

MANGANESE CONCENTRATION IN LOBSTER (*HOMARUS AMERICANUS*) GILLS AS AN INDEX OF EXPOSURE TO REDUCING CONDITIONS IN WESTERN LONG ISLAND SOUND

ANDREW F. J. DRAXLER,^{1*} ROBERT M. SHERRELL,² DANIEL WIECZOREK,¹
MICHELE G. LAVIGNE² AND ANTHONY J. PAULSON³

¹NOAA-Fisheries, Howard Marine Laboratory, Highlands NJ 07732

²Institute of Marine and Coastal Sciences, and Department of Geology, Rutgers, The State University of New Jersey, New Brunswick NJ 08901. ³USGS, Tacoma, Washington.

ABSTRACT We examined the accumulation of manganese (Mn) in gill tissues of chemically naïve lobsters held *in situ* at six sites in Long Island Sound (LIS) for up to six weeks to evaluate the possible contribution of eutrophication-driven habitat quality factors to the 1999 mass mortality of American lobsters (*Homarus americanus*). These western LIS lobster habitats experience seasonal hypoxia, which results in redox-mobilized Mn being transferred to and deposited on the tissues of the lobsters. Manganese accumulated in gill tissue of lobsters throughout the study, but rates were highest at western and southern LIS sites, ranging from 3.4–0.8 $\mu\text{g/g/d}$ (~16 $\mu\text{g/g}$ initial). The Baden-Eriksson observation that Mn accumulation in Norway lobsters (*Nephrops norvegicus*) is associated with ecosystem hypoxia is confirmed and extended to *H. americanus*. It seems likely that, after accounting for molting frequency, certain critical values may be applied to other lobster habitats of the NE US shelf. If a high proportion of lobsters in autumn have gill Mn concentrations exceeding 30 $\mu\text{g/g}$, then the habitats are likely experiencing some reduced oxygen levels. Manganese concentrations above 100 $\mu\text{g/g}$ suggest exposure to conditions with the potential for lobster mortality should the temperatures of bottom waters become elevated, and gill concentrations above some higher level (perhaps 300 $\mu\text{g/g}$) indicate the most severe habitat conditions with a strong potential for hypoxia stress.

KEY WORDS: lobster, *Homarus americanus*, manganese, hypoxia

INTRODUCTION

American lobsters (*Homarus americanus* H. Milne Edwards, 1837) in LIS are at the southern extremity of their inshore range. In 1999, a mass mortality struck lobsters in the western one third of the Sound. Among the scenarios considered to explain the event (Pearce & Balcom 2005) was a biologic infection exacerbated by environmental stressors (high temperature, low dissolved oxygen and high concentrations of sulfide and ammonium).

Seasonal hypoxia in the bottom waters of western Long Island Sound (LIS; Fig. 1), followed by sequential increases in sulfide and ammonium (Valente & Cuomo 2001, Cuomo & Valente 2003), arises as a natural consequence of cultural eutrophication emanating from the New York City metropolitan area. The organic matter resulting from the nutrient-stimulated planktonic overproduction falls out of the lighted zone (Parker & O'Reilly 1991), enriching the habitat of the benthic microbial community, which then depletes the bottom waters of the dissolved oxygen, normal in mid August. After dissolved oxygen is exhausted, Mn(IV) is the next most plentiful terminal electron acceptor permitting the continuation of microbial oxidation of organic detritus. The resulting reduced Mn(II) is soluble and enters the water overlying the sediment where it is available to the biota (Aller 1994, Baden et al. 1994).

Manganese is a required metabolic element and although excess concentration can have negative physiologic effects (e.g., Holmes et al. 1999), it is relatively nontoxic to marine animals. For example, the 48-h LC₅₀ of 16 mg/L for oyster (*Crassostrea virginica*) embryos (Calabrese et al. 1973) and ~59 mg/L for a sea star (*Asterias rubens*; Hansen & Bjerregaard 1995) are about 120 and 430 times the Mn concentration we observed in LIS bottom water. Higher temperatures are associated with increased rates of Mn accumulation in biota (Eriksson 2000), but low dissolved oxygen concentrations alone are not (Baden et al. 1995). In principle,

externally deposited Mn is lost on molting although a portion may be included in the structure of the shell when the animal ingests the old exoskeleton. Eriksson and Baden (1998) reported that Mn in gill tissues of the Norway lobster, *Nephrops norvegicus*, was associated with areas of hypoxia along the Swedish coast and these same workers have suggested (Baden et al. 1995) that the Mn concentration in decapod tissue may be a useful indicator of exposure to hypoxia. The mass mortality of lobsters in western Long Island Sound (LIS) in 1999 provided an opportunity to examine this speculation. The study reported here was designed to extend the Baden-Eriksson idea of Mn as an indicator of lobster habitat quality from *N. norvegicus* to *H. americanus*. We hypothesized that the results could also help to validate reference values for *H. americanus* that would be applicable in other areas of the United States coast where hypoxia-related conditions are of concern for loss of the lobster resources.

METHODS

During the first week of July 2001 and 2002, we deployed between 9 and 24 chemically naïve lobsters in individual cages at each of six locations in western Long Island Sound (Fig. 2). All lobsters were commercially caught from >100 m depth in Atlantis Canyon, approximately 280 km southeast of the eastern entrance to Long Island Sound. They were ~570 gm in weight, 225–270 mm in length and were at molt stage B or C. In 2001, an approximately equal mixture of males and females was used whereas in 2002 all were males. Sites were selected to expose the lobsters to the west-to-east eutrophication gradient emanating from the New York metropolitan area, and to be on either sandy (Sites A1, B1, C1) or muddy (Sites A2, B2, C2) sediments based on Poppe et al. (1992). Each site was equipped with a temperature recorder and up to four sites had temperature-salinity-dissolved oxygen recording instruments. At 2-wk intervals, divers retrieved cages from each site, collected sediment and water column samples, exchanged hydrographic instruments and then fed western LIS clams to the remaining lobsters. In the shipboard laboratory, habitat samples were split into

* Corresponding author: andrew.draxler@noaa.gov

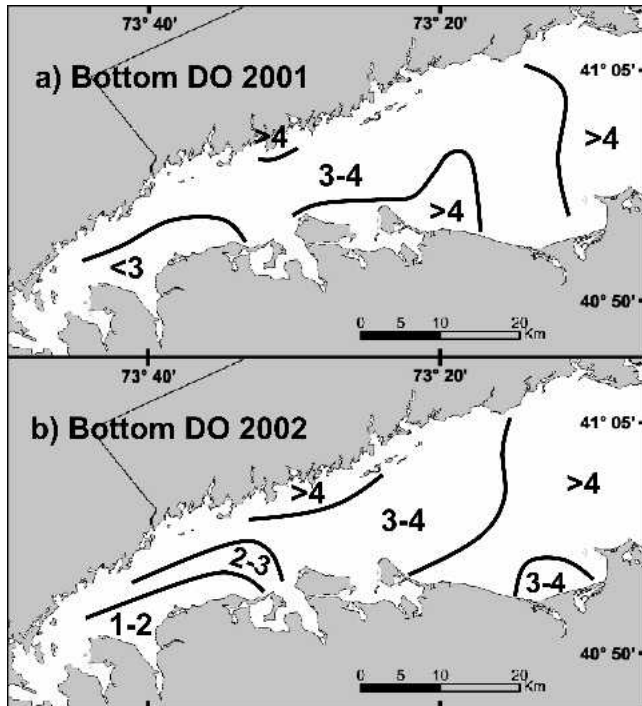


Figure 1. Hypoxia in western Long Island Sound. Average bottom water dissolved oxygen concentrations (mg/L) during which caged lobsters were deployed. Data courtesy of Connecticut DEP cruises in 2001 (6–11 and 24–26 July, 6–9 and August 20–22) and 2002 (8–10 and 22–25 July, 5–8 and August 22–23).

aliquots for biogeochemical and contaminant analyses. Retrieved lobsters were dissected on board in a HEPA clean air hood. Tissues were also preserved for analyses of metals, chlorinated hydrocarbons, bacteria and other pathogens, the data from which will be reported elsewhere. In addition, a small number of native LIS lobsters, which were captured nonsystematically in mid August of each year in traps deployed with our cages, were processed in the same way.

Gill tissues were returned to the analytical laboratory frozen where they were archived for analysis. All sampling materials and analysis tubes were acid-leached prior to use. Digest vials and caps were boiled in aqua regia and heated with nitric acid, then rinsed thoroughly in Milli-Q deionized water. Frozen gill samples were further cooled in a -80°C freezer, freeze dried, and manually homogenized with an acid-cleaned glass stir rod and stored in a desiccator cabinet. The following digestion method was used; a subsample of ~ 50 mg (± 5 mg) of homogenized gill powder was digested in Teflon vials with $700\ \mu\text{L}$ concentrated trace metal grade HNO_3 at 100°C to 105°C for 8 h on a hotplate, allowed to cool overnight, and heated with an addition of $700\ \mu\text{L}$ of 30% H_2O_2 for 1 h with lids removed for the first 30 min. Clear digest solutions were cooled to room temperature, weighed and diluted to $\sim 10\%$ HNO_3 with Milli-Q deionized water, and stored in 15-mL polypropylene centrifuge tubes until ready for analysis. Digestion blanks were carried through the procedure and replicate samples of homogenized dry tissue were digested approximately every 25 samples.

Gill samples and digestion blanks were analyzed for Mn by magnetic sector inductively coupled plasma mass spectrometry in medium resolution (ELEMENT-1, Finnigan MAT, Bremen, Germany) by the standard addition technique described by Cullen et

al. (2001). In brief, digested samples were diluted by a factor of 10 for analysis with 3% ultrapure nitric acid and standardized against a 4-point standard addition curve made with certified standards (High-Purity Standards, Charleston, South Carolina) added to a representative sample. Digest blank Mn concentrations were subtracted from sample concentrations to correct for Mn blank introduced through sample preparation by acids, contact with Teflon vials and test tubes and handling.

Mean RSD for replicate gill digestion Mn measurements was 5.9% ($n = 10$). We determined Mn in a lobster hepatopancreas standard reference material for trace metals (TORT-2; National Research Council of Canada; certified at $13.6\ \mu\text{g/g}$ Mn $\pm 1.2\ \mu\text{g/g}$) to be 14.0 ± 0.2 (1 sigma; $n = 8$). Mean Mn blank concentration of lobster gill digestion procedure blank solutions ($1.85 \pm 1.4\ \text{ng/g}$ 1 sigma; $n = 15$) was 0.4% of the average Mn value for gill digest solutions ($523 \pm 713\ \text{ng/g}$ 1 sigma; $n = 126$). Manganese concentrations in gill tissue are reported on a dry weight basis throughout this report.

Manganese concentrations determined for gill tissue were fitted with a linear functional geometric mean regression (Ricker 1973) for each site over time to derive the slope as an estimate of accumulation rate (Table 1). Individual Mn gill concentrations were regressed stepwise on 17 habitat variables using Sigmastat 2.03 (SPSS, Inc., Chicago, Illinois).

RESULTS AND DISCUSSION

Return rates of deployed lobsters were lowest (55%) in the second and third retrievals in 2001 as a result of methodologic problems (lost gear, female lobsters extruding eggs, unsealed terrapin excluder openings) and mortality. Addressing these issues (e.g., using exclusively males) in 2002 deployments raised returns to between 79% and 96% with an overall return of 77% for the 2-yr study. Despite gaps in specimen availability (most notably at B1 in 2001), Mn accumulation in gills was strongly related to duration of deployment at all sites (Table 1). Most temporal trends were statistically significant at the $P < 0.01$ level. Even in the case of site C1 in 2001 where an outlier was excluded from the statistics reported, the regression was significant at the $P < 0.05$ level with all data included. We used a linear model to determine the relationship between Mn accumulation and duration of deployment, however, all cases except C1 in 2002 contained a higher degree of association (higher r^2) when fitted with

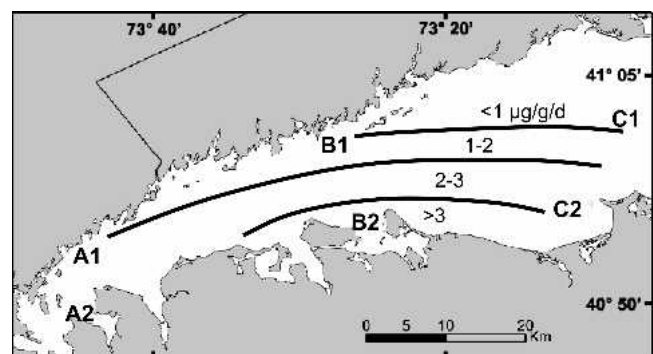


Figure 2. Locations of caged lobster deployments in western Long Island Sound and isopleths of mean manganese uptake rates by gill tissue ($\mu\text{g/g/d}$). Sites A1, B1, and C1 were sandy sediment and A2, B2, and C2 were muddy sediments.

TABLE 1.

Rate of manganese accumulation ($\mu\text{g/g/d}$) on gill tissue of chemically naïve lobsters from Atlantis Canyon held up to 6 wk in western Long Island Sound during July and August of 2001 and 2002.

Site	2001	n	R ²	2002	n	R ²	Site Mean
A1	2.52	11	0.71**	1.10 ^c	13	0.56**	1.81
A2	0.99	8	0.77**	1.51	15	0.41**	1.25
B1	1.12 ^a	7	0.72*	1.16	12	0.68**	1.14
B2	5.17 ^b	9	0.70**	1.56	14	0.67**	3.36
C1	1.09 ^c	8	0.78**	0.46	13	0.32*	0.78
C2	4.10 ^b	8	0.72**	1.00	12	0.54**	2.55
Mean by year	2.50			1.13			Study Mean = 1.82

Sites were aligned west to east from A to C. "1" sites were chosen to have sand substrate and "2" sites selected to have muddy sediment. All deployments were for 41 to 45 days except a = 15 days, b = 30 or 31 days. An outlying data point was excluded from the regressions noted with c (see text). Linear regressions significant at * $P < 0.05$, ** $P < 0.01$ levels.

a second order polynomial model. Because balance with the surrounding medium is likely to become established within a few days (Baden et al. 1999), the escalation in accumulation implied by the second order (convex up) fit likely reflects changing environmental conditions in the bottom waters of LIS. It is consistent with increases of Mn (II) supply, rather than the nonlinear accumulation kinetics, reflecting the onset of severe hypoxia beginning several weeks after the start of experiments and returning conservative estimates of late summer accumulation rates.

Manganese accumulated in gill tissue of the caged lobsters at all sites throughout the study. Rates ranged from 0.8–3.4 $\mu\text{g/g/d}$ beginning at an average initial concentration of 16 $\mu\text{g/g}$ for animals that spent no time in LIS. Mean accumulation isoclines (Fig. 2) partition the western LIS study area into four regions (<1, 1–2, 2–3 and 3–4 $\mu\text{g/g/d}$) with higher rates in western and southern areas, which are more seasonally hypoxic (Fig. 1), and lower rates to the north and east where dissolved oxygen conditions tend to be higher. It should be noted that the northern ("1") site in each pair was selected (based on USGS reports, Poppe et al. 1992) to have coarser sediment. A detailed examination of the biogeochemistry influencing Mn accumulation (A. Draxler & R. Sherrell, in preparation) is beyond the scope of this report. However, a stepwise regression on six significant habitat variables together explained more than 48% of the variation in Mn concentration on gill tissues in 2002. These are: (a) redox potential (E_h) in the sediment and water overlying core samples—temporal integrators of the oxygen condition at the sediment-water interface (SWI); (b) concentrations of phosphate and Mn in discrete bottom water samples—indicators of eutrophication and SWI exchange and (c) the length of time and the temperature during which the lobsters were held in the cages. Eleven other sediment characteristics (including grain size, measures of organic matter concentration, pH, pore water Mn) and bottom water variables (including pH, salinity and dissolved oxygen concentrations) did not significantly contribute to the explanation of gill Mn variation. Because sediment physical characteristics used as site selection criteria were not significantly correlated with gill Mn accumulation rates, we do not believe they bias the results.

We can apply our measured rates of Mn accumulation to a hypothetical 10-wk hypoxia season in LIS. The resulting range of calculated concentrations from 46–360 $\mu\text{g/g}$ (Fig. 3a), approximates the range of Mn concentrations (19–385 $\mu\text{g/g}$) found in gills of native LIS lobster trapped during the study (Fig. 3b).

The Mn concentrations found in gills of native LIS lobsters suggest that the accumulation rates for the caged lobsters are conservative and consistent with what might be expected for the early part of the hypoxia season. The range of measured accumulation

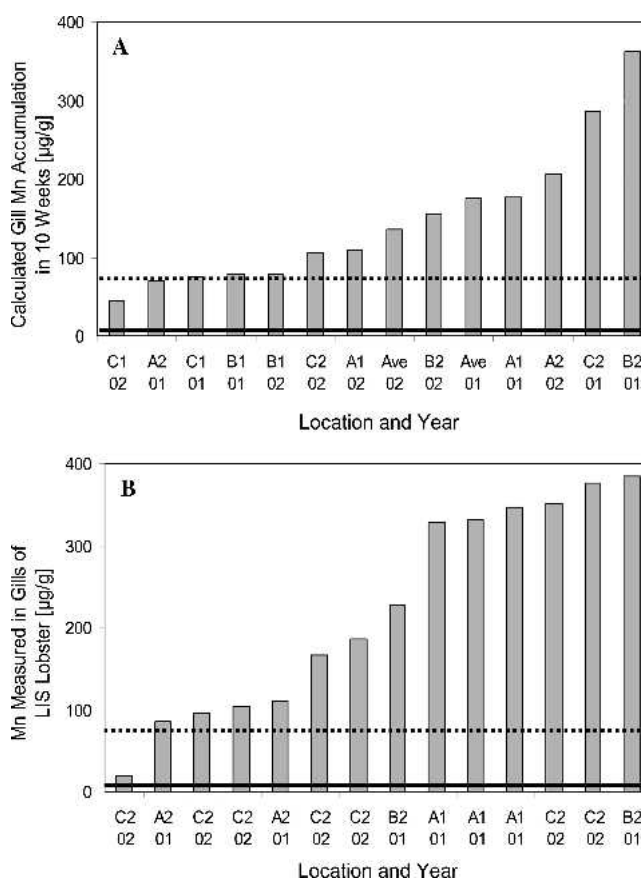


Figure 3. Manganese concentrations in lobster gill tissue for: (A) manganese calculated to accumulate in 10 weeks of exposure to rates determined in this study for American lobsters (*Homarus americanus*) deployed in LIS; (B) manganese measured in native lobster collected opportunistically from LIS in mid August of 2001 or 2002. Lines indicate manganese concentrations in *Nephrops norvegicus* (Eriksson & Baden 1998) from Faeroe Islands (8 $\mu\text{g/g}$, solid) and areas of SW Sweden not known to experience hypoxia (74 $\mu\text{g/g}$, dotted). Laholm Bay in the Kattegat west of Sweden is frequently hypoxic and gill manganese averaged 1560 $\mu\text{g/g}$.

rates (0.8 $\mu\text{g/g/d}$ at C1 and 3.4 $\mu\text{g/g/d}$ at B2) can be applied to the Mn concentration in the native lobsters to estimate the time required to reach the Mn level observed in the native lobsters. Calculated intervals average 4.03 mo and range from 6 days for the lowest concentration if the LIS individual were at Site B2–1.35 y if the lobster with the highest concentration had been at Site C1. Further, the time required to accumulate the mean concentration at site B2 calculates to 2 mo of exposure as might be expected for the most reducing habitat of the study.

Calculated and observed gill concentrations of American lobsters in LIS may be compared with results for Norway lobsters (Eriksson & Baden 1998) from sites with a variety of eutrophication levels. Norway lobsters were collected in spring and autumn from a relatively pristine site (Faeroe Islands), seven commercially fished sites along the west coast of Sweden not experiencing hypoxia, and Laholm Bay in the Kattegat in which hypoxia is known to develop seasonally in the bottom waters. The mean Mn concentrations in gills tissues were 8, 74 and 1,560 $\mu\text{g/g}$, respectively. Even though our LIS observations were made during the early part of the hypoxia season, they fall at the upper end of this range. We therefore conclude that the relationship between lobster gill Mn concentration and degree of hypoxia was roughly similar between regions and species.

These results for LIS extend the use of Mn as a proxy for exposure to hypoxic conditions in *H. americanus* and suggest criteria for application that may be useful in delineating and monitoring lobster habitats elsewhere along the northeast United States coast. Noting that external Mn is lost at each molt, as a first approximation, the gill Mn data suggest three discriminating values: 30, 100 and 300 $\mu\text{g/g}$. The first interval (to 30 $\mu\text{g/g}$) arises from observed gill concentrations of <8 $\mu\text{g/g}$ in *N. norvegicus* from the Faeroe Islands (Eriksson & Baden 1998), offshore lobsters used in this study that ranged from 12–22 $\mu\text{g/g}$ (mean 16 $\mu\text{g/g}$) and two low values from this study of 14 and 19 $\mu\text{g/g}$ in native LIS lobsters. If a high proportion of lobsters in autumn has gills containing less than 30 $\mu\text{g/g}$, they are likely reflecting a normoxic habitat. The second level (30–100 $\mu\text{g/g}$) suggests incipient exposure to hypoxic conditions. Gill Mn concentration of

~100–300 $\mu\text{g/g}$, the range of most data in our study, suggest the potential for catastrophic lobster mortality should temperatures of bottom waters be uncharacteristically high (as occurred in western LIS in 1999). This temperature threshold is likely ~24°C for LIS lobsters (Draxler et al. 2005), but would be expected to be lower at higher latitudes. Under these conditions, excess sulfide and ammonium would be expected in bottom water, along with the hypoxia exposure evidenced by the elevated Mn accumulation. Gill concentrations >300 $\mu\text{g/g}$ might suggest exposure to severe habitat conditions such as western LIS in 1999 with a strong potential for mortality. Baden and Neil (2003) have recently concluded that Mn concentrations in mobile appendages may be a stronger and more specific biomarker for hypoxia than gill concentrations. Antennules may also be more easily collected with less damage to the animal than collection of gill tissue.

CONCLUSION

Manganese accumulation in lobster gills was strongly related to duration of deployment at all sites. Accumulation was highest at sites in the west and south of the LIS study area and lowest at sites to the east and north. The reasoning of Baden et al. (1995) that Mn accumulation is an indicator of hypoxia exposure in lobsters is extended to *H. americanus*, and it may be a useful measure in lobster habitats of the northeast United States shelf. Habitats where autumn Mn concentrations in lobster gills exceed 30 $\mu\text{g/g}$ are likely experiencing some reduced oxygen levels; >100 $\mu\text{g/g}$ may be the beginning of the potential for lobster mortality; and more than ~300 $\mu\text{g/g}$ indicates habitat conditions with a strong potential for hypoxia stress.

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