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Seasonal Variation in Soft Tissue Weights and Trace Metal Burdens in the Bay Mussel, *Mytilus edulis*

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Trace elements of anthropogenic origin are ultimately deposited in the marine environment where they are considered a potential hazard to both lower animals and man (FORSTNER and WITTMANN 1979).

Environmental monitoring programs such as the U. S. "Mussel Watch" (GOLDBERG et al. 1978) and the California State Mussel Watch (STEPHENSON et al. 1978) have utilized several species of bivalve molluscs, with special dependence on *Mytilus edulis*, as monitors for detecting environmental pollutants. Some programs have employed the approach of sampling many sites at rather infrequent intervals. Such a protocol may not be sensitive to variations associated with possible physiological differences between populations investigated or within a population sampled during different times of the year.

It is important to have knowledge about the changes in metal levels that should generally be considered within a normal range. In order to obtain this type of information for bivalve populations, sampling must be relatively frequent, and encompass a time period sufficient to permit the analysis of specimens in different physiological states.

Ideally, data should be available for animal populations in locations relatively free from anthropogenic input, so that a baseline may be established. If complete and accurate data were provided, such a baseline would not consist of merely one value for each pollutant considered, but would be a profile, spanning at least the major seasons of one year, and perhaps even several years. Without such baseline information for a monitoring species, it cannot be determined whether or not an observed increase in a metal concentration was due to pollution, or was within an expected range.

The purposes of the present study were to measure the levels of 6 trace metals in *M. edulis* soft tissues during a 9-month period, establish patterns of seasonal variation and determine the normal ranges of metal burdens in mussels. The metals were vanadium (V), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), and cadmium (Cd); Ni, Cu, Zn, and Cd are EPA Priority Pollutants.

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MATERIALS AND METHODS

A population of *M. edulis* in Yaquina Bay (Newport, Oregon) was sampled at regular intervals between October 23, 1979 and June 17, 1980. The site consisted of pilings and cross-members of an abandoned railway trestle to which the mussels were attached, and was relatively isolated from anthropogenic sources of contaminants. The site was destroyed during construction of a marina during June, 1980. All specimens used were taken from cross-members which were at a height of +3 MLLW. Specimen size was maintained between 50 and 60 mm throughout the experiment to reduce variation due to size and age and to assure that sexually mature specimens were used throughout the study.

After collection and transfer to the laboratory, attached organisms were removed and the shells were then opened and the contents drained for 10 min. Whole mussels, including shell, were weighed, and their lengths and heights measured. Next, that portion of the gonad contained within the mantle was dissected out (with the mantle), weighed, and reserved in a polyethylene vial. The balance of the soft tissues were removed, weighed and retained in an additional vial. From these measurements, the condition index and gonad index were subsequently calculated.

Tissues were then combined in two pools, "somatic" or "gonadal" tissue, and homogenized in a Waring blender equipped with a carbon bearing. Tissue moisture was determined by drying small quantities of homogenized somatic and gonadal tissue at 100°C for 24 hrs. A wet procedure was used for digesting tissue to minimize any possible loss of volatiles; 2.5 g quantities of wet tissue were placed into screw-top culture tubes (150 mm x 25 mm) and 4 ml conc HNO_3 added (reagent grade). The tubes were tightly capped and placed in a heat block at 90°C for 1 hr. The tissue digest was then filtered through glass wool and made up to a volume of 10 ml with 2% HNO_3 in 18 MΩ distilled water.

Flame atomic absorption was used to determine concentrations of Mn, Ni, Cu, and Cd; the digest solution was aspirated directly into the flame. Vanadium was determined by neutron activation analysis (LATOUCHE et al. 1981).

Data from the regularly acquired samples provided mean values (n = 20 mussels) for determining average dry gonad tissue weight (AGW), average dry somatic tissue weight (ASW), and gonad index (GI = AGW/ASW mg g^{-1}). A "between sample" comparison was made for all samples taken over the 9-month sampling period, for each of these categories.

Metal recoveries were assessed by analyses of NBS Oyster Tissue (SRM 1566).

RESULTS AND DISCUSSION

Table 1 lists collection dates, day numbers, tissue weights, gonad indices, and tissue metal burdens. Each value is for a pooled sample of 20 mussels; analyses were done in triplicate.

Average gonad weight (AGW) was the mean gonad weight from the dry weights of 20 specimens; similarly, ASW was the mean somatic weight. These means varied over the sample period, and this was verified statistically by a one-way analysis of variance, in which day number was regarded as a "treatment." The calculated F-values for AGW and ASW were 5.50 and 5.92, respectively, and these values were greater than $F_{(0.01,12,247)} = 2.26$.

The gonad index (GI) has been used to minimize differences in soft tissue weights in bivalves so that their reproductive states can be compared (ELVIN 1975). The GI is a ratio of dry gonadal tissue weight (mg) to dry somatic tissue weight (g): hence, it provides a weight-independent index of reproductive state. This index is valid for mature specimens, but obviously would not be indicative of the reproductive state of immature specimens. Significant between-sample differences in gonad index were revealed by an analysis of variance as with AGW and ASW. The calculated F-value was $7.39 > F_{(0.01,12,247)} = 2.26$. It was evident that the gonad index reached a maximum on March 24 after which it declined, probably indicating that a major spawning had occurred. Table 1 also shows that somatic tissue weights declined before March 24 whereas gonadal tissue weights increased during this period; this change suggests a shift of material from the somatic to the gonad compartment during gametogenesis.

The use of specimens in the same size range permitted comparisons of body burdens. Body burden data provides the same result as normalized concentration data based on the same average weight for each sample.

Metal concentrations can be easily determined from Table 1 by dividing any metal value for a particular tissue by its average weight (e.g. for Mn concentration in somatic tissues on 5/21/80, 3.8 µg (MnS) ÷ 0.588 g (ASW) = 6.46 µg g^{-1}). Recoveries were determined by analyses of NBS Oyster Tissue and are summarized below (metal [% recovery ± s.d.]): V(80 ± 4); Mn(81 ± 4); Ni (not available); Cu(87 ± 3); Zn(83 ± 0); and Cd(87 ± 2). No value for Ni was obtained because concentrations in the SRM were very close to the detection limit of flame atomic absorption.

With the exception of Cu, all gonadal tissue burdens were greatest in late winter or early spring, which corresponded to the time at which gametogenesis occurred. This was verified by histological examination of other samples. The gonadal burden of Cu did not show an obvious seasonal change. Somatic burdens of Ni, Mn and Zn were maximal in early spring; however, Mn appeared to increase again shortly before termination of the study. Somatic burdens of Cu, Cd, and V fluctuated in an irregular

TABLE 1. Tissue burdens of vanadium, manganese, nickel, copper, zinc and cadmium in gonadal (G), somatic (S), and total soft (T) tissues of *M. edulis*. Burden is the total content of a metal, in µg, within a specific tissue compartment (i.e., gonadal or somatic). GI - gonad index, AGW - average gonad weight, ASW - average somatic weight.

Date	Day no.	GI (mg/g)	AGW (g)	ASW (g)	VG	VS	MnG	MnS	NiG	NiS	CuG	CuS	ZnG	ZnS	CdG	CdS
10/23/79	1	328	0.154	0.467	ND	0.39	0.48	2.5	0.0	1.0	2.0	5.0	6.3	69.5	0.26	7.2
11/14/79	22	257	0.178	0.692	ND	0.73	0.59	3.3	0.0	0.8	2.3	7.3	6.6	112.5	0.44	10.4
1/14/80	82	286	0.191	0.680	ND	0.70	0.84	4.4	0.0	3.6	1.1	9.9	8.0	128.5	0.42	9.0
2/11/80	110	236	0.151	0.626	ND	1.26	0.69	4.4	0.47	3.0	1.4	5.6	11.2	126.2	0.49	5.8
2/26/80	125	285	0.190	0.647	0.06	1.70	0.80	5.4	0.80	4.9	1.8	6.5	14.1	121.1	0.57	8.4
3/10/80	138	288	0.169	0.581	0.07	1.02	0.66	4.6	0.63	3.2	1.0	4.8	9.2	89.5	0.44	5.6
3/24/80	152	384	0.235	0.607	0.07	1.20	0.87	3.7	0.87	3.9	1.6	6.1	11.4	94.9	0.55	6.3
4/8/80	167	332	0.209	0.630	--	2.11	0.50	3.6	0.46	2.5	1.4	5.0	10.6	100.6	0.46	7.6
4/23/80	182	318	0.197	0.620	0.11	1.17	0.63	3.6	0.51	2.5	1.7	5.1	9.0	92.4	0.37	5.7
5/7/80	196	183	0.112	0.616	0.03	1.52	0.27	3.1	0.29	2.4	0.8	4.3	5.0	99.8	0.34	5.5
5/21/80	210	250	0.152	0.588	0.04	1.16	0.53	3.8	0.33	2.0	1.0	9.0	7.0	85.7	0.35	5.5
6/2/80	222	198	0.128	0.646	0.07	1.43	0.47	3.9	0.31	3.1	0.9	7.9	6.6	92.2	0.34	6.4
6/17/80	237	312	0.258	0.818	ND	2.30	1.01	5.7	0.54	3.0	1.3	7.2	11.6	132.0	0.49	7.0

manner and did not appear to have any relationship to gametogenesis or spawning.

Zinc was considered to be a key element with respect to possible relationships with the reproductive cycle, because it is biologically important and concentrated to orders of magnitude above ambient seawater concentrations by most bivalves. Gonadal tissue burdens of Zn reached a maximum on February 26, several weeks after a similar maximum in somatic tissues (January 14). Again, this may indicate that materials associated with gametogenesis, in this case, Zn, are transferred from somatic to gonadal tissues.

GABBOTT and BAYNE (1973) found that in *M. edulis*, an increased rate of gametogenesis was accompanied by elevations in energy demand and oxygen consumption. These processes, in turn, required an accelerated filtration rate and increases in phytoplankton ingestion. PHILLIPS (1979) believed that levels of trace metals in *M. edulis* were chiefly related to metal levels in the phytoplankton that they consumed, and not to metals associated with inorganic particulates or in solution. Thus, the physiological demands during gametogenesis might be expected to lead to a net increase in metal concentrations in the soft tissues of *M. edulis*.

SIMPSON (1979) suggested that the reproductive cycle had an effect on uptake and loss of zinc and lead by *M. edulis*, but provided no specific supporting data. COSSA et al. (1979, 1980) noted a relation between cadmium concentration and body weight of *M. edulis*, and also observed that sexual maturation introduced some variation into an otherwise predictable relationship. STEPHENSON et al. (1979) were not able to demonstrate that the sexual cycle had any effect on trace metal concentrations in *M. californianus*. However, they initiated that practice of removing gonadal material prior to analysis of soft tissues so as to eliminate a possible variable.

From this study, and others, it seems clear that somatic and gonadal tissues should be considered separately when measuring metal levels. Otherwise, the changing quantity of gonadal material, which may comprise as much as 40% of the dry soft tissue in completely ripe *M. edulis*, will make a significant transient contribution (i.e., the large reduction in soft tissue weight on spawning) at certain times of the year.

The zinc data suggest that levels in somatic and gonadal tissues were related to the reproductive cycle; the change in Zn burdens may have been indicative of imminent spawning. Concentrations in both tissues increased dramatically between days 22 and 80. Such increases may be predictive for the onset of gametogenesis, since maximum Zn burdens were reached in both somatic and gonadal tissues before the time when gametogenesis was thought to occur (ca. day 152). It would seem probable that Zn burdens would increase first in somatic tissues if zinc were

an essential constituent for gamete production. GABBOTT (1976) noted that the mantle has an active role in gametogenesis; basically, the gonad develops within mantle tissue. Glycogen is accumulated and stored largely in the mantle during the non-reproductive period before gametogenesis. Protein and lipid are accumulated in non-mantle tissues. Meanwhile, as nutrients are taken up, the digestive gland controls their distribution to other tissues. This distribution pattern may explain why Zn appeared to be taken up first by somatic tissues (which included the digestive gland) and subsequently by the gonadal tissue. The Zn increase in gonadal tissues may have corresponded to the period of vitellogenesis when stored glycogen was converted to the lipid reserve of developing eggs. GABBOTT (1975) suggested that after this conversion, there was a movement of nutrients from the digestive gland to the mantle for gonad development. Also, there may be a metallothionein constituent in developing eggs that is zinc-specific, and in which certain other divalent metals such as Cd may be substituted.

The relationships between Zn, Cd, and Mn burdens in gonadal tissues were significant. For Zn and Cd, the r-value (correlation coefficient) was 0.93, and Mn was only slightly less well correlated with the other two metals ($r = 0.74$).

The data demonstrated that for metals included in this study, levels were always higher in the somatic tissues of *M. edulis* than in the gonadal tissues. There were clear indications that the somatic burdens of a metal influenced gonadal burdens in the context of seasonal fluctuations (Table 1). For example, patterns for gonadal and somatic Zn burdens were similar. Too, there was a temporal delay in fluctuations of gonadal burdens which supports the view that Zn was transferred from somatic to gonadal tissues. The rise in Zn burdens, in both compartments, between May 7 and June 16, may have indicated another period of gametogenesis prior to a second spawning.

There were not appreciable variations in gonadal burdens of Cu, although there were two rapid increases in somatic tissue Cu levels between October 1979 and June 1980. This supports the view of PHILLIPS (1976) concerning the unsuitability of *M. edulis* for monitoring Cu.

From results of the present study, it is concluded that utilization of *M. edulis*, as an environmental biomonitor, necessitates the use of a sampling and evaluation regime which differs from that commonly used in certain monitoring programs. The two major findings of our study which support this view are: (1) metals were more abundant in somatic tissues than in gonad tissues of mussels; and (2) increases in tissue weights, related to the reproductive cycle, were associated with increases in the amount of metal-poor gonadal material and thus, corresponding decreases in metal concentrations. These results have several implications. Infrequent sampling (i.e. fall and/or spring one year, winter and summer a second year) may result in the comparison of ripe

mussels with spawned out mussels. If soft tissues from such samples were analyzed, differences could be incorrectly interpreted as being related to increased environmental contamination. This type of variation is illustrated by the following data from our studies:

Date	Dry gonad wt. (g)	Dry somatic wt. (g)	Zinc conc. for total tissue ($\mu g\ g^{-1}$)
3/24/80	0.39	0.67	100
5/7/80	0.06	0.64	150

The effect of increased gonad weight on reducing Zn concentrations is obvious in this example. This type of error can be avoided or greatly reduced by increasing the sampling frequency; samples collected monthly or at least quarterly would yield the best data. If that is not possible, efforts should be made to collect animals at the same time(s) of year from the same population. In addition, they should have approximately the same size and degree of sexual maturity.

Finally, gonadal and somatic tissues should be analyzed separately. More meaningful results would also be provided if tissue weights are recorded since predictive relationships between tissue weights and metal contents could then be developed.

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