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Heavy Metal Levels in *Nucella lapillus* (Gastropoda: Prosobranchia) from Sites with Normal and Penis-Bearing Females from New England[1]

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Pseudohermaphroditism, as manifested by reproductively normal females possessing non-functioning penises, has been reported for more than thirty species of dioecious neogastropods (Smith 1981a). In Ilyanassa obsoleta from the Connecticut shoreline of Long Island Sound, Smith (1971, 1980) reported female snails possessing one or more of the following male secondary sexual characteristics, penises with a penial duct, vas deferens, or convoluted gonadal oviducts. Snails with any of these characters, were said to exhibit the condition "imposex". This anomaly was shown to be unrelated to age or parasitism (Smith 1980), but the geographic distribution of this phenomenon indicated that it was environmentally induced rather than genetically controlled (Smith 1981a). Eventually, Smith (1981b,c,d) demonstrated that this abnormality was associated with marinas, and that the etiological agents were organo-tin biocides leaching from anti-fouling paints. Feral (1976, 1980a) reported penis-bearing Ocenebra erinacea females from the coast of France, and that the development of this abnormality was induced environmentally. However, it was not indicated whether "imposex" conditions were observed. Feral and Le Gall (1982), presented experimental evidence that this anomaly in female O. erinacea could be induced by tributyltin.

Populations of abnormal female Nucella lapillus (L.) have been reported from Great Britain (Blaber 1970), France (Feral 1980b), and the coast of Maine (Osborne 1977). Histological sections of the penis-like structure reported by Blaber (1970) showed no evidence of a penial duct. In a survey of N. lapillus populations along the New England Coast, Pondick (in prep.) found abnormal females at sites directly under the influence of industrial discharges, sewage effluent, or vessel-related activities. Histological sections revealed the presence of a penial duct, which provided evidence for the existence of a second "imposex" species.

The presence of "imposex" and its pattern of distribution in N. lapillus populations strongly suggested the involvement of heavy metal pollution associated with boating activity.

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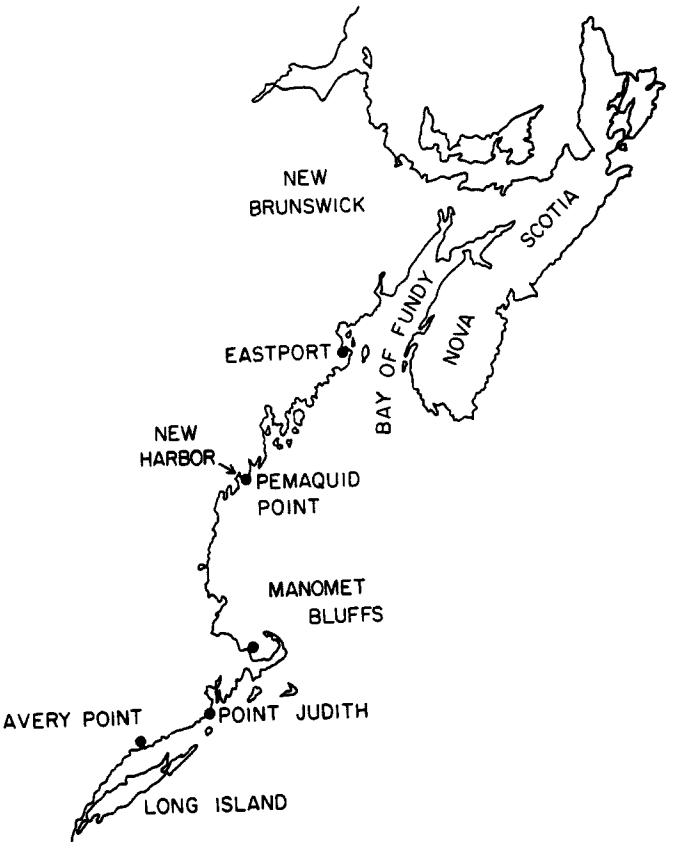

Figure 1. Sampling sites along the New England coast.

The purpose of this study was to determine if there were differences in metal levels in the snail populations studied by Pondick (in prep.), with particular attention to metals associated with boating activity.

METHODS AND MATERIALS

Variability of heavy metal levels in molluscan tissues within a single population is common and produced by such factors as size, sex, season of collection, and position of the animal in the water column (See Phillips 1976 for review). Appropriate sampling and handling precautions were followed to minimize these effects.

Snails were randomly collected from the lower shores of sites along the New England Coast (Eastport {EP}, Pemaquid Point {PEM}, New Harbor {NH}, Maine; Manomet {MAN}, Massachusetts; Point Judith {PJ}, Rhode Island; and Avery Point {AP}, Connecticut) from July-Oct. 1982 (Figure 1). Site characteristics such as exposure to

Table 1. Characterization of Sites.*

Site	Sources of Anthropogenic Contaminants: Exposure to Industrial Effluent	Exposure to Sewerage Effluent	Proximity to Harbors and Marinas	Habitat	Prevalence of Abnormal Females
Eastport, ME	-	-	-	Large Embayment	0
Pemaquid Pt., ME	-	-	-	Exposed Coastal	0
New Harbor, ME	-	-	++++	Sheltered Harbor	94%
Manomet, MA	-	-	-	Large Embayment	0
Pt. Judith, RI	++	-	+	Exposed Coastal	97%
Avery Pt., CT	++	++	++	River Mouth	100%

* From Pondick (in prep.)

industrial effluent and proximity to marinas are presented in Table 1.

Snails from each site were randomly divided into five subsamples each containing eight individuals. Snails were measured with dial calipers, crushed, sexed, and checked for the the presence of a penis and parasites with a dissecting microscope. Minimum size limits for each site were established from previous work (Pondick unpbl. data) to insure that only mature snails were used. Snails not meeting size requirements were discarded and randomly replaced. Sex was determined by the method described by Hall and Feng (1976) for Urosalpinx cinerea, which is applicable to N. lapillus (Osborne 1977; Pondick unpbl. data). It is based on the texture of the gonads, which are smooth in males and granular in females. Both sexes were used since the ratio of males to females was approximately equal in all groups. Subgroups were then placed in glass jars and frozen for subsequent analysis.

Each subsample was dried at 90°C for 24 hours and then homogenized with a mortar and pestle. Aliquots of 0.8 g of tissue were removed from each subsample and digested as described by Greig et al. (1975). The tissue levels of the following metals were examined: cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), tin (Sn), and zinc (Zn) and reported as micrograms per gram dry weight (parts per million (ppm)) The Cr, Cd, and Sn contents of each subsample were determined using a flameless atomic absorption spectrophotometer (Perkin-Elmer 5000) equipped with a graphite furnace and background corrector. The Cu, Fe, and Zn contents of the tissues were determined using a flame atomic absorption spectrophotometer (IL 151).

A Shapiro-Wilk's test was performed to test the normality of all metal data. These were all normally distributed except for Cd, which was $\{X\}^2$ transformed. A one-way analysis of variance (ANOVA) was used to determine differences in metal levels amongst sites. Only metals showing significant differences in the ANOVA were analyzed further using Tukey's multiple comparison test. All differences reported are significant at $\alpha=0.05$. Descriptive statistics and analyses were performed using SAS (1982) procedures on an IBM 3081 computer.

RESULTS AND DISCUSSION

The metal concentrations for six populations of Nucella lapillus from New England are presented in Table 2. Tin levels were below the detection limit (<0.5ppm) of the analytical method employed here for all samples tested.

Results of the ANOVA showed significant differences in the levels of Cd, Cu, and Zn amongst sites, while Fe and Cr levels were not significantly different (Table 3).

Table 2. Mean metal[1] concentrations in whole body tissues of *Nucella lapillus* from New England (ppm ± S.D.). (N = 5 groups of eight individuals per site)

Site	Metal				
	Cd	Cr	Cu	Fe	Zn
Eastport, ME	11.8 (±2.4)	3.0 (±0.2)	32.6 (±6.6)	147.6 (±29.6)	153.8 (±39.1)
Pemaquid Pt., ME	25.1 (±5.9)	3.0 (±1.1)	37.8 (±9.6)	121.0 (±38.4)	241.0 (±63.7)
New Harbor, ME[2]	25.7 (±5.1)	2.7 (±0.4)	85.0 (±19.2)	147.8 (±25.6)	331.0 (±95.2)
Manomet, MA	11.9 (±3.4)	2.3 (±1.0)	34.4 (±9.8)	159.2 (±59.9)	207.4 (±68.1)
Pt. Judith, RI[2]	18.4 (±5.3)	2.3 (±0.8)	105.2 (±30.9)	177.4 (±37.8)	443.4 (±109.7)
Avery Pt., CT[2]	8.1 (±0.6)	2.1 (±0.9)	80.6 (±6.4)	200.2 (±50.3)	382.8 (±58.3)

1. Sn levels were below the detection limit (<0.5ppm) of the analytical method employed here for all samples tested.
2. Sites with abnormal females.

Sources of heavy metal enrichment in nearshore waters have natural and anthropogenic origins (Bryan 1976; Forstner and Wittman 1979; Young *et al.* 1975). Table 1 presents potential sources of heavy metal addition at the six sites studied, and indicates that only those populations exposed to anthropogenic contaminates had penis-bearing females. Furthermore, the occurrence of "imposex" in a population is most strongly associated with that population's proximity to marinas and harbors. In these areas metals contained in paints, ship hulls, and sacrificial anodes are leached from boat bottoms into the seawater (Young *et al.* 1975). Bottom paints represent a significant potential source of metal contaminates (Cd, Cr, Cu, Pb, Hg, Sn, and Zn), as reviewed by Young *et al.* (1979). Metal levels and composition vary in paints depending on the brand influencing both the amount and rate at which metals are added to the water. For example, antifouling paints, released from boat hulls, use Cu, Hg or Sn as toxicants either separately or in combination. Furthermore, bottom primer paints may contain Cr, Pb, or Zn. Zinc is also used extensively in sacrificial

Table 3. Results of the ANOVA[1] and Tukey's Multiple Comparison Test[2] (D.F.=24) for mean metal concentrations (ppm) in tissues of Nucella lapillus from New England (N=30).

Cd[3]

Site	NH[4]	PEM	PJ[4]	MAN	EP	AP[4]
Mean	25.7	25.1	18.4	11.9	11.8	8.1

ANOVA F=15.51, p=0.0001, D.F.=29

Cu

Site	PJ[4]	NH[4]	AP[4]	PEM	MAN	EP
Mean	105.2	85.0	80.6	37.8	34.4	32.6

ANOVA F=18.57, p=0.0001, D.F.=29

Zn

Site	PJ[4]	AP[4]	NH[4]	PEM	MAN	EP
Mean	443.4	382.8	331.0	241.0	207.4	153.8

ANOVA F=10.65, p=0.0001, D.F.= 29

1. Results of the Cr and Fe comparisons were not significantly different ($p>0.05$) and are not included in this table.
2. Sites connected by lines are not significantly different ($p>0.05$).
3. $\{X\}^2$ transformation.
4. Sites with abnormal females.

anodes attached to vessel bottoms to prevent corrosion of metal parts.

Metal enrichment of nearshore waters associated with heavy vessel activity was demonstrated for coastal Southern California Young et al. (1979). This study showed that mussels, Mytilus edulis, from populations associated with heavy boating activity had significantly higher metal levels in tissues than mussels from areas without this activity. This held true for all the metals previously mentioned, but was particularly pronounced for Cu. This supports the findings of the present study where populations of N. lapillus with abnormal females (NH, PJ, and AP) had significantly higher levels of Cu than populations with normal females (EP, PEM, and MAN) (Table 3).

Elevated Zn concentrations appeared to be clearly associated with the presence of penis-bearing females in a population (Table 2), but results of a multiple comparison test (Table 3) showed that this association was not as distinct as that shown for Cu. Specifically, the Point Judith (PJ) population had significantly higher Zn levels than any of the sites with normal females (PEM, EP, and MAN), and Zn levels in the Eastport population were significantly lower than any site with abnormal females (NH, PJ, and AP). However, the levels of Zn at NH and AP (with abnormal females) are not different from sites with normal females (PEM and MAM).

There was a significant difference in the levels of Cd between sites. However, no distinct pattern emerged with respect to the occurrence of "imposex". The highest and lowest Cd levels (NH and AP respectively) were found in areas with penis-bearing females. A possible explanation for this is that the potential sources of Cd identified in Table 1 were not adding significantly more of this metal to the water than is added naturally. A second possibility is that the uptake of Cd is largely dietary and that the two major food items of this snail, Mytilus edulis and Balanus balanoides, may differ significantly in availability and metal content. This is a strong possibility as Peden et al. (1973) suggested that the major route of Cd uptake by N. lapillus is through the food chain, as has been demonstrated for Zn and Fe (Young, 1977).

Iron-hulled boats and bottom primer paints offer potential sources of Fe to marine waters. Bottom paints are also a potential source of Cr to marine waters. However, both Fe and Cr levels in snail tissues were not significantly different amongst sites. This suggests that either these metals were not added in significant amounts from the sources identified in Table 1, or that N. lapillus is not a good indicator of Cr or Fe addition.

The undetectable levels of Sn in all samples of snail tissues examined may indicate that this metal is in extremely low levels at these sites compared with other metals examined, or could reflect the snails inability to concentrate Sn in significant amounts. The results presented here do not offer additional support for organo-tin compounds as the etiological agents responsible for "imposex". However, this possibility cannot be ruled out and indicates the need for more sensitive methodology.

Results of this study show Cu and Zn to be indicative of vessel-related contamination. Elevated levels of Cu are indicative of "imposex" in populations of N. lapillus, while the association of Zn and "imposex" is not as clear. Cadmium, Cr and Fe are not indicative of imposex in N. lapillus. These findings provide evidence for the suggestion by Pondick (in prep.) that substances associated with vessel related activities could be implicated as causes of imposex in N. lapillus.

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REFERENCES

Blaber SJM (1970) The occurrence of a penis-like outgrowth behind the right tentacle in spent females of Nucella lapillus (L.). Proc Malac Soc Lond 39:231-233

Bryan GW (1976) Heavy metal contamination in the sea. In: Johnston R (ed) Marine Pollution. Academic Press, London, pp. 185-302

Feral C (1976) Etude statistique de la présence d'un tractus génital mâle externe chez les femelles d'un Mollusque Gastéropode gonochorique: Ocenebra erinacea (L.). Cah Biol Mar 17:61-76

Feral C (1980a) Influence de la qualité de l'eau de mer sur la différenciation d'un tractus génital mâle externe chez les femelles d'un Mollusque Gastéropode gonochorique: Ocenebra erinacea L.. C R Acad Sc Paris Ser D 291:775-778

Feral C (1980b) Variations dans l'évolution du tractus génital mâle externe des femelles de trois Gastéropodes Prosobranches gonochoriques de stations atlantiques. Cah Biol Mar 21:479-491

Feral C, Le Gall S (1982) Induction expérimentale par un polluant marin (le tributylétain), de l'activite neuroendocrine contrôlant la morphogenese du pénis chez les femelles d'Ocenebra erinacea (Mollusque, Prosobranche gonochonique). C R Acad Sc Paris Ser III 295:627-630

Forstner U, Whitman GT (1979) Metal Pollution in the Aquatic Environment. Springer Verlag, New York, 486 pp

Greig RA, Nelson B, Nelson D (1975) Trace metal content in the American oyster. Mar Pollut Bull 6:72-73

Hall JG, Feng SY (1976) Genital variation among Connecticut populations of the oyster drill, Urosalpinx cinerea Say (Prosobranchia: Muricidae). Veliger 18:318-321

Osborne CM (1977) Ecology of Shell Color Polyphenism in the Marine Intertidal Gastropod, Thais lapillus, in New England. Ph.D. Dissertation. Yale University, New Haven, 337 pp

Peden JD, Crothers JH, Waterfall CE, Beasley J (1973) Heavy metals in Somerset marine organisms. Mar Pollut Bull 4:7-9

Phillips DJH (1976) The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead, and copper. I. Effects of environmental variables on uptake of metals Mar Biol 38:59-69

SAS Institute Inc (1982) SAS User's Guide: Statistics. SAS Institute Inc., Cary, 584 pp

Smith BS (1971) Sexuality in the American mud snail Nassarius obsoletus Say. Proc Malac Soc Lond 39:377-378

Smith BS (1980) The estuarine mud snail, Nassarius obsoletus: Abnormalities in the reproductive system. J Moll Stud 46:247-256

Smith BS (1981a) Male characteristics in female Nassarius obsoletus: Variations related to locality, season and year. Veliger 23:212-216
Smith BS (1981b) Reproductive anomalies in stenoglossan snails related to pollution from marinas. J Appl Toxicol 1:15-21
Smith BS (1981c) Male characteristics on female mud snails caused by antifouling bottom paints. J Appl Toxicol 1:22-25
Smith BS (1981d) Tributyltin compounds induce male characteristics on female mud snails Nassarius obsoletus = Ilyanassa obsoleta. J Appl Toxicol 1:141-144
Young DR, McDermott DJ, Hessen TC, Jan TK (1975) Pollutant inputs and distributions off southern California. In: Church TM (ed) Marine Chemistry in the Coastal Environment. American Chemical Society, Washington, D.C., pp. 424-439
Young DR, Alexander GV, McDermott-Ehrlich D (1979) Vessel-related contamination of southern California harbours by copper and other metals. Mar Pollut Bull 10:50-56
Young ML (1977) The roles of food and direct uptake from water in the accumulation of zinc and iron in the tissues of the dog-whelk, Nucella lapillus (L.). J Exp Mar Biol Ecol 30:315-325

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