Bull. Environ. Contam. Toxicol. (1991) 46:473–478

Environmental Contamination and Toxicology

Effect of Starvation on Trace Metal Levels in Blue Mussels (*Mytilus edulis*)

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The use of bivalves as monitors of marine coastal contamination has become widespread since its suggestion by Goldberg (1975). However, many investigations (reviewed by Phillips 1980) have shown that numerous factors affect contaminant concentrations, in particular trace metals, in mussel tissues. This is not surprising since some metals, e.g., copper and zinc, have well-established physiological roles, while others, such as cadmium, are toxic to higher animals and have no known physiological function. The situation within lower phyla, however, is less clear. Lobsters (*Homarus americanus*) accumulate high cadmium concentrations in their digestive glands in both clean and contaminated situations with no demonstrable negative effects (Uthe and Zitko 1980). Chou et al. (1986) reported no effect on growth or survival from feeding cadmium-fortified diets (up to 45 mg $Cd \cdot kg^{-1}$ wet wt.) to juvenile lobsters. Dietary copper and silver have an optimum dietary ratio for lobster growth and survival (Chou et al. 1981) suggesting a biochemical role for silver in this species either as a micronutrient or as a detoxification factor for excess copper.

An organism can serve as a quantitative indicator of environmental contamination only if a tissue contaminant concentration or burden (defined as the tissue contaminant concentration times the total tissue weight) reflects the contamination of the animal's environment in a rational way. Results with radionuclides (e.g., Pentreath 1973) have been used to calculate uptake and depuration rate constants and biological half-lives. Researchers recognize that these calculated rates and half-lives are dependent upon both the physiology of the animal and its external environment. This is particularly true for metals that are under some sort of biological control. Cadmium, for example, is tightly bound to protein in shellfish (Highman et al. 1986) and is not lost from lobster when attempts are made to remove it by holding in uncontaminated water or through chelation treatment (Uthe 1980). Cadmium was not lost from sea scallops (*Placopecten magellanicus*) collected offshore and held in filtered seawater for fourteen months in spite of a 55.3% loss in soft-tissue weight

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(Uthe and Chou 1987). Although cadmium burden was tightly related to shell height, nutritional factors, i.e., slower growth and ration, were suggested as the underlying reasons for cadmium concentrations and burdens in scallops from an offshore, uncontaminated area being significantly higher than in equivalently sized scallops from an inshore, cadmium-contaminated area. Similar findings could be expected with contaminants which, once taken up, were slowly, if at all, excreted. We have investigated the effect of starvation on a number of trace elements in blue mussels (*Mytilus edulis*) to determine which elements were not eliminated as the animal starved (a "burden" control model) and which elements were excreted (a "concentration" control model) in response to decreasing tissue weight.

MATERIALS AND METHODS

Mussels (approximately 5.0 cm shell length) were collected during autumn at half-tide height in the vicinity of Petit Rocher, New Brunswick, Canada. The area is located approximately 10 km from a coastal lead smelter. Animals were transported in seawater collected at the sample site and held overnight in flowing, filtered seawater. After draining, length and total weight of mussels were determined. The soft tissue was removed from each of 25 mussels, weighed in a graduated Folin-Wu tube and gently refluxed (1.5-2 hr.) in 5 mL nitric acid . After making up to volume with quartz-distilled water, elemental concentrations were determined using atomic absorption spectrophotometry (Perkin-Elmer model 403) with deuterium arc background correction. Furnace methodologies and the method of standard additions were used as required. A second group was held in filtered, flowing seawater for 3 months without feeding prior to autopsy and trace elemental determinations.

Trace metal concentrations in filtered aquarium seawater were measured (Dr. P.A. Yeats, Marine Chemistry Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia) (Table 1). With the exception of iron, the trace metal concentrations were in the low range of reported coastal seawater concentrations (Yeats et al. 1978).

Table 1. Trace metal concentrations ($\mu g \cdot L^{-1}$) in filtered aquarium seawater.

Trace Metal	Concentration	Trace Metal	Concentration
Cadmium	0.030	Arsenic	3.4
Nickel	0.24	Zinc	1.0
Iron	8.2	Copper	0.24
Manganese	2.4	Lead	0.022

RESULTS AND DISCUSSION

Starvation had no effect on either shell length or total weight (Table 2), however, a substantial decline (43.3%) in soft tissue weight was observed. The same response to starvation has been reported in scallop (Uthe and Chou 1987) and shows that starving bivalves with a hard calcareous shell make up for the decreasing soft tissue mass by fluid replacement.

Substantial differences were observed in the responses of trace metal concentrations and burdens to starvation (Table 2). Cadmium was not eliminated during starvation, resulting in a significant increase in concentration in the soft tissue mass but without any significant change in its cadmium burden. This is the response that was observed in scallops (Uthe and Chou 1987). Uthe and Chou (1987) suggested that the binding of cadmium in scallop digestive gland (the tissue which contains >90% of the cadmium burden) may be slow compared to cadmium uptake under certain conditions. Thus, it is easy to reconcile the apparently conflicting evidence of rapid cadmium excretion and a long half-life with a two-compartment model, one of which can excrete cadmium or relocate it slowly to the second compartment which retains it.

Table 2. The effect of three months starvation on mean trace metal concentrations ($\mu g \cdot g^{-1}$ wet wt ± sd), burdens (μg ± sd), and size parameters in blue mussel soft tissue.

	Unstarved		Starved	
	Conc.	Burden	Conc.	Burden
Cd	2.62±0.64	7.28±3.38	4.59±1.44^{s} (+74.0)	6.83±2.05^{ns} -
Zn	34.1±18.9	90.1±51.0	32.4±13.9^{ns} -	50.3±28.6^{s} (-44.3)
Cu	1.56±1.07	4.86±6.41	2.17±0.47^{s} (+39.1)	3.49±1.44^{ns} -
Ag	0.02±0.01	0.05±0.03	0.05±0.03^{s} (+178)	0.08±0.03^{s} (+61.0)
Se	0.63±0.10	1.79±0.81	0.71±0.13^{s} (+12.8)	1.08±0.26^{s} (-39.7)
Mn	2.14±0.81	5.79±2.65	2.91±1.12^{s} (+36.0)	4.33±1.59^{s} (-25.2)
As	1.43±0.31	4.11±2.16	1.52±0.38^{ns} -	2.28±0.65^{s} (-44.5)
Fe	33.3±6.00	96.7±55.4	52.3±12.8^{s} (+57.1)	77.6±17.0^{s} (-19.8)
Mg	597±99	1602±526	1013±116^{s} (+69.8)	1539±307^{ns} -
Pb	2.08±1.84	7.23±9.15	2.44±0.81^{ns} -	3.68±1.17^{s} (-49.1)

	Unstarved	Starved
Soft Tissue Weight (g)	2.87±1.30	1.54±0.38^{s} (-46.3)
Shell Length (cm)	5.45±1.08	5.12±0.45^{ns}
Total Weight (g)	22.38±13.75	18.36±4.42^{ns}

[figures in parentheses are percent change; ^{s} - significant, ^{ns} - not significant, starved vs unstarved, P = 0.05, modified t test (Snedecor and Cochran 1967), outliers removed; ±2.35 x sd; n = 25]

Zinc showed a very different response to starvation. Starvation resulted in a significant drop in the tissue burden, whereas the zinc concentration remained unchanged at approximately 35 times that of seawater. These results suggest that zinc concentrations are rather controlled within the animal through a dynamic mechanism. Pentreath (1973) has shown that radioactive zinc is rapidly taken up by mussels. The role of zinc in metalloenzymes may be reflected in a need to maintain intracellular zinc concentrations. Zinc concentrations in mussels were independent of tissue weights (Phillips 1980). These results must be contrasted to the report of high zinc concentrations in membrane-limited electron dense granules (amoebocytes) in renal tissue of oyster (*Ostrea edulis*) (George et al. 1978) and blue mussels (George and Pirie 1980). The latter authors reported that approximately 30% of the total zinc burden in the soft tissues is in the kidney. The loss of almost one-half of the zinc burden by starvation suggests that the zinc in these granules is readily metabolized.

Copper, like cadmium, appears to be under a "burden" control mechanism, i.e., starvation did not result in significant copper loss from the tissue. George et al. (1978) reported that copper is present in amoebocytes in oyster. Either mussel amoebocytes differ from those of the oyster or one must postulate a mechanism allowing for zinc, but not copper, loss during starvation.

Starvation resulted in an increase in both silver concentration and burden. The underlying reason for accumulation of silver under starvation conditions is unclear. It might simply reflect an increase in silver concentration in the holding seawater compared with that at the collection site. Chou et al. (1981) suggested a role for silver in the metabolism of copper, and Chou and Uthe (1978) reported a strong correlation between silver and copper concentrations in marine crustacean tissues.

The response of selenium was that of both a significant increase in concentration and a significant decrease in burden during starvation, suggesting a more complex control mechanism than a burden or concentration one. This may simply reflect the number of selenium compounds that occur in tissue (Diplock 1976), coupled with a slow elimination rate compared to starvation.

Manganese concentration increased significantly over the starvation period while the burden showed a significant decrease. It appears that the animal is unable to excrete manganese as rapidly as required to maintain a constant concentration in the soft tissues. The decrease in burden could also be accounted for by elimination of gut contents, since elimination of all gut contents probably takes longer than the 1-2 day depuration period.

Arsenic burdens fell significantly during starvation with no significant change in concentration. It is known (Freeman et al.

1978) that arsenic in many marine animals is present in a water-soluble form which is rapidly taken up from the diet and rapidly eliminated in the urine. A similar mechanism may be present in mussels to maintain concentrations in equilibrium with coastal seawater.

Iron concentration increased while burden decreased during starvation, thus falling into the group characterized by selenium, manganese and, possibly, arsenic. This is not to suggest a biochemical commonality in response. The decrease in burden could also have been due to elimination of gut contents over the period of starvation.

The results for magnesium, i.e., a constant burden and increased concentration with starvation, were surprising since magnesium is known to play an important role in the stabilization of polymeric phosphate-containing molecules such as DNA, phosphoryl-transfer enzymes and ATP (reviewed by Nieboer and Richardson 1980) and one might have reasonably expected tissue concentrations to be constant. However, if the majority of magnesium is present in pearls within the tissue, a concentration control mechanism within the tissue could have been masked.

The results for lead showed no significant change in concentration and a significant decrease in burden. While this suggests that lead concentrations are controlled, the fact that no biological role for lead is known renders such a mechanism unlikely. Rather, these results may simply reflect the effect of transferring the animals to cleaner water and lead removal at a rate similar to the starvation rate. Seargent and Westlake (1980) report that mussels transferred from lead-contaminated water to cleaner water lost lead.

These results show that trace metals in mussels are under the influence of a variety of mechanisms, some of which are likely to be biochemical in nature, while others, e.g., for arsenic, simply may reflect the physicochemical nature of the element. The results suggest that modeling the mussel system, particularly in situations which are not severely contaminated, will be complex and may necessitate dealing with trace metals on both an individual and interactive basis. The occurrence of these additional biochemical effects on trace metal levels in biota, in addition to those such as sex, salinity, age, tide height of collection, etc. (Phillips 1980), shows the need for a clear understanding of trace metal metabolism in bivalves under normal and stressful conditions. There is no reason to assume that the same control mechanism will dominate in both clean and contaminated situations.

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Received August 13, 1990; accepted August 24, 1990.