived from clean sediments (Figure 7). Thus, there was no price paid to evolving resistance, at least in terms of



**Figure 7.** Measure of cost of evolution of resistance. Growth of *Lumnodrilus hoffmeisteri* collected from cadmium-polluted Foundry Cove and grown in Foundry Cove sediments (FC/FC), South Cove sediments (FC/SC), as compared with South Cove-collected worms grown in South Cove sediments (SC/SC). Growth is measured as length of worms that started at the same approximate length. After Montero and Levinton (1995).



somatic growth rate, although growth rate was clearly reduced by the presence of metals. We can thus conclude that there is a physiological cost paid by the population exposed to metals but the cost is not fixed genetically, as individuals genetically adapted to metals can perform well when placed in clean sediment.

A lack of cost of the evolutionary response to metals may be explained by the hypothesis of gene duplication and induction of resistance. As mentioned above, Foundry Cove worms exposed to metals had high levels of metal-binding protein, probably metallothionein. The direct exposure to toxic cadmium or the costly production of metal-binding proteins may have contributed to reduced growth of the Foundry Cove worms when exposed to metals. In the absence of metals, the induction may cease and the cost of responding to metal proteins may be that of having several copies of the gene at the ready for induction. This hypothesis, however, does not explain why such genotypes failed to invade over a geographic distance of a few hundred metres in over 20 years. It is possible that non-genetic responses maintain metal-binding proteins at high levels for a time and this may be the resistant genotypes that are invading clean sediments at a disadvantage relative to the resident non-resistant genotypes.

The cost of living in cadmium-rich sediments may have affected *L. hoffmeisteri* in other ways. The cost experiment mentioned above was used also to examine differences in reproductive effort. Montero and Levinton (1995) found that the worms from the cadmium-polluted Foundry Cove produced more offspring than worms from clean sediments of South Cove. This may be an adaptation to counteract lower growth rates and perhaps somewhat higher adult mortality rates, which we speculate are likely (Klerks and Levinton, 1989a).

If there was a high cost of adaptation to cadmium, we would expect that the resistance trait would be lost rapidly from Foundry Cove populations, following a clean-up. The US SuperFund Act-enforced clean-up of 1994 provided just such an experiment. As a control we also have followed resistance in South Cove worms after the clean-up, where no dredging was done. Sediments were dredged to a depth of ca. 30 cm and removed from the cove (J. Rod, personal communication). Following the clean-up, a small survey (Sokol *et al.*, 1996; Suatoni and Levinton, 1997) demonstrated that cadmium concentrations throughout the formerly polluted and relatively non-polluted areas of the cove were far lower (ca. 80 ppm) than those concentrations that caused detectable mortality in worms naive to natural selection for cadmium resistance (Klerks and Levinton, 1989a,b).

In 1996, L. Suatoni (Suatoni and Levinton, 1997; L. Suatoni, unpublished data) measured mortality patterns of *L. hoffmeisteri* in Foundry Cove for two years after the clean-up, which could be compared to previous survivorship studies (Klerks, 1987; W. Wallace, unpublished data), using a simple statistical test for comparing survivorship (Gehan's Generalized Wilcoxon Test; Lee, 1980). Two sites in Foundry Cove were compared with a non-polluted site in South Cove. Site 1 (ca. 500 ppm before clean-up, ca. 80 ppm after) in Foundry Cove was dominated by non-resistant individuals before the clean-up, whereas Site 2 (ca. 5000 ppm before clean-up, ca. 80 ppm after) was formerly dominated by very resistant individuals. In the first post-clean-up sampling, worms from both Foundry Cove sites were equally resistant to cadmium, but were more resistant than worms from the South Cove site. Their Cd body burdens (Table 1), however, were even lower than the control site. The Foundry Cove worms, however, were now significantly less resistant than the worms found in the high-cadmium sites before the clean-up, but even the Foundry Cove site 1 worms were more resistant than worms that had formerly lived at the same site. Clearly the clean-up, which involved extensive dredging, caused a strong degree of population mixing.

There are three possible explanations for the decrease in resistance levels following the removal of cadmium: (1) mixing of previously metal-resistant and non-metal-resistant populations within Foundry Cove; (2) the immigration of non-metal-resistant worms from surrounding populations; and (3) the existence of costs associated with metal resistance, resulting in a decline of resistance. Evidence

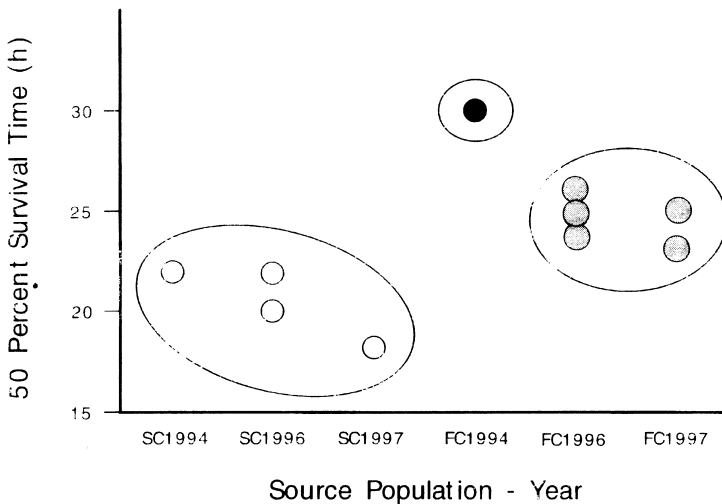
**Table 1. Body burdens of total Cadmium in *Limnodrilus hoffmeisteri*, before the clean-up (data from Klerks and Bartholomew, 1991) and after the clean-up in the summer of 1996 (data from Suatoni and Levinton, 1997).**

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

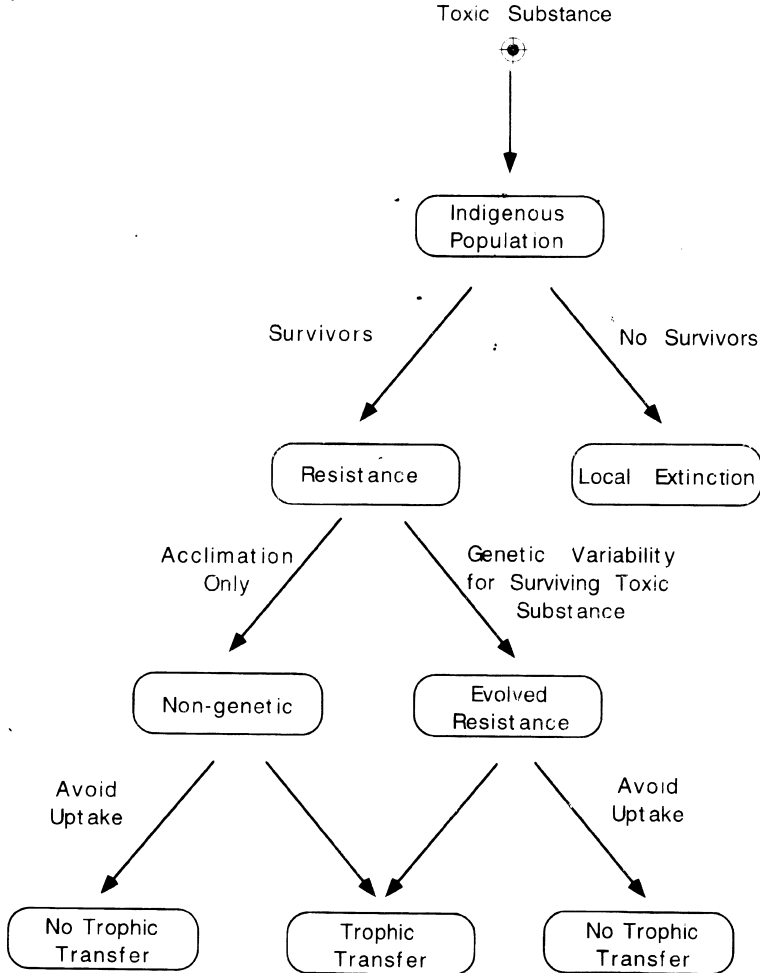
reported above does not support the hypothesis of costs. An unpublished study (C. Sturmbauer, personal communication) demonstrates that there are strong differences in frequencies of 16s rRNA genotypes between Foundry and South Coves, which militates against hypothesis 2. Thus hypothesis 1 is supported weakly as the only one not rejected.

In 1997, L. Suatoni (unpublished data) estimated mortality of worms from South Cove and Foundry Cove. Interestingly, resistance had not declined from 1996, further suggesting that there was no strong evolutionary or physiological cost to adapting to cadmium (Figure 8).

We will not discuss here in detail any other aspects of recovery at Foundry Cove. Of interest is the strikingly larger number of species that are now found in soft bottoms in Foundry Cove, relative to South Cove (Sokol *et al.*, 1996), whereas the reverse was true before the clean-up (Klerks and Levinton, 1992). Oligochaetes, moreover, are now less abundant in Foundry Cove relative to South Cove (Sokol *et al.*, 1996; Suatoni and Levinton, 1997) whereas they were more abundant before the clean-up (Klerks and Levinton, 1989a). The diversity may relate to the disturbance by dredging, which may have created new microhabitats, including a generally deeper cove. A fringing marsh was replanted but grazing by birds has forestalled recovery, and some weedy species have become dominant (personal observations).



**Figure 8.** Survivorship of Foundry Cove worms and South Cove worms before the clean-up (1994) and after the clean-up (1996 and 1997). Ovals enclose groups of points that are statistically homogeneous and significantly different from other groups. Data combined from Suatoni and Levinton (1997) and L. Suatoni (unpublished data)



**Figure 9.** A flow chart for examining polluted habitats for resistance to toxic substances and potential for trophic transfer.

## Reprise — what needs to be done with toxicology testing?

Our work may be summarized in a flow chart that may be applied to other natural communities exposed to toxic substances (Figure 9). Importantly, this approach requires an understanding of indigenous populations and their responses to the stress imposed by the toxic substance. It would be inappropriate to expose, as has often been done, a standard stock culture of organisms, reared and perhaps inbred under laboratory circumstances, to predict the fate of the population in any specific field site. The laboratory stock may or may not have the non-genetic capacity for individuals to acclimate to the introduction of the toxic stress. Geographic variation and even cryptic species are often found, which makes it dangerous to generalize from results found using one stock to an entire species, especially in different localities. Differences in species-specific and between-population toxicities must be taken into account (Luoma, 1996; Duan *et al.*, 1997).

Bioassay tests used by government agencies, furthermore, never include a multigenerational selection component. Even if they did, the genotypic makeup of the laboratory stock culture, taken from one geographic locality, may be quite different from the target population found in another. In an extreme case, we would expect that the laboratory stock would lack resistant genotypes that could be selected to high frequency. But in polluted environments we might expect that pollution in the region might have preserved resistant genotypes at some large frequency. Thus, there might be a storehouse of resistant genotypes, ready for natural selection. Furthermore, cross-resistance between toxic substances (e.g., between Cu and Cd reacting to metallothionein) might confer genetic resistance more frequently than expected, even to generalized stress and from exotic chemical toxins (Fisher, 1977). Laboratory stocks might be quite homogeneous and might even have been selected for a lack of variation, which makes the choice of laboratory stocks in toxicology a subject of continuing controversy (Forbes and Forbes, 1994).

Another important consideration is the physiological cost of responding to the toxic substance. We have mentioned cost above but here define it in a physiological and genetic context. The physiological cost is the reduction of viability when an individual is exposed to the toxic substance. Thus, in Foundry Cove sediments, both survival and growth are reduced strongly by the presence of a high concentration of cadmium (Klerks and Levinton, 1989a; Montero and Levinton, 1995). This reduction in fitness might be debilitating for the lifetime of the animal but it might disappear entirely if it dispersed to a clean sediment and depurated. On the other hand there might also be a cost of evolution, which would make genetically resistant populations perform more poorly when introduced into clean environments, as compared to populations that had not ever been selected for resistance. In Foundry Cove, there is evidence for a reduction of viability owing to toxicity effects but no evidence for a cost of fitness during the evolution of resistance.

We believe that future studies must take the local context of pollution, acclimation and evolution into account. Without an understanding of indigenous evolution for resistance in Foundry Cove, it would have been impossible to understand the spectrum of changes and potential of trophic transfer of metals through benthic food webs.

## Acknowledgements

This project could not have been completed without the continuing support of the Hudson River Foundation and the unfailing courtesy and support of James Rod of the Constitution Marsh Audubon Sanctuary. We dedicate this paper to his uncommon abilities as a naturalist and environmentalist and, sadly, report his untimely death in 1998. The members of a field course from the State University of New York at Stony Brook collected some of the data on recovery following the clean-up of Foundry Cove.

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