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IN A SMALL HAWAIIAN ESTUARY.

University of Hawaii, Ph.D., 1974
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ASPECTS OF THE DYNAMICS OF MERCURY CYCLING
IN A SMALL HAWAIIAN ESTUARY

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
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ABSTRACT

The environmental dynamics of mercury were studied in Ala Wai Canal, a small Hawaiian estuary. Three indicator species were used in the study - the detritus feeding polychaete Nereis succinea and shrimp Palaemon debilis and the predaceous decapod Thalamita crenata. The study employed laboratory experiments, utilizing $^{203}\text{-HgCl}_2$, and analyses of total mercury concentrations in sediment and biota samples collected periodically from the estuary. The two detritus feeders concentrated dissolved $^{203}\text{-Hg}$ from 160 to 310 times over the concentration in seawater. Reduced salinity inhibited $^{203}\text{-Hg}$ uptake by the worm but had little effect on uptake by the shrimp. Dissolved organic material also reduced $^{203}\text{-Hg}$ accumulation. Little $^{203}\text{-Hg}$ was accumulated from labelled estuarine sediment by either species - the steady state concentrations of $^{203}\text{-Hg}$ in the animals ranged from only 0.25% to 1.5% of the concentration in the sediment. However, $^{203}\text{-Hg}$ bound to sediment originating from urban street runoff was more available to both species than was $^{203}\text{-Hg}$ bound to estuarine sediment. The availability of $^{203}\text{-Hg}$ from runoff sediment declined as the sediment aged in seawater. Net loss of mercury occurred by way of two mono-exponential processes in both organisms and was slow relative to net accumulation. Total mercury analyses illustrated that mercury concentrations in sediment in Ala Wai Canal showed no obvious pattern of temporal variation. However, there was an obvious decrease in total mercury in the two detritus feeders between the rainy season and the dry summer months. Samples of P. debilis were collected at five day intervals during the rainy season. The equations describing accumulation and

loss of mercury in this species were used with these samples and rainfall data to construct a simple model of biotic mercury dynamics in the estuary. The model illustrated a strong correlation between rainfall and biotic mercury concentrations, and indicated that the primary source of mercury for the shrimp, and probably the worm, occurred in dissolved form.

To further study the trophic transfer of mercury the predator, T. crenata, was fed 203-Hg labelled N. succinea over a two week period. The activity of 203-Hg in the crab bodies appeared to reach a steady state after four to seven days. At ingestion rates of up to 3% of body weight per day the concentration of 203-Hg in the muscle of this species never exceeded 26% of the concentration of 203-Hg in the food of the crab. Total mercury and organic mercury were also determined in crabs collected from Ala Wai Canal. Of the total mercury in crab muscle 25% was in organic form. It appeared that dissolved mercury was an important source of this ion for T. crenata and that no food chain biomagnification of mercury occurred at this trophic level.

In their natural habitat the shrimp and the worm are exposed to mercury concentrations in their food several times greater than the minimum lethal dose of dissolved mercury. However, neither these species nor the predator T. crenata concentrated mercury to whole body concentrations greater than 5% of that in their food. It is hypothesized that natural selection might favor processes which result in inefficient assimilation of cations from food by aquatic species. Toxic cations occur in much higher concentrations in the food of aquatic species than in solution. In ecosystems where a sufficient

supply of essential cations exists in solution, an inefficiency in removing cations from food might reduce assimilation of toxic cations from their most concentrated pool at no expense to essential cation regulation.

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INTRODUCTION

Objectives

Determination of the fate of mercury within aquatic ecosystems is a problem of considerable importance. An epidemic of mercury poisoning between 1954 and 1959 among villagers consuming seafood in Minamata, Japan, illustrated the potential toxic effects of this metal to humans (Nelson, Kurland, Kolbye and Shapiro, 1971). More recently several species of marine pelagic fish, important as human food resources, have been shown to contain significant mercury contamination (e.g. Rivers, Pierson, and Schultz, 1972). Mercury is not only important as a potential contaminant of man's aquatic food resources, but also may have significant potential for disruption of aquatic ecosystems themselves (Harriss, White and MacFarlane, 1972).

Many studies have considered the physical and biotic distribution of mercury within aquatic systems. However, it is also important to understand why mercury is distributed as it is. Such understanding may facilitate prediction of both the effects of any further increase in the rate of the release of mercury into aquatic environments by man's activities, and the fate of mercury already deposited in aquatic ecosystems. Despite the importance of this type of knowledge, many aspects of the dynamics of mercury cycling within aquatic communities have not been clarified.

The purpose of this research was to develop some understanding of mercury cycling within an estuarine ecosystem. The study emphasized mercury dynamics within a simple detritus-based food web from Ala Wai

Canal, a small eutrophic estuary. An estuarine ecosystem was chosen for study because, in general, these systems are subject to greater influence from human activities, including toxic metal input, than are most other coastline ecosystems (DeFalco, 1967). Estuaries are an important source of human food resources. Eutrophic estuaries, such as Ala Wai Canal, also are characterized by simple food chains, amenable to both laboratory and field study of metal fluxes.

The dominant food web in productive estuaries is often based upon detritus (Darnell, 1967). Mercury has a strong affinity for organic particulates (Reimers and Krenkel, 1972). Thus the highest concentrations of mercury (and many other metals) within the physical portion of an estuarine ecosystem occurs associated with detritus and other particulate materials (Cranston and Buckley, 1972). This concentrated particulate-bound reservoir of trace metals has been described by several authors as a major source of these pollutants for detritus feeders (Duursma and Gross, 1971; Lowman, Rice and Richards, 1971; Wolfe and Rice, 1972). Yet, the extent to which such organisms can assimilate bound metals is still open to question. Bryan (1971) determined the concentrations of Cu, Zn, Pb, Mn, and Fe in the polychaete Nereis diversicolor from two different estuaries. Sediment concentrations of these metals varied by an order of magnitude between the estuaries, but the concentrations of the metals in the worm varied only a small percentage between the two locations. Cross, Duke and Willis (1970) studied concentrations of Zn, Fe and Mn in a variety of particulate feeding annelid worms from several locations in Chesapeake Bay. Although metal concentrations in sediment varied from

four- to tenfold between stations, concentrations within the polychaetes showed little variation of this type. Several authors have studied the flux of mercury from sediment to water using biotic indicators (e.g. Fagerstrom and Jernelov, 1971; Gillespie, 1972), but no direct studies of the biotic availability of sediment-bound mercury have been reported.

Likewise, the extent to which mercury is passed by detritus feeders to their predators is not well understood. General statements are found frequently in current scientific and popular literature which indicate that concentrations of trace contaminants are magnified as the contaminants are passed from one trophic level to the next (Kneip and Lauer, 1973). Woodwell, Wurster, Jr. and Isaacson (1967) suggested food chain biomagnifications explained concentrations of DDT found in the biota of an East Coast estuarine ecosystem. However, Kneip and Lauer (1973) have suggested that most studies to date show that food chain biomagnification of mercury and other heavy metals may occur only exceptionally in aquatic ecosystems. Conclusions concerning trophic magnification of metal concentrations are usually based upon studies of contaminant concentrations in organisms from different trophic levels. A few authors have directly studied the trophic availability of metals to predators (e.g. Rice and Baptist, 1970; Jernelov, 1972; Pentreath, 1973a,b,c). However, in no instance has laboratory study of accumulation and retention of mercury by species from a given ecosystem been combined with a comprehensive field study of the dynamics of metal concentrations in those same species. One purpose

of this study was to combine these two techniques to approach the problem of food chain transfer of mercury within Ala Wai Canal.

Concurrent with any study of food chain transfer of mercury it is essential to understand how other sources might contribute to the body burden of the metal observed in various species. Many authors have shown that aquatic biota readily concentrate heavy metals from solution (e.g. see review by Pentreath, 1971). The relevance of these findings, however, depends upon the concentration and the availability of dissolved metals in nature. The combination of laboratory and field work used in this research pointed out some characteristics of biotic accumulation of dissolved mercury and suggested how this form of the metal contributed to biotic mercury concentrations observed in Ala Wai Canal.

Because of the dynamic nature of many environmental parameters in estuaries, mercury accumulation by estuarine biota may be influenced by more factors than just the concentration of the ion in various sources. Salinity changes have been shown to influence the uptake of several heavy metals by estuarine organisms (Bryan and Hummerstone, 1973b,c; Wolfe and Coburn, 1970; Townsley, 1967). Salinity also affects the degree to which some metals are bound by sediment (Forster, Wolfe, Lowman and McClintock, 1971). Salinity-induced desorption or adsorption of sediment-bound metals may significantly affect biotic concentrations of those ions (Lentsch, Kneip and Wrenn, 1971). Productivity in estuaries is usually high, and often varies seasonally (e.g. Harris, 1972). Thus dissolved organic compounds, which may reduce the toxicity of dissolved metal ions (Lewis, Whitfield and Ramnarine, 1972), may occur in

relatively high concentrations during certain times of the year in productive estuaries. Estuaries also receive large amounts of allochthonous organic and inorganic particulates. Variations in the geological characteristics of particulates may affect the way they bind metals and hence the availability of metals to particulate ingesting biota. The effects of salinity, the effects of dissolved organic matter, and the results of variations in some characteristics of mercury-particulate binding have all been considered in relation to biotic accumulation of mercury in this research.

Finally, experiments considering ecological aspects of mercury cycling must include study of biotic accumulation and retention of the ion. Such studies cannot be fully interpreted without some understanding of the physiology of biotic mercury regulation. Many of the experiments in this study were interpreted in terms of both their ecological and their physiological implications.

Thus, in studying the cycling of mercury between the physical environment and a detritus based food chain in an estuary, several questions seemed of special significance:

- 1) To what extent is sediment bound mercury available to detritus feeding biota? Do detritus feeders cycle this metal from the sediment into the macrobiotic portion of the ecosystem?
- 2) To what extent are mercury concentrations within detritus feeders passed on to higher trophic levels?
- 3) What are the relative contributions of assimilation of mercury associated with food and absorption of mercury from solution to the body burden of the metal observed in estuarine organisms?

- 4) How is biotic uptake of mercury from both sources affected by variations in environmental parameters? Can indicator species be used to study these dynamic phenomena?
- 5) What physiological mechanisms play a role in determining the concentration of mercury observed within estuarine biota?

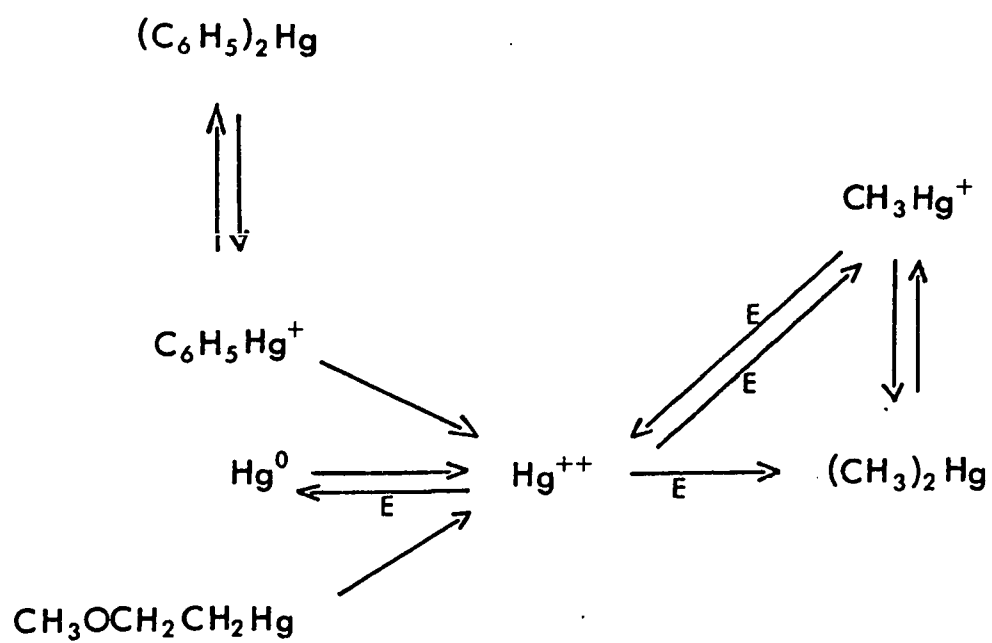
The specific objective of this research was to provide information that might help answer these questions.

The Chemistry of Mercury in Aquatic Environments

The chemistry of mercury in aquatic environments is complex. In general, the chemical cycle of mercury in aqueous systems seems to follow the model shown in Figure 1 (Baughman, Gordon, Wolfe and Zepp, 1973). All the species of organic mercury shown in this figure represent methyl groups bonded by covalent processes to the mercuric ion.

Mercury usually enters the aquatic environment as Hg^0 , Hg^{++} , $\text{C}_6\text{H}_5\text{Hg}^+$ (often as phenyl mercuric acetate) or as $\text{CH}_3\text{OCH}_2\text{CH}_2\text{Hg}$ (Jernelov, 1972; Wood, 1974). Both elemental mercury (Hg^0) and dimethylmercury ($(\text{CH}_3)_2\text{Hg}$) are volatile species of the metal (Kolb, Porcella and Middlebrooks, 1973; Wood, 1974). At pH 7 and above equilibrium reactions seem to favor formation of these moieties and the eventual vaporization of mercury (Imura, 1971; Jernelov, 1972), although the extent to which these conclusions apply in nature are largely unknown (Kolb, Porcella and Middlebrooks, 1973). Divalent mercury (Hg^{++}) and monomethylmercury (CH_3Hg^+) are the forms of the metal most readily accumulated by living organisms. Although both are strong toxins, monomethylmercury (a neurotoxin) is approximately an

Figure 1. A model of the environmental mercury cycle (adapted from Baughman et al, 1973). Divalent mercury (Hg^{++}) may also form soluble and insoluble chemical complexes as described in the text. One way arrows indicate reaction rates in the opposite direction are extremely slow and are probably insignificant. Reactions lettered E have been shown to be enzymatically catalyzed.



order of magnitude more dangerous to living organisms than the inorganic divalent form of the metal (Nelson et al, 1971). Methylation of inorganic mercury to CH_3Hg^+ can occur by chemical reactions alone (Imura et al, 1971), however, it appears catalysis of this reaction by microorganisms is important in aquatic ecosystems (Jernelov, 1969, 1970; Wood, 1974). Even under optimum laboratory conditions ($\text{pH} < 7$; 20°C ; sediment constantly shaken) bacterial methylation of mercury in sediments occurs very slowly (Jernelov, 1969; Fagerstrom and Jernelov, 1971). Extrapolating from such conditions Jernelov (1969) has estimated it would take bacteria 10 to 100 years to methylate all the mercury in the sediments of a lake if input of the metal into the lake ceased. The rate of bacterial methylation of mercury is further reduced by anaerobic conditions and any resultant formation of HgS within the sediments (Jernelov, 1972; Gillispie, 1972). Once methylated, organic mercury may be demethylated within aquatic sediments. Bacteria have been found in lake sediments which may catalyze the degradation of CH_3Hg^+ to Hg^0 (Spangler, 1973). Methylmercury bound to organic sulfhydryl groups may be demethylated to HgS in the presence of sunlight (Baughman et al, 1974).

The relative contribution of the various methylation and demethylation processes, and thus the net methylation of mercury in aquatic sediments in nature, is unknown. Reimers and Krenkel (1972) and Baughman et al (1974) have shown that methylmercury has a strong affinity for sulfhydryl groups which occur in organic matter associated with sediments. Thus if net methylation of mercury occurs to any significant extent within the sediments, the methylated form of the

metal should concentrate in sedimentary organic material. Yet, Andern and Harriss (1973) found that the concentration of methylmercury in various estuarine sediments around the Gulf of Mexico never exceeded 0.07% of the concentrations of total mercury in those sediments. Jernelov (personal communication) has suggested concentrations of methylmercury in aquatic sediments in Sweden seldom exceed a few ppb.

Despite the very low concentrations of methylmercury observed in aquatic sediments this more toxic form of the metal makes up 70 - 95% of the concentration of total mercury observed within the tissues of larger aquatic organisms (e.g. Westoo, 1973). Jernelov (1972, 1973) and Wood (1974) suggest bacterial methylation of inorganic mercury in the sediments is the source of the methylmercury observed in the large aquatic species. Wood (1974) suggests the kinetics of methylmercury formation are such that the rate of methylmercury uptake by aquatic macrofauna determines the rate of bacterial methylation; thus low methylmercury concentrations in sediment are actually not an indication of the rate of synthesis of the organic form of the metal. However, Andern and Harriss (1973) suggest bacterial methylation of sedimentary mercury is insignificant, and that intraorganism methylation of mercury by macrofauna results in the observation of the organic form of the metal in aquatic species. Ukita (1972) has shown that homogenized tuna liver will slowly convert Hg^{++} to CH_3Hg^+ . Macrobiotic methylation of mercury has not been adequately investigated over sufficient periods of time to determine if this process is significant in nature.

The chemistry of divalent mercury (Hg^{++}) alone may also be complex. This form of the metal may occur in solution or in association with the sediments. Dissolved mercury may occur in several forms: a) as dissociated Hg^{++} ; b) an undissociated salt or chemical complex (e.g. $\text{Hg}(\text{Cl}_2)_2^-$, HgOH^+ , $\text{Hg}(\text{OH})_2$ - Baughman et al, 1974; Reimers and Krenkel, 1972); or c) bound by ligand formation to dissolved organic molecules (Martel, 1971). The relative abundance of the various forms of the dissolved metal in aquatic ecosystems is a subject of controversy. Inorganic mercury associated with the sediments may exist in an insoluble form (e.g. HgS) or may be bound to particulates. Mercury may bind to inorganic sites on sedimentary particulates (e.g. by cation exchange) or it may complex with inorganic molecules by electrostatic coordination (i.e. ligand formation) to form mercury-organic complexes or mercury-organic chelates (Martel, 1971).

The laboratory studies conducted in this research all involved use of inorganic divalent mercury (Hg^{++}) in chloride form. Samples collected from the field study site were analyzed for total mercury (the sum of all forms of the metal in Figure 1), except in a few cases where organic mercury analyses were performed. This approach was chosen because:

- 1) It is unlikely significant bacterial methylation of mercury occurs at the site of this study in Ala Wai Canal. The sediments in this area are characterized by high sulfide concentrations and anaerobic conditions, which would favor the formation of HgS and would reduce any bacterial methylation

of the metal (Fagerstrom and Jernelov, 1971). The generally high pH of the estuarine water would favor formation of volatile $(\text{CH}_3)_2\text{Hg}$ over the formation of CH_3Hg^+ (Jernelov, 1972), and penetration of light to the substrate in this shallow portion of the estuary should degrade any organically bound CH_3Hg^+ to HgS (Baughman et al, 1974).

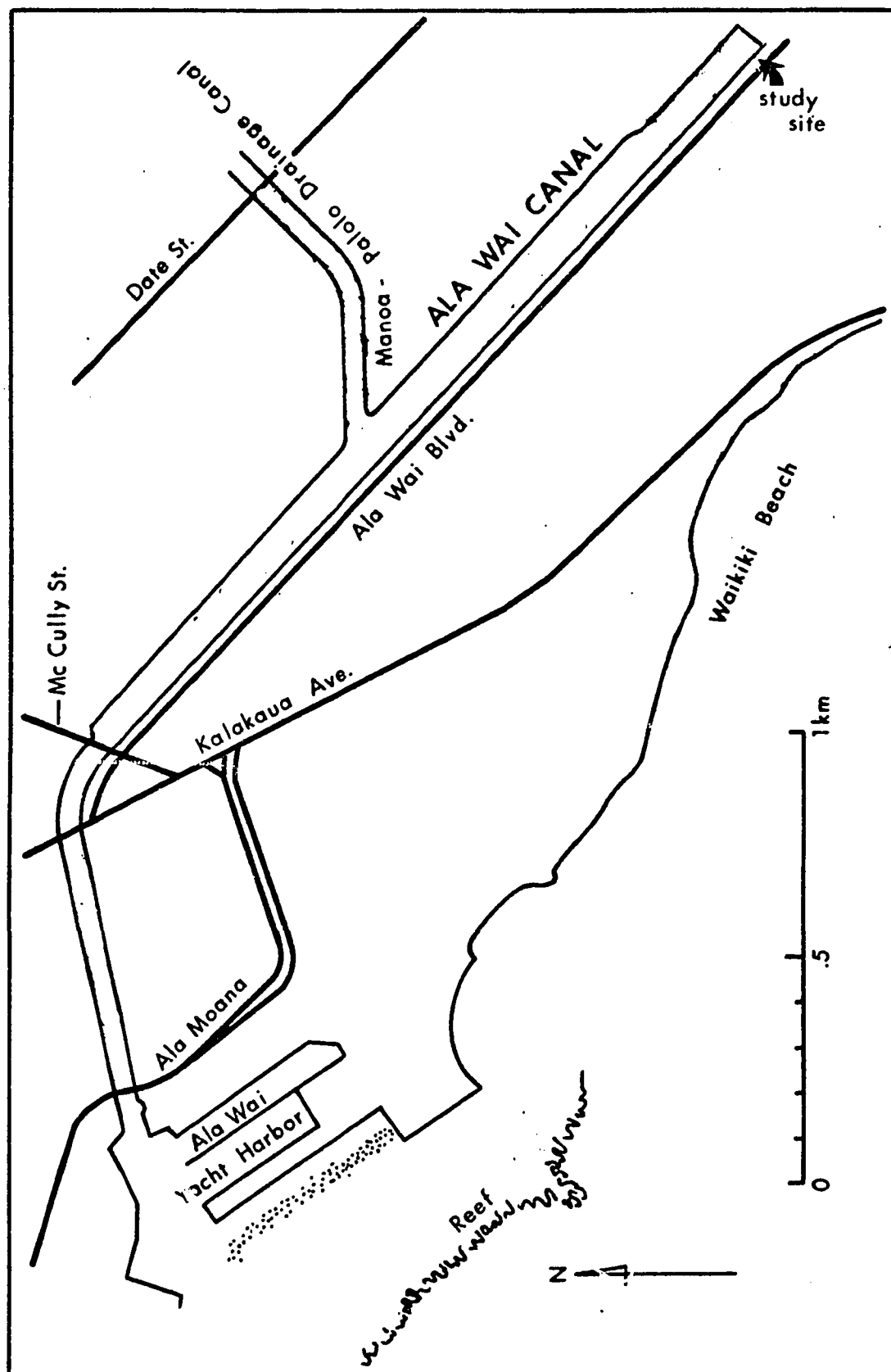
- 2) If the occurrence of methylmercury in large aquatic species results from macrofaunal methylation of the metal, then the environmental cycling of inorganic mercury would be the most important factor determining the biotic distribution of methylmercury.
- 3) Inorganic divalent mercury may be a significant toxin itself (Harriss et al, 1970).

All references to mercury in this report then refer to either inorganic mercury or total mercury unless otherwise noted. All references to dissolved mercury indicate the ion occurs in one (or more) of the three forms defined above; however, the exact form(s) of the metal may be unknown. Reference to organically bound or organically complexed mercury indicates either dissolved or adsorbed mercury fixed to organic matter by ligand formation. Reference to organic mercury, on the other hand, indicates mercury to which a methyl group(s) is covalently bonded.

Study Site

The area chosen for this investigation was a brackish water community at the head of Ala Wai Canal (Figure 2). This is a small,

Figure 2. Location of study site in Ala Wai Canal.



eutrophic estuary running through urban Honolulu. Ala Wai Canal opens to the ocean through Ala Wai Boat Harbor. It receives freshwater from two sources:

- 1) Manoa-Palolo stream enters the canal approximately one kilometer from the blind inland termination of the estuary (Figure 2);
- 2) During rainstorms untreated storm runoff from the dense urban area surrounding Ala Wai enters the estuary directly through large storm drain outfalls located at the blind end of the canal. Storm runoff from urban Manoa and Palolo valleys enters Manoa-Palolo stream and also makes its way into Ala Wai Canal during rainstorms.

This study was conducted within 150 meters of the blind inland termination of Ala Wai Canal (Figure 2). All samples analyzed for total mercury and all organisms used in the laboratory experiments were collected from within this study site.

In the area of the study site the canal is shallow, less than one meter deep at MLLW. Salinity in this area fluctuates with rainfall in the Ala Wai watershed (Gonzalez, 1971). During summer months salinities may reach 36‰ in this part of the canal, while, during rainstorms, salinities as low as 6‰ have been reported (Gonzalez, 1971; Harris, 1972). Mean water temperatures range from 28°C in June-July to 25°C in December through March (Gonzalez, 1971). Productivity fluctuates seasonally, reaching a peak in the summer months (Harris, 1972). The residence time of surface water in the study area varies as a function of the intensity of storm runoff. During heavy rains in the Ala Wai

watershed residence time may be as low as three hours at the inland termination of the canal. During drier seasons surface water in the study area may exchange only once every 36 hours (Gonzalez, 1971). The influx of urban storm runoff also results in a high rate of sedimentation at the study site (personal observation). Surface sediments within the study area were of two types: a) fine silt which was 24.5% organic material, or b) more coarsely grained sediment which was 10.5% organic matter. (Sediment was dried at 60°C to a constant weight; organic content represents weight lost after ashing this sediment for 12 hours at 550°C.) All organisms used in this investigation were found associated with both types of substrate.

Organisms

Four aquatic animals characteristic of the study site were chosen for investigation. Preliminary studies of gut contents and the work of other authors (Hiatt, 1944; Goerke, 1971) showed three of these species were detritus feeders - the polychaete, Nereis succinea, the shrimp (opae), Palaemon debilis, and the poeciliid fish, Mollienesia latipinnia. The three detritus feeders were chosen principally because of their large numbers, ease of collecting and relatively small size. All three were easily maintained in closed laboratory aquaria. Observations in the study area and laboratory feeding experiments indicated the fourth species, the decapod Thalamita crenata, was primarily a predator and a carnivorous scavenger. The choice of these four organisms allowed study of a three trophic level, detritus-based food web amenable to both field and laboratory investigation.

Since T. crenata is the subject of a sport fishery in Ala Wai Canal, one recipient of mercury accumulated in this food web is man.

METHODS AND MATERIALS

Mercury Analyses

Total Mercury

Samples of all four organisms and of sediment were collected periodically from the study site during 1973-74 and analyzed for total mercury. For each of the detritus feeding species (polychaete, shrimp, and fish) one sample represented a number of individuals pooled to a weight of 2 - 5 grams. Thus, while only three samples of each species were usually taken at each time period, the mean of the mercury concentration in these three samples represented the mean whole-body mercury concentration from 10 to 50 individual organisms of that species. The crabs were dissected so that four organs or groups of organs were analyzed from each individual - chela muscle, body muscle, viscera, and gills.

Sediment samples were scraped from the upper 5 mm of intertidal sediment, and 1 gm (wet weight) was separated for digestion and analysis. The water content of the sediment was determined by drying 5 gm of the material. These values were also used to calculate dry weight mercury concentrations. Oven dried sediment samples were not analyzed for total mercury directly because such treatment may result in volatilization of mercury from the sample (Thompson and McComas, 1973).

The analysis for total mercury in samples employed the digestion techniques suggested by Knauer and Martin (1972). To each sample 7 ml of concentrated sulfuric:nitric acid (2:1) was added. After the

reaction had cooled, the mixture was kept overnight at 80°C (in a drying oven), then samples were stored, if necessary. Prior to analysis, an excess of 6% potassium permanganate (usually about 10 ml) plus 2 ml potassium persulfate were added to each sample to assure oxidation of all organic matter. This mixture was left overnight. The excess permanganate was then reduced with several drops of hydrogen peroxide and back titrated with permanganate until light pink. (Knauer and Martin (1972) found this process important in removing gaseous oxides of nitrogen which otherwise caused peak suppression during mercury determination.) Samples were then washed into standard BOD bottles and distilled water was added to a total volume of 100 ml. At this point all mercury was in oxidized form. Hydroxylamine hydrochloride was added to reduce the permanganate and remove any interference from Fe^{+++} (Thompson and McComas, 1973). To these samples 5 ml of stannous chloride (88 g/l in 0.5N H_2SO_4) were added to reduce all Hg^{++} to the volatile species Hg^0 , and the BOD bottle was immediately connected to a closed cold vapor mercury analysis chamber (Manning, 1970). The Hg^0 was purged from solution in this system by bubbling air through the mixture. Mercury was determined by flameless atomic absorption using either a Perkin-Elmer Atomic Absorption Spectrophotometer (model 305A) fitted with a hollow cathode mercury lamp or by using a Coleman Mercury Analyzer. Preliminary experiments in this laboratory and work by other authors (Siegel, 1973; Tuna Research Foundation, 1971) indicated similar results are obtained from either type of instrumentation. The detection limit of mercury was 0.01 μg . All samples were corrected

for contamination using reagent blanks. Instruments were calibrated before and during every run using Hg^{++} in chloride form. Recoveries, using digested animal tissue spiked with known concentrations of mercury, showed this method to be $103 \pm 5\%$ (s.d.) efficient.

Organic Mercury

Organic mercury analyses were conducted according to the methods of Rivers, Pierson and Schultz (1972). Briefly, tissue samples were homogenized in a Waring Blender with an equal volume of distilled water. Each sample was made up to a volume of 70 ml with 10 g of NaCl and distilled water. The sample was then extracted in 65 ml of benzene, and centrifuged until the benzene phase was clear. From the benzene phase 50 ml was pipetted into a 125 ml separatory funnel with 7 ml of 1% cysteine solution (1 g L-cys, 0.775 g sodium acetate, and 12 g sodium sulfate in 100 ml distilled water). After extraction and phase separation (by centrifuging several times), 4 ml concentrated HCl and 10 ml 6% potassium permanganate were added to 4 ml of the cysteine extract. After 30 minutes oxidizing time the samples were analyzed for Hg^{++} using cold vapor atomic absorption techniques.

Radiomercury

All laboratory experiments utilized the radioisotope $^{203}\text{-Hg}$ in chloride form (HgCl_2). The specific activity of the $^{203}\text{-Hg}$ was such that no carrier mercury other than that in the processed radioisotope was needed to reach the desired concentrations in the medium.

Liquid Scintillation Techniques

The ^{203}Hg activity in individuals from all three species of detritus feeders, in dissected crab organs, in water, and in sediment were measured using liquid scintillation. The ^{203}Hg activity in water was determined from 1 ml Millipore filtered ($0.45\ \mu$) samples without digestion. The polychaetes were individually digested at each sampling interval in scintillation vials using 75% nitric:perchloric acid (17:3) for three hours at 50°C . Crab organs and individual shrimp and fish were digested in 2 or 3 ml concentrated nitric:perchloric acid at room temperature. The sediment was digested in 5 ml of the concentrated acid at 50°C for 24 hours. No loss of mercury occurred using these procedures, because at low pH all mercury is present as non-volatile Hg^{++} . Recoveries of known amounts of ^{203}Hg added to sediment were $99\pm 1\%$ (s.d.) using these techniques.

After digestion or extraction, liquid scintillation was used to measure the ^{203}Hg in an aliquot of tissue digest or extract. The liquid samples were all made to 1 ml with distilled water or ascorbic acid and 10 ml of the liquid scintillation cocktail Aquasol (New England Nuclear) was added to each. The samples were allowed to stand (to stabilize any chemiluminescence) and then the activity was measured using a Beckman LS100 ambient temperature liquid scintillation counter. Because of the wide variation in quench between samples the widest Beckman isoset window (covering the energy spectrum from tritium to ^{32}P) was used for counting. The background resulting from use of such a wide window was normally 50 - 70 cpm, whereas sample counts were usually at least an order of magnitude greater.

Counting efficiency was determined by plotting the external standard ratio from quenched standards, made by adding varying amounts of acid digested, nonradioactive, animal tissue, to known 203-Hg samples. Disintegrations per minute were corrected for decay, then converted to parts per billion (ppb) mercury using the formula

$$\text{ppb } 203\text{-Hg} = \frac{(\text{DPM/Decay}) \times \text{SpAct}}{\text{gm worm}} \quad (1)$$

where DPM = disintegrations per minute

Decay = the percent of activity remaining after decay since the nuclide was processed (all samples were corrected to the activity on the day of processing)

SpAct = specific activity or ng Hg/dpm 203-Hg on the day of processing

gm worm = weight of the worm analyzed.

During digestion animal tissues displaced a quantity of acid, the volume of which was dependent upon the weight and type of tissue. In samples where only an aliquot of digest was used for 203-Hg analysis, correction was made for volume displacement before whole sample 203-Hg concentration was calculated.

Gamma Scintillation Techniques

The time course of 203-Hg whole body uptake by the crab, T. crenata, and by the shrimp, P. debilis, were determined by placing whole animals on a lead shielded NaI gamma scintillation crystal and counting by gamma scintillation on a Nuclear Chicago Model D181 gamma counter. Although no variable pulse height analyzer was used, the resulting

background (2000 - 5000 cpm) was insignificant in terms of total activity of the samples.

Experimental Procedures

To minimize variations in the quality of water used in laboratory experiments seawater was collected from the open ocean source used by the Kewalo Basin Marine Laboratory in Honolulu. The inlet pipe for this seawater source is located at a depth of 12 meters offshore. This water was filtered through a 0.45 μ Millipore filter and (unless otherwise noted) diluted to 32‰ salinity for experimentation. All animals were acclimated in laboratory seawater with food for at least a week before use in experiments. All experiments were run at 25°C.

Uptake of Dissolved ²⁰³Hg

Prior to studies of ²⁰³Hg uptake from seawater animals were placed in clean seawater for 3 - 12 hours to remove the sediment from their alimentary tract. During an experiment at least five individuals were analyzed at each sampling interval. Studies of uptake of dissolved ²⁰³Hg were all made with starved animals. After sampling from an experiment, animals were briefly rinsed in clean seawater, blotted dry, weighed to the nearest 0.1 mg and prepared for radio-assay.

Uptake from Food by Detritus Feeders

In experiments involving uptake from food by detritus feeders sediment from the study area in Ala Wai Canal was labelled by exposing it to approximately 500 ml of seawater containing the appropriate

quantity of ^{203}Hg . The mixture was incubated at room temperature for 24 hours. Within this period at least 99% of the mercury originally present in the water became bound to the sediment. The original ^{203}Hg contaminated water was poured off and replaced by fresh seawater. Water samples were Millipore filtered ($0.45\ \mu$) to remove particulate fractions before counting. After sediment and seawater had equilibrated for one to three hours the animals were added to the mixture. At each sampling interval at least five individuals were removed from the experimental aquarium and placed in clean seawater. The detritus-feeding species defecated the radioactive sediment from their digestive tract rapidly (3 - 10 hours) if the filtered water was changed often enough to prevent reingestion of feces. Any failure of an individual to totally eliminate the sediment was easily detectable: 1) due to the translucent nature of the worms and shrimp; 2) from the obvious presence of sediment in the tissue digest; and 3) from excessively high ^{203}Hg content. Such samples were discarded. Any net loss of mercury by the organisms during the period of sediment defecation was corrected by extrapolating from data collected in efflux experiments. Such losses usually amounted to less than 10% of the body load of the animal.

Worm and fish feces were analyzed for ^{203}Hg by drying fecal pellets on tared filter paper at room temperature, weighing pellets to the nearest 0.1 mg, then digesting and counting them using liquid scintillation techniques. No volatilization of mercury was observed when pellets were dried at room temperature (similar results have been observed by Thompson and McComas, 1973).

Loss of 203-Hg

To study the efflux of 203-Hg from the three detritus feeders, animals were pre-loaded with the radionuclide by placing them in 203-Hg labelled seawater for the previously determined time necessary to reach steady state. Efflux experiments were conducted in closed aquaria using unlabelled seawater and sediment from Ala Wai Canal. The sediment served as food for the organisms and also served to remove from solution most 203-Hg lost by the animals. At each sampling interval at least five organisms were removed from the aquaria, digested and 203-Hg activity was determined. The water was changed every other day in these experiments. Preliminary experiments indicated this interval was sufficient to prevent backflux of 203-Hg into the animals.

Autoradiography

Autoradiographs were made of the polychaete, N. succinea. Cross-sections of the worm (1-2 segments) were fixed in neutral formalin or glycerinaldehyde fixative. Fixed chunks of tissue were dehydrated, step-wise, in alcohol, cleared using cedarwood oil, embedded in paraffin, sectioned and placed on slides pretreated with subbing solution (0.5% gelatin in distilled water; 0.05% chromium potassium sulfate). Slides were dipped in Kodak NTB liquid emulsion (five coats of emulsion seemed to yield optimum results), and placed in a light free box for three to five weeks. After this time period the autographs were developed using D-19 developer and Kodak Rapid Fix fixative (diluted 2:1). Photomicrographs of histological and autoradiographical preparations were taken with a Zeiss Photomicroscope.

Uptake of ^{203}Hg from Food by Crabs

The accumulation of mercury from food by T. crenata was studied by feeding crabs ^{203}Hg labelled N. succinea. The crabs were maintained individually in one gallon jugs in 2 liters of seawater. The seawater was analyzed daily for dissolved ^{203}Hg and was changed every other day. Worms were labelled by exposing them for 24 hours to ^{203}Hg in seawater. To maintain as constant a concentration of ^{203}Hg as possible in the crab food, a fresh batch of worms was labelled daily, and exposure conditions (dose of ^{203}Hg per volume of seawater; number of worms per volume of seawater) were kept as constant as possible. Each day a preweighed mass of worms (daily dose varied from 200 - 400 mg) was given to each crab. After four hours any uneaten worms were removed from the crab aquaria and weighed. A record was maintained of the weight of worms ingested daily by each crab.

At the completion of the experiment all crabs were frozen, then dissected. Organs were separated into the four groups used in stable mercury analysis and counted using liquid scintillation techniques.

RESULTS

Volatilization/Adsorption of Mercury

Between pH 7.0 and 8.2 (the conditions of these experiments) the concentration of mercury dissolved in seawater tends to be somewhat unstable over time. Corner and Rigler (1957) noted that when mercuric chloride was added to seawater significant amounts disappeared from solution due to either volatilization (presumably due to the formation of volatile Hg^0 at this pH) or to uptake by bacteria adsorbed to the sides of their experimental vessel. They found that the addition of glucose to such a system inhibited the loss of mercury from solution.

Table 1 describes the concentration of dissolved 203-Hg in Millipore filtered (0.45μ) seawater: 1) during a study of the time course of uptake of 203-Hg by the worm N. succinea; and 2) in a control vessel to which no animals were added. The presence of the worm appeared to inhibit the rate at which mercury was lost from solution. In the aquarium containing the worm only 12 - 17% of the mercury originally present was lost from solution after 50 hours. In a control aquarium, left without any worms, 64% of the 203-Hg originally present was lost after 50 hours. A reduction in the number of worms in the experimental aquarium over time (due to sampling) probably prevented extensive organismic depletion of the mercury, which occurred in such experiments at higher animal density/water volume ratios.

Organismic depletion and/or significant volatilization/adsorption of mercury resulted during initial studies of the time course of shrimp uptake. Because reduction in the level of 203-Hg in the medium

Table 1

A comparison of the loss of 203-Hg from Millipore filtered seawater containing the polychaete N. succinea versus loss from seawater without animals

Time (hrs.)	Aquarium containing <u>N. succinea</u>		Control aquarium	
	ppb 203-Hg	% lost	ppb 203-Hg	% lost
0.25	1.90		1.91	
0.50	1.85	2.6	1.80	5.8
1.0	1.79	5.8	1.82	4.7
3.0	1.77	6.8	1.80	5.8
5.0	1.75	7.9	1.73	9.4
13.0	1.73	8.9	1.24	35.1
25.0	1.56	17.9	0.92	51.8
50.0	1.67	12.1	0.69	63.9

affected calculations of rate constants describing the time course of uptake, this experiment was repeated. To hold the concentration of 203-Hg constant it was necessary to monitor 203-Hg activity every 12 - 24 hours and add label when activity decreased.

In experiments testing changes in steady state organismic 203-Hg levels as a function of the concentration of 203-Hg in seawater, small numbers of animals were exposed to 203-Hg in relatively large volumes of seawater to prevent depletion of mercury by the animals. In these experiments some disappearance of mercury (probably due to volatilization/adsorption) occurred. In these instances 203-Hg activity was determined before and after every experiment, and concentration factors were based upon the initial concentration of 203-Hg in the water.

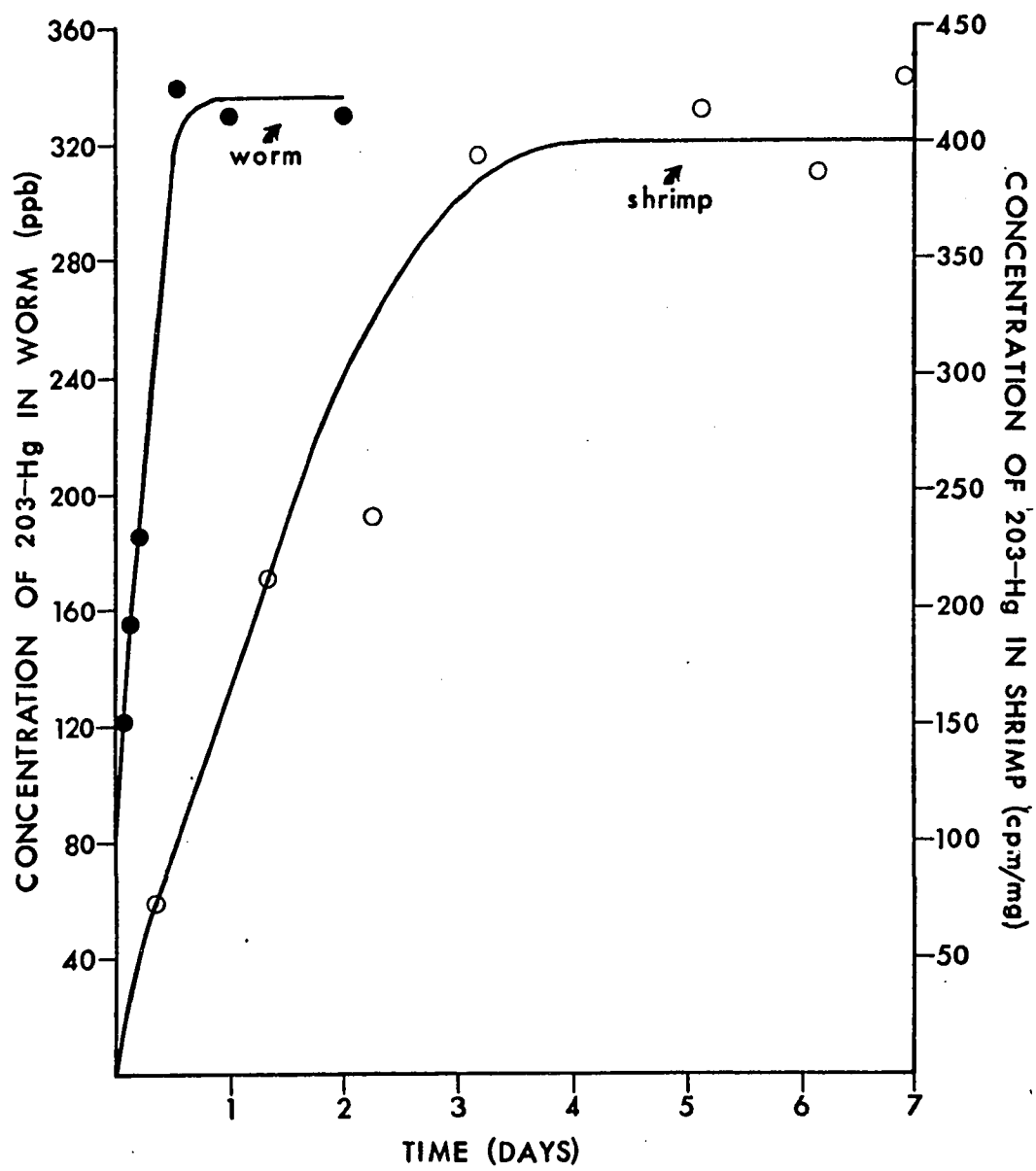
Loss of 203-Hg through volatilization or through organismic depletion of the metal were not problems in feeding experiments. A combination of large amounts of bound mercury and low biotic accumulation appeared to prevent both processes in these instances.

Accumulation and Retention of Mercury by Detritus Feeders

Uptake from Water

The uptake of dissolved 203-Hg by two detritus feeders, the polychaete worm N. succinea, and the shrimp P. debilis, as a function of time is shown in Figure 3. It can be seen that the worm achieved a steady state whole body concentration of mercury within 13 hours, while the shrimp reached steady state within three days. In these experiments the concentration of 203-Hg in the medium was held constant at concentrations of 1.88 ppb for the worm and 1.00 ppb for the shrimp.

Figure 3. Uptake of ^{203}Hg from seawater by N. succinea and P. debilis as a function of time. The medium contained 1.0 ppb ^{203}Hg in experiments with shrimp and 1.88 ppb ^{203}Hg in experiments with worms. Each point represents the mean mercury concentration in at least five animals. Lines were fit by eye.



To determine if the magnitude of the steady state organismic mercury concentrations were dependent upon the concentration of dissolved mercury, worms were exposed to different concentrations of the dissolved metal for 24 hours, and shrimp were exposed to the nuclide for 4 days. These data are presented in Figure 4 and Table 2. Both species accumulated 203-Hg to concentrations from 160 to 310 times greater than observed initially in seawater. The concentration factor¹ for dissolved 203-Hg uptake by the worm increased with increasing 203-Hg in the water (Table 2). The curve describing the concentration dependence of 203-Hg accumulation in the worm fit a power function.

$$C_{SS} = 135.6C_{wo}^{1.285} \quad r = 0.970 \quad (2)$$

where C_{SS} = the concentration of 203-Hg in the worm at steady state

C_{wo} = the initial concentration in the water

and r = the correlation coefficient for the relationship.

The correlation between the data and the function was significant ($p < 0.010$, testing the correlation coefficient). Accumulation of 203-Hg by the shrimp followed a more complex function.

Steady state body concentrations of 203-Hg accumulated by both species from solution showed an inverse correlation with body size (Fig. 5a). The correlation showed a significant fit ($p < 0.01$ as determined from the correlation coefficient, r) to a log-log power function for the worm

¹The ratio C_{SS}/C_{wo} where C_{SS} is the whole body concentration of 203-Hg in the animal at steady state and C_{wo} is the initial concentration of 203-Hg in seawater.

Figure 4. The steady state concentration of ^{203}Hg in N. succinea and P. debilis as a function of the concentration of ^{203}Hg in seawater. Lines were fit by eye.

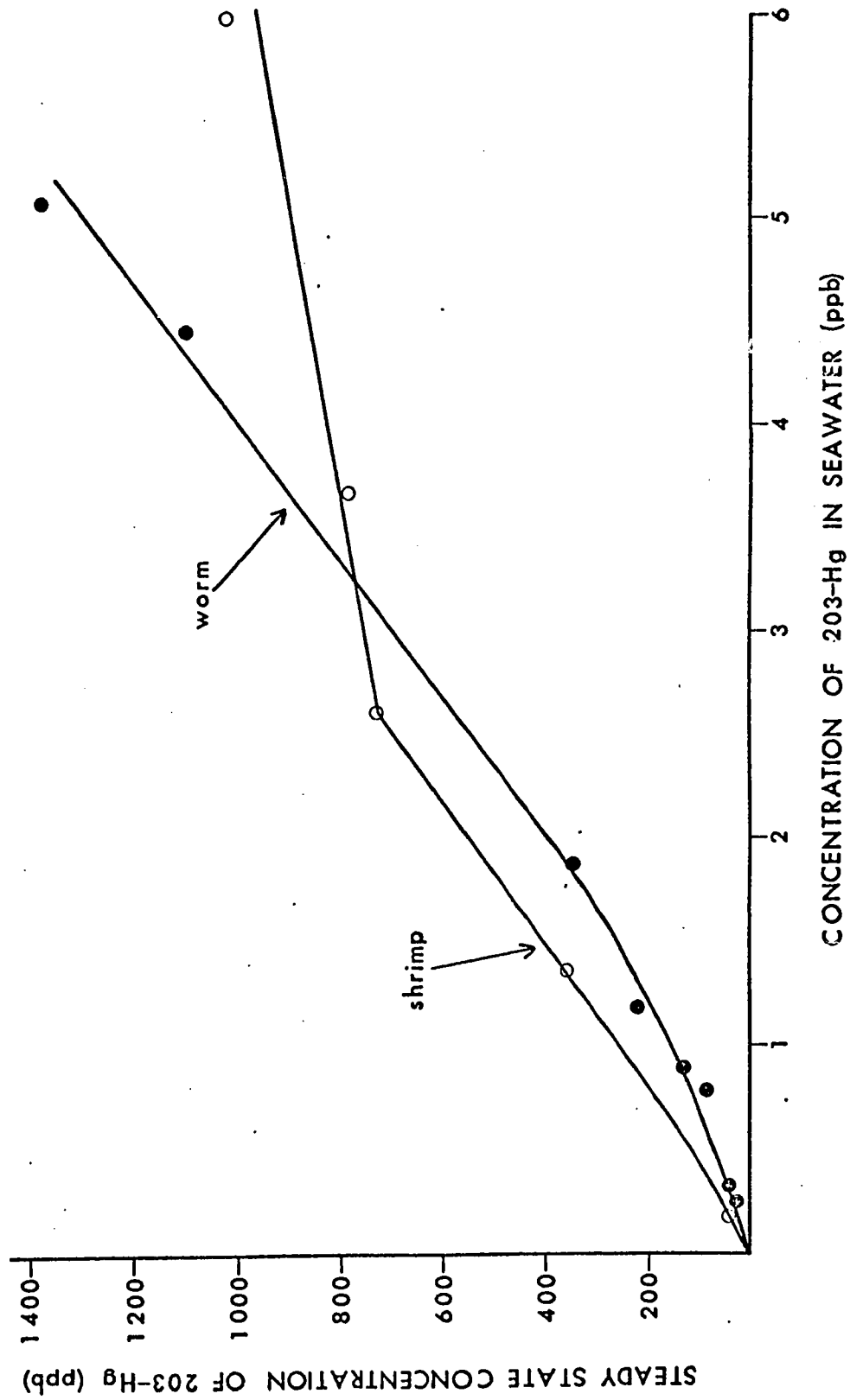


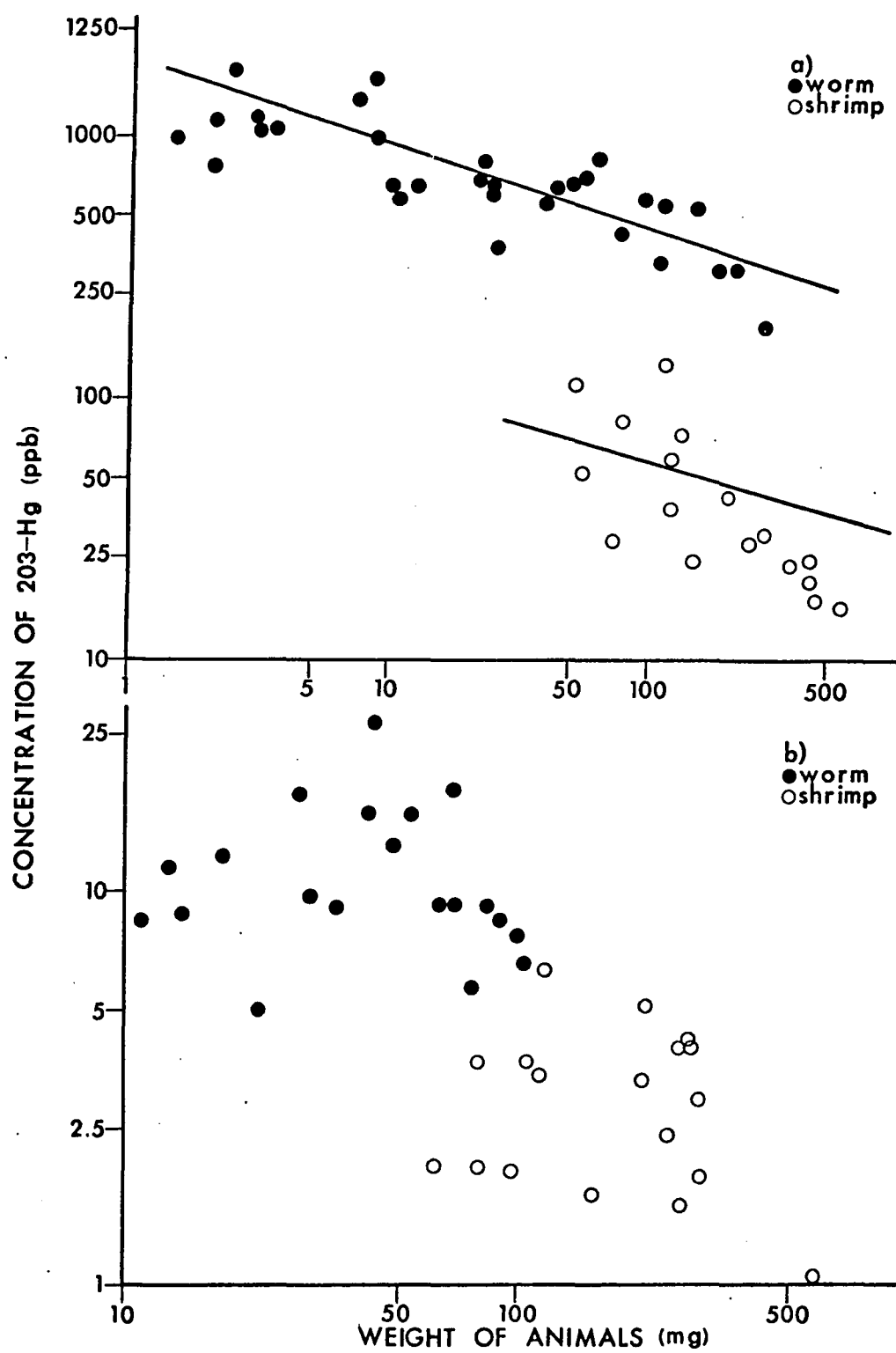
Table 2

The concentration factor, C_{ss}/C_{wo}^* , for the worm, N. succinea and the shrimp, P. debilis, as a function of the concentration of 203-Hg in seawater (calculated from data presented in Fig. 2)

Concentration of 203-Hg in seawater (ppb)	C_{ss}/C_{wo}^* (<u>N. succinea</u>)	C_{ss}/C_{wo} (<u>P. debilis</u>)
1	160	270
2	180	275
3	203	240
4	236	200
5	310	181

* C_{ss} = the organismic steady state concentration of 203-Hg; and
 C_{wo} = the initial concentration of 203-Hg in seawater.

Figure 5. The steady state concentration of ^{203}Hg as a function of weight in the polychaete worm N. succinea and the shrimp P. debilis. a) Exposure to dissolved ^{203}Hg . b) Exposure to ^{203}Hg in food.



$$C_{ss} = 1975W^{-0.33} \quad r = -0.84 \quad (3)$$

and for the shrimp

$$C_{ss} = 1123W^{-0.654} \quad r = -0.776 \quad (4)$$

where W = weight of the animal in mg.

Using worms weighing less than 50 mg the toxicity of dissolved mercury was also investigated. The results are shown in Figure 6 plotted on a log-probit scale. The LD₅₀ of divalent mercury to the worms was 65 ppb. The minimum dose at which mortality was observed was 50 ppb. These experiments were conducted over exposure periods of 96 hours. Mortality occurred through the first 36 hours, but beyond that time no additional mortality was ever observed.

Uptake from Food

Not all mercury available to an aquatic organism occurs in soluble form. The availability of mercury from the food of an organism must also be taken into account. Both the shrimp and worm are detritus feeders. Thus the affinity of mercury for particulate material could significantly affect mercury concentrations in the different sources of the metal to which these species are exposed. The loss of mercury from solution as a function of time in the presence of sediment is shown in Figure 7. This experiment compared absorption of mercury by Ala Wai sediment at salinities of 32‰ and 16‰, and adsorption of mercury by terrestrial sediment characteristic of storm runoff. In all three experiments more than 90% of the dissolved mercury was adsorbed to the sediment within two minutes after exposure. The time course of adsorption of the final 10% of the mercury varied between the three

Figure 6. The toxicity of divalent mercury in seawater to the worm, N. succinea.

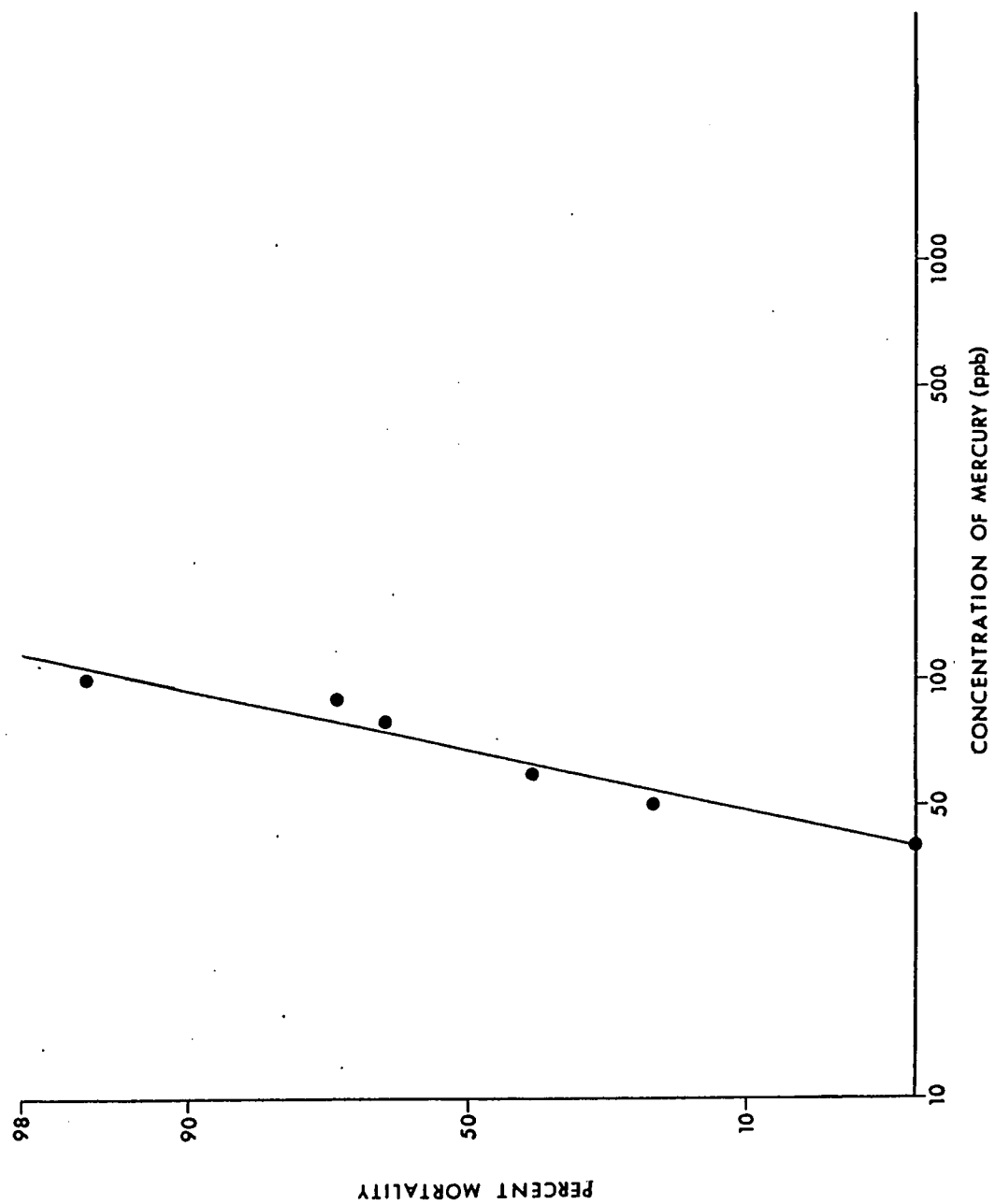
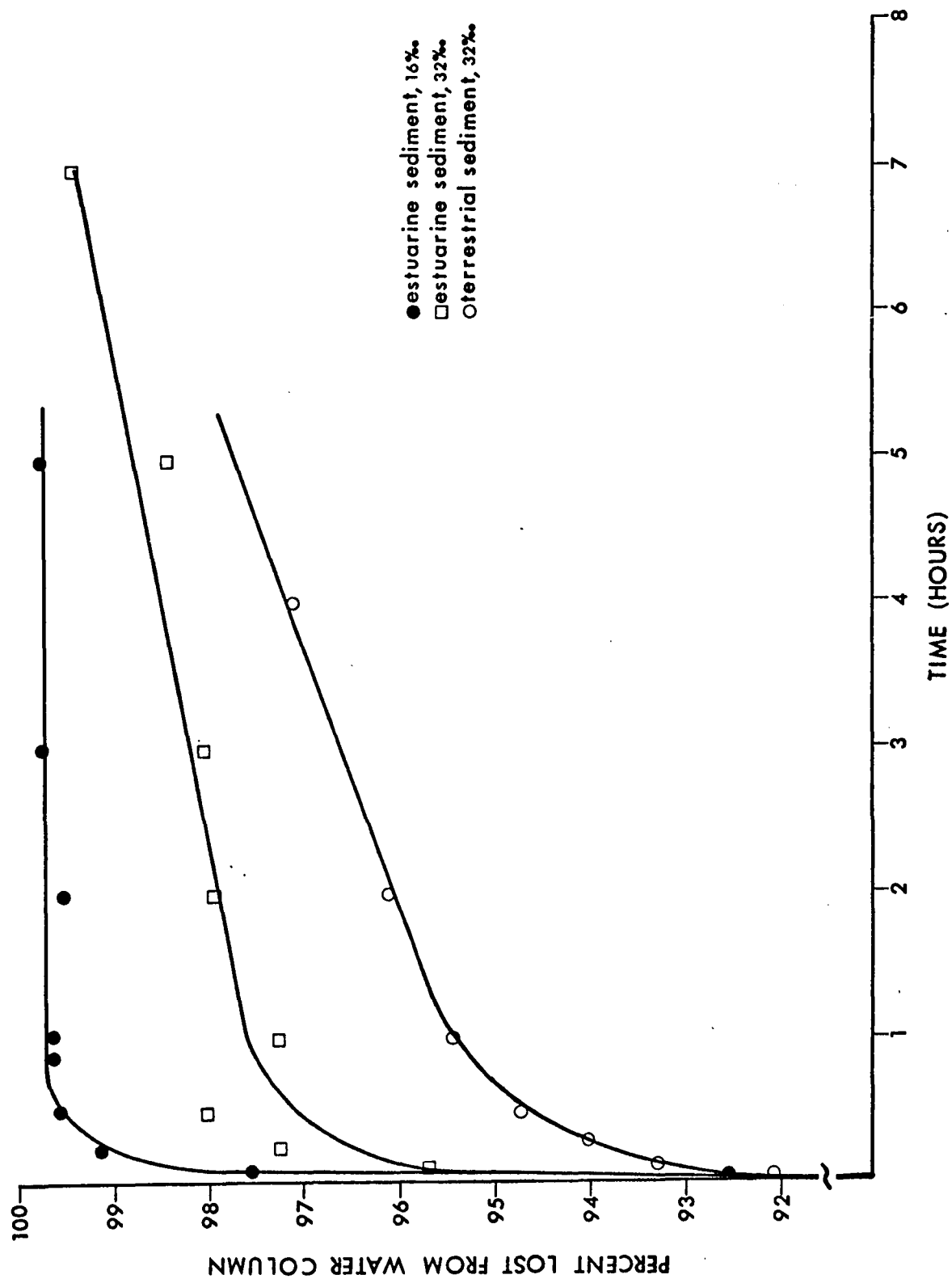


Figure 7. The loss of ^{203}Hg from seawater after exposure to sediment as a function of time.



treatments. However, in all experiments, after 20 hours of exposure to the sediment, only approximately 0.5% of the initial 203-Hg activity remained in the water column (Table 3). This fraction was similar over a 180-fold difference in the initial concentration of dissolved 203-Hg. When the contaminated seawater was removed and the labelled sediment was immersed in fresh seawater the distribution coefficient² of 203-Hg averaged, in eight experiments, $1.42 \pm 0.82 \times 10^5$ (s.d.). The range of distribution coefficients observed in these experiments is shown in Table 4.

The biological availability of the concentrated particulate-bound pool of mercury was studied by providing 203-Hg labelled estuarine sediment to the deposit feeders N. succinea, P. debilis, and M. latipinnia. The uptake of 203-Hg as a function of time from the labelled sediment is shown in Figure 8. The three species reached a steady state body concentration of mercury after several days exposure to the labelled food - as was previously found with uptake from water alone. However, Table 5 shows that the concentration of 203-Hg achieved by feeding animals was only a small fraction of the concentration of 203-Hg observed in the food they ingested - unlike accumulation from water alone (Table 2). At sediment 203-Hg concentrations of 1475 ppb the worm accumulated a steady state 203-Hg concentration of only 22 ppb; 1.5% of the dry weight 203-Hg concentration in the sediment. Similarly, the shrimp and the fish accumulated

²A ratio of the amount of bound element per unit dry weight of sediment to the amount of element per unit weight of seawater (Duursma and Gross, 1971).

Table 3

The percentage of ^{203}Hg bound to sediment 20 hours
after dissolved ^{203}Hg and sediment were mixed

Experiment	Concentration of ^{203}Hg in water (ppb)		% bound at 20 hrs.
	Initial	After 20 hrs.	
Terrestrial sediment	115	0.54	99.54
Estuarine sediment			
32%	1.14	0.0067	99.41
	1.14	0.0064	99.44
	1.14	0.0043	99.62
16%	183	0.76	99.58

Table 4

A comparison of the concentration of 203-Hg in sediment and the concentration in seawater in experiments using Ala Wai Canal sediment. Seawater was added to 203-Hg labelled sediment and measurements were taken 24 hours of equilibration. The distribution coefficient is the ratio of dry weight 203-Hg concentration in sediment versus the concentration of 203-Hg in an equal weight of seawater.

Concentration of 203-Hg (ppb)		Distribution coefficient
Sediment	Seawater	
344	0.122	2.82×10^4
914	0.096	9.52×10^4
801	0.076	1.05×10^5
1171	0.071	1.65×10^5
1292	0.086	1.50×10^5
1425	0.068	2.10×10^5
1834	0.225	8.10×10^4
2086	0.070	2.98×10^5

Figure 8. The uptake of ^{203}Hg by the detritus feeders N. succinea (worm), P. debilis (shrimp), and M. latipinnia (fish) from labelled estuarine sediment as a function of time.

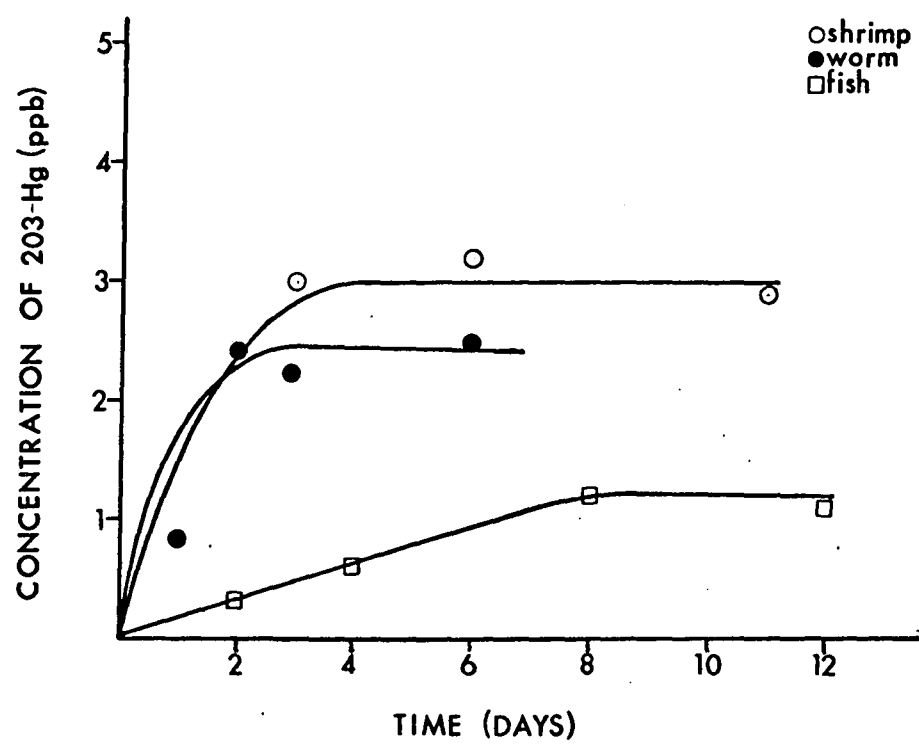


Table 5

The uptake of 203-Hg from labelled sediment by three detritus feeders, the worm, N. succinea the shrimp, P. debilis, and the poecilid fish, M. latipinnia. The organic content of the sediment was 24.5% weight lost after ashing, except where otherwise noted. Numbers in parentheses indicate the number of samples from which each value was determined.

Species	Mean concentration of 203-Hg \pm one std. dev. (ppb)		CF _{es} *
	Sediment (dry wt.)	Feeding organism at steady state (wet wt.)	
Worm	344 \pm 93 (4)	2 \pm 1 (14)	0.010
Worm	915 \pm 397 (4)	11 \pm 5 (16)	0.012
Worm ⁻	1475 \pm 75 (2)	22 \pm 7 (7)	0.015
Shrimp	1172 \pm 268 (3)	3 \pm 2 (28)	0.0025
Fish [#]	1354 \pm 125 (2)	2 \pm 2 (18)	0.0016
Worm [#]	1292 \pm 439 (3)	12 \pm 5 (11)	0.0094

*The steady state concentration of 203-Hg accumulated by the organism relative to the concentration of the nuclide in estuarine sediment (concentration factor relative to estuarine sediment).

⁻The concentration of 203-Hg in the feces of the worm was 2487 ppb (dry weight).

[#]The concentration of 203-Hg in the feces of the fish was 3325 ppb (dry weight).

[#]The organic content of the sediment in this experiment was 11.5% weight lost after ashing.

only 0.26% and 0.15%, respectively, of the mercury concentration in their food. A 50% reduction in the organic content of the labelled sediment resulted in approximately a 50% reduction in ^{203}Hg uptake (Table 5). Table 5 also indicates that high concentrations of ^{203}Hg occurred in the feces of N. succinea and M. latipinnia, indicating the animals were ingesting the label.

The relationship between steady state body concentrations of ^{203}Hg achieved through ingestion and weight of individual worms and shrimp is shown in Figure 5b. These data also indicated that the availability of ingested mercury was different from that observed for uptake of dissolved ^{203}Hg . There was insignificant ($p > 0.05$) correlation to a power function for either the worm ($r = 0.03$) or the shrimp ($r = -0.25$); nor was there obvious correlation with any other type of function.

The toxicity of particulate-bound mercury to detritus feeders was tested by exposing a group (15) of small (10 mg) N. succinea to sediment contaminated with 5000 ppb Hg^{++} and another group (15) to uncontaminated sediment in a laboratory experiment. The mortality over a period of two months was identical for both conditions (16.7%). As shown in Table 6, the growth of the worms varied as a function of worm density, but was not significantly different between mercury contaminated and uncontaminated sediment. As a further test five jugs of sediment from Ala Wai Canal were covered with 2 - 3 cm of Hg^{++} contaminated sediment (5000 ppb) and implanted in the canal with five jugs of uncontaminated sediment. Jugs with contaminated and uncontaminated sediment were implanted in pairs. Each jug contained worms

Table 6

The growth rate of N. succinea after 2 months exposure in the laboratory to sediment contaminated with 5000 ppb Hg^{++} compared to growth rate of worms over a 2 month period in uncontaminated sediment. All worms initially weighed less than 10 mg.

Density (worms/vessel)	Growth rate in contaminated sediment (mg/month)	Growth rate in uncontaminated sediment (mg/month)
3	8.2	8.7
5	5.9	5.6
10	1.4	2.8

from a 200 cm² quadrat in the canal. Table 7 shows that after two months in the estuary the density, biomass and the size frequency distribution of the populations in the jugs were not significantly different between the two treatments ($p > 0.40$, t-test for paired data).

Environmental Factors Affecting Accumulation

Estuaries such as Ala Wai Canal are characterized by large fluctuations in salinity, a relatively large amount of dissolved organic matter in the water, and a seasonal influx of sediment of terrestrial origin. All three of these factors could potentially affect the accumulation of mercury by estuarine organisms.

Salinity

To study the effect of salinity on the uptake of dissolved 203-Hg by the worm and the shrimp the animals were first acclimated to reduced salinity for four hours. Some weight gain resulting from a net influx of water over this time was observed at salinities of 16‰ and below. Any dilution of the concentration of 203-Hg in the worm by such a weight gain was subtracted out using data presented by Ogelsby (1965) for net water influx into N. succinea after four hour exposures to various salinities. It can be seen in Figure 9 that salinities below 16‰ had no effect on the rate of 203-Hg uptake. Salinities as low as 6.4‰ had no significant effect on the rate of uptake of 203-Hg by shrimp acclimated for four hours. Similar results were obtained when worms and shrimp were acclimated for three days. The steady state 203-Hg concentrations did not differ in salinities of

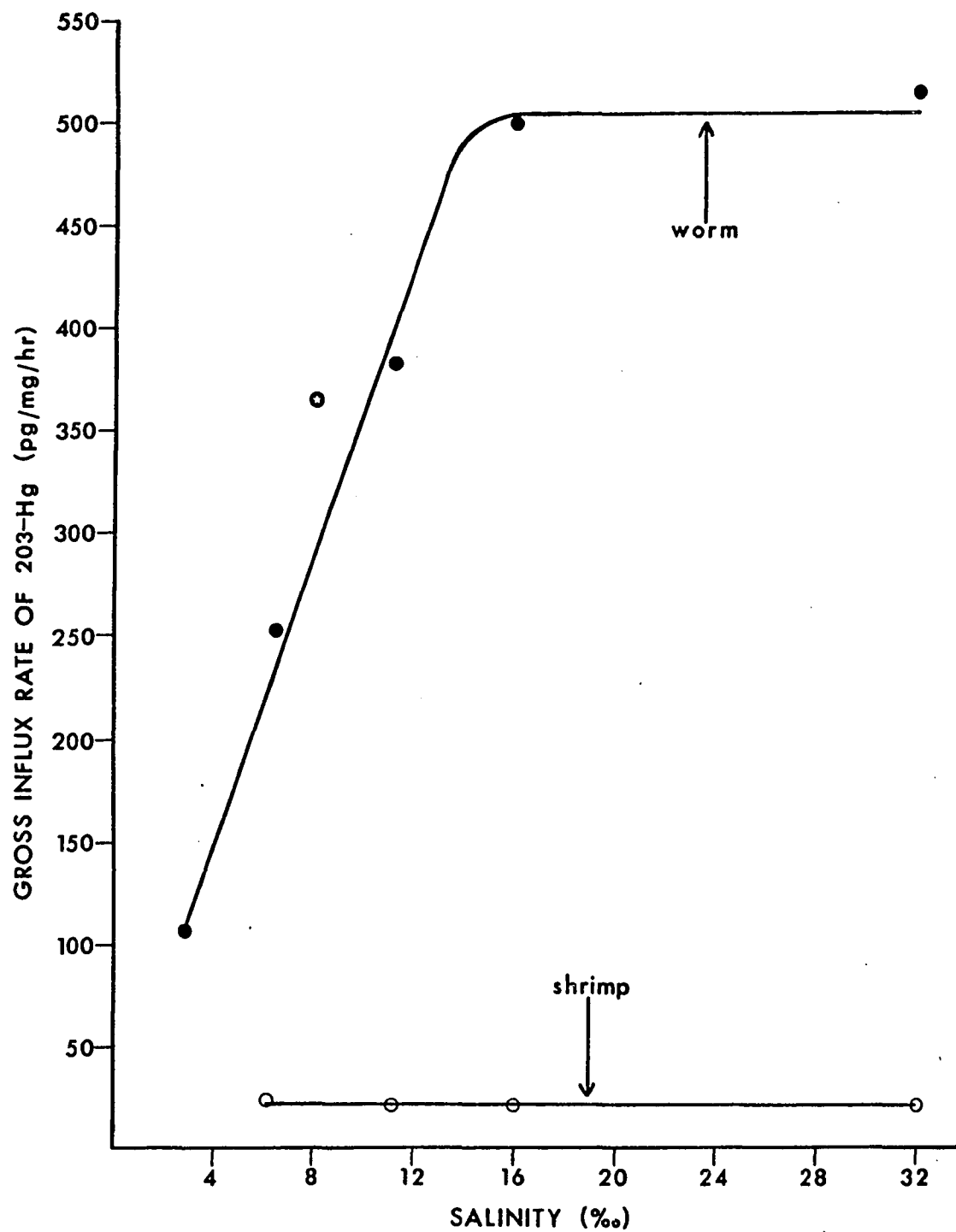
Table 7

A comparison of density, biomass, and mean weight between a population of N. succinea in jugs containing surface sediment contaminated with 5000 ppb of Hg^{++} , and a population of the worm in jugs with uncontaminated sediment

		Treatment sites (0.04 m ² quadrats)				
		2	2	3	4	5
Hg^{++} contaminated sediment	Density	50	42	23	17	25
	Biomass (mg)	2.88	1.93	1.77	1.64	1.60
	Mean weight (mg)	49	46	77	97	64

Uncon- taminated sediment	Density	38	42	32	8	23
	Biomass (mg)	1.68	2.27	1.71	0.68	1.50
	Mean weight (mg)	44	54	53	65	65

Figure 9. The rate of uptake of dissolved ^{203}Hg by N. succinea and P. debilis as a function of the salinity of the medium after animals were acclimated to a given salinity for four hours (concentration of ^{203}Hg in the medium was 1 ppb). Starred point represents the rate of uptake of ^{203}Hg by N. succinea after four days acclimation to the given salinity.



16°/oo and 32°/oo for either species. However, when worms were exposed to 203-Hg at a salinity of 8°/oo, the rate of uptake was reduced 30.8% (Fig. 9) and the steady state concentration of 203-Hg was reduced 52.2% over that observed at 32°/oo.

Salinity also affected the rate of uptake of 203-Hg by the worm from food. At a salinity of 16°/oo after three days of acclimation, the steady state 203-Hg concentration observed in N. succinea feeding on labelled detritus was reduced approximately 50% below that observed at 32°/oo.

Effect of Dissolved Organics

The effect of dissolved organics on the uptake of 203-Hg was investigated by dissolving cysteine (1 mM) and bovine serum albumin (BSA - 100 mg/l) in seawater and labelling with from 1 nM (0.2 ppb) to 25 nM (4 ppb) 203-Hg. BSA is a large protein characterized by large numbers of sulfhydryl side chains. Cysteine contains a sulfhydryl group. Vallee and Ulmer (1972) have cited the affinity of mercury for Hg-S binding when sulfur groups are present. Because there was a high ratio of organic molecules to Hg^{++} in these experiments, it was presumed that most of the 203-Hg was bound to the dissolved organic molecules. The ability of N. succinea to concentrate mercury in these experiments is shown in Table 8. It can be seen that the binding to dissolved organics reduced 203-Hg accumulation by the worm by nearly two orders of magnitude.

Figure 10 presents a comparison of mercury accumulation by N. succinea from 203-Hg labelled open ocean seawater and from Ala Wai Canal water. Salinity in both treatments was the same. Both water

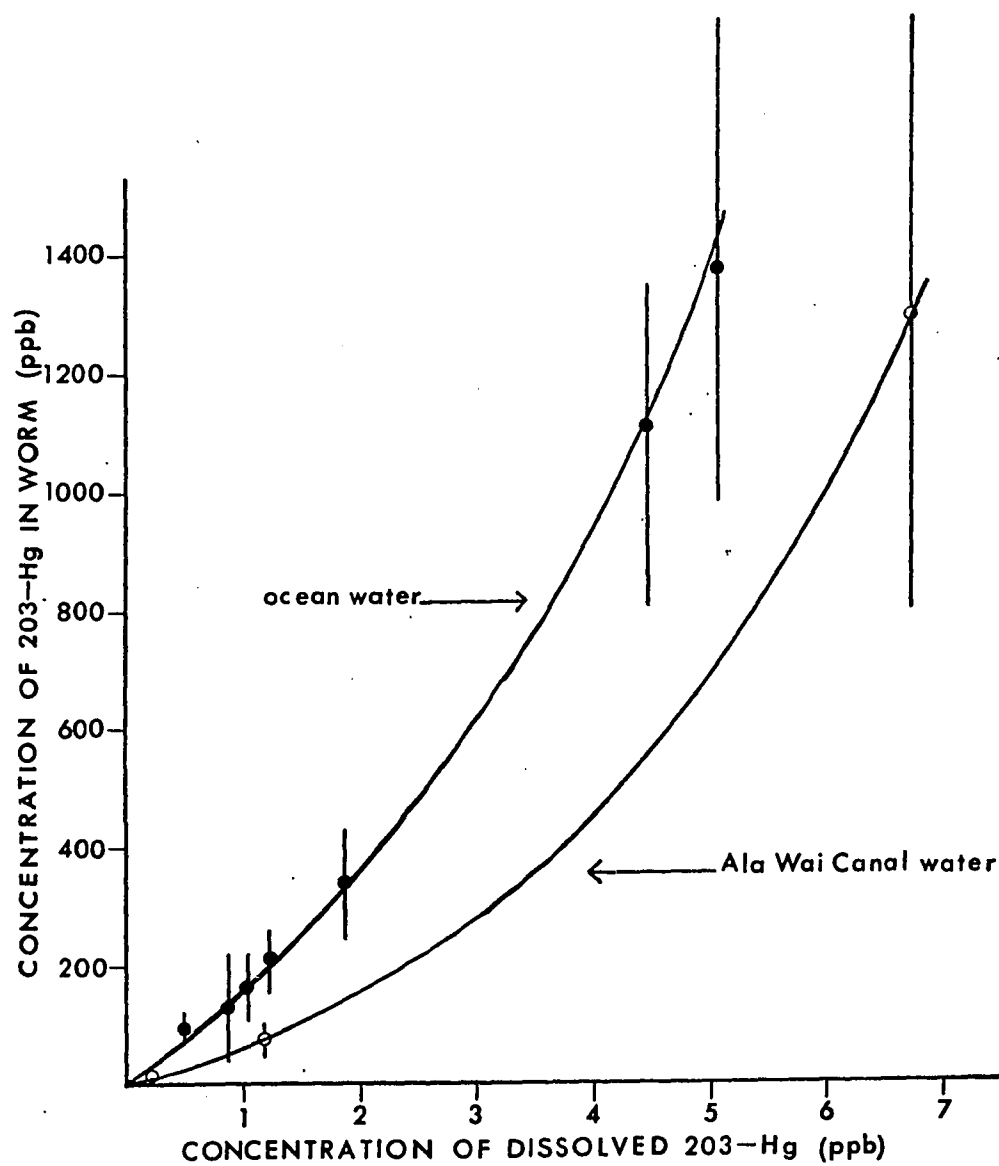
Table 8

The concentration of 203-Hg accumulated by N. succinea relative to the initial concentration of the nuclide in seawater (CF_w^*) when the worm was exposed to 203-Hg in seawater containing dissolved cysteine (1 mM) or dissolved BSA (100 mg/l), compared with the degree to which the worm concentrated 203-Hg from seawater alone

C_{wo}^* (ppb)	Cysteine - bound 203-Hg (CF_w^*)	BSA - bound 203-Hg (CF_w^*)	203-Hg in sea- water alone (CF_w^*)
0.21		7	124
0.45	4		129
1.14		5	158
2.12	4		180
4.12		4	235

* CF_w is the concentration factor of 203-Hg from seawater, C_{ss}/C_{wo} , where C_{ss} = the organismic steady state concentration of 203-Hg, and C_{wo} = the initial concentration of 203-Hg in seawater.

Figure 10. The concentration dependence of steady state ^{203}Hg concentrations in N. succinea exposed to labelled Ala Wai Canal water and labelled open ocean water. Vertical bars represent \pm one standard deviation.



samples were Millipore filtered ($0.45\ \mu$) before labelling. The canal water sample was taken in summer when productivity was high (Harris, 1972), but the influence of runoff on water quality (e.g. the presence of dissolved metals which might compete with the transport of $^{203}\text{-Hg}$) was at a minimum. It can be seen that $^{203}\text{-Hg}$ uptake by the worm from the more eutrophic canal water was considerably less than uptake from the more oligotrophic open ocean water over a range of dissolved $^{203}\text{-Hg}$ concentrations from 0.2 to 6.8 ppb.

Organic Content of Sediment

Sedimentary particles of terrestrial origin which enter an estuary often lack the adsorbed coat of organic material, microflora and microfauna which characterize estuarine sediment (Darnell, 1969). This difference between the two types of particulates could affect the way in which metals are bound to the particles, and thus affect the biological availability of the metals. Because most of the terrigenous material which enters the study area in Ala Wai Canal originates from street runoff, sediment swept from streets around the canal was labelled in seawater and used to compare $^{203}\text{-Hg}$ uptake from terrigenous sediment by detritus feeders with uptake from estuarine sediment. Only terrigenous particulates smaller than 1 mm were used in these experiments. The street sediment was washed twice in seawater before being labelled.

The worms and shrimp exposed to terrigenous material labelled with $^{203}\text{-Hg}$ reached steady state concentrations of the nuclide in approximately 6 days ($t_{1/2} = 2.84$ days) and 9 days ($t_{1/2} = 3.59$ days) respectively (Fig. 11). Table 9 shows that the concentration of $^{203}\text{-Hg}$

Figure 11. The uptake of ^{203}Hg by N. succinea and P. debilis provided labelled terrigenous sediment as food as a function of time.

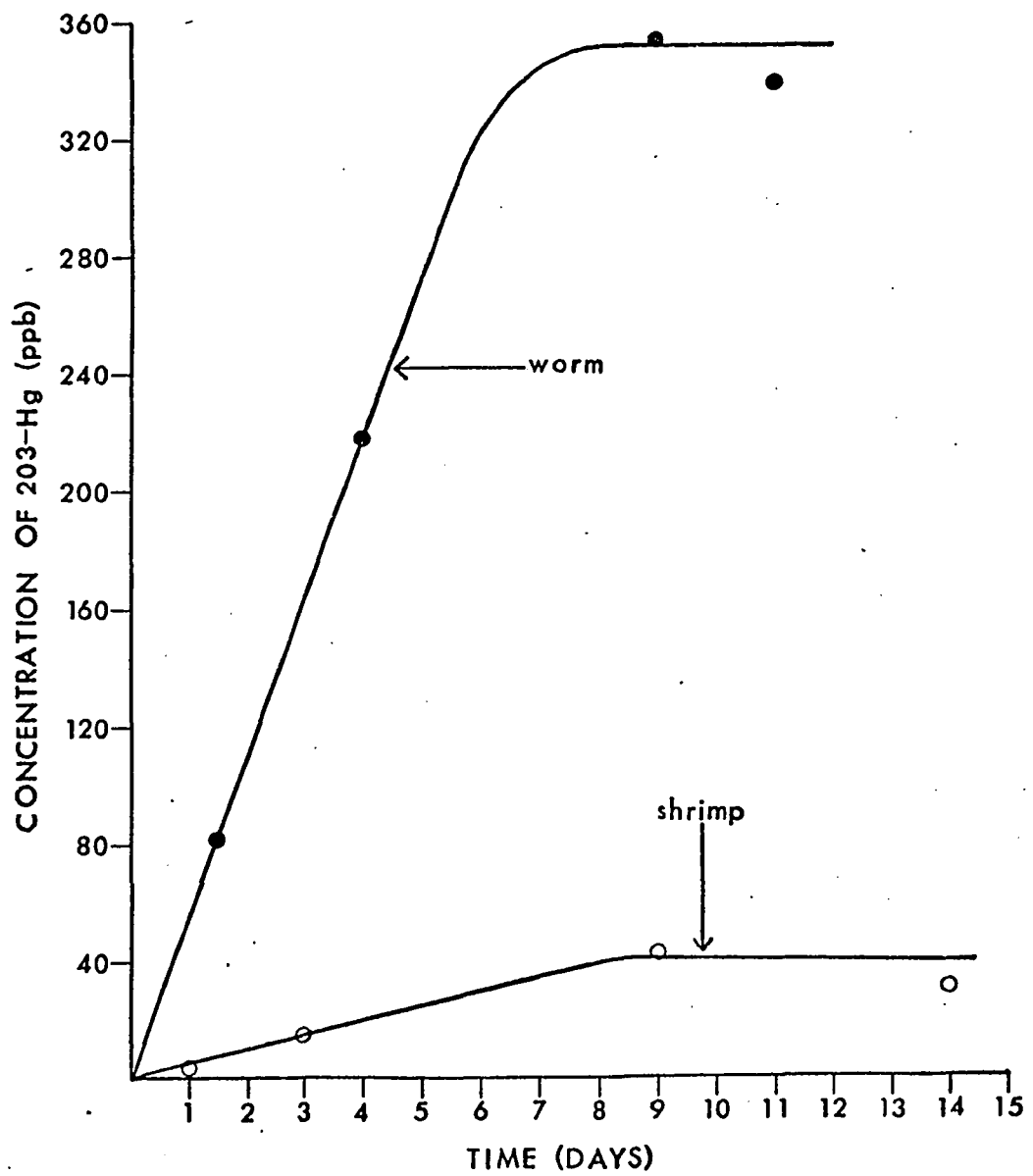


Table 9

The steady state whole body concentrations of ^{203}Hg in the worm, *N. succinea*, and the shrimp, *P. debilis*, after feeding on ^{203}Hg labelled sediment of terrestrial origin. Numbers in parentheses indicate the number of samples from which each value was determined.

Organism	Concentration of ^{203}Hg \pm one std. dev. (ppb)			CF_{ts}^-	$\text{CF}_{\text{ts}}/\text{CF}_{\text{es}}^\#$
	Sediment	Organism	IW_O^*		
Worm	1625 ± 628 (5)	810 ± 232 (9)	0.380	0.498	33.2
Worm	391 ± 141 (3)	343 ± 90 (13)	0.374	0.878	87.8
Shrimp	321 ± 93 (2)	35 ± 35 (11)		0.109	44.0

* IW_O = the concentration of ^{203}Hg in the interstitial water 4 days after the experiment was begun.

$^- \text{CF}_{\text{ts}}$ = the steady state concentration of ^{203}Hg accumulated by the organism relative to the concentration of the nuclide in terrigenous sediment (concentration factor relative to terrigenous sediment).

$^\# \text{CF}_{\text{ts}}/\text{CF}_{\text{es}}$ = a comparison of the concentration factor relative to terrestrial sediment and the concentration factor relative to estuarine sediment.

in the worm at steady state ranged from 0.50 to 0.88 of the concentration in the sediment. The steady state concentration of ^{203}Hg in the shrimp was 0.11 of the nuclide concentration in the sediment. These two species appeared to concentrate mercury bound to terrestrial sediment from 33 to 88 times more effectively than they concentrated mercury from estuarine sediment (comparing Table 9 and Table 5).

Table 9 also shows that the concentration of ^{203}Hg in the interstitial water in these experiments ranged around 0.4 ppb. In the water above the sediment-water interface ^{203}Hg concentrations were only 0.03 to 0.1 ppb throughout these experiments. A reproductively metamorphosed, non-feeding epitokous worm was exposed to the labelled terrestrial sediment during the first week of one experiment. This individual took up ^{203}Hg at a rate that was 55.2% of that observed in feeding worms. Two epitokes were exposed to the labelled sediment during the fourth week of a similar experiment. The steady state concentration of ^{203}Hg attained by these individuals averaged 56.7% of that observed in feeding worms. All three epitokes lived in the interstitium during their periods of exposure to ^{203}Hg and presumably accumulated the nuclide only from the interstitial water.

As the terrigenous sediment "aged" in seawater, the availability of mercury to the polychaetes decreased. Table 10 shows that the rate of uptake of ^{203}Hg by the worm (one-day exposures) decreased exponentially between the beginning of one experiment and an exposure run 40 days after the sediment and seawater were initially mixed. It is shown in Table 11 that the steady state mercury concentrations in worms

Table 10

The change in the rate of uptake of ^{203}Hg by the worm, N. succinea, from sediment of terrestrial origin as a function of the age of the sediment/seawater mixture

Age of sediment/seawater mixture (days)	Rate of uptake of ^{203}Hg by feeding worms (pg/mg/day)	Percent decrease in uptake rate
1	161 ± 100	
9	70 ± 20	56.5
17	25 ± 15	84.5
40	22 ± 4	86.3

Table 11

The steady state concentration of ^{203}Hg in N. succinea feeding on sediment of terrestrial origin, as a function of the age of the sediment/seawater mixture and the concentration of ^{203}Hg in interstitial water

Experiment	Age of sediment/seawater mixture (weeks)	C_{ss}^* (ppp)	IW^- (ppo)	Percent decrease in C_{ss}
II	1	810	0.442	
II	6	111	0.102	86.3

III	1	353	0.374	
III	4	61	0.107	83.7

* C_{ss} = the steady state concentration of ^{203}Hg in the worm.

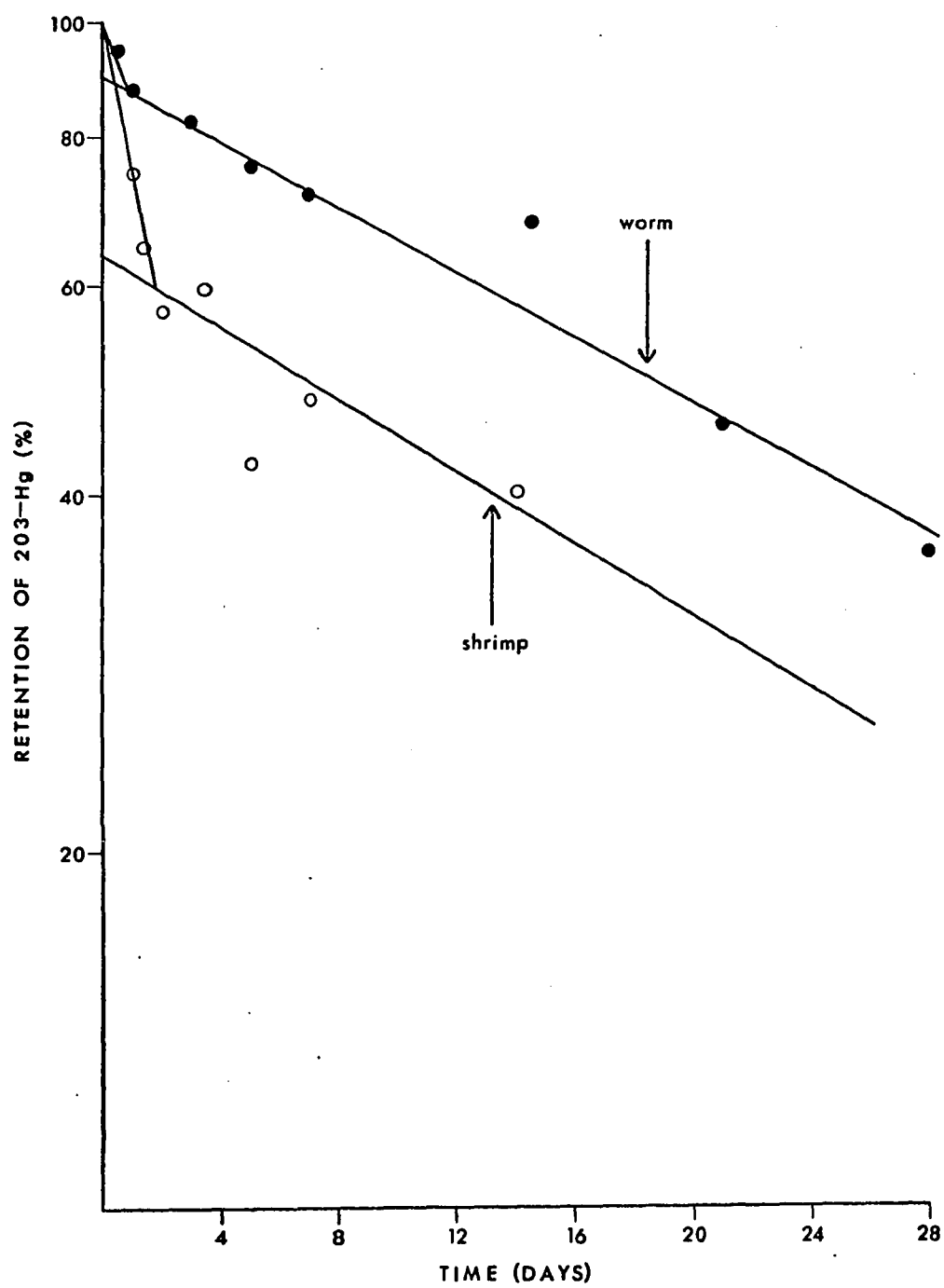
^-IW = the concentration of ^{203}Hg in the interstitial water.

also decreased in two different exposures when terrigenous sediments aged in seawater were used. The concentration of 203-Hg in the sediment did not change significantly over these time periods; however, the concentrations of 203-Hg in the interstitial water decreased (Table 11).

The Rate of Loss of 203-Hg

Figure 12 describes the rate of loss of 203-Hg from feeding polychaetes and feeding shrimp as a function of time. Efflux from any biochemical compartment within an organism occurs exponentially (Neame and Richards, 1972). When the loss of 203-Hg is plotted on semi-logarithmic graph paper, compartments losing the label at different rates can be observed as straight lines with different slopes. Efflux appeared to be biphasic for both the worm and the shrimp. During biphasic efflux in the early part of the experiment, the rate of loss is rapid. The slope of the curve during this period represents loss from both compartments. To determine the rate constant describing this fast phase of loss (k_f) the contribution of the slowly exchanging component to this process is subtracted out by plotting the difference between the observed steeper phase of the efflux curve and a line extended to the ordinate along the slope of the slower phase of the efflux function (Riggs, 1963). The resultant function represents the short-lived pool of 203-Hg in the animal and its slope is k_f . Because the short-lived pool of the ion empties rapidly (in equation 5 the first term, $AC_0e^{-k_ft}$ goes to zero), the latter, less steep phase of the curve represents loss from only the more slowly exchanging long-lived pool. The rate constant of this component of efflux can be

Figure 12. The loss of ^{203}Hg from P. debilis and N. succinea as a function of time.



calculated directly from the slope of less steep phase of the curve (Riggs, 1963). Loss of 203-Hg from an organism then can be mathematically described as the sum of two mono-exponential processes by the equation

$$C_t = AC_0 \cdot e^{-k_f t} + BC_0 \cdot e^{-k_s t} \quad (5)$$

where k_f = the rate constant of the fast component of loss, after subtraction of the contribution of the slow component;
 k_s = the rate constant describing the slow component of loss;
 A and B = constants representing the proportion of the total body burden of mercury of which each compartment is composed;
 C_0 = the initial concentration of 203-Hg in the organism, i.e. the concentration of 203-Hg in the animal when efflux began.
 C_t = the concentration of 203-Hg in the animal at any time t.

To find the half-times of a component of loss requires that

$$\frac{C_t}{C_0} = 0.5 = e^{-kt} \quad (6)$$

Hence

$$\ln (0.5) = -k_a t \quad (7)$$

$$\text{or} \quad \ln (2) = + k_a t \quad (8)$$

$$\text{or} \quad t_{1/2} = \frac{0.693}{k_a} \quad (9)$$

The curves shown in Figure 12 were fit by log regression using the method suggested by Riggs (1963). The fit of the slow component and the fast component of efflux to the lines shown in Figure 12 was significant for both the worm and the shrimp ($p < 0.05$ in both cases),

suggesting the data did fit the two compartment loss model. In the worm loss of 203-Hg was shown to fit the equation

$$C_t = 0.084 e^{-3.44t} + 0.92 e^{-0.031t} \quad (10)$$

Loss from the shrimp best fit the equation

$$C_t = 0.39 e^{-1.49t} + 0.63 e^{-0.044t} \quad (11)$$

The biological half-time of each component of the efflux process and the proportion of the whole body load of 203-Hg lost by way of that component are shown in Table 12.

Total Mercury Dynamics in Ala Wai Canal

Sediment

The concentrations of total mercury found in sediment from various marine and estuarine ecosystems around Hawaii are presented in Table 13. It can be seen that total mercury in sediments from Ala Wai Canal, both in 1971 (Siegel, 1973) and in 1973, were significantly higher than were found in other Hawaiian aquatic environments. This suggested that there was some localized input of mercury into the canal. The range of mercury concentrations observed in monthly samples of sediment collected from Ala Wai Canal between February, 1973, and February, 1974, are presented in Table 14. The mean concentration of total mercury in the monthly samples are plotted as a function of time in Figure 13. Three to five sediment samples were collected at each sampling date. It was found that total mercury values were quite variable both within and between sampling periods over this interval. The minimum mercury concentration observed was

Table 12

The rate constants and biological half-lives of the components of loss of ^{203}Hg for N. succinea and P. debilis as observed during efflux experiments compared with those constants observed during accumulation experiments

Species	Accumulation experiment					Efflux experiment				
	Short-lived pool			Long-lived pool		Short-lived pool			Long-lived pool	
	k_a (day ⁻¹)	$t_{1/2}$ (days)	% of total	k_a (day ⁻¹)	$t_{1/2}$ (days)	k_f (day ⁻¹)	$t_{1/2}$ (days)	% of total	k_s (day ⁻¹)	$t_{1/2}$ (days)
Worm	103.4	0.0067	25.0	2.1	0.33	3.47	0.20	8.4	0.031	22.4
Shrimp			0	0.91	0.76	1.50	0.46	36.5	0.044	15.9

Table 13

Total mercury concentrations in sediment in several Hawaiian aquatic ecosystems. Numbers in parentheses indicate the number of samples from which means were calculated.

Location	Type of system	Total Hg in sediment (ppb dry wt.)
Kahana Bay, Oahu*	Marine (pristine)	72 \pm 65 (12)
Kalihiwai Bay, Kauai*	Estuarine (agricultural discharge)	125 \pm 75 (11)
North Kauai*	Marine (agricultural discharge)	94 \pm 83 (27)
Pearl Harbor, Oahu*	Estuarine (harbor)	194 \pm 102 (7)
Waikiki, Oahu*	Marine (urban discharge)	128 \pm 234 (22)
Ala Wai Canal, Oahu ⁻	Estuarine (urban runoff)	386 \pm 383 (30)
Ala Wai Canal, Oahu [#]	Estuarine (urban runoff)	334 \pm 108 (31)

*Water Resources Research Center. 1972. The Quality of Coastal Waters; First Annual Progress Report. Tech. Rept. No. 60, Sea Grant Program No. UNIHI SEAGRANT-72-01, University of Hawaii.

⁻Siegel, S. M. 1973. Mercury: Aspects of its ecology and environmental toxicity.

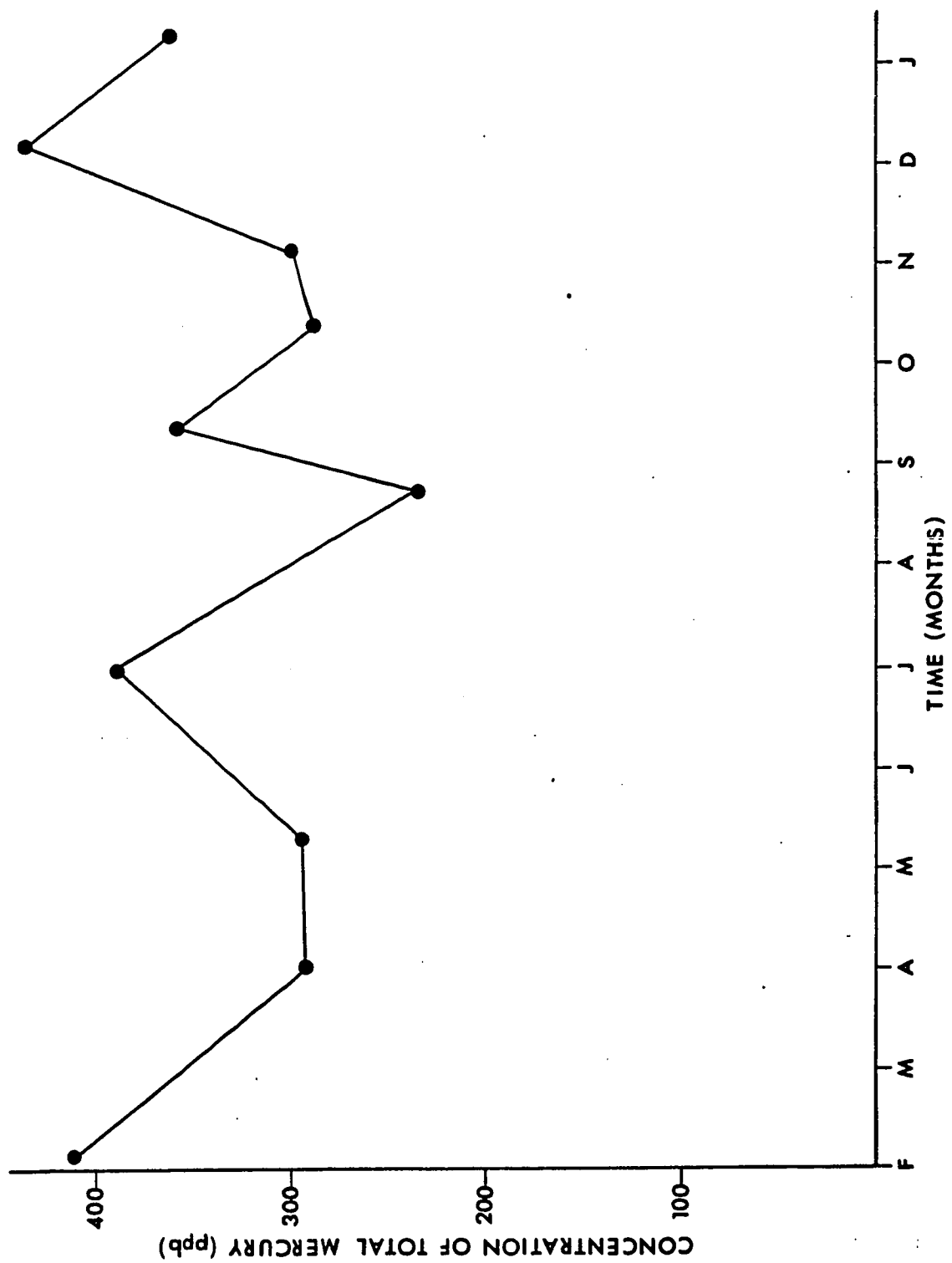
[#]This study.

Table 14

Total mercury concentration in monthly sediment samples collected from Ala Wai Canal between February, 1974 and January, 1974. Vertical bar represents generally drier months of the year.

Date collected	Total mercury concentration (ppb dry wt.)	
	mean	range
Feb. 4, 1973	410	371-449
Apr. 2, 1973	291	222-380
May 10, 1973	294	208-408
July 1, 1973	389	231-682
Aug. 22, 1973	234	219-263
Sept. 11, 1973	356	303-433
Oct. 12, 1973	288	248-345
Nov. 4, 1973	298	223-339
Dec. 6, 1973	436	384-485
Jan. 10, 1974	362	280-451

Figure 13. Total mercury concentrations in sediment from Ala Wai Canal over 1973-74. Each point represents the mean of at least three samples.



208 ppb while the maximum was 682 ppb. There was no readily obvious temporal pattern of variation in total mercury concentrations in sediment.

Detritus Feeders

The total mercury concentrations (ppb - wet weight) observed over the 1973-74 sampling period in the two detritus feeders N. succinea and P. debilis are shown in Figure 14. There was an obvious trend in the temporal variability in mercury concentrations measured in these two organisms. During winter months (December - May) rainstorms in the Ala Wai watershed generally occurs more frequently and monthly precipitation is of greater magnitude than during the summer. In 1973, little rain fell between July 10 and November 10 in the watershed of the canal (see lower portion of Fig. 16). During this period total mercury concentrations in the shrimp and the worm were considerably less than concentrations observed in other months of the year (Fig. 14). Likewise, during months when rainfall was more frequent and of greater intensity, total mercury concentrations in the two species were elevated (compare Fig. 14 with the lower portions of Figs. 15 and 16). Thus total mercury concentrations in the worm and the shrimp appeared to bear some relationship to the occurrence of rainfall.

A Model of Mercury Dynamics in Ala Wai Canal

The hypothesis was made then, that if organismic mercury concentrations in Ala Wai Canal were following some function of rainfall those values should show some short term temporal variability. The

Figure 14. Total mercury concentration in *P. debilis* and *N. succinea* periodically collected from Ala Wai Canal through 1973-74 (mercury values represent wet weight concentrations).

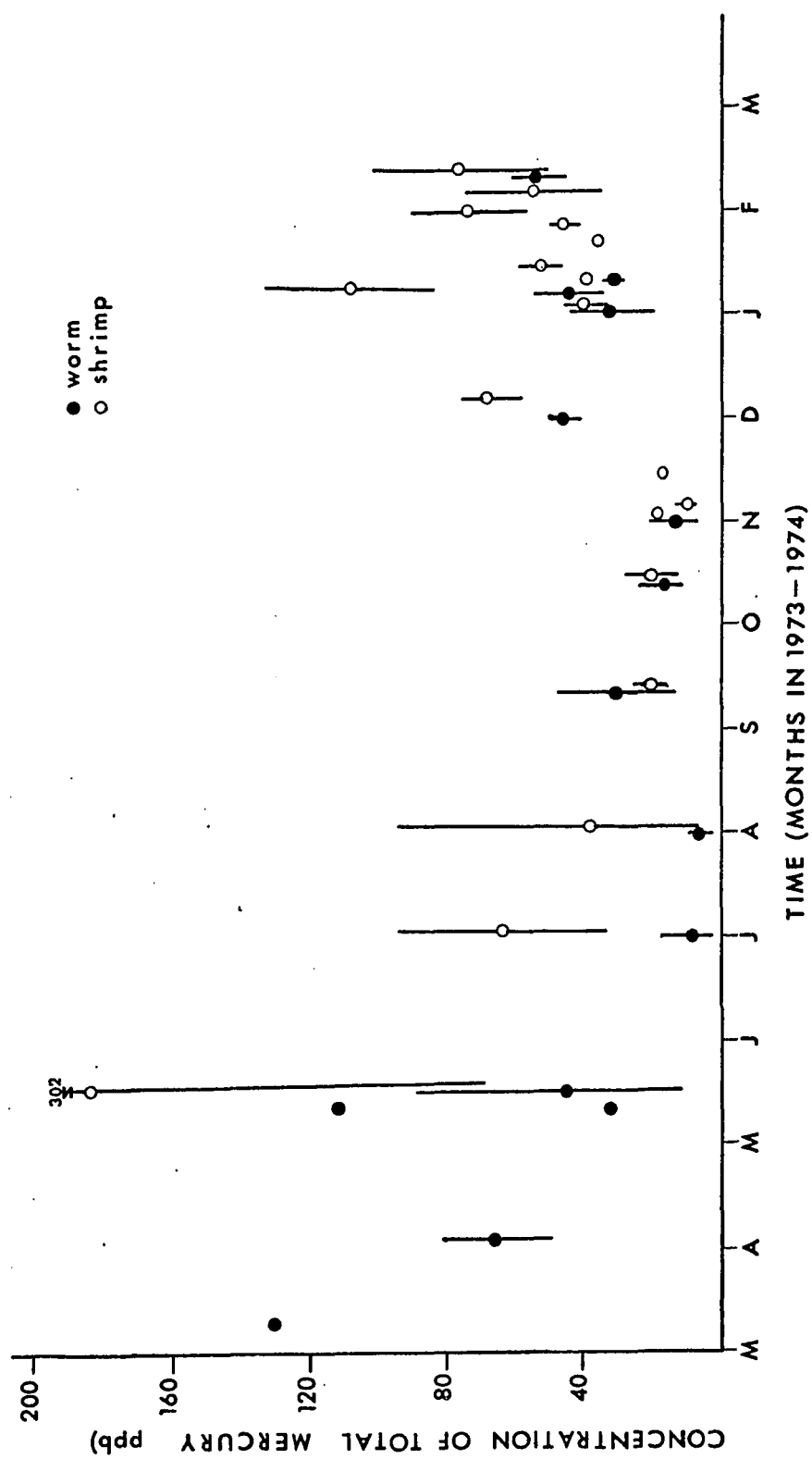


Figure 15. Mercury concentrations in P. debilis in January-February, 1974, as observed at nine collecting dates, and calculated from the model of mercury accumulation and retention in the shrimp. Vertical lines in the figure represent the range of observed total mercury values. Lower histogram gives daily rainfall in Manoa and Waikiki watersheds of Ala Wai Canal.

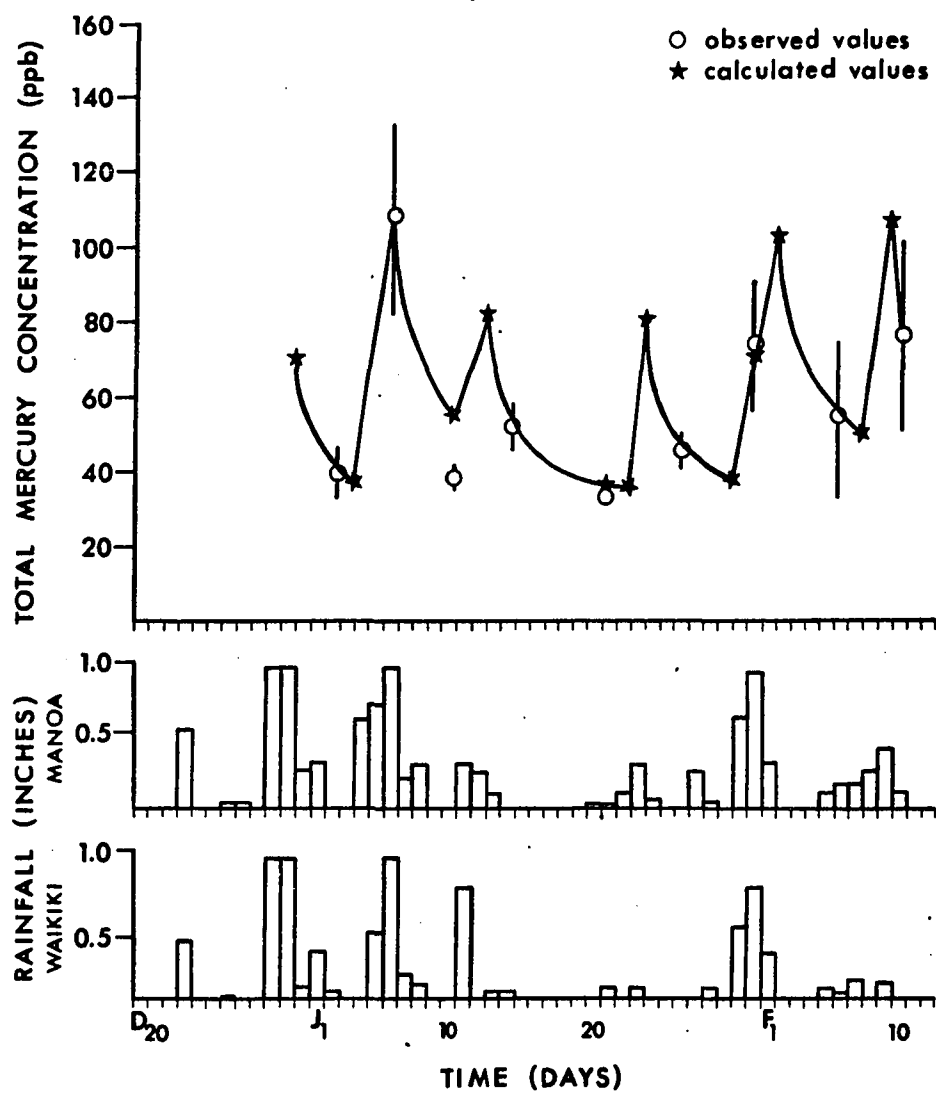
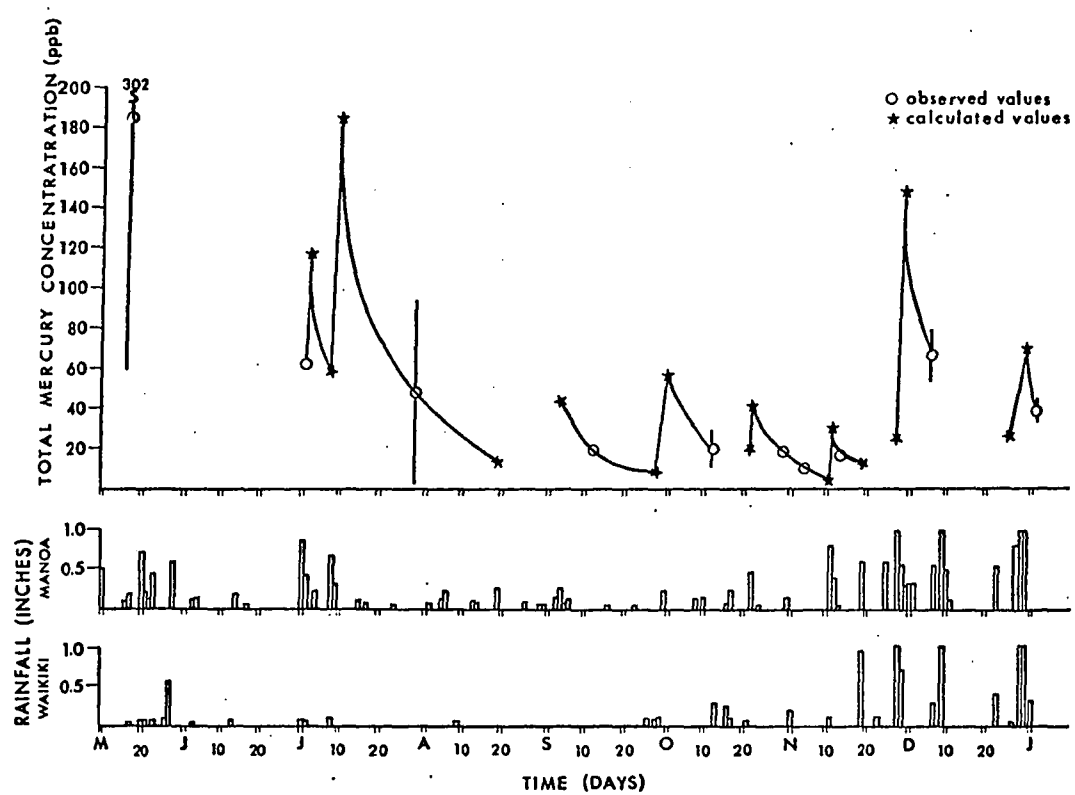


Figure 16. Mercury concentrations in P. debilis in 1973 as observed at ten collecting dates, and calculated from the model of mercury accumulation and retention in the shrimp. Vertical lines represent the range of observed total mercury values. Lower histogram gives daily rainfall in the Manoa and Waikiki watersheds of Ala Wai Canal.



determination of the characteristics of any such variability was made by collecting and analyzing shrimp for total mercury at nine sampling times between January 2 and February 10, 1974. Samples of N. succinea were also collected four times during this interval. The mercury concentrations in both animals for this time interval are shown in Figures 14 and 15. It can be seen that total mercury concentrations in the detritus feeders varied noticeably over the January-February sampling period. There was indication of some correlation of higher biotic mercury concentrations with the occurrence of rainfall.

It was further hypothesized that fluctuations in biotic mercury concentrations indicated that the biological availability of mercury in Ala Wai Canal fluctuated with time. In such an instance any measurement of the mercury concentration in an organism from the canal would then represent a point during either a period of net influx or a period of net efflux of mercury, rather than measurement of a steady state value. The equations describing the accumulation and loss of mercury by the shrimp were derived from the laboratory experiments previously reported. Using these equations, the mercury concentrations measured in samples collected in January-February, 1974, and the rainfall data collected from the same period, it was possible to:

a) develop a model of mercury accumulation and retention by the shrimp, P. debilis; b) determine the characteristics of any correlation between total mercury concentrations in P. debilis and rainfall or storm runoff; c) generate a curve describing mercury dynamics in P. debilis for the January-February sampling period; and d) predict the nature of mercury variability in P. debilis for 1973-74. The detailed methods

used to develop and test the resultant curves are described in Appendix A.

The model described in Appendix A was used to test the hypothesis that the variability of total biotic mercury concentrations in the shrimp could be explained by the discharge into Ala Wai Canal of a pulse of biologically available mercury, followed by the subsequent flushing of this mercury from the canal. It was further hypothesized that any pulse of mercury had some association with rainfall. The excellent fit between calculated and observed data in two different tests of these hypotheses suggested that the hypotheses approached reality (see Appendix A for more detail). The same hypotheses were then used to calculate the curves describing the dynamics of total mercury concentrations in the shrimp over the entire 1973-74 sampling period (Figs. 15 and 16). The curves shown in Figures 15 and 16 were calculated from total mercury concentrations observed in the shrimp over this period using rainfall data shown in the lower portion of the figures. The calculated curves showed that the correlation between mercury in the shrimp and rainfall was much stronger than was obvious from field data alone. These figures show that higher mercury concentrations appeared to occur in shrimp after heavier and longer storms than after shorter or less intense storms. In two instances mercury concentrations were observed in the shrimp at the termination of rainstorms (May 16 and January 6). These peak concentrations of mercury were similar to maximum mercury concentrations predicted after rainstorms of similar intensity which occurred during the same time of year (Figs. 15 and 16).

The relationship between the magnitude of maximum mercury concentrations in P. debilis observed or predicted after each rain-storm, and the sum of rainfall occurring in the Waikiki and Manoa watersheds of Ala Wai Canal for three days prior to peak biotic mercury concentrations is shown in Figure 17. The variation between data points is large in this figure (probably due to the influence of other environmental parameters; see discussion) but a relatively obvious asymptotic relationship appeared to exist between the two variables.

Trophic Transfer of Mercury to an Estuarine Predator

To fully understand the mechanisms of mercury flux through a simple detritus-based food chain it is necessary to know to what degree detritus feeders pass their body burden of mercury on to their predators. To study this problem 203-Hg labelled worms (N. succinea) were fed, in the laboratory, to the predaceous decapod Thalamita crenata. A control crab, fed non-radioactive worms, was maintained for two days in water in which crabs feeding on radioactive food had lived for two days. The 203-Hg content (whole body) of the control crab was less than 1% of the 203-Hg content of the crabs eating labelled worms. Thus it appeared that essentially all the 203-Hg observed in experimental T. crenata was taken up from their food.

The results of daily ingestion of the labelled worms by the crabs are shown in Figure 18. The whole body counts of 203-Hg in the crabs appeared to approach a steady state after four to seven days of exposure to the labelled food. The magnitude of the whole body steady

Figure 17. The maximum total mercury concentration in P. debilis observed or calculated after each rainfall, as a function of the sum of the precipitation observed in the Waikiki and Manoa watersheds of Ala Wai Canal over the three day period prior to each total mercury peak. (Mercury values represent wet weight concentrations.) Line was fit by eye.

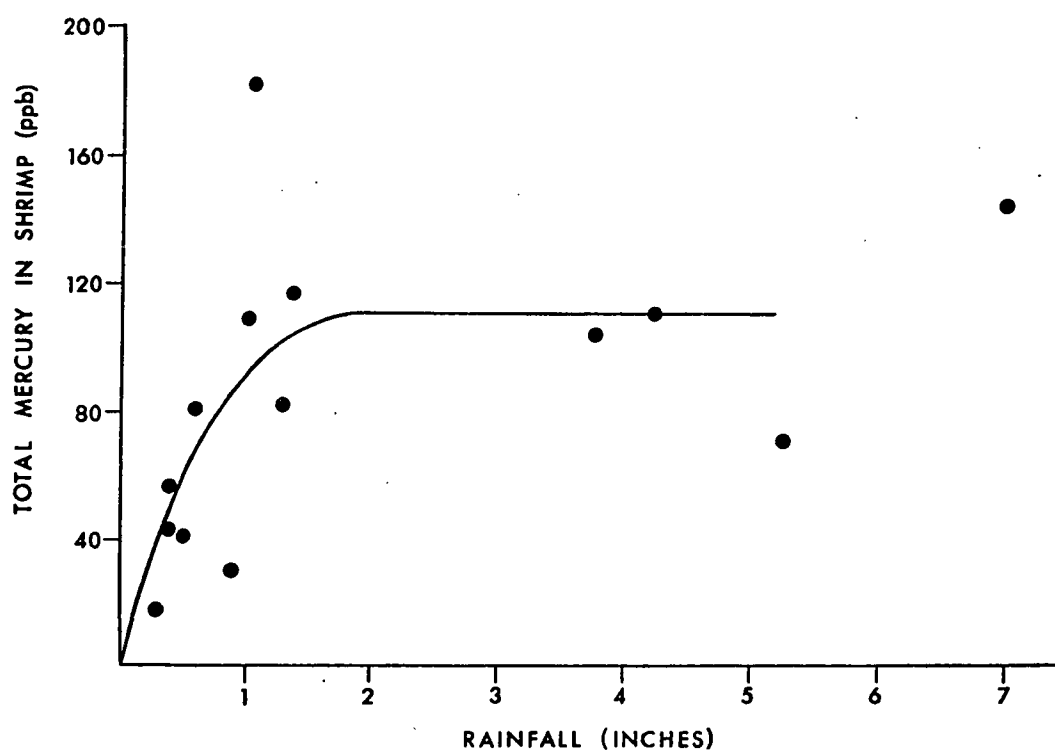
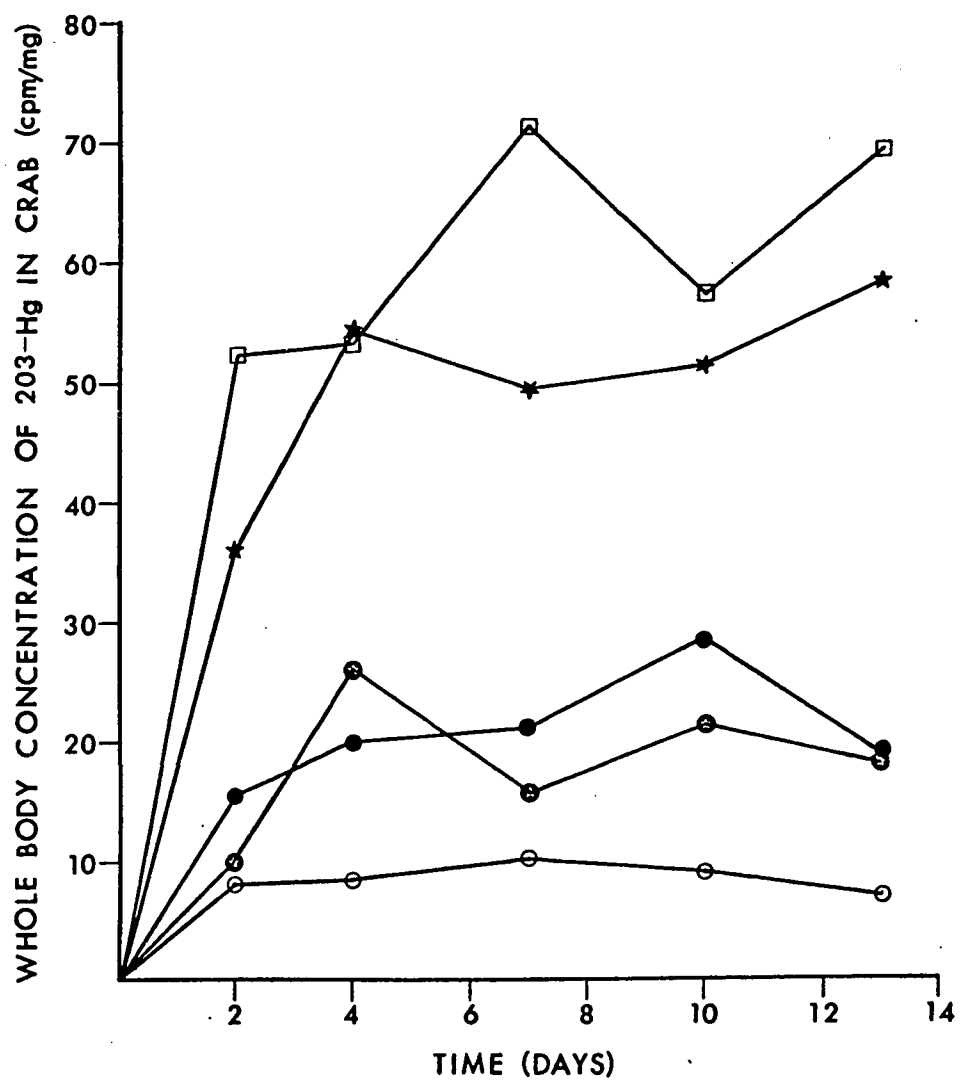


Figure 18. The accumulation of ^{203}Hg from food (N. succinea) by Thalamita crenata as a function of time. Each curve represents one animal fed 200 - 400 mg of worms per day.



state activity observed in Figure 18 varied between individuals. Since a similar mass of worms was, in general, ingested by crabs of different weight, each crab ingested a different percentage of its body weight per day, or:

$$Z = \frac{W/B}{t} \quad (12)$$

where Z = the percent of its body weight in worms that each crab ingested per day;

W = the mass of worms ingested by a crab in gms;

B = the whole body weight of the crab in gms;

and t = time in days.

Since the concentration of mercury in the worms was held constant (71 ± 20 ppb) each crab then ingested 203-Hg at a different rate in relation to its size. That is:

$$R = Z \times C_w = \frac{W/B \times C_w}{t} \quad (13)$$

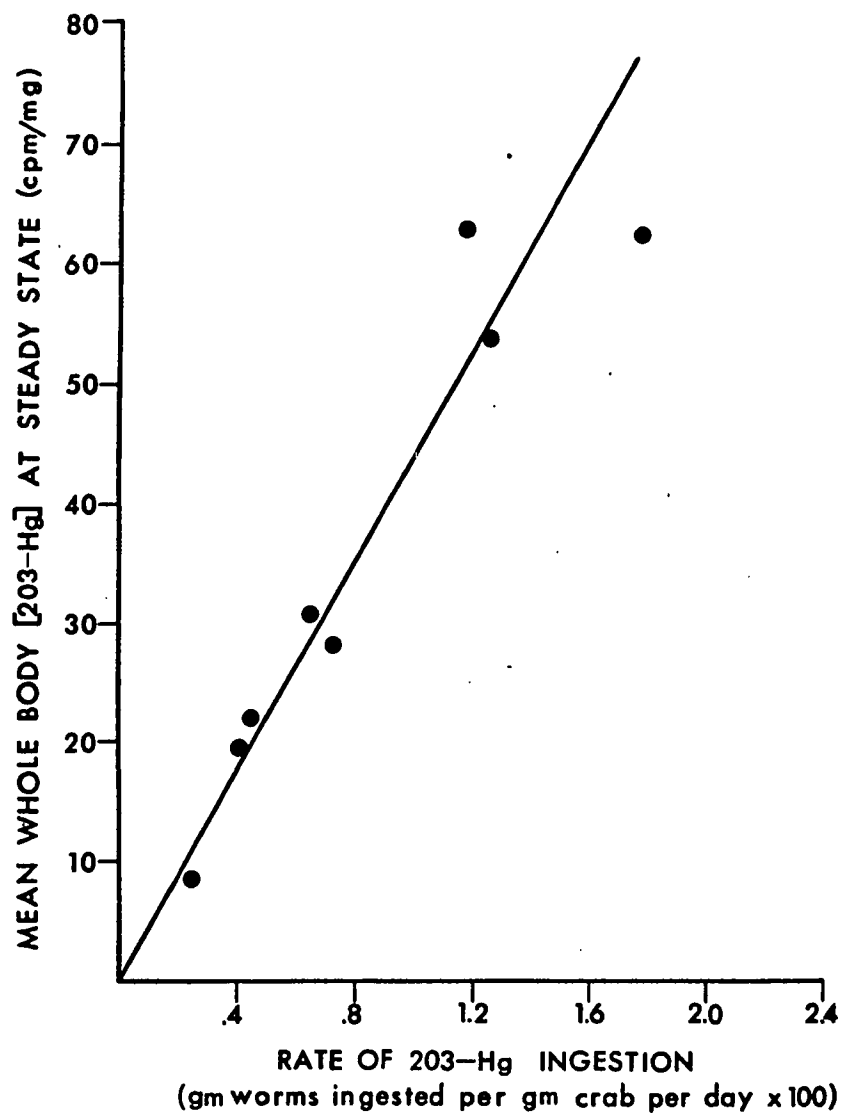
where R = the rate of 203-Hg ingestion by each crab in ng 203-Hg per gm crab per day;

and C_w = the concentration of 203-Hg in the worms in ng/mg.

The variation in the magnitude of steady state whole body activity then resulted from a difference in the rate of mercury ingestion.

The percent body weight ingested per day of labelled worms ranged from 0.26% for the largest crab to 1.44% for a smaller crab. No weight loss was observed over the 13 day study period in any crab. Figure 19 shows that the whole body steady state concentration of

Figure 19. The whole body activity of ^{203}Hg in Thalamita crenata as a function of the rate of ^{203}Hg ingestion by the crab. The concentration of ^{203}Hg in the worms was held constant at 71 ng Hg/gm worm. Each point represents ^{203}Hg activity in a single crab at steady state.



203-Hg attained by the crabs was a linear function of the rate of 203-Hg ingestion, expressed in Figure 19 as gm worm ingested per gram crab per day $\times 100$ (i.e. percent body weight ingested per day). The crabs were fed 203-Hg labelled food daily for 13 days. All crabs were killed and the organs were dissected into four groups at the end of this interval - chela muscle, body muscle, viscera, and gills. The 203-Hg concentration observed in each organ or organ group was directly related to the rate of mercury ingestion by individual crabs, as shown in Figure 20. The degree to which each organ group concentrated mercury in these experiments, relative to the concentration of the metal in the food of the crabs are presented in Table 15. These data show that the concentration of 203-Hg within the different organ groups generally followed the order viscera > gills > body muscle > chela muscle, after the 13 day exposure. Although the crabs were dissected at least 12 hours after feeding it is possible that unassimilated labelled food remained in the viscera. The contribution of 203-Hg in the unassimilated food to the concentration of 203-Hg observed in the viscera of the crabs in these experiments is unknown. The regression equations describing the different curves observed in Figure 20 are shown in Table 15. The slopes of the different curves serve as an indicator of sensitivity of an organ to any change in the ingestion rate of mercury. The sensitivity of response to a change in dose within the various organs also followed the order viscera > body muscle > chela muscle.

Total mercury concentrations were determined in crab samples collected from the sampling area in Ala Wai Canal at five different

Figure 20. The concentration of ^{203}Hg in organs of T. crenata as a function of the rate of ^{203}Hg ingestion, after feeding the crabs ^{203}Hg labelled worms for 13 days. The concentration of ^{203}Hg in the worms was 71 ng Hg/gm worm. Each point represents a single carb. a) viscera; b) gills; c) body muscle; d) chela muscle.

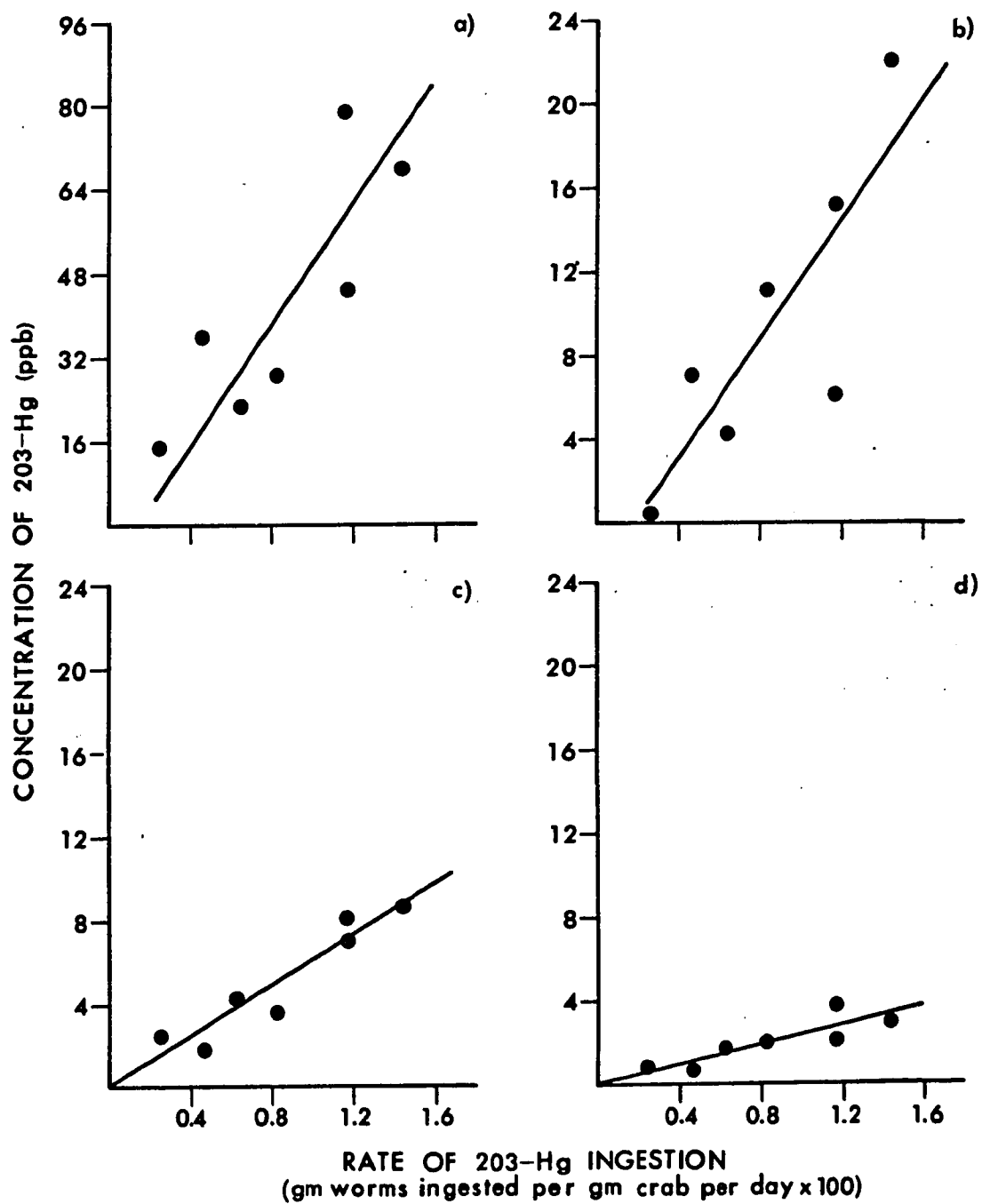


Table 15

The range of concentrations of 203-Hg observed in organs of T. crenata, relative to the concentration of the nuclide in the food of the crab, after 13 days of feeding on 203-Hg labelled worms. Minimum concentrations represent an ingestion rate of 0.24% of body weight per days or 0.17 ng 203-Hg per gram crab per day. Maximum concentrations represent an ingestion rate of 1.44% body weight per day or 1.02 ng 203-Hg per gram crab per day.

Organ	Range of C_{organ}/C_{food}^* observed	Regression equation (ppb 203-Hg)
Viscera	21.1 - 111.2	$Y = 2.5 + 46.4X$ $r = 0.84$
Gills	0.6 - 31.0	$Y = -2.7 + 14.2X$ $r = 0.83$
Body muscle	2.7 - 12.4	$Y = -0.007 + 6.1X$ $r = 0.94$
Chela muscle	0.9 - 5.3	$Y = -0.05 + 2.3X$ $r = 0.88$

*The concentration of 203-Hg in the organ of the crab relative to the concentration of the nuclide in the food of the crab.

times between August 15, 1973, and February 6, 1974. These results are shown in Table 16 and Figure 21. Mercury concentrations in body muscle increased at each sampling between August and February. Mercury concentrations were higher in chela muscle and viscera in February than in August. In all samples but one the distribution of mercury in the crabs from Ala Wai Canal was such that body muscle > chela muscle > viscera. Concentrations of total mercury in the gills of crabs from the canal were extremely variable over the sampling period.

Organic mercury concentrations were determined in body muscle from crabs collected on December 5 and February 6. These results are also shown in Figure 21. The concentrations of organic mercury in body muscle on the two dates were 14 ppb and 15 ppb respectively. This constituted 24.5% and 24.6% of the total mercury observed in December and February in this organ. The concentration of organic mercury in the chela muscle of a crab collected on December 5 was 11 ppb - 55% of the total mercury observed in that organ on that date.

The total mercury concentrations in crab muscle did not exceed the federal standard for edible materials of 500 ppb in any crab sample collected from Ala Wai Canal over the interval of this study.

Nereis succinea - Population Dynamics

To study the population dynamics of Nereis succinea, samples of the polychaete were collected at monthly intervals between December, 1972, and November, 1973. At each collection from 3 to 5 0.04 m² quadrats of sediment from an area 0.1 to 0.3 feet above mean low water

Table 16

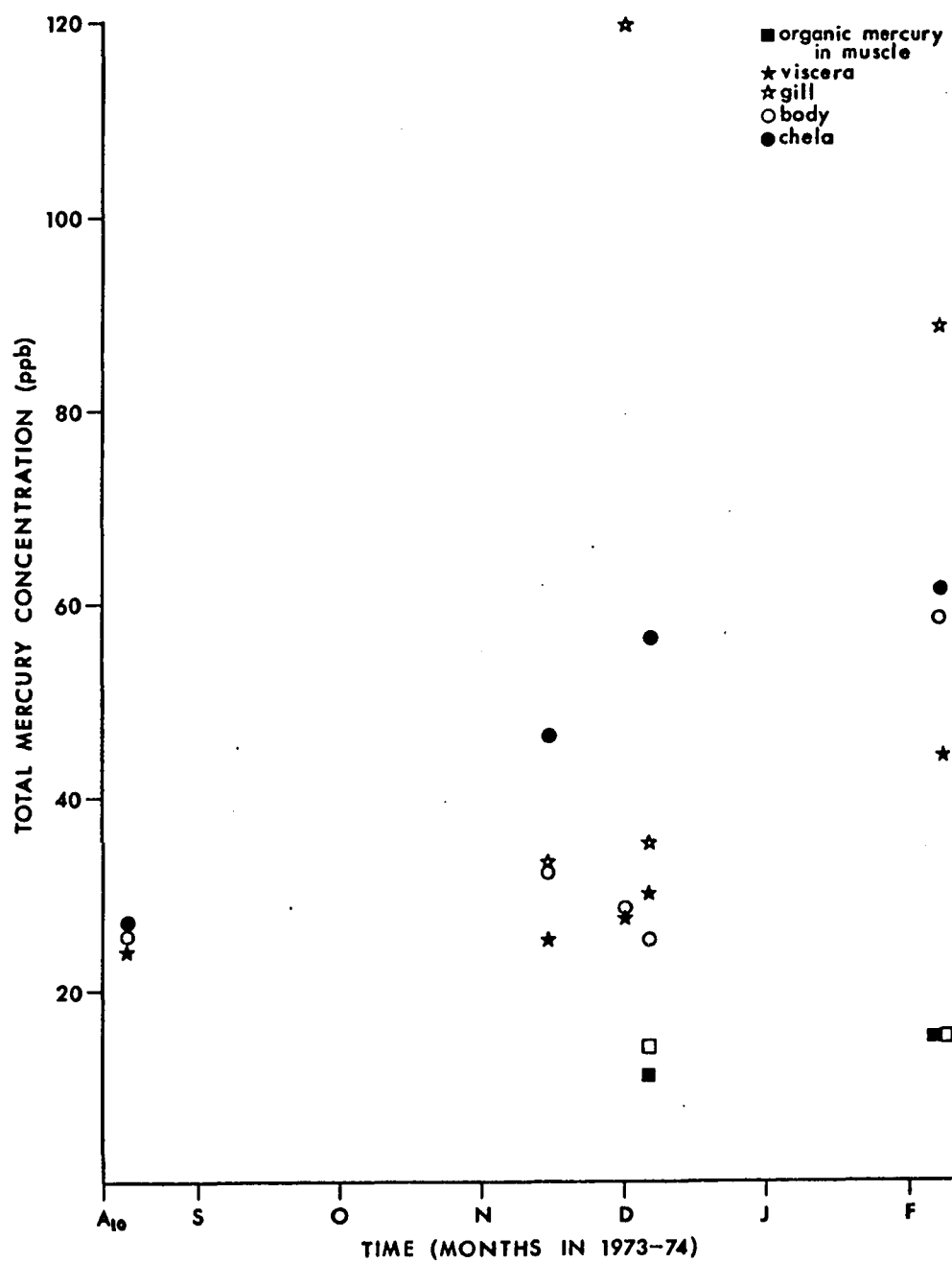
Total mercury concentrations in organs from crabs collected between August 15, 1973, and February 6, 1974 from Ala Wai Canal. Numbers in parentheses indicate the number of samples from which each mean was calculated.

Date	Mean total mercury concentration \pm one std. dev. (ppb)			
	Chela muscle	Body muscle	Viscera	Gills
Aug. 15	26 \pm 8 (8)	27 \pm 13 (5)	21 \pm 16 (6)	
Nov. 14	32 \pm 18 (3)	46 \pm 32 (3)	25 \pm 5 (4)	33*
Nov. 30		28- (1)	25 (1)	119 (1)
Dec. 5	20 \pm 7 (2)	57 \pm 37 (2)	30 \pm 8 (3)	35 \pm 5 (2)
Feb. 6	58 \pm 22 (3)	61 \pm 20 (3)	44 \pm 6 (3)	88 \pm 30 (3)

*Pooled sample of gills from three animals.

~Pooled sample of body muscle and chela muscle from one animal.

Figure 21. The total mercury and organic mercury in organs from T. crenata from Ala Wai Canal collected between August 15, 1974 and February 6, 1974.



were sieved using 1 mm mesh. Worms from each quadrat were counted and weighed individually to determine monthly changes in density, biomass and size-frequency distribution. Population dynamics were studied in two microhabitats from within the study area. One habitat, at the periphery of the study site was characterized by highly organic sediment (24.6% weight lost after ashing) and composed of mostly silt. The second site, an island that emerged at low tide, had sediment of lower organic content (11.6% weight lost after ashing) and generally larger particle size.

Diagrams of the size-frequency distribution observed within populations of N. succinea collected from the two different microhabitats within the study area in Ala Wai Canal are shown in Figures 22 and 23. The density, biomass, and mean weight of the worms from the microhabitats observed over the same time interval are listed in Table 17. The worms seemed to have two reproductive peaks in the year. In both November-December and May-June smaller individuals (< 40 mg) dominated the population and densities were highest. For the next four months, after each reproductive period, density declined, and the mean weight of the individuals in the population increased as the cohort grew. Large changes in biomass were not recorded over the period, implying that the growth rate (in gm/day) nearly equaled the rate of loss from the population (in gm/day - Table 17).

The predation rate on N. succinea was determined by implanting 10 one-gallon polyethylene jugs with an upper surface area of 200 cm² in Ala Wai Canal at -0.2 feet MLLW. A 200 cm² (surface area) hole was dug at low tide. A jug was placed in the hole and sediment from

Figure 22. Size-frequency distribution of N. succinea collected from 0.04 m² quadrats in Ala Wai Canal over 1972-73. Samples were collected from the island microhabitat within the study area.

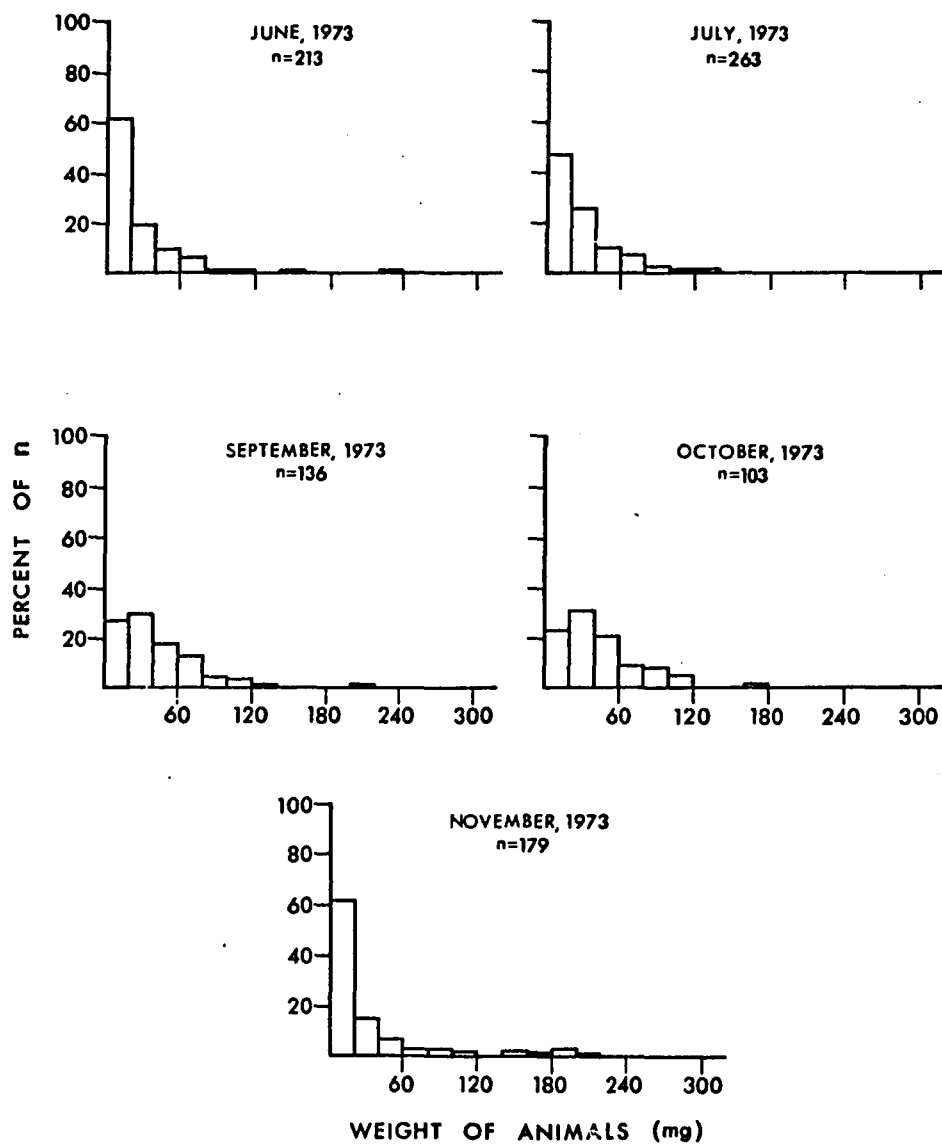


Figure 23. Size-frequency distribution of N. succinea collected from 0.04 m² quadrats in Ala Wai Canal over 1972-73. Samples were collected from the microhabitat at the periphery of the study site.

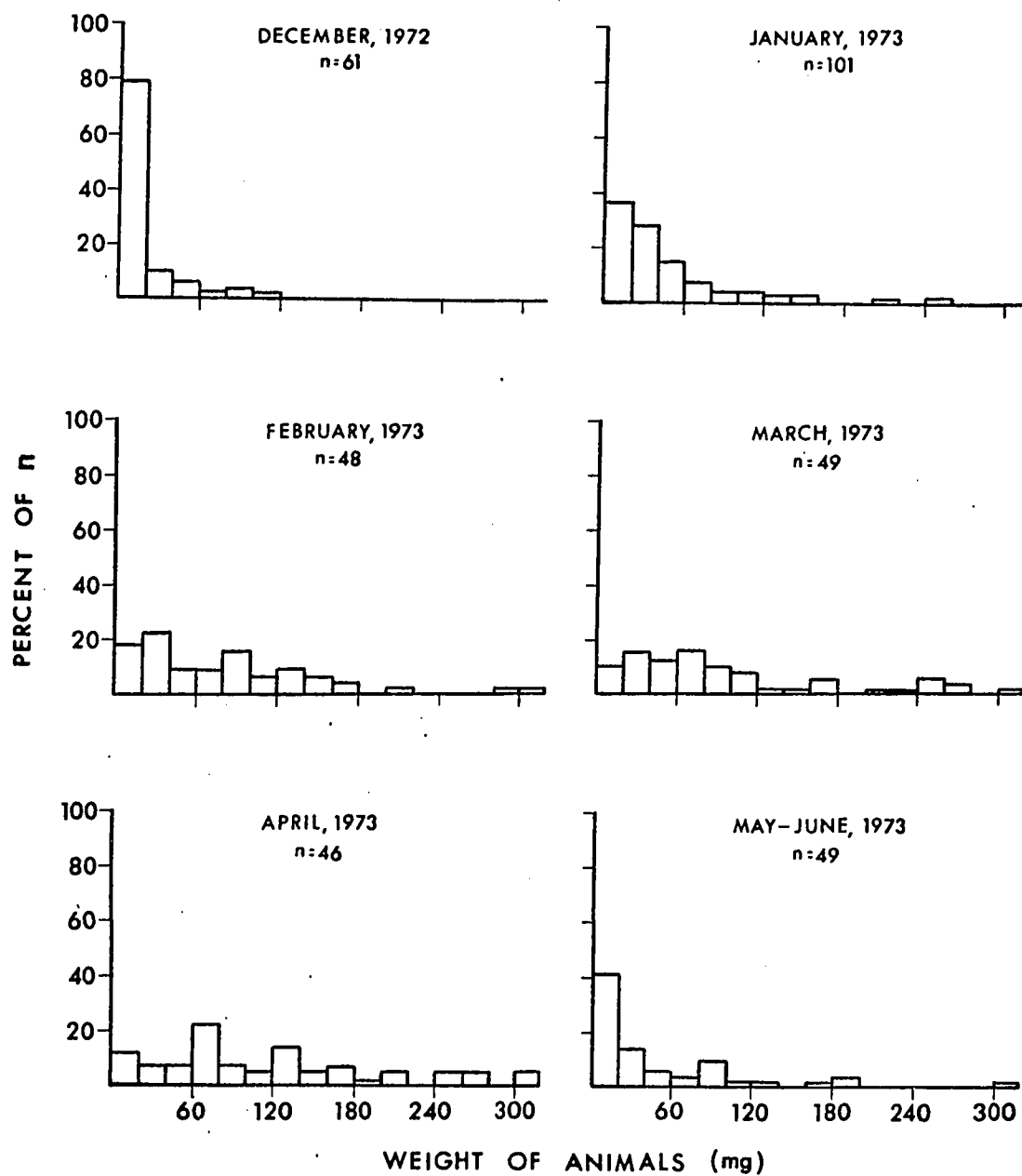


Table 17

Density, biomass and mean weight of two populations of the polychaete N. succinea as determined from 400 cm² quadrates collected from two microhabitats in Ala Wai Canal between December, 1972 and November, 1973

Month	Study site periphery			Island		
	Density	Biomass (mg)	Mean wt. (mg)	Density	Biomass (mg)	Mean wt. (mg)
Dec.	60	1040	17			
Jan.	34	1555	47			
Feb.	15	1555	98			
Mar.	16	1530	104			
Apr.	11	1359	124			
May	13	1168	78	70	1565	23
Jul.				61	2297	34
Sept.				38	1670	44
Oct.				28	1343	49
Nov.				60	2095	35

the hole was placed in the jug. Worms were not removed from the sediment. Thus each implanted jug contained the population of worms existing in the 200 cm² surface prior to implantation. Five of the jugs were covered with 2 mm rubber mesh to prevent predation. A jug covered with mesh and a jug open to predation were implanted at each of five different sites. Two months after implantation all 10 jugs were removed from the canal, sieved through 1 mm mesh and the worms were counted and weighed individually. Differences in density and biomass between age classes in covered and uncovered jugs were used to calculate predation rate.

A comparison of the size-frequency distribution between the two treatments at the five treatment sites is shown in Figure 24. Results from the five treatment sites are pooled into two histograms comparing the two treatments in Figure 25. A comparison of density, biomass, and mean weight are presented in Table 18. Biomass and mean weight differed significantly between treatments (using a t-test for paired data, $p < 0.025$) while the differences in density were insignificant ($p > 0.40$). The size-frequency histograms illustrated in Figures 24 and 25 show that the heaviest predation appeared to occur on individuals weighing greater than 120 mg. After the experiment, the biomass of worms weighing less than 40 mg was only 20 mg/200 cm² higher in the mesh covered jugs than biomass in jugs open to predators. The biomass of worms weighing between 40 mg and 120 mg was 168 mg/200 cm² higher in jugs open to predation than in mesh covered jugs. However, the biomass of worms weighing greater than 120 mg was 964 mg/200 cm² higher in mesh covered jugs than in open jugs. The net difference

Figure 24. The size-frequency distribution of N. succinea in mesh-covered, predation-free jugs in Ala Wai Canal and in jugs left open to predation in the canal. Jugs were implanted in pairs at five treatment sites in the canal. a) Mesh-covered jugs protected from predation. b) Jugs left open to predation.

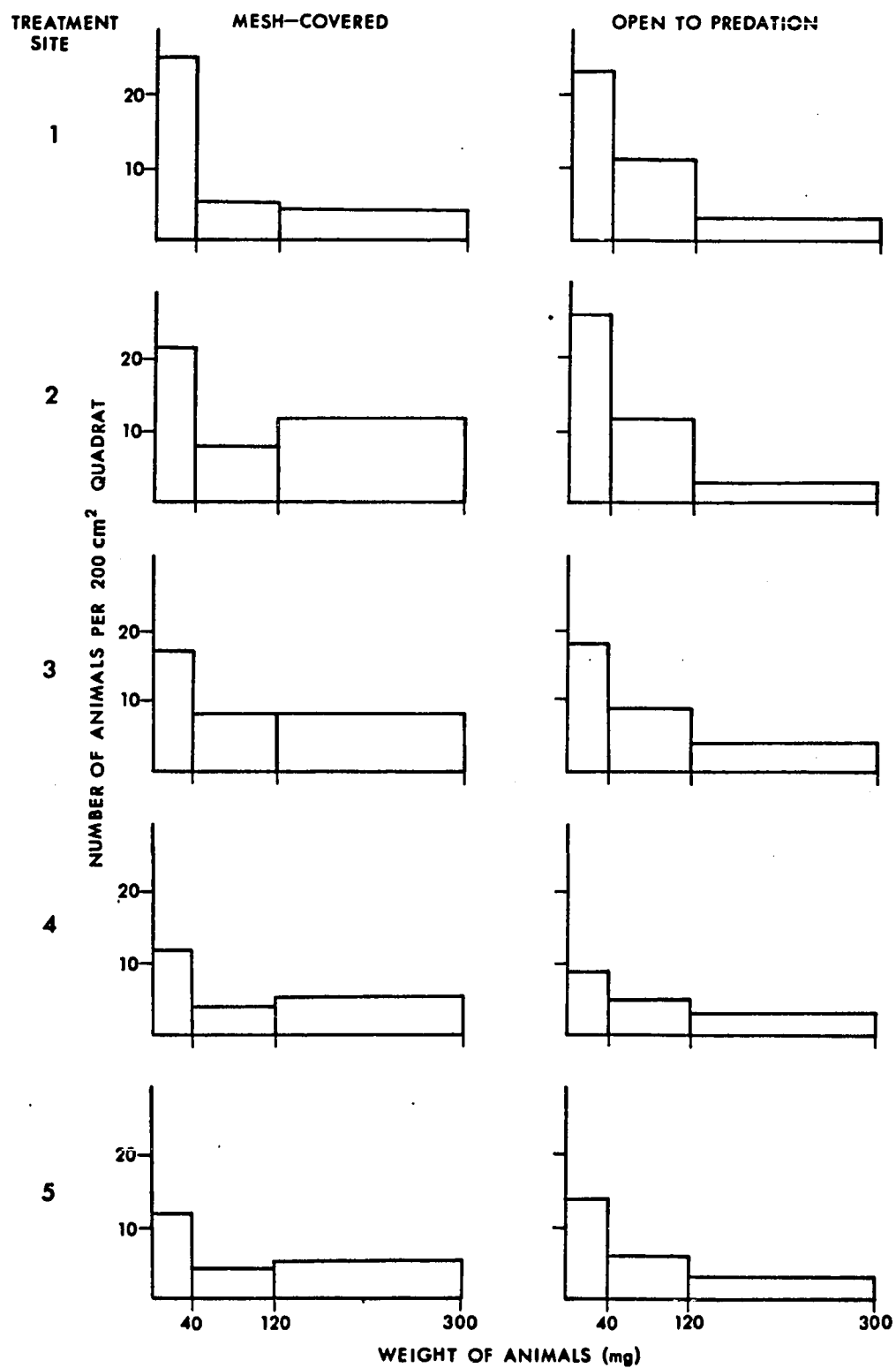


Figure 25. The size-frequency distribution of populations of N. succinea pooled from five mesh-covered, predation-free jugs in Ala Wai Canal and from five jugs left open to predation in the canal. a) Mesh-covered jugs protected from predation. b) Jugs left open to predation.

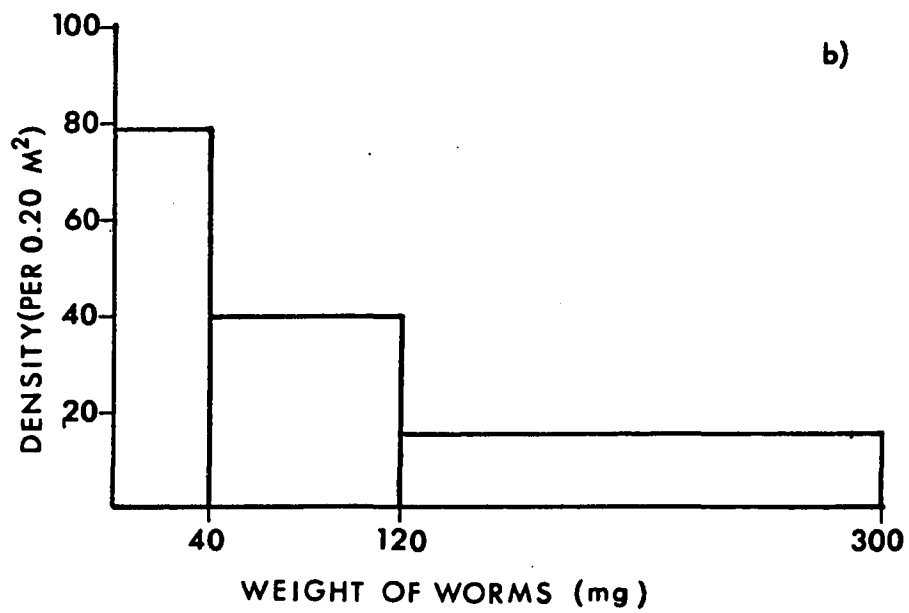
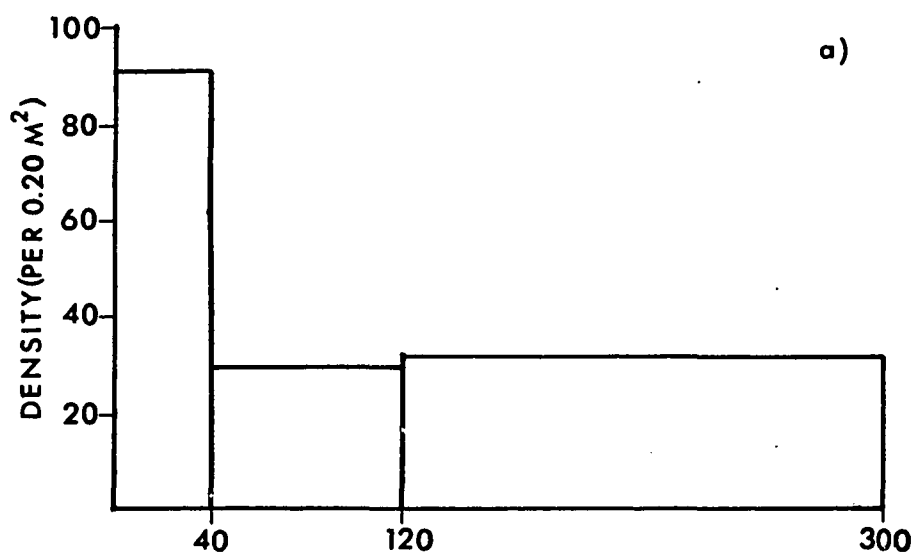


Table 18

A comparison of density, biomass and mean weight between a population of N. succinea protected from predation in mesh-covered jugs and a population of the worm in jugs open to predation

		Treatment site (0.04 m ² quadrates)				
		1	2	3	4	5
Mesh-covered	Density	36	42	34	21	22
	Biomass (mg)*	1.97	4.41	2.95	1.68	2.34
	Mean weight (mg)*	55	105	87	80	107

Open to predation	Density	38	42	32	8	23
	Biomass (mg)*	1.68	2.27	1.71	0.68	1.50
	Mean weight (mg)*	44	54	53	65	65

*Differ significantly between treatments ($p < 0.025$, t-test for paired data).

between the two treatments indicated that, over 61 days, 846 mg/200 cm² of N. succinea were ingested by predators.

Physiological Regulation of Mercury in Estuarine Organisms

Many of the results presented previously provided information about the physiology of mercury regulation by species used in this study. A few additional experiments were conducted to further study physiological aspects of this problem.

Ethanol Extraction

To determine the degree to which 203-Hg might be bound to ethanol-insoluble macromolecules within N. succinea, samples of the worm were extracted in ethanol during studies of the accumulation of dissolved 203-Hg. Individual organisms were extracted in 10 ml of 70% ethanol for 24 hours and a 1 ml aliquot of the extract was counted using liquid scintillation techniques. (Extraction for 48 hours or double extraction did not result in any increase in ethanol-soluble 203-Hg.)

Mercury occurred in both ethanol-soluble and ethanol-insoluble forms within the worm, as shown in Table 19. The ethanol-soluble fraction of the 203-Hg body burden in the species equaled the size of the short-lived component of efflux observed in Figure 12 and Table 12. Prior to the efflux experiments control worms were pre-loaded at a dissolved 203-Hg concentration in seawater of 1.26 ppb. As can be seen in Table 19, the ethanol-extractable fraction within these worms comprised 8.4% of the total body burden of the nuclide. The short-lived component of efflux also accounted for 8.4% of the total body burden of

Table 19

The fraction of 203-Hg in the worm N. succinea which is ethanol-soluble and the ethanol insoluble fraction, as a function of the concentration of 203-Hg dissolved in seawater

C _{wo} (ppb)	Concentration of 203-Hg (ppb)		Ethanol soluble fraction of total 203-Hg (%)
	Soluble	Insoluble	
0.10	0.9	4.0	18.4
0.53	6.5	75.4	7.9
1.19	17	191	8.2
1.26	22	244	8.4
1.88	46	282	14.0
5.07	228	1152	16.5

203-Hg in the worms (Table 12). Thus the more readily exchangeable pool of mercury in N. succinea appeared to occur in an ethanol-soluble form. The ethanol-soluble proportion of the total 203-Hg body burden was always relatively small in the worm, but it increased as the concentration of 203-Hg to which the worm was exposed increased (Table 19).

Figure 26 shows that the ethanol-soluble fraction of 203-Hg seemed to fit a steeper negative function of body size

$$C_{ss} = 426W^{-0.597} \quad r = 0.893 \quad (14)$$

than did the ethanol-insoluble portion of the body burden

$$C_{ss} = 2387W^{-0.409} \quad r = 0.864 \quad (15)$$

where W = the weight of the animal

The fit to a power function was significant in both cases ($p < 0.01$ - determined from the correlation coefficient, r). Thus smaller animals appeared to contain proportionately more readily exchangeable than did larger animals.

The time course of dissolved 203-Hg accumulation for both ethanol-insoluble and ethanol-soluble 203-Hg within the worm is shown in Figure 27. Because of the variability between samples interpretation of the time course of uptake of ethanol-soluble 203-Hg was difficult. However, the data seemed to indicate this fraction of the mercury in the worm reached steady state at the same time as the ethanol-insoluble fraction, after 13 hours exposure to the nuclide.

Figure 26. The size dependence of the ethanol-soluble and ethanol-insoluble ^{203}Hg in the polychaete N. succinea at steady state.

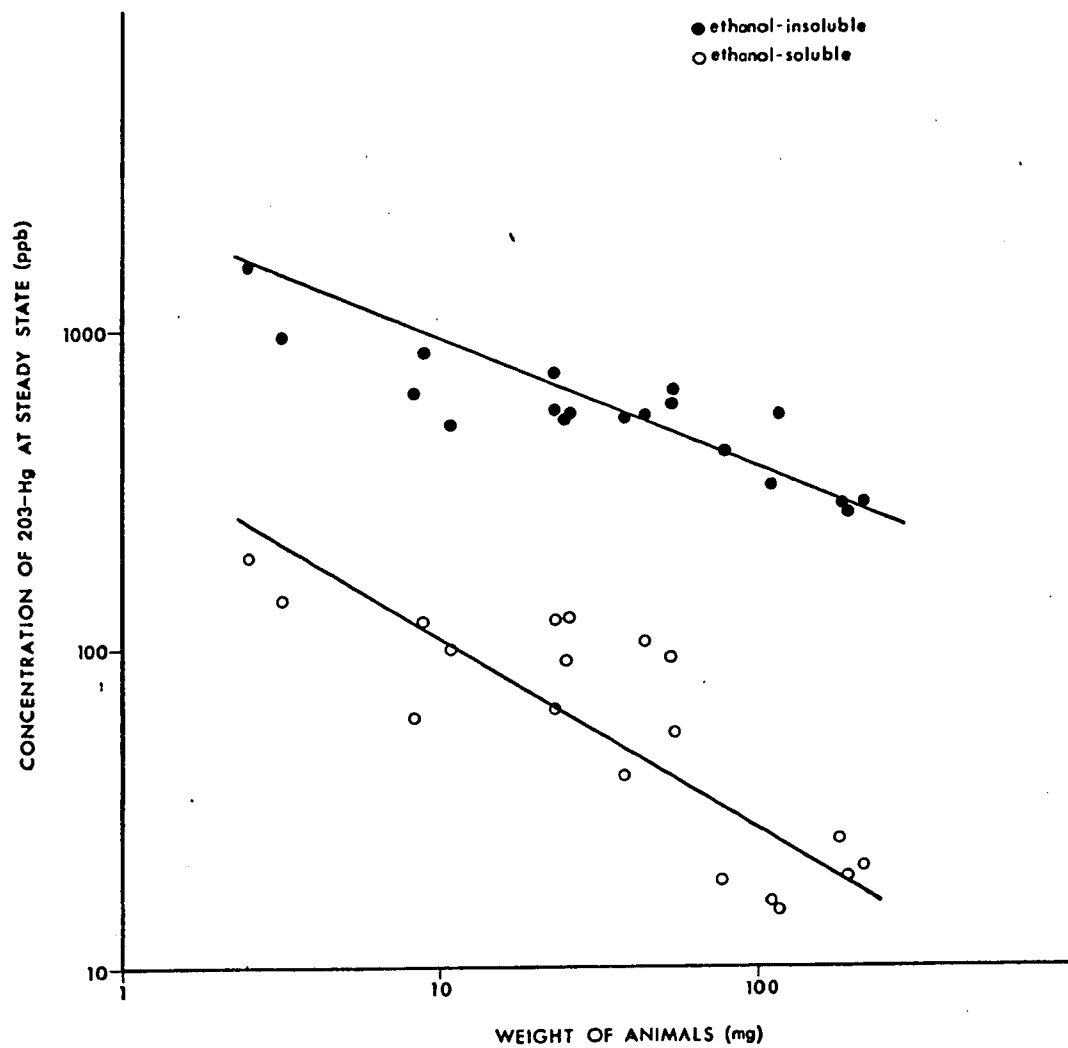
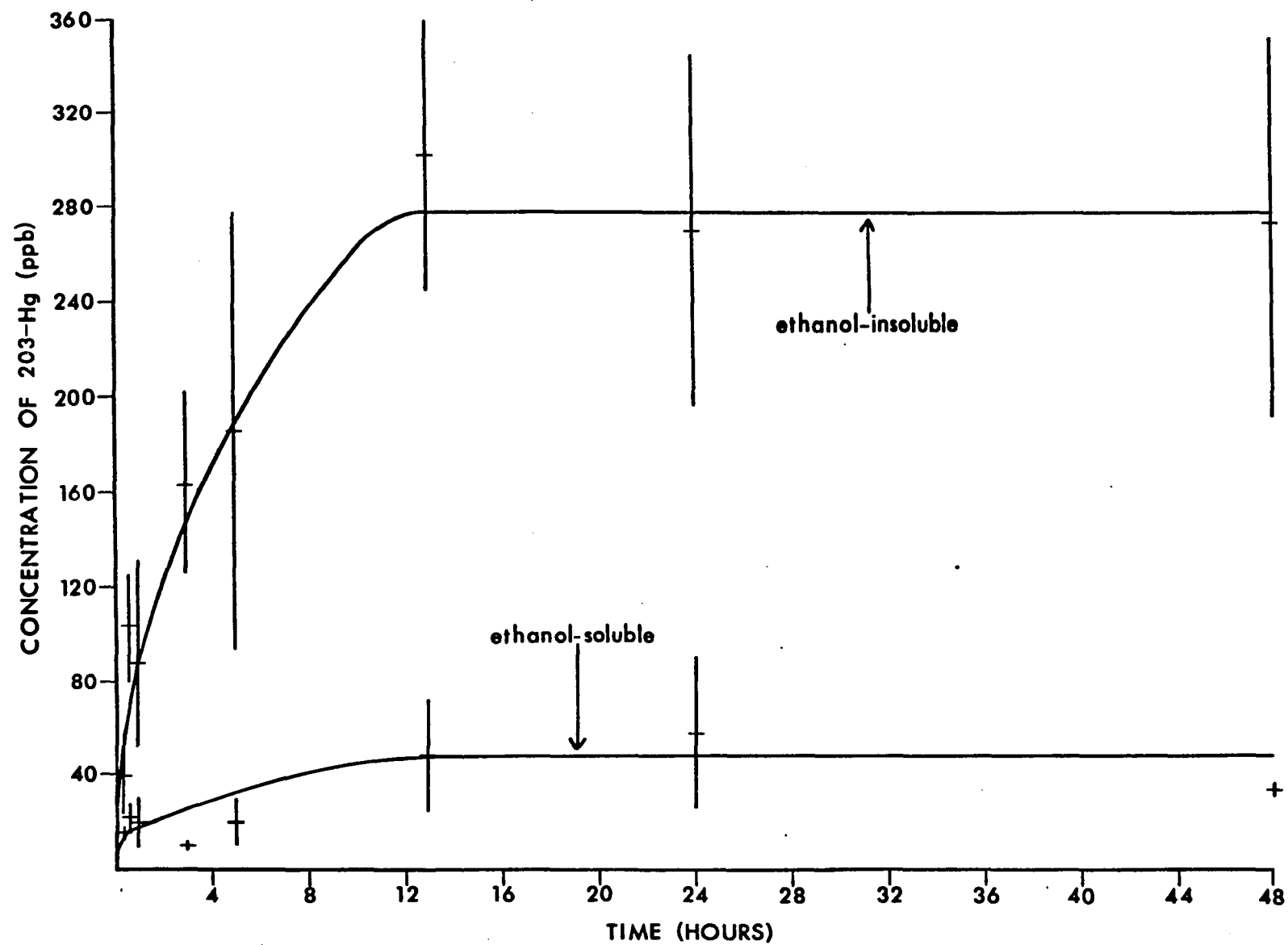


Figure 27. The accumulation of ethanol-extractable and non-ethanol-extractable fractions of ^{203}Hg in the worm, N. succinea, as a function of time. Vertical bars represent \pm one standard deviation.



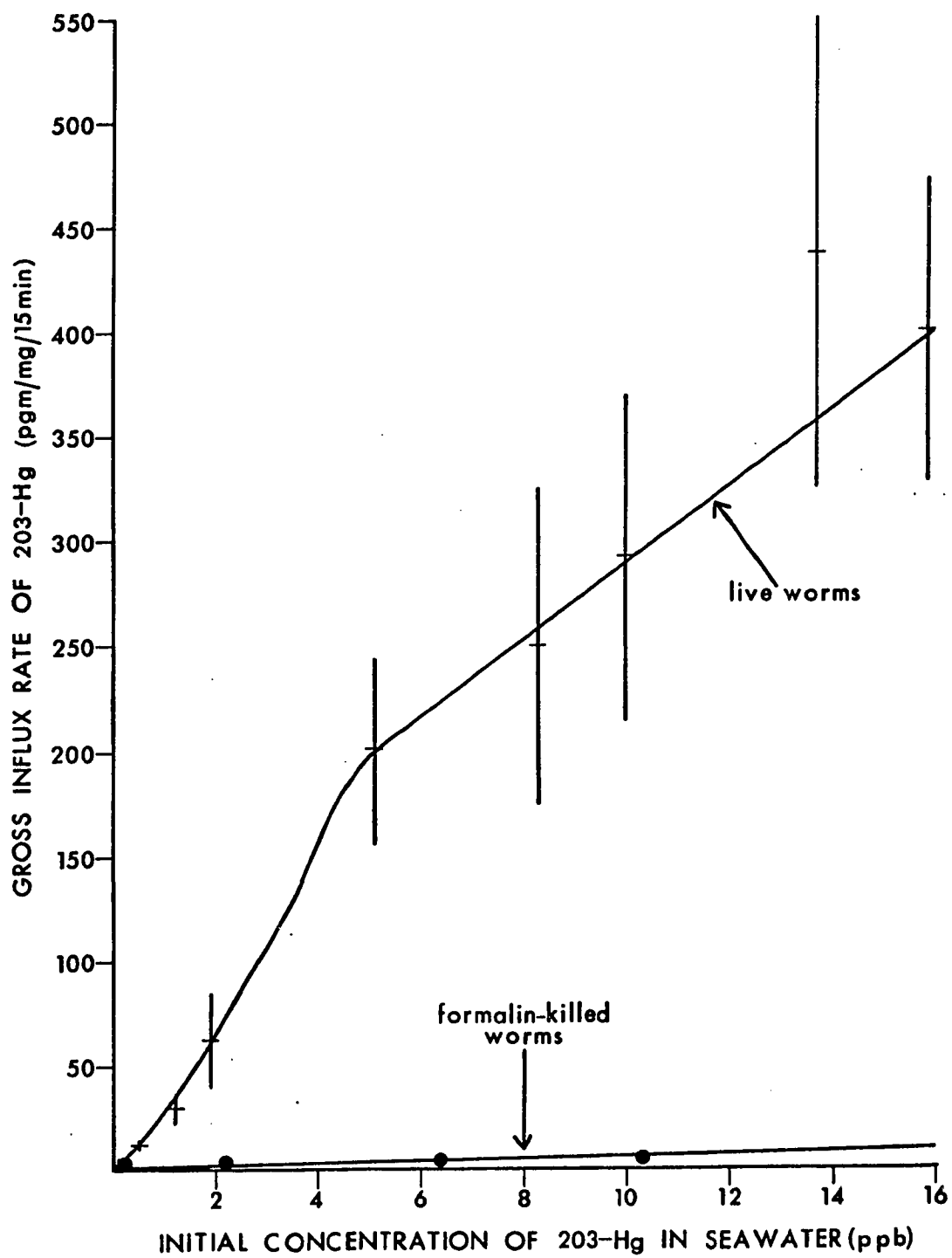
Absorption of 203-Hg by N. succinea

To determine if the accumulation of 203-Hg from solution by N. succinea represented adsorption to the surface of the worm or absorption through the body wall of the animal the gross influx rate of dissolved 203-Hg observed in formalin-killed worms was compared to the gross influx rate of the nuclide observed in living worms. The gross influx rate was defined as the whole body concentration of 203-Hg within the worm after a 15 minute exposure. For the initial 15 minutes of 203-Hg accumulation the slope of the curve describing the time course of accumulation was linear. Hence, over this time period the contribution of gross efflux to the observed concentration of 203-Hg in the animal was minimal, and observed mercury concentrations were used to represent influx alone (Neame and Richards, 1972). It is seen in Figure 28 that between 0.5 ppb and 11 ppb the rate of uptake of 203-Hg by the formalin-killed worms was insignificant compared to the rate of influx into the living worms. The concentration dependence of the influx rate in living worms appeared to change slope at 5 ppb dissolved 203-Hg. Variation in this data, within samples, was large. However, a change in slope in this type of curve is characteristic of ion transport, and indicates uptake of 203-Hg by the worm may have occurred by way of at least one saturable mechanism (Neame and Richards, 1972).

Autoradiography

Since formalin-fixation might affect the passive permeability of N. succinea, autoradiographic preparations were conducted on ethanol dehydrated sections of the worm to further determine the nature of the

Figure 28. Rate of ^{203}Hg influx into N. succinea as a function of ^{203}Hg concentration in the medium. Vertical bars represent \pm one standard deviation. Curves were fit by eye.

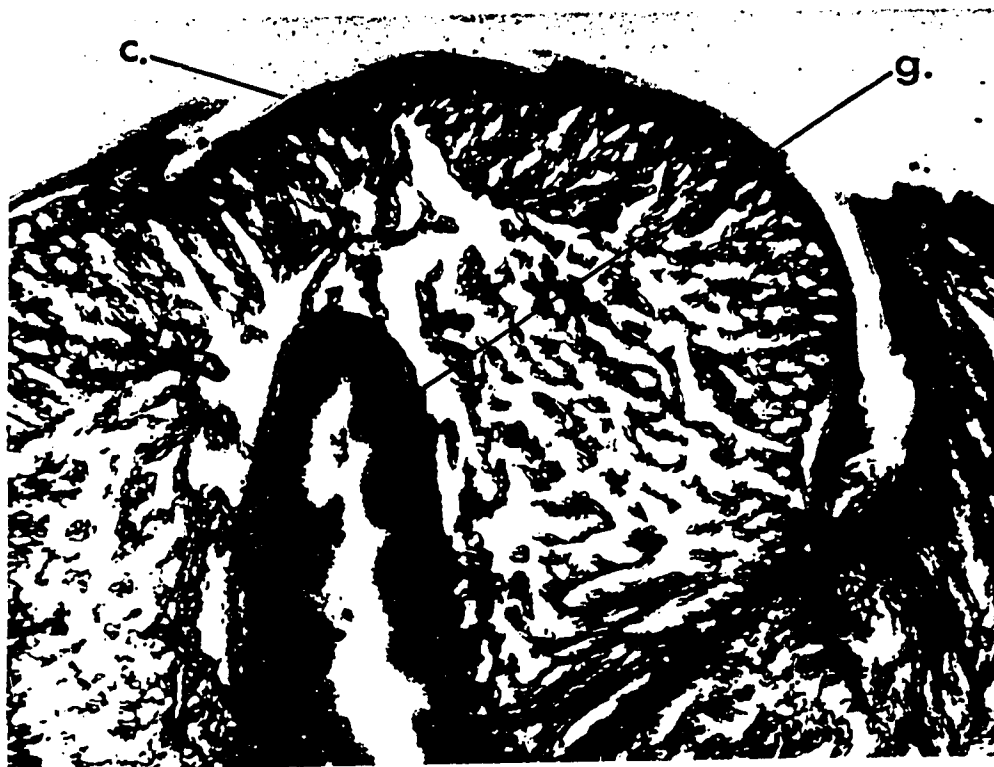


accumulation process. Plate 1 shows that intense concentrations of ^{203}Hg occurred at the periphery of the worm (mostly inside the cuticle), however, significant concentrations of label were present also in the gut cells and in organs throughout the animal.

Methylation of ^{203}Hg by N. succinea

These experiments were conducted to determine the degree to which macrofauna, such as the worm, might methylate mercury over short time periods, and to assure that inorganic mercury was the species of the metal accumulated by the worm in these two experiments. Organic ^{203}Hg analyses were conducted on N. succinea by extracting animals for 4 hours or 48 hours in benzene, centrifuging, and counting an aliquot of the benzene extract using liquid scintillation techniques (time of extraction did not affect the values). In both cases a small quantity of organic mercury was detectable, but it represented less than 1% of the total body burden of the nuclide in worms.

Plate 1. Autoradiograph of a cross-section of *N. succinea* exposed to ^{203}Hg dissolved in seawater. Slides were unstained. The dense black material represents radioactivity. g = gut; c = cuticle.



DISCUSSION

Accumulation and Retention of Mercury by Detritus Feeder

Uptake from Food and Water

The ability of the worm and the shrimp to concentrate dissolved inorganic mercury (Figure 4, Table 2) and the time necessary for these animals to reach steady state concentrations of mercury (Figure 3) were similar to observations of these phenomena in other organisms. From a field study, Knauer and Martin (1972) estimated a mercury concentration factor of 220 times for all three trophic levels of an open ocean pelagic food chain. Burrows and Krenkel (1973) observed that blue-gill accumulated CH_3Hg^+ 270 times over that initially dissolved in seawater. This species reached steady state mercury concentrations of the ion in approximately three days. McKane, Young, Bache and Lisk (1972) found goldfish concentrated ionic mercury 200 times over an initial dissolved mercury concentration of 250 ppb in fresh water, approaching steady state in 100 hours. Dissolved mercury concentrations were not held constant in the latter two experiments as they were in experiments with N. succinea and P. debilis.

Since $^{203}\text{-Hg}$ concentrations in seawater were held constant in the experiments with the worm and the shrimp the time course of accumulation of mercury by these two species could be described mathematically. A constant concentration of $^{203}\text{-Hg}$ in the medium means the gross influx rate of the metal was a constant. Thus net accumulation to an asymptote over time resulted from an exponential

increase in the efflux rate as a function of the concentration of 203-Hg within the organism. As the rate of efflux approached the rate of gross influx the concentration of mercury in the organism approached a steady state. Thus net uptake of 203-Hg in these experiments followed the equation

$$\frac{dC}{dt} = I - k_a C \quad (16)$$

where C = the concentration of 203-Hg in the organism at any time t ;

I = the influx rate (a constant);

k_a = the rate constant describing the efflux process which determines the nature of the hyperbolic net influx curve.

Integrating and solving for C_t we get

$$C = \frac{I}{k_a} (1 - e^{-k_a t}) \quad (17)$$

Since I and k_a are both constants we may combine them to one constant $I/k_a = C_{ss}$. As t approaches infinity in the equation C_t approaches C_{ss} . Thus C_{ss} is the concentration of 203-Hg in the animal at equilibrium and

$$C_t = C_{ss} (1 - e^{-k_a t}) \quad (18)$$

By taking the logarithm of equation (18) we obtain

$$\log (C_{ss} - C_t) = \log (C_{ss}) - k_a t / 2.3 \quad (19)$$

This equation simply describes a semi-log plot of $C_{ss} - C_t$ where the slope of the line equals k_a . The half-time of this process then can be calculated as in equations (6) and (9) by substituting

$$\frac{C_{ss} - C_t}{C_{ss}} \text{ for } \frac{C_t}{C_o}.$$

Mathematical analysis of the accumulation of 203-Hg by the worm indicated uptake was regulated by the sum of two exponential efflux processes. The rate constant of each process was determined as in equation (18), and the half-times of loss for the processes were calculated to be 0.33 days and 0.0067 days (Table 12). The efflux process which controlled 203-Hg accumulation by the shrimp appeared to be a single exponential with a half-time of 0.759 days (Table 12).

The concentration dependence of steady state mercury accumulation by the worm, N. succinea, fit a log-log power function (Figure 4), i.e. the concentration factor increased with increasing concentrations of dissolved 203-Hg (Table 2). Because of the rate at which N. succinea achieved the steady state, volatilization/adsorption of mercury appeared to have little effect on the magnitude of the steady state concentration of the ion in this species. Power function concentration dependence of mercury uptake has also been shown for the unicellular alga Chlorella pyrenoidosa (Matsui and Gloyna, 1972) and for the red blood cells of trout (Olson, Bergmann and From, 1973). An increasing organismic concentration factor as a function of dissolved ionic concentration has not been shown before in multicellular organisms exposed to dissolved mercury, or with metals other than mercury.

The steady state concentration factor of 203-Hg in the shrimp (Table 2) was 30% higher in the time course experiment (where 203-Hg concentrations were held constant) than in concentration dependence

experiments at a similar concentration of ^{203}Hg . This reduction was probably a result of volatilization/adsorption of mercury over the four day exposure period in the latter experiments. Observations within the samples of various experiments indicated that the reproductive state of shrimp did not affect the accumulation of mercury. Egg-bearing females tended to take up concentrations of ^{203}Hg which were similar to concentrations accumulated by individuals that were not carrying eggs.

Like N. succinea, the shrimp rapidly accumulated mercury to concentrations 180 - 270 times the concentration of the metal in seawater. Thus in the case of either the worm or the shrimp a small short-term change in the concentration of dissolved mercury to which an organism is exposed would result in a large change in the body burden of mercury in the organism.

The ability of small organisms to rapidly concentrate mercury from water probably facilitates the high susceptibility of such species to the toxic effects of dissolved mercury. For the polychaetes Ophryotrocha sp. (Brown and Ahsanullah, 1971) and N. succinea (Figure 6), LD_{50} 's are less than 75 ppb. Roesijidi, Petrocelli and Anderson (1973) found an LD_{50} of 50 ppb for the small porcellanid crab Petrolisthes armatus. A larger species, the fiddler crab, Uca pugilator, was generally not able to survive chronic exposures to dissolved mercury concentrations greater than 180 ppb (Vernberg and O'Hara, 1972). Most studies of the LD_{50} of dissolved mercury in larger species have used 96 hour exposures, without control of volatilization/adsorption of the ion. Such studies generally suggest

mercury lethality at doses from 10 to 2000 ppm Hg^{++} (e.g. Knapik, 1969). Loss of mercury from solution in such experiments probably reduces organismic mercury uptake. More important, such short exposure times are probably not long enough for these larger species to achieve steady state whole body mercury concentrations, much less allow any tissue redistribution of Hg which might affect toxicity (e.g. Vernberg and O'Hara, 1972). Thus such studies probably grossly underestimate the susceptibility of larger species to the toxic effects of the mercuric ion.

While laboratory studies have shown the potential biological significance of dissolved mercury, the role of the dissolved ion in natural ecosystems is still a major question. Like most toxic heavy metals, mercury has a strong affinity for both organic and inorganic particulates (Reimers and Krenkel, 1972). Dissolved divalent mercury was bound extremely rapidly by estuarine or terrestrial sediment (Figure 7). The distribution coefficient of the ion (1.42×10^5) implied that if a pulse of dissolved ionic mercury entered an estuary little of it would remain in solution (Table 4). Thus, by far, the most concentrated physical pool of mercury in an estuarine ecosystem occurs in the sediment. Most of this bound mercury is probably complexed with organic matter in highly productive estuaries such as Ala Wai Canal (Siegel, 1971). As a result the organic portion of the sediment - the food of detritus feeders - is potentially a significant pathway for cycling sediment-bound heavy metals such as mercury into the macrobiotic portion of aquatic ecosystems (Duursma and Gross, 1971). However, few studies have

directly approached the biotic availability of this particulate bound fraction of metals. Based upon laboratory experiments with the polychaete worm Nereis diversicolor, Renfro (1973) estimated that this species could remove 0.08% of the 65-Zn from the sediment in its habitat. No measure of the concentration of the nuclide in the worm relative to that in the sediment was attempted since all worms analyzed for activity contained radioactive sediment in their alimentary tract. Fagerstrom and Jernelov (1971) and Gillespie (1972) have investigated the mobilization of mercury from sediment into freshwater, using fish as indicators. Again, the availability of bound mercury was not studied.

The laboratory experiments using 203-Hg in this study showed that little of the mercury bound to highly organic estuarine sediment was available to deposit feeding biota (Fig. 8, Table 5). Three deposit feeding species representing three different phyla, assimilated steady state body concentrations of 203-Hg ranging from only 0.16% to 1.5% of the concentration of 203-Hg in the sediment they were ingesting. At the concentration of mercury observed in the sediment of Ala Wai Canal (334 ppb) the concentration factors for the different species (Table 5) suggested that the polychaete, Nereis succinea would assimilate from its food a steady state mercury concentration of only 1-4 ppb total mercury. The shrimp, P. debilis, and the poeciliid fish, M. latipinnia, would assimilate concentrations of only 1 ppb. Obviously these organismic concentrations of the metal are insignificant in terms of contamination of the organisms. The relatively innocuous nature of sedimentary bound mercury was

further demonstrated by the failure of bound mercury concentrations of 5000 ppb Hg^{++} to affect the growth of N. succinea in either field or laboratory experiments (Tables 6 and 7). (The minimum dose at which dissolved Hg^{++} resulted in overt toxicity to N. succinea was 50 ppb - two orders of magnitude lower than the concentration of bound mercury showing no effect on the worm.)

Unlike accumulation of dissolved mercury, very large changes in the concentration of estuarine sediment-bound mercury would be necessary before any significant change in organismic mercury concentrations would occur. Small changes (less than 1 ppm) in mercury concentrations in sediments of this nature probably have little effect on mercury contamination in deposit feeders ingesting these sediments.

The presence of ^{203}Hg in the feces of the worm and the fish during feeding experiments verified that both species were ingesting the nuclide. The ratio of mercury concentration in feces to mercury concentration in the sediment was 1.70 for the worm, N. succinea, and 2.45 for the fish, M. latipinnia (Table 5). Boothe and Knauer (1972) observed a feces/food mercury ratio of 1.45 when the crab, Pugettia producta, was fed algae containing 340 ppb mercury. They concluded that the inability of this primary consumer to assimilate heavy metals from its food resulted in an increase in the concentration of metals in the sediment and an increase in metal concentrations to which detritus feeders are exposed. Like this herbivorous crab, deposit feeders in this study appeared to contribute to the biomagnification of sedimentary mercury

concentrations. However, they also demonstrated a marked inability to assimilate the resulting elevated concentrations of organically bound sedimentary mercury.

Effect of Environmental Factors on Mercury Accumulation

Salinity

Salinity fluctuations in the Ala Wai Canal study area occur mainly as a function of rainfall in the watershed of the canal (Gonzalez, 1971). As a result the intensity and duration of salinity changes in the study area are quite variable. To assess the influence of salinity on mercury accumulation by detritus feeders from this area two different acclimation times were used. Organisms were acclimated to a given salinity for four hours before experimentation to determine the effects of short term changes in salinity on the mercury uptake rate. An acclimation period of four days was used to determine the effects on mercury uptake of longer term salinity changes.

The rate of uptake of dissolved 203-Hg by the polychaete N. succinea followed the same function of salinity after either four hours or four days of acclimation (Fig. 9). Below 16‰ salinity the uptake rate of 203-Hg decreased as salinity decreased. Salinities above 16‰ had no effect on 203-Hg uptake. During periods of storm runoff into Ala Wai Canal salinity decreases and dissolved mercury concentrations appeared to increase (see p. 70 - 76). The response of N. succinea to any increase in dissolved mercury in this situation would be inhibited by a decrease in the rate of mercury uptake by the worm resulting from decreased salinities.

The salinity-induced decrease in the uptake of mercury by the worm was opposite from the effects of salinity on the accumulation of other cations as observed by other authors. Townsley (1967) showed that the uptake of $^{90}\text{-Sr}$ by Tilapia sp. was approximately 10 times faster in freshwater than in seawater. Bryan and Hummerstone (1973a,b) reported that $^{54}\text{-Mn}$ uptake and Zn uptake by the polychaete Nereis diversicolor increased as salinity decreased. Both N. diversicolor and N. succinea maintain hyperosmotic extracellular body fluids at salinities below approximately $12^{\circ}/\text{oo}$ (Ogelsby, 1972). This is accomplished by a reduction in the permeability of the body wall to water and to some salts, plus active inward transport of other essential salts (Ogelsby, 1972). The reduction in the rate of mercury accumulation by N. succinea at salinities below $16^{\circ}/\text{oo}$ could result from a general decrease in the permeability to some cations at these salinities. At lower salinities a reduced concentration of an ion(s) which has membrane or cytoplasmic allosteric effects on the mechanism responsible for the transport of mercury could reduce the permeability of the worm to mercury. The general curve describing the allosteric effects of ions on other carrier-mediated transport mechanisms (Pappas and Read, 1972) is similar in shape to the curve in Figure 9 describing the uptake rate of mercury as a function of salinity. Manganese and zinc, biologically essential metals, may enter N. diversicolor by processes uninfluenced by such a reduction in body wall permeability.

In contrast, the rate of $^{203}\text{-Hg}$ uptake by P. debilis did not seem to be affected by changing salinity at greater than $6.4^{\circ}/\text{oo}$

after four hours acclimation. The steady state ^{203}Hg concentrations were also the same in shrimp exposed to the metal after four days acclimation and exposure at salinities of $16^{\circ}/\text{oo}$ and $32^{\circ}/\text{oo}$. Wolfe and Coburn (1970), and Bryan and Hummerstone (1973a,b) have shown that the effects of salinity on biotic cation accumulation increased most rapidly below salinities of $5 - 10^{\circ}/\text{oo}$. Mercury uptake by the shrimp may be affected by salinities below $6.4^{\circ}/\text{oo}$. However, salinities below $6^{\circ}/\text{oo}$ have never been recorded in the portion of Ala Wai Canal used in this study (Gonzalez, 1971; Harris 1972; personal observation). Very low salinities in this area may occur after extremely intense storms in the watershed of the canal, but such situations are probably exceptional and short-lived. From these experiments it appears that most salinity changes in Ala Wai Canal would have little effect on mercury accumulation by the shrimp.

Dissolved Organic Material

Dissolved organic molecules tend to reduce the toxicity of metal ions. Morris and Russel (1973) found that the toxicity of copper to marine algae was reduced when the chelating agent EDTA was added to seawater. The LD_{50} of mercury to crustacean larvae increased when reduced glutathione was added to the medium (Corner and Rigler, 1958). Reductions in the toxicity of organically bound dissolved metals probably result from the reduced biological availability of metal ions in this form. When the uptake of dissolved ^{203}Hg was studied in the presence of high concentrations of either a dissolved protein (BSA) or an amino acid (cysteine)

dissolved in seawater, N. succinea accumulated the metal to steady state concentrations that were only 4 to 7 times higher than the concentration of 203-Hg in the seawater (Table 8). In contrast, dissolved mercury was concentrated 124 - 235 times over concentrations in seawater when organic molecules were not added to the water.

Because of the low concentration of dissolved organic material in the open ocean, the extent and significance of dissolved organic complex formation with metals remains in question (Robertson, 1971). However, in highly productive waters the organic chelation of dissolved metals appears to influence the biological availability of those metals. Both a seawater extract of soil and a similar extract of raw sewage reduced the toxicity of copper to copepods (Lewis et al, 1972, 1973). Many productive aquatic ecosystems receive large discharges of soil and/or sewage. D'Itri, Annett and Fast (1971) found less mercury in trout raised in an eutrophic lake than in trout raised in an oligotrophic lake, when both bodies of water were subject to the same mercury input. Mercury in water from Ala Wai Canal was less available to N. succinea than was mercury dissolved in less productive open ocean water (Figure 10). Results from these experiments indicate that in highly productive waters the biological significance of the concentration of dissolved mercury may be modified by the physico-chemical form of the ion. In such instances conclusions based on the concentration of the dissolved metal alone may be misleading. In any study of the significance of mercury contamination both concentration and biological availability of the dissolved pool of the metal should be considered.

Highly productive ecosystems appear to have a mechanism which buffers the effects of dissolved forms of mercury and other toxic heavy metals. High biological productivity and/or the influx of terrestrial materials increase the amount of dissolved organic material in the water (Lewis et al, 1972). Subsequently, the biological availability of dissolved mercury may be decreased. In oligotrophic waters this protection is reduced. Hence, any small increase in the concentration of dissolved mercury in oligotrophic water could result in a larger increase in biotic mercury concentrations than would occur in eutrophic waters.

Sediment Type

The organic and inorganic particulates of which estuarine sediment is composed are characterized by an adsorbed "coat" of microflora, microfauna and organic complexes (Darnell, 1967). Inorganic particulates of terrestrial origin, which enter estuaries during periods of storm runoff, probably initially lack much of this organic coating. After such particulates enter the estuary adsorption of organic compounds and the microorganisms responsible for breakdown of those compounds may eventually result in development of such an organic envelope (Khailov and Finenko, 1970). Any physico-chemical differences between terrestrial and estuarine particulates could affect the processes by which metals are bound to those particles. Reimers and Krenkel (1972) reported that mercury may be found either electrostatically to clays in sediment or to the organic humus in sediments. The affinity of the ion for

organic matter containing sulfhydryl bonds greatly exceeded its affinity for the clays. Jenne (1968) proposed that sorption of heavy metals by high-surface area hydrous oxides of manganese and iron provides the principal control of heavy metal fixation to sediments; except in highly organic sediments where the overwhelming quantity of organic matter resulted in dominance by organic chelation or complex formation.

Terrestrial sediment-bound ^{203}Hg was more available to deposit feeding biota than was ^{203}Hg bound to estuarine sediment in the experiments reported here (Table 9). A 50% decrease in organic matter associated with estuarine sediment resulted in a 50% decrease in ^{203}Hg assimilation by the worm, *N. succinea*. Thus the increase in the availability of mercury observed with terrestrial sediment was not a simple function of the organic content of that material. Rather these results suggested that a biologically significant proportion of the ^{203}Hg bound to terrestrial sediment was bound by a process with low enough affinity to allow biotic desorption and assimilation of the ion. In the highly organic estuarine sediment the binding of ^{203}Hg occurred in a way that prevent such assimilation.

As the terrestrial sediment "aged" in seawater, the ability of the organisms to assimilate the bound mercury decreased. The "aging" of terrestrial sediment in seawater probably involved the accumulation of an organic coat around the terrestrial particulates; as has been suggested by Khailov and Finenko (1970) for sediment in natural ecosystems. As the organic coat developed, it may have

enveloped the mercury bound to the low affinity sites, thereby reducing access to the ion during digestion by the organism. A more likely supposition is that suggested by the work of Reimers and Krenkel (1972). The higher affinity of mercury for organic complexes may have resulted in a shift of the metal from binding sites on inorganic particulates to organic binding sites as organic matter adsorbed to the inorganic particulates. Whatever the specific process(es) involved, these results suggested that as an organic coat developed around particulates the biological availability of mercury decreased. This provided further evidence that: a) little organically bound mercury was assimilated by detritus feeders; and b) the relatively high biotic availability of ^{203}Hg bound to terrestrial sediment resulted from mercury, binding by processes other than organic complex formation.

A significant proportion of the mercury accumulated by worms from the labelled terrestrial sediment-seawater system came from dissolved mercury in the interstitial water of this system. Three non-feeding epibenthic polychaetes accumulated an average of 56% of the ^{203}Hg taken up by feeding worms. Assuming that reproductive metamorphosis does not involve changes in organismic permeability to mercury, this meant only 44% of the ^{203}Hg taken up by N. succinea from terrestrial sediment was assimilated through ingestion of particulates. Even if 56% of the body burden of ^{203}Hg observed in feeding worms resulted from uptake of ^{203}Hg dissolved in interstitial water, the remaining 44% of the concentration of mercury in these animals would still represent an increase in the biological

availability of ^{203}Hg bound to terrestrial sediment of 15 to 38 times over the availability of ^{203}Hg fixed to estuarine sediment.

As the terrestrial sediment aged in seawater, ^{203}Hg concentrations in the interstitial water declined. Diffusion of ^{203}Hg from the interstitial water into the water column above the sediment-water interface and subsequent volatilization of mercury may have accounted for some of the ^{203}Hg loss from interstitial water in these experiments. However, after the beginning of an experiment, ^{203}Hg concentrations in the water column did not increase over the concentrations observed in experiments with estuarine sediment. Moreover, the fraction of the body burden of ^{203}Hg in feeding animals attributed to uptake of dissolved mercury from interstitial water remained proportionately the same as the terrestrial sediment aged in seawater, although the total body burden of mercury in these animals declined over the course of an experiment. After terrestrial sediment aged one day in seawater a non-feeding heteronereid accumulated 55.2% of the ^{203}Hg observed in feeding worms. In a similar sample taken 25 days after the beginning of an experiment two heteronereids accumulated a mean of 56.7% of the mercury detected in feeding worms. The accumulation of bacterial populations on and within the terrestrial sediment could have resulted in fixation to the sediment of ^{203}Hg dissolved within the interstitial water. By the same process and thus at the same rate, biologically available mercury may have been converted to a less available form. Little biotically available dissolved mercury existed within the interstices of the highly organic estuarine sediment since little ^{203}Hg was

accumulated by worms exposed to this type sediment. Thus, in sediments where particulates are enveloped by a heavy organic coating, the formation of mercury-organic matter complexes may reduce the biotic availability of the metal, not only by binding the ion in a form unavailable for assimilation, but also by preventing dissolved concentrations of the metal from accumulating in interstitial water.

It is possible that both the mercury taken up from the terrestrial sediment and the mercury observed in the interstitial water of these experiments occurred in a chemical form other than divalent mercury. Jernelov (1969) stressed the potential importance of bacterial methylation of mercury and the increased availability of the ion in this form. However, benzene extraction of worms exposed to ^{203}Hg labelled terrestrial sediment showed less than 1% of the label existed in extractable organic form. Other inorganic forms of mercury have been described. Andern and Harriss (1973) cited thermodynamic calculations that suggested the existence of mercury in dissolved polysulfide complexes. Reimers and Krenkel (1972) and D'Itri (1973) stressed the importance of mercury-chloride or mercury-hydroxide complexes in salt water ecosystems. While chloride, sulfide, or hydroxide complexes of mercury may reduce the adsorptive nature of the ion, their effect on the biotic accumulation of mercury has not been determined.

Enrichment of mercury within interstitial water has been observed in aquatic ecosystems in nature. Andern and Harriss (1973) reported pore water concentrations of dissolved mercury in

some Florida estuaries exceeded water column concentrations of the ion by as much as 30 times. No correlation between sediment organic matter and fluctuations in pore water mercury content was mentioned. Mercury concentrations in the open ocean often are higher in bottom water than in surface water (D'Itri, 1973) - possibly due to diffusion from high concentrations in the interstitial water. However, such a correlation is not characteristic of all aquatic ecosystems (D'Itri, 1973).

Results reported here suggest the concentration of biologically available mercury in interstitial waters and the biological availability of bound mercury may be elevated in aquatic ecosystems subject to significant sedimentation of particulates containing mercury adsorbed by processes other than organic complex formation. In oligotrophic aquatic ecosystems, where energy cycles are extremely efficient, sufficient inorganic binding sites for mercury in sediments may also exist to permit increased biotic availability of bound mercury and enrichment of mercury concentrations in interstitial waters. Several studies suggest this is the case:

- 1) In such a system benthic detritus feeding species may obtain significant concentrations of mercury by both ingestion of particulates and by contact with interstitial water. Armstrong and Hamilton (1973) found that detritus feeders in a Northwest Ontario lake contaminated with mercury contained higher concentrations of mercury than other trophic groups.

- 2) Armstrong and Hamilton (1973) also reported that benthic species in the lake, in general, contained 10 times more mercury than did organisms living higher in the water column. Klemmer and Luoma (1972) found organisms associated with food chains linked directly to the substrate contained higher concentrations of mercury than other biota sampled from a near-shore open ocean ecosystem off Hawaii.
- 3) Some goatfish continually disturb the substrate in their search for food, passing sediment over their gills as they search (Mahi, 1969). These species could be exposed to both enriched mercury concentrations in interstitial water and low affinity sedimentary bound mercury. Joyner (1962) found goatfish maintained consistently higher levels of 65-Zn than other biota from near Eniwetok Atoll. A survey of mercury concentrations in the biota of a near-shore marine Hawaiian ecosystem showed goatfish consistently had higher mercury concentrations than other near shore fish (Water Resources Research Center, 1972).

The experiments reported in this section provide only an initial glimpse of a phenomenon that may be important in understanding trace metal contamination. Many questions remain unanswered. Nevertheless, the results included in this discussion emphasize the susceptibility of oligotrophic ecosystems to heavy metal contamination. In highly productive, eutrophic systems the biological availability of dissolved mercury may be reduced, organically rich sediment may complex mercury and inhibit biotic assimilation of the ion, and interstitial

water concentrations of biologically available mercury may be depleted by organic uptake. In oligotrophic ecosystems the extent to which these mechanisms protect biota may be reduced. Any increase in mercury contamination is likely to have much more significant biological effects in oligotrophic waters than in a more eutrophic ecosystem. Any massive disturbance of oligotrophic sediments (e.g. dredging, deep sea mining) may disperse throughout the water column both readily concentrated dissolved mercury from the interstitial water, and biologically available bound mercury. The extent to which these conclusions apply can only be determined by further research emphasizing an understanding of any characteristics of sedimentary binding which might increase the biotic availability of bound ions, and a determination of the frequency with which that type of binding occurs in nature.

Retention of Mercury by Detritus Feeders

Knowledge of the ability of an organism to retain a metal after exposure to the ion is important in assessing how physiological factors may affect the biotic concentrations of the metal observed in animals collected from the field. In the case of mercury, retention of the ion is affected by the form of the ion within the organism. Organic mercury is excreted much more slowly than are other forms of this metal. Lockhart, Uthe, Kenney and Mehrle (1972) released methylmercury-laden pike into a lake that was not polluted with mercury and estimated a half-time of loss of approximately two years. In a laboratory experiment Burrows and Krenkel (1973) found

blue-gills exposed to methylmercury lost the metal by way of two components - a faster component comprising 40% of the body burden, with a half-time of 38.5 days and a slower component with a half-time of 130 days.

In contrast, inorganic mercury is lost more rapidly by aquatic organisms. Weisbart (1973) injected goldfish with $^{203}\text{-HgCl}_2$ and, beginning measurements 24 hours after injection, found that loss occurred by way of a single component with a half-time of 23.7 days. Seymour and Nelson (1971) exposed oysters to dissolved $^{203}\text{-HgCl}_2$ for one day then followed the efflux of the metal. Loss occurred by way of four components, with half-times ranging from 1.4 to 44 days.

Pentreath (1973b) has shown that injection of a radionuclide (versus assimilation of the label) alters the size of the components of efflux but has no effect on the rate constants describing the various components. Baptist, Hoss and Lewis (1970) demonstrated that the route of uptake of $^{65}\text{-Zn}$ (food or water) had a small effect on the size of the various efflux components in the croaker, but again, did not affect the rate constants of loss. Bryan (1967), Cross, Dean and Osterberg (1969) and Takahashi and Hirayama (1971) have shown that feeding animals excrete metals faster than starved organisms. Thus in experiments reported here animals were pre-loaded with $^{203}\text{-Hg}$ by exposure to the dissolved metal over the time period necessary to reach steady state, and efflux experiments were conducted in the presence of unlimited food. Loss of mercury from the shrimp and the worm occurred by way of two components whose half-lives agreed well with those observed by other authors (Table 12).

The slow component of loss dominated efflux in both species. The half-time of this component greatly exceeded the half-time of loss as observed during accumulation. Thus while N. succinea and P. debilis accumulated mercury rapidly to a steady state, it took significantly longer to excrete most of the body load accumulated (compare Figs. 3 and 12). This disparity between rapid accumulation and slow elimination has been reported for mercury excretion by the American oyster, Crassostrea virginica, (Cunningham and Tripp, 1973) and for other metals (Harrison, 1973; Pentreath, 1973c,e). Cunningham and Tripp (1973) emphasized the significance of this phenomena to fisheries, where only a short period of biotic exposure to mercury could result in a significantly longer period of contamination of the fishery.

The slow rate at which mercury is lost, relative to the rate of accumulation, also means that in systems where available mercury concentrations fluctuate, organisms will seldom be at steady state metal concentrations. Many interpretations of metal concentrations found in aquatic biota assume steady state organismic concentrations (e.g. determination of concentration factors, Polikarpov, 1966). For mercury such assumptions may not be valid due to the long biotic retention time of the ion, if dissolved mercury concentrations fluctuate.

Size

Within larger species of fish and crabs collected from various types of aquatic ecosystems older and/or larger individuals show

higher concentrations of total mercury and organic mercury than do younger or smaller individuals (Bache, Gutenmann and Lirke, 1971; Barber, Vyeyakumar and Cross, 1972; Rivers et al, 1972; Cross, Hardy, Jones and Barber, 1973; Parsons, Bauden and Heath, 1973; Westoo, 1973). Only at organismic mercury concentrations below 100 ppb and above 1000 ppb has this relationship not applied in reported studies (Johnels, Westermarck and Berg, 1967). In similar species other metals show either an inverse intraspecific correlation between size and metal concentration, or no correlation between the two parameters (Cross et al, 1973). Cross et al (1973) have hypothesized that the correlation between mercury concentrations and organismic size is different from this correlation in other metals because loss of mercury is so slow in aquatic organisms that steady state concentrations of the ion are never achieved over the life-time of the individual. Burrows and Krenkel (1973) demonstrated that blue-gill reached a steady state concentration of $\text{CH}_3^{203}\text{-Hg}$ within three days after exposure to the dissolved ion. The polychaete, N. succinea, and the shrimp, P. debilis reached steady state rapidly when exposed to $^{203}\text{-HgCl}_2$ (Fig. 3). In both the worm and the shrimp, however, steady state mercury concentrations fit an inverse power function with size when exposed to dissolved mercury. No relationship between mercury concentration and size was evident when mercury was obtained from food (Fig. 5ab).

Obviously evidence conflicts between field and laboratory observations, and between work with small and large species. Further studies with mercury regulation in larger animals, more knowledge of

the dynamics of intraorganism tissue fluxes of mercury and an understanding of the role of long-term macrofaunal methylation of this element may clarify some of the contradictions.

Mercury Dynamics in Ala Wai Canal

An obvious pattern of temporal variation in total mercury concentrations was observed during the 1973-74 sampling period within samples of the deposit feeders N. succinea and P. debilis collected from the study area in Ala Wai Canal. The model describing mercury dynamics in the shrimp suggested the biotic variations in mercury concentrations showed a direct correlation with the occurrence of rainfall (Figs. 15 and 16). Rainfall might influence mercury concentrations in detritus feeders in a secondary way by decreasing salinity, changing water temperatures or affecting mercury concentrations in the food of the two species being studied. Other studies have attributed fluctuations in biotic metal concentrations to changes in one or more of these parameters:

- 1) Food. Knauer and Martin (1973) observed that metal concentrations in phytoplankton increased after periods of upwelling along the California Coast. Bryan (1973) suggested temporal variations in metal concentrations in scallops occurred as a result of variations in the metal content of their phytoplankton food. In Ala Wai Canal, however, there was no correlation between temporal variations in total mercury levels in estuarine sediment and variations in sediment-ingesting deposit feeders. These results

substantiated laboratory observations that estuarine sediment-bound mercury had little influence on mercury concentrations accumulated by the deposit feeders.

- 2) Salinity. Bryan and Hummerstone (1973a,b) attributed spatial variations in the Mn, Zn and Cd content of the estuarine polychaete Nereis diversicolor to spatial variations in the salinity of the estuary. Wolfe and Coburn (1970) gave similar reasons for spatial fluctuations in ¹³⁷-Cs content of the clam, Rangia cuneata. Although salinity in Ala Wai Canal fluctuates with rainfall, laboratory experiments implied that salinity was probably not a significant factor determining mercury accumulation by the shrimp under conditions characteristic of the canal. However, decreases in salinity during rainstorms may have inhibited mercury uptake by the worm.
- 3) Temperature. The net accumulation of metals by aquatic poikilotherms increases as a function of temperature (Duke, Willis, Price and Fischler, 1969; Wolfe and Coburn, 1970; Pentreath, 1971). Atmospheric temperatures from near Ala Wai Canal varied inversely with the general trend of biotic mercury concentrations (higher temperatures occurred from July through October than from November through March). Thus, while the small seasonal temperature changes may have increased the variability in concentrations of metals observed in the deposit feeders, temperature alone could not have accounted for the general trend observed in the data.

Rainfall may have directly influenced available concentrations of mercury in Ala Wai Canal: a) through mercury contamination in the rain itself; b) by depositing terrestrial sediment in Ala Wai Canal which contained mercury bound in a biologically available manner; or c) through a small increase in dissolved mercury originating from urban storm runoff.

Because of the presence of active volcanoes the atmosphere of the Hawaiian Islands contains unusually high concentrations of mercury (Eschelmann, Siegel and Siegel, 1971). Mercury in rainfall on the island of Hawaii, where volcanoes remain active, has been reported in concentrations as high as 0.25 ppb. On the island of Kauai rainfall mercury concentrations of 0.15 ppb have been measured (Siegel, 1973). The concentrations of dissolved mercury in rainfall on Oahu may have contributed to the mercury burden of the biota in Ala Wai Canal. However, such concentrations were probably not large enough to totally explain the mercury peaks observed in the species studied here. Mercury concentrations in the sediment of Ala Wai Canal exceeded concentrations in the sediment of other estuaries on Oahu subject to similar rainfall (e.g. Kahana Bay; West Loch Pearl Harbor). Therefore it is likely that Ala Wai Canal receives mercury from a source(s) not present in the other estuaries. The source(s) of mercury is probably associated with the unique urban location of the canal.

During rainsotrms runoff from a large, densely populated urban area enters Ala Wai Canal directly (see description of the study site p. 12 - 16). The smaller fractions of particulates found on

urban streets can contain as much as 1000 ppb mercury (Sartor and Boyd, 1972). Smaller particles are usually the fraction of street sediment most easily washed by rainfall from city streets (Sartor and Boyd, 1972). The suspended sediment load in urban street runoff increases asymptotically with the intensity of rainfall (Sartor and Boyd, 1972); i.e. the sedimentary load of the runoff increases with increased rainfall intensity to the point where the maximum possible amount of sediment has been washed from the street. Any increase in intensity beyond that point removed little or no further sediment. Predicted peak mercury concentrations in the shrimp showed a similar asymptotic relationship to the amount of rain which fell in a given storm in the urban Ala Wai watershed (Fig. 17). Temperature, the effects of the tide on the residence times of storm runoff in the canal, and variation in the intensity of rainfall during each storm probably all contributed to the large amount of variation in these data. However, the similarity between the nature of the relationship describing sediment loads in urban street runoff as a function of rainfall of different intensities, and the general relationship describing peak mercury concentrations in the shrimp as a function of precipitation, suggested the increase in biologically available mercury in Ala Wai Canal occurred as a result of mercury associated with sediment originating from urban streets.

The previously described laboratory experiments (Table 9) have shown that the biological availability of particulate-bound mercury may increase when mercury is bound by processes other than organic complex formation. Such low affinity binding phenomena may

characterize the way mercury is bound to terrestrial sediment occurring in urban storm runoff. Two factors suggested storm runoff-related increases in total mercury in shrimp did not occur by way of this phenomenon:

- 1) The rate constant derived for the uptake of dissolved 203-Hg by the shrimp provided an extremely close fit to the observed mean mercury concentration in the shrimp in the January 31 test of the model of mercury dynamics in Ala Wai Canal (Appendix A). The rate constant describing 203-Hg accumulation by the shrimp from terrestrial sediment (0.19 day^{-1}) was much smaller than was the rate constant describing accumulation of the dissolved ion (0.55 day^{-1}), i.e. uptake from sediment was much slower than uptake from solution. To attain the mercury concentration observed on January 31, terrestrial sediment mercury concentrations greater than 3000 ppb would have been necessary. Measurements of mercury concentrations in the sediment of Ala Wai Canal never approached this value. In order that the calculated value fall close to the observed concentration of the metal on this data the shrimp must have obtained mercury from a dissolved source, or a combination of dissolved and bound mercury, but not from bound mercury alone.
- 2) Two separate tests of the model of mercury dynamics showed that after the end of a rainstorm, mercury was lost from P. debilis at a rate described by efflux experiments in the laboratory. Had backflux of the metal occurred during the

period of net efflux (i.e. had any significant gross influx occurred due to assimilation of dissolved mercury or mercury bound to terrestrial sediment) mercury concentrations predicted from efflux equations would have significantly underestimated observed concentrations. Since such an underestimation was not observed it appeared that at the end of each rainstorm all biologically available mercury was flushed from the canal. Terrestrial sediment is not flushed from the canal. Terrestrial sediment is not flushed from Ala Wai Canal after each rainstorm. The depth of runoff sediment accumulated in the study area of Ala Wai Canal over the 18 month period of this study was as high as 30 cm. Thus it appeared that dissolved mercury alone was responsible for the mercury concentrations observed in the shrimp.

There are two reasons why the increased biological availability of terrestrial sediment-bound mercury observed in the laboratory may not have affected total mercury levels in shrimp from Ala Wai Canal:

- 1) The method used to bind ^{203}Hg to sediment in the laboratory may not have simulated the physico-chemical form by which mercury was bound to the sediment entering Ala Wai Canal in storm runoff; and
- 2) In the terrestrial sediment laboratory experiments only terrestrial material was available for ingestion by P. debilis. However, in the field shrimp are probably selective detritus feeders, choosing only the most heavily organic

particles as food (personal observation). In Ala Wai Canal shrimp may ingest few terrestrial particulates (until an organic coat develops around these particles) and be exposed to little of the bound ion when it occurs in its most biologically available form.

Conclusions to this point indicate that increases in biotically available mercury were associated with the influx into Ala Wai Canal of storm runoff (and probably sediment) from urban streets. Biotic concentrations of mercury observed in the estuary did not appear to occur as a result of secondary effects of the runoff on accumulation of the metal. Little of the mercury content in *P. debilis* appeared to originate from the sediment-bound pool (terrestrial or estuarine) of the metal. The model showed that the pulse of available mercury occurring in Ala Wai Canal appeared to be flushed out of the estuary after each rainstorm and that the rate constant of dissolved ^{203}Hg uptake fit well with observed field observations of total mercury accumulation by the shrimp. These conclusions all indicate an increase in dissolved mercury occurred in conjunction with the influx of storm runoff into Ala Wai Canal.

It is likely that dissolved mercury in such a pulse does not occur in divalent form. The rapid rate by which divalent mercury is bound by organic material and sediment would prevent this form of the ion from remaining in solution for a biologically significant period of time.

Salinity changes have been shown to affect sedimentary adsorption, desorption and dissolution of manganese (Lentsch et al, 1971),

and other divalent cations (Forster et al, 1971) in estuarine ecosystems. Desorption of some forms of particulate-bound mercury appears to occur in the presence of increasing levels of chloride or increasing salinity (Feick, Horn and Yeaple, 1972; Reimers and Krenkel, 1972). Reimers and Krenkel (1972) showed that mercury was more easily desorbed from sands than from clays, while no desorption of organic complexes of the metal occurred. The desorbed form of mercury in seawater appeared to be a chloride complex of the ion $(\text{Hg}(\text{Cl}_4)^{-2})$, which showed little resorption to particulates in saline water (Reimers and Krenkel, 1972). Thus desorption of a portion of the mercury bound to inorganic terrestrial particulates could have occurred as the sediment in the storm runoff (freshwater) entered the saline waters of Ala Wai Canal. If the desorbed mercury occurred in a form that was not resorbed in the estuary, sufficient exposure time for biotic uptake of the metal could occur, and would explain all the results observed in these experiments. Because of the ability of the worm and shrimp to accumulate dissolved mercury to concentrations a hundred times (or more) greater than observed in seawater, a pulse of mercury less than 1 ppb could account for the concentrations of the ion observed in the shrimp. Measurement of dissolved mercury in the study area used in this work was impeded by interference from aromatic hydrocarbons in the street runoff. However, at the end of a storm in July, 0.24 ppb was measured in Ala Wai water. Siegel (1973) detected 0.6 ppb dissolved mercury in March, 1971, below Manoa-Palolo stream. Mercury in the water of the

canal was not detectable between storms in this study or in the study conducted by Siegel (1973).

The degree to which these conclusions apply to mercury concentrations in the polychaete, N. succinea, is not certain. The worm was probably a less selective detritus feeder than was P. debilis (Goerke, 1971). The uncertainty of the rate of mercury exchange between the water column and the interstitial habitat of the worm make predicting mercury dynamics in this species difficult. However, in instances where both organisms were sampled simultaneously, the same general trends in mercury concentrations seemed to occur (e.g. Fig. 14). Thus, while desorbed, dissolved mercury may not have been the sole source of this metal for the worm, it probably affected the values observed in worm samples collected from Ala Wai Canal.

The model describing temporal mercury dynamics in the shrimp (Figs. 15, 16; App. A), P. debilis, was important in defining the principal source of mercury for shrimp in Ala Wai Canal. The simulation curve derived from the model illustrated the extremely dynamic nature of biotic mercury concentrations in organisms from Ala Wai Canal. Interpretation of the significance of mercury contamination problems may be significantly influenced by the temporal variations in biotic mercury concentrations. Conclusions drawn from samples of biota from the canal taken only at one time of the year could have resulted in significant misinterpretations of the meaning of the observed mercury concentrations. Measurement of mercury concentrations in water and/or in sediment alone may not detect factors resulting in changes in biotic mercury concentrations. This was obviously the

case in Ala Wai Canal where the largest and most obvious physical pool of mercury (the sedimentary bound pool) had little effect on mercury concentrations in detritus feeders; while small short term changes in dissolved mercury appeared to be primarily responsible for observed biotic mercury fluctuations. Previous experiments have emphasized that important changes in biotic mercury uptake may also occur without changes in concentrations in physical mercury pools if the biological availability of the metal changes. Detection of changes in the biological availability of mercury, and interpretation of the significance of observations of mercury in physical pools of the ion are difficult using analytical techniques alone. The relatively rapid flux rates of mercury in the worm and the shrimp used in this study made these species excellent indicators of even short-term changes in the biological availability of mercury in Ala Wai Canal. These results show that indicator species can be an important aid in interpreting the biotic importance of complex physical processes; and that such organisms may be useful in enhancing an understanding of the role of these processes in ecosystems contaminated with mercury.

Trophic Transfer of Mercury to a Predaceous Decapod

The relative contribution of uptake from food and uptake from water to the total body burden of metals in aquatic organisms is a current subject of controversy (Pentreath, 1973a). After a laboratory study of uptake of $^{137}\text{-Cs}$ and $^{65}\text{-Zn}$ from both sources by the fathead minnow, Lipke (1971) concluded that the dominant source will vary

according to concentrations in food, and that cases should be considered individually. Preston (1971) showed oysters could accumulate ^{51}Cr from both phytoplankton and water, but concluded higher concentrations in the food of the oysters probably made that the dominant source. Baptist and Lewis (1969) found that, at steady state, mummichog (*Pisces*) reached whole body concentrations of ^{65}Zn and ^{51}Cr that were 9.7% and 42.8%, respectively, of the concentration of the two nuclides in post-larval fish upon which they fed. However, Hoss and Baptist (1971) found mummichog accumulated ^{65}Zn to concentrations five times that in their food when fed labelled shrimp. More recently Pentreath (1973a,b,c,d) has concluded that body concentrations of Fe, Mn, Zn, and Co in both plaice and thornback rays come primarily from food. In these experiments isotopes of the four ions, in dissolved form, were concentrated by the fish by factors which represented only a small fraction of the total body pool of the elements. Plaice were fed labelled nereid polychaetes and, after four days exposure to the labelled food, normalized organ concentrations of the nuclides showed that whole body predator retention of ^{65}Zn was 36%, ^{59}Fe was 30%, ^{54}Mn was 40% and ^{58}Co was 3.5%. The turnover time for each ion was then calculated assuming an exponential accumulation of the ion at this retention rate (Pentreath, 1973b,c). Jernelov and Lann (1971) have cited experiments showing 10% of the mercury in the prey of predator fish was retained by the predator. From this they calculated that the contribution of food to mercury concentrations in full-grown pike should equal the mercury concentration in the prey of the animal

(assuming 10% of the mercury in each gram of food ingested by pike over its lifetime is assimilated by the predator).

In contrast to these results Polikarpov (1966) concluded that the primary mode by which cations are accumulated by aquatic organisms is through uptake from water. Harrison (1973) has shown that clams exposed to ^{60}Co labelled food and water accumulated no more of the nuclide than clams exposed to the dissolved form of the nuclide alone. Experiments cited in the previous section illustrated that the primary source of mercury for two detritus feeders in Ala Wai Canal was dissolved mercury.

In the study reported here ^{203}Hg labelled polychaetes were fed to predaceous crabs. Whole body activity in the crabs appeared to reach steady state after six to nine days (Fig. 18). Thus Jernelov and Lann's (1971) assumption that a given proportion of the mercury in the prey would be accumulated indefinitely by the predator did not hold in these experiments. After dissection of these crabs a regression equation was derived for each of four organ groups, describing the concentration of ^{203}Hg attained by each organ group as a function of the mean ingestion rate over the 13 day exposure period (Table 15). No work has been found on the ingestion rate of portunid crabs in nature, but this rate probably lies between 1% and 3% of their body weight per day. Terrestrial isopods have been shown to ingest food amounting to 0.8% of their body weight per day (Hubbell, Sikora and Parks, 1965). Demersal fish are said to ingest 2-3% of their body weight per day in food (Pentreath, 1973c). Table 20 lists the concentration of mercury that would be attained, relative

Table 20

The concentration of ^{203}Hg attained in crab organs relative to the concentration of the nuclide in the food (worms) of the crabs at ingestion rates of 1%, 2%, and 3% body weight per day, after an exposure to labelled worms for 13 days. Values are calculated from regression equations in Table 15. The concentration of ^{203}Hg in the food of the crabs was 71 ± 20 ppb.

Organ	$C_{\text{organ}}/C_{\text{food}}^*$ at given ingestion rate		
	1% day ⁻¹	2% day ⁻¹	3% day ⁻¹
Chela muscle	0.033	0.066	0.10
Body muscle	0.086	0.17	0.26
Gill	0.16	0.36	0.56
Viscera	0.70	1.34	2.00

*The concentration of ^{203}Hg in the organ of the crab relative to the concentration of the nuclide in the food of the crab.

to mercury concentration in food, by each organ group of the crab, under the conditions of this experiment, at ingestion rates of 1%, 2% and 3% per day. In all organs except viscera the concentration of mercury that would be accumulated by the organ would be less than the concentration of the ion in food, and in all cases but one the ratio between the two would be less than 0.5. Mercury concentrations in viscera were affected by an undetermined amount of radioactive food in the gut. Obviously, under these conditions, food chain bio-magnification did not occur at this trophic level.

When T. crenata were fed 203-Hg labelled worms the viscera of the crabs contained higher concentrations of the label than the gills or muscle. In contrast, the highest concentrations of mercury in crabs (Uca pugilator) exposed to the dissolved ion were found in the gills (Vernberg and Vernberg, 1972). Thus relative mercury concentrations in gills and viscera may serve as indicators of the availability of mercury to a crab from food or from water.

Mercury concentrations in the gills of crabs collected from Ala Wai Canal between August and February were extremely variable (Fig. 21). High tissue concentrations occurred in the gills of a single crab collected on November 30 and in the gills of crabs collected on February 6 (Fig. 21, Table 16). Both samples were taken during rainstorms in the Ala Wai Canal watershed. Lower concentrations of mercury were found in the gills of crabs collected on November 14 and December 5. Both these samples were collected several days after any rain had fallen in the watershed. Some of the mercury in the gills may have originated from food (see Fig. 20),

but low total mercury concentrations in viscera implied this amount was probably very small. Like the studies of mercury dynamics in detritus feeders, these data suggested that during periods of storm runoff concentrations of dissolved mercury in Ala Wai Canal increased. Previous experiments (p. 155) indicated concentrations of dissolved mercury increased less than 1 ppb during such mercury pulses. This mercury appeared to be rapidly concentrated by the gills of T. crenata to concentrations exceeding that of the dissolved ion by many times, and rapidly lost by the gills after rainfall ceased.

Flux rates of mercury in the viscera of aquatic animals are rapid, as they are in the gills (Romeril, 1971; Harrison, 1973; Pentreath, 1973b,c). However, while gills concentrate mercury many times over the concentrations of the dissolved ion, the viscera are much less efficient in assimilating mercury from food (Table 20). Visceral mercury concentrations on the five dates crabs were collected from Ala Wai Canal followed the same general trend as mercury concentrations in shrimp and worms on those dates, i.e. concentrations of total mercury were similar on August 15 and November 14, but increased at each sampling date between November 30 and February 6 (Fig. 21). However, the extreme variations in mercury concentrations observed between November 30 and December 5 in the shrimp (and in crab gills) were not obvious in samples of crab viscera (Figs. 16 and 21).

Total mercury concentrations observed in gills or viscera of crabs from Ala Wai Canal, like mercury concentrations in detritus feeders, probably seldom represent steady state values due to

periodic fluctuations in biologically available mercury within the canal. Since the gills respond directly to dissolved mercury concentrations in the environment the mercury content of this organ was quite labile. The dynamics of the metal in crab gills probably are similar to those observed in the smaller, lower trophic level organisms. Fluctuations in the mercury content of crab viscera, however, will likely be more damped, due to the lower efficiency of accumulation of mercury from food. Such fluctuations may also be damped by temporal integration of fluctuations in the mercury content of the food resulting from the time necessary for digestion and assimilation of food by the predator (i.e. residence time in the digestive tract).

Ion flux into the muscle of aquatic organisms tends to be slower than flux rates in organs exposed directly to mercury (Bryan, 1973; Harrison, 1973; Pentreath, 1973b). In T. crenata, mercury concentrations in the muscle of the crab represent a complex integration of mercury influx from the gills, where concentration of the metal is subject to strong temporal fluctuations; and an influx of mercury from the viscera, where concentrations of mercury were generally lower than those observed in the gills (Fig. 21) and fluctuations in metal content are probably damped. Several factors suggested that mercury from the gills (i.e. dissolved mercury) was the major contributor to the body muscle burden of mercury observed in T. crenata from Ala Wai Canal:

- 1) The organ distribution of ^{203}Hg in crabs fed labelled worms followed the order viscera >> body muscle > chela muscle.

In field samples, however, body muscle concentrations of total mercury exceeded concentrations in the viscera. A difference in the primary source of mercury in these two instances could have resulted in the differences observed in the distribution of the metal between organs of the crab.

- 2) At whole body steady state the concentration of ^{203}Hg in the muscle of crabs fed labelled worms for two weeks was only a small proportion of the concentration of the label in the food (Table 15). Total mercury concentrations in potential prey organisms from Ala Wai Canal were never sufficiently high to account for more than a minor portion of the total mercury concentrations observed in the body muscle of T. crenata from the canal.
- 3) The two previous cases assume the concentrations of ^{203}Hg in body muscle observed after the 2-week feeding exposure represented steady state concentrations. Long term exposures to mercury could result in more efficient assimilation of the metal by crab muscle than was observed here. If this were the case determination of the relative contribution of food and water to muscle burdens of mercury would require comparing laboratory and field data over similar exposure periods. Between November 14 and December 5 total mercury concentrations in crab body muscle increased by 10 ppb (Fig. 21). To attain such an increase from food alone would require exposure to an increase in the ingestion rate of

mercury of 114 ng Hg/gm crab/day. (From Figure 20c, a 10 ppb concentration of mercury in body muscle occurs at an ingestion rate of 1.60 gm worm/gm crab/day. At the 203-Hg concentration of 71 ppb in worms used in these experiments this is equivalent to $71 \text{ ng Hg/gm worm} \times 1.60 \text{ gm worm/gm crab/day}$ or 114 ng Hg/gm crab/day.) Yet the difference in total mercury concentrations in viscera between the two dates (5 ppb - Fig. 21) indicated the ingestion rate of mercury was only 8 ng Hg/gm crab/day greater on December 5 than on November 14. These calculations estimate that the ingestion rate of mercury by the crab was only 7% of the rate necessary to account for the increase observed in total mercury concentrations in body muscle. The ingestion rate of mercury, as indicated by concentrations of the metal in the viscera, may have fluctuated over the period from November 14 to December 5. However large increases in mercury concentrations in the viscera (which would be necessary if the mercury in the body muscle came from food) were not observed in any of the crab samples collected during this study (Table 16). Thus it appears that the major portion of mercury responsible for the increased concentration observed in the body muscle entered that organ by way of a source other than the viscera.

The experiments with organisms from Ala Wai Canal allow several conclusions about the relative importance of food and water as biotic sources of mercury:

- 1) Dissolved mercury was the primary significant source of this metal for shrimp and possibly for polychaetes in Ala Wai Canal.
- 2) At the next highest trophic level, predaceous crabs appeared to assimilate more mercury from their food in laboratory experiments than did the detritus feeding shrimp and worm (compare Tables 20 and 5). Because of the physiological complexity of interorgan translocation of mercury in these crabs, and because observed concentrations of mercury in the organs of crabs collected from Ala Wai Canal probably seldom represented steady state values it was not possible to precisely quantify the relative contribution of ingested and dissolved sources of the metal to mercury concentrations in this predator. Nevertheless, several factors suggested dissolved mercury contributed the major proportion of the body burden of this pollutant to the crab.
- 3) Mercury concentrations in aquatic organisms collected from field study sites do not represent only trophic influx of mercury into the organisms. Because of the physiological ability of aquatic species to concentrate mercury from solution, dissolved mercury may also contribute significantly to observed body burdens of the metal. Concentrations of mercury in species from different trophic levels, then, cannot be used to compare the concentration of mercury along food chains (e.g. Johnels et al, 1967) unless differences in the ability to assimilate dissolved mercury are taken into account.

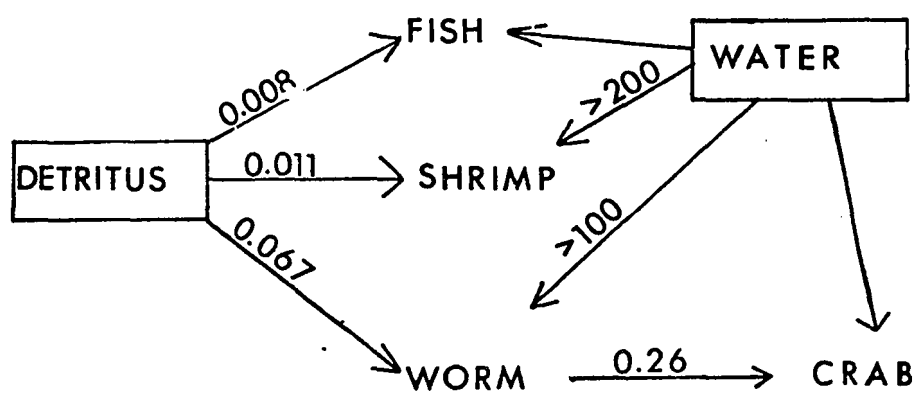
The efficiency of trophic assimilation of mercury and the efficiency of uptake of dissolved mercury within the simple detritus based food chain considered in this study is shown in Figure 29. Trophic assimilation of this metal was extremely inefficient at all trophic levels considered, relative to accumulation of the dissolved ion. Biomagnification of mercury concentrations by trophic transfer of the metal from one trophic level to the next was not observed at any level of this simple food chain. Although food chain biomagnification of heavy metals has been a widely accepted theory for over five years, this study and other recent work (e.g. Kneip and Lauer, 1973) indicate this phenomenon probably occurs only exceptionally, if at all, between aquatic trophic levels.

Population Dynamics - Nereis Succinea

An understanding of the transfer of any material between two trophic levels may be enhanced by knowledge about the population dynamics of the prey population. The results of studies of population dynamics of N. succinea showed that the worms seemed to breed twice a year (Figs. 22 and 23). Worms heavier than 120 mg did not comprise a significant proportion of the population until several months after a new cohort of young individuals appeared in population samples. The heaviest rate of predation appeared to occur on these heavier individuals.

Selective predation mainly upon worms larger than 120 mg has some significance toward the dynamics of the trophic transfer of mercury. The mercury content of N. succinea varied as a function of the size

Figure 29. A comparison of the efficiency of mercury transfer from food versus transfer from water along a simple detritus-based food chain. Fish, shrimp and worm values represent steady state whole body concentrations (dry weight) relative to mercury concentrations in food (dry weight) or water. Crab values represent muscle concentrations of mercury at whole body steady state relative to mercury concentrations in food at an ingestion rate of 3% body weight per day.



of individuals when the source of the ion was dissolved mercury (Fig. 5a). Smaller individuals also lived closer to the sediment surface than did larger worms (personal observation). Thus fluctuations in mercury content in the water column that do not penetrate deep into the substrate may still affect smaller N. succinea. Four samples taken on May 16, 1973 indicated smaller worms had higher concentrations of mercury than larger individuals at this time (Table 21). In predation experiments smaller individuals suffered little predation. Thus any transfer of mercury from N. succinea to its predators may occur primarily when the concentration of mercury in the food of the predator is at a minimum.

The population dynamics of N. succinea and the selective predation upon only larger worms also suggested that during possibly as much as one-third of the year (four months) there is little flux of mercury from N. succinea to its predators. Only as a cohort grows to the point where individuals exceed 120 mg does that flux occur.

Physiological Regulation of Mercury by Estuarine Organisms

Regulation of the Uptake of Dissolved Mercury

While many studies have considered uptake of toxic non-essential cations, like mercury, few workers have studied the physiological mechanisms by which such ions are accumulated. An understanding of such mechanisms might facilitate prediction of the relative importance of various sources of mercury available to aquatic biota. A number of the experiments in the research reported here can be interpreted in terms of the physiological regulation of mercury by the animals involved.

Table 21

Total mercury in four samples of N. succinea taken May 16, 1973

Number of worms per sample	Mean weight (mg)	Concentration of total Hg (ppb)
70	11.4	77
63	13.3	89

6	212	5
8	185	10

Dissolved mercury was accumulated by both N. succinea and P. debilis to concentrations more than 100 times higher than the concentration of the ion in seawater (Table 2, Fig. 4). Because of the strong affinity of mercury for organic compounds (Vallee and Ulmer, 1972), this ability to accumulate the metal could result simply from adsorption to the surface of animals exposed to dissolved mercury. This appears to be the case with the accumulation of dissolved Zn by some species. Fowler and Small (1969) found that dead euphausiids accumulated as much 65-Zn from seawater as did living animals. Cushing and Watson (1968) discovered that formalin-killed plankton accumulated more 65-Zn from the Columbia River than did living plankton. However, the uptake of 203-Hg by formalin-killed polychaetes occurred at an insignificant rate compared to the uptake rate of the ion by living worms (Fig. 28). This suggested the accumulation of mercury by the worm may have been the result of more than physical adsorption alone. Formalin fixation may have affected the nature of the biochemical sites to which mercury binds. Thus autoradiographs of living N. succinea exposed to dissolved 203-Hg were conducted. These procedures showed that heavy concentrations of label could be observed in the peripheral epithelial cells of the worm (Plate 1). However, nearly all of this portion of the label was inside the cuticle. Significant amounts of label also appeared in internal organs of the worm and in cells of the alimentary tract (Plate 1). Preparation of the worm tissue prior to and after sectioning may have resulted in some desorption of mercury from the worm. Thus surface adsorption may account for a portion of the mercury concentrated by

N. succinea, but most of the metal accumulated by this species appeared to be absorbed across the cuticular layer and/or the lumenal membrane of the gut into the tissues of the animal.

The rate at which ^{203}Hg was taken up by N. succinea was dependent upon the concentrations of the dissolved ion in seawater (Fig. 28). The shape of the concentration dependence curve as illustrated in Figure 28, was characteristic of the transport of many ions (Neame and Richards, 1972) and implied that absorption was at least partially dependent on at least one saturable, possibly carrier facilitated, process. Since mercury has no known biological function it is possible that the carrier involved in absorption of the metal is primarily responsible for the transport of another ion(s), and that mercury is transported because of its structural resemblance to the normal substrate. Similar hypotheses have been used to explain biotic uptake of other cations (e.g. the uptake of Sr^{++} is thought to be by way of the process responsible for Ca^{++} uptake - Polikarpov, 1966). Experiments determining if other ions compete with mercury transport could illuminate such a process.

Once mercury was accumulated by either the worm or the shrimp it is likely that most of the metal taken up became bound to proteins within the organism. Vallee and Ulmer (1972) have cited the affinity of mercury for carboxyl groups and histidyl protein side chains. They also emphasized the strong affinity of mercury for cysteinyl side chains of proteins, and the ubiquity of Hg-S interactions within living systems. Ukita (1972) showed that all the mercury in rat red blood cells was bound to the cysteinyl fraction of the globin fraction

of the hemoglobin. The largest proportion of ^{203}Hg in N. succinea exposed to the dissolved ion existed in a slowly exchanging, alcohol insoluble pool (Fig. 12; Table 12, 19). Both ethanol insolubility and slow exchange could result from mercury complex formation with large proteinaceous macromolecules. This binding appeared to be a dynamic process rather than an irreversible phenomena, since depletion of the slowly exchanging ^{203}Hg occurred after long periods of efflux.

Bryan (1971) has suggested that the polychaete N. diversicolor may regulate its body burden of zinc (an essential ion) at somewhat similar levels regardless of the concentration of the ion to which the worm is exposed. Nereis succinea did not regulate the size of its body burden of mercury. Up to dissolved concentrations of 10 ppb ^{203}Hg the whole body concentration of mercury in the worm increased as a power function of dissolved mercury concentration (Fig. 4). The worms reached a steady state whole body concentration of mercury after sufficient exposure to a given concentration of the ion (Fig. 3). This steady state was probably not the result of a saturation of all available proteinaceous binding sites, because its magnitude was concentration dependent (Fig. 4). Thus the steady state was most likely the result of a dynamic balance between the influx and efflux of mercury.

The efflux experiments with N. succinea and P. debilis indicated that mercury existed within at least two biochemical compartments with both species. A typical two-compartment model (Ruzic, 1972) which might explain how these organisms achieved a concentration

dependent dynamic steady state, at organismic mercury concentrations 100 times (or more) greater than the concentration of the dissolved ion is shown in Figure 30. The specific biochemical processes governing mercury accumulation are more complex than can be readily resolved by efflux experiments or accumulation experiments alone. Each of the two compartments described in the model presented here are probably composed of biochemical subcompartments. The analysis of the simple two compartment model which follows was undertaken to facilitate a general understanding of why the worm and the shrimp concentrate dissolved mercury, without thoroughly defining the specific biochemical or transport processes involved. In Figure 30, then, compartment 2 represents a large, slowly exchanging, bound pool of mercury, while compartment 1 represents a more rapidly exchanging ethanol-soluble pool of the ion. The rate constants describing loss by way of the fast component of efflux (k_f) and the slow component of efflux ($k_s = k_{21}$) are listed in Table 12.

Efflux of mercury can also be observed from the time course of mercury accumulation in Figure 3. In these experiments the gross rate of influx of mercury was held constant. The accumulation of mercury by the organism followed an asymptotic function over time because the efflux rate of the ion increased exponentially as a function of the mercury concentration within the organism. Steady state occurred when the efflux rate equaled the influx rate of the metal. By subtracting $C_{ss} - C_t$ (see p.127) one can observe the nature of the efflux process which determined the time course of accumulation, and calculate the rate constant(s), k_a , describing

Figure 30. A model of the regulation of mercury uptake by the polychaete worm N. succinea where

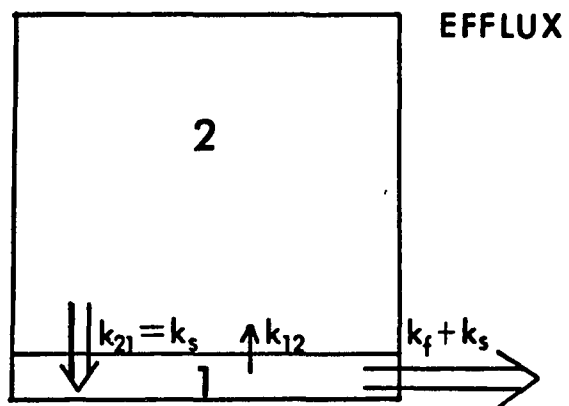
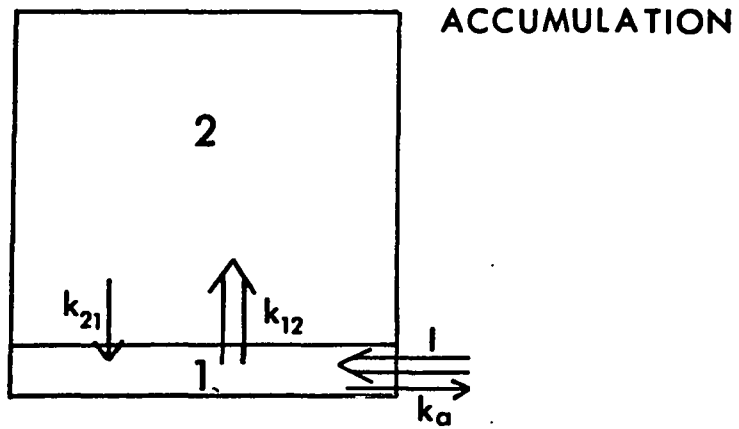
I = the gross influx rate of mercury (a constant);

k_a = the rate constant of loss as observed during accumulation;

$k_f + k_s$ = the two component loss observed during efflux experiments alone, where k_f = the loss from the short-lived pool (compartment 1), and k_s = the loss from the long-lived pool (compartment 2);

$k_{21} = k_s$ = the rate constant describing mercury movement from compartment 2 to compartment 1;

k_{12} = the rate constant describing mercury movement from compartment 1 to compartment 2.



that process. The rate constant(s) observed during ^{203}Hg uptake by N. succinea and P. debilis are also listed in Table 12. The efflux rate observed during ^{203}Hg accumulation by the shrimp followed a single exponential. However, accumulation of ^{203}Hg by the worm appeared to be governed by an efflux rate that was the sum of two mono-exponential processes. The shorter-lived process was small (it contributed to only the initial 25% of efflux during accumulation) and extremely rapid ($t_{1/2} = 0.0067$ days). Extremely rapid exchange of this nature has been shown to be characteristic of surface adsorption phenomena (Neame and Richards, 1972). For the purposes of this general discussion this component of loss will be considered as characterizing a sub-compartment within compartment 1 of the model.

The rate constant(s) of loss determined during accumulation (k_a) was more than 20 times larger than the rate constant describing efflux from the large bound pool of mercury (k_s) in both the shrimp and the worm (Table 12). That is, during accumulation, efflux increased more than 20 times more rapidly as a function of the concentration of mercury in the animal than it would have increased had this efflux been from the bound pool of mercury in compartment 2. Thus the time course of mercury accumulation appeared to be controlled by loss of mercury from a faster exchanging biochemical pool than the bound pool in compartment 2.

The half-life of loss from compartment 1 (as observed during efflux alone) was much shorter in both species than was the half-life of loss from compartment 2 (Table 12). If the loss of mercury which determined the hyperbolic nature of the accumulation curve was

dependent upon mercury concentrations in compartment 1 then k_a should have equaled k_f . However, the half-time of loss determined from k_a exceeded the half-time of loss calculated from k_f by 1.65 times in the worm and by 1.67 times in the shrimp (Table 12). That is, the efflux observed during accumulation was a slower function of mercury concentrations in the animal than it should have been if mercury concentrations in compartment 1 alone determined the dynamics of accumulation. This can be explained if (as described in Fig. 30), during accumulation, most of the mercury was initially assimilated into compartment 1 then rapidly transported from compartment 1 to compartment 2. Translocation of the ion from compartment 1 to compartment 2 would decrease the rate at which compartment 1 filled with mercury. For determination of the efflux rate from accumulation the influx rate must be held constant; i.e. mercury must accumulate at a constant rate within the animal. While this was true for the whole animal in these experiments it was not true for compartment 1 alone, due to translocation of mercury to compartment 2. If mercury concentrations in compartment 1 determined the time course of accumulation (i.e. if $k_f = k_a$) any reduction in the rate of increase in mercury concentration in compartment 1 would appear as a decrease in the efflux rate from compartment 1. The rate constant describing efflux as calculated from accumulation (k_a) would then be reduced and the half-time of loss from that compartment would appear longer than in efflux experiments alone. Thus, because of translocation, k_a should have been smaller than k_f if the model in Figure 30 held. Eventually steady state must have occurred in both compartment 2 and compartment 1. This could

occur by way of a concentration dependent, exponential increase in backflux from the bound compartment of mercury to compartment 1 (governed by k_{21}).

Figure 27 provides further evidence that suggests this model may generally describe regulation of mercury accumulation by N. succinea. Both the ethanol-soluble pool of mercury in N. succinea and mercury lost by the fast component of efflux made up 8.4% of the total concentration of 203-Hg in worms used in the efflux experiment (Tables 12, 19). This similarity in size suggested ethanol-soluble mercury represented that pool of mercury which exchanged most readily with the environment (compartment 1 of the model). During the time course of accumulation ethanol-soluble mercury appeared to increase gradually to a steady state (although there may be alternative interpretations of this curve, due to variation between sampling points, they would not significantly change the substance of the following discussion). Both ethanol-soluble and ethanol-insoluble mercury reached steady state at 13 hours. If the ethanol-soluble pool of mercury represented compartment 1, these results would be consistent with a net translocation of mercury from compartment 1 to compartment 2 during the period of net accumulation in the whole animal. Prior to achievement of steady state this translocation appeared to occur nearly as rapidly as assimilation of the ion - since mercury concentrations in compartment 1 increased only slowly, while whole body metal concentrations were increasing rapidly. The slowly increasing mercury concentrations in compartment 1 resulted from influx from the medium plus a slow backflux from

compartment 2 governed by k_{21} . When the rate of loss from compartment 1 via the k_f component equaled the rate of influx into that compartment via the I component the whole animal would be at steady state (since the flux rates via k_{12} and k_{21} must be equal before the k_f component could equal I). Although the binding in compartment 2 was not irreversible, k_{21} , the rate constant governing flux from compartment 2 to compartment 1, must have been very small to permit such a large accumulation of bound mercury. Laboratory experiments proved that, indeed, this was the case, since k_{21} equaled the k_s rate constant observed during efflux.

Thus the ability of these two species to concentrate mercury appeared to be greatly facilitated by the inherent affinity of mercury for organic complexes. This affinity appeared to result in a rapid binding of the mercury once it entered an organism (during net accumulation the ion appeared to be bound nearly as rapidly as it entered the organism) and a slow reversal of the binding process once mercury formed an organic complex. The rapid translocation to a bound form, and the slow reversal of the binding process allowed rapid concentrative uptake of mercury by constantly moving the ion from a dynamically active compartment to a compartment less important in controlling the dynamics of accumulation. Since the slowly exchanging bound pool of mercury made up the largest proportion of mercury in the animal, loss of the total body burden of mercury from both N. succinea and P. debilis took much longer than the time necessary to accumulate that concentration of the metal.

Results to this point then have shown that:

- 1) Dissolved mercury is concentrated by N. succinea and P. debilis to a steady state at least a hundred times greater than observed in the medium to which the organism was exposed.
- 2) The size of the steady state body burden of mercury cannot be regulated; it increases with the concentration of dissolved mercury in the medium.
- 3) The concentration of mercury by N. succinea is an assimilative process, possibly carrier facilitated, and is not totally the result of surface adsorption of the ion.
- 4) Attainment of a steady state mercury concentration in both the worm and the shrimp was governed by the kinetics of a rapidly exchanging pool of mercury within the organism.
- 5) The achievement of a concentrated steady state body burden of mercury was greatly facilitated by a) the rapid movement of mercury to a macromolecular bound pool after uptake, and b) the slow rate at which mercury was lost from this bound pool.

These results all emphasize how the inherent affinity for organic complexes of strongly electropositive divalent cations, like mercury, may result in the biotic concentration of these ions to many times higher concentrations than occur in the medium to which an organism is exposed. It is this concentrative potential that probably accounts for a great deal of the susceptibility of aquatic biota to toxic effects resulting from very low concentrations of the dissolved form of these ions.

Uptake of Ingested Mercury

Most ions to which estuarine organisms are exposed play essential roles in the life processes of the organisms. The problem facing an estuarine species in terms of these ions is primarily one of regulation of the proper biochemical concentrations to maintain necessary functions. Unlike these essential ions, mercury has no known biological function. Dissolved mercury in all its chemical forms is extremely toxic to aquatic biota. The LD_{50} of dissolved divalent mercury for the polychaete *N. succinea* was 65 ppb or 325 nM (Fig. 6). Most aquatic organisms studied show some effects after sufficient exposure to dissolved divalent mercury in concentrations greater than 200 ppb or 1 μ M (D'Itri, 1973).

In terms of ionic transport and regulation, the development of mechanisms which would maximally avoid accumulation of naturally occurring divalent toxins (such as mercury) may have played a role in determining the evolutionary success of some estuarine species. At the same time these organisms must have maintained mechanisms allowing accumulation of sufficient amounts of more essential but similarly divalent cations (e.g. Ca^{++} , Mg^{++} , Mn^{++} , or Zn^{++}).

It is obvious that the organisms included in this study had little control over the body burden of mercury they accumulated when presented with the ion in dissolved form. However, the chemical nature of mercury is such that little dissolved mercury is present under most circumstances in most aquatic ecosystems. The most common physico-chemical forms of dissolved mercury are removed rapidly from solution by sediment or any other available particulate organic

matter (including biota). Because of this affinity for organic particulates mercury concentrations in the food of aquatic biota are probably always much higher than the concentrations of dissolved mercury to which these organisms may be exposed. For example, the sediment of Ala Wai Canal, from which the detritus feeder N. succinea obtains its nutrition, has an average total mercury concentration of 334 ppb. This concentration is more than five times greater than the concentration of dissolved mercury at which 50% of the worms die. In fact, worms exposed to sediment contaminated with as much as 5000 ppb mercury showed no difference in growth from worms living in uncontaminated Ala Wai Canal sediment (Tables 6,7). Obviously, if this species concentrated mercury from its food to the same extent that it concentrated dissolved mercury, it would not be able to survive in the estuary where it now flourishes.

The organisms included in this study could withstand higher concentrations of mercury in their food than in water, because most of the mercury ingested by these animals passed through the digestive tract without assimilation, and was eliminated in the feces (Tables 5, 22). The concentration of 203-Hg in the feces of the polychaete, N. succinea, the poecilid fish, M. latipinnia, and the predaceous decapod, T. crenata, exceeded that in the food by at least 1.5 times (Tables 5, 22). This suggested organic matter was removed from the food, but little of the mercury associated with that organic matter was assimilated. As a result of this inefficient absorption the whole body steady-state concentration of mercury achieved in all four species was only a fraction of the concentration of mercury in

Table 22

A comparison of ^{203}Hg in the food, feces and bodies
of shrimp, worm and crab at steady state

Species	Concentration of ^{203}Hg		
	Food	Whole body	Feces
Worm	1425 ppb	22 ppb	2487 ppb
Shrimp	1172 ppb	3 ppb	
Crab	2449 cpm/mg	63 cpm/mg	6823 cpm/mg

the food they ingested. There may be several possible reasons why mercury in the food of these species was absorbed so much less efficiently than was dissolved mercury. These include:

- 1) The gut of these animals may have been impermeable to mercury. Pentreath and Jeffries (1971) have shown that the gut of plaice (Pleuronectes platessa) is impermeable to the insoluble radio-nuclides $^{106}\text{-Ru}$, $^{95}\text{-Zr}/^{95}\text{-Nb}$ and $^{144}\text{-Ce}$. However, when the polychaete, N. succinea, or the shrimp, P. debilis, was exposed to $^{203}\text{-Hg}$ labelled terrestrial sediment, feeding organisms accumulated concentrations of mercury many times greater than animals ingesting estuarine sediment. Steady state concentrations of $^{203}\text{-Hg}$ in organisms exposed to labelled terrestrial sediment still did not exceed concentrations of the nuclide in the sediment. However, the increase in availability of $^{203}\text{-Hg}$ bound to terrestrial sediment suggested that the physico-chemical form of the bound mercury, not the permeability of the gut wall of the organism, played a role in limiting mercury uptake from food.
- 2) Digestive processes may not have broken down mercury-organic matter complexes and, thus, free mercury may not have been released during digestion. When dissolved $^{203}\text{-Hg}$ was bound to either cysteine or BSA, the efficiency with which it was accumulated by N. succinea was greatly reduced. It appeared that the processes which facilitated rapid concentration of free dissolved mercury did not work when dissolved

mercury was bound to an amino acid (possibly because the electro-positive nature of mercury would be neutralized by such binding, and the amino acid-mercury complex would probably be less rapidly transferred to a macromolecular binding site after assimilation). If digestive processes in aquatic organisms broke proteins into amino acids but did not break the amino acid-mercury bond, these species would avoid the concentrative phenomena that appeared to result from the presence of unbound mercury, at no cost to nutritional efficiency.

- 3) The most heavily mercury contaminated portions of the food of aquatic biota may be those portions least efficiently digested. Greenaway (1972) has suggested that bacteria bind mercury to cellular membranes, as a means of reducing the toxicity of the ion. (Mercury toxicity is usually attributed to Hg-S interactions at the active site of enzymes important in oxidative phosphorylation, although membrane-related mechanisms have also been suggested (Vallee and Ulmer, 1972).) The heaviest concentrations of mercury in N. succinea were associated with the gut and body surface epithelium (Plate 1). The macromolecules associated with cellular membranes (and macromolecules in general) would be, energetically, the most expensive source of nutrition within a given food source. Any reduction in the efficiency with which such molecules were digested might also reduce the assimilation rate of mercury and similar

divalent toxins which tend to be primarily associated with these portions of the food.

To little is known about the digestive physiology of aquatic organisms to determine if any, or all, of these mechanisms function in nature. It seems possible, however, that there might be selective pressure toward maintenance of some such mechanisms. The food of aquatic biota present a highly concentrated source of the most toxic heavy metals, relative to the concentrations of the metals found in solution. Where a sufficient supply of essential divalent cations exists in solution some organisms which become efficient in extracting cations from food may be at a selective disadvantage, if such efficiency resulted in efficient extraction of natural divalent toxins from their most concentrated pool. Terrestrial animals and air breathing organisms must obtain essential cations from their food. In open ocean marine ecosystems mercury concentrations are higher in marine mammals and in sea birds than in most trophically similar gill-breathing marine biota (D'Itri, 1973). In Hawaii, the open coast sand crab Ocypode ceratophthalmus (which lives on beaches above the high water-line and seldom enters the water) consistently contains mercury concentrations many times higher than are observed in trophically similar aquatic species from the same area (Water Resources Research Center, 1972).

These terrestrial portions of marine food webs provide evidence which suggests that when a dissolved source of essential cations is lacking, food chain biomagnification of toxic metals may occur. However, when a sufficient supply of essential cations exists in

solution, food chain biomagnification occurs only exceptionally, if at all (Kneip and Lauer, 1972). The lack of food chain biomagnification of metals between aquatic trophic levels may have resulted from selective pressure toward a reduced efficiency in accumulating cations from food in instances where such ions are available from sources where the most toxic cations are less concentrated.

SUMMARY

In terms of the questions asked at the beginning of this report, the research reported here showed:

- 1) Little of the mercury bound to highly organic, estuarine sediment was assimilated by the deposit feeding worm, shrimp or fish. Large changes in mercury concentrations in estuarine sediment would be necessary to significantly affect concentrations of mercury within these organisms. Detritus feeders did not appear to cycle mercury from sediment into the macrobiotic portion of the Ala Wai Canal estuarine community.
- 2) Mercury concentrations in detritus feeders were passed on to predators in a relatively inefficient manner. At ingestion rates which probably characterize predaceous portunid crabs in nature, the muscle of the crab accumulated concentrations in the food of the crab. In Ala Wai Canal mercury from food did not appear to be the primary source of this metal for the predaceous decapod T. crenata.
- 3) Absorption of dissolved mercury appeared to be the only significant source of mercury for the shrimp, P. debilis, and possibly the polychaete N. succinea. Dissolved mercury also appeared to be the major source of mercury for the predator T. crenata. In the crab the physiology of inter-organ mercury transfer appeared to be complex. Mercury concentrations in various organs from crabs collected from Ala Wai Canal seldom, if ever, represented steady state values.

- 4) Variations in environmental parameters played a major role in determining biotic mercury concentrations in Ala Wai Canal. Biotically significant, short-term increases in dissolved mercury concentrations appeared to result from salinity-induced desorption of mercury from the sediment in storm runoff which periodically entered Ala Wai Canal. These pulses of dissolved mercury resulted in strong temporal fluctuations in biotic mercury concentrations in the Canal. Salinity changes alone, as observed in the Ala Wai estuary, appeared to have little effect on biotic mercury accumulation. Sufficient quantities of dissolved organic material existed in Ala Wai Canal during the summer to reduce the biotic availability of divalent mercury. Mercury accumulation from food by detritus feeders was increased when mercury was bound by processes other than organic complex formation. A worm and a shrimp, small indicator species, proved useful in determining and interpreting the complexities of those physical processes which were important in estuarine mercury cycling. Results from experiments with these species suggested oligotrophic aquatic ecosystems would be much more susceptible to mercury contamination than would eutrophic systems subject to a similar input of the metal. The abundance of organic matter in the sediments and water of the latter systems may bind metals in a manner which reduces the biological availability of these toxins. Likewise, mercury in oligotrophic ecosystems may exist in

physical or chemical forms readily available for accumulation by biota.

- 5) Dissolved mercury was rapidly accumulated by the species studied here to steady state concentrations 100 times or more greater than concentrations in seawater. Thus small changes in concentrations of dissolved mercury would result in large changes in biotic concentrations of the metal. The steady state concentration of mercury observed in the worm was maintained by a dynamic balance between influx and efflux. Its magnitude was dependent upon the concentration of dissolved mercury. The ability of aquatic biota to concentrate mercury appeared to be facilitated by the strong affinity of the metal for organic binding sites.

Mercury in food was inefficiently assimilated by all species included in this study. It was hypothesized that natural selection might favor inefficient assimilation of cations from food. In ecosystems where a sufficient supply of essential cations exists in solution, such inefficiency would reduce assimilation of toxic cations which concentrate in organic material.

APPENDIX

APPENDIX A

Model of Mercury Dynamics in Ala Wai Canal

This model is based on the hypothesis that the mercury concentrations observed in shrimp collected at different times from Ala Wai Canal did not represent steady state values, but represented points during periods of net accumulation or net loss of mercury in the shrimp. Using laboratory results described in Section I, mercury accumulation and loss in the shrimp can be described by the model observed in Figure 31. In that model

k_w = the rate constant describing net accumulation of dissolved mercury;

k_{es} = the rate constant describing accumulation of mercury from estuarine sediment (Since the degree of accumulation from this source is negligible - Table 3 - this process can be ignored);

k_{ts} = the rate constant describing accumulation of mercury from terrigenous sediment;

and $k_f + k_s$ = the rate constants describing loss of mercury from the shrimp.

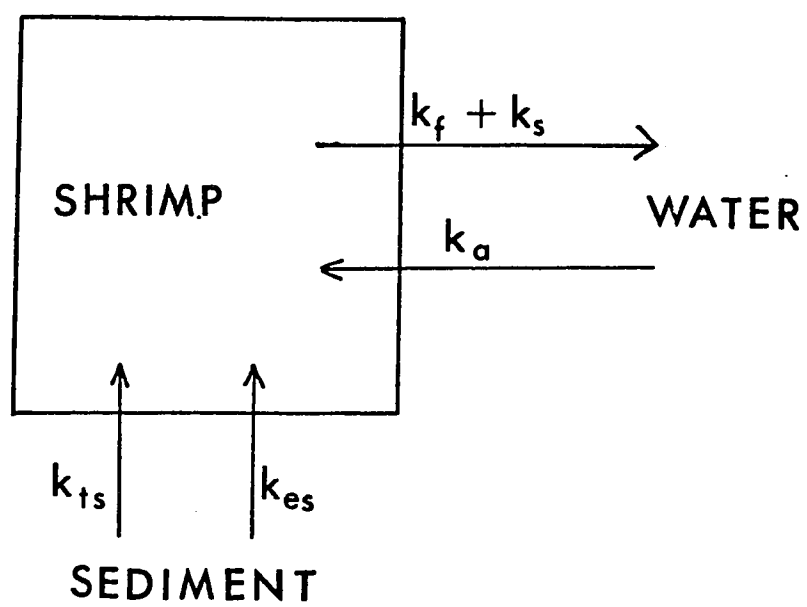
According to this model the influx of mercury into the shrimp could come from either of two sources; food or water. Accumulation from either source could be described by the equation

$$C_t = C_{ss} (1 - e^{-kt}) \quad (18)$$

where

k = either k_w (0.759 days⁻¹) or k_{ts} (3.59 days⁻¹), or a combination of the two;

Figure 31. A model of the dynamics of the accumulation and retention of $^{203}\text{-Hg}$ by the shrimp, P. debilis.



C_t = the concentration of mercury observed in field samples;

C_{ss} = the steady state concentration of mercury reached after exposure to a given concentration of mercury;

and t = the time of exposure.

The loss of mercury from the shrimp can be described by the equation

$$C_t = 0.39C_0e^{-1.49t} + 0.635C_0e^{-0.044t} \quad (11)$$

from equation (15).

These equations were used to correlate mercury concentrations in the shrimp from Ala Wai Canal with rainfall in the watershed of the canal. The simplest hypothesis with which the model might be used to test any correlation between rainfall and the concentration of total mercury in the shrimp would be that after a rainstorm ended, all significant biologically available mercury was flushed from the canal. In this case, during storms influx of mercury into the shrimp would occur as described in equation 18. During periods without rain equation 11 alone would describe mercury flux in the animal. To test this hypothesis, two observations of mercury concentrations in the shrimp were necessary, between which there was no rainfall. Over such a period mercury flux in the shrimp should follow equation 11 (i.e. efflux alone should be observed) if the hypothesis is correct. Two such determinations were made on January 14, and January 21 (see Fig. 15). The mercury concentrations observed in shrimp samples on January 14 ranged from 47 - 59 ppb with a mean of 53 ppb. Mercury on January 21 ranged from 34 - 37 ppb with a mean concentration of 36 ppb. There was no significant rainfall in

either Waikiki or Manoa between these two dates, thus loss of mercury over this interval should have followed equation 11. To test this the mean mercury concentration in the shrimp on January 14 was made equal to C_t in the equation. The time of efflux (t) prior to the observation of C_t was taken from the morning of January 14, since the last rain observed in the watershed fell prior to the morning of this date. The January 14 shrimp sample was taken in the afternoon, so $t = 0.25$ days. Equation 11 was solved for C_0 and predicted $C_0 = 58$ ppb on the morning of January 14. Applying this C_0 it was then possible to solve equation 11 for C_t on January 21, using $t = 7$ days, and to compare this value the mercury concentration observed in the shrimp on that date. The calculated value of C_t on January 21 was 27 ppb and the observed concentration of mercury observed in the shrimp on that date was 36 ppb. The calculation appeared to underestimate the observed concentration of the metal. However, only a small amount of rain fell in the Ala Wai watershed on January 13 (0.15 inches). If this rainfall was not sufficiently intense to result in a measurable release of mercury into the canal, then net loss of mercury from the shrimp would have begun on the morning of January 13. Under this condition, using $t = 1.25$ days equation 11 was then solved to $C_0 = 82$ ppb on the morning of January 13. Applying this value of C_0 , the equation predicted $C_t = 37$ ppb on January 21. The excellent fit between the observed (36 ppb) and the calculated (37 ppb) data in this case implied the basic hypothesis of the model - the disappearance of biologically available mercury after rainfall ended - could explain the observed variability in organismic mercury content.

It was possible to test the model and the hypothesis in a second case. Mercury concentrations were determined in shrimp on January 31, during a three day rainfall, and on February 6, four days after the storm ended. The mercury concentrations in shrimp ranged from 92 - 56 ppb on January 31 with a mean of 74 ppb. On February 6 mercury values ranged from 75 to 35 ppb with a mean of 55 ppb. If it is assumed efflux (following equation 11) began on the morning of February 2, then using $C_t = 55$ ppb (observed on February 6) and $t = 4$ days, C_0 on February 2 should have equaled 104 ppb. Three days elapsed between the beginning of this storm and the morning of February 2. Shrimp reach steady state when exposed for three days to dissolved mercury (Fig. 3). Thus C_0 on February 2 could have equalled C_{ss} in equation 18. It would then be possible to solve equation 18 for C_t on January 31 using $t = 1.5$ days (the time from the beginning of the storm until the January 31 sample was taken) and k_w , and compare this value with the mercury concentration observed in the shrimp on that date. The solution showed $C_t = 71$ ppb, compared to an observed concentration of 74 ppb mercury on January 31. Again the fit between values predicted by the model and those observed in the field was excellent, suggesting the validity of the model and the hypothesis that biotically available mercury was flushed from the canal after each rainstorm.

If the hypothesis of post-rainstorm flushing of mercury from the canal were incorrect (if there was a constant supply of available mercury beyond the date chosen for C_0) the calculated C_t would always underestimate the observed C_t , because of the inhibition of net

efflux due to backflux from the source of mercury. The excellent fit observed in both test cases implied there was no backflux of mercury from any source into the shrimp after rainstorms over the January - February sampling period. Thus, this hypothesis and equations 18 and 11 were used with rainfall data to generate the curves observed in Figures 15 and 16. There were several instances where there was insufficient field data to calculate mercury peaks in Figure 16. In these instances blanks were left in the curve.

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