Costs of Adaptation to Cadmium in *Limnodrilus hoffmeisteri*, a common Hudson River invertebrate.

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Abstract

Heavy metals are toxic substances to most aquatic invertebrates. However, the oligochaete *Limnodrilus hoffmeisteri* from a metal-polluted area on the Hudson River (Foundry Cove) has developed genetic resistance to cadmium. Growth, as well as reproduction experiments were conducted with *L. hoffmeisteri* from metal-free (South Cove) and metal-rich areas (Foundry Cove), in order to test the hypothesis that there are costs associated with the evolution of resistance to metals. Three treatments were tested: (1) worms from Foundry Cove in Foundry Cove sediments, (2) worms from Foundry Cove in South Cove sediments, and (3) worms from South Cove in South Cove sediments (control). Results showed that *L. hoffmeisteri* from metal-polluted areas grew in clean sediments as well as their conspecifics from clean sediments. Growth results showed that there were costs associated with the maintenance of the resistance to metals (such as lower somatic growth). Foundry Cove worms in Foundry Cove sediment accelerated reproduction with respect to the other treatments and seemed to produce more offspring. Results are interpreted in terms of life history of this and other species.

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Introduction

Stress is defined as any environmental condition that acts to reduce Darwinian fitness of organisms, reducing chances of survival, reducing fecundity, or increasing the time between life-cycle events. Stress would act as a selection pressure and organism can respond adaptively to it (Sibly & Calow 1989). The response would depend on the amount of genetic variation that is available for selection. The stress response is dynamic and involves an alteration of function properties over time by processes of homeostatic physiological compensation (Koehn & Bayne 1989). The relative fitness of an individual with respect to others of the same species has several components and it can be investigated in terms of comparative growth, survival, and offspring production.

Heavy metals have been demonstrated to be toxic under both field and laboratory conditions. Toxicity, however, differs between metals (Reish 1978). Metals stress organisms, impairing physiological as well as "strategic" costs (e.g., lower competitive ability) (Wilson 1988). Populations exposed to pollutants can develop mechanisms to resist such toxicity but these are likely to be energetically expensive and occur at the expense of somatic growth. Physiological mechanisms capable of increasing an organism's resistance to metals can be grouped into three categories: mechanisms that reduce the concentration of the bioavailable metal externally, those that reduce the net accumulation at unchanged metal levels, and internal detoxification mechanisms.

Foundry Cove is a tidal freshwater bay located near Cold Spring, New York on the east side of the Hudson River, approximately 100 km upriver from the Verrazano Narrows. Foundry Cove sediment exhibits high levels of heavy metals (Ni, Co, and especially Cd) caused by waste discharged from a nearby battery factory. Cd levels are particularly high and an estimated 179 MT of cadmium were discharged into the cove from 1953 to 1979. concentrations (before cleanup) were as much as 250,000 $\mu g\ g^{-1}$ dry sediment near the factory outfall and varied between 10, 000 $\mu g\ g^{-1}$ in the zone close to a marsh to 500 $\mu g\ g^{-1}$ in the outer part of the cove. Foundry Cove was declared a "Superfund" site by the U.S. Environmental Protection Agency and was dredged in an attempt to restore its pristine condition.

Despite the high levels of pollutants seen in Foundry Cove, some species of invertebrates are present in relatively high densities. This is the case for the tubificid oligochaete *Limnodrilus hoffmeisteri*, which is the most abundant species showing a uniform distributions in the area (Klerks 1987). Bacteria act as intermediates for the transfer of heavy metals from liquid effluents and are a valuable food source for deposit feeders such as tubificids (Sager & Pucsko 1991). Tubificids can take up cadmium in solution from pore waters. The uptake, transport, and sequestering of metals is known to occur in this species and the spread of metals throughout communities by transfer to higher trophic levels has also been demonstrated (Wallace 1992). Previous studies (Klerks & Levinton 1989) reported the evolution of genetically-based resistance to metals in *L. hoffmeisteri*. The resistance is probably mediated by metallothionein-like proteins.

The pollution in Foundry Cove offers a unique opportunity to study the evolution of metal resistance. The aim of this work was to compare some aspects of fitness between populations of the oligochaete *Limnodrilus hoffmeisteri* from metal-rich and metal-free environments. My hypothesis was that there are costs associated with the maintenance of the resistance to metals. The organism would pay a price for resistance such as lower somatic growth or lover reproductive rate. Such costs would be reflected in tradeoffs in the life history of this species.

Materials and Methods

The two study sites were the polluted Foundry Cove and South Cove, a nearby bay in the Hudson River that has no significant levels of contamination and which was used as a control (Figure 1). In order to test the cost hypothesis I set up cultures of *L. hoffmeisteri* in the laboratory from natural populations growing in metal-free and metal-rich sediments. Although at this moment the cleaning up of Foundry Cove is almost completed, stocks of worms and sediment were collected before dredging in November 193. Sediment from Foundry Cove had an average cadmium concentration of 3,400 ppm (W. Wallace, personal communication). Worms and sediment from South Cove were collected in December 1993. Cultures were maintained in the laboratory under constant conditions of 22°C, 12:12 hours (light:dark) and all cultures were aerated. A fraction of sediments was kept frozen at -20°C until the moment prior to the experiments.

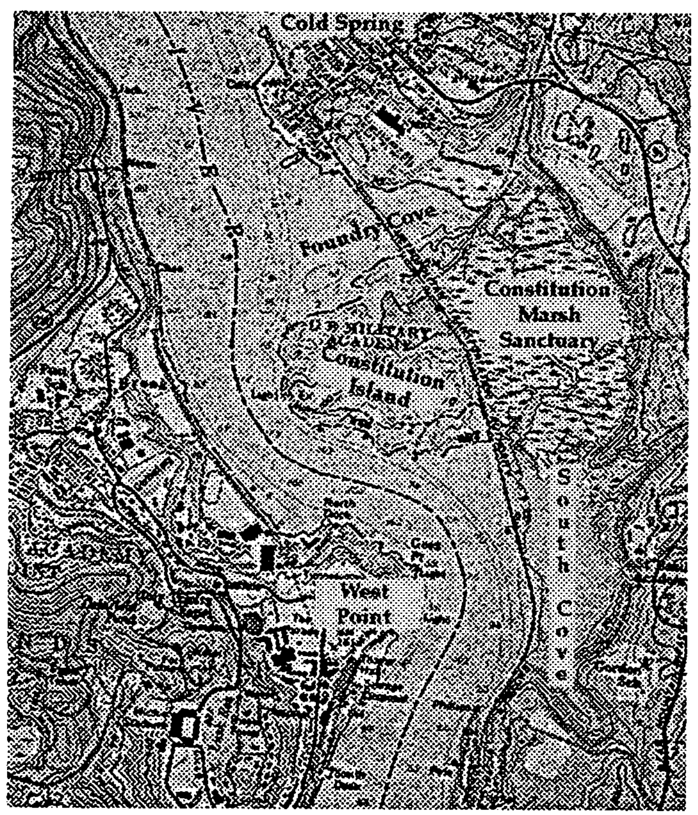


Figure 1. Map of the Hudson River in the vicinity of Cold Spring, New York, showing the Foundry Cove (SuperFund site) and South Cove source sites for worms.

I performed the following experiments:

1. Growth experiments:

In *L. hoffmeisteri*, after fertilization, eggs are deposited in protective albuminous cocoons, 2 to 10 embryos per cocoon. The embryonic period lasts from 15 to 17 days (Poddubnaya 1979) after which juvenile worms emerge from the cocoon. Cocoons were obtained from cultures of both populations and were hatches in the following conditions:
(1) cocoons from Foundry Cove in Foundry Cove sediment (FC/FC)
(2) cocoons from Foundry Cove in South Cove sediment (FC/SC)
(3) cocoons from South Cove in South Cove Sediment (SC/SC)
Emerged worms were grown individually in separate bowls (25 ml volume) with approximately 1 cm of pre-frozen sediment and previously aerated tap water. Three treatments were tested:
(1) worms from Foundry Cove growing in Foundry Cove sediment (FC/FC)
(2) worms from Foundry Cove growing in South Cove sediment (FC/SC)
(3) worms from South Cove growing in South Cove sediment (SC/SC)
The SC/SC treatment was used as a control. Total body length was used as a measure of size. Measurements were performed weekly by video monitoring using a color video camera (Canon L1). Sediment was replaced after each check. Images were stored and analyzed with the program NIH Image in a Power Macintosh 8100/80 AV computer. Results were analyzed using one-way ANOVA (Sokal & Rohlf 1981). Measurements were performed only up to the fifth week because worms showed a tendency to coil when the reached a certain size, which makes the video method imprecise.

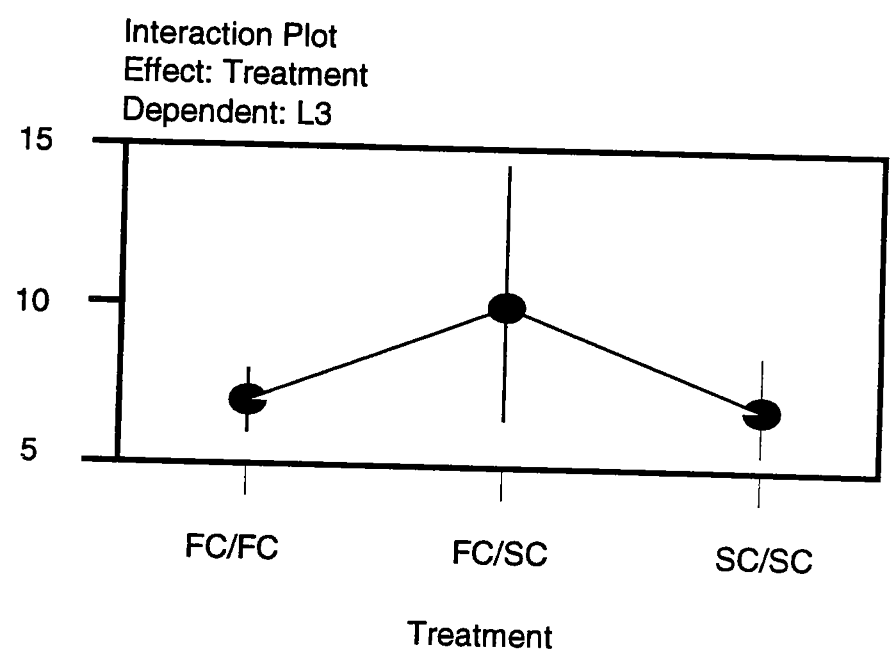
2. Reproduction experiments :

Mature adults of *L. hoffmeisteri* from South Cove and from Foundry Cove were randomly collected from the stock cultures and put in couples in glass bowls (100 ml volume) with 2 cm of pre-frozen sediment according to the treatments: FC/FC; FC/SC; SC/SC. A total of 54 pairs were used, 18 for each treatment, arranged in three trays with six replicates within each tray. Reproducing pairs were checked weekly and the number of offspring was recorded. Sediment was not replaced but new sediment was added after each check. The experimental design was analyzed as a nested balanced ANOVA (Sokal & Rohlf 1981). In addition, I estimated the u number of eggs per cocoon. Samples of 30 cocoons from each population were dissected and eggs were counted (regardless of their stage of development). A t-test of sample means was used to test for differences between groups.

Results

1. Growth experiments.

No significant differences ($p < 0.05$) in total length were observed at the beginning of the experiments between worms hatched under the different treatments (Table 1). Lengths (log transformed) were significantly different by the third week (Table 2) and were maintained up to the fifth week, when the experiment was terminated. A Tukey-Kramer a posteriori pairwise comparison showed significant differences between FC/SC and both FC/FC and SC/SC treatments (Figure 2). Worms from Foundry Cove growing in South Cove sediments were the largest. Growth rate between first and third weeks (standardized by initial length) was also significantly higher in the FC/SC treatment (Table 3), no significant differences were observed between the FC/FC and SC/SC treatments.



	Contrast	Diff.	Critical Diff.	Significant?
SC/SC	FC/FC	0.115	3.11	No
SC/SC	FC/SC	3.648	3.50	Yes
FC/FC	FC/SC	3.533	3.308	Yes

Figure 2. Upper: Length at third week of the growth experiments; bars are 95% confidence limits. Lower: Tukey-Kramer a posteriori comparisons. Significance test at 5 % level.

Table 1. Length at the beginning of growth experiments. $p > .05$ = not significant.

Source	df	Sum of Squares		Mean Square	F-value	P-value
Treatment	2	17.143	8.572	1.945	0.15	
Residual	80	352.590	4.407			

Table 2. Length at third week of growth experiments. $p < .05$ = significant.

Source	df	Sum of Squares		Mean Square	F-value	P-value
Treatment	2	123.616	61.808	4.129	0.02	
Residual	47	703.614	14.971			

Table 3. Growth rate between first and third week of the growth experiments. $p < .05$ = significant.

Source	df	Sum of Squares		Mean Square	F-value	P-value
Treatment	2	2.732	1.366	6.092	0.005	
Residual	46	10.314	0.224			

2. Reproduction experiments.

Reproduction was measured as the number of offspring produced by a pair of mature worms after three and five weeks. I assumed that all hatched worms were *L. hoffmeisteri* because classification is based upon adult traits.

There was a tendency for a higher number of offspring per pair in the FC/FC treatment (Figure 3). When data, however, were analyzed by ANOVA, treatments were not significant due to a tray effect, which adds significant variance to the model (Table 4).

Table 4. Number of offspring per treatment at the third week of reproduction experiments.

Source	df	Sum of Squares		Mean Square		F-value	P-
value	Error Term						
Treatment	2	7.546	3.773	1.174	0.3713	Tray (Treatment)	
Tray	6	19.281	3.214	2.946	0.017	Residual	
Residual	46	48.000	1.091				

The number of reproducing pairs in each treatment was estimated after three and five weeks (Table 5). A larger number of worms form the FC/FC treatment reproduced at both dates, but the G-test reveals independence of the number of reproducing pairs from the treatment. However, when only the FC/FC and SC/SC treatments (which represent the natural conditions) were compared, significantly more worms reproduced in the FC/FC treatment than in the SC/SC treatment ($G = 4.130$, corrected for small samples by Williams correction, $p < 0.05$, (Sokal & Rohlf 1981). The number of eggs per cocoon was not significantly different between the samples (t-test = 1.23 $p > 0.05$).

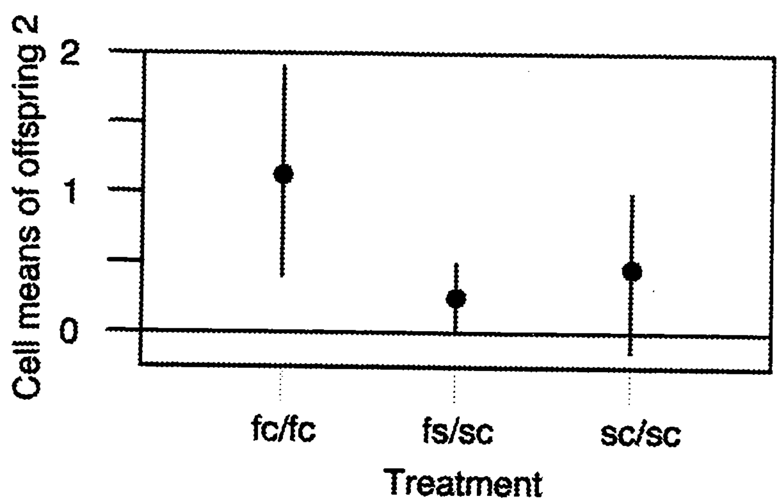


Figure 3. Number of offspring per reproducing pair of *Limnodrilus hoffmeisteri;* bars are 95 % confidence limits.

Table 5. Number of reproducing replicates per treatment at the third and fifth week of the reproduction. experiments. For the third week, G = 2.16 (not significant); for the fifth week, G = 4.92 (not significant). See text for further analysis.

Treatment	Third Week Reproductive	%	Fifth Week Reproductive	%
FC/FC	7	38.8	10	55.5
FC/SC	5	27.8	5	27.8
SC/SC	3	16.7	4	22

Discussion

L. hoffmeisteri is a cosmopolitan aquatic oligochaete (Brinkhurst 1965) that is known to be extremely tolerant to pollution (Finogenova & Lobasheva 1987). The most important of the local conditions that can influence its life cycle appears to be the quality of the habitat (Kennedy 1966). At present we don't know if foundry Cove and South Cove sediment differ in characteristics other than their metal content, but heavy metals probably play a major role in determining the conditions for *L. hoffmeisteri* populations.

Results from growth experiments reflect the presence of a cost associated with the evolution of resistance to metals in the Foundry Cove populations, given the potential of worms to grow more and faster when environmental conditions are more favorable (absence of heavy metals). Studies on marine invertebrates have shown that "scope for growth" (energy available for somatic production) is lower in marine mussels at sites exposed to heavy metals (Bayne et al 1979). However, (Klerks 1987) did not find evidence for costs of resistance in *L. hoffmeisteri*. Indeed, Foundry Cove worms grew as well as those from the control area and did not seem to have fewer offspring. But he also observed a reduction in the second generation of Foundry

Cove offspring relative to field-collected worms. The result could imply that there is a cost associated with adaptation, due to a decreased resistance in the absence of a continued selection pressure. Exposure to metals does not always result in reduction of growth. In *Orchesella cincta* (Collembola) the smallest growth reduction occurs in populations where selection was assumed to be the strongest. In this species the absence of growth reduction was considered to be advantageous since clutch size is positively correlated with the weight of the mother (Posthuma 1990).

Resistance to metals is not always heritable. F_2 populations of *Onychiurus armatus* (Collembola) feeding upon metal-contaminated fungi reached the same maximum length as the parental generation but growth rate and survival were decreased (Bengtsson et al 1983; Bengtsson et al 1985). Life cycle traits can be affected in different ways by the stress imposed by heavy metals; in *O. armatus* lowered egg production was observed but eggs developed normally (Bengtsson et al 1985). This is not the case in *L. hoffmeisteri* where metal-rich populations seem to compensate for the costs of growth by accelerated reproduction keeping egg production constant. Results can be interpreted in terms of the mode of reproduction in this species. *L. hoffmeisteri* is a semelparous species, after reproduction most individuals die and very few of the survivors reproduced again (Kennedy 1966). As reproduction is an energetically expensive process, costs of reproduction are probably increased under stress conditions. In such a case one might expect organisms under unfavorable conditions to evolve or adopt the reproductive strategy (life cycle) that maximized Darwinian fitness (Sibly & Calow 1989). This can be achieved in two ways:
(1) Starting reproduction earlier. A short generation time is favored since it reduces the probability of death between birth and breeding.
(2) Allocating a high amount of resources to reproduction in order to produce more offspring. The latter seems to be the case for *L. hoffmeisteri* from Foundry Cove in Foundry Cove sediment where worms start reproduction earlier and also produce a larger number of offspring than the other treatments. In fact, it has been shown that *L. hoffmeisteri* has the ability to accelerate growth and development (Finogenova & Lobasheva 1987). These characteristics allow them to predominate under conditions of pollution, especially organic matter that is easily assimilated. (Eggers-Schumacher 1983) found that resistant clones were superior to susceptible clones in both fecundity and developmental period. The high variability between trays within treatments can be explained by the fact the resistance in *L. hoffmeisteri* is not an all-or-nothing phenomenon. Much variation is present within populations (Klerks 1987).

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