Arch. Environm. Contam. Toxicol. 11, 539–545 (1982)

Archives of Environmental Contamination and Toxicology

# A Study of Metal Concentrations in Relation to Gill Color and Pathology in the Rock Crab

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**Abstract.** Gills of adult rock crabs, *Cancer irroratus* Say, collected near a sewage disposal site in the New York Bight apex were analyzed for copper, lead, cadmium, and silver. One gill from each animal was processed for histologic study to document pathological conditions within the tissues, and the presence or absence of fouling organisms on gill surfaces. Comparisons are made between metal concentrations in rock crab gills and published data on crustaceans from other locations.

Waste disposal sites in the apex of the New York Bight have received considerable attention by scientists, planners, and managers responsible for environmental quality and the protection of aquatic natural resources (Gross *et al.* 1976). The sewage sludge disposal site, located within the Bight apex and bordered by New York and New Jersey, is of particular concern because it has been closed to the harvesting of commercially valuable shellfish and harbors high numbers of sewage-associated bacteria of public health concern. Pearce *et al.* (1976) conducted extensive surveys on the distribution of benthic macroinvertebrates in the Bight apex and reported that during the period August 1973 to August 1974 the average number of individuals per station decreased from 417 to 174. The same authors reported that rock crabs, *Cancer irroratus* Say, were found at 46 stations in 1973 and only 10 stations in 1974. Young and Pearce (1975) previously had described shell disease in rock crabs and lobsters from the apex and noted that specimens taken near the sewage disposal site had a hydrogen sulfide odor, blackened discoloration of the gills, and black discoloration of the articulations between their jointed appendages. The end result of apparent environmental stress on crustacean health is, however, difficult to measure quantitatively, because weakened or distressed animals are likely to be consumed by predators and removed from populations that are subsampled for statistical studies.

Benthic surveys and visual estimates have served as useful indicators of temporal changes in marine invertebrates that are affected by ocean disposal practices. Analytical procedures for chemical pollutants, such as PCBs, DDT, and heavy metals have the added advantage of providing statistically valid qualitative and quantitative measurements of pollutant concentrations in biological systems. Greig *et al.* (1977) have documented heavy metal concentrations in zooplankton collected from the Bight apex, and Wenzloff *et al.* (1979) have conducted similar studies on ocean quahogs, *Arctica islandica,* and surf clams, *Spisula solidissima,* collected from Cape Cod Bay, MA, to Cape Hatteras, NC. The present report summarizes the observations on gill pathology, gill fouling, and heavy metal concentrations (copper, lead, cadmium, silver) in gills of rock crabs collected from stations near Sandy Hook Bay, NJ, and the New York sewage-sludge dumpsite. A synopsis of biological data on *C. irroratus* may be found in a recent publication by Bigford (1979).

## Methods

Ninety-seven crabs were collected along a 13.5 nautical mile transect beginning at the mouth of Sandy Hook Bay, NJ (74°

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0090–4341/82/0011–0539 $01.40

**Table 1.** Summary of *C. irroratus* collections, December 1978–June 1980

| Date | Location | No. crabs[a] | No. male[b] | No. female[c] | Comments |
|---|---|---|---|---|---|
| Dec. 1978 | Mouth of Sandy Hook Bay | 24 | 20 | 4 | 1 new molt (F) |
| Mar. 1979 | Mouth of Sandy Hook Bay | 25 | 22 | 3 | 16 new molt (M) |
| Oct. 1979 | N.Y. Bight Sewage Site | 14 | 12 | 2 | — |
| Jan. 1980 | N.Y. Bight Sewage Site | 11 | 8 | 3 | — |
| June 1980 | N.Y. Bight Sewage Site | 25 | 17 | 8 | 3 new molt (M) |
| Totals | | 99 | 79 | 20 | 20 new molt |

[a] Two crabs lost—metal analyses on 97
[b] Carapace width in cm. Range—5.5–13.0, Mean—9.1
[c] Carapace width in cm. Range—6.5–8.5, Mean—7.3

00.9′N, 40°28.9′W) and extending eastward to the inshore edge of the sewage disposal site (73°43.3′N, 40°27.5′W). Sixty-six specimens were taken at the sewage site and 31 at the mouth of the Bay; station depth ranged from 20 to 30 m. Collections were scheduled to coincide with seasonal molting times to obtain both intermolt and newly molted animals (Table 1). At the time of collection, each crab was sexed, measured, and opened to record gill condition as clean (white, yellow), discolored (tan to dark brown), <50% black, or >50% black. One gill was placed in Davidson's or Bouin's fixing solution for histological processing, and the other 9 were placed in separate clean, washed plastic vials and frozen until processed for heavy metal analyses. Gill tissue was analyzed for metal content (copper, lead, cadmium, silver) by placing them first in 50 ml Pyrex beakers and then digesting them for 1 to 2 days in quartz-distilled nitric acid. After evaporating the samples to dryness, they were heated in 2 to 3 portions of 1 ml each hydrogen peroxide and taken again to dryness. The residue was taken up with 7% (v/v) $H_2O$-nitric acid, filtered through Whatman No. 2 FP, and brought to a final volume of 10 ml. The nitric acid solution was analyzed directly with a graphite furnace (Model 2100) on a Perkin-Elmer atomic absorption instrument using conditions recommended by the manufacturer. Metal concentrations were calculated on a ppm wet weight basis. Preserved gills were processed by routine histological methods, sectioned at 6 and 12 $\mu$m, and stained with Harris' hematoxylin staining solution and by the Feulgen reaction for DNA. Stained sections were examined for evidence of melanized nodules within the tissue, epiphytic bacteria, diatoms, ciliate protozoa, amoebae, and ectoparasitic copepods. Additional records were made to list crabs with swollen or ballooned lamellae, necrotic lamellae, and coagulated hemolymph. Each crab was categorized as severely affected by black gill disease, moderately affected by gill discoloration other than black, or apparently healthy with clean gills. The three groups were then evaluated with respect to heavy metal burdens to determine whether there was an association between gill pathology and metals concentrations. Separate tabulations were made to determine differences in metal concentrations between intermolt and post-molt specimens.

## Results

Rock crab collections during the period 1978 to 1980 (Table 1) showed that seasonal differences had a direct influence on the number(s) of crabs caught at different times of the year. In March 1979, newly molted animals that were leaving Sandy Hook Bay were plentiful at the station located at the mouth of the Bay, and again were caught in abundance in June 1979 and 1980 when most of them had returned to the ocean (June 1979 specimens were lost in transit and a replacement collection was made in June 1980). In October 1979 and January 1980, crabs were scarce at the ocean stations, possibly because of shoreward migrations which precede the winter molting period for adult males (Sawyer in press). All of the crabs selected for study were adults that measured from 5.5 to 13.0 cm in carapace width, 77 males and 20 females (Table 1). Twenty of the 97 animals processed for histology and metal analyses were recently molted papershells; 19 were males and 16 of them were caught in March 1979. Although black gills were observed only in one specimen (Table 2), gill fouling organisms and tissue pathology were not uncommon (Table 3). The incidence of fouling organisms was unremarkable with respect to earlier observations (Sawyer *et al.* 1979; Bodammer and Sawyer 1981).

### *Heavy Metal Analyses*

*Copper:* Copper (Table 4) ranged from 2.6 to 110 ppm (mean—22.5) with 75% (73/97) of the gills showing 30.0 ppm or less. Except for two high values of 65.8 and 110.0 ppm, 98% (95/97) of the specimens did not exceed values of 41.7 ppm. Twenty-five percent (24/97) of the crabs had less than 12.0 ppm of which 58% (14/24) were recently molted papershells. Among the remaining 10, 6 were females and 4 were males. Copper in papershell crabs ranged from 2.6 to 14.5 ppm and only

**Table 2.** Summary of *C. irroratus* gill color, December 1978–June 1980

| Date | No. crabs | Clean (%) | Discolored (%) | <50% Black (%) | >50% Black (%) |
|---|---|---|---|---|---|
| Dec. 1978 | 24 | 17 | 6 | 1 | 0 |
| Mar. 1979 | 25 | 17 | 8 | 0 | 0 |
| Oct. 1979 | 14 | 10 | 2 | 2 | 0 |
| Jan. 1980 | 11 | 8 | 0 | 2 | 1 |
| June 1980 | 25 | 16 | 8 | 1 | 0 |
| Totals | 99 | 68 (68) | 24 (24) | 6 (6) | 1 (1) |

**Table 3.** Summary of histological findings on gills of *C. irroratus,* December 1978–June 1980

| Date | No. crabs | Debris (%) | Bacteria (%) | Diatoms (%) | Nodules (%) | Ciliates (%) | Amoebae (%) | Copepods (%) |
|---|---|---|---|---|---|---|---|---|
| Dec. 1978 | 24 | 21 | 16 | 1 | 5 | 1 | 1 | 3 |
| Mar. 1979 | 25 | 23 | 8 | 1 | 4 | 3 | 3 | 2 |
| Oct. 1979 | 14 | 6 | 10 | 1 | 2 | 2 | 2 | 4 |
| Jan. 1980 | 11 | 4 | 4 | 3 | 2 | 0 | 1 | 2 |
| June 1980 | 25 | 18 | 14 | 1 | 17 | 2 | 4 | 7 |
| Totals | 99 | 72 (72) | 52(52) | 7 (7) | 30 (30) | 8 (8) | 11 (11) | 18 (18) |

**Table 4.** Heavy metal concentrations (ppm wet wt.) in gills of *C. irroratus,* n = 97

| | | All Crabs | | Mean value for |
|---|---|---|---|---|
| Metal | Range | Mean | S.D. | newly molted crabs |
| Copper | 2.6–110.0 | 22.5 | ±14.4 | 8.55 |
| Lead | 0.2– 52.0 | 3.8 | ± 6.9 | 0.85 |
| Cadmium | <0.1– 2.8 | 0.7 | ± 0.4 | 0.45 |
| Silver | <0.1– 5.1 | 0.9 | ± 0.7 | 0.30 |

4 of them had over 10.9 ppm. The range of copper (2.6 to 110 ppm) in gill tissue was extremely wide within the sample size of 97 animals, thus precluding the possibility of obtaining meaningful results from statistical tests. The data suggest that further testing of 2 or 3 more comparable collections will provide sufficient observations to confirm our present findings. Data analyses suggested a range of approximately 10 to 50 ppm as occurring most frequently in intermolt crabs with values of 10 ppm or less being most frequent in newly molted animals. There was no distinct association between sex or size of crabs and differences in copper concentrations.

*Lead:* Lead (Table 4) in all crabs ranged from 0.2 to 52.0 ppm (mean—3.8). Seven specimens (7%) had more than 10 ppm lead (10.4 to 52.0), and 19 had more than 5.0 (5.8 to 52.0). Among those with more than 5.0 ppm, 12 were males and 7 were females, indicating that, as with copper, the higher values did not differ with sex or size. Approximately 35% of the animals had less than 1.0 ppm and 80% had less than 5.0 ppm.

*Cadmium:* Cadmium (Table 4) levels ranged from <0.1 to 2.8 ppm (mean—0.7) with over 1.0 ppm occurring in 17% (17/97) of the crabs, of which 5 were females and 12 males. Three animals had over 2.0 ppm (2.1 to 2.8); 2 of them were females and 1 was male. As with copper and lead, the size and sex of the crabs did not influence cadmium concentrations.

*Silver:* Silver (Table 4) in all crabs ranged from less than 0.1 to 5.1 ppm (mean—0.9) with over 1.0 ppm occurring in 25% (24/97), 9 males and 15 females. There was no influence of size or sex on silver concentrations below 1.0 ppm, but the three values over 2.5 ppm (2.6, 3.0, and 5.1 ppm) occurred in females.

### *Gill Pathology*

Dense growths of fouling organisms or extensive gill pathology were found in 40% (12/31) of the crabs with discolored, partially blackened, or completely blackened gills. Thus, direct visual observations, supported by histological studies, showed that discoloration or blackening of the gills was not always associated with microbial fouling or tissue abnormalities. Similarly, metal concentrations exceeding 40 ppm copper, 5 ppm lead, or 1 ppm cadmium or silver were found in less than 50% of the crabs with discolored or blackened gills. Among 9 specimens with blackened or severely discolored gills (Table 5), only one had 110 ppm copper, the highest value obtained for this metal (Table 4), and none of them yielded the maximum value obtained for lead, cad-

**Table 5.** Heavy metal concentrations (ppm wet wt.) in nine individuals of *C. irroratus* with slight to extensively black or dark brown gills[a]

| Sex | Width | Cu(22.5) | Pb(3.8) | Cd(0.7) | Ag(0.9) |
|---|---|---|---|---|---|
| M | 11.5 | 33.4 | 2.8 | 0.9 | 2.0 |
| M | 12.0 | 28.5 | 3.7 | 0.5 | 0.3 |
| M | 10.5 | 26.2 | 1.7 | 0.9 | 0.8 |
| M | 11.0 | 110.0 | 11.6 | 1.1 | 1.5 |
| M | 9.5 | 4.9 | 2.6 | 0.49 | 0.35 |
| M | 8.5 | 33.7 | 8.6 | 1.1 | 0.31 |
| M | 10.0 | 34.8 | 0.6 | 0.51 | 0.81 |
| M | 7.0 | 39.7 | 1.3 | 1.7 | 0.79 |
| M | 7.0 | 34.1 | 4.6 | 2.1 | 0.81 |

[a] Note overall mean value from 97 *C. irroratus* given in ( ) after each metal

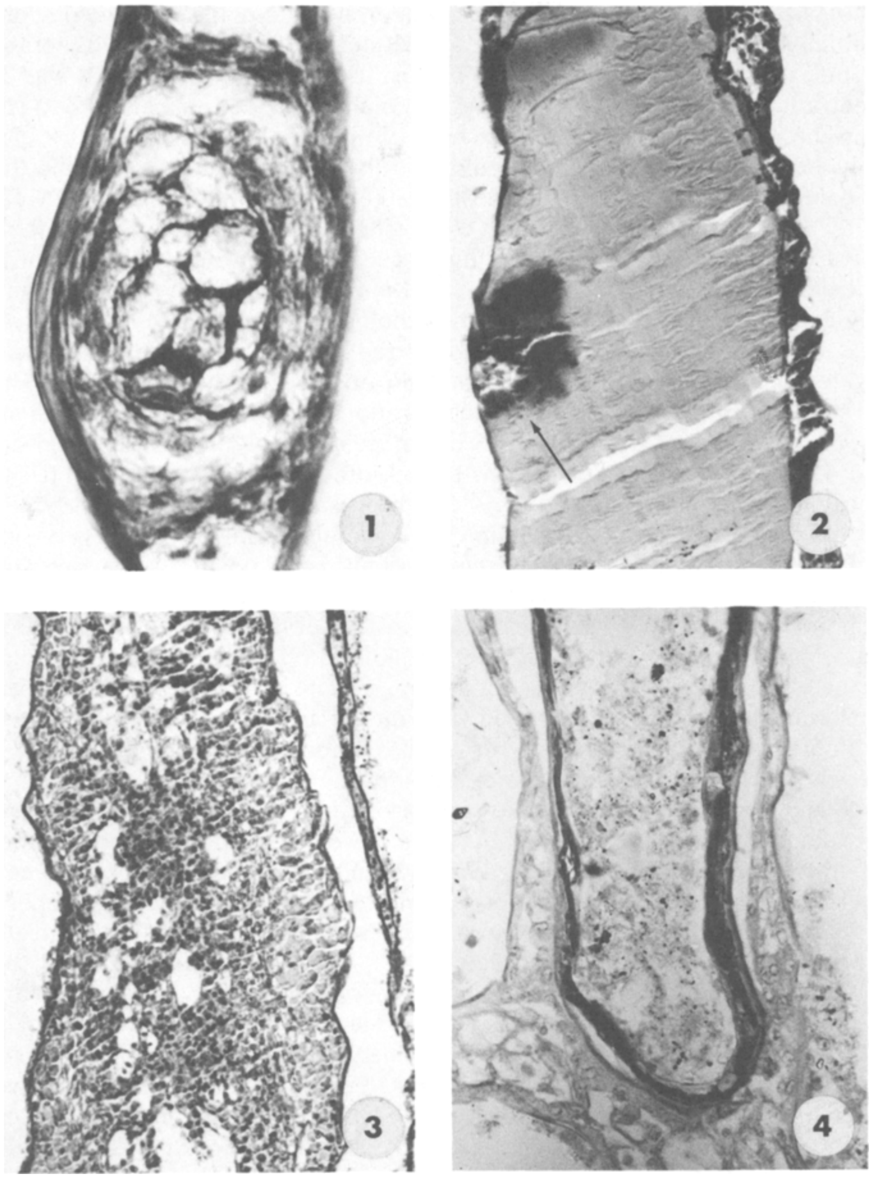
mium, or silver (Table 4). Gill color was useful for monitoring the incidence of black gill condition in *C. irroratus* but was not associated with the incidence of microbial fouling, tissue pathology, or heavy metal concentrations.

Four distinct types of pathological response were noted in the gill filaments (Figures 1–4) but none of them could be attributed to specific biological or chemical agents. The first response (Figure 1) consisted of cell clumps or aggregates (nodules) that progressively became necrotic and melanized. The second response (Figure 2) was the swelling of individual gill filaments filled with coagulated hemolymph and sometimes showing a small area of focal necrosis. The third response (Figure 3) was the swelling of individual filaments that showed extensive hemocyte infiltration rather than coagulation of the hemolymph. The fourth response (Figure 4) was melanization and associated blackening of the gill epicuticle. Similar melanization sometimes involved entire filaments that were blackened, brittle, and broken. The four types of lamellar pathology are well known in various species of crustaceans experimentally exposed to a variety of chemical and biological agents. The four illustrations document advanced stages of response to disease or injury in wild-caught specimens that could not be attributed to a specific cause. Small cell clusters (nodules) were observed in 30% of the specimens (Table 3). They were present in small numbers, usually 1 to 4 per 6 $\mu$m section, except in one specimen in which 20 were counted. Swollen filaments, coagulated hemolymph, hemocyte infiltration, or melanization and necrosis were noted in approximately 20% of the individuals examined.

## Discussion

Collections were made in locations known to be contaminated with heavy metals from barge-delivered wastes, inputs originating from industrial, commercial, and residential sources from Raritan Bay, NJ, and the Hudson River in the north and northwest. Greig and McGrath (1977) analyzed sediments from Raritan Bay and found dry weight values that ranged up to 1,230 ppm copper, 985 ppm lead, and 15 ppm cadmium. Further east and across the mouth of Sandy Hook Bay, NJ, the same authors reported decreased sediment values of 13 to 330 ppm copper, 16 to 24 ppm lead, and less than 0.3 to 13 ppm cadmium, values which were well within the wet weight range obtained from *C. irroratus* gills. Greig *et al.* (1977) studied metal concentrations in zooplankton samples from the New York Bight and found that those samples containing 95–100% *C. irroratus* larvae had dry weight values of up to 54.4 ppm copper, 21.5 ppm lead, 2.6 ppm cadmium, and 12 ppm silver, all considerably less, except for lead, than that found in adult intermolt crabs. Wenzloff *et al.* (1979) found that heavy metal levels (copper, silver, zinc, arsenic) in the surf clam, *Spisula solidissima,* and the ocean quahog, *Arctica islandica,* decreased southward from Montauk Point, NY, to Cape Hatteras, NC. Maximum wet weight concentrations found in one or the other mollusc species were: copper—1.16 ppm, lead—1.8 ppm, cadmium—0.54 ppm, and silver—2.62 ppm, all considerably less, except for silver, than that found in rock crabs. It was not possible to estimate the range for each metal that represents the naturally occurring *in situ* value for healthy crabs since there are few published accounts of metals in physiologically healthy cancroid crabs.

Copper and zinc have received some attention in recent studies on *C. irroratus*. Martin (1974) analyzed whole body tissue levels of copper in 18 intermolt adult crabs and obtained a mean value of 19.9 ± 18.8 ppm. In a later study, Martin (1975) analyzed copper in the exoskeleton, hepatopancreas, hemolymph, hypodermis, gills, and muscles of *C. irroratus* throughout the prepost- and intermolt cycle; the highest copper concentration was found (wet wt.) in the hemolymph (45 ppm), followed by the exoskeleton (42 ppm), hepatopancreas (27 ppm), hypodermis (23 ppm), gill (22 ppm), and muscle (17 ppm). Intermolt specimens and those in the peeler stage which precedes molting and ecdysis had higher copper in the hemolymph (45 to 46 ppm) than did crabs in other stages of growth. Gills of newly molted animals had 9 to 10 ppm copper and gills of intermolt stages had 12 to 22 ppm. Bryan (1968) studied *Cancer pagurus* and found a mean copper concentration in the gills of 32 ppm and stated that most decapod crustaceans contained approximately 20 to 35 ppm copper in their gills. Until larger numbers of *Cancer* crabs are studied, our



**Figs. 1–4.** Gill pathology in rock crabs, *Cancer irroratus*, collected from waters of the New York Bight apex. Harris' hematoxylin-eosin stain.
**1.** Focal nodule occluding gill filament; note "honeycomb" appearance of the nodule. (640×).
**2.** Swollen gill filament with dense coagulated hemolymph and focal area of necrosis (arrow). (640×).
**3.** Gill filaments with extensive hemocyte infiltration. (640×)
**4.** Swollen filament with black melanization of cuticle. (300×)

data are in agreement with previous reports; lowest copper values were found in post-molt adults and with a mean value of 20 to 30 ppm obtained from intermolt adults. Within the limits of sample size, and previously published data, the two highest copper values (65.8 and 110.0 ppm) probably represent levels that exceed normal physiological limits for *C. irroratus*.

Lead showed an uneven distribution of less than 0.2 to 52.0 ppm, with a mean of 3.8. Fourteen males and 9 female crabs had lead concentrations exceeding the mean. It was not possible to locate previously published data on lead concentrations in *C. irroratus* and it must be speculated that concentrations greater than the mean of 3.8 ppm lead suggest atypical metal burdens. Chow *et al.* (1976) examined lead levels in *Cancer antennarius* from California and reported a wet weight value of 0.75 ppm in the shell. They also analyzed for lead in 14 species of molluscs and found a maximum concentration of 2.05 ppm in the cockle, *Prototheca staminea*. Wenzloff *et al.* (1979) reported less than 0.7 ppm lead (wet wt.) in surf clams, *S. solidissima*, and up to 1.8 ppm in ocean quahogs, *A. islandica*. Greig *et al.* (1976) analyzed 7 species of fish and found a maximum lead concentration of 2.4 ppm in the liver of *Seriola* sp. We have analyzed *C. irroratus* gills from the Philadelphia-Camden sewage dumpsite (work in progress, unpublished) and 6.8 ppm lead is the highest value obtained to date. Because of the relatively low levels of lead reported previously in vertebrates and invertebrates in the

New York Bight apex (Greig *et al.* 1976, 1977; Wenzloff *et al.* 1979), we did not initially appreciate the potential value of lead as an indicator of high metal levels in *C. irroratus*. Concentrations of over 10 ppm in 7 of the 97 animals from the New York dumpsite suggested that lead might be one of the more important metals to be considered for further study.

Cadmium concentrations ranged from less than 0.2 to 1.0 ppm in 82% (80/97) of the crabs and from 1.0 to 2.8 ppm in the others. Only 3 specimens (1 male, 2 females) had high values, 2.1, 2.3, and 2.8 ppm, respectively. The relatively low concentrations of cadmium in gill tissue did not show differences with respect to sex of crabs, or to seasons of the year and molting activity. Overnell and Trewhella (1979) have shown that cadmium may be about four times higher in the hepatopancreas than in the gills of *Cancer pagurus*. They found that maximum cadmium concentrations (mean values) in tissues from five specimens increased in the following order: hemolymph 0.3, claw muscles 0.9, gills 1.1, dermis 4.0, and hepatopancreas 7.0 ppm, respectively. They also showed that crabs had copper and cadmium binding metallothionein enzyme in the hepatopancreas which, after feeding experiments, yielded values of over 148 ppm cadmium (mean value) in that organ. Further studies with *C. irroratus* are in progress in our laboratory to determine whether cadmium in the hepatopancreas is present in higher concentrations than in gills. The importance of cadmium in the environment has been recognized in Sweden where the Swedish government has banned major uses of the metal and has stopped the importation of most cadmium-containing products (Nilsson 1979).

Silver ranged from <0.1 to 1.0 ppm in 75% of the crabs (73/97), and from 1.1 to 5.1 ppm in the others. The three highest concentrations, 2.6, 3.0, and 5.1, occurred in females, suggesting a sex-related difference in silver burdens. Further studies are necessary to test this assumption, including analyses of tissues other than gills. Only 4 of 20 females examined had discolored gills and 3 of these had the highest values determined for silver. In contrast, 3 males with 2.0 ppm or more of silver (2.0, 2.0, and 2.3) did not show a relationship between the metal and gill color; 2 had clean gills and 1 had black gills. Greig *et al.* (1977) found less than 0.5 to 1.1 ppm (dry wt.) of silver in zooplankton samples made up primarily of rock crab larvae, and Wenzloff *et al.* (1979) found concentrations of up to 2.62 ppm (wet wt.) in ocean quahogs, *Arctica islandica*. Silver uptake, like cadmium and lead, must be studied in *C. irroratus* from different geographical locations before it is possible to distinguish levels that might be indicative of atypical metal burdens.

Gill blackening, fouling, and tissue pathology did not show a well-defined association with crabs having the highest levels of heavy metals. It was of interest that the highest copper concentration (110 ppm) was found in gills of a large male (11.0 cm) with discolored gills and tissue necrosis, while the next highest level (65.8 ppm) was found in a 9.0 cm male with clean gills and no observed tissue pathology. The highest lead concentration (52 ppm) was found in a small male (5.5 cm) with clean gills and no observed pathology, while the next highest level (31.3 ppm) occurred in a 7.0 cm female with discolored gills and no observed tissue pathology. The highest concentrations of both cadmium and silver (2.8 and 5.1 ppm, respectively) occurred in 7.5 cm females with discolored gills and no observed tissue pathology. Gill necrosis, gill swelling, hemolymph coagulation, and nodule formation have been induced experimentally by exposing various crustaceans to heavy metals (Couch 1978). Similar responses have been observed in wild-caught crabs, but it is not possible to relate them to heavy-metal uptake for several reasons: (1) gill necrosis has been illustrated (Bodammer and Sawyer 1981) in association with severe gill fouling by epiphytic bacteria, diatoms and microscopic debris; (2) nodule formation may be associated with parasitic amoebae, *Paramoeba perniciosa*, in rock crabs and lobsters (Sawyer 1976); (3) gill swelling or "ballooning" may be associated with tissue damage due to copepods which possibly cause puncture wounds while feeding (Ruddell 1979; Sawyer in press); and (4) the presence and numerical abundance of nodules are determined by chance, since only small amounts of tissue are examined by microscopical techniques. Thus, cause and effect relationships between heavy metals and tissue pathology which may be demonstrated under laboratory conditions may be difficult to detect in nature because of additive factors, such as gill fouling and non-specific tissue responses to disease. Heavy metal concentrations, pathology, and gill blackening, however, may be recorded on a geographical basis and cumulative data analyzed to compare populations of animals from clean and polluted study sites. Further studies are now in progress to compare gill condition and metal burdens in *C. irroratus* from several adjacent ocean-disposal sites, and from sites that are not used for ocean dumping. Preliminary findings (now in preparation for publication) indicate that silver and lead will show discrete distributional patterns in *C. irroratus* gills, depending on their source.

*Acknowledgments.* The authors gratefully acknowledge the Office of Marine Pollution Assessment, NOAA, U.S. Department of Commerce, Rockville, MD, for encouragement and support during the conduct of the work reported herein. Further appreciation is expressed to the Histology Unit, NMFS Laboratory, Oxford, MD, for the excellent quality of stained sections of gill tissues.

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*Manuscript received August 17, 1981; accepted December 30, 1981.*