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# Heavy Metals in Tissues of the Norway Lobster *Nephrops Norvegicus*: Effects of Sex, Size and Season M. Canli<sup>a</sup>; R. W. Furness<sup>a</sup>

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# HEAVY METALS IN TISSUES OF THE NORWAY LOBSTER NEPHROPS NORVEGICUS: EFFECTS OF SEX, SIZE AND SEASON

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Concentrations of mercury, cadmium, copper, zinc and iron were determined in the carapace, hepatopancreas, gills, tail muscle, ovary and eggs of 288 Norway lobsters, *Nephrops norvegicus*, caught in the Clyde Sea area, south of the Isle of Cumbrae, Scotland. Metal levels in males and females were examined separately in relation to size and the season in which the animals were caught.

Cadmium, copper and zinc concentrations were highest in the hepatopancreas whereas mercury and iron concentrations were highest in the gill. Levels of metals showed variations between months, with highest levels tending to occur during molt. Metal levels were influenced by lobster size with pronounced size-related increases in mercury levels in the tail muscle and cadmium levels in the hepatopancreas. Levels of several metals in the various tissues differed between the sexes.

### INTRODUCTION

Metals occur naturally in sea water and many, such as copper, cobalt, iron, manganese, nickel, selenium and zinc, are essential for marine organisms. Crustaceans require trace metals for their metabolism and must accumulate them from ambient water and food.

Tissue levels of essential metals can be regulated by decapod crustaceans at concentrations of dissolved metals below a threshold level (Bryan, 1964; White and Rainbow, 1982; Rainbow and White, 1989). Thus, the concentrations of essential metals in water may not be a problem to the animals until such threshold concentrations are reached. However, metals reach the sea in industrial effluents, sewage and atmospheric pollution (Nolting, 1986; Mance, 1987; Langston, 1990; Guieu *et al.*, 1991). Experimental studies have shown that excess amounts of essential metals can cause elevated concentrations in crustaceans and cause mortality depending on concentration, time and animal species (White and Rainbow, 1982; Rainbow, 1985; Nugegoda and Rainbow, 1988).

Mercury and cadmium have no known role in biological systems. In addition to being present at low natural levels, they are contaminants of aquatic systems when they are released by human activities such as discharge from chlor-alkali plants, the use of fungicides, pesticides, antifouling preparations, and from mining and smelting activities (Campbell *et al.*, 1986; Mance, 1987; Langston, 1990). Berk and Colwell (1981) showed that mercury can be bioamplified through the food-chain by marine animals. Food can be the dominant route for accumulation of cadmium in Crustacea (Jennings and Rainbow, 1979; Davies *et al.*, 1981). It is well known that accumulations of non-essential metals are greatly dependent on concentrations in ambient water, period of exposure and species. There is no evidence that non-essential metals can be regulated by crustaceans. These metals are accumulated and

stored and their concentration factors may reach many thousand-fold (Jennings and Rainbow, 1979; Meadows and Erdem, 1982; Krishnaja et al., (1987).

The Norway lobster, *Nephrops norvegicus*, is a widely distributed crustacean on the continental shelf of Europe. In economic terms, the Norway lobster is the most important shellfish species in the United Kingdom (Howard, 1989). The Clyde Sea area receives inputs of pollutants (Mackay *et al.*, 1972; Steele *et al.*, 1973; Mackay, 1986), like most British waters (Allen and Rae, 1986; Campbell *et al.*, Nolting, 1986; Langston, 1990; Cossa and Fileman, 1991), and supports a large fishery for *Nephrops* (Bailey *et al.*, 1986). This paper reports levels of mercury, cadmium, copper, zinc and iron in *Nephrops norvegicus* sampled from this region and investigates variations in metal concentrations in relation to sex, size and season.

## MATERIALS AND METHODS

A total of 288 Norway lobsters were caught in different months of the year from south of the Isle of Cumbrae in the Clyde Sea area by trawling. Samples were brought to the laboratory and frozen in a -20 °C freezer if the analyses could not be done immediately; frozen samples were thawed prior to use. Carapace length (from the rear of the eye socket to the mid dorsal edge of the carapace) was measured to the nearest 0.1 cm. Samples were carefully dissected using clean equipment to separate the tissues. Each tissue sample was weighed using a Precisa 300MC (Metagram Instrument Ltd.) top-pan balance and dried to constant dry mass in an oven at 60 °C for six days.

Total mercury was analysed in the tissues of *Nephrops* by the method of Furness *et al.* (1986), using a cold vapour, atomic absorption spectrophotometer (DA 1500-DP6 mercury vapour detector, Data Acquisition Ltd.). The other metals (Cd, Cu, Zn and Fe) were determined by flame AAS using a Philips 9200 atomic absorption spectrophotometer after digesting dry tissues with concentrated nitric acid. Standard reference materials were included in each run to check the consistency of results; in the range of metal concentrations found in this study all metal levels were measured to within 10%.

## STATISTICAL ANALYSES OF DATA

Trace metal concentrations in the tissues of 288 *Nephrops norvegicus* were statistically analysed to investigate if there were seasonal differences. As levels of some metals (e.g. mercury, Davies and McKie, 1983) are known to differ between sexes, the male and female animals were separated. Trace metal concentrations may vary with size, so effect of carapace length was also investigated.

Linear regression (e.g. Draper and Smith, 1981) was used to investigate the relationship between trace metal concentration (c), and carapace length (1) in relation to month (m). For each metal, tissue and sex, five hypotheses were considered.

Hypothesis 1:  $c = a \pm error$ . Concentration does not depend on month or carapace length.

Hypothesis 2:  $c = a + b(l - I) \pm error$ 

Concentration varies linearly with carapace length and this linear relationship is the same in each month.

Hypothesis 3:  $c = a_m \pm error$ .

There is no relationship between concentration and carapace length; however, concentration varies with month.

Hypothesis 4:  $c = a_m + b(l - I) \pm error$ .

Concentration varies linearly with carapace length. The slope is the same each month. However, the intercept varies with month.

Hypothesis 5:  $c = a_m + b_m (l - I) \pm error$ .

Concentration varies linearly with carapace length. The slope and intercept both vary with month.

In the five models above, I is the mean carapace length of whole sample (4.2 cm). The parameter  $a_m$  is the mean concentration in month m (adjusted, if necessary, to correspond to *Nephrops* of length 4.2 cm), and the parameter  $b_m$  is monthly variation in mean concentration or slope, then  $[a_m]$  and  $[b_m]$  reduce to a and b respectively. The errors are assumed to be independent and normally distributed with zero mean and constant variance; this assumption appears consistent with the data.

For each metal, tissue and sex combination, the most parsimonious description of the data was found as follows; Model 5 is first fitted to the data. To test whether model 4 is an adequate simplification, model 4 is then fitted to the data and the residual sums of squares from both models are compared using an F-test (Draper and Smith, 1981). If model 4 is an adequate simplification, then we can subsequently test whether we can simplify still further (e.g. model 3 or 2) by further F-tests. In this way, the most suitable model for each data set is obtained. Tissues which show trace metal concentration according to model 1 or model 5 are not further examined in seasonal graphs. They are shown only as mean metal concentrations and standard deviations of tissues, because metal concentration does not depend on either month or size in model 1 and month and size show an interaction in metal concentration in model 5. Tissues which are suitable for model 3 and model 4 are shown in seasonal variation graphs giving estimated mean values of metals in the tissues and standard errors. In the case of model 3, mean metal concentrations and standard errors are shown in graphs since carapace length is not a significant factor to take into account. But in the case of model 4, concentrations of metals were adjusted for carapace length (a carapace length of the whole sample is 4.2 cm as a standard) with associated standard errors. In this way, expected concentrations of the metals are calculated for model 4. Metal concentrations (on a dry mass basis) in the tissues of male and female animals were reported as mean levels and standard deviations of the whole sample throughout the sampling period (Table 1). However, they could not be compared statistically due to effects of size and season. Where required for comparison with other published studies, wet mass equivalent concentrations can be estimated by using the wet to dry mass conversion ratios determined for each tissue (Table 2).

Table 1 Means and standard devii periods. Carapace lengths (cm) oi	ttions of conce samples were	entrations ( $\mu g g^{-1} d.w.$ ) of e 4.0 $\pm$ 0.66 (n=197) and	f metals in the tissues of r d 4.7 $\pm$ 1.06 (n=91) for	nale and female <i>Neph</i> male and female anin	<i>rops norvegicus</i> th nals respectively.	roughout sampling
Tissues	Sex	Mercury	Cadmium	Copper	Zinc	Iron
Carapace Carapace	Male Female	$\begin{array}{c} 0.13 \pm 0.08 \\ 0.12 \pm 0.07 \end{array}$	$1.68 \pm 2.36 \\ 1.85 \pm 1.56$	47 ± 26 48 ± 24	37 ± 17 32 ± 16	214 ± 151 156 ± 148
Hepatopancreas Hepatopancreas	Male Female	$0.29 \pm 0.14$ $0.22 \pm 0.11$	$10.30 \pm 7.93 \\ 14.25 \pm 11.60$	$503 \pm 242$ $731 \pm 265$	242 ± 91 237 ± 74	$138 \pm 84 \\ 102 \pm 53$

Tissues	Sex	Mercury	Cadmium	Copper	Zinc	Iron
Carapace Carapace	Male Female	$\begin{array}{c} 0.13 \pm 0.08 \\ 0.12 \pm 0.07 \end{array}$	$1.68 \pm 2.36 \\ 1.85 \pm 1.56$	47 ± 26 48 ± 24	37 ± 17 32 ± 16	$214 \pm 151$ $156 \pm 148$
Hepatopancreas Hepatopancreas	Male Female	$0.29 \pm 0.14$ $0.22 \pm 0.11$	$10.30 \pm 7.93 \\ 14.25 \pm 11.60$	503 ± 242 731 ± 265	242 ± 91 237 ± 74	$138\pm84\\102\pm53$
Gil Gil	Male Female	$0.42 \pm 0.25$ $0.75 \pm 0.35$	$8.43 \pm 5.89$ $13.52 \pm 6.62$	$\begin{array}{c} 250 \pm 105 \\ 207 \pm 106 \end{array}$	$161 \pm 104$ $156 \pm 148$	$916 \pm 999$ 1196 ± 998
Tail muscle Tail muscle	Male Female	$0.37 \pm 0.12$ $0.60 \pm 0.27$	$1.12 \pm 1.07$ $1.69 \pm 1.44$	25 ± 12 27 ± 15	$\begin{array}{c} 59 \pm 10 \\ 63 \pm 13 \end{array}$	$37 \pm 46$ $21 \pm 24$
Ovary Eggs		$0.08 \pm 0.05$ $0.12 \pm 0.11$	$1.85 \pm 1.01$ $2.26 \pm 1.61$	$126 \pm 38$ 113 ± 61	$99 \pm 21$ 131 ± 33	$37 \pm 30$ 411 ± 889

	Carapace	Hepatopancreas	<i>Gill</i>	<i>Tail muscle</i>	Ovary	Eggs
	N=107	109	113	122	34	18
ratio	2.24	2.90	9.65	4.70	3.67	4.35
s.d.	0.37	0.83	1.96	0.49	1.45	1.15

Table 2 Ratios of wet to dry mass of Nephrops norvegicus tissues.

# RESULTS

The significant relationships between tissue metal concentrations, month, carapace length and sex are shown in summary in Table 3. Except for carapace of females and gills of males, all tissues showed variation in metal levels between months (Figures 1-10).

**Table 3** Results of linear regression analyses. The best suitable model for the tissues of each sex is described in this table with P values. CL = carapace length. C&CL = Relationship (+ or -) between carapace length and metal concentration in a tissue; int = Interaction between carapace length and month.

Tissues	Metal	Sex	C&CL	CLP	Month P	Model
Carapace Hepatopancreas Gill Tail muscle	Hg	Males	+	ns ns int ★★★	*** *** int ***	3 3 5 4
Carapace Hepatopancreas Gill Tail muscle	Hg	Females	+ +	int ns ★ ★★★	int ★★★ ★★★ ★★★	5 3 4 4
Carapace Hepatopancreas Gill Tail muscle	Cd	Males	+	int ★★★ ns int	int ★★★ ★★ int	5 4 3 5
Carapace Hepatopancreas Gill Tail muscle	Cd	Females	+	int ★★★ ns int	int ns ★★ int	5 2 3 5
Carapace Hepatopancreas Gill Tail muscle	Cu	Males	+ - -	ns ★ ★ ★★	★★ ★★★ ns ★★★	3 4 2 4
Carapace Hepatopancreas Gill Tail muscle	Cu	Females	+ - -	ns ★ ★★	★★ ★ ns ★★★	3 4 2 4
Carapace Hepatopancreas Gill Tail muscle	Zn	Males	-	★ ns ns ns	★ ★★★ ns ★★★	4 3 1 3
Carapace Hepatopancreas Gill Tail muscle	Zn	Females		ns ns ns int	★ ★ ns int	3 3 1 5
Carapace Hepatopancreas Gill Tail muscle	Fe	Males		ns ns ★	★★★ ★★ ★★★ DS	3 3 3 2
Carapace Hepatopancreas Gill Tail muscle	Fe	Females		ns ns ns ns	★★★ ns ns ns	3 1 1 1

A relationship was found between carapace length and mercury concentration of tail muscle for males and females (p < 0.001). Concentrations of mercury in the gills of female animals also showed a relationship with size (p < 0.05) (Table 3). Female animals had higher mean concentrations of mercury in their gill and tail muscle than male animals, while males showed higher mean concentrations of mercury in their hepatopancreas. Mercury concentrations of carapace were similar in the two sexes (Figures 1 and 2, Table 1).

Mean cadmium concentrations in females were also higher than in males in all tissues (Table 1). Regression analyses revealed that males showed seasonal cadmium variations in their hepatopancreas and gill tissues, while females showed seasonal variation in cadmium levels only in their gill tissue (Table 3). These variations are shown in Figure 3. A positive relationship was found between carapace length and hepatopancreas cadmium for both sexes (p < 0.001) (Table 3). Cadmium levels in tail muscle and carapace of males and females did not show any seasonal variation or size related relationship (Table 3).

Mean copper concentrations in the hepatopancreas were higher in females than in males, but for gill tissue males showed higher concentrations than females, while in the carapace and tail muscle levels were similar (Table 1). Carapace, hepatopancreas and tail muscle copper levels showed seasonal variations for both sexes. These variations are shown in Figures 4, 5, 6. Negative relationships were found between carapace length and copper concentrations of the gill and tail muscle of males and females, while copper levels in the hepatopancreas showed positive relationships with carapace length (Table 3).

Mean concentrations of zinc in the tissues of males and females were very similar (Table 1). Table 3 shows that concentrations of zinc in all tissues of males varied seasonally except in the case of gill tissue, while only carapace and hepatopancreas showed seasonal variations in zinc level in female animals (Figures 7 and 8). Carapace length and zinc concentration in the carapace showed a negative relationship in males (Table 3).

Mean iron concentrations in the carapace, hepatopancreas and tail muscle of males were higher than in females (Table 1). Table 3 shows that the carapace, hepatopancreas and gill tissues of males showed seasonal variation in iron levels, while females showed seasonal variation in iron levels only in the carapace (Figures 9 and 10). The only relationship between carapace length and iron concentration was a negative relationship for iron in the tail muscle of males.

#### DISCUSSION

Mean mercury concentrations in the tail muscle of *Nephrops norvegicus* in this study were similar to levels presented by Davies and McKie (1983), also for the Clyde Sea area, and by Lima (1984) for Portuguese waters. Studies in Italian waters found higher levels of mercury in the tail muscle (Renzoni, 1980; Capelli *et al.*, 1983, Viviani *et al.*, 1983). A positive relationship between body size and mercury concentration in the tail muscle of *Nephrops norvegicus* is in agreement with results of Renzoni (1980); Capelli *et al.* (1983); Davies and McKie (1983); Lima (1984). Davies and McKie (1983) and Lima (1984) found differences in mercury concentrations of tail muscle in the same size specimens of female and male *Nephrops*. In this study differences between sexes were not statistically compared due to seasonal and size dependent variations. Mean values of tissue metal











Figure 3 Seasonal cadmium variations in male and female Nephrops.











Figure 6 Seasonal copper variations in male and female Nephrops.











Figure 9 Seasonal iron variations in male and female Nephrops.

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Figure 10 Seasonal iron variations in male Nephrops.

concentrations in Table 1 show the difference in tail muscle mercury concentrations between the sexes. This difference may be related to growth rate differences between male and female *Nephrops*. Male *Nephrops norvegicus* grow much faster than females (Davies and McKie, 1983; Howard, 1989). Therefore, animals of the same age would have different sizes; males being larger than females of the same age. This means that females of the same size as males have lived in ambient mercury concentrations for a longer time. However, higher levels of mercury were found in the hepatopancreas of male *Nephrops* than in females. Our unpublished data show that males have higher feeding rates than females and distribution of mercury to the hepatopancreas is more by the food route than by direct uptake from water, so higher concentrations of mercury in the hepatopancreas of males might come from their greater food intake.

Howard (1989) indicated that *Nephrops* moults at any time of the year, though peak occurrence is in March-April and from July to November. These peak periods of moulting activity are generally more pronounced in females, which must moult before mating takes place. Our results show that mercury levels in all tissues varied seasonally. Female carapace and male gills showed interactions between size and season which did not allow us to look at seasonal and size dependent variations.

Moulting might cause the biggest variation in mercury levels in tissues, but this may also be influenced by varying conditions of ambient sea water or diet. Tugrul *et al.* (1980) indicated that mercury can vary in marine organisms with season. Sivadasan and Nambisan (1988) also showed that mercury levels varied with season in the prawn, *Metapenaeus dobsoni.* 

Cadmium distribution among the tissues of *Nephrops norvegicus* was similar to that reported in some other studies on decapod crustaceans (Overnell and Trewhella, 1979; Davies *et al.*, 1981; Ray *et al.*, 1981; Uthe *et al.*, 1982). Ray *et al.* (1981) indicated that there can be wide variations in cadmium concentrations in the same tissues of different animals, even within the same size class. They also indicated that individuals with high cadmium levels in one tissue generally tend to have high levels in other tissues. Positive correlation between size and hepatopancreas cadmium in this study agrees with the results of their study. White and Rainbow (1987) related the variations in cadmium concentrations of *Systellaspis debilis* to

dietary enrichments of cadmium from different areas. Cadmium concentrations in the tail muscle of *Nephrops* were also different in material from different areas (Murray, 1981; Capelli *et al.*, 1983; Viviani *et al.*, 1983 Schuhmacher *et al.*, 1990). Hepatopancreas cadmium showed size and seasonal variations in males, but it showed only a size effect in females. Higher concentrations of cadmium in the hepatopancreas of female animals could be due to higher carapace length of the animals. It has been shown that food is a major uptake route for cadmium, especially for that taken up in the hepatopancreas (Jennings and Rainbow, 1979; Davies *et al.*, 1981). As indicated earlier, male *Nephrops* have higher feeding rates than females, so seasonal changes in dietary conditions would affect cadmium concentrations of males' hepatopancreas more than that of the hepatopancreas of females. Seasonal variations in cadmium concentrations of the gill should come from the effects of moult and seasonal variation of ambient water conditions, because food does not affect cadmium concentrations of the gill (Davies *et al.*, 1981).

Copper concentrations in the tail muscle of Nephrops norvegicus have previously been found to be similar to the present results (Murray, 1981; Capelli et al., 1983). Copper concentrations vary greatly between tissues of decapod crustaceans, even within the same species (Bryan, 1964, 1968; Murray and Portmann, 1984). Copper concentrations in the tissues of Nephrops vary far more than those for zinc, as Bryan (1964) found for Homarus vulgaris. The distribution of copper among the tissues of Nephrops was similar to that found in other decapod crustaceans, as summarized by Bryan (1968). A positive relationship has frequently been found between size and copper concentrations in decapod crustaceans (White and Rainbow, 1987; Rainbow and Abdennour, 1989; Darmono and Denton, 1990). Higher hepatopancreas copper concentration could markedly raise the total body copper concentrations due to its large size. Copper levels in gill tissue did not show any seasonal effects in either sex, while levels in the carapace and tail muscle showed seasonal variations in both sexes. Bryan (1967) indicated that most of the copper in muscle tissues in Crustacea is the result of contamination of cellular spaces by the blood. Seasonal changes in the blood concentrations of copper could also affect copper concentrations of the tail muscle. Moulting affects copper concentrations of the tissues, especially the carapace and hepatopancreas. Belleli et al. (1988) indicated that haemocyanin concentrations from the Mediterranean lobster, Palinurus elephas, change with season and may thus show seasonal copper variations. Engel (1987) also found significant variations in copper concentrations of the haemolymph and digestive gland of the blue crab, Callinectes sapidus, among moult, premoult and intermoult stages, having highest levels in premoult stage and lowest in soft crab stage.

Zinc concentration shows a relatively low variation within tissues of *Nephrops* norvegicus compared to the other essential metals studied. Variations among different species of decapod crustaceans can be enormous. Ober *et al.* (1987) found zinc concentrations in decapod crustaceans from 36 to 464  $\mu$ g g<sup>-1</sup> d.w., and Bryan (1968) found variations from 18 to 19  $\mu$ g g<sup>-1</sup> w.w. among a large variety of species. Zinc concentrations in the tissues of *Nephrops norvegicus* were found to be within this range. Murray (1979) and Capelli *et al.* (1983) found zinc concentrations in the tail muscle of *Nephrops norvegicus* similar to the present results. Zinc concentrations of the tissues of *Nephrops norvegicus* similar between male and female animals. Cuadras *et al.* (1981) indicated that there were no differences in concentrations of zinc in the organs studied between male and female hermit crab, *Dardanus arisor*. White and Rainbow (1987) indicated that zinc concentrations decrease slightly with increasing dry weight of *Systellaspis debilis*. Rainbow and Abdennour (1989) found

that total body zinc concentrations did not change significantly in the same species. In this study, there was a small size effect on zinc concentrations of the tissues. Seasonal variations were found to be significant for all tissues except the gill. This could be a result of moulting. Engel (1987) found significant variations in zinc concentrations of haemolymph and digestive gland of the crab, *Callinectes sapidus*, among moult, premoult and intermoult stages, with highest levels in premoult stage and lowest in soft crabs, and Engel and Brouwer (1987) found that levels varied seasonally in relation to both moult and ambient temperature.

Iron concentrations in the tissues of male and female *Nephrops* were very different and enormous variations were found within the same sex. Iron concentrations also varied widely in other studies of crustaceans (Ober *et al.*, 1987; Depledge, 1989; Ridout *et al.*, 1989). White and Rainbow (1987) indicated that iron concentrations decreased with increasing weight of *Systellaspis debilis*. The present study showed that size affected iron concentrations only of the tail muscle of male animals. Seasonal variations were found to be more important for all tissues.

### CONCLUSIONS

Heavy metal concentrations varied with season and size of male and female Nephrops. Seasonal variations were a very important factor for all metals. This could be related mainly to the timing of moult, though variations in metal concentrations of sea water may explain some of the variance. Although size was a significant factor affecting levels of most metals, it was a much more important factor for non-essential metals (Hg and Cd). Positive relationships between size and concentrations of mercury and cadmium could be interpreted as the result of continuous accumulation of these metals with no regulation. Differences in metal levels between the sexes might be related to differences in metabolic activity, feeding and growth rates of the sexes. There was no evidence from this study that ambient levels of essential metals in the Clyde Sea area exceeded levels that can be regulated by Nephrops norvegicus and thus essential metals are unlikely to present a toxic hazard to this population. Levels of non-essential metals were similar to those reported in several other studies of Nephrops norvegicus and other Crustacea and appear to be unlikely to present a toxic hazard. However, it should be noted that there are substantial variations (sometimes more than doubling) in the metal levels in tissues due to season, size or sex, and this might have important implications for regulatory practice. These large differences between seasons and sizes of animals also make comparison between studies more difficult, since data are often presented without reference to the size or moult stage of animals sampled.

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