BLACK-GILL DISEASE IN THE ROCK CRAB, CANCER IRRORATUS ASSOCIATED WITH SEWAGE-DUMPING IN COASTAL WATERS OF MARYLAND, DELAWARE, AND NEW JERSEY

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INTRODUCTION

Intensive research by state, federal, and private institutions has been directed to determining the effects, or lack of effects of ocean-dumping practices on coastal marine resources. A considerable amount of information derived from such studies has been accumulated on the build-up of heavy metals, chemical wastes, and sewage-associated bacteria in bottom sediments but very limited historical data are available for estimating the extent of environmental deterioration or improvement with time. The pros and cons of the impact of ocean-dumping on the health of the ocean and its natural resources, therefore, remain a matter of considerable debate and general agreement is the exception rather than the rule. Ocean-disposal activities along the Atlantic coast of the northeastern United States provide a unique opportunity to study shortterm and long-term effects of sewage-dumping because in the New York Bight apex such practices have existed for over 40 years and in the present Philadelphia-Camden site for only about 5 years.

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Our report concerns a direct comparison of the occurrence of "black gill" disease in rock crabs, <u>Cancer irroratus</u>, collected from the Philadelphia-Camden sewage dump-site, and from the New York Bight dump-site. Data to be presented show that the black-gill condition varies seasonally with molting activity of the crabs, and is virtually absent in specimens collected from areas away from the dump-sites. The principal cause of the black color is believed to be due to the accumulation of black sewage-associated silt between the gill lamellae. Our conclusions are based on direct examinations of gills from over 4,000 rock crabs, and on microscopic studies of gill diseases in over 2,000 of them. The present report provides a detailed summary of data from

- 1 -

animals collected in the Philadelphia-Camden sites and discusses such observations in terms of their relationship to other findings in different geographical areas.

METHODS

Rock crabs were collected with an otter trawl or by recovering them from a rocking-chair dredge used for collecting clams, scallops, or oysters. Collections were made during the period December, 1975 to April, 1979 (Table 1) and all crabs were measured and sexed. One gill lamella from each crab was preserved in Davidson's fixing solution and returned to the laboratory for histological processing and staining with Harris's hematoxylin and by the Fuelgen-reaction. Each crab was then frozen and stored for future heavy metals analyses. Immediately after opening each crab the gills were observed and recorded as clean, discolored, partly black, or completely black. All stations sampled were between the 20 and 40 fathom-lines and were situated within the sewage dump-site or peripheral to it. The sewage stations were considered to be those that were located within the area that has been closed to shellfishing (Fig. 1, Table 2). A total of 610 animals of which 406 were processed for histological study, were collected during the 10 cruises. Records were kept of all microscopical findings including, fouling organisms, diseased lamellae, parasites, and copepods (Table 3). Molting activity was taken into account to explain variations in the intensity of gill-fouling.

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RESULTS

Gills of the rock crab were remarkably clean throughout the study area when collections coincided with molting activity. The number of crabs collected was not adequate to support conclusions on molting behavior of C. irroratus in the open ocean but it was noted that among 60 newly molted specimens collected during a cruise in September 1978, 27 were males and 33 were females. The data from the September cruise suggested that adult C. irroratus in offshore waters molt in late summer or early fall, and that both sexes molt at approximately the same time. Discolored or blackened gills seldom were observed during seasons which coincided with molting activity. In contrast, intermolt crabs had gills which ranged from clean to discolored or black (Table 4). Some difficulty was experienced in categorizing gill conditions as clean or discolored because each condition varied from slight to intense. In order to remove some of the subjectivity that was inherent to visual evaluations by different observers, all data were recorded in 1 of 4 categories: (1) clean, (2) discolored or with black tips or bases, (3) black for up to 50 percent of the total gill surface, (4) all gills completely black. Cumulative data for 610 crabs (Table 4) showed that 60 percent had clean gills, 38 percent had discolored or partially black gills, and 2 percent had completely black gills. When seasonal influences were taken into account, the number of crabs with black gills ranged up to 6 percent (Table 5).

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Further data analyses showed that the black gill condition varied seasonally because of the molting season in which all fouling conditions were discarded with the molted cuticle, and because of the fact that juvenile crabs molted more than once during a single year. Size frequency data (Table 6) showed that 95 percent of all crabs collected measured more than 5.5 cm in carapace width and were adults. Data in Table 6 also showed that males and females were about equally represented in the study (54 percent males, 46 percent females).

Particulate matter from between the gill lamellae showed that clean gills contained very fine sand grains or silt particles, and black gills contained fine black silt which resembled organic detritus or sewage sludge. Histologically, some of the gills showed that blackened lamellae sometimes were dead, melanized, and necrotic. Thus, some black color was due to the presence of dead gill tissue in seriously affected animals. All crabs were collected at stations located between the 20 and 40 fathom lines which run east and west of the Philadelphia-Camden dump-site. Six of the 14 crabs with black gills were caught in the immediate vicinity of the dump-site and the remaining 8 were caught within a 12 mile radius of the site. Gross and microscopic observations on gills that were severely blackened showed that such blackening was due to the accumulation of black silt between the lamallae, or to extensive disease, melanization, and death of gill tissue.

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The seasonal incidence of fouling conditions on <u>C</u>. <u>irroratus</u> as observed in histological sections of 406 of the 610 crabs is summarized in Table 7. Microscopic debris, fouling bacteria, and copepods were observed throughout all seasons of the year while epiphytic diatoms, sessile ciliates, and amoebae rarely were detected. The number of ciliates per gill section ranged from 1 - 10 and moderate to heavy infestations never were observed. The number of copepods per stained section ranged up to 39 indicating that fairly heavy infestations probably were not uncommon. Individual gill filaments sometimes were swollen with extensive accumulations of agranular hemocytes indicating that such pathology may have been associated with localized reactions to puncture wounds by ectoparasitic copepods. Swelling and congestion was noted in 13 percent (65/406) of the crab gills. The cause of such swelling

- 4 -

and congestion could not be determined solely on the basis of histologic studies but it was of interest to note that such pathology has been observed in shrimp exposed to heavy metals such as copper and cadmium. Gills with obvious congestion were necrotic and melanized to the extent that hemolymph flow was interrupted and gill structure was distorted.

The incidence of black-gill disease in <u>C</u>. <u>irroratus</u> collected in or near the Philadelphia-Camden dump-sites represented a very conservative estimate for several reasons, (1) although 10 separate collections were made, there were only 4 trips in which 50 or more specimens were collected, and (2) 3 of these 4 trips were made in August or September when it was likely that molting activity resulted in the collection of low numbers of intermolt specimens. Analyses of all data collected during the study showed that a higher incidence of black-gill disease probably would be found by timing collections to coincide with the intermolt seasons.

DISCUSSION

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Attempts to understand the cumulative effects of ocean-dumping on benthic animals often yield inconsistent results, or results that are difficult to reproduce on a predictable basis. Variable results are not unexpected when animals selected for study are migratory species, or when such species vary in abundance from year-to-year. Results from our studies with the rock crab, <u>C. irroratus</u>, suggest that it is ideally suited for pollution-related studies for several reasons, (1) it resides on the sea-bottom with little or no vertical migration, (2) it walks rather than swims and, therefore, is in constant contact with bottom sediments, and (3) seasonal molting activity by adults is predictable and field studies may be scheduled to yield intermolt crabs with

- 5 -

with relatively long-standing gill-fouling communities. Gross and microscopic observations on gills of the rock crab showed clean sand or silt accumulated between lamellae when crabs were collected from unimpacted environments. In contrast, black silts were observed when crabs were collected from stations where sediments similarly were altered or impacted by substrates that were undergoing organic decay.

Environmental data have shown that the impact of ocean disposal of chemical or sewage-wastes may not be immediately apparent in areas that have suffered only short-term effects. For example, the New York Bight apex sewage disposal sites have been used for over 40 years and much of the seabottom is covered with a "sludge-blanket" which has the consistency of "black-mayonmaise." In contrast, the present Philadelphia-Camden sewage-disposal site has been used for less than 5 years and sewage wastes have not yet accumulated to the extent that a black "sludge-blanket" has accumulated. Nevertheless, Lear & O'Malley (personal communication) have identified areas in the Philadelphia-Camden dump-site where organic carbon values are 3 to 4 times higher than background or control levels and much of the area has been closed to shell-fishing because of contamination by fecal-coliform bacteria. Because of the relative "youth" of the Philadelphia-Camden sewage disposal site, it was not surprising that only 2 percent of the rock crabs examined had "black" gills in contrast to an overall 6 percent in the New York Bight apex.

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Recent experimental studies have shown that shrimp and lobsters may react to a variety of biological and chemical agents by forming blackened or melanized foci in areas of tissue destruction (Fontaine et al, 1975; Couch, 1979). Other workers have shown that similar blackening may be caused by parasitic ciliates (Johnson and Bradbury, 1976), or certain nutritional deficiencies such as a lack of ascorbic acid (Magarelli, et al, 1979). Our studies have shown that

- 6 -

"black-gill" condition in the New York Bight and Philadelphia-Camden ocean dump-sites may be caused by the accumulation of black detritus between adjacent gill lamellae, or by extensive melanization of dead gill tissue. Affected crabs from the dump-sites often have gills in which entire lamellae may be blackened. In contrast, shrimp and lobsters which are affected by known parasitic or chemical agents often have the black color limited to discrete localized foci. Future studies on black-gill from ocean dump-sites are needed in which data are supported by analyses for fecal-coliform bacteria, coprostanol, and heavy metals.

A wide variety of studies on the influence of ocean-dumping on bottom sediments and benthic animals conducted by private, state, and federal agencies has shown that measurable affects or impacts have occurred. Uncertainties which remain to be defined include measurements of the significance of the impacts and their long-term effects, and estimates of the ability of the marine benthos to recover after the cessation of dumping practices. Our extensive surveys on gill condition in rock crabs (Sawyer et al, 1979) support conclusions from other studies on species abundance and diversity, total organic carbon levels, coliform-bacteria populations, and heavy metal concentrations in bottom sediments which document the progressive deterioration of ocean environments that are affected by ocean dumping activities.

- 7 -

CONCLUSIONS

1. The ocean disposal of sewage-wastes contributes to the progressive deterioration of coastal and offshore seabottoms.

2. Brown to black sediments composed of sewage-sludge and containing the fecal sterol coprostanol and fecal coliform bacteria are present in collecting sites which yield rock crabs, <u>Cancer irroratus</u>, with blackened gills.

3. Black gills in rock crabs may be found in 2-6 percent of the specimens collected in or near sewage disposal-sites.

4. Fouling communities made up of bacteria, diatoms, protozoans, and copepods occur on gills of rock crabs, possibly throughout their geographic range. Black gills, however, are statistically more prevalent in ocean-dumping areas than in clean or control areas.

5. Black-gill condition and tissue pathology in rock crabs from New York Bight sewage-disposal sites, and Philadelphia-Camden dump-sites were remarkably similar with the exception of the following:

a. Copepods infesting gill surfaces were considerably more abundant and frequent on crabs from the Philadelphia-Camden site than from the New York site.

b. Sessile ciliate protozoans were considerably more abundant and frequent on New York crabs than on Philadelphia-Camden crabs.

c. Diatoms on gill surfaces were considerably more abundant and frequent on New York crabs than on Philadelphia-Camden crabs. 6. Spatial and temporal differences in gill condition and fouling may be related to several factors that deserve further study:

a. New York collecting sites were close to shore and ranged from approximately 10-20 fathoms in depth.

b. Philadelphia-Camden collecting sites were offshore and ranged from
20-40 fathoms.

c. Nearshore rock crab populations molt at different times of the year than do offshore populations.

Cruise Name	Date	Number Males	Number Females	Total Examined
TOUCHSTONE	Dec. 1975	46	37	83
PICK-UP	Jun. 1976	8	10	18
HOT SPOT	Aug. 1976	34	30	64
MOGUL	Feb. 1977	16	6	22
HOMERUN	Aug. 1977	61	28	89
COLIFLOWER	Oct. 1977	11	10	21
ENCORE	Jan. 1978	10	a i ta <u>n</u> ta man	10
RIDGERUNNER	Apr. 1978	13		13
HELMALEE	Sep. 1978	120	145	265
ENFIN	Apr. 1979	12	13	25
Totals		331	279	610

Table 1. Summary of Rock Crab, <u>Cancer irroratus</u>, Collections from Philadelphia-Camden Ocean Dump-Site Stations.

Note: 54 percent males and 46 percent females or approximately a 1:1 ratio (1:0.85). Histological examinations were made on gills from 406 of the 610 specimens. Table 2. Gill Condition in Rock Crabs, <u>Cancer irroratus</u>, Collected from Philadelphia-Camden Ocean Dump-Site Stations. Percent Incidence in ().

Number of Stations	Number Crabs Examined	Clean Gills	Discolored Gills	Black Gills
		Control Statio	ons	10
28	335	230 (68.7)) 103 (30.7)	2 (0.6)
	A	cid-Waste Star	tions	
4	80	36 (45.0)) 40 (50.0)	4 (5.0)
		Sewage Static	ons	
9	195	95 (48.7)) 94 (48.2)	6 (3.1)
41	610 Mean	- 361 (59.2)) 237 (38.9)	12 (1.9)

Note: Clean gills were present in approximately 20 percent more of the crabs from control stations than in those from the dump-sites.

Table 3. Cumulative Summary of Histological Observations on Fouling-Conditions on Gills of Rock Crabs, <u>Cancer irroratus</u>, Collected from Philadelphia-Camden Ocean Dump-Site Stations. Percent Incidence in ().

No. Examined	Debris	Bacteria	Diatoms	Ciliate Protoz oa	Amoebae	Copepods
406	(62)	(4)	(3.5)	(5)	(8)	(47)
				ween section		nellae.
	naviculoi		tached to g	gill cuticle. gill cuticle		g tissue
Ciliate Pı	rotozoa – s gill cuti		rich or suc	ctorian cilia	ites attache	ed to
Amoebae -	free-livi	ng amoebae f	eeding on h	oacteria atta	ached to cut	cicle.
Copepods -	and cause	ectoparasiti localized f ed gill fila	oci of hemo	which punctu ocyte infilt	re gill and ration and s	l cuticle swelling

Table 4. Cumulative Summary of Gill Condition in Rock Crabs, <u>Cancer irroratus</u>, from Philadelphia-Camden Ocean Dump-Site. Percent incidence in ().

No.	No.	No.	No. < 50%	No. > 50%
Examined	Clean	Discolored	Black	Black
610	361 (59)	200 (33)	35 (6)	14 (2)

Note:

Clean - white, cream-color, tan, or light-mahogany.

Discolored - dark brown, gray-brown for part or all of gill filament.
< 50% black - clean or discolored with blackening at tips or bases of gill lamellae.

> 50% black - black for 50% or more of the length of the gill filament.

Table 5. Comparative Seasonal Incidence (percent) of Black-Gill Condition in Rock Crabs, <u>Cancer irroratus</u>, from Philadelphia-Camden Ocean Dump-Sites and New York Bight Apex Dump-Sites.

Location	Winter (%) JanMar.	Spring (%) AprJun.	Summer (%) JulSept.	Fall (%) OctDec.	
PhilaCamden	2/32 (6)	1/56 (2)	5/418 (1)*	6/104 (6)	
New York Bight	1/200 (0.5)	55/780 (7)	11/290 (4)	17/177 (10)	

* Includes 67 soft- or papershell newly-molted crabs. Incidence of blackgill condition low during this season because of molting activity.

Table 6. Size Distribution of Rock Crabs, Cancer irroratus,

		8	2	10
2.5 - 3.5		8	3	11
3.6 - 4.5		4	6	10
4.6 - 5.5 5.6 - 6.5		6	16	22
6.6 - 7.5		21	62	83
7.6 - 8.5		70	133	203
8.6 - 9.5		80	46	126
9.6 - 10.5		85	9	94
10.6 - 11.5		30	1	31
11.6 - 12.5		14	0	14
11.6 - 12.3 12.6 - 13.0		5	1	6
12.0 - 10.0	Totals	331	276	610

Collected from Philadelphia-Camden Ocean Dump-Sites.

*Carapace width.

Note: Based on the assumption that all crabs over 5.5 cm wide were adults then at least 95 percent (579/610) were adult specimens.

Table 7. Comparative Seasonal Incidence (percent) of Fouling Conditions in Rock Crabs, <u>Cancer irroratus</u>, from Philadelphia-Camden Ocean Dump-Sites and New York Bight Apex Dump-Sites. New York Data in ().

Number Examined	Debris	Bacteria	Diatoms	Ciliate Protozoa	Amoebae	Copepods
		Januar	y - March			
32	63 (28)	34 (37)	3 (15)	9 (4)	9 (11)	50 (13)
		Apri	1 - June			
56	55 (70)	40 (57)	0 (23)	3 (25)	3 (16)	50 (20)
		July -	September			
220	64 (65)	48 (50)	5 (21)	3 (13)	11 (18)	40 (18)
		October	December			
98	64 (79)	37 (60)	6 (30)	4 (10)	7 (35)	46 (13)
406 (472)	62 (61)	40 (51)	3.5(22)	5 (13)	8 (20)	47 (16)
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Note:

Debris - present in most gills during both molt and intermolt seasons in Philadelphia-Camden crabs but not in New York crabs.

Bacteria - same as above.

Diatoms - considerably lower incidence in Philadelphia-Camden crabs than in New York crabs.

Ciliates - same as above.

Amoebae - same as above.

Copepods - considerably higher incidence in Philadelphia-Camden crabs than in New York crabs.

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