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Some aspects of mercury accumulation by the purple shore crab Hemigrapsus nudus Dana (Crustacea: decapoda) : a thesis ...

Ralph Gene Swanson

University of the Pacific

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SOME ASPECTS OF MERCURY ACCUMULATION BY THE
PURPLE SHORE CRAB HEMIGRAPSUS SPINEX Dana.
(CRUSTACEA: DECAPODA)

A Thesis
Presented to
The Graduate Faculty
University of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Ralph Gene Swanson
August 1973
This thesis, written and submitted by

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Dated August 1, 1973
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Dillon Beach, Summer, 1973
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INTRODUCTION

The indiscriminate release of mercurial compounds to the marine environment embodies an array of serious problems for many biological ecosystems. With the recognition that concentrations of heavy metals are orders of magnitude higher in the marine biosphere (Vinogradov, 1953) and the recent elucidation of possible pathways of biotransformation of mercury residues to highly toxic compounds (Jensen and Jernelov, 1969; Landner, 1971), there has been increased attention given to environmental monitoring. Research, in some regions of the world, has revealed alarmingly high values of mercury in both fresh and marine waters as well as the fauna that inhabits them. To date, most information concerning mercury hazards has come from Japan (Kurland, et al., 1960, Irukayama, 1961) and Sweden (Otterlind and Lennerstedt, 1964; Johnels, et al., 1967), principally as a result of crisis situations. Canada (Wobser, et al., 1970) and the United States (Anon. 1970a, b, c) have only started to recognize and focus attention on the problem.

The phenomenon of heavy metal concentration by organisms to the point of toxicity is not new. Traditionally heavy metal chemistry, biochemistry and physiology have overlapped marine research only insofar as they pertained to antifouling paints for the shipping industry and in naval forces of the world. Jones (1935, 1937, 1938 and 1940) working in Great Britain was particularly productive in regard to a variety of organisms. A large gap corresponding to the years of World War II is evident in the literature of Jones as well as others. Such information may well have been held classified and this reflects the military importance attached to this type of research. Following the war, papers relating to the effects of
heavy metals on both larvae and adults of fouling organisms again appeared in the literature (Clarke, 1947; Jones, 1947; Weiss, 1947, Pyefinch and Mott, 1948).

In more recent years heavy metals, mainly mercury, have come into use in industries unrelated to the commercial or military utilization of the high seas. Principally, in relation to its fungicial, bactericidal and slimicidal properties, mercury has been found valuable in agriculture where the pre-treatment of cereal grains significantly reduced crop loss due to fungal diseases; the pulp and paper industry where the use of mercury as phenyl mercuric acetate (PMA) counteracts the clogging slimes on screens as well as preventing decomposition of pulp during long term storage; and in chlorine and caustic soda production where mercury is involved as a catalyst in the reaction process.

The net result of this is that like the by-products of most of man's activities, heavy metals find their way, via many different routes, into the world's oceans. This can be particularly disadvantageous to marine organisms since, unlike the terrestrial animals that, perhaps, will come into brief contact with harmful materials, aquatic organisms, by virtue of their immersion, are continually exposed to a given toxic substance.

While all elements are taken up to some degree by marine plants and animals, the concentration factor is usually unity (Bowen and Sutton, 1951). It has long been known, however, that insofar as toxicants such as pesticides and heavy metals are concerned the phenomenon of biological concentration is effective in bringing about whole body levels far in excess of environmental concentrations. This is particularly true in
fish and shellfish which are able to accumulate high levels of toxic materials harmlessly through biotransformation or shunting of the toxic material to certain anatomical storage areas.

This becomes particularly evident when cases involving man occur:

1. In the late 1950's in a Japanese fishing village on the shores of Minimata Bay a number of unexpected cases of a nervous crippling disorder began to appear. By 1965, 111 cases of what came to be called "Minimata Disease" had occurred and 41 deaths had been attributed to it. Perhaps, the real tragedy involved the many infants born with congenital defects due to their mothers eating the food products from the bay. The cause of the problem was traced to a nearby vinyl chloride-acetaldehyde plant that was dumping mercury contaminated effluent directly into the bay (Kurland, et al, 1960). Fish and shellfish from the bay were shown to contain tissue levels of up to 20 ppm. The people of the village, dependent on the bay for food and economic resources, ate seafood from the bay three to five times a day.

2. Shortly after the disaster at Minimata and with that experience to draw on, 26 cases of mercury poisoning were detected in Nigata, Japan, another small bayshore village dependent on the sea for its welfare. The pollution of the bay was again traced to a local industrial complex. Fortunately, the discharge was subjected to treatment, but not before the consequences were manifested in six deaths.

3. In Sweden, the first observable sign of poisoning was a decrease in specific bird populations (Otterlind and Lennerstedt, 1964). These changes were eventually correlated with the increased mercury content
in nearly all the tissues of the animal. Further research traced the source of the problem to the birds' diet of seed, 80% of which had been mercury treated by 1964 (Swedish Royal Comm., 1966). The mercury contaminants in the discharge from Swedish pulp mills were believed responsible for the elevated levels of mercury found in the tissues of fish eating birds at about the same time.

4. Closer to home, on May 7, 1971, the Food and Drug Administration advised the U. S. public not to eat swordfish as 811 of 853 fish tested showed levels in excess of the maximum allowable limit for food stuffs (0.5 ppm). In December of 1972, 2.5 million cans of tuna-fish were removed from the market when 1% showed higher than standard limits.

These last two incidents scuttled U. S. public confidence in fish and other seafood products and sparked an interest on the part of the citizenry into research that previously had been only of academic interest. Survey information, on an international basis, began to show that environmental levels were suffering from man's impact.

Comparing the mercury content of 100 year old feathers from Swedish museum birds with feathers from freshly killed game birds (Berg, et al, 1966) determined that a ten to twenty fold increase in mercury content began taking place in the 1940's. This correlates well with the first widespread use of alkyl mercury compounds for seed dressing in Sweden.

Weiss (1971) studied the mercury content in layers of Greenland ice and, using the ice sheets as a time clock, noted significantly higher rates of deposition due to aerial fallout after 1952.

Foote (1972) has related such things as the point with which rooms
were painted and the elapsed time since painting, to his findings of higher than normal levels of mercury in offices, homes and laboratories in some large U. S. cities.

Only recently have studies on the absorption, distribution, tissue level and depuration of heavy metals begun (Bryan, 1973; Pentreath, 1973; Cunningham and Tripp, 1973). Of particular interest is the transport of the metals, its chemical form and the site of storage. Considerable importance is attached to their turnover rates and toxic action in vertebrates including man. Additionally, the possibility of defense mechanisms such as metabolic detoxification or the formation of various metal-protein complexes is gaining attention. Clarkson (1972) has reviewed nearly 150 papers on the aspects of the toxicology of mercury. Only three predate 1960 and few are earlier than 1965. It is abundantly evident, with regard to mercury (also lead), that the toxicology is effected to a great extent by its chemical form (whether incorporated in an organic or inorganic molecule) and on the route of accumulation. The organometallics of mercury are lipid soluble and are usually associated with central nervous system damage, while the metal ions are generally absorbed in the serum and distributed to hepatic and renal sites where they exert their toxic effects.

The present study has attempted to investigate some of the more basic questions concerning the toxicity and accumulation of mercury in the purple shore crab, Hemigrapsus nudus. This rocky intertidal denizen is found in abundance along the entire Pacific coast of the United States from Sitka, Alaska, to the Gulf of California (Ricketts and Calvin, 1968) where it dominates the mid-tide pool region. Local specimens were
collected just north of the Pacific Marine Station, Dillon Beach, Marin County, California. The animal is hearty and can be kept in a state of good health for long periods of time in the laboratory with minimum care and negligible mortality. Most importantly, as a middle intertidal inhabitant (Zone 3, Ricketts and Calvin, 1968), *H. nudus* would be among the first groups of organisms to feel the stress of environmental contamination from an industrial source located on the open coast. This, coupled with its wide distribution, indicates its possible use as an environmental monitor.

Three avenues of approach have been attempted using mercuric chloride as the source of mercuric ions. First, an estimation of the upper tolerance limit or lethal dose was accomplished. This served to define workable mercury concentrations for use throughout the rest of the study as well as to provide information concerning the level of environmental stress that a given, defined population of *H. nudus* can withstand. Second, the histopathological consequences related to long term (chronic) exposure to sublethal concentrations of mercury were determined using standard histological techniques. Last, and perhaps most important, examinations of the absolute levels of mercury in the various tissues of the experimental animal were carried out to determine the rate and pattern of mercury accumulation, anatomical storage sites and possible detoxification capabilities.
PART I

Acute Toxicity Testing with Hemigrapsus nudus
INTRODUCTION

Certain metal ions are an essential part of every living organism since they are involved in the tertiary molecular structure of enzymes and also act as acid catalysts (activators) in enzyme systems (Lehninger, 1950). Both a deficiency and an excess of a physiologically important metal ion can cause pathological results. The beneficial and adverse effects have been studied for centuries but their biochemical roles have only been examined during the last forty years.

Manifestations of metal deficiencies have been discerned in microorganisms, plants and man. As a result, the list of biologically important elements has grown steadily (Vallee and Ulmer, 1972).

Conversely, nearly every element is toxic to living systems when presented in high enough quantities. The acutely toxic effects of mercury on organisms has been amply demonstrated and the concern over the potential harm to man has been well justified.

At present, there seems to be no information concerning the possible beneficial actions of mercury, but the complexity of the mode of action may obscure a possible beneficial biologic roles.

Since a deficiency state, against which to assess an essential role, has not been technically defined for mercury, it is not surprising that the major emphasis has been placed on the toxic action of elevated levels of the ion.

As previously stated, the need for an inexpensive but effective anti-fouling compound suitably miscible with marine paints has stimulated research that has been centered on copper, mercury, zinc lead and cadmium (approximately in that order) with the major emphasis on the first two.
Concurrent attempts to find a suitable organism with which to test these compounds has led to the study of a variety of phyla including protzoans, ctenophores, platyhelmenthes, crustacea, molluscs and teleosts (Weiss, 1947). Sessile organisms naturally dominated researchers' efforts, and in this regard the larval stages and their ability to attach under conditions of exposure drew most of the attention.

Clarke (1947) devotes the greatest portion of his paper to copper but touches on mercury long enough to go on record as stating that copper is the more toxic to barnacles. He continues by establishing that the metamorphosing Balanus improvisus is the heartiest stage requiring much higher concentrations of mercury (16 ppm) to prevent metamorphosis than would be required to kill the adult.

However, much lower concentrations of copper were required to kill the newly metamorphosed barnacle than were necessary to prevent the metamorphosis. He laments the considerable variation in his data but concludes that while the degree of toxic action is proportional to the concentration, the prevention of initial attachment of cyprid barnacle larvae probably can not be prevented by any concentration of copper or mercury derivable from paint.

Weiss (1947) has compared the tolerance of animals from a number of phyla based on the sequence of attachment to copper and mercury paints. The most tolerant organisms studied were the red alga, Polysiphonia sp. and the barnacle Balanus amphitrite. Less tolerant were B. improvisus, Hydroclides parvus, Bugula neriting, Anoma sp., Enteromorpha sp., tunicates and hydroids.

Pyefinch and Mott (1948) pointed to the considerable differences in sensitivity to copper and mercury during stages of a life cycle within a
single species or between species and emphasized that results can not be applied even to closely allied species. They support this hypothesis with further evidence demonstrating that the sensitivity of barnacles drops markedly at the point of metamorphosis as compared to the presettlement or post metamorphosis stages. In work paralleling that of Clarke, they supported the view that copper is more toxic than mercury but only insofar as it relates to the settlement of barnacles.

Dosages of copper and mercury are again examined for their lethal limit to 50% of the population (LD₅₀) of Nitocra spinipes by Barnes and Stanbury (1948). The extreme difference in the LD₅₀ values for the two metals led the authors to suggest differing modes of toxic action. Also, bipartite mixtures of the two metals exhibited powerful synergistic toxicities. They suggested that one metal may interfere with the detoxification of the other and vice versa.

Shortly thereafter, (Russell-Hunter, 1950) more than additive toxicity was, again, demonstrated with Marinogammarus marinus when copper was added to mercury but not for the reverse order. Russell-Hunter was the first to comment on other parameters of vitality such as oxygen consumption. He noted that while copper decreases oxygen uptake, after an initial increase due to increased activity of the test organism, mercury does not. He was also one of the earliest to mention the increased effectiveness of mercury in diluted seawater over that of normal seawater.

As an experimental animal, Artemia salina, has proven to be by far the most resistant to mercurials (Corner and Sparrow, 1956-1957). However, its resistance could be lessened by pretreatment with sub-lethal doses of copper. Corner and Sparrow (1957) and Corner and Rigler (1958) made one of the first
concerted attempts to examine a homologous series of organomercurial compounds with respect to their toxicity in the presence of sulfhydryl containing compounds to marine invertebrates. Use of cysteine and reduced glutathion considerably lessened the toxicity of mercury to Elminius modestus and Artemia salina. These results are not totally surprising in light of the fact that the binding affinities for the mercuric ion and mercaption (Sulfur) containing compounds are very great in comparison to other chelating agents. Thus, the commonly chosen antidote in human mercury poisoning is a mercaptol compound (Dimercaperol, British Anti-Lewisite) which competes successfully for the heavy metal ions.

In recent years the approach taken in studying heavy metal toxicity has shifted. The trend has moved away from the type of experiments designed merely to contribute LD$_{50}$ values for increasingly large numbers of marine animals. An ecological tact that relates toxicity testing to such parameters as the sub-lethal effects of mercury on the photosynthesis of plankton (Harriss, et al, 1970; Boney, 1971) and the inhibited growth rates of marine organisms (Brown and Ahsanullah, 1971) has commenced. These reports have emphasized the need for chronic exposure studies of sub-lethal concentrations of toxicants before sound environmental criteria can be implemented.

The larger decapod crustaceans have been somewhat neglected in toxicity studies with mercury. Portmann (1968) made use of the brown shrimp Crangon crangon, the pink shrimp Pandalus montagui and the shore crab Carcinus maenas in his surveys of lethal limits. DeCoursey and Vernberg (1972) and Vernberg and Vernberg (1972) have initiated extensive
studies concerning the effects of mercury on the larval and adult stages of the fiddler crab Uca pugilator covering survival, metabolism, and accumulation. The also introduced for probably the first time, the combination of temperature and salinity stress conditions on systems already subjected to mercury toxicity.

The present study constitutes only a portion of the larger overall study of the effects of mercury on H. nudus. Before any meaningful work could be instigated, a range of acceptable mercury concentration values needed to be quantified. This first section was designed, then, as a starting point on which to base the other portions of the investigation.

Specifically, this section attempts to examine to toxicity of mercury to H. nudus under the conditions of normal seawater and the additional stress of reduced salinity. This not only adds to the already voluminous data on upper lethal limits for marine organisms (Portmann, 1968 and 1971) but will, hopefully, contribute information to the growing awareness of the synergistic effects of extraneous environmental stresses on the ability of an animal to withstand a toxic foreign substance.

MATERIALS AND METHODS

Throughout the fall of 1972 and early 1973, a large number of experiments were conducted to assess the effects of acute exposure to mercury as mercuric chloride on the mortality of H. nudus in 100% (32%) seawater and dilutions thereof.

H. nudus could easily be collected in abundance along the rocky coast north of Dillon Beach on medium to low tides (Figure 1). On each collection trip an attempt was made to move further north along the coast
so as to ease any deliterious effects collecting pressure may have on any single area. In the field, medium sized males and non-gravid females (approximately 2-3 cm across the carapace) were collected from their habitats under rocks. In the laboratory, animals were held in glass aquaria with sufficient water. Animals were generally fed chopped fresh frozen fish two to three times a week. Twenty-four hours prior to an experiment they were not fed and were, subsequently, starved during the actual experimentation. Twenty-four hours before scheduled experiments, a suitable number of individuals were placed in running seawater to bring them all to approximately the same physiological state. These animals were selected on the basis of vigor and healthful appearance and were assumed to be in the intermolt stage. These assumptions were borne out by dissections that failed to reveal resorption and thinning of the exoskeleton or calcium gastrolith formation (Eliss, 1968).

At the appropriate time, enough animals were removed from the holding tanks to allow five individuals to be subjected to a given condition, be it experimental or control. Data on the sex and width of the carapace were taken: the mean size of all animals for all experiments was 2.38 ± .06 cm. The mean size of all males was 2.39 ± .03 cm and the mean size of the females used was 2.24 ± .03 cm.

A constant flow assay system that would eliminate changes in the external concentration of mercury through uptake by the animals, absorption to the walls of the vessels and possible changes in the chemical form of the toxicant was not possible due to logistical problems and the inability to dispose, properly, of the large volumes of contaminated water that would be generated. A static system was,
therefore, devised with procedures designed so as to minimize reductions in the levels of mercury. Corner and Rigler (1957) determined that loss of mercury from seawater solutions was negligible up to 48 hours but that after that bacterial uptake and absorption to vessel walls was considerable. Accordingly, the present experiments were designed to allow for a total change of the environmental solution after just 24 hours of exposure. Atomic absorption analysis of the media indicated variable success in maintaining a constant external media. The measured change over a 24 hour period was 20-25% at times.

Plastic dishpans (composition unknown) measuring 30 x 25 x 12 cm, each holding five individuals under a total volume of three liters of seawater of the appropriate salinity and mercury concentration, were used throughout the study and proved satisfactory.

Double filtered seawater from the marine station seawater system was diluted to the desired salinity with glass distilled water.

Mercury as mercuric chloride was mixed to a concentration of 1000 ppm as the metal ion (not as total salt) in 5% nitric acid in distilled water. This solution has been shown to be stable for a month or more (Thorpe, 1971). It was, nevertheless, made up fresh at the start of every experiment and at the 24 hour point at which the solutions were changed. Aliquots of the stock containers to produce the desired concentrations. All concentrations, not including background levels, have been reported as parts per million of metal ion in the seawater media at the start of each experiment (or at the change of the solution).

Temperature, salinity, pH and dissolved oxygen were monitored throughout the course of the experiments. Temperatures ranged from 13 to
18.5°C for all experiments. During a given 48 hour run of any particular experiment, however, temperatures usually did not vary more than one or two degrees. Salinity, measured with an AO Refractometer varied between 32 and 34 °/oo for 100% seawater and between 8-9 °/oo for 25% seawater. A Corning pH meter with a Markson Single Electrode was used to measure pH changes. These readings ranged from 7.0 to 8.0 for all salinities. Dissolved oxygen was measured with a Yellow Springs Instruments Model 51 Oxygen Meter with a low sensitivity membrane. No attempt was made to change the membrane other than when it showed visible signs of deterioration due to use. Values were consistently highest (9.0-9.5 ppm) at the start of an experiment or just following a change of the solution and lowest just prior to the change (4.4-5.06 ppm). Aeration was not attempted due to technical difficulties involved and the consistent vigor of most animals under the control conditions. Lighting was subdued at all times but a definitely controlled cycle was impossible due to the multi-purpose use of the laboratory by other investigators at all hours of the day and night. The cycle, however, approximated fifteen hours of light and 9 hours of darkness.

Russell-Hunter (1950) has discussed different methods of assessing the toxicity of foreign chemicals on animals. He points out that the major fallacy of each method lies in its requirement of a determination of the "death" of an animal. In complex animals this tends to yield variable results. Frequently, in the case of H. nudus, following cessation of all movement, dissection revealed a beating heart. Therefore, an activity rating system of points was patterned after the method of Russell-Hunter (1950). Under this system, each animal could earn a
maximum of 5 points. At intervals of 12 hours following the start of each experiment, readings were taken on the experimental animals and the controls. Each animal was scored on a scale of 5 points making each container capable of attaining 25 points at each reading. The scale of activity points was as follows:

5 points - The animal is totally active and able to right itself immediately when placed on its back.
4 points - The animal is able to right but is lethargic.
3 points - The animal is barely able to right when placed on its back or rights only after a long struggle (1 minute or more).
2 points - An animal is unable to right itself but makes active to feeble movements of the legs, mouth parts and/or antennae.
1 point - Animal displays no movement other than feeble movements of mouth parts and antennae.
0 points - The animal shows no movement of the appendages whatever when repeatedly probed with a blunt instrument.

The total score for each container of animals is plotted against time to show the declining activity of the animals exposed to the various concentrations of mercury. From this graph, the time taken to reach 50% activity of the group in a given concentration was interpolated. This was, in turn, plotted against concentration to give the time in hours to reach a 50% reduction of activity. Whatever statistical importance is attached to a Mean Activity Time, it is at least a reliable and representative measure of the effect of a
poison on a given population. It serves to eliminate the more variable part of a curve based on a determination of the death of an organism. (Russell-Hunter, 1950).

RESULTS

Extensive preliminary experimentation was conducted using the data of Portmann (1971) for Carcinus as a reference point. Results of the preliminary work indicated that a range from 0.1 to 2.0 ppm mercury would produce a 50% reduction in the activity of H. nudus within 48 hours. It quickly became apparent that the diluted seawater media brought about a much more rapid toxic effect.

Animals showing a reduction in activity were not observed to recover when placed in fresh running seawater. Rather, toxic symptoms progressed rapidly to the point of death within six to twelve hours. Additionally, post-treatment mortality of apparently healthy animals was heavy during the entire experimentation period. In this respect, the level of Mean Activity discussed here can be likened to the commonly used lethal dose for 50% of the tested population (LD$_{50}$).

Animals serving in parallel control experiments at both salinities exhibited negligible mortality during the period of experimentation. In every case, this could be attributed to combat or cannibalism.

The cumulative results of five replicate experiments at each of four mercury concentrations in 100% seawater (three replicates for 0.5 ppm) are depicted in Figure 3. The cumulative results of five replicate experiments at each of three mercury concentrations in 25% seawater are given in Figure 4.
DISCUSSION

The elucidation of a clearly defined pattern of toxic activity for a particular agent is the first and most important step in dealing with a harmful toxicant. These curves (Figures 3 and 4) give a satisfactory representation of toxicity of mercuric ion to Hemigrapsus.

Usefulness of the toxicity curves can not be stated to extend very far beyond the range of mercury concentrations actually tested. For instance, extrapolation of the curves to their Y intercepts will give the false assumption that, in natural 100% seawater, H. nudus survives for only 68 hours (Figure 3), and that in 25% seawater Hemigrapsus will live only 61 hours (Figure 4). Obviously, this is not the case for, in truth, these curves represent small portions of a larger curve, a square hyperbola, the tails of which become almost asymptotic to the X and Y axes. Thus, at very low concentrations of mercury there is essentially no reduction in activity in both salinities while at very high concentrations there is very little difference in the time taken for the populations to reach a 50% reduction in activity, ultimately ending in death. Thus, from these curves, under the salinity conditions included here, one can relate the time and concentration of mercuric ion necessary for a population of H. nudus to reach a 50% reduction in its activity.

The converse situation holds true and is often useful. Exposure times between 48 and 96 hours have been employed by many in the field of toxicity testing (Portmann, 1968,1971; Eisler, 1971; LaRoche, et al, 1970) as a reasonable period over which to assay the toxicity of environmental contaminants. A 48 hour period was arbitrarily chosen for
this study and, applying this to Figure 3, interpolation shows that a concentration of mercuric ion equal to 1.2 ppm should cause 50% of a population of _H. nudus_ to suffer a severe reduction in the activity and an ensuing rapid death. This is comparable to the published LD<sub>50</sub> value for _Carcinus maenas_ of 1.3 ppm (Portmann, 1971).

From Figure 4, a 48 hour toxicity value of 0.24 ppm can be derived. Thus, under the stress of 25% seawater, the lethal limit of mercury for a 48 hour exposure, decreases to one sixth of its value in normal seawater salinities.

The slopes of the curves are proportional to the rate of toxicity of a substance on the test animal. In comparing slopes between the two curves, it is immediately apparent that for the 25% seawater solutions (m = -73), a small increase in the mercury level brings about a rather rapid lethal response of large magnitude. The curve describing the effects in 100% seawater has a much more gradual slope (m = -11.7). Note that a relatively greater increase in the environmental mercury concentration is necessary to produce similar changes in the toxicity of the mercuric ion to _Hemigrapsus_.

It is evident that the ratio of the slopes to that of the metal concentrations necessary for a 48 hour toxicity period are roughly inversely proportional. Thus, the additional stress of reduced salinity seriously effects the animal's capabilities to cope with the toxicant. In this regard, a pollutant present in sub-lethal concentrations may have no effect on a given population while other environmental parameters are optimal. When one or more extraneous conditions become abnormal, it may potentiate the toxicity of an agent and an organism or whole population of organisms may die.
Environmental stress factors are only beginning to be studied. Under conditions of low temperature (5°C) and low salinity (5%/00) along with sub-lethal amounts of mercury as mercuric chloride, survival of *Uca pugilator* is reduced below control levels (Vernberg and Vernberg, 1972). Only slightly better survival is obtained at high temperature (35°C) and low salinity. Unfortunately, a single environmental variable was never isolated as to its effects on the survival of *Uca* in mercury concentrations. The authors also investigated the metabolic effects of mercury toxicity by way of oxygen consumption measurements only to find a decrease in oxygen uptake under conditions of low temperature and low salinity. Obviously, the animals were expiring and, thus, decreasing their respiratory rate.

The larval stages of *Uca* have also been examined (DeCoursey and Vernberg, 1972) and the picture is now complete for this animal. Zoea I, III and V were exposed to 0.18 ppm mercury for 24 hours and, at the end of that time, all stage V and most of stage I and III zoea were dead. After 6 hours, metabolism and swimming had decreased markedly. Ten and one-hundred fold dilutions of the media were sub-lethal to the larvae but still exerted effects on metabolism and swimming. They concluded that toxicity of mercury increases with larval age for *Uca*.

In terms of water quality criteria, it does little good to state that half a population will survive 48 hours or 96 hours given certain acute toxic conditions. Questions as to the delayed or prolonged chronic effects must be considered equally important. The more fragile larval stages may be destroyed by concentrations that are well under the lethal tolerance of the adult.
Figure 1. The location of the collecting sites along the central California coast just north of Dillon Beach, California.
Figure 2. Hemigrapsus nudus (Dana)
FIGURE 3
Toxicity of mercury to H. nudus in 100% seawater
True Regression Line
\[ y = -1.7x + 61.4 \]
FIGURE 4

Acute toxicity of mercury to *H. nudus* in 25% seawater

True Regression Line

\[ y = -73x + 68.7 \]
PART II

Histological Studies
INTRODUCTION

To date a tremendous amount of data and a substantial volume of literature has accumulated on the lethal limits of heavy metals on organisms. The first section of this paper has already dealt with this topic and the literature reviews should be consulted. Little, however, is known about the effects of low levels of heavy metal toxicants and almost nothing is published concerning the physiological response to or the consequent changes of animals to such concentrations. Emphasis must, again, be placed on the fact that with its diluent capacity, the marine environment is not likely to deliver up the lethal levels discussed earlier in connection with lethal limits.

The inter- and intracellular distribution of heavy metals has been studies in various ways. Unfortunately, each has its own drawbacks. General dissection and analysis of the anatomical structures provides a picture of the gross distribution and is usually the first method of approach. However, it will not yield a resolution of various cell types that may be involved. Autoradiography will provide resolution down to 5-15 microns but is not reliable beyond the cellular level (Littman, et al., 1966). Histochemistry may identify the distribution down to intracellular loci but lacks total sensitivity and is open to interference from other metals.

The use of the latter, histopathological techniques, to investigate pollution effects is, perhaps, the best but is only beginning to be employed. Medical pathologists have contributed greatly to the available information concerning cellular response to toxicants. Both gross dissections and histological examinations have been employed in the present
work.

With histopathology, as with investigations of acute toxicity, most attention has centered around copper and zinc with only a few recent studies venturing away from those two metals. Nevertheless, it is apparent from the few papers available that heavy metals do effect changes in, both, vertebrate and invertebrate tissue architecture.

With respect to copper, Vogel (1959) has shown that goldfish exposed to 100 ppb (0.1 ppm) exhibit neurotoxic and nephrotoxic effects with severe necrosis of the renal tubule epithelium. Hubschman (1967) found that long exposures of the freshwater crayfish Orconectes rusticus to low concentrations of copper as copper sulphate produced a progressive break-down of histological organization in the antennal gland. A thirty day exposure to 0.5 ppm induced heavy vacuolation of the cells of the labyrinthic liver and complete destruction of the antennal gland in the moribund animal. The author could detect no such changes under conditions of short exposure even to higher concentrations of the metal. In Canada, where the emphasis is on the vertebrate teleost, Baker (1969) has subjected Pseudopleuronectes americanus to approximately the same levels of copper sulphate as used by Hubschman. During exposures of up to 700 hours in 0.18 and 0.56 ppm copper, Baker purported to show a decrease in the size of and an increase in the vacuolation of the epithelial layer of the gill lamellar cells. Under conditions of 1.0 ppm for up to 500 hours, the epithelial layer became detached from the basement membrane while at 3.2 ppm complete cell destruction with fusion of the lamella was noted. Accompanying the exposure to the higher concentrations were changes in other internal organs such fatty deposits in the liver and generalized
necrosis of the kidney.

Gardner and Yevich (1970) have shown histopathological changes in the intestinal tract, kidney and gills of Fundulus heteroclitus after 1-20 hours exposure to 50.0 ppm cadmium. A change in environmental parameters (pH, salinity, temperature) altered the sequence of appearance and degree of lesions. It was concluded, however, that morphological changes were indistinguishable from those observed under natural seawater conditions.

In the single published work pertaining to mercury, histological considerations are only briefly mentioned. Rucker and Amend (1969) have stated that gill hyperplasia was the only condition noted in rainbow trout and chinook and sockeye salmon after repeated weekly exposures to 2 ppm Timsan (6.25% Ethyl mercury phosphate). Tissue levels were upwards of 39 ppm for the kidneys. No changes from the normal were noticed in the liver, red blood cells or kidney.

Beyond the brief account of Rucker and Amend, no further published work has been unearthed or otherwise brought to this author's attention. As such, the results contained in the present study probably constitutes the first organized effort to differentiate structural changes in invertebrate tissues in response to the stress of the mercuric ion.

MATERIALS AND METHODS

In a series of experiments carried out during the winter and spring of 1973, the shore crab Hemigrapsus nudus was subjected to long term exposure to low concentrations of mercury under two salinity regimes to determine the effects on the general histology of representative tissues.

H. nudus was collected from the same general area as described earlier and held in glass aquaria for varying lengths of time in the laboratory.
Only vigorously active animals with no signs of physical impairments to health were selected for use. Adult individuals of approximately 2 cm across the carapace were selected without regard to sex.

Plastic containers measuring 25 x 15 x 12 cm were used throughout the course of the work, and when filled with three liters of seawater, served to hold three individuals adequately without crowding or undue stress.

Stock solutions of mercury were prepared each week according to the procedures described in Section 1. The previous acute toxicity experiments were valuable in choosing the appropriate range of concentrations for chronic exposure studies. It was evident that increased survival of virtually the entire population would be possible at concentrations of 0.1 ppm and less in 100% seawater while extended survival would be possible in 25%. To bracket this value, concentrations of 0.05, 0.1, 0.3 and 0.5 ppm Hg\(^{+2}\) were chosen for use. Subsequent experiments as well as controls in normal 100% or 25% seawater, were set up and repeated until at least ten individuals from each of the various regimes had been examined histologically. Water in the containers was changed every 24 hours, and with each change, fresh mercury stock solution was added to maintain the concentration at the desired level.

Temperature, salinity, pH and dissolved oxygen were monitored as in the toxicity studies and values conformed to the ranges given in that section. Throughout the extended course of the experiments animals were fed two to three times per week on chopped fresh frozen fish. Animals were allowed to consume as much as they wanted for 6-12 hours before food was removed.
Successful chronic exposure was arbitrarily chosen to be survival for 200 hours or longer under the experimental conditions. Under the stress of lowered salinity and the higher mercury concentrations, survival did not exceed the 200 hour minimum and, therefore, fewer animals were examined (Figures 5 and 6).

Toward the conclusion of the study it was believed beneficial to balance the results of the chronic exposure studies with very short term, acute exposures to extremely high concentrations of mercury. Under these conditions enough individuals were exposed to 50.0 and 100.0 ppm in 100% seawater so that at least three active animals could be sacrificed at 6, 12, and 24 hours after the start of the experiment for histological study.

Portions of gill filaments, hepatopancreas, stomach, heart and antennal glands were removed from healthy animals sacrificed during the course of the experiments as well as at the end of a trial. Moribund animals were dissected immediately upon presenting signs of imminent death, i.e. inability to right themselves, feeble movements of the legs, mouthparts and antennae. When these signs were ignored animals did not recover. Upon dissection of these animals, or any other animals during the study, if beating of the heart was not detected the animals were considered dead and unsuitable for microscopic examination (See Figures 5 and 6).

Immediately upon removal, tissues were placed in 0.15% Propylene phenoxytol for 5-10 minutes and then fixed in Smith's alcoholic Bouin's solution (Guyer, 1953) to which had been added 1% calcium chloride. Variations on this scheme involved elimination of the narcotization as well as fixation in standard Bouin's. No differences in tissue
morphology could be attributed to these procedural changes. Tissue was dehydrated in a graded series of isopropyl alcohols and embedded in 52.2°C paraffin wax, sectioned at 10 microns and stained regressively with Delafield's Iron Hematoxylin and counterstained with Putt's Eosin (Humason, 1962).

RESULTS

Following careful removal of the carapace and the delicate tissue lining it, one exposes the total body cavity. The vivid color differentiation of the organs makes identification rather easy. Figure 7 shows the typical control animal after such a dissection procedure and illustrates relationships of the various tissues sampled. Shortly after the study began it became apparent that little would be gained by continuing to investigate the histology of the gut, and heart as little cellular structure could be recognized in either organ. Study of these organs was then abandoned and attention focused on the gills, hepatopancreas and antennal glands.

Gill

Control: On either side of the body cavity immediately posterior to the hepatopancreas, the gill filaments lie compactly tucked into lateral depressions of the exoskeleton (Figure 7). Eight distinct pairs of filaments of varying size conform to the oval branchial chamber. With the carapace intact there is just enough room for the movement of the gill raker to provide circulation of seawater throughout the branchial chamber.
The gill filament is formed by a large number of lamellae (Figure 10,1) or broad flattened plates serially arranged in pairs along the central gill axis (Figure 10,c). The axis provides support for the lamellae and is the pathway for afferent and efferent branchial blood vessels. The entire outer surface of the gill is covered by a 1 micron thick chitin layer.

Underlying the chitin is a layer of living epithelial cells. Contiguous to the epithelial layer and lining the lumen are the pillar cells (Figure 11,p) whose projecting processes delimit lacunae within the lamellae. In life these are filled with blood and communicate with the afferent and efferent branchial vessels of the filament axis (Figure 10). The tips of the lamellae are also expanded into broad lacunae for circulation (Figures 10 and 11).

Experimental: During the course of the study 25 animals were removed from the salinity stress of 25% seawater in combination with various mercury concentrations prior to the 200 hour exposure point. Of these 25 individuals, only four exhibited what was considered to be gill morphology different from the control animals. One of the four was deemed to be only marinally different from the normal.

Fifteen animals were removed from 100% seawater in combination with various mercury concentrations prior to the 200 hour exposure point. None of these animals, in the opinion of the author, were observed to have gill structure varying from that of the control individuals.

Following extended (greater than 200 hours) exposure to low concentrations of mercury there is a generalized breakdown of the gill structure roughly proportional to the concentration and length of
exposure. Figure 8 illustrates the results pertaining to the gill filaments on a gross anatomical level. The most striking result is the blackening of the lamellae. This appears to be both localized and generalized with respect to the individual filaments. The effects on a single filament are shown more clearly in Figure 9.

On the cellular level these blackened lamellae take up the counterstain to a great extent and while nuclei are present, cell structure is virtually obliterated (Figure 12). The tips of the filaments often exhibited this effect and upon sectioning seemed to fracture as if solidification of the living tissue had taken place (Figure 13). Those areas untouched by the necrosis have a shredded and extracted appearance (Figures 12 and 14). A total breakdown of the cellular structure is evident. The epithelial layer and pillar cells are ragged in appearance and in complete disarray after 1000 hours in 0.1 ppm. Vacuolation of both the central axis and lamellae is extensive and there is widespread deformation of the nuclei (Figure 14).

No experimental animals in 25% seawater were able to survive long enough to qualify as chronic exposure specimens. Controls, however, were active for more than 900 hours at which time the experiments were terminated. Only in a single case was a moribund individual considered unsuitable for use after 700 hours.

Survival was very good up to a concentration 0.1 ppm mercury in 100% seawater. Many individuals were active for well over 600 hours. Even animals sacrificed after 1000 hours of exposure showed no outward signs of ill health. Ten such animals qualified as chronic exposure victims. All of these specimens showed some degree of gill damage ranging from
mild vacuolation in those sampled between 200 and 600 hrs. to the massive tissue destruction pictured in the accompanying photos (Figure 14).

Of the animals subjected to severe acute exposure of 50.0 and 100.0 ppm none showed changes from the normal.

**Hepatopancreas**

Control: The hepatopancreas, a bilateral evagination of the midgut, functions in secreting enzymes and absorbing and metabolizing food stuffs. The gland is composed of innumerable tubules separated from each other by loose connective tissue and proliferates throughout the body cavity in the space not occupied by the stomach, heart or branchial chambers (Figure 7).

A longitudinal section of a tubule shows the lumen to be lined with evenly staining columnar cells having basily oriented nuclei (Figure 15, cl). Cross sections reveal a multiradiate lumen that is variable in diameter. This variation in both size and shape of the lumen is possibly related to the nutritional state of the animal.

Experimental: Of the 25 animals from 25% seawater-mercury regimes examined prior to the 200 hour point, four displayed changes in the general histology of the hepatopancreas considered to be different from the controls. One of these individuals had undergone exposure for nearly 100 hours while the others were all below 50 hours.

In the group of 15 animals from 100% seawater and 0.05 to 0.5 ppm mercury, three showed changes from the normal before 200 hours had elapsed. One of these, however, had been exposed for 190 hours to 0.1 ppm, and one for 122 hours at the same concentration. The remaining one underwent an
exposure for only 48 hours but at the highest concentration of mercury 0.5 ppm.

The most predominant feature of chronic exposure observed in the hepatopancreas is manifested as a widespread vacuolation of the lumen wall (Figure 16). It is possible that the column cells (Figure 16, cl) may participate in that they are greatly swollen or they may suffer extreme displacement as the vacuolation of the wall proceeds. This vacuolation was found to be severe in some cases such as the one depicted in Figure 17. Pronounced vacuolation has taken place to the point where the lumen wall is no longer recognizable. In this particular example, 190 hours had elapsed at the 0.1 ppm level of mercury when the tissue was taken for analysis.

Of the ten individuals examined between 200 and 1000 hours of exposure, all but two displayed this typical exposure morphology, while none of the animals subjected to the extreme acute exposure of 50.0 or 100.0 ppm could be considered to show it.

Antennal Gland

The antennal glands could invariably be found, upon careful removal of the stomach and hepatopancreas, against the inner wall of the carapace immediately below the base of the eyestalks and in close proximity to the urinary openings. The gland was observed as a small mass of translucent tissue and could be removed as discrete body if care was taken.

Control: The histology of the decapod antennal gland has been well treated (Marchal, 1892) (Pearson, 1908). The antennal gland of H. nudus conforms to prior descriptions. Four distinct structures form the renal
complex; the end sac, labyrinth, renal tubule and bladder. The lumen of the end sac communicates with that of the renal tubule which empties excretory products via the bladder to the outside. Throughout the duration of the study, tissue identified as antennal gland consistently proved to be the labyrinthine portion of the complex as typical sections through the material revealed the interdigitation of the convoluted tubules.

Tubules cells are cuboidal in shape (Figure 18, cb). The nuclei stain deeply with the iron hematoxylin and the cytoplasma takes up the counterstain uniformly. Intertubule spaces are filled with non-nucleated connective tissue (Figure 18, it).

**Experimental:** A marked variance from the previous pattern of results is found in the 25 animals examined from 25% seawater. In this instance, antennal glands from 11 of the individuals show a divergence from the normal. Seven of these occur following exposures of 48 hours or less, with the remaining four coming after an exposure of just under 100 hours.

As for the animals from 100% seawater, only two out of 15 were considered to be abnormal. These came after exposure to 0.1 ppm for more than 100 hours.

Under chronic conditions of up to 1000 hours in 100% seawater and mercury stress, just half of the ten animals could be considered to possess changes in the antennal gland structure from that of the normal control animals. This was manifested in two general ways. Figure 19 illustrates the first and most common observable variation, that of a lack of a loss of staining characteristics and, thus, a loss of differentiation of the tubule cells. The cells have lost nearly all distinction
from the connective tissue surrounding and supporting them, and it is
difficult to assess whether the cuboidal shape has been maintained.
Second, a vacuolation of the tubule cells was apparent in only a few
cases.

None of the short term exposures to high concentrations of mercury
were successful in producing recognizable changes in the morphology of
the crab antennal gland tissue.

DISCUSSION

A toxic agent may be exogenous or produced by the organism itself
as a result of a deficient metabolic cycle (Baker, 1969). It is probable
that the data accumulated by medical investigators on pathological changes
may be relevant to pollution studies only insofar as exogenous toxicants
are concerned. Nevertheless, concerted efforts on a cooperative basis
between medical practitioners and marine environmental investigators can
not fail to reap benefits in the future.

Within the limits of light microscopy and the facilities available
for gross histological studies, this research has attempted to define some
of the more obvious histopathological changes taking place in various
tissues under the stress of sub-lethal mercury concentrations in seawater.
As a result of man's activity, it is no doubt possible that mercury levels
in a restricted marine environment, such as a confined estuary or tidal
marsh, could approach the lowest levels incorporated in this portion of
the study.

While toxicity of heavy metals is normally measured in acute tests,
the low level effects may be at least as damaging (Brown and Ahsanullah,
1971). Worrisome to many, and justifiably so, are the prospects of long range consequences now almost entirely unknown. This study has begun to bring some of those consequences to light.

It is to be concluded that in the course of extended exposure to very low environmental levels of mercury an organism such as *Hemigrapsus* will begin to show tissue damage between 200 and 1000 hours. The gill filaments and the lamellae are the most affected regions of the anatomy. This is reasonable due to the very extensive surface area of the lamellae and the vigorous metabolic activities carried on in this organ. Gray (1957) has determined gill areas in many species of crabs including two species of related *grapsids*. In *Sesarma cinerea*, thirteen determinations yielded a mean gill area of 638 mm$^2$/gram body weight. For *S. reticulata* the mean area of eight animals was 579 mm$^2$/gram body weight. *H. nudus* is no doubt within this range of values and when considering the weight of a medium sized adult of 2 cm carapace width to be approximately 7-15 grams, this provides an area somewhat equivalent to that of a 3 x 5 inch index card. This is a large amount of surface to be presented to a toxicant with the absorptive as well as the adsorptive capabilities of mercury.

Metabolically, it has been demonstrated by Ayers (1938) and Verberg (1956) that intertidal and land crabs have the highest oxygen consumption on both a whole animal and isolated gill tissue basis. Furthermore, they have shown that there is a progressive decrease in the oxygen consumption as the habitat approaches the ocean depths.

Additionally, Flemister (1959) has discussed the role of the gill as the chief portal of entry of the chloride ion into the body of the crab.
The epithelial cells were shown by her to be rich in mitochondria, a recognized sign of metabolic activity.

It is not surprising, then, to find the gill so devastatingly attacked by the mercuric ion. After approximately 600 hours, when the blackened necrosis sets in and the lacunar structure begins to breakdown, the efficiency of the gill as a respiratory organ and ion pump must surely decrease markedly. Only by virtue of the vast gill surface area provided can the animal compensate for the deficient mechanisms and carry on in a state of apparent health and vigor.

Whether these changes are primary or secondary is difficult to assess. The mercuric ions are probably taken up by the gill epithelial cells and act primarily on cellular systems resulting in the formation of lysosomes, vacuoles, and vesicles. Alternately, simple adsorption to the gill surface may be causing damage to metabolic transport systems.

The hepatopancreas with its numerous diverticula is the most important region of the midgut from a functional and nutritional point of view. While it is known to be responsible for the secretion of enzymes and storage, in many cases, of glycogen and probably other food reserves at least one textbook author (Meglitsch, 1967) has pointed out that it is not possible to discuss specific roles played in digestion and absorption. Andrew (1959) has described column cells as well as pyriform cells lining digestive diverticula. He attributes storage and absorption responsibilities to the former and enzyme secretion roles to the latter. These enzyme cells conform in structure to what appear as vacuolated areas of the lumen wall in experimental animals under chronic toxicity stress. Nutritional data was carefully recorded during the course of all the experiments, thus, making
it possible to estimate the probable presence of food in the gut and digestive gland and compare it to the time of tissue sampling. At no time was feeding in close proximity to a sampling. In all aspects possible experimental animals were in a nutritional state closely paralleling that of the controls. While the control individuals (Figure 15) show a few scattered cells of the form described by Andrew, the experimentals (Figure 16) demonstrate these structures to a much greater degree. Eight out of ten animals exposed for more than 200 hours were considered positive in this regard. This poses the question as to whether the two areas-chronic mercury toxicity and nutritional physiology—could possibly be related in such a way as to produce a response in the enzyme secreting mechanisms of the hepatopancreas in the presence of a mercurial toxicant. Eubschman (1967) has shown that detoxification of copper by fresh water crayfish proceeds at such a rate as to prevent inhibition of succinate utilization or oxygen consumption of homogenates of the hepatopancreas. His histological work on chronically exposed animals, however, was confined to the antennal glands and he can not provide a visual picture of what is actually occurring at the cellular level in the hepatopancreas.

The antennal glands of decapods have long been intimately linked with the processes of osmotic and ionic regulation (Riegel and Kirschner, 1960; Riegel, 1961, 1963). In his 1963 paper, Riegel firmly established this through micropuncture studies. Flemister (1959) provided evidence by means of the Leschke silver nitrate test, that chloride ion is excreted from the body via the antennal gland.

Other evidence (Krugler and Burkner, 1948) demonstrates the high
level of metabolic activity concentrated in the cells of the antennal gland complex. Here, as with the gill filaments, it is not surprising to find cellular damage.

Detoxification mechanisms will be discussed in the following section of this paper. As the antennal gland is primarily involved with excretion in the decapod, it is not unreasonable to think of it as playing a role in possible detoxification of harmful exogenous materials in the environment, such as pesticides, detergents and heavy metals.

If active excretion of mercury is taking place in the antennal glands, then the histological changes noted prior to the 200 hour point may be explained. Particularly with regard to the animals in 25% seawater trying to keep pace with the salinity stress by producing large amounts of hypotonic urine, the additive effects could be taking their toll before 200 hours. Animals in 100% seawater show much less change (2 of 15 prior to 200 hours and 5 of 10 up to 1000 hours). This could be related to the absence of the need to balance body osmotic and ionic conditions. Total attention could be given to detoxification of mercury ions.

Hubschman (1967) purported to show damage to the labyrinth portion of the crayfish antennal gland in the form of vacuolated cytoplasm and deformed nuclei following 30 days exposure to 0.5 ppm copper sulfate. After more than 40 days exposure to sub-lethal concentrations of mercuric chloride in the present study, no change in the nuclear form could be detected in the antennal gland cells of any other tissue examined. Vacuolation, however, seemed to be general result in all tissues examined.
after chronic stress.

All of the tissues sampled in the course of this study are linked in one way or another to important physiological systems that all make use of intricate enzymatic pathways. Likewise, all show some degree of histological change from the normal when placed in low concentrations of mercury in seawater. As authors discussing proposed modes of toxic action of heavy metals repeatedly make mention of effects on enzyme systems (Pyefinch and Mott, 1948; Corner and Sparrow, 1956; Vallee and Ulmer, 1972), one can only wonder what further extensive use of histochemistry and histologic techniques might reveal about the mechanisms of toxicity of the heavy metals.
Figure 5. Chronic exposure of _H. nudus_ to low concentrations of mercury as HgCl₂ in 100% seawater.

Each circle represents a single animal taken for histological study. Circles with an X represent animals that had died and were not examined for histopathological changes.
Figure 6. Chronic exposure of H. nudus to low concentrations of mercury as HgCl₂ in 25% seawater.

Each circle represents a single animal taken for histological study. Circles with an X represent animals that had died and were not examined for histopathological changes.
Figure 7. Typical specimen of a healthy *H. nudus*. The carapace has been removed along with the underlying tissue to expose the body cavity. Tissues taken for histological analysis are as marked: g-gill, hp-hepatopancreas, he-heart, s-stomach. The antennal glands are not observable.

Figure 8. Experimental animal following approximately 800 hours in 100% seawater and 0.05 ppm mercury. Note widespread blackening of the filaments as opposed to the gills in Figure 7. Extracted appearance of body cavity due, in part, to removal of hepatopancreas and storage effects.
Figure 9. Isolated gill filament from a crab exposed to 100% seawater and 0.05 ppm for 1000 hours. Regions of necrosis of the lamellae are distinctly apparent. Scale is in millimeters. 6.3X.

c - central axis   l - lamellae
Figure 10. Section of a normal gill filament. The central axis traverses the picture with lamellae alternately arranged. Note (arrow) lacunae of lamellae communicating with central axis spaces. The tips of lamellae are expanded into lacunae. 63X.

c - central axis  l - lamellae

Figure 11. Individual lamellae of a normal gill filament. Pillar cell processes delimit lacunae within the lamellae. 160X.

p - pillar cells  la - lacuna
Figure 12. Portion of a gill filament from an animal exposed to 100% seawater and 0.05 ppm mercury for 800 hours. Distal parts of the lamellae have lost all cell structure while portions of the lamellae proximal to the axis show breakdown of the regular orientation. 63X.

Figure 13. The tip of a gill filament effected by 800 hours exposure to 0.05 ppm in 100% seawater. Note fractured appearance. 63X.
Figure 14. Following exposure for 1000 hours to 0.1 ppm mercury in 100% seawater, lamellae have lost all semblance of organized structure. Total breakdown in the cell structure is accompanied by deformation of nuclei (arrow). Central axis is to the left. 250X.
Figure 15. Typical view of normal *H. nudus* hepatopancreas. Longitudinal sections show columnar cells with basal nuclei lining the lumen. Cross sections show tetraradiate lumen configuration. Presence of vacuolated cells is rare. 63X.

1.s - longitudinal section  
cl - column cells  
xs - cross section  
lu - lumen

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Figure 16. Hepatopancreas from an experimental. Heavy vacuolation of the lumen is apparent after 802 hours exposure to 0.05 ppm mercury in 100% seawater. 63X.

cl - column cells  
v - vacuoles
Figure 17. Hepatopancreas from an experimental animal having undergone 190 hours exposure to 100% seawater containing 0.1 ppm mercury. Note the almost total obliteration of the lumen walls. Column cells have been heavily vacuolated. 250X.

cl - column cells
Figure 18. Section through the antennal gland of a control animal. Convoluted tubules interdigitate. Tubule walls are lined with cuboidal cells and intertubule spaces are filled with non-nucleated connective tissue. 160X.

ct - convoluted tubule  it - intertubular
cb - cuboidal cells  connective tissue

Figure 19. Section through the convoluted tubules of an experimental animal after 12 hours exposure to .3 ppm mercury in 25% seawater. Note lack of distinction of tubule wall cells. Intertubule spaces contain nucleated material (arrow). 160X.
Note on Photography

Figures 2, 7, 8 and 9 were all photographed using a macro-lens at f/22. Exposure time for all was 1/2 second.

Figures 10 through 19 were photographed through a Leitz Ortholux scope at the magnifications indicated using a green filter. Exposure time for all was 1/8 second.
PART III

Accumulation and Distribution of Mercury by Hemigrapsus nudus
Marine invertebrates, especially filter feeders or other animals collecting calcium for their shells, are renown biological accumulators of heavy metals. Tissue levels of 10,000 to 100,000 times greater than that of seawater are common (Korringa, 1968; Pringle, 1968). Invertebrates nearly always exceed vertebrates in this regard (Goldberg, 1957).

The most important route of accumulation appears to be directly from the water through the outer epithelia, i.e. direct absorption (Hannerz, 1967). This dictates that the aquatic environment is of basic interest in mercury studies, as one of the most important factors governing levels and rates of buildup in marine organisms.

Possible modes of uptake of heavy metal ions are numerous (Brooks and Humshby, 1965; Pringle, 1968). These include: (1) Particulate ingestion of suspended material from seawater to which ions may be adsorbed; (2) Ingestion of elements pre-concentrated in food material; (3) Coordinate complexing of metals with appropriate ligands in tissues; (4) Incorporation of metal ions into physiologically important systems; (5) Uptake by exchange, for example, onto mucous sheets of bivalves.

Lehninger (1950) in a general review article approached the problem of the biological specificity of metal ions for organic molecules such as proteins and carbohydrates on the physical basis of ion structure and properties. Until then, little had been done on these physiologically important substances with respect to the complexes formed under the chemical and physical variables of a biological system (Williams, 1953).

Lately, considerable material has accumulated concerning the biochemistry of trace metal uptake and its fate within the living organism.
Clarkson (1972) has discussed the binding of mercury with proteins and the biotransformations that it must undergo, while Rothstein and Hayes (1960) in Clarkson (1972) have examined, from a theoretical point of view the important factors that determine the transport of mercury from the environment to the tissues. They emphasized that tissue uptake is determined by chemical affinities between cell binding sites and diffusable ion complexes. As variation in affinities and diffusability occurs, not all complexes contribute to tissue accumulation.

The literature relating to accumulation of heavy metals by organisms is diverse with no emphasis on any single species apparent. However, the attention has seemed to center on generalized groups of animals and enough material is available to begin to draw meaningful relationships. Swedish biologists have worked almost exclusively on bird and coastal fish populations. Their interest has been intense since the early sixties when questions concerning the effect of mercury treated seed grains were first raised. The earliest surveys (Tjening, 1967) immediately revealed accumulation in the liver, muscles, kidney and plumage of pheasants.

Techniques designed to distinguish between the forms of mercury revealed that transformation from the less toxic phenyl mercury and alkoxyalkyl mercury to the more deadly methyl mercury had taken place within the bodies of the birds. Phenyl mercuric acetate (PMA), clearly from pulp mill effluents, was found at high concentrations in the yolks of quail eggs (Swedish Royal Comm., 1966).

Since 1965 when mercurials were totally banned in agricultural use, residues in eggs and adult tissues have dropped (Ackefors, et al, 1971). The problem remains acute, however, for fish and birds that eat fish.
Sample concentration levels of 10 ppm are still being recorded (Jernelow, 1968).

The first evidence of elevated levels in fish from Swedish waters came in 1965 (Ackfores, et al., 1971). The highest levels were found in fresh water fish. Seventy to 100% of the total mercury present was determined to be in the methylated form. Johnels, et al., (1967) found mercury content of the axial muscle of pike to be directly proportional to the age and weight of the fish except at high levels where it leveled off to a somewhat constant value. The proportion of methyl mercury also appears to rise with age (Bache, et al., 1971).

Portions of the work of Johnels, et al (1967) involved monitoring animal tissue levels above and below a dam belonging to a pulp factory. Pike above the dam (upstream from the point of effluent discharge) registered 0.3 - 0.6 ppm. Members of the same species caught and monitored below the dam had 2.4 - 8.0 ppm mercury in their tissues. The common leech taken from above the dam contained 0.025 ppm mercury while leeches below the dam had 3.1 - 4.4 ppm.

The accumulation rate for fish was found to be very high and the elimination rate low producing high concentration factors. Highest levels in the fish were found in the brain, kidneys, liver and spleen (Hannerz, 1967). Varying amounts of mercury accumulate in the gills, muscle and skin depending on the method of intake, i.e. food, contaminated water, injection (Swedish Royal Comm., 1966).

Canadian research has centered almost exclusively on coastal fish surveys and laboratory experimentation with common teleosts. Wobeser, et al., (1970) first noted elevated levels in fish from the Saskatchewan River system. He has published results ranging up to 21 ppm for kidney which
appears to be the sight of heaviest absorption. Concentration factors for teleost kidney may be as high as 9000 for methyl mercury (Hannerz, 1967). As much as 92% of the total mercury content is methyl mercury (Zitko, 1971).

Other Canadian surveys have not been nearly so alarming. Uthe and Bligh (1971) sampled fish from both heavily industrialized and non-industrialized areas for 13 toxic substances. For Pb, As, Cu, and Zn there was no significant difference in tissue levels from the two areas. Only mercury exceeded the recommended limits. Tissue levels in fish from the industrialized Great Lakes Basin ran up to 0.7 ppm.

Gillespie and Scott (1971) and Gillespie (1972) studied the mobilization of mercury from sediments into guppies only to find differences in the degree of accumulation and the percentage of methyl mercury for different compounds of mercury studied. Under both aerobic and anaerobic conditions mercury accumulated significantly. Tissue levels rose to 3.0 ppm after 50 days exposure over sediment laced with HgCl₂. Methyl mercury constituted 30-45% of the total burden when the sediments were spiked with metallic mercury, mercuric Chloride or HgS. Lignosulphonate nutrient enrichment of the sediments stimulated mobilization and methyl mercury totals in tissue, apparently by increasing sediment microbial activity, already known for its methylation capabilities (Jensen and Jernclov, 1969; Wood, et al, 1968).

American efforts have concentrated on fish and shellfish in an effort to maintain a healthy and viable economic resource. Nucker and Amend (1969) investigated the effects of a forty year practice in United States hatcheries of using various mercurials to control intestinal protozoa as
well as Pridylmercuric acetate and Ethyl mercuric phosphate as a bath to control external parasites. Depending on the concentration pattern of exposure employed, levels in the blood, liver, kidney and muscle rose to as much as 40 ppm (Kidney) but then fell off to lower, but not control, levels when returned to a clean environment.

Very recently Cunningham and Tripp (1973) studied the uptake of mercury by the oyster *Crassostrea virginica* in 0.01 and 0.1 ppm solutions of mercuric acetate. After 45 days of exposure, tissue homogenates contained 28.0 and 140.0 ppm respectively. Vernberg and Vernberg (1972) published the only work to date employing brachyuran decapod in studies on mercury accumulation. They detected 0.03 ppm total mercury in the hepatopancreas of their control *Uca pugilator* but none in any other tissues nor in the seawater off the coast of South Carolina. Following 24 hour exposures to an environmental concentration of 0.18 ppm in seawater, gill levels rose to 1.73 ppm. Tissue levels continued to rise for 28 days after which the gill tissues contained 0.14 ppm and the hepatopancreas 3.75 ppm. Later, Vernberg, this time in collaboration with O'Hara (1973), used a different technique of mercury analysis to determine that 82% of the entire mercury burden in *Uca* was to be found in the gills. Certain environmental parameters such as salinity and temperature were varied to determine the possible synergistic effects of stress on mercury accumulation (See Discussion).

The third and final portion of the present research was intended as an overall study of the patterns of accumulation of total mercury with respect to individual tissues by *Hemigrapsus nudus*. The study began with an in-depth survey of natural environmental levels of mercury found in the
crab itself as well as the seawater of Bodega and Tomales Bays. Laboratory experiments were designed and carried out to determine the absolute accumulation from a seawater medium in relation to its mercury concentration and salinity. These values were supplemented by an examination of the accumulation by dead animals.

Additional routes of possible accumulation were studied. First, sediment was dosed with a known amount of mercury and animals were allowed to roam freely atop the moist substrate for a period of time. Secondly, mercury "spiked" food material was offered, underwater, to starved individuals for a specified period of time and then removed. At varying intervals animals were taken for analysis.

Possible mechanisms for the detoxification of crab tissues were examined by exposing the animals for a period of time and then removing them to fresh running seawater and sampling the population at specified intervals.

It was hoped that this part of the study would, in conjunction with the histology portion, shed additional light on the events as they transpire at the tissue level.

MATERIALS AND METHODS

During the late winter and early spring of 1973, the series of experiments comprising this portion of the study were carried out.

Tissues were collected and stored at the Pacific Marine Station, Dillon Beach, California. Tissues were then transported to the Cell Physiology Laboratories of the University of the Pacific, Stockton, California where analysis of total mercury was conducted by Flameless Atomic Absorption Spectrophotometry.
Control material was selected from a number of sources: (1) Animals used as controls in various experiments; (2) Animals taken from the laboratory holding aquaria; (3) Animals taken straight from the field environment. None could be considered to have been exposed to anything but natural mercury levels. As a check on the possible causes of variation observed for the control animals, several were allowed to feed on chopped Mytilus californianus for two to twelve hours before their tissues were collected. Thus, results in Figure 21 are broken down into values for fed and starved animals (see Discussion).

Much of the experimental detail is identical to previous sections. Specimens of H. nudus were collected, held, fed and otherwise treated identically as heretofore described. The containers used for these accumulation studies were those employed for the toxicity work. Mercury stock solutions and experimental dilutions thereof were prepared using accurate volumetric techniques and paralleled the procedures already described for the toxicity work. Environmental monitoring was carried on as before with comparable ranges of values.

Employed for studies of accumulation from the liquid media were mercury concentrations of 0.001 and 0.01 ppm in 100% seawater as well as 0.1 and 0.5 ppm in conjunction with 25% and 100% seawater.

At the beginning of an experiment three individual crabs were placed into each dishpan containing three liters total volume of the appropriately mixed (with respect to salinity and mercury content) experimental solution. This medium was changed after 2½ hours as in the toxicity work. After 12, 24 and 48 hours an animal was removed from each medium and placed under -15°C conditions until the animal had expired or was sufficiently moribund to allow for dissection. The
carapace was cut with stout scissors around its perimeter and removed exposing the interior of the body cavity (Figure 7). A non-metallic brush was used to gently remove soft tissue adhering to the carapace and was rinsed briefly in distilled water and allowed to drain on absorbent towelling. Portions of the gill and hepatopancreas were also removed, rinsed and allowed to drain. The stomach and its contents were removed intact, rinsed and drained. Individual tissues were placed in tared vials and the tissue wet weight was determined. Thus, all data presented is on a wet weight basis. The vials were then marked and refrozen to await analysis. Three replications of each experiment allowed for at least triplicate analysis for each condition. Carapace, gill, hepatopancreas and stomach were collected and identically treated for each experiment except for the accumulation by dead animals in which the soft internal tissues were pooled.

In experiments using dead *H. nudus*, the animals were sacrificed under the narcotic effect of low temperatures (-15°C) before being introduced into the experimental media. The experiments were carried out using 0.5 ppm mercury in 100% and 25% seawater. At the start of an experiment each dishpan contained three animals. After 12, 24 and 36 hours a single animal was taken and immediately dissected. A change of the solution was made after 24 hours. Three replications of the experiment were conducted.

In examining mercury accumulation from moist substrate 100% seawater containing 0.1 or 0.5 ppm mercury was mixed with 2 liters of freshly washed sediment from the field areas normally inhabited by *H. nudus* to produce a slurry. This was allowed to stand for 15 minutes following which excess water was drained to leave a glistening sediment. Over this substrate, six *H. nudus* were released. At 12, 24 and 48 hour periods after the start
of the experiment two animals were removed from each dishpan and sacrificed by freezing and the tissues collected and stored. During the course of the experiment no attempt was made to replace or recharge the sediment-mercury mixture. Duplicate analysis of each tissue comprise the graphical material. Sediment samples were taken at 0, 12, 24 and 48 hours for analysis of mercury content.

In order to examine the pathways and distribution of mercury within the crab as a function of its method of administration, suitable food material in the form of healthy Mytilus californianus was exposed to 100% seawater containing 0.1 ppm mercury for 96 hours (water and mercury replaced every 24 hours). Six individual H. nudus that had been totally starved for a week or more were placed in each of two dishpans containing 4 liters of clean seawater. A suitable amount of the dosed mussel meat (mantle, gonad and viscera were included in the fare) was offered and all crabs were noted to feed vigorously. Samples of the mussel tissue consumed by the crab were taken for proper analysis of their mercury content and triplicate analysis revealed a mean value of 3.51 ppm.

Following four hours of feeding, the remaining food was removed, along with a single animal for each dishpan for determination of initial levels of mercury. The water was replaced with fresh seawater for the remaining crabs and they were left undisturbed. After 12, 24, 48, 96 and 114 hours a pair of animals was removed and their tissues collected. A minimum of two analyses are available for each tissue at each time.

To determine the presence of detoxifying capabilities for H. nudus, six crabs were placed in each of two dishpans containing 3 liters of 100% seawater of 0.1 ppm mercury content. Each solution was changed after 24
hours and the entire batch of animals in both dishpans was allowed to sit for 48 hours at which time a pair of individuals was removed and frozen for analysis. The remaining animals were placed in fresh running seawater. After 24, 48, 96 hours and three weeks, a pair of animals was removed and the tissues collected. Duplicate determinations are included along with spot values for a detoxification experiment involving dosing the animals at 0.5 ppm. High mortality both during and after treatment did not allow for the successful completion of that experiment.

The Flameless method for atomic absorption analysis of total mercury based on the techniques of Hatch and Ott (1968) is becoming the method of choice for accurate measurement of the mercury content of biological materials. It was employed in this study using a Perkin-Elmer Model 290-B Atomic Absorption Spectrophotometer with a hollow cathode lamp and the standard Mercury Analysis System specifically designed by Perkin-Elmer for their instruments.

A modification of the methods of Uthe, et al (1970) was used for handling and preparing of the tissues. Reagent grade chemicals were used throughout. Mercury was not detectable in any of the reagents used (Khanna, pers. comm.). The mercuric ion is complexed in the tissue, but is gently and effectively liberated by a wet acid digestion which hydrolyzes and/or partially oxidizes tissues. The freshly thawed tissue sample was transferred to the bottom of a 125 ml erlenmeyer oxygen flask and 30 mls of concentrated sulfuric acid (hydrochloric acid for carapaces) and 5 mls of concentrated nitric acid were added followed by 1-2 hours on a shaker bath at 50-60°C. When a totally clear, slightly colored solution resulted, it was transferred to a BOD bottle containing 50 ml of cooled, double
distilled water. All remaining macromolecular structures (carbohydrates, amino acids, fatty acids, organomacurials, the mercurous ion or its salts) were oxidized with granular potassium permanganate. Such oxidation eliminates nitric oxides which interfere with elemental mercury determinations. The BOD was allowed to heat in the water bath for 1 hour. Excess oxidizer was reduced with hydroxylamine hydrochloride and the sample containing mercuric ion and no organic chelating agents, was then ready for analysis.

Seawater samples were handled in much the same manner, however, reagents were diluted in double distilled water rather than being added in the solid form as the tissue preparation. To 100 mls of the seawater sample in a BOD was added 10 mls of 5.6 N nitric acid and 5 drops of 5% potassium permanganate. This was heated for 5-10 minutes in a water bath at 50-60°C. Five mls of 1.5% hydroxylamine hydrochloride was then added and the sample was ready to be analysed.

For both tissues and seawater samples, reduction of mercury to its zero valence state was accomplished with 5 mls of 10% stannous chloride and samples were immediately aerated with a peristaltic pump into a cold vapor quartz cell. Absorption was read at 152.7 mu with a thoroughly warm lamp at a current of 4 mamp. The maximum concentration setting was at full counterclockwise rotation to provide maximum needle stability.

Standards mixed immediately before use from a stock solution of 1000 ppm, and spanning the optimal working range of 1-10 micrograms of mercury, were used in setting up a standard curve. A magnesium perchlorate dessicator and an activated charcoal mercury scrubber were employed. Fresh standards were run coincidental with a change of the dessicant. This generally
followed about twenty unknown determinations. Also, at that time, lamp and cell alignment was checked and wavelength settings were adjusted if needed to insure maximum transmittance.

Standards run with a given dessicant both fresh and exhausted yielded the same results indicating good reliability in the readings although standard curves varied with a change of the dessicant. The scrubber was not changed.

Recovery of internal standards from "spiked" material was excellent with a mean value of 81.65% (range 64.8-96.5%) over a range from 3 to 9 micrograms of added mercury.

RESULTS

In a study of this type, a starting point must necessarily be a determination of the natural levels found in the environment and in the animal in question. Accordingly, numerous replicates of control animal tissues were carefully analysed using scrupulously clean and mercury free glassware and utensils. These control determinations and the values for seawater were all obtained from material collected during the months of April through June, 1973. This approach makes no allowance for seasonal trends or changes in the natural levels of mercury that may be taking place. Rather it will average the values of a small portion of the curve describing those changes. Though unforeseeable at the start of the study, it is reasonable to assume that, in an area as untouched by potential polluters as the Dillon Beach coast, little if any changes would be taking place throughout the course of a year other than contributions made by currents. It is believed that the values for the natural levels given here, based on
replication, yield a genuine picture of the mercury distribution within
the test animal and the marine environment in which it lives.

This particular situation, significant or not, is rectifiable to a
degree. Mr. Vijay Khanna of the School of Pharmacy of the University of
the Pacific has been conducting heavy metal studies with marine organisms
concurrent with this study. His efforts included a period of nearly monthly
sampling of seawater from the Dillon Beach coast. Through his generosity,
the data relating to the mercury content of the seawater has been included
here (Figure 20B).

The monitoring data for November 1972 to June 1973, shows a sharp drop
in the mercury content of the seawater off the Dillon Beach coast between
December 1972 and January 1973. Values of 0.5 ppb for November and Dece-
ember, fall to 0.2 ppb in January. The trend is reversed during the months
of February to April, as a slow increase takes place to 0.3 ppb but this is
followed by another drop to 0.075 ppb in June.

The rainfall data, recorded at the Pacific Marine Station, is included
so as to correlate the climatic conditions with the monitoring data (Figure
20A). The drop in the mercury content of the seawater corresponds with
January-February heavy rainfall of over 30 inches. There does not seem to
be any explanation along this line for the drop in mercury observed to
begin in April as rainfall remains at a low level unless the 0.2 ppb reading
for January is in error.
The information derived from this study concerning the natural levels of mercury found in the selected tissues of *Hemigrapsus* compared to mercury concentrations in the seawater is broken down in a number of different ways in Figures 21 and 22. Each graph treats, individually, the data for each tissue examined using a standard statistical format. This is easily compared to the seawater data pooled from numerous samples taken between April and June, 1973. Note the units for each vertical axis. Tissue is in parts per million parts while seawater is in parts per billion. Figure 21 compares the data for starved animals with that for animals feeding on fresh *Mytilus* and Figure 22 combines all the data for each tissue.

In Figure 21, it is evident that there is no statistically significant difference in mercury between the feeding and starved animals within each tissue category i.e. fed carapace is not different from starved carapace and so on. Likewise, a comparison of all the tissue mercury, feeding and starved, in Figure 21 shows the 95% Confidence Limits overlapping indicating a lack of significant difference. For the fed animal the high mean value is about 0.22 ppm for the stomach while the low is in the hepatopancreas with a mean of 0.04 ppm. In starved animals the gill is well above the other tissues at 0.58 ppm. Lowest in this case is the stomach with 0.1 ppm. In Figure 22, on a combined basis the results for the control animals. Here, again, the lack of significant difference prevails except in one instance. The mercury content of the hepatopancreas seems to be less than that of the gill tissue. Even in this case the difference is marginal.

On the basis of the data in Figure 22, it is apparent that the natural levels of mercury found in *Hemigrapsus*, along this portion of the west coast, show mean readings of 0.2 ppm in the carapace, 0.43 ppm in the
gills, 0.09 ppm in the hepatopancreas and 0.1 ppm in the stomach. Shortly
we shall see how the gill tissue continues to dominate over the other
tissues in its accumulation of mercury from the experimental media.

Turning attention to the seawater concentrations, we see that a mean
value of 0.24 ppb is derived from 10 separate determinations. The narrow
confidence limits of the mean indicate the excellent reproducibility of the
results. If it were possible to properly display both the data for the
seawater and the tissue using the same units for the vertical axis, the
histogram for the seawater would barely be visible at the bottom of the
graph. The mean value of the seawater is about three to four orders of
magnitude below the tissue means. The difference in the mercury concen-
trations in the water is extremely significant and illustrates the capacity
for accumulation of heavy metals within an organism.

The concentration factors for tissue accumulation of mercury over that
of seawater are given in Table 1. Again, they are broken down to the feeding
animals, starved animals and the combined. On a combined basis the concen-
tration factors range from almost 400 (hepatopancreas) to nearly 1800 (gill)
times that of seawater.

Accumulation of Mercury From Seawater

Four different concentrations of mercury using two different salinities of
seawater were employed in experiments designed to measure the accumulation
of mercury when the test animals were totally submerged for a specific
period of time. The data is presented here on an ascending basis with
respect to mercury concentration and a descending basis with respect to
salinity since only at the higher mercury concentrations of 0.1 and 0.5
ppm was 25% seawater used.
The procedure for all the graphical material presented in this section has been to use, as the Y intercept, the control mercury value for that tissue.

In Figure 23A graphs the mean values, of total mercury from triplicate analysis are plotted in the various tissues of crabs exposed to 0.001 ppm mercury in 100% seawater. Figure 23B displays the same type of data from experiments in 0.01 ppm. In Figure 23A a peaking of the curve for gill tissue seems to occur at 3.1 ppm after 24 hours and is followed by a decline to 2.2 ppm at 48 hours. The remaining tissues rise from their natural levels but stay below 1 ppm for 48 hours.

Results for the crabs subjected to mercury concentrations of 0.01 ppm (Figure 23B) show a parallel effect that is more pronounced. A peak of the curves for the gill and stomach occurs between 12 and 24 hours, while the carapace and hepatopancreas appear to rise to about 1 ppm and hold this level up to 48 hours. The gill again attains the highest concentration of all the tissues at about 24 hours, registering a mercury level of 32 ppm.

Figure 23B graphs the results of accumulation studies at the 0.1 ppm level of external mercury. As in the previous experiments, readings were taken on tissues up to the 48 hour point. Additionally, single determinations on tissues from animals exposed for extended periods of time at 0.1 ppm are included here. Most of the animals that underwent extreme exposure contributed a portion of their tissues to the histology study, and in this way the two sections can be related.

It is strikingly evident upon examination of Figure 24 that at this level of environmental mercury, a tremendous accumulation occurs within the body of *Hemigrapsus* just within the first 48 hours. Again, the
distribution is heavily weighted towards gills. In the first 12 hours alone there is a jump to 26 ppm. This is followed by an increase to over 100 ppm after 48 hours of continuous exposure. The spot checks made after 92.5 and 700 hours indicate that the levels in the gill tissues are still rising and are about to pass the 300 ppm mark.

Every other tissue examined for total mercury content displayed the same trend toward a continual rise. The hepatopancreas reaches the second highest level - 7 ppm - after 48 hours. Following exposure for 700 hours the hepatopancreas seems to level off at just over 100 ppm. This is still far below the burden carried by the gill tissues. Carapace and stomach both rise steadily to just over 3 ppm at the end of 48 hours. This is continued through the 92.5 hour reading up to 700 hours. These tissues, also, show no sign of leveling off with respect to their mercury accumulation, after a thirty day exposure.

In Figure 25, the mercury concentration of the seawater is five times that of the previous conditions 0.5 ppm. While it is not likely that such a level would ever be reached in the natural environment it is of interest to examine the performance of the test animal in relation to this high concentration and to compare the results of the previous data. It is immediately notable that the pattern of increase in the mercury content of the tissues in 0.5 ppm is not appreciably different from the results reported for 0.1 ppm, both in relation to the mean values and the general trend of absorption. Of the tissues tested, the gills, again, accumulate the greatest proportion. They exhibit an immediate and rapid rise within 24 hours to a level of 75 ppm. In the next 24 hours a doubling of that figure occurs to a level just under 150 ppm. The other tissues follow suit as in the previous manner. The hepatopancreas exhibits a steady uptake of mercury to
about 12 ppm within 48 hours, and the stomach reaches about half that value in the same time. Carapace mercury appears to level off at about 2.7 ppm.

Figures 26 and 27 both represent the case for accumulation of mercury from dilute seawater regimes. In both instances readings were carried only as far as 48 hours. The pattern of extreme accumulation by the gill over other tissues is continued in both of these experimental conditions. In terms of the number involved and the overall trend, both graphs are similar to the previous results as well as to each other. For this reason, experiments with 25% seawater were not carried on extensively beyond this point. The gills, in both 0.1 and 0.5 ppm show a prominent increase in the first 12 hours. While the accumulation by the gill at the first reading (12 hours) is higher in the 0.5 ppm. Gill tissue levels appear to become near equivalent in the 0.1 and 0.5 ppm media after 48 hours of exposure with one (0.1 ppm) just under 100 ppm and the 0.5 just over that amount. The remaining tissues follow the customary trend toward a slow but steady rise in their mercury levels. In the 0.1 ppm experiment, all of these tissues range between 1-3 ppm while for the animals exposed to 0.5 ppm only the carapace acquired a level above this amount as it reaches 0.6 ppm after 48 hours.

Table 2 presents the concentration factors calculated from experiments relating to mercury accumulation from seawater. At the two lowest mercury concentrations - 0.001 and 0.01 ppm - the factors for tissues, except gills, are in the range of 100 to 1000. The carapace is generally lower than the hepatopancreas and stomach which have about equal levels of concentration. The gill tissue is, of course, greater being in the range 1200 to 3700 and more.

At the highest concentrations of environmental mercury - 0.1 and 0.5 -
it is apparent that the concentration factors are universally lower. For carapace, hepatopancreas and stomach, factors are all between 1 and 100. Only gill tissue exceeds 100 and then only rarely does it approach a factor of 1000. Extreme exposure conditions are required to elevate concentration factors for gill tissue over 2000.

**Accumulation By Dead Animals**

In examining the accumulation of mercury by the tissues of dead animals it was hoped that simple adsorption or passive diffusion of mercury could be divorced from actual metabolically related uptake, transport or excretion mechanisms. In this respect, both 100% and 25% seawater were used in conjunction with the highest level of mercury - 0.5 ppm. Animals were exposed for only 36 hours in these experiments to lessen the effects that decomposition and microbial uptake might have on the situation.

Figures 28A and 28B represent the mean results from triplicate analysis. Three tissue groups are used here, the soft internal parts being taken together for analysis. As regards the general form of accumulation, these graphs closely resemble the others we have examined to this point. However, the actual values of mercury attained are far lower for a 36 hour exposure. The gills, as usual, lead the way picking up just over 6.0 ppm in 36 hours in both salinities. The carapace curves show some divergence from each other but appear to become more nearly equivalent, after 36 hours of exposure, with values of 4.2 ppm and 5.5 ppm for the 25% and 100% seawater respectively. Only after 24 and 36 hour periods do the internal soft tissue parts accumulate mercury levels that could be considered above the normal range. During the early hours of the experiments the levels remain well below the 1.0 ppm point and even below 0.05 ppm.
Examination of the concentration factors (Table 3) reveals that for the internal tissues of the dead animal, values of unity with the media are rarely achieved. For the gill and carapace, both directly exposed to the environment, concentration factors exceed two only occasionally.

**Accumulation of Mercury From a Moist Substrate**

Field observations and collections of *H. nudus* will quickly demonstrate that the animal is a mid-intertidal denizen. It inhabits a region near enough to the water's edge to be covered by the tide or scurry into pools to moisten its gill chambers, but spends a good deal of its time under rocks above the water line where it enjoys cool temperatures and moist sediment. As a result of this, encounters with mercury containing effluent would most likely be over damp substrate. Accordingly, experiments were designed to measure the accumulation of mercury under these conditions.

Accumulation from the sediment is obviously quite low in comparison to other modes of uptake (Figure 29A and 29B). In fact, for the most part, the readings cannot be distinguished from the normal range of values in unexposed control animals. Only at the highest concentration in the sediment, 0.5 ppm (Figure 29B) is an increase in the gill mercury level observed. After the 48 hour limit of exposure, the gills were determined to contain 2.5 ppm.

Concentration factors for the accumulation from a moist sediment constitute Table 4. As in Table 3, they remain low throughout the exposure period, being highest in the gill tissues.
Mercury Accumulation From Food

As a third and final pathway for possible accumulation of mercury metals by *H. nudus*, that of uptake from food sources was examined. It was hoped that the distribution of mercury through the body tissues with time could be elucidated by allowing starved animals to feed on food material that had been adequately dosed with mercury. Figure 30 displays the results of the sampling of the four tissue groups at specified times following termination of feeding. A noticeable variation occurs in this situation with respect to the gill tissue. It no longer accumulates the highest amount of mercury. Rather the stomach, as would be expected since it was removed and analyzed with contents intact, recorded the greatest amount. Within 24 hours the values for the stomach are seen to drop from over 3 ppm to just over 1.0 ppm and from there the mercury content appears to level off at something over 0.5 ppm.

The hepatopancreas, next to receive ingested mercury via normal digestive processes, is variable with respect to the measured mercury. At 24 hours it remains near 2 ppm and then seems to fall only to begin a slow rise upward past 2 ppm that shows no sign of abating after 144 hours.

The gill tissue mercury levels behave in a manner opposite to that of the stomach. As the stomach is falling off in its mercury content, the gills begin a slow rise that approached 3 ppm after 96 hours.

Carapace mercury content remains well within the range of control values. There is little, if any, variance in the readings and no observed tendency to change even after 140 hours following feeding.
Detoxification

The possibility that _H. nudus_ might be able to rid itself of harmful heavy metal burdens was examined by exposing a number of animals to seawater containing 0.1 ppm mercury for 48 hours. Following specific periods of time in fresh seawater, tissues were sampled to determine what changes had taken place. Figure 31 is a graphical representation of those results.

The gill, as might be anticipated, has the highest mercury level after 48 hours (about 65 ppm). Wide variation occurs in the readings at 24 and 48 hours indicating that some animals still carry very high amounts. The trend is for a decrease in the mercury content after 96 hours and this appears to continue for at least three weeks, where, at that time, a level of 42 ppm if approached.

For the hepatopancreas and stomach the mean values give no consistent trend. Instead they vary between 3.0 and 10.0 ppm and after 3 weeks are about equal to levels found at the beginning. These data, by their variation, suggest a redistribution of mercury with time, in the soft body tissues of _H. nudus_. Excretion may also be at work here but evidence of this is not clear.

The carapace is the only tissue that displays a solid trend of change one way or the other, perhaps, because redistribution is not a factor here. After an initial reading 1.7 ppm an elimination of mercury appears to take place with the levels dropping through 1.03 ppm and 24 hours and leveling off in the neighborhood of 0.7 - 0.8 ppm from then until the termination of readings.
DISCUSSION

Natural Environmental Mercury

Monitoring the natural environmental levels of mercury is valuable in that one can determine not only what the prevailing levels are, but what cyclic changes may be taking place over an extended period of time. Also, identification of extraneous environmental parameters may prove useful in explaining the changes. In Figure 20A and 20B the rainfall data coincides so closely with the precipitous drop in the mercury that the strong suggestion that the two might be related cannot be taken lightly. The limited nature of the January monitoring data may abrogate this possibility.

The extremely wet and cold winter of 1972-73 began in earnest in January with measured rainfall of close to 17 inches for Dillon Beach in that month alone. At that time, personal observations of the situation along the rocky coast north of the town revealed a tremendous runoff of rainwater with concomitant large scale erosion and movement of huge amounts of soil into the sea. Portions of the cliffs began to slide away and great crevices are still visible. For miles up and down the coast the siltation of the inshore waters was evident and extended out from the coast for a mile or more. The heavy rains continued throughout February and March, 1973 and further widened great gullies in the cliffs contributing to extensive dilution and siltation of the water. The rains did not abate substantially until early April (Figure 20A).

Heavy rainfall and runoff carrying great quantities of soil into the coastal waters coupled with the fact that just inland in the area of Sonoma, California, is one of the few mercury mining sights along the coast,
would lead one to hypothesize that mercury levels would show an increase. This study appears to reveal a contrary situation for the Marin coast. Whether the sediments reaching the ocean contain substantial amounts of mercury is unknown, but there is no registering, by the methods employed here, of increased levels. Even if appreciable amounts of mercury were being swept into the sea with the soils, it is probable that the heavy influx of water and the constant mixing by tides, offshore currents and upwelling (Marcus and Houston, 1970) diluted the element to an extent that a decrease was recorded.

The second sharp decline in mercury from April to June does not have the evidence of rainfall to support or explain it. No other satisfactory explanation can be devised for the drop except to suggest that some facet of the spring plankton bloom that occurs along the coast starting in April may be responsible.

A mean level of all the readings from April through June has been determined to be 0.24 ppb (Figure 21 and 22). In contrast with this are numerous published values for mercury in the open seas. Leatherland, et al (1971) recorded 0.013 - 0.018 ppb in surface waters of the Northeast Atlantic and Hosohara (1961) has determined surface waters off Japan to contain 0.1 ppb. Williams and Weiss (1973) have contributed much higher values for the waters Southeast of San Diego. Their published results for water less than 10 meters deep is 0.27 ppb. It is clear that wide variation in the values can occur due, in part, to the differences in the analytical techniques used to measure mercury, but also due to genuine regional and depth related differences. The presence of a relatively close inland mercury mine could clearly be contributing to elevated
mercury levels in a nearby coastal environment. These will most likely be reflected in the fauna also.

Mercury levels in the unexposed animals as determined by this study are reasonably satisfactory in their repeatability and the standard errors are small in most cases. It is hoped that these figures will lend information to our knowledge concerning the scope of natural levels to be found in marine organisms. Only through an understanding of what is "normal" in the ecosystem can we begin to assess what is abnormal and dangerous.

One important consideration notable in the scrutiny of these natural concentrations is the variance in the distribution of mercury throughout the tissues of the animal (Figures 21 and 22). The gill tissue is by far the heaviest accumulator having more than twice the amount of mercury of any of the others examined. Considered anatomically, the gills are the soft body organs most in contact with the environment. We have already noted the tremendous surface area (100 cm$^2$) presented by the lamellae to the exterior as well as the important metabolic activity going on there. We have also seen (Section II) that the gill is potentially the sight of the most extensive destruction by exposure to inorganic mercuric ion. Based on these considerations and the results already presented concerning the huge ability of the gills to attract and hold mercury, the results for the control animals are not unreasonable. The mean level recorded for gill tissue - at 0.43 ppm - is the closest of all the tissues to the United States Public Health standard of 0.5 ppm in food. This compared to Sweden's standard of 1.0 ppm and the United Nations FAO/WHO standard limit of 0.05 ppm.

The range of the individual determinations for mercury in the gills
also shows the widest variation of all the tissues. The lowest amount
recorded was 0.13±0.11 ppm and the highest 1.09±0.11 ppm. This could
be indicative of the variation that occurs between animals of the same
species.

The remaining tissues, taken as a whole, show as much as a 10 fold
difference in total mercury. The stomach contained as little as 0.03±
0.04 ppm while the carapace had as much as 0.35±0.06 ppm. The exoskeleton,
being totally exposed to the external medium might be expected to contain
a high level of mercury. The mean of 0.2±0.06 ppm is, however, identical
with that which Zitko (1971) suggests as a naturally occurring level for
organic matter. Carapace determinations might well reflect adsorption of
mercury although with time deposition of mercury into the substructure
of the exoskeleton may take place.

In examining the mercury levels in the stomach of the starved animal
as opposed to the individual actively feeding, it is obvious that the
food material is contributing a substantial amount and is no doubt respon-
sible for the wide range of readings for the organ (0.09-0.46±0.11 ppm).
Only in this instance did the mean for the fed animal exceed that of the
starved (Figure 21) and in no case are the values for the feeding animal
significantly different from those of the starved one. Food, then, may
increase the values in the stomach for a period of time, but not appreciably
in other tissues of the body.

Given that the seawater off the coast of Marin County shows higher
than some published values for mercury in the open oceans, it is reasonable
that the organisms inhabiting the region would accumulate higher natural
levels within their tissues. Pringle, et al (1968) has pointed out that
temperature, salinity, dissolved oxygen and the general physiological condition of the animal could be related to uptake, to say nothing of species differences.

Khanna (1973) has recorded whole body mean values for invertebrates from the Marin coast that are commonly in close association with H. nudus. He found a low of 0.04 ppm in Balanus species and a high of 0.89 ppm in the anamuran Petrolisthes cinctipes (See Appendix B).

The enrichment factors for mercury in the various tissues of the control animals range from a low of 390 in the hepatopancreas to 1796 in the gills. These are low when compared to published results for concentration factors for other heavy metals. Pringle, et al. (1968) in extensive studies using atomic absorption techniques determined natural concentration factors in bivalves to be from 450 for copper to 318,000 for cadmium. Brooks and Rumsby (1965) using spectrographic analysis of cadmium, reported a factor of more than 2 x 10^6 for scallops. Their work centered totally on bivalves which, by virtue of their continual pumping of the environment across their soft body parts, could potentially accumulate these metals to degrees higher than Hemigrapsus which spends probably less than half of its time submerged.

**Accumulation From Artificial Media**

The 0.001 - 0.5 ppm mercury concentrations chosen for this portion of the study represent a suitable range of values extending from below the contamination level of Minimata Bay (0.0016 - 0.0036 ppm) to far above what any reasonably foreseeable contamination could produce. In this regard, contrasts and comparisons can be drawn from many quarters.
The curious trend toward a rise and a fall of tissue mercury within the 48 hours of an experiment is exhibited in Figure 23A. While a study of the possible detoxification mechanism is included for the greater levels of mercury later on, no attempt was made to examine elution capabilities at extremely low concentrations of mercury such as 0.001 ppm. Khan (pers. comm.) has concluded that both molluscs and echinoderms are capable of divesting themselves of all measurable mercury (as well as some measurable lead and cadmium) when placed in seawater made up from commercial "instant ocean" salts which was shown not to contain measurable amounts of these three metal ions. However, his data suggests that accumulation may be passive flux rather than an active process. It is possible, and suggested by the results in Figures 23A and 23B, that a delayed mechanism may come into play that is capable of actively ridding the tissues of excess mercury and that it is effective at levels comparable to the lowest ones used here. Extended exposure to these levels and continued monitoring of tissue levels beyond the 48 hour point might be useful in determining if the trend towards a reduction of mercury is continued or is just a short lived phenomenon.

While statistical analysis is difficult on even triplicate determinations, it is worthwhile to examine the results of such analysis.

The gill tissue immediately exhibits a rise in mercury content that is significantly above the combined control values (Figure 22). Within 24 hours it peaks and begins to decrease, however, it stays significantly \((p<0.05)\) above the controls throughout the experiment. The other tissues are more variable. None show consistently significant differences from the combined control. Carapace tissue is significantly higher only at the
24 hour mark corresponding to its peak mercury content. Hepatopancreas rises immediately beyond the range of the control and holds a significantly elevated level for 24 hours but in the decline to 48 hours, it no longer is different from the controls. The stomach exhibits the same pattern as the hepatopancreas, being significantly higher than controls only for 24 hours.

Perhaps, the most important point to be derived from Figure 23A is that, regardless of the detoxification mechanisms that may be operative, significant accumulation of mercury into living tissues can take place within 24 hours from very low levels of external mercury. These induced levels surpass not only the control values but can, for some of the tissues, climb well above the 0.5 ppm limit acceptable in food. That the maximum acceptable limit of mercury in water (0.005 ppm) set by the United States Bureau of Water Hygiene is no less than five times higher than the amount used to derive Figure 23A is a disquieting thought. The unmistakable conclusion is that, given the opportunity, M. nudus, and possibly related decapods suitable for consumption, are capable of concentrating unsafe levels of mercury in their tissues from an aquatic environment deemed safe by government standards.

By the time the 0.01 ppm external level is reached there is no question but what substantial accumulation occurs over and above the control values. Figure 23B conforms in every case to the trend of a rise and fall in tissue mercury. For all four tissues examined there is a peak at either 12 or 24 hours and the mean for the 48 hour reading is not only below this peak point but is lower than the initial 12 hour determination. While the extensive replication needed to establish solid statistical support is lacking,
the general trend, that is so apparent here, gains credence. The possibility that sometime during the early stages of exposure pathways of detoxification are mobilized and reach their peak effectiveness between 24 and 48 hours, is strongly suggested.

Only the most heavily contaminated effluent discharged into an extremely confined environmental setting could approach the levels of mercury depicted in Figure 24 and 25. They have been employed here to determine what the distribution of mercury is within the body under conditions of extreme stress. Only by pushing an animal to its limit of tolerance, can we determine its level of ability to cope with physiological stress.

The pattern of mercury accumulation is obvious in Figures 24 and 25. The gill tissue remains, from the beginning, the major depot of storage of the ion. Even after nearly a month of exposure the internal tissues have not caught up with the gills (Figure 24). There is no apparent tendency for an equilibrium to be established with respect to the gill tissues. Even after 700 hours of exposure it is still increasing its heavy metal burden. The stomach and hepatopancreas tissue appear to be approaching each other only after extended exposure. In Figure 25 this same equilibrium is, perhaps, developing but at a lower level.

Figures 26 and 27 follow the same pattern with the only difference being an apparent leveling off of the values for the gill tissue after 48 hours of exposure.

In comparing these results in various other fashions, their resemblance to each other is continued. By grouping the curves by tissue, little, if any, outstanding variation is noted. Divergent tendencies blend back into the common pattern. All curves for gill tissues, whether
from 100% or 25% seawater, 0.1 or 0.5 ppm mercury show a steep climb.
For carapace, hepatopancreas and stomach the rise is much slower but just as steady.

The accumulation of mercury into the tissues of Uca pugilator has been described (Vernberg and Vernberg, 1972). The results pertaining to the general patterns of uptake correspond to the present study, however, the absolute values of accumulation are far lower. For instance, gill tissue was determined to accumulate just under 3 ppm after seven days in 0.18 ppm mercury as mercuric chloride. It is believed by this author that their use of a large number of animals (30) in a single experimental container would effectively increase the competition for ions, and, therefore, lower the amount that any single individual could accumulate. Vernberg and Vernberg make no mention of this possibility nor do they comment on the reason for utilizing such a large number of animals at one time. In the absence of experiments with a smaller number of individuals, such as this study employs, it is not inconceivable that the point could be overlooked.

Vernberg and O'Hara (1972), based on similar results only slightly more extensive than those here, have concluded that a pathway exists by which mercury is transported to the hepatopancreas from the gill. This author is not convinced that the data warrants such a conclusion. At levels of 0.1 ppm (Figure 24) - lower than that employed by Vernberg and O'Hara, both the curve for the gill and the hepatopancreas rise steadily but it is not apparent that mercury formerly in the gill has been shunted to the hepatopancreas. It may well be possible that such a route of transport is operative, but it is doubtful whether the data proves or even
suggests it. Nutritional pathways, for instance, could be at work. The present study required feeding of the animals during extended exposure. In the course of the feeding, adsorbed ions could easily have been ingested. We have already seen the effects of *Mytilus* tissue on the control stomach mercury values (Figure 21). Feeding on chopped fish in a mercury contaminated media must certainly contribute to the intake of ions that would not only account for the rise in the curve for the stomach but the hepatopancreas as well.

Unlike the potentiated effect that reduced salinity had on survival, there is no apparent effect on the picture of actual absorption of mercury into the tissues of the body. In this respect it has been impossible to correlate internal tissue mercury with toxicity. Many of the animals recording the highest limits following extreme exposure, were active and appeared in good health at the time they were sacrificed for analysis. Conversely, those that expired under salinity-mercury stress within 48 hours had values comparable to the results in Figures 26 and 27.

The synergistic or additive effects of temperature and salinity were also studied by Vernberg and O'Hara (1972). They concluded that the regime influenced the distribution of mercury. At low temperatures and low salinities, less mercury was to be found in the hepatopancreas while at the higher temperatures more mercury accumulated in that organ. They concluded that high temperature increased the transport efficiency. This author remains skeptical about the existence of transport at all let alone the efficiency of same.

Concentration factors for the tissues under the various regimes show conclusively that accumulation of mercury is lower at higher concentrations of the media. Only in the case of gill tissue at the 0.001 and 0.01 ppm
do the concentration factors correspond. Concentration of mercury (as \( \text{HgCl}_2 \)) in oysters has recently been shown to behave in a similar manner (Cunningham and Tripp, 1973). At 0.1 ppm \textit{Crassostrea virginica} concentrated mercury in its tissues 1400 times that of the media while at 0.01 ppm the factor was 2800. The same pattern has been noted for copper (salt not indicated) in bivalves. The trend for lead (salt not indicated), in the same animals, is for an almost direct dose (external media concentration) response (accumulation) relationship, although total linearity does not exist (Pringle, \textit{et al}, 1968).

The deposition and distribution of artificially induced mercurials varies with the compound and the species being tested (Clarkson, 1972). Inorganic forms are characterized by non-uniform distribution in vertebrates. The renal cortex and proximal convoluted tubules of the kidneys may concentrate the element to a degree 300 times greater than that found in the blood. In comparison, short chain alkyl mercury compounds exhibit a much more uniform distribution throughout the blood, brain and kidneys. This group of compounds is able to penetrate placental and blood-brain barriers resulting in high levels in the brain and the fetus.

Intake by way of inhalation leads to rapid accumulation of mercury by the brain. The distribution within the brain is uniform immediately after exposure but changes with time as elimination rates vary within the brain (Clarkson, 1972).

The great weight of biochemical evidence favors the conclusion that mercury in organic materials binds to thiol groups (Clarkson, 1972). The affinity for the sulphur atom of the thiol group is many orders of magnitude greater than for binding with any other ligands. It has been
previously mentioned that in the presence of thiol containing compounds mercury toxicity is reduced. Corner and Rigler (1958) further demonstrated that washing tissues of the lobster *Homarus americanus* that had been exposed to mercurials, in glutathione solutions, caused little or no loss of mercury. They concluded that the majority of mercury actually penetrated into the tissues rather than having been adsorbed to their surface. However, this does not rule out the fact that surface chelating sites may have far larger binding constants than the glutathione-mercury complex.

**Accumulation by Dead *H. nudus***

Figures 28A and 28B lend evidence to support the conclusion that dead animals, regardless of the salinity, do not accumulate mercury on nearly the scale that the live animals are capable of. As the only difference is the absence of metabolic pathways and enzymatic reactions of the life processes, we might conclude that what is displayed here represents that amount of mercury that will passively diffuse into or adsorb onto tissues, thus, the dead animal presents less "surface area" than the live animal as transportation of the ion to potential sites of accumulation is eliminated. Conversely, the tremendous accumulation of mercurials from the far lower concentrations, that we have just examined, is probably due primarily to the normal life processes which may include active as well as passive modes.

**Accumulation From the Sediment***

No other conclusion can be forthcoming from Figures 29A and 29B than accumulation from a moist sediment is by far the lowest of the conditions investigated. Although the means are elevated somewhat above the control
values, the ranges of readings are such that there is no significant difference from the controls.

Gill tissue again, accumulated the greater proportion of mercury under these conditions - even more than the carapace which might be expected to adsorb quite substantial amounts.

Suggested disposal of mercury residues by burying them under sediment at the bottom of the oceans should be carefully examined in light of marine microbial roles in releasing toxic compounds into the water column (Wood, et al, 1968; Landner, 1971; Gillespie, 1972). However, the results presented here suggest that a return of the element to the sediment such as dry sumping waste mercurials might be the safest method of disposal from the standpoint of animal uptake.

**Accumulation From Food**

Barring uptake by direct absorption from the environment, i.e. through the epithelia, the only remaining possible method of obtaining heavy metal ions is by ingesting them.

Rucker and Amend (1969) fed fingerlings dosed to 3 ppm mercury with Timsan to chinook salmon and recorded mercury levels in the various tissues with time. After 30 days they determined the highest levels to be in the liver and large intestine. Lower levels were found for the kidneys, blood and small intestine.

During feeding on dosed food material it was reasonable to record elevated levels of mercury in the gut. Depicted in Figure 30 is the decrease of mercury as the gut is voided and food material passes into the hepatopancreas for absorption and distribution to the other tissues. Hepatopancreas and gill tissues show a consequent rise in their mercury content.
as nutrient products, combined with mercurials, are distributed to them over a period of time.

Carapace tissue is apparently left virtually untouched by this pathway of mercury uptake. It is completely bypassed as a major site of mercury accumulation. Those readings that do vary from the control levels are no doubt, due to slight adsorption that could take place as the animal crawled over or manipulated the food material in its mouthparts.

Concentration factors for this mode of uptake seem to peak at near unity. Mussel tissue (food) was determined to have 3.51 ppm and the crab gill tissue approaches 3.0 ppm after four days. This is in sharp contrast to the huge concentration factors examined prior to this point.

The long held belief that concentration factors of environmental contaminants, particularly heavy metals, rise along successively higher levels in the marine food chain has come under attack very recently, with respect to mercury. Williams and Weiss (1973) have published results showing that while zooplankton at 30-500 meters contained less mercury than zooplankton taken at greater depths, mercury in the higher trophic levels of organisms collected at the greater depths was not significantly greater than the concentration of mercury in zooplankton at these depths.

**Detoxification**

The ability to eliminate mercurials from the tissues has been shown for a number of animals. Pheasants have probably the most ingenious method of detoxification. Mercury is mobilized to the plumage where it is shed in the regular molt cycle (Tejning, 1967). Pike are able to ride their bodies of up to 30% of their total mercury in a year if given a "clean" environment (Lockhart et al., 1972). Distribution within the body remains
the same, however. Under conditions of short exposure, i.e. 1 hour or less, rainbow trout are able to remove all traces of mercury from the blood and liver in 12 - 24 weeks when placed in clean media (Rucker and Amend, 1969). When treatments were administered on a regular basis one week apart liver, kidney and muscle levels rose to as much as 40 ppm (kidney) and would not return to completely normal levels even after 20 weeks. In general, repeated treatments by mercurials maintained high levels in the tissues of the trout and prevented any detoxification.

Excretion rates for mercury in vertebrates depends on the body burden species and form of the compound (Clarkson, 1972). In man the half life of a mercurial may be as much as 70 days while in the mouse, the half life is just 8 days. Fish and shellfish take about 1000 days for a half reduction of the compound.

Pringle, et al., (1968) showed a variation in the detoxification of copper between species of bivalves. Cunningham and Tripp (1973) concluded that a drop of one third the body burden took place in C. virginica after 45 days.

The sufficient variation depicted in Figure 31 would lead one to conclude that if detoxification is going on it is not a generalized phenomenon. If some animals are experiencing a drop in tissue mercury levels, clearly many are also maintaining very high levels for long periods of time, thus, mean determinations have little value. No consistent trend towards a return to normal levels is evident after 3 weeks. This argues strongly against the existence of a definite pathway for detoxification of mercury from Hemigrapsus. However, the three week period allowed may not have been sufficient in the face of the time periods mentioned above.

From spot determinations of detoxification from 0.5 ppm, it is possible
that partial detoxification may be taking place. This may be the best that can be hoped for from an animal that has been subjected to such high accumulations of mercurials.

The steady drop in carapace values defines the ideal curve for the release of adsorbed ionic material. It appears as if only partial release of mercury has taken place as the curve levels off well above control values after 48 hours and remains fairly constant.

Consideration of Figures 23A and 23B, suggests the possibility of an active removal of mercury from the tissues by virtue of the rise and definite fall in so many of the curves. In the present situation, with internal mercury levels so high, it is reasonable to think of the detoxification mechanism becoming overwhelmed by such a rapid accumulation of such a large quantity of mercury. The breakdown of the hypothetical mechanism appears to have been complete as detoxification has apparently not commenced even after three weeks.
Figure 20A. Rainfall measured at the Pacific Marine Station November, 1972 to June, 1973.

Figure 20B. Mercury in the seawater off Dillon Beach, California. November, 1972 to June 1973. △ Data after Khanna, ○-data by author. Mean, S.E. and 95% C.I.
Figure 21. Mercury levels (ppm) in selected tissues of both fed and starved H. nudus compared to the mean seawater mercury concentration (ppb).
C-carapace
G-gill
H-hepatopancreas
S-stomach
SW-seawater
f-fed
s-starved

The diagram shows the concentration of mercury (ppm) in different tissues of an organism, categorized as fed (f) or starved (s), under different conditions (C, G, H, SW). The vertical bars represent the range of mercury concentrations, with the whiskers indicating the variability. The specific tissues and conditions are as follows:

- **C** (carapace): Mercury levels range from 0.1 to 0.7 ppm, with various fed and starved conditions.
- **G** (gill): Mercury levels range from 0.3 to 0.6 ppm, with similar fed and starved conditions.
- **H** (hepatopancreas): Mercury levels range from 0.2 to 0.5 ppm, with various fed and starved conditions.
- **S** (stomach): Mercury levels range from 0.1 to 0.4 ppm, with similar fed and starved conditions.
- **SW** (seawater): Mercury levels range from 0.0 to 0.2 ppm, indicating a lower concentration compared to the tissues.

The concentration of mercury in seawater is significantly lower than in the tissues, with values ranging from 0.0 to 0.2 ppm.
Figure 22. Combined data on naturally occurring levels of mercury (ppm) in selected tissues of H. nudus compared to the mean seawater concentration (ppb).
C - carapace
G - gill
H - hepatopancreas
S - stomach
SW - seawater

Mercury

(ppm)

Tissue

C  G  H  S  SW
Table 1
Concentration Factors for the Accumulation of Mercury from the Natural Environment by *H. nudus*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Feeding</th>
<th>Starved</th>
<th>Combined</th>
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<tbody>
<tr>
<td>Carapace</td>
<td>333</td>
<td>1158</td>
<td>833</td>
</tr>
<tr>
<td>Gill</td>
<td>750</td>
<td>2430</td>
<td>1796</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>194</td>
<td>508</td>
<td>390</td>
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<tr>
<td>Stomach</td>
<td>835</td>
<td>416</td>
<td>609</td>
</tr>
</tbody>
</table>
Figure 23A. Accumulation of mercury from 100% seawater—0.001 ppm mercury by various tissues of H. nudus.

□ - Carapace     △ - Gill     ○ - Hepatopancreas     X - Stomach

Figure 23B. Accumulation of mercury from 100% seawater—0.01 ppm mercury by the tissues of H. nudus.

□ - Carapace     △ - Gill     ○ - Hepatopancreas     X - Stomach
Figure 24. Accumulation of mercury from 100% seawater-.1 ppm mercury by the tissues of H. nudus.

- Carapace  △ - Gill  ○ - Hepatopancreas  X - Stomach
Figure 25. Accumulation of mercury from 100% seawater-.5 ppm mercury by the tissues of H. nudus.

☐ - Carapace  △ - Gill  0 - Hepatopancreas  X - Stomach
Table 2
Concentration Factors for the Accumulation of Mercury from 100% Seawater - Mercury Regimes

<table>
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<tr>
<th>Tissue</th>
<th>Hg Con. (ppm)</th>
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<th>24</th>
<th>48</th>
<th>92.5</th>
<th>700</th>
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</tbody>
</table>
Figure 26. Accumulation of mercury from 25% seawater-.1 ppm mercury by the tissues of H. nudus.
☐ - Carapace  △ - Gill  ○ - Hepatopancreas  X - Stomach
Figure 27. Accumulation of mercury from 25% seawater-.5 ppm mercury by the tissues of H. nudus.

□ - Carapace △ - Gill ○ - Hepatopancreas X - Stomach
Table 3
Concentration Factors for the Accumulation of Mercury from 25% SW - Mercury Regimes

<table>
<thead>
<tr>
<th>Tissue</th>
<th>External Hg Con (ppm)</th>
<th>Exposure (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Carapace</td>
<td>.1</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Gill</td>
<td>.1</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>120.0</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>.1</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Stomach</td>
<td>.1</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Figure 28A. Accumulation of mercury from 25% seawater - 0.5 ppm mercury by selected tissues of dead H. nudus.

- Carapace  △ - Gill  ○ - Hepatopancreas  X - Stomach

Figure 28B. Accumulation of mercury from 100% seawater - 0.5 ppm mercury by selected tissues of dead H. nudus.

- Carapace  △ - Gill  ○ - Hepatopancreas  X - Stomach
Table 4
Concentration Factors for the Accumulation of Mercury by Dead Animals
Media Mercury = .5 ppm

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Salinity (‰ SW)</th>
<th>Exposure (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carapace</td>
<td>100</td>
<td>5.38 11.3(\frac{1}{4}) 11.1</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7.3 5.34 8.4</td>
</tr>
<tr>
<td>Gill</td>
<td>100</td>
<td>3.68 4.32 12.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7.3 5.56 12.26</td>
</tr>
<tr>
<td>Internal Soft Tissue</td>
<td>100</td>
<td>.68 2.14 1.32</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>.64 .54 .6</td>
</tr>
</tbody>
</table>
Figure 29A. Accumulation of mercury from a moist substrate dosed to .1 ppm mercury by various tissues of H. nudus.

- Carapace  △ - Gill  0 - Hepatopancreas  X - Stomach

Figure 29B. Accumulation of mercury from a moist substrate dosed to .5 ppm mercury by various tissues of H. nudus.

- Carapace  △ - Gill  0 - Hepatopancreas  X - Stomach
Table 5
Concentration Factors for the Accumulation
of Mercury from a Moist Substrate

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Substrate Mercury (ppm)</th>
<th>Exposure (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Carapace</td>
<td>.1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>1.68</td>
</tr>
<tr>
<td>Gill</td>
<td>.1</td>
<td>.98</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>3.82</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>.1</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>.34</td>
</tr>
<tr>
<td>Stomach</td>
<td>.1</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>.5</td>
<td>.76</td>
</tr>
</tbody>
</table>
Figure 30. Prevailing levels of mercury in selected tissues of *H. nudus* following four hours of feeding on dosed *M. californianus*. Means of 2 to 4 determinations.

- Carapace
- Gill
- Hepatopancreas
- Stomach
Figure 31. Patterns of detoxification of the tissues of *H. nudus* following a 48 hour exposure to 0.1 ppm mercury.

- Carapace  △ - Gill  ○ - Hepatopancreas  X - Stomach

Spot determinations on detoxification from 48 hour exposure to 0.5 ppm.
SUMMARY

1. Three aspects of mercury poisoning were studied using *Hemigrapsus nudus* as the test animal. These were studies of the upper lethal limit of acute toxicity, histopathological changes occurring in various tissues following prolonged exposure to low mercury levels, and patterns of mercury accumulation in selected tissues balanced against naturally occurring levels.

2. Studies reveal a definite additive effect on acute toxicity when the salinity stress of 25% seawater (8-9 ppt) accompanies the presence of mercury as mercuric chloride.

3. The slope of the toxicity curve for 100% seawater (32 ppt) is -11.7 with a 50% reduction in activity (virtually equivalent to an LD$_{50}$) of the animal occurring in 48 hours at a mercury concentration of 1.2 ppm mercuric ion (not total salt).

4. The slope of the toxicity curve for 25% seawater is -73 with a 50% reduction in activity of the animal occurring in 48 hours at a mercuric ion concentration of only .24 ppm.

5. The gill lamellae show the earliest and most extensive destruction when subjected to 200-1000 hours exposure to mercury concentrations in the range of 0.05 to 0.1 ppm. Damage is evidenced as severe vacuolation and derangement of the lamellar structure accompanied by localized corrosive blackening of the tissue. Deformation of nuclei is commonly observed in extreme cases.
6. Hepatopancreas tissue exhibits a vacuolated appearance of the lumen wall after extended exposures approaching 1000 hours in 0.05 ppm.

7. Antennal glands also show changes from the normal with extended exposure to mercury. Loss of distinction and vacuolation of the cells lining the convoluted tubules are the most prominent changes observable.

8. No histological deviations from the controls can be detected in animals undergoing short term (12-48 hours) exposure to very high concentrations of mercury (0.1 to 100.0 ppm).

9. Mercury in the seawater off the coast of Dillon Beach, California, as determined by this study, shows a mean level of 0.24 ppb. A cycle of mercury levels in the seawater may be occurring although more complete monitoring data is necessary to confirm this.

10. Naturally occurring levels of mercury in H. nudus determined by this study are as follows: Carapace-0.2 ppm; Gill-0.43 ppm; Hepatopancreas-0.09 ppm; Stomach-(with contents) 0.15 ppm. These levels are not significantly different (p 0.005) from one another except in the case of gill and hepatopancreas. Animals feeding on fresh Mytilus californianus did not show levels of mercury significantly higher than starved animals for all tissues examined. All tissue mercury levels were significantly greater than hydrospheric concentrations. This indicates the tremendous capacity for concentration of mercury by H. nudus.

11. Accumulation experiments employing elevated levels of mercury in both
100% and 25% seawater show concentrations to be greatest in the gills. Carapace, hepatopancreas and stomach tissues display less pronounced patterns of accumulation.

12. In 0.001 ppm mercury ion in 100% seawater, a concentration considered safe by current public health standards, all tissues examined accumulate mercury above the maximum limit allowable for food products in the United States.

13. At 0.001 and 0.01 ppm mercury ion the characteristic trend of a rise followed by a fall of mercury levels within 48 hours suggests a possible detoxification mechanism at work.

14. At concentrations of 0.1 and 0.5 ppm in both 100% and 25% seawater, concentration of mercury in the gill tissue is rapid and extreme reaching levels of 100 ppm in 48 hours. Carapace, hepatopancreas and stomach tissues rise to between 1.0 and 15.0 ppm given the same conditions.

15. Exposure to 0.1 ppm in normal seawater for 700 hours reveals apparent continued increases in the mercury content of all the tissues examined. Carapace and stomach concentrations rise to about 10.0 ppm and 70.0 ppm respectively while hepatopancreas levels exceed 100 ppm. Gill tissues approach 300 ppm without showing signs of abating.

16. Dead crabs accumulate very little mercury from an external concentration of 0.5 ppm compared to live animals. Concentrations for all tissues examined from 100% and 25% seawater are less than 7.0 ppm after 36 hours of exposure.
17. Live animal accumulation of mercury from dosed sediment is extremely low. For tissues other than the gills, concentrations can not be distinguished from control levels. Only for the highest concentration of mercury in the sediment—0.5 ppm—will gill tissue levels exceed 2.0 ppm.

18. Concentration factors for live animal mercury accumulation from the external environment were universally higher for animals exposed to lower concentrations of the toxicant.

19. Mercury accumulation and distribution from contaminated food sources appears to follow the expected route from the stomach to the gills and hepatopancreas. Levels in the stomach drop over a period of 144 hours while those in the gills and hepatopancreas rise. Carapace mercury levels are not distinguishable from controls.

20. The presence of a mechanism for the elution of mercury in "clean" seawater is unclear. Levels of mercury in the carapace seem to show a steady drop after a 48 hour exposure to 0.1 ppm, while those in the gill, hepatopancreas and stomach are variable.

21. Circulation of mercury through the environment is discussed and previously recorded levels of mercury in the hydrosphere, atmosphere and biosphere are included.
LITERATURE CITED


*Original published paper not seen
APPENDIX A

Balance Sheet of Environmental Mercury Levels
BALANCE SHEET OF ENVIRONMENTAL MERCURY LEVELS

A naturally occurring element in the earth's crust, mercury exists mostly as a sulfide with an average content of $5 \times 10^{-6}\%$ (50 ppb) of which .02% is found in ore (Swedish Royal Comm., 1966) but, due to its unequal distribution, there can not be a universal "background" level. Nevertheless, concentrations of 0.2 ppm and below are considered by most to be due to naturally occurring environmental mercury (Zitko, 1971).

Millions of kilograms of mercury are mined, produced and "consumed" annually. However, there is little information on where this ends up or the concentrations of mercury at specific points in the environment. World production of mercury in 1968 was about 10,000 tons (Weiss, 1971), a doubling from the production in 1953 (Swed. Royal Comm., 1966). The United States alone consumes 27% of the total (3X more that is mines) which amounts to about 165 million pounds in this centry (Abelson, 1972). Four to five thousand tons per year of this amount becomes unintentional or uncontrolled discharge (Klein and Goldberg, 1970).

For instance, the burning of coal releases 3000 tons of mercury per year, a third of which comes from United States furnaces (Billings and Mattson, 1972). An equivalent amount is released by industries such as concrete and cement production (Joensuu, 1971; Weiss, 1971). The results of all this is that surveys after 1940 are reading at least slightly elevated levels.

Mercury enters the environment largely as water borne compounds that pass from suspension or solution to the sediments. Published data on seawater concentrations characteristically vary from 10 to 50 fold decending on the region of the world, depth and method of analysis.
In general, increased are shown to occur when going from the surface to the depths. Hosohara, (1961 in Klein and Goldberg, 1970) has recorded 0.1 ppb in surface waters and 0.15-0.27 ppb at depths in the Lamapo Deep off Japan. Other studies (Erickson, in Swed. Royal Comm., 1966) have recorded 0.03 ppb presumably off the Scandinavian coast although the specific area is not cited.

The present thesis research has shown a mean level of 0.24 ppb off the coast of Marin County, California. Some states of the United States have reported up to 0.2 ppm (Sport Fishing Inst. Bull. No. 221, pp. 4-7).

Pelagic sediments on the Pacific Ridge (Postrom and Fisher, 1969 in Klein and Goldberg, 1970) range from 1-400 ppb. In the sediments, bio-transformation of mercury to the dimethyl form takes place (Weiss, 1971) and the mercury once again rises into the water column and, being highly lipid soluble, is available to organisms or is liberated into the atmosphere.

Data from Sweden indicates that airborne mercury may be substantial. (Tejning, Ms, 1967 in Weiss, 1971). The presence of mercury in the atmosphere has been demonstrated in industrial areas. Air over San Francisco Bay (Williston, 1968 in Klein and Goldberg, 1970) was shown to vary with wind speed and direction, temperature, and season, being .5-25 ng/m³ in winter and 1-50 ng/m³ in the summer. United States maximum allowable limit is 100 ng/m³. Rain contributes heavily to the "wash out" of atmospheric mercury causing a turnover rate of less than 2 years. Limited data for rain and ground water show 0.2 and 0.02 ppb respectively (Erickson, in Swed. Royal Comm., 1966 pp. 13).

Organic matter in soils, mainly the sulphur containing compounds,
accumulate the 1000 mg/hectare of mercury that falls along with the rain. Approximately 100 mg/hectare immediately runs off. Mercury storage in the soil has been measured to be 1.3 lb/acre (1.5 Kg/hectare) with the majority being within the first three feet of depth, well within the reach of plant roots which absorb $10^{-4}$ Kg/hectare (Sed. Royal Comm., 1966 pp. 13).

Regardless of the biochemical form of discharge into the environment, mercury is able to undergo extensive biotransformation to many different compounds which are able to circulate widely throughout the ecosystem.
APPENDIX B

Previously Recorded Values for Mercury in the Environment
<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HYDROSPHERE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seawater</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scandnavia</td>
<td>Total</td>
<td>Unknown</td>
<td>3 x 10^{-5}</td>
<td>Eriksson, E. (in SRC, 1966)</td>
</tr>
<tr>
<td>(Unknown location)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE Atlantic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>Total</td>
<td>Unknown</td>
<td>1.3 x 10^{-6}</td>
<td>Leatherland, 1971</td>
</tr>
<tr>
<td>1000m</td>
<td></td>
<td></td>
<td>1.7 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>2000m</td>
<td></td>
<td></td>
<td>6.0 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>4000m</td>
<td></td>
<td></td>
<td>2.0 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>430 Km SE of San Diego</td>
<td>Total</td>
<td>NAA</td>
<td>3.0 x 10^{-5}</td>
<td>Williams and Weiss, 1972</td>
</tr>
<tr>
<td>10m</td>
<td></td>
<td></td>
<td>2.7 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>100m</td>
<td></td>
<td></td>
<td>9.6 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>950m</td>
<td></td>
<td></td>
<td>3.5 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>4000m</td>
<td></td>
<td></td>
<td>5.5 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Bottom Sediment (4300m)</td>
<td></td>
<td></td>
<td>.018</td>
<td></td>
</tr>
<tr>
<td>Japan, Lampoo Deep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>Total</td>
<td>Unknown</td>
<td></td>
<td>Hoschara, 1961 (in Klein and Goldberg, 1970)</td>
</tr>
</tbody>
</table>
### Recorded Values for Mercury in the Environment

<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific, Dillon Beach Coast</td>
<td>Total</td>
<td>AAS</td>
<td>$5 \times 10^{-5}$, $3 \times 10^{-4}$</td>
<td>This study</td>
</tr>
<tr>
<td>LaHave River Estuary, Nova Scotia, Canada</td>
<td></td>
<td></td>
<td></td>
<td>Cranston, Buckley, 1972</td>
</tr>
<tr>
<td>River System</td>
<td>Total</td>
<td>AAS</td>
<td>$3.6 \times 10^{-5}$, $1.7 \times 10^{-4}$, 2-34.4</td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended (particulate matter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td>.09-1.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paper mill effluent nearby</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td></td>
<td></td>
<td>$8 \times 10^{-5}$, $3 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Suspended</td>
<td></td>
<td></td>
<td>5.6-10</td>
<td></td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlor-alkali plant nearby</td>
<td>Total</td>
<td>AAS</td>
<td>$8 \times 10^{-2}$, 2.0</td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilizer Plant nearby</td>
<td></td>
<td></td>
<td>.002-.064</td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td>.56</td>
<td></td>
<td></td>
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</table>
# Recorded Values for Mercury in the Environment

<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Clair River, Canada (contaminated)</td>
<td>Total</td>
<td>AAS</td>
<td>9-120</td>
<td>Gillespie, 1972</td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saskatchewan River, Canada (contaminated)</td>
<td></td>
<td></td>
<td>7-60</td>
<td>Klein, Goldberg, 1970</td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern California Coast</td>
<td>Total</td>
<td>Spectrographic Analysis</td>
<td>3.5-25 ng/m³</td>
<td>Klein, Goldberg, 1970</td>
</tr>
<tr>
<td>Bottom Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATMOSPHERE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Francisco Bay</td>
<td>Total</td>
<td>Unknown</td>
<td>5-25 ng/m³</td>
<td>Williston, 1968</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td>1-50 ng/m³</td>
<td>(In Klein, Goldberg, 1970)</td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home (painted 2 yrs. before)</td>
<td>Total</td>
<td>Unknown</td>
<td>68.2</td>
<td>Foote, 1972</td>
</tr>
<tr>
<td>Study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td></td>
<td></td>
<td>66.5</td>
<td></td>
</tr>
<tr>
<td>Home (painted 7 dys. before)</td>
<td>Total</td>
<td>Unknown</td>
<td>1560.0</td>
<td></td>
</tr>
<tr>
<td>Livingroom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td></td>
<td></td>
<td>3070.0</td>
<td></td>
</tr>
<tr>
<td>Dentists Office</td>
<td>Total</td>
<td>Unknown</td>
<td>5550.0</td>
<td></td>
</tr>
</tbody>
</table>
### Recorded Values for Mercury in the Environment

<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital Ward</td>
<td></td>
<td></td>
<td>336.0</td>
<td></td>
</tr>
<tr>
<td>Washington D.C.</td>
<td></td>
<td></td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td><strong>BIOSPHERE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marine Fish</td>
<td>Total</td>
<td>Unknown 2.5 ( \times 10^{-2} ) - 1.1 ( \times 10^{-1} )</td>
<td>Stock and Cucel, 1934 (in Johnels, et al, 1967)</td>
<td></td>
</tr>
<tr>
<td>Marine Fish</td>
<td>Total</td>
<td>Unknown 4.4 ( \times 10^{-2} ) - 1.5 ( \times 10^{-1} )</td>
<td>Reader, Snekvik, 1941 (in Johnels, et al, 1967)</td>
<td></td>
</tr>
<tr>
<td>Fiske (<em>Esox lucius</em>)</td>
<td>Total</td>
<td>NAA</td>
<td></td>
<td>Johnels, et al, 1967</td>
</tr>
<tr>
<td>4 yrs. old</td>
<td></td>
<td>.19-.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 yrs. old</td>
<td></td>
<td>.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 yrs. old</td>
<td></td>
<td>.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near paper mill</td>
<td></td>
<td>.015-9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowry (near Los Angeles)</td>
<td>Total</td>
<td>Spectrographic Analysis</td>
<td>21</td>
<td>Klein, Goldberg, 1970</td>
</tr>
<tr>
<td>Sea Cucumber (near Los Angeles)</td>
<td>Total</td>
<td>AAS</td>
<td>.4</td>
<td>Vernberg, Vernberg, 1972</td>
</tr>
<tr>
<td><strong>Uca pugilator</strong></td>
<td>Total</td>
<td>AAS</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>Total</td>
<td>AAS</td>
<td>1.0</td>
<td>Gillespie, Scott, 1971</td>
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<tr>
<td>Guppies (FW)</td>
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<td>.11</td>
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## Recorded Values for Mercury in the Environment

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<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>North Atlantic Finfish</td>
<td>Total</td>
<td>AAS</td>
<td>.1-4.5</td>
<td>Windom, et al, 1973</td>
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<tr>
<td>(inshore, pelagic)</td>
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<td>Osteichthys</td>
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<td>Chondrichthys</td>
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<tr>
<td>Conads (single sample)</td>
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<td></td>
<td>9.0</td>
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<td>Zooplankton (430 Km SE of San Diego)</td>
<td>Total</td>
<td>NAA</td>
<td>.006-.016</td>
<td>Williams, Weiss, 1973</td>
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<tr>
<td>Surf.</td>
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<td>30-500m</td>
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<tr>
<td>3200m</td>
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<td>.036</td>
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<td>Decapods (600m)</td>
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<td>.03</td>
<td></td>
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<tr>
<td>Hatchet Fish (500m)</td>
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<td>.022</td>
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<td>Brittle Star (4300m)</td>
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<td>Sponge (4300m)</td>
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<td>Tune (surface)</td>
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<td>Khanna, 1973</td>
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<td><em>Mytilus californianus</em></td>
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<td>.047(.03-.62)</td>
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<td>.06(.03-.09)</td>
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<td></td>
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<td><em>Carolina sp ()</em></td>
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<td></td>
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<tr>
<td><em>Petrolisthes sp.</em></td>
<td></td>
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<td>.89(.83-.93)</td>
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<td>Technique of Measurement</td>
<td>Levels (ppm)</td>
<td>Reference</td>
</tr>
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<td>Lake Erie Finfish</td>
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<td>10-12 yrs.</td>
<td>%Methyl-Hg</td>
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<td>Fish, Saskatchewan River,</td>
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<td>Wobeser, et al., 1970</td>
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<tr>
<td>Southern Part</td>
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<td>muscle</td>
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<td>kidney</td>
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<td>1.2-21.1</td>
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<tr>
<td>Fish, &quot;clean&quot; lakes, Canada</td>
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<td>.07-.11</td>
<td>Uthe, Bligh, 1971</td>
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<td>Fish, industrialized lakes,</td>
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<td>.05-.7</td>
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<tr>
<td>Canada</td>
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<tr>
<td>American eel (Anguilla rostrata)</td>
<td>Methyl-Hg</td>
<td>GC</td>
<td>.01-.76</td>
<td>Zitko, et al., 1971</td>
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<td>Bass (Micropterus dolomieu)</td>
<td>Methyl-Hg</td>
<td>GC</td>
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<tr>
<td>Trout (Salvelinus fontinalis)</td>
<td>Methyl-Hg</td>
<td>GC</td>
<td>.08-1.2</td>
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</table>
### Recorded Values for Mercury in the Environment

<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Pike (from contaminated lake, Canada)</td>
<td>Total</td>
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<td>6.29-16</td>
<td>Lockhart, et al., 1972</td>
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<tr>
<td>Northern Pike (from clean lake, Canada)</td>
<td>Total</td>
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<td>Spiny dogfish (Squalus acanthias-Sw)</td>
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<td>.01-.05</td>
<td>Forrester, et al., 1972</td>
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<tr>
<td>Embryo</td>
<td>Total</td>
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<td>.01-.05</td>
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<tr>
<td>Adult males</td>
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<td>0-1.7</td>
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<tr>
<td>Adult females</td>
<td></td>
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<td>0-2.0</td>
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<tr>
<td>Harbor propois (Phocoena - phocoena) Bay of Fundy</td>
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<td></td>
<td>Gaskin, et al, 1972</td>
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<tr>
<td>Males - muscle</td>
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<td>.75(.21-1.92)</td>
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<tr>
<td>Females - muscle</td>
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<td></td>
<td>1.02(.26-2.58)</td>
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<tr>
<td>Males - liver</td>
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<td>.89-18.3</td>
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<tr>
<td>Females - liver</td>
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<td>.55-91.3</td>
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</tr>
<tr>
<td>Muscle - male/female</td>
<td>%Methyl-Hg</td>
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<td>100%</td>
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</tr>
<tr>
<td>Liver - male/female</td>
<td>%Methyl-Hg</td>
<td></td>
<td>7.4-41%</td>
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<tr>
<td>Whale (Globicephala scammoni)</td>
<td>Total</td>
<td>AAS</td>
<td>8.5-23.9</td>
<td>Anon. Mar. Poll.</td>
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<tr>
<td>Range of means of six animals' livers</td>
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<td></td>
<td>8.5-23.9</td>
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<tr>
<td>Range of values</td>
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<td></td>
<td>7.4-25.9</td>
<td>Bull. 1971</td>
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# Recorded Values for Mercury in the Environment

<table>
<thead>
<tr>
<th>Subject or Area of Study</th>
<th>Form of Hg Measured</th>
<th>Technique of Measurement</th>
<th>Levels (ppm)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>MISCELLANEOUS</strong></td>
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<tr>
<td>Greenland ice</td>
<td>Total</td>
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<td></td>
<td>Weiss, et al., 1971</td>
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<tr>
<td>800 B.C.</td>
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<td></td>
<td>6.2 x 10^{-5}</td>
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</tr>
<tr>
<td>1892</td>
<td></td>
<td></td>
<td>6.6 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>1965 (spring)</td>
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<td></td>
<td>2.3 x 10^{-4}</td>
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</tr>
<tr>
<td>1965 (summer)</td>
<td></td>
<td></td>
<td>9.8 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Pheasants</td>
<td>Total</td>
<td>DE</td>
<td>.29-1.0</td>
<td>Tjening, 1967</td>
</tr>
<tr>
<td>Organs, Claws, and Plumage</td>
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</table>

Methyl-Hg  Methy mercury  
AAS  Atomic Absorption Spectrophotometry  
NAA  Neutron Activation Analysis  
GC  Gas Chromatography  
DE  Dithizone Extraction
APPENDIX C

Means and Value Range Data from Mercury Accumulation Experiments
Control *H. nudus*

Means and Range of Values for Mercury in Selected Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Feeding (n=3)</th>
<th>Starved (n=5)</th>
<th>Combined (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carapace</td>
<td>.06 (0.04-.11)</td>
<td>.27 (0.09-.49)</td>
<td>.20 (0.04-.49)</td>
</tr>
<tr>
<td>Gill</td>
<td>.18 (0.13-.21)</td>
<td>.58 (0.22-1.09)</td>
<td>.43 (0.13-1.09)</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>.04 (0.04-.06)</td>
<td>.12 (0.05-.21)</td>
<td>.09 (0.04-.21)</td>
</tr>
<tr>
<td>Stomach</td>
<td>.22 (0.09-.46)</td>
<td>.10 (0.05-.19)</td>
<td>.15 (0.05-.46)</td>
</tr>
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Mean and Range for Seawater-Mercury
Central Pacific Coast-Dillon Beach, CA
(April - June, 1973)

(ppb) n=10

.24
(.05-.3)
<table>
<thead>
<tr>
<th>Media Conditions</th>
<th>Tissue</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>92.5</th>
<th>700</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%SW-.001ppm</td>
<td>Carapace</td>
<td>.13</td>
<td>.88</td>
<td>.12</td>
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<tr>
<td></td>
<td></td>
<td>(.06-.18)</td>
<td>(.28-.1.29)</td>
<td>(.1-.14)</td>
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</tr>
<tr>
<td></td>
<td>Gill</td>
<td>2.55</td>
<td>3.74</td>
<td>2.28</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(2.42-2.84)</td>
<td>(3.38-3.98)</td>
<td>(1.84-2.94)</td>
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<tr>
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<td>.67</td>
<td>.2</td>
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<td>Stomach</td>
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<td>.89</td>
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<td></td>
<td>(.81-1.8)</td>
<td>(.8-.94)</td>
<td>(.28-.44)</td>
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<td>100%SW-.01ppm</td>
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<td>(.63-1.4)</td>
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<td>Gill</td>
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<td>(26.04-32.1)</td>
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Mercury Accumulation from Seawater Means and Range of Values

(n=3)
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<tr>
<th>Media Conditions</th>
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<th>48</th>
<th>92.5</th>
<th>700</th>
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</thead>
<tbody>
<tr>
<td>100%SW-.1ppm</td>
<td>Carapace</td>
<td>.94 (4)</td>
<td>1.62</td>
<td>3.48</td>
<td>2.86 (1)</td>
<td>11.04 (1)</td>
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<td></td>
<td>(.4-1.39)</td>
<td>(.6-2.58)</td>
<td>(1.25-5.22)</td>
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<tr>
<td></td>
<td>Gill</td>
<td>27.34 (5)</td>
<td>32.66</td>
<td>122.85</td>
<td>275.0 (1)</td>
<td>296.0 (1)</td>
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<td>(14.25-37.95)</td>
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<td>Hepatopancreas</td>
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<td>3.15</td>
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<td>113.0 (1)</td>
<td>62.57 (1)</td>
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<td>(2.04-7.16)</td>
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<td>Stomach</td>
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<td>3.02</td>
<td>13.1 (1)</td>
<td>62.57 (1)</td>
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<td>(.33-6.31)</td>
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<td>25%SW-.1ppm</td>
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<td>1.52</td>
<td>2.22</td>
<td>88.97</td>
<td>(16.28-176.5)</td>
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<td>97.59</td>
<td>88.97</td>
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<td>2.91</td>
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<td>Stomach</td>
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<td>2.0</td>
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### Mercury Accumulation from Seawater
#### Means and Range of Values

(n=3)

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<th>92.5</th>
<th>700</th>
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<tbody>
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<td>100%SW-.5ppm</td>
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<td>2.6</td>
<td>(2)</td>
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<td>(.26-1.39)</td>
<td>(1.83-3.11)</td>
<td>(1.56-3.7)</td>
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<tr>
<td></td>
<td>Gill</td>
<td>29.50</td>
<td>76.26</td>
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<td>(13.29-43.2)</td>
<td>(48.76-92.03)</td>
<td>(98.83-184.0)</td>
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<tr>
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<td>3.21</td>
<td>12.15</td>
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<tr>
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<td></td>
<td>(1.09-2.41)</td>
<td>(.68-5.35)</td>
<td>(2.9-17.92)</td>
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<td>2.02</td>
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<td>(.64-1.87)</td>
<td>(1.52-2.62)</td>
<td>(.91-7.9)</td>
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<tr>
<td>25%SW-.5ppm</td>
<td>Carapace</td>
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<td>4.24</td>
<td>8.67</td>
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</tr>
<tr>
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<td>(2.08-3.48)</td>
<td>(2.47-6.57)</td>
<td>(4.5-13.93)</td>
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<tr>
<td></td>
<td>Gill</td>
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<td>100.61</td>
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</tr>
<tr>
<td></td>
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<td>(27.83-114.64)</td>
<td>(82.0-113.19)</td>
<td>(77.41-151.0)</td>
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<tr>
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<td>Hepatopancreas</td>
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<td>1.54</td>
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<tr>
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<td>(1.05-1.81)</td>
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<tr>
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<td>(1.17-1.38)</td>
<td>(1.06-3.91)</td>
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<td>Tissue</td>
<td>Mercury (ppm) (range)</td>
<td>Exposure (Hrs.)</td>
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<td>2h</td>
<td>36</td>
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<tr>
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<td>100% SW .5 ppm</td>
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<tr>
<td>Carapace</td>
<td>2.69 (1.77-3.67)</td>
<td>5.67 (1.83-10.52)</td>
<td>5.55 (2.3-9.87)</td>
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<tr>
<td>Gill</td>
<td>1.84 (1.14-2.55)</td>
<td>2.16 (2)</td>
<td>6.15 (2)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Internal Soft Tissue</td>
<td>.34 (.18-.49)</td>
<td>1.07 (.12-2.16)</td>
<td>.66 (.56-.76)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Hepatopancreas and Stomach)</td>
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</tr>
<tr>
<td></td>
<td>25% SW .5 ppm</td>
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</tr>
<tr>
<td>Carapace</td>
<td>3.65 (2.5-4.42)</td>
<td>2.67 (1.93-3.82)</td>
<td>4.2 (2.6-6.21)</td>
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</tr>
<tr>
<td>Gill</td>
<td>3.65 (2)</td>
<td>2.78 (2)</td>
<td>6.13 (2)</td>
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<tr>
<td>(1.91-5.4)</td>
<td>(2.25-3.32)</td>
<td>(4.2-8.09)</td>
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<tr>
<td>Internal Soft Tissue</td>
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<td>.27 (.16-.375)</td>
<td>.30 (.21-.37)</td>
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<tr>
<td>(Hepatopancreas and Stomach)</td>
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</table>
### Mercury Accumulation from Sediment

Means and Range of Values

*(n=2 except where indicated by number in parenthesis)*

<table>
<thead>
<tr>
<th>Sediment Concentration (ppm)</th>
<th>Tissue</th>
<th>Mercury (ppm) (range)</th>
<th>Exposure (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Carapace</td>
<td>0.26 (0.23-0.30)</td>
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<tr>
<td></td>
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<td>0.17 (0.16-0.19)</td>
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<tr>
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<td>0.15 (0.06-0.24)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>0.98 (3)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16 (0.93-1.39)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.23 (0.17-0.3)</td>
<td>48</td>
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<tr>
<td></td>
<td>Hepatopancreas</td>
<td>0.42 (0.3-0.55)</td>
<td>12</td>
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<tr>
<td></td>
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<td>0.32 (0.3-0.35)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16 (0.15-0.18)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>0.34 (0.19-0.5)</td>
<td>12</td>
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<tr>
<td></td>
<td></td>
<td>0.21 (0.2-0.22)</td>
<td>24</td>
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<tr>
<td></td>
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<td>0.09 (0.06-0.1)</td>
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<tr>
<td>0.5</td>
<td>Carapace</td>
<td>0.84 (1)</td>
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<tr>
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<td>0.27 (0.26-0.29)</td>
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<tr>
<td></td>
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<td>0.28 (0.08-0.49)</td>
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<td></td>
<td>Gill</td>
<td>1.91 (1)</td>
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<tr>
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<td>2.0 (1.38-2.63)</td>
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<td>2.5 (2.21-2.76)</td>
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<tr>
<td></td>
<td>Hepatopancreas</td>
<td>0.17 (0.11-0.23)</td>
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<tr>
<td></td>
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<td>Stomach</td>
<td>0.38 (1)</td>
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<td></td>
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<td>0.23 (0.09-0.37)</td>
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<tr>
<td></td>
<td></td>
<td>0.54 (0.3-0.78)</td>
<td>48</td>
</tr>
<tr>
<td>Tissue</td>
<td>4</td>
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<td>24</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Carapace</td>
<td>0.21 (4)</td>
<td>0.01 (2)</td>
<td>0.35 (2)</td>
</tr>
<tr>
<td></td>
<td>(0.04-.58)</td>
<td>(0.01-.2)</td>
<td>(0.29-.41)</td>
</tr>
<tr>
<td>Gill</td>
<td>1.5 (4)</td>
<td>0.95 (2)</td>
<td>2.01 (4)</td>
</tr>
<tr>
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<td>(0.5-3.28)</td>
<td>(0.7-.21)</td>
<td>(1.14-1.33)</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>1.73</td>
<td>1.72 (2)</td>
<td>2.22 (4)</td>
</tr>
<tr>
<td></td>
<td>(0.2-4.17)</td>
<td>(1.25-2.19)</td>
<td>(0.34-5.16)</td>
</tr>
<tr>
<td>Stomach</td>
<td>3.32 (4)</td>
<td>2.34 (3)</td>
<td>1.08 (2)</td>
</tr>
<tr>
<td></td>
<td>(.64-8.74)</td>
<td>(2.11-2.64)</td>
<td>(.99-1.18)</td>
</tr>
</tbody>
</table>

Mercury Accumulation from Food-Underwater
Means and Range of Values for Mercury
in Selected Tissues
("n" in parenthesis)
## Depuration of Mercury from Tissues of *H. nudus*
### Means and Range of Values

(n=2 except where indicated by number in parenthesis)

<table>
<thead>
<tr>
<th>Dose Conditions</th>
<th>Tissue</th>
<th>Mercury (ppm) (range)</th>
<th>Time Following Termination of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Hrs.</td>
<td>24 Hrs.</td>
</tr>
<tr>
<td>48Hrs./.1ppm</td>
<td>Carapace</td>
<td>1.73</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>65.85</td>
<td>82.53</td>
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<tr>
<td></td>
<td>Hepatopancreas</td>
<td>9.98</td>
<td>6.98</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>6.49</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.40-3.07)</td>
<td>(0.89-1.18)</td>
</tr>
<tr>
<td>48Hrs./.5ppm</td>
<td>Carapace</td>
<td>9.22</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>240.65</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>45.57</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>19.25</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
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<td>(5.87-14.92)</td>
<td>(186.19-338.37)</td>
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</table>